# THE DIFFERENTIATION AND SPECIFICITY OF CORRESPONDING PROTEINS AND OTHER VITAL SUBSTANCES IN RELATION TO BIOLOGICAL CLASSIFICATION AND ORGANIC EVOLUTION:

## THE CRYSTALLOGRAPHY OF HEMOGLOBINS.

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# PREFACE.

This research was begun by me in October, 1902, and after considerable preliminary laboratory investigation I found that in the solution of my problems the crystallographic method promised at the present time to be the most likely to yield satisfactory results. Not being an authority in the science of crystallography, I associated with me in 1904 one of my colleagues, Professor Amos Peaslee Brown, upon whom has fallen especially that portion of the work which demanded the services of an expert crystallographer.

The trend of modern biological science seems to be irresistibly toward the explanation of all vital phenomena on a physico-chemical basis, and this movement has already brought about the development of a physico-chemical physiology, a physico-chemical pathology, and a physico-chemical therapeutics. The striking parallelisms that have been shown to exist in the properties and reactions of colloidal and crystalloidal matter *in vitro* and in the living organism lead to the assumption that protoplasm may be looked upon as consisting essentially of an extremely complex solution of interacting and interdependent colloids and crystalloids, and therefore that the phenomena of life are manifestations of colloidal and crystalloidal interactions in a peculiarly organized solution. We imagine this solution to consist mainly of proteins with various organic and inorganic substances. The constant presence of protein, fat, carbohydrate, and inorganic salts, together with the existence of protein-fat, protein-carbohydrate, and protein-inorganic salt combinations, justifies the belief that not only such substances, but also such combinations, are absolutely essential to the existence of life.

The very important fact that the physical, nutritive, or toxic properties of given substances may be greatly altered by a very slight change in the arrangement of the atoms or groups of molecules may be assumed to be conclusive evidence that a trifling modification in the chemical constitution of a vital substance may give rise to even a profound alteration in its physiological properties. This, coupled with the fact that differences in centesimal composition have proved very inadequate to explain the differences in the phenomena of living matter, implies that a much greater degree of importance is to be attached to peculiarities of chemical constitution than is universally recognized.

The possibilities of an inconceivable number of constitutional differences in any given protein are instanced in the fact that the serum albumin molecule may, as has been estimated, have as many as 1,000 million stereoisomers. If we assume that serum globulin, myoalbumin, and other of the highest proteins may have a similar number, and that the simpler proteins and the fats and carbohydrates, and perhaps other complex organic substances, may each have only a fraction of this number, it can readily be conceived how, primarily by differences in chemical constitution of vital substances, and secondarily by differences in chemical composition, there might be brought about all of those differences which serve to characterize genera, species, and individuals. Furthermore, since the factors which give rise to constitutional changes in one vital substance would probably operate at the same time to cause related changes in certain others, the alterations in one may logically be assumed to serve as a common index of all.

In accordance with the foregoing statement it can readily be understood how environment, for instance, might so affect the individual's metabolic processes as to give rise to modifications of the constitutions of certain corresponding proteins and other vital molecules which, even though they be of too subtle a character for the chemist to detect by his present methods, may nevertheless be sufficient to cause not only physiological and morphological differentiations in the individual, but also become manifested physiologically and morphologically in the offspring.

Furthermore, if the corresponding proteins and other complex organic structural units of the different forms of protoplasm are not identical in chemical constitution it would seem to follow, as a corollary, that the homologous organic metabolites should have specific dependent differences. If this be so it is obvious that such differences should constitute a preëminently important means of determining the structural and physiological peculiarities of protoplasm.

It was such germinal thoughts that led to the present research, which I began upon the hypothesis that if it should be found that corresponding vital substances are not identical, the alterations in one would doubtless be associated with related changes in others, and that if definite relationships could be shown to exist between these differences and peculiarities of the living organism, a fundamental principle of the utmost importance would be established in the explanation of heredity, mutations, the influences of food and environment, the differentiation of sex, and other great problems of biology, normal and pathological.

To what extent this hypothesis is well-founded may be judged from this partial report of the results of our investigations: It has been conclusively shown not only that corresponding hemoglobins are not identical, but also that their peculiarities are of positive generic specificity, and even much more sensitive in their differentiations than the "zoöprecipitin test." Moreover, it has been found that one can with some certainty predict by these peculiarities, without previous knowledge of the species from which the hemoglobins were derived, whether or not interbreeding is probable or possible, and also certain characteristics of habit, etc., as will be seen by the context. The question of interbreeding has, for instance, seemed perfectly clear in the case of *Canidæ* and *Muridæ,* and no difficulty was experienced in forecasting similarities and dissimilarities of habit in *Sciuridæ, Muridæ, Felidæ*, etc., not because hemoglobin is *per se* the determining factor, but because, according to this hypothesis, it serves as an index (gross though it be, with our present very limited knowledge) of those physico-chemical properties which serve directly or indirectly to differentiate genera, species, and individuals. In other words, vital peculiarities may be resolved to a physico-chemical basis.

EDWARD TYSON REICHERT.

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# INTRODUCTION.

The extraordinarily large number of researches suggested by the hypothesis stated in the Preface are so vast in their scope as to enter every domain of the science of living matter, normal and abnormal; and they are so diversified and exacting in their technical requirements that at the outset of our work we realized the necessity, for the time being, of limiting our studies by means of practically a single method of investigation and to a single substance. The crystallographic method was selected, for the reasons stated; and one of the proteins was selected because these substances are universally recognized as constituting the most important class of the body constituents, and also because it would seem that modifications in their molecules would be more likely to occur and to be of a more far-reaching influence and importance than in those of other vital substances. Unfortunately, however, the study of the chemistry of proteins has proven so extraordinarily abstruse that our knowledge is still in an early formative stage. It has only been within very recent years that any really important progress has been made; and notwithstanding a large amount of laboratory investigation and the accumulation of a voluminous literature, our information is still largely of a rudimentary and fragmentary nature.

The undeveloped state of the science of proteins is perhaps nowhere more evident than in the absence of any classification that seems to be other than of a purely tentative character, in the absence of satisfactory knowledge of such fundamental subjects as the molecular constitution of even the best-known proteins, and in our very incomplete data of the primary dissociation products. The methods of analysis of proteins into the primary dissociation products are not only not strictly quantitative, but also very imperfect qualitatively. The figures of different investigators for a given protein often differ quite as much as for different substances, and there are yet large percentages to be accounted for, such as 30 per cent of globin, 74 per cent of egg albumin, 57 per cent of serum albumin, 55 per cent of serum globulin and of fibrin, 59 per cent of lactalbumin, 83 to 90 per cent of proteoses, 39 to 54 per cent of plant globulins, 40 to 65 per cent of glutelins, 25 to 50 per cent of gliadins, etc. In fact, only one of the proteins, salmine, which is one of the simplest, has been fully accounted for, but even here the data are unsatisfactory, chiefly because a larger per cent (110.5) has been recorded than can exist; Abderhalden found leucin and alanin, which is not admitted by Kossel and Dakin; and there is doubt as to the purity of the substance analyzed, there being several similar bodies in the spermatozoa of the same species. The data in regard to the other protamines are very incomplete, almost wholly qualitative, and by no means conclusive.

Whether or not the corresponding proteins of different species of animals or of plants are chemically identical had not, up to the inception of this research, in any instance been conclusively determined. It is true that in certain instances differences have been noted, but these, as a rule, could readily be accounted for without the assumption of chemical differences inherent in the molecules; and in the exceptional cases it is not certain whether we are dealing with normal individuals or with several individuals or with contaminated individuals, etc. For instance, the fact that the egg-white of the eggs of certain species remains perfectly clear upon boiling, while that of other species becomes opaque, might be taken as meaning a difference in chemical composition, but the difference has been shown to lie in the different amounts of alkali and saline present. That the egg-albumin of the chicken, pigeon, and seed-crow has been crystallized, while the experimenter has not been successful with the egg-albumin of other species, may imply nothing more than different conditions extrinsic to the molecules. The caseinogens of woman's and cow's milk are looked upon as being not identical, yet the primary dissociation products show a great similarity and the elementary analyses of the caseinogen of the cow, goat, and rabbit are identical. The centesimal analyses of corresponding albumins and globulins have failed to show any positive differences. Oppenheimer states, from the results of a recent study of the serum albumins of man, the horse, and ox, that serum albumin is a uniform and specific substance, and that the elementary analyses point to one serum albumin. In the case of the hemoglobins the differences in the analyses of the hemoglobins of different species are not, with rare exceptions, greater than those of the hemoglobins from different individuals of the same species. Osborne's assertion that the glutenins of wheat and rye are not chemically alike is founded on the fact that "wheat flour readily yields gluten consisting of gliadin and glutenin, whereas rye flour does not." (Extract from a letter from Professor Osborne in response to an inquiry for data justifying his statement.)

Crystallographic differences believed by Gürber to be shown by the serum albumins of the horse and rabbit were disproved by his pupils; the crystals of lactalbumin closely resemble or are even identical with those of serum albumin. The statements made from time to time that the hemoglobins of different species can be distinguished by differences in crystalline form have been contradicted, and have been based upon manifestly inadequate investigation. Positive differences have been noted, but the differences between the forms of the crystals of different species have not been greater than the differences in the forms obtained from specimens of blood from individuals of the same species that have been recorded by the same or by different investigators. Preyer, in his well-known monograph Die Blutkrystalle, Jena, 1871, refers in a table (see Chapter V) to the crystalline forms of the hemoglobin from 44 species, and with rare exceptions the crystals are described as prisms, rods, plates, and needles, and occasionally as six-sided rods or plates. All of the crystals, when classified according to system, have been assigned to the rhombic system, except one, and possibly three other instances where they are classified as hexagonal. Indeed, in these exceptional instances we have proven that the crystals do not belong to the hexagonal system. While seeming differences have been recorded in the shapes of the crystals of different species, differences quite as marked have been described in the crystals of the same species by different observers. Thus, the crystals from the blood of the guinea-pig have been recorded by one as occurring occasionally as octahedra, by another as tetragonal, by another as tetrahedra and rhombic plates and prisms, and by another as six-sided plates; squirrels' crystals are almost invariably described as hexagonal plates, but they have also been seen as needles and rhombic prisms; crystals of the mouse are variously referred to as fine needles, or six-sided plates, or small prisms; rats' crystals are described as tetrahedra, prisms, rods, plates, needles, or hexagons.

Even the processes for preparing protein crystals are, except those of the hemoglobins, very limited in their range of usefulness, as has been shown by the failure to obtain crystals of even corresponding proteins save in a very limited number of instances. Moreover, the processes are so far from perfect that crystalline condition *per se* is not a guarantee that we have necessarily either a pure or a *normal* body; nor are such crystals free albumin, free globulin, etc., but acetates or sulphates or other forms of combination, etc. Moreover, when purification of proteins has been sought by repeated recrystallization it is by no means clear that the processes have not given rise to abnormal substances through a stripping off of very unstable or feebly combined radicals which constitute normal components of the molecules, and which of course must contribute to giving the substance its peculiar properties. In the case of the hemoglobins it has been shown that decomposition products are formed at each step of recrystallization. It has even been found that crystalline habit may be so affected by recrystallization that a hemoglobin which normally appears in the form of hexagons may crystallize only in rhombic needles and tetrahedra.

We are not, however, without certain data which indicate differences of corresponding proteins. The very fact of the breaking down of the serum albumin and the serum globulin of the food during the processes of digestion, when these substances are in a natural state, can not be due to a non-absorbability, because in such form they may be rapidly absorbed under appropriate conditions, and it is at least suggestive that the degradation of the protein molecules must be, in part at least, for some important purpose in relation to the synthesis of the proteins of the individual. To what extent this disintegration is carried out we do not know, but from our present knowledge it is probable that the molecules are broken down into essentially the primary dissociation products. In what ways and from what derivatives the proteins of the individual are built up is a matter of speculation, but it seems at least that such analyses and syntheses mean a differentiation of the proteins of the food and the corresponding proteins of the animal.

The best instance on record which positively indicates or shows chemical differences in homologous proteins was brought to light by Kossel and others several years (1904 *et seq.*) after this research was begun. The differences in the protamines in elementary composition, in rotatory power, and in the primary dissociation products in both percentage and groups, seem to be conclusive that these substances (assuming their purity) are not identical. If Griffiths's work on the achroglobulins be confirmed, we have another such instance. Other indications of differentiation are shown in the difference in the globin of the horse, bullock, and dog on the one hand and that of the goose on the other; the "precipitin test," by which can readily be shown certain zoölogical differentiations of the blood, milk, and flesh extracts, is admitted to depend upon specificities of proteins; hemoglobins of different species are recorded as differing in solubility, decomposability, water of crystallization, crystallizability, color intensity, and absorptive power in relation to O and $CO_2$; in contradiction to Hüfner, who describes such a striking identity and constancy of the extinction coefficients and quotients of the oxyhemoglobin of all species, we have sufficient evidence in literature to show that these coefficients do vary in different species. Differences have been noted in several hemocyanins in regard to the degree of dissociability of the O and $CO_2$, and to the temperature of coagulation; and there are indications of differences in echinochromes and chlorocruorins.

If chemical differences exist in corresponding proteins they seem to be of so subtle a nature, except in rare instances, as to be beyond the possibilities of the present methods of chemical distinction. It was therefore believed that some other method might bring success where the chemist has failed. As it is recognized that crystalline form may depend upon either chemical composition or constitution, it seems that the method of investigating microscopic crystals as developed by Sorby, and later by Zirkel, Rosenbusch, and others, and the resulting lithological microscope with its various attachments, might afford the means of obtaining satisfactory results. By this method of investigation an entire science, the science of petrography, has been built up. The "optical reactions" thus obtained are often as distinctive, and even as exact, as the chemical reactions, and this instrument is now used by the petrographer and chemist alike for the study of crystals too small to be examined in the usual way. Thus the crystals may be studied in the solution in which they are formed, and fairly accurate measurements may be obtained of their plane angles and various optical properties. Inasmuch as the optical properties, which are dependent upon the internal tensions of the crystal, are often more distinctive than the exterior form, and since even "isomeric substances possess different crystal structures" (Groth), it will be readily seen that this method of investigation may show differences which at present may be or are too obscure for the chemist.

Thus far only a very limited number of the proteins have been obtained in crystalline form. A number of hemoglobins and hemoglobin compounds and derivatives, serum albumin, lactalbumin, casein, vitellin, a number of globulins from seeds and nuts (some of them being recorded as albumins), the albumin and globulin of egg-white, hyalin, two proteins from abnormal urines (one of which is a casein-like body and the other probably a heteroproteose), ichthulin (probably a lecithoprotein) from the eggs of fish, glutokyrin, hemocyanin, and phycoerythrin and phycocyanin of algæ, include all, as far as we have been able to find, that have been obtained in crystals. Excepting hemoglobin, the crystallographic studies of proteins have been very limited and inconclusive, and in so far as this substance is concerned it is clear, from a study of the literature of the subject, that the inquiries have with rare exceptions been of so superficial a character as to possess little or no intrinsic value in indicating positive chemical differentiation. Among the literature on hemoglobin we have found only very rare instances where an adequate study was reported of the geometric characters of the crystals, and we have failed to find any quantitative data of any value in regard to the optical characters of the crystals in polarized light that might be of service in showing zoölogical differentiations; yet these very characters, we believe, will be found to prove the best and most easily applied means of differentiating the crystals of hemoglobin and of showing the identity or non-identity of chemical composition or chemical constitution. The comparative readiness with which hemoglobin can be crystallized, together with the exceptional importance of this protein in animal life, led to its selection as the subject of study.

The important problems next demanding our attention were in regard to the methods to be adopted to obtain graphic records of the crystals, to the methods for preparing the crystals, and to the sources of supply of the numerous and diverse kinds of blood required in order to yield the necessary data. As to the first, experience has demonstrated that line-drawings, lithography, perspective drawings, photomicrography, etc., each has its advantages and disadvantages, yet it goes without saying that while line-drawings are absolutely essential in the geometric descriptions of crystals, the only means of reproduction which eliminates the personal factor and gives at the same time a permanent and faithful record for verification and further study lies in the photomicroscope and its accessories. The generally very poor reproductions of photomicrographs of crystals that have appeared in print, together with the usual extreme unstability of hemoglobin crystals, those of certain bloods melting at temperatures scarcely above the freezing-point, seemed to us upon first thought to render this method impracticable, except to a limited degree. Moreover, we feared that, owing to the fact that the crystals and the solution in which they have been formed are generally of so nearly the same color and tint, satisfactory reproductions for printing would be found to be practically impossible; but these difficulties we overcame.

All of our photomicrographic negatives were made with ordinary laboratory apparatus. We used a standard Bausch and Lomb microscope, and almost without exception a 2-inch eyepiece and a $\frac{2}{3}$ objective, which gave us a magnification in our negatives of about 250. Occasionally we used higher powers, giving us a magnification of about 500, 800, and 1,200. Many of our negatives are not up to the standard we sought, because of the great sensitivity of many of the crystals to the slightest increase of temperature during the focusing and exposure in the photomicrographic apparatus, or to the little or no contrast in the color of the crystals and solution, in many instances the crystals being discernible solely by the shadows of their outlines. The difficulties of the last instance were usually successfully met by the selection of a proper quality of gelatin plate and by careful development and printing, which often brought out marked contrasts. We occasionally resorted to the use of color screens with excellent results, but in general they were not found necessary or of any particular advantage. The negatives, about 2,500 in number, were made by Dr. Reichert, from which we have selected 600 to illustrate the text.

The line-drawings, numbering 411, were made by Charles Travis, Ph.D., instructor in geology and mineralogy in the University of Pennsylvania, to whom we are especially indebted for the great care and accuracy with which the work was done.

While a large number of methods for preparing hemoglobin crystals in large or small quantities have been published, it was found that in order to obtain satisfactory results we should have to devise means whereby we could have better control over the rapidity of crystallization, and also to avoid any method which might injuriously affect the hemoglobin molecule. We therefore devised methods for promoting or retarding crystallization. By the former we have obtained crystals from small quantities of blood which had not heretofore been obtained; and by retarding crystallization we have secured measurable crystals from blood in which, on account of their rapid crystallizability, it has heretofore been impossible or difficult to develop them. Moreover, by modifications of our processes we have in specimens of blood of certain species been enabled to crystallize at will one or another of several forms of oxyhemoglobin normally present in the same blood. The pernicious effects of alcohol and of recrystallization led to the avoidance of these agents.

Finally, it was obvious that a successful outcome of our research demanded an examination of specimens not only from a large number of species, but also from species related and unrelated, so as to permit of a critical examination of possible generic, family, and other peculiarities. Such supplies as might be obtained from domesticated animals and such small wild animals as could be secured within the possibilities of our grant from the Carnegie Institution of Washington we realized could not meet our necessities. We therefore sought the coöperation of those in authority at the various zoölogical gardens of this country for specimens of blood from animals that died. A circular letter was forwarded by President R. S. Woodward to the management of each garden, and from a number of them we obtained assistance. We are also indebted to Dr. S. Weir Mitchell and to Dr. Charles D. Walcott for assistance in securing material. Specimens were received from Mr. Stone, Rochester Park, N. Y.; Mr. M. P. Hurlbut, commissioner of parks and boulevards, Detroit; Dr. H. H. Donaldson, Wistar Institute of Anatomy; Mr. Ernest Tretow, Highland Park, Pittsburg, Pa.; Mr. P. P. Randolph, Zoölogical Gardens, Seattle, Washington; Mr. R. G. Rau, Zoölogical Park, St. Joseph, Mo.; Dr. Herbert Fox, pathologist of the Zoölogical Society of Philadelphia; Mr. Charles H. Townsend, superintendent of the New York Aquarium; Mr. H. A. Surface, State zoölogist of Pennsylvania; Dr. Frank Baker, National Zoölogical

Park, Washington, D. C., and Dr. W. Reid Blair, pathologist of the New York Zoölogical Park. To Dr. John R. Mohler, chief of the division of pathology, U. S. Department of Agriculture, Washington, D. C., we are especially indebted.

We have had at our disposal specimens from about 200 species of mammals, most of them received from the zoölogical gardens, and usually in various stages of putrefaction. It would have been advantageous in many ways if in every case we had had not only fresh blood, but also blood from healthy animals and in larger quantities. Yet in so far as the specificity of the crystals is concerned we believe that neither the presence of putrefactive processes in the blood nor diseased conditions generally have any important influence. The greatest disadvantage of putrid blood consists in a greater unstability of the preparations and in the difficulty of securing some of the more evanescent forms of oxyhemoglobin. Owing to an absence of preliminary knowledge of the peculiarities of the hemoglobins of different bloods as regards the degree of crystallizability, or to the exceedingly small quantities we usually had to work with, or to extreme putrefaction or other conditions over which we had no control, we occasionally failed absolutely to obtain any evidence of crystallization, and many of our specimens were lost owing to the perishability of the crystals of certain species or to the very pressing demands of teaching. In fact, what work we have accomplished has been through the utilization of such scattered hours as could be taken from the exacting requirements of the class-room and of routine work. Moreover, owing to the extreme solubility of many of the crystals, our investigations were largely limited to the cooler months, and much of our work was done at temperatures at or near the freezing-point.

It was our expectation to include in this memoir the results of a few preliminary studies of certain other corresponding vital substances, especially of plant proteins. Our data are not, however, more than sufficient at present to justify the announcement that we believe that the zoölogical distinctions we have found to be shown by hemoglobins will be demonstrated in other primary organic substances.

This research has proved of exceptional fertility and importance in crystallography. It has brought to light the most extraordinary isomorphous series known; and it has yielded not only the crystallographic data we have recorded in this memoir, but also much that has been omitted because chiefly of its essentially technical character. This latter we will include in a separate memoir in the near future.

We have not in the present memoir attempted to support Dr. Reichert's hypothesis beyond the mere presentation of our discoveries. The problems pertaining to the origin of species, heredity, mutations, sex, and the influence of food and environment are of such extraordinary importance as to have engaged the master minds in biological inquiry, and the task of presenting so important a matter in the form which we believe is necessary to be acceptable to the critical student has seemed too formidable for us to undertake at present. After all, perhaps, it is sufficient and better that we merely state the important hypothesis upon which we have worked, together with the unique facts we have brought to light, with the hope, in thus pausing in our research to make this partial announcement of our results, that their publication may be the means of exciting original thought and investigation along the same or collateral lines, especially in the utilization of the extraordinarily rich supply of material that must be available in the great zoölogical gardens of Europe and in the marine laboratories of this country and abroad.

The first grant for this research was made in April, 1904, and the second grant in January, 1908. The tentative title of our research was announced in the Year Book of the Carnegie Institution of Washington, 1904. Preliminary Reports of our investigation appeared in the Year Book of 1908, 218; in the Proceedings of the Society for Experimental Biology and Medicine, 1907–08, v, 66; and in the Proceedings of the American Philosophical Society, 1908, XLVII, 298.

In conclusion, we take pleasure in acknowledging our great indebtedness to President R. S. Woodward, whose interest and assistance have been invaluable; and especially, to Dr. S. Weir Mitchell, who has been a most valuable co-worker with us throughout our investigations.

Edward Tyson Reichert.
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# THE DIFFERENTIATION AND SPECIFICITY OF CORRESPONDING PROTEINS AND OTHER VITAL SUBSTANCES IN RELATION TO BIOLOGICAL CLASSIFICATION AND ORGANIC EVOLUTION:

## THE CRYSTALLOGRAPHY OF HEMOGLOBINS.

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# CHAPTER I.

## THE DISTRIBUTION OF HEMOGLOBIN AND ALLIED SUBSTANCES IN THE ANIMAL KINGDOM.

Specific respiratory substances are universally distributed throughout plant and animal life, except probably in some of the very lowest organisms. For the most part they are colored, and of a variety of colors and tints, and they exhibit decided variations in their respiratory capacities and other properties. In plant life chlorophyl is preëminently the pigment concerned in the interchange of O and $CO_2$; while in animal life hemoglobin occupies an analogous place, but they are undoubtedly very different in their manner of functionating. In each kingdom the major pigment may be represented or supplemented by physiologically allied bodies, which may or may not be closely related chemically.

The alliance between chlorophyl and hemoglobin that was first suggested by Hoppe-Seyler has been convincingly shown by the investigations of Schunk and Marchlewski (Annal. d. Chem. u. Pharm., 1894, No. 278, 349; 1895, No. 284, 81, and No. 288, 209; 1896, No. 290, 306; and Marchlewski, Bull. de l'Acad. des Sciences de Cracovie, etc., 1902; Biochem. Centralbl., 1902–03, I, 215), who obtained from chlorophyl a derivative coloring matter which they termed phylloporphyrin ($C_{16}H_{18}ON_2$—new formula $C_{34}H_{38}O_2N_4$), which bears a striking resemblance to hematoporphyrin, $C_{34}H_{38}O_6N_4$, an iron-free derivative of hemoglobin, and differing from it only in 4 atoms of O. Moreover, Marchlewski and Zaleski obtained hemopyrrol by reduction from both chlorophyl and hemoglobin. Nencki and Zaleski (Berichte d. deutsch. chem. Ges., 1901, XXIV, 997) attempted to convert hemoporphyrin into phylloporphyrin by reduction, but succeeded in removing only two atoms of O, producing a crystalline intermediate body which they named mesoporphyrin ($C_{16}H_{18}O_2N$—new formula $C_{34}H_{38}O_4N_4$), and which they believe to be identical with hematoidin. Zaleski (Zeit. f. phys. Chemie, 1902, XXVII, 74) found that from mesoporphyrin and hematoporphyrin similar salt and ester compounds can be obtained; and Marchlewski, in examining the spectra of hemoporphyrin, mesoporphyrin, and phylloporphyrin, found them to be very similar, and distinguishable from one another only by a slight displacement of the absorption bands towards the violet end of the spectrum. According to Schunk and Marchlewski, both chlorophyl and hemoglobin are pyrrol derivatives.

Chlorophyl in granular form (chloroplastids) has been found in a large number of invertebrates and vertebrates. In certain of these animals, especially in the lowest types, as in the *Protozoa,* it or an almost identical body is a normal functionating constituent, while in others it is an incidental inactive body, being introduced as food, etc. According to Marchlewski (Die Chemie d. Chlorophyll, 1895, 63), chlorophyl isolated from whatever plants is identical, but Montverde (Acta horti Petropolit., 1893, XIII, 176) and Etard (Compt. rend. soc. biolog., 1895, CXX, 275) hold that there are various kinds. It is probable that the bodies studied by Montverde and Etard were impure. Whichever may be true, there is no doubt that isolated chlorophyl *pigment* is physiologically inert (page 22) and that the chloroplastids (chlorophyl-protein combinations) differ chemically, physically, and biologically, and that the functionating chlorophyl granule of animal life is not identical with that of plant life. There is evidence of intermediate bodies between chlorophyl and histohematin, as MacMunn has found in *Helix pomatia.*

## STATEMENT OF THE DISTRIBUTION.

Hemoglobin is entirely absent from *Protozoa, Porifera,* and *Cœlenterata;* it is rare in *Echinodermata;* it is quite common in certain classes of *Annelida;* and it is comparatively rare in *Arthropoda.* (See page 63, Chapter II.) It is distributed among the invertebrates in a remarkably sporadic and inexplicable way, appearing in only certain classes of a series, or in certain members of a class, etc., sometimes exclusively as a constituent of blood corpuscles or blood plasma, or in nervous matter, or in parts of the musculature, etc. It may be present in members of a certain class, as for instance the *Chætopoda,* but in certain of them it is found as a constituent of special blood corpuscles, and in others in solution in the blood plasma. It may be present in certain members and absent in others, and in the latter it may be represented by closely allied bodies, chemically and physiologically, such as the chlorocruorins, histohematins, etc.

The close chemical relationship of chlorocruorin to hemoglobin is shown in its yielding hematin as a decomposition product, while the histohematins are in the nature of modified forms or derivatives of hemoglobin. Chlorocruorin probably exists in several forms or modifications and seems to have a very restricted distribution, limited to the invertebrates, while histohematins and myohematins are very numerous among both invertebrates and vertebrates. Closely related to hemoglobin, chiefly physiologically, is hemocyanin, which probably exists in several modified forms. Hemocyanin is albuminous and contains copper in the molecule in place of the iron of the hemoglobin molecule; it is distributed solely among the invertebrates, but more widely and quite as erratically as hemoglobin. A large number of lipochromes, some closely allied to chlorophyl and hemoglobin, have been found in both invertebrates and vertebrates.

In all vertebrates, except the *Leptocephalus* and probably the *Amphioxus,* hemoglobin is present in the red blood corpuscles, but is never normally in solution in the blood plasma. In addition to hemoglobin, we find modifications, compounds, and derivatives as normal constituents of various body fluids and solids.

### HISTOHEMATINS AND MYOHEMATINS.

MacMunn's investigations (Philosoph. Trans., 1886, I, 235, 267; Journal of Physiology, 1886, VII, 240, and 1888, IX, 1) have demonstrated a wide distribution of the histohematins and allied bodies among both invertebrates and vertebrates, including porifera, echinoderms, molluscs, arthropods, worms, amphibia, fishes, reptiles, birds, and mammals. In his studies of the chromatology of the British sponges he has shown the presence of coloring matters which are closely related to hemoglobin, and which he groups under the term histohematins. Out of 12 specimens examined by him, 7 by Krukenberg, and 1 by Ray Lankester, making 20 in all, 18 contained chlorophyl, nearly all contained lipochromes, and 7 contained histohematins. In a previous communication on the chromatology of the *Actiniidæ*, MacMunn (Philosoph. Trans., 1885, II, 641) reports a coloring matter in *Actinia mesembryanthemum*, *Bunodes crassicornis*, and other *Actiniidæ*, which can be changed into a hemochromogen and a hematoporphyrin, which are indistinguishable from the corresponding bodies obtained from hemoglobin.

In the echinoderm *Ophiactis viriens*, Foettinger (Archiv d. Biologie, 1880, I, 405) states that he found hemoglobin, and although the correctness of this statement was questioned by Krukenberg (*loc. cit.*) it was subsequently stated by MacMunn to be justified (Journal of Physiology, 1886, VII, 240). MacMunn found hematoporphyrin in *Uraster rubens*. All of the star-fish showed the presence of histohematins from which hematoporphyrin could be obtained. Hematoporphyrin he found in the integument of slugs, *Limax flavus*, *Limax variegatus*, and *Arion ater*. In molluscs he noted enterohematin in the bile and histohematins in various tissues and organs. He also reports histohematins in *Littorina littorea*, *Purpura lapillus*, *Trochus cinerarius*, *Patella vulgata*, *Limnæus stagnalis*, *Paludina vivipara*, *Mytilus edulis*, *Ostræa edulis*, *Unio*, *Anodonta*, *Limax*, *Arion*, *Helix aspersa*, and *Helix pomatia*.

In arthropods MacMunn (Philosoph. Trans., 1886, I, 235, 267) determined that the histohematins are the same as those of the echinoderms and molluscs, and he records finding myohematins in *Hydrophilis piceus*, *Dytiscus marginalis*, *Lucanus cervus*, *Periplanate orientalis*, *Bombus terratus*, *Apis mellifica*, *Cerambyx moschatus*, *Creophilis maxillosus*, *Carabus violaceus*, *Coccinella bipunctata*, *Staphylinus olens*, *Geotropes stercorarius*, *Gryllus domesticus*, *Tipula oleracea*, *Musca domestica*, *Musca vomitoria*, *Musca chlora*, *Vespa vulgaris*, *Acrida viridissima*, *Pieris rapæ*, *Epeira diadema*, *Tegenaria civilis*, and others. In dipterous, hymenopterous, and lepidopterous insects he made the interesting observation that those which use their wing muscles actively have the greatest amount of myohematin in these structures. The presence of histohematins was found to be well marked in the *Crustacea*, of which he examined *Homarus vulgaris*, *Cancer pagurus*, *Carcinus mænas*, *Astacus fluviatilis*, and *Pagurus bernhardus*. Among *Vermes*, in *Lumbricus* and *Hirudo* all organs which in other species show histohematin spectra appear to contain a small amount of hemoglobin, which he believes functionates in a similar manner to the histohematins.

Mosely (quoted by MacMunn) described polyperythrin, which MacMunn regards as being identical with hematoporphyrin, in *Ceratotrochus diadema, Flabellum variable, Flabellum* sp.?, *Fungia symmetrica, Stephanophyllia formosissima, Stephanophyllia* sp.?, *Actinia* with a coriaceous test and in *Discosoma* sp.?, and also in *Cassiopea* (a rhizostomean acaleph).

### ECHINOCHROME, HEMERYTHRIN, AND CHLOROCRUORIN, ETC.

Echinochrome, hemerythrin, and chlorocruorin are very close to hemoglobin. Delle Chiaje (Memoria sulla storia e notomia degli animali senza vertebre del regno di Napoli, I, 33, 127; MacMunn, Quar. Jour. Microscop. Science, 1885, xxv, 476) noted in *Sipunculus balanorphus* and *Echinorhynchus* that the arterial blood is red and the venous blood brown. Schwalbe (Archiv f. mikros. Anat., 1869, v, 248) describes the body fluid of *Phascolosoma elongatum* as being a bright rose or grayish-red color which became darker and darker until it was of an intense Burgundy-red. A similar or identical coloring matter was found by Krukenberg (*loc. cit.*) in the blood of *Sipunculus nudus*, who found that the change of color was due to oxidation and that $CO_2$ restored the original color. The coloring matter, which he termed hemerythrin, he found was decomposed by $H_2S$, and that the O seemed to be more firmly combined than in oxyhemoglobin. Geddes (Gamgee's Physiological Chemistry, 1880, 134; Proc. Roy. Soc., 1880) also observed the color changes in the body fluid of echinoderms upon exposure to the air.

The most important investigations of this coloring matter, or of what are closely identical substances, were made by MacMunn (Proc. Birmingham Philosoph. Soc., 1883, III, 380; Quar. Jour. Microscop. Science, 1885, xxv, 482), who found in various parts of the body, and in the perivisceral fluid, of *Echinus* (*esculentus?*) and *Sphæra* a brown coloring matter which deepened in color upon exposure to the air and which reacted to reducing agents. In the later article he reports studies of the coloring matter of the perivisceral fluid of *Strongylocentrotus lividus*, which he found is capable of existing in two states of oxidation, and which therefore was regarded by him as being respiratory. To it he gave the name echinochrome. He states that it differs from the blood pigments of most invertebrates, and that it can be obtained in solution by two methods:

(*a*) The fresh blood clot can be extracted by water or by alcohol in which it is partially soluble, or by glycerin, ether, chloroform, benzine, bisulphide of carbon, or petroleum ether, in which it is more soluble, the extract upon evaporation yielding an amorphous precipitate.

(*b*) The clot can be separated from the serum by filtering, the clot dried at room temperature, and then extracted with one of the solvents in a test-tube in the dark.

The latter method gives the better results.

MacMunn in other articles (Philosoph. Trans. Royal Soc. London, 1886, I, 267; Journal of Physiology, 1886, VII, 240) records the presence of various pigments in the tissues of echinoderms, certain of which he identified as hematoporphyrin, hemochromogen, or other bodies very close to hemoglobin.

An elementary analysis of echinochrome was made by Griffiths (Compt. rend. soc. biol., 1892, CXIV, 419, 669, 738). This substance he obtained from *Echinus esculentus, Strongylocentrotus lividus, Echinus sphæra,* etc. He showed its very close relationship to hemoglobin, and he gives the following as the molecular formula:

$$C_{102}H_{99}N_{12}FeS_2O_{12}$$

Griffiths also analyzed hemerythrin, and gives to it the formula

$$C_{427}H_{761}N_{135}FeS_2O_{153}$$

Kobert (Archiv f. ges. Physiologie, 1903, XCVIII, 411) states that the hemerythrin from the corpuscles of *Sipunculus nudus* contains the iron, unlike in hemoglobin, in loose combination. He failed to obtain hemin crystals, hemochromogen, or hematoporphyrin by means of the ordinary processes. $H_2O_2$ was decomposed by it, but he did not find any blue coloration with guaiac.

The chlorocruorins, which from the molecular formula of Griffiths are more closely related to hemoglobin than either echinochrome or hemerythrin, have been studied by a number of investigators. The green coloration of the blood of certain annelids was first pointed out by Milne-Edwards (Ann. des Sciences Natur., 1838, x, 190) and later by Quatrefages (quoted by Ray Lankester, *loc. cit.*) in *Siphonostoma.* Krukenberg (Vergleich. physiol. Studien, 1 Rh., 3 Abth., 1882, 87) noted this green pigment in *Spirographis* and *Branchiomma.* Ray Lankester (*loc. cit.*) studied the chlorocruorins of *Siphonostomum* and *Sabella.* He found that, like hemoglobin and oxyhemoglobin, there exist chlorocruorin and oxychlorocruorin, which show different absorption spectra; and he states his belief that hemoglobin and chlorocruorin have a common base in a so-called cyansulphaem (an undetermined body), or perhaps in Stokes's reduced hematin.

MacMunn (*loc. cit.*) subsequently studied the optical properties of oxycruorin and reduced cruorin. The green fluid of *Sabella,* he found, had a reddish tinge with reflected gaslight, and in most cases it was green with transmitted daylight and reddish with transmitted gaslight. On dilution with water the solution gave two bands: the first between C and D from $\lambda$ 618 to $\lambda$ 593; and second between D and E from $\lambda$ 576 to $\lambda$ 554.5. On then adding ammonium sulphide the first of these extended from $\lambda$ 625 to $\lambda$ 596, but it and also the second bands were very faint. "If now caustic soda were added to this solution a dark band was seen covering D, which recalls to mind the band of alkaline hematin, and this band extended from $\lambda$ 595 to $\lambda$ 576."

He also studied the blood of *Serpula contortuplicata,* which he found presents some resemblance to that of the *Sabella.* An aqueous solution obtained from 9 serpulæ was of a reddish-yellow color by gaslight and yellow by daylight. The band before D was from $\lambda$ 620.5 to $\lambda$ 593, the second about $\lambda$ 583.5 to $\lambda$ 572, the third uncertain (about $\lambda$ 551 to $\lambda$ 532). After adding sulphide of ammonium the only band seen with certainty was that before D, which seemed slightly nearer the violet.

Griffiths (*loc. cit.*) gives the elementary composition of chlorocruorin as

$$C_{54\cdot23}H_{6\cdot82}N_{16\cdot16}Fe_{0\cdot45}S_{0\cdot78}O_{21\cdot56}$$

and the empirical formula as

$$C_{560}H_{845}N_{143}FeS_3O_{167}$$

### RESPIRATORY METAL-FREE COLORLESS PROTEINS.

The absence of colored respiratory substances from certain of the invertebrates has been noted by a number of observers. Colored respiratory pigments in such animals, except in certain of the very lowest forms, are doubtless represented by those without color. Griffiths (*loc. cit.*) has described several colorless metal-free proteins which through their behavior towards O and $CO_2$ are to be regarded as being respiratory, and which he believes are widely distributed among the invertebrates. From the blood of *Patella vulgata* he states he obtained a colorless globulin which he distinguishes as α-achroglobulin. He gives to it the formula $C_{523}H_{761}N_{196}SO_{140}$, and states that 100 grams at 0° and 760 mm. combine with 132 c.c. of O and 315 c.c. of $CO_2$. Its rotatory power in dilute magnesium sulphate solution he found to be $[a]_D = -48°$.

From *Chiton* he obtained another form of respiratory globulin which he designates β-achroglobulin, to which he ascribes the formula

$$C_{621}H_{814}N_{175}SO_{169}$$

Its combining capacity for 100 grams he determined to be at 0° and 760 mm., 120 c.c. of O and 281 c.c. of $CO_2$. In dilute magnesium sulphate solution, its rotatory power was $[a]_D = -55°$.

TABLE 1.—*The achroglobulins of Griffiths, and their empirical formulas, oxygen capacities, and rotatory powers.*

| Source. | Variety of achroglobulin. | Formula. | Capacity per 100 grams. | Rotatory power. |
|---|---|---|---|---|
| | | | *c.c.* | ° |
| Patella vulgata... | α-achroglobulin | $C_{523}H_{761}N_{196}SO_{140}$ | 132 | −48 |
| Chiton .......... | β-achroglobulin | $C_{621}H_{814}N_{175}SO_{169}$ | 120 | −55 |
| Tunicates ....... | γ-achroglobulin | $C_{721}H_{915}N_{194}SO_{183}$ | 149 | −63 |
| Doris ........... | δ-achroglobulin | $C_{659}H_{792}N_{165}SO_{153}$ | 125 | −54 |

A third form, distinguished as γ-achroglobulin, he prepared from the blood of tunicates (*Ascidia, Mogula, Cyanthia*). To this he gives the formula $C_{721}H_{915}N_{194}SO_{183}$. Its rotatory power he found to be $[a]_D = -63°$. Its O-capacity was 149 c.c. per 100 grams at 0° and 760 mm. It also combined with methane, CO, and acetylene. A fourth form, δ-achroglobulin, he obtained from the mollusc *Doris*. The formula he gives as $C_{659}H_{792}N_{165}SO_{153}$ and the combining capacity for O as 125 c.c. and the rotatory power as $[a]_D = -54°$. This globulin combines with methane, acetylene, and ethylene to form yellowish, greenish, and brownish compounds, respectively, which are dissociable in vacuum. Similarities and dissimilarities of composition, O-capacity, and rotatory power are shown in table 1. It is of particular interest to note that the O-capacities compare most favorably with the O-capacity of hemoglobin (134 c.c.—Hüfner).

## THE DISTRIBUTION OF HEMOCYANIN.

Hemocyanin is closely allied to hemoglobin, physiologically and chemically, but it is farther removed chemically than the histohematins, echinochrome, chlorocruorins, and the colorless respiratory proteins referred to. It is distributed solely among the invertebrates and, like hemoglobin and the allied bodies mentioned, in an erratic and as yet inexplicable way; but unlike hemoglobin it is found solely in the blood, and as far as known there are no closely related bodies in the form of compounds or derivatives, except oxyhemocyanin, that represent or supplement it in various body tissues and fluids. It has the respiratory function of hemoglobin, but chemically it is not nearly so closely related as chlorophyl, as will be seen by the context. Hemocyanin is colorless, and it is the analogue of reduced hemoglobin; while oxyhemocyanin is blue, and is the analogue of oxyhemoglobin, the oxygen being loosely bound as in oxyhemoglobin, but not so readily displaced.

The blue color of the blood of certain invertebrates was first observed by Ermann, in 1816, in the pulmogasteropod *Helix* (Abhandl. d. k. Akad. d. Wissensch. z. Berlin, 1819, 199); he described it as an opalescence. A few years later Carus (Von d. äussern Lebensbedingungen d. weiss- u. kaltblut. Thiere, Leipzig, 1824, 85) noted the blue color of the bloods of *Helix* and the crayfish *Astacus*. Harless and von Bibra (Archiv f. Anat. u. Physiologie, 1847, 148) examined the blue bloods of *Eledone, Sepia, Cancer pagurus*, and *Helix pomatia*. They studied the influences of exposure to the atmosphere, O, N, and $CO_2$, and they also made elementary analyses. They found that when the colorless blood of *Helix* was exposed to the air it became blue, and that it became colorless when exposed to an atmosphere of $CO_2$; but they state that the bloods of the cephalopods *Eledone* and *Loligo* are affected in the opposite ways by these gases, becoming blue upon exposure to $CO_2$ and colorless when exposed to O, which, however, has since been shown to be incorrect. Ammonia, they found, removed the blue color, which reappeared upon neutralization with hydrochloric acid. In their analysis of the coloring matter of the blood of *Helix* they found

$$C_{45\cdot79}H_{5\cdot05}N_{13\cdot23}O_{35\cdot93}$$

and also copper, but no iron.

Genth (Annalen d. Chemie u. Pharmacie, 1852, LXXXI, 68) found that the blood of *Limulus cyclops* became blue upon exposure to the air, and that it contained both copper and iron. In his analyses of the ash he found in one case 0.081 per cent of oxide of iron and 0.085 per cent of oxide of copper; and in another only a trace of iron and 0.297 per cent of oxide of copper.

Haeckel (Archiv f. Anat. u. Physiologie, 1857, 511) observed that the colorless blood of *Homola cuvieri* upon withdrawal from the animal became gradually gray, and finally an intense blue; and that the bright bluish blood of *Homarus* became after many hours a dark violet. Witting (Jour. f. pract. Chemie, 1858, LXXIII, 121) refers to the bluish tinge of the blood of *Unio pictorum*. He also examined the blood of *Astacus*, but failed to note a blue coloration which was subsequently found by Krukenberg (*loc. cit.*) in specimens of the same species. Rouget (Jour. de la Physiologie, 1859, 660) in his studies of the colored corpuscles of the bloods of tunicates and *Actinozoa* found scarlet, orange, yellow, blue, and violet corpuscles.

The bluish blood of *Sepia* was noted by Bert (Compt. rend. soc. biologie, 1867, LXV, 300). He showed that the color belongs to the plasma, and that it was intensified by exposure to the air, and not destroyed by boiling. Color changes of the same kind were recorded by Rabuteau and Papillon (Compt. rend. soc. biologie, 1873, LXXVII, 135) in the bloods of *Octopus* and crabs. They observed not only the effects of the air, but also of $CO_2$, and they discovered the fact that the blue blood does not give spectral absorption bands. The statement by Genth that the blue blood of *Limulus* contains copper received confirmation in the analyses of the blue blood of *Helix* by Gorup-Besanez (Lehrbuch d. physiologischen Chemie, 1878, 379), and by those of Müller and Schlossberger (quoted by Gorup-Besanez), who found copper in the bloods of *Sepia* and *Octopus*.

Jolyet and Regnard (Archives de Physiologie, 1877, XLIV, 584) found two coloring matters in the blood of crabs, one blue and the other reddish, the former (hemocyanin) being precipitated by alcohol, while the latter (tetronerythrin) remained in solution. They found that agitation of the blood with air developed an ultramarine blue as seen by reflected light, and a brownish coloration as seen by transmitted light, and that upon the removal of O by the gas-pump the blood became rosy and finally yellowish, and that upon shaking the blood with O the blue color was restored. In opposition to the statement of Harless and von Bibra (*loc. cit.*) that $CO_2$ caused the blood to become blue, they found it to be without influence. They studied the percentages of O, N, and $CO_2$ (free and combined) in the bloods of crabs, and noted the low capacity for O. In the blood of *Astacus* they found 3.5 per cent; in the common crab, 3 to 3.2 per cent; and in *Pagurus*, 2.4 to 4.4 per cent of O. They were the first to suggest that the blue coloring matter is in protein form. The bloods of *Scorpio* and *Limulus* were found by Ray Lankester (Quar. Jour. Microscop. Science, 1878, XXI, 453; 1881, XXIV, 151) to become blue upon exposure to the air.

Tetronerythrin seems to be widely distributed: Merejkowski (Compt. rend. soc. biologie, 1881, XCIII, 1029) found it in 104 species, and this list has been largely increased by the investigations of others.

The term hemocyanin we owe to Frédericq (Bull. de l'Acad. roy. de Belgique, 1878, XVI, 4; Compt. rend. soc. biolog., 1879, LXXXVII, 996), who definitely showed in the blood of *Octopus* that the copper, to which the blue coloration is due, is in combination with protein. He recognized the analogy between hemoglobin and its oxide and hemocyanin and its oxide, and that the copper in the hemocyanin molecule plays a similar rôle to that of the iron of the hemoglobin molecule. He noted that the venous blood was colorless and the arterial blood blue, and that the latter becomes decolorized as the weakly combined O is withdrawn *in vacuo* or driven off by $CO_2$ or $H_2S$, and that the color is restored by O. The statement of several previous observers that hemocyanin does not give absorption bands was confirmed. In a subsequent research (Compt. rend. soc. biologie, 1897, XVII, 47) he proved the identity of the blue coloring matter of the bloods of *Octopus* and *Homarus*, and in the latter he found two coloring matters corresponding to those described by Jolyet and Regnard (*loc. cit.*) in the blood of crabs. He confirmed their observations of the behavior of hemocyanin towards reflected and transmitted light, and he showed that the reddish coloring matter (tetronerythrin) takes no part in the change of color caused by oxygenation and deoxygenation. Hemocyanin was found by Frédericq in the bloods of the gasteropods *Arion* and *Helix*.

The list of animals whose blood contains hemocyanin was materially added to by the investigations of Krukenberg (Vergleich. physiol. Studien, 1 Rh., 3 Abth., 1880, 66; 1881, 49; 1882, 87, 182; Centralb. f. med. Wissensch., 1880, VIII, 417), and he added information regarding the behavior of hemocyanin towards $CO_2$, CO, and $H_2S$, and especially in the direction of indicating the existence of several forms or modifications of hemocyanin which has since received support by the investigations of Howell (p. 10), Cuénot (p. 12), and Couvreur (p. 13). He showed that the blue blood of two cephalopods (*Eledone moschata* and *Sepia officinalis*) and of a number of species of crabs (*Homarus vulgaris, Carcinus mœnas, Eriphia spinifrons, Portunus depurator, Graspus marmoratus, Maia verrucosa, Pilumnus villosus,* and *Squilla mantis*) became more or less intensely blue upon agitation with air or oxygen, and more or less decolorized by shaking with $CO_2$. He found that the blood of *Limnæus stagnalis* was scarcely affected by shaking with $CO_2$, and he believes that in this species, and also in *Helix pomatia* and *aspera*, the coloring matter exists as a body very closely related to hemocyanin. Marked differences were noted in the degree of coloration of the blood and fixity of the O. The blood of *Portunus depurator* was a very light blue, while the bloods of *Homarus, Eriphia spinifrons,* and *Squilla mantis* were a deep indigo blue. In the gasteropod molluscs, crabs, and cephalopods, he noted such differences in the behavior of the hemocyanin towards O as to lead him to the belief that this gas is in firmer combination in crabs and cephalopods than in molluscs. He also compared hemocyanin and hemoglobin in their behavior towards certain gases. He found, for instance, that after decolorization of the blood with $CO_2$ the original color was restored by shaking with air, and that when subjected to $SO_2$ or sulphide of ammonium the blood of crabs and *Eledone* became yellowish, and that the color could not be restored by agitation with O, both of which are the opposite to the behavior of oxyhemoglobin. He failed to find any evidence of the presence of hemocyanin in the blood of a number of molluscs.

Little of importance was added to our knowledge of this important substance during the following decade. Gotch and Laws (British Association Reports, 1884; quoted by Lankester, *loc. cit.*) found hemocyanin or a body closely identical with it in *Limulus polyphemus;* Halliburton (Journ. Physiology, 1885, VI, 300) reported hemocyanin in the crustacean *Nephrops*, and he gives the following list of animals in which hemocyanin, hemoglobin, chlorocruorin, hemerythrin, chlorophyl, and tetronerythrin have been found in the blood. The name of the authority is given in parentheses or brackets.

A. Hemocyanin.
- Crustacea:
  - Homarus (Frédericq).
  - Astacus (Krukenberg).
  - Cancer (Krukenberg).
  - Carcinus (Jolyet and Regnard).
  - Nephrops [Halliburton].
  - Eriphia (Krukenberg).
  - Squilla (Krukenberg).
  - Maia (Krukenberg).
- Arachnida:
  - Scorpio (Lankester).
  - Limulus (Lankester).
- Gasteropods:
  - Cassidaria (Krukenberg).
  - Fissurella (Krukenberg).
  - Haliotis (Krukenberg).
  - Helix (Frédericq).
  - Murex (Krukenberg).
  - Turbo (Krukenberg).
- Cephalopods:
  - Octopus (Frédericq).
  - Sepia (Krukenberg).
  - Eledone (Krukenberg).
  - Loligo (Krukenberg).

B. Hemoglobin.
- Vertebrata:
  - In special corpuscles in all except Amphioxus (Lankester).
  - Leptocephalus (Lankester).
- Crustacea:
  - Daphnia (Lankester).
  - Chirocephalus (Lankester).
  - Apus (Regnard and Blanchard).
  - Lernanthropus (Van Beneden).
  - Clavella (Van Beneden).
  - Cypris (Regnard and Blanchard).
  - Marine parasitic crustacean, undescribed (Van Beneden).
- Insecta:
  - Chironomus (Lankester).
  - Musca domestica (MacMunn).
- Mollusca:
  - Planorbis (Lankester).
  - Arca (Lankester).
  - Solen (Lankester).
- Chætopoda:
  - Lumbricus (Lankester).
  - Eunice (Lankester).
  - Cirrhatulus (Lankester).
  - Nereis (Lankester).
  - Terebella (Lankester).
  - Tubifex (Lankester).
  - Arenicola (Lankester).

B. Hemoglobin.—Continued.
- Chætopoda—Continued.
  - Limnodrilus (Lankester).
  - Lumbriculus (Lankester).
  - Nais (Lankester).
  - Chætogaster (Lankester).
  - Glycera (Lankester).
  - Capitella (Lankester).
  - Euchytrachus (Lankester).
  - Aphrodite (MacMunn).
- Gephyrea:
  - Phoronis (Lankester).
  - Thallasena (Lankester).
  - Hamingia (Lankester).
- Nemertina:
  - Polia (Lankester).
  - Other nemertines (Hubrecht, 1875).
- Hirudinea:
  - Nephilis (Lankester).
  - Hirudo (Lankester).
- Echinodermata:
  - An ophiurid (Foettinger, 1880).
  - In all invertebrates hemoglobin occurs in solution in the blood plasma, except in Solen, Glycera, Capitella, Phoronis, where it is contained in special corpuscles.

C. Chlorocruorin.
- Chætopoda:
  - Siphonostomum (Lankester).
  - Sabella (Lankester).
  - Chloronema (Quatrefages).

D. Hemerythrin.
- Gephyrea:
  - Phascolosoma (Schwalbe).
  - Sipunculus (Krukenberg).
  - Phoronis (Krukenberg).

E. Chlorophyl.
- Insecta:
  - Various butterflies and moths (Poulton)

F. Tetronerythrin.
- Crustacea:
  - Homarus [Halliburton].
  - Carcinus [Halliburton].
  - Astacus [Halliburton].
  - Nephrops [Halliburton].

G. Various colored granules are described in the corpuscles of Holothurians and Sea-urchins (Geddes). The blood of Patella is described as being of an orange color (Krukenberg).

This list has been increased by subsequent communications, as will be seen by the context.

Howell (Studies from the Biological Laboratory, Johns Hopkins University, 1884, III, 284) studied the hemocyanin of the blood of *Limulus polyphemus*, *Callinectes hastatus*, and *Cucumaria* sp.?, and in comparing the condition of the respiratory oxygen he found the O to be in more stable combination in the first, and also that the hemocyanin of this organism coagulates at higher temperature. MacMunn (Quart. Jour. Microscop. Science, 1885, xxv, 469) in his studies of the chromatology of the bloods of certain invertebrates, chiefly of the spectroscopic characters, states that the blood of *Helix aspersa* is bluish-white by daylight and of a purplish tinge by gaslight; he found that the blood of *Limnæus stagnalis* on exposure to the air assumed a bluish-white color and that the blood of *Paludina vivipara* is of a blue color. None gave absorption bands.

Griffiths (Compt. rend. soc. biol., 1892, CXIV, 496, 840, 1277; CXV, 669) made elementary analyses of the hemocyanin of the bloods of *Homarus*, *Sepia*, and *Cancer*, which he obtained by precipitating with magnesium sulphate, dissolving the precipitate in water, again precipitating with alcohol, and finally drying in vacuum at 60°. Frédericq, Krukenberg, and Henze state that magnesium sulphate causes either little or no precipitation of hemocyanin. This statement is opposed by Griffiths, Couvreur (Compt. rend. soc. biologie, 1902, LIV, 125), and Halliburton (Journal of Physiology, 1885, VI, 300). The latter states that "just as precipitation by heat is slow, so is also precipitation with salts; to effect complete saturation with either of the above-mentioned salts (magnesium sulphate or sodium chloride) the serum must be shaken in an engine for 12, 24, and in some experiments 36 hours, with the finely powdered salt." Probably the difference in the results of Griffiths, Couvreur, and Halliburton from those of Frédericq, Krukenberg, and Henze may be explained by differences in species and freshness of the blood and other incidental conditions.

Griffiths' analyses showed

$$C_{54\cdot06-54\cdot23}H_{7\cdot00-7\cdot14}N_{16\cdot21-16\cdot35}Cu_{0\cdot31-0\cdot36}S_{0\cdot60-0\cdot69}$$

the mean being

$$C_{54\cdot155}H_{7\cdot095}N_{16\cdot268}S_{0\cdot647}O_{21\cdot507}Cu_{0\cdot328}$$

The empirical formula he calculated to be

$$C_{867}H_{1363}N_{223}CuS_4O_{258}$$

Interesting in this connection is his analysis of the brown coloring matter of the blood of the lamellibranch *Pinna squamosa*. This substance he describes as a body very closely related to hemocyanin, but in which there is manganese instead of copper. Krukenberg (*loc. cit.*) had already found that the blood of this animal was rich in manganese. Griffiths named this pigment pinnaglobulin. His elementary analysis showed

$$C_{55\cdot07}H_{6\cdot24}N_{16\cdot24}S_{0\cdot81}O_{21\cdot29}Mn_{0\cdot35}$$

and the empirical formula he calculated to be

$$C_{729}H_{985}N_{183}MnS_4O_{210}$$

In the blood-ash of *Pinna* he found manganese but not iron, while in that of *Sabella* and *Sipunculus* he found iron but not manganese. The centesimal analyses and empirical formulas of hemocyanin, pinnaglobulin, echinochrome, chlorocruorin, hemerythrin, and hemoglobin are given by Griffiths as follows:

TABLE 2.—*Empirical formulas of certain respiratory substances, according to Griffiths.*

| | | | | | | |
|---|---|---|---|---|---|---|
| Hemocyanin | $C_{857}$ | $H_{1363}$ | $N_{223}$ | Cu | $S_4$ | $O_{258}$ |
| Pinnaglobulin | $C_{729}$ | $H_{985}$ | $N_{183}$ | Mn | $S_4$ | $O_{210}$ |
| Echinochrome | $C_{102}$ | $H_{99}$ | $N_{12}$ | Fe | $S_2$ | $O_{12}$ |
| Hemerythrin | $C_{427}$ | $H_{761}$ | $N_{135}$ | Fe | $S_2$ | $O_{153}$ |
| Chlorocruorin | $C_{560}$ | $H_{845}$ | $N_{143}$ | Fe | $S_3$ | $O_{167}$ |
| Hemoglobin | $C_{600}$ | $H_{960}$ | $N_{154}$ | Fe | $S_3$ | $O_{179}$ |

Heim (Compt. rend. soc. biolog., 1892, CXIV, 772) found in decapods that hemocyanin is not the only albuminous substance of the blood, and that dialysis does not yield a pure hemocyanin. He states that the blood of crustacea does not contain a higher percentage of O than pure water, except that of *Palinurus vulgaris,* which contains about one-third more. He does not look upon the copper as existing in the form of an albuminate.

Cuénot (Compt. rend. soc. biolog., 1891, CX, 724) found in *Aplysia punctata* a colorless hemocyanin (?) that is not colored blue upon exposure to the air, which he regards as being without respiratory function. He likewise found (Compt. rend. soc. biolog., 1892, CXV, 127), as had Jolyet and Regnard (*loc. cit.*) and others, a low oxygen capacity of hemocyanin. Frédericq (Centralb. f. Physiologie, 1899, XIII, 147) in experiments with the blood of crabs showed that hemocyanin is the only protein of the blood that contains copper, that it is coagulated between 65° and 70°, that it is present in the proportion of 4.4 to 3.78 per cent and that the percentage is decreased during fasting. In a previous article (*loc. cit.*) he gives the coagulation point as 68° to 69°. That the blue coloring matter is a copper compound was reaffirmed by Couvreur (Compt. rend. soc. biolog., 1900, LII, 395), who found that after precipitation of the hemocyanin by magnesium sulphate, alcohol, or heat, the filtrate does not contain any copper. The spontaneous decolorization of hemocyanin upon keeping seems, according to Phisalix (Compt. rend. soc. biolog., 1900, LII, 729), to be due to bacterial action, for he found that this color change may be hindered by chloroform, ether, 10 per cent formaldehyde, or fluoride of sodium, and that the blood of *Helix pomatia,* if kept antiseptically, retained its blue color for a year.

TABLE 3.—*Quantities of copper in the blood of certain invertebrates, according to Dhéré.*

| Name. | Copper in milligrams. | | Name. | Copper in milligrams. | |
|---|---|---|---|---|---|
| | 100 c.c. fresh blood. | 100 grams dried substance. | | 100 c.c. fresh blood. | 100 grams dried substance. |
| Cancer pagurus | 3.5 | .... | Helix pomatia | 7.0 | 175 } * |
| Do | 7.5 | .... | Do. | 7.5 | 205 } |
| Do | 13.5 | .... | Do. | 8.0 | .... |
| Palinurus vulgaris | 7.5 | .... | Do. | 11.5 | .... |
| Do | 10.5 | 70 | Do. | 12.5 | .... |
| Do | 11.0 | 75 | Octopus vulgaris | 18.0 | .... |
| Homarus vulgaris | 9.5 | .... | Do. | 18.0 | .... |
| Do | 10.5 | .... | Do. | 20.0 | 153 |
| Astacus fluviatilis | 4.0 | .... | Do. | 23.5 | .... |
| Do | 8.0 | | | | |

* Hibernating.

In studies of the distribution of copper in invertebrates and fish, Dubois (Compt. rend. soc. biolog., 1900, LII, 392; 1903, LV, 1161) found that the proportions are very variable, not only in different species and individuals, but also in different organs. Ascidians are very poor in copper, and fish contain less than invertebrates. In the blood of *Palinurus vulgaris* he found 22.97 mg. of copper per 100 grams, and in muscle 4.47 mg. The egg was copper-free. In the blood of *Helix pomatia* there were 24.39 mg.

per 100 grams. Coincident with the appearance of Dubois's first article, Dhéré (Compt. rend. soc. biolog., 1900, LII, 458) reported the results of his investigations of the quantity of copper in the blood of certain invertebrates and the respiratory capacity of hemocyanin. His analyses were made with 10 c.c. of fresh blood in each case, and he gives the figures shown in table 3.

It will be noted that the copper content varied much, not only in different species, but also in members of the same species. He also found that the intensity of blue coloration is in relation to the quantity of copper. The respiratory capacities in relation to the quantity of copper and hemocyanin are shown in table 4.

TABLE 4.—*Respiratory capacities in relation to the quantity of copper and oxygen in the blood, according to Dhéré.*

| Name. | 100 grams blood. | | | Remarks. |
|---|---|---|---|---|
| Helix pomatia | 17° | 1.45 c.c. O | 6.5 mg. Cu. | Blood filtered. |
| Do | 19° | 2.2 | 11.5 | Do. |
| Homarus vulgaris | 17° | 3.0 | 9.5 | Blood defibrinated and filtered. |
| Do | 18.5° | 3.1 | 10.5 | Do. |
| Astacus fluviatilis | 22° | 2.4 | 8.0 | Blood with fluoride filtered. |
| Octopus vulgaris | 18° | 4.2 | 28.5 | |
| | | 3.9 | 23. | |
| Carcinus mœnas | 18° | ... | 8.5 | |
| | | ... | 10.0 | |
| Cancer pagurus | 18° | 1.6 | 5.5 | |
| | | ... | 7.0 | |
| Maia squinado | 18° | ... | 3.0 | |
| | | ... | 4.5 | |

Couvreur (Compt. rend. soc. biolog., 1900, LII, 395), in his studies of coagulative and other phenomena of the blood of the snail, states, in opposition to Heim (*loc. cit.*) and in support of Jolyet and Regnard (*loc. cit.*), that hemocyanin should be looked upon as being a combination of copper with protein. In a later article (*ibid.*, 1902, LIV, 125) he made comparative studies of the bloods of certain marine gasteropods (*Murex brandaris, Murex trunculus*, and *Tritonium nodiferum*) with the blood of the snail. The hemocyanin of these bloods, like that of the snail, was precipitated by saturation with magnesium sulphate. He also notes that the hemocyanin of marine gasteropods seems to be more stable than that of the snail. Couvreur and Rougier (*ibid.*, 1902, LIV, 1476), in comparing hemocyanin and hemoglobin, note that hemoglobin is not broken down by putrefactive processes while hemocyanin is, and that the normally blue blood becomes dark and forms an insoluble product of a dark tint which is a derivative containing copper. Dhéré (*ibid.*, 1903, LV, 1161) kept the bloods of *Octopus vulgaris, Cancer pagurus, Carcinus mœnas*, and *Maia squinado* in sealed tubes for three years. The fluids were discolored, and upon being shaken with air only the blood of *Octopus* became blue, while that of the crustaceans became a slate-gray. He also made determinations of the percentages of copper.

In other articles Dhéré (*ibid.*, 1903, 1012, 1338) and Couvreur (*ibid.*, 1247) discuss the effects of dialysis, heat, alcohol, etc., on hemocyanin. Henze (Zeit. f. physiolog. Chemie, 1901, XXXIII, 370) found in the hemocyanin of *Octopus vulgaris* 0.38 per cent of copper. The blood contained

9 per cent of hemocyanin, which would give 34.2 mg. of copper per 100 c.c. of blood.

According to Henze, 1 gram of hemocyanin combines with 3 to 3.7 per cent of O, or about 0.4 c.c., which is about one-fourth the capacity of hemoglobin. Henze was the first to obtain crystals of hemocyanin. These he prepared according to the Hofmeister (Zeit. f. physiolog. Chemie, 1892, XIV, 165) and the Hopkins-Pinkus methods (Journal of Physiology, 1898–99, XXIII, 130) for obtaining crystals of egg albumin. In following out the first method the blood of *Octopus vulgaris* was centrifugalized and filtered, and the filtrate mixed with a sufficient amount of ammonium sulphate to cause a slight precipitation. The solution was placed in shallow vessels, when upon evaporation clusters of little needles were deposited.

Much better results were obtained by the Hopkins-Pinkus method: Ammonium sulphate was added to the centrifugalized blood until the appearance of cloudiness; this slight precipitate was dissolved by the addition of distilled water; acetic acid was then added until the appearance of cloudiness. After standing for half a day the precipitate increased very much and settled to the bottom. A crystalline mass formed at the bottom of the vessel and consisted of excellently formed, doubly refractive, microscopic prisms. There appeared in two preparations egg-shaped leaflets, some of which were 3 to 4 mm. in diameter, but which were difficult to isolate from the crystalline mass owing to their fragility. Although Henze found it possible to recrystallize hemocyanin, the second recrystallization was rendered impure because of amorphous admixtures. He also noted that crystallization took place perfectly only when fresh blood from healthy animals was used. The mean elementary analysis he gives is

$$C_{53\cdot66}H_{7\cdot33}N_{16\cdot09}S_{0\cdot86}Cu_{0\cdot38}O_{21\cdot67}$$

(Compare with Griffiths's analysis of the amorphous hemocyanin of *Sepia*, *Homarus*, and *Cancer*, p. 11.) In this article he reports studies of the manner in which the copper is in combination in the protein molecule, of the reactions, and of the O-capacity of hemocyanin, etc. He states that hemocyanin behaves in certain ways differently from hemoglobin, that the copper is in loose combination, that the substance behaves like a copper albuminate, and that the O-capacity is only about one-fourth that of hemoglobin.

In a later contribution (Zeit. f. physiolog. Chemie, 1904–05, XLIII, 290) Henze reports that hemocyanin can not be separated into a protein and a component free of protein, as in the case of hemoglobin, and that it behaves like a copper albuminate from which the masked copper can easily be separated. By hydrolysis he found tyrosin, leucin, histidin, lysin, probably glutaminic acid, and possibly a minimal amount of arginin. He failed to find evidence of a carbohydrate group, although the fresh blood of *Octopus* gave a positive reaction with Molisch's test. In determining the distribution of its N he found the following: as mono-amino-N, 10.20 (63.39 per cent); diamino-N, 4.45 (27.65 per cent); humin-N, 0.43 (2.67 per cent); and ammonia-N, 0.93 (5.78 per cent).

Kobert (Archiv f. ges. Physiologie, 1903, xcviii, 411), in experiments with the blood of *Eledone moschata*, confirmed Henze's statement of the loose combination of the copper in the form of a copper albuminate, and also the differences in the chemical behavior of hemocyanin in comparison with hemoglobin. He found that CO, for instance, does not form a compound similar to CO-hemoglobin, but seemingly a cyanhemocyanin, and that while hemocyanin decomposes $H_2O_2$ it does not cause a bluing of guaiac solution. He found that from hemocyanin neither hematin nor hematoporphyrin could be obtained, and he states that on this account there can not be a close chemical relationship between hemocyanin and hemoglobin. He writes that crystals from *Eledone* blood were examined by Prof. O. Leudecke, who states "that they are optically uniaxial and positive, apparently hexagonal." Kobert reports that hemocyanin is absent from the blood of *Aplysia limacina*, but present in *Maia verrucosa.*

### THE DISTRIBUTION OF HEMOGLOBIN IN THE INVERTEBRATES.

Hünefeld (Der Chemismus in der thierischen Organization (prize essay), Leipzig, 1840, 160) discovered hemoglobin crystals in the blood of the common earthworm that had been placed between plates of glass. Rollett (Sitz. d. k. Akad. d. Wissensch., Wien, 1861, xliv, 615) identified the blood crystals of the earthworm and those of the insect *Chironomus* with those of vertebrates. Nawrocki (Centralblatt f. Wissensch., Feb. 8, 1867, xv, 195) and Ray Lankester (Jour. Anat. and Physiology, 1867–68, ii, 114) also identified the substance of the blood crystals of invertebrates with the hemoglobin of vertebrates. The latter, in a series of articles (Jour. Anat. and Physiology, 1867–68, ii, 114; 1869–70, iv, 119; Archiv f. ges. Physiologie, 1871, iv, 315; Proceedings Royal Soc. London, 1872–73, xxi, 70), studied the area of distribution of hemoglobin in the animal kingdom, especially in the invertebrates. In his earlier articles he notes that he detected hemoglobin in the non-corpuscular saccular fluid of annelids *Lumbricus, Eunice sanguinea,* and *Hirudo;* in the plasma of the blood of the larva of the insect *Chironomus plumosus;* in the plasma of the blood of the mollusc *Planorbis corneus* and of the crustacean *Chirocephalus diaphanus;* and also in larvæ allied to *Chironomus.* In the last article he takes exception to the statement of Preyer that hemoglobin is found in all vertebrates, and he shows the absence of hemoglobin from the *Leptocephalus,* which possesses corpuscles corresponding to erythrocytes, which animal is perfectly colorless and glass-like except the black-pigmented eye. He also failed, after repeated attempts with the spectroscope, to find evidence of hemoglobin in the *Amphioxus,* although, as he states, Wilhelm Müller, of Jena, found that this vertebrate has corpuscles of a pale red color. The facts ascertained as to the distribution of hemoglobin (and myohematin) Lankester summarizes as follows:

(1) *In special corpuscles:*

- (*a*) In the blood of all vertebrates, except *Leptocephalus* and *Amphioxus* (?).
- (*b*) In the perivisceral fluid of some species of the vermian genera *Glycera, Capitella,* and *Phoronis.*
- (*c*) In the blood of the lamellibranchiate mollusc *Solen legumen.*

(2) *Diffused in a vascular or ambient liquid:*
   (*a*) In a peculiar vascular system of the chætopodous annelids very generally, but with apparently arbitrary exceptions.
   (*b*) In the vascular system (which represents a reduced perivisceral cavity) of certain leeches, but not of all (*Nephelis, Hirudo*).
   (*c*) In the vascular system of certain turbellarians, as an exception *Polia.*
   (*d*) In a special vascular tissue (distinct from the general blood system) of a marine parasitic crustacean (undescribed) observed by Prof. Edouard van Beneden.
   (*e*) In the general blood system of the larva of the dipterous insect *Chironomus.*
   (*f*) In the general blood system of the pulmonate mollusc *Planorbis.*
   (*g*) In the general blood system of the crustaceans *Daphnia* and *Chirocephalus.*

(3) *Diffused in the substance of the muscular tissue:*
   (*a*) In the voluntary muscles generally of *Mammalia,* and probably of birds, and in some muscles of reptiles.
   (*b*) In the muscles of the dorsal fin of the fish *Hippocampus,* being generally absent from the voluntary muscular tissue of the fish.
   (*c*) In the muscular tissue of the heart of *Vertebrata* generally.
   (*d*) In the unstriped muscular tissue of the rectum of man, being absent from the unstriped muscular tissue of the alimentary canal generally.
   (*e*) In the muscles of the pharynx and odontophore of gasteropodous molluscs (observed in *Limnæus, Paludina, Littorina, Patella, Chiton, Aplysia*) and of the pharyngeal gizzard of *Aplysia,* being entirely absent from the rest of the muscular and other tissues and the blood of these molluscs. See as to *Planorbis* above (2, *f*).
   (*f*) In the muscular tissue of the great pharyngeal tube of *Aphrodite aculeata,* being absent from the muscular tissue and from the blood in this animal, and absent from the muscular tissue generally in all other annelids as far as yet examined.

(4) *Diffused in the substance of the nervous tissue:*
   (*a*) In the chain of nerve-ganglia of *Aphrodite aculeata.*

Since Lankester's researches, the list of invertebrates in which hemoglobin exists has been largely increased. Hubrecht (Niederland. Archiv f. Zoologie, 1876, II, Heft 3; Maly's Jahresbr. ü. d. Fort. d. Thierchemie, 1876, VI, 92) found by spectroscopic examination hemoglobin in the oval blood corpuscles of *Drepanophorus* and in the red brain ganglia of nemertean worms which are without colored corpuscles.

Krukenberg (*loc. cit.*) found hemoglobin in *Planorbis* and *Apus.*

Van Beneden (Zoologischer Anzeiger, 1880, III, 55) reports hemoglobin in *Planorbis corneus,* in sea-water gasteropods, in tunicates, in parasitical copepods (*Lernanthropus* and *Clavella*), and in an undescribed parasitic crustacean.

Foettinger (Archiv d. Biologie, 1880, I, 405) discovered hemoglobin in an ophiuridean echinoderm *Ophiactus virens.*

Regnard and Blanchard (Compt. rend. soc. biolog., 1883, XCVII, 197) found in the blood of certain phyllopods (*Apus productus* and *Cancriformis,* and probably *Branchipus*), of *Cladocera* (*Daphnia*) and *Ostracoda* (*Cypris*), that the hemoglobin is dissolved in the plasma.

Howell (Studies from the Biological Laboratory, Johns Hopkins University, 1884, III, 284) found hemoglobin in the blood of a holothurean echinoderm (*Thyonella gemmata*).

MacMunn (Proc. Birmingham Philosoph. Soc., 1883, III, 385) observed hemoglobin in lamellibranchs, leeches, a turbellarian, and insects. In a later article (Quar. Jour. Microscop. Science, 1885, XXV, 469) he reports hemoglobin in *Lumbricus, Arenicola,* and *Eunice.*

Eisig (Die Capitelliden; Maly's Jahresber. ü. d. Fort. d. Thierchemie, 1887, XVII, 336) in studying the bloods of a group of annelids (*Capitella*) obtained hemoglobin crystals, mostly in the form of four-sided prisms or rhombic plates, some of which were very large. He used methods that are employed to obtain blood crystals from the higher animals. Sometimes crystallization took place spontaneously, intraglobular and extraglobular, and more abundantly in *Dasybranchus caduceus* than in the other species. He also found evidence of hemoglobin derivatives.

While the hemoglobin of invertebrates and vertebrates had been identified microscopically and spectroscopically, Griffiths (Proc. Roy. Soc. Edinburgh, 1891; Physiology of the Invertebrates, 1892, 147) was the first to show by elementary analyses that the hemoglobins of invertebrates and vertebrates are comparable chemically. The blood of 500 earthworms (*Lumbricus terrestris*) was treated with benzene, which lakes the blood. The mixture was allowed to stand for 24 hours at 0° C., when it separated into two layers. The one containing the coloring matter was then separated from the other, and about one-sixth of its volume of pure alcohol was added. After filtration the alcoholic extract was exposed to −12° C., when red crystals were obtained. These crystals yielded, on analysis, the figures given in table 5, which he compares with those of dog's hemoglobin recorded by Hoppe-Seyler.

TABLE 5.—*Analyses of crystals from blood of earthworm, compared with those of dog hemoglobin.*

| | I. | II. | III. | Dog. |
|---|---|---|---|---|
| Carbon | 53.91 | 53.85 | .... | 53.85 |
| Hydrogen | 7.02 | 7.10 | .... | 7.32 |
| Nitrogen | .... | .... | .... | 16.17 |
| Sulphur | 0.41 | 0.37 | .... | 0.39 |
| Iron | .... | .... | 0.39 | 0.43 |
| Oxygen | .... | .... | .... | 21.84 |

Velichi (Inaug. Dissert., Berlin, 1900; Centralblatt f. Physiologie, 1900, XIV, 679; Deutsch. med. Wochenschr., 1900, XXVI, Juni 21, 148), with the microspectrophotometer, made determinations of extinction coefficients and the percentages of hemoglobin and oxyhemoglobin in the bloods of several annelids (*Arenicola piscatorum, Terebella nebrelosa, Lumbricus terrestris*) and certain other invertebrates. In annelids he found the percentage to be similar to that of the frog, namely, 3.465 per cent. When, however, he made his determination by carbonic-oxide hemoglobin he always obtained lower values, only 3.02 per cent. From the blood of *Arenicola* he prepared hemin crystals. He also states that the hemoglobins of different classes of animals are not identical, because their extinction coefficients differ. He found hemoglobin in the pharyngeal muscles of gasteropods and also in the blood of certain crustacea.

Dhéré (Compt. rend. soc. biolog., 1903, LV, 1162) records by the colorimetric method, in comparing the bloods of the dog and *Planorbis corneus*, that the oxygen capacity of the latter is the higher—in the dog 1.34 c.c. of O per gram (Hüfner) and in *Planorbis* 1.92 and 2.24 c.c.

### CAUSES OF THE PECULIARITIES IN THE DISTRIBUTION OF RESPIRATORY PIGMENTS.

The extraordinarily erratic manner in which hemoglobin, hemocyanin, and other respiratory pigments are distributed among invertebrates has not unnaturally given rise to inquiries of the reasons. Ray Lankester (*loc. cit.*) from such an investigation writes as follows:

> From a consideration of the facts with regard to the mode of occurrence and distribution of hemoglobin in animal organisms, the following general statements may be made, which are in accordance with the now thorough establishment, by chemical investigation, of its peculiar oxygen-carrying property. Hemoglobin is irregularly distributed throughout the animal kingdom, being absent entirely only in the lowest groups.* It may be present in all the representatives of a large group, with but one or two exceptions, or it may be present in only one out of the numerous members of such a group; or, again, it may be present in one and absent in another species of the same genus. It may occur in corpuscles in the blood, or diffused in the liquor sanguinis, or in the muscular tissue, or in the nerve tissue. The same apparent capriciousness characterizes its occurrence in tissues as in specific forms. It may be present in one small group of muscles and absent from all the rest of the tissues of the body, or it may occur in one part only of a tissue, histologically identical throughout its distribution in the organism.
>
> The apparently arbitrary character of this distribution is to be explained (though only partially) by a reference to the chemical activity of hemoglobin. Wherever increased facilities for oxidation are requisite, hemoglobin may make its appearance in response; where such facilities can be dispensed with or are otherwise supplied, hemoglobin may cease to be developed. The *Vertebrata* and the annelids possess a blood containing hemoglobin in correlation with their greater activity as contrasted with the *Mollusca*, which do not possess such blood. The actively burrowing *Solen legumen* alone amongst lamellibranchiate molluscs, and amongst gasteropods only *Planorbis*, respiring the air of stagnant marshes, possess blood containing hemoglobin. In the former the activity, in the latter the deficiency of respirable gases are correlated with the exceptional development of hemoglobin. But we can not as yet offer an explanation of the absence of hemoglobin from the closely allied species of *Solen*, and from Limnæi which accompany *Planorbis*. The crustaceans *Chirocephalus* and *Daphnia*, and the larva of *Chironomus*, possessing, as exceptions in their classes, hemoglobin in their blood, inhabit stations where the amount of accessible oxygen must be small (that is to say, stagnant ponds), the last living in putrescent mud; whilst the possession of abundant hemoglobin in its vascular fluid may be supposed to be one of the chief properties which enables the oligochæte annelid *Tubifex* to hold its ground in the foul, and therefore much deoxygenated, water of the Thames at London.
>
> The known chemical properties of hemoglobin furnish a more complete explanation of its peculiar distribution in tissues. That it should occur in a circulating fluid which is the medium of respiration is obviously related to those properties. Its occurrence in the voluntary muscles of the most active of *Vertebrata*, and in the most active muscles of some others (as in the case of the dorsal-fin muscles of *Hippocampus*), is equally so; so also its occurrence in the most powerfully acting part of the intestinal

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* It is perhaps of some significance that hemoglobin has only been found in that great group of the animal kingdom which in the course of its development gives rise to a middle layer of blastodermic cells or mesoderm, and in examples from nearly every great branch of this stem.

muscles, those of the rectum, and in the only rapidly and constantly acting muscles of the gasteropods, namely those used in biting and rasping.

To connect its occurrence in the nervous chain of *Aphrodite aculeata* with its properties is more difficult, since we have no knowledge that this annelid is remarkable for nervous energy. The large bulk of the animal in proportion to the size of the nervous system, and the deficient respiration, indicated by the very slightly developed vascular system and the total absence of hemoglobin from the fluids of the worm, may be a reason for the endowment of the nervous center which has to control such a large and complicated organism with a special facility for appropriating what little oxygen may come in its way.

The complete absence of hemoglobin from *Leptocephalus* is an example of the submission of an auxiliary, but not an essential, structural attribute to an all-powerful necessity—that of transparency. The absence of hemoglobin from the transparent annelid *Alciope* may be similarly correlated.

MacMunn (*loc. cit.*) in his studies of the distribution of hemoglobin, myohematins, and histohematins in insects attributes a relationship between the degree of activity of the musculature of the wings and the quantity of coloring matter. Likewise, Velichi (*loc. cit.*) found hemoglobin in the most used muscles of gasteropods, which are the pharyngeal.

In a recent article (Archives de Zoologie expérimentale, 1903, 31) on the respiratory pigments in relation to the alkalescence of the blood, Gautrelet states that hemocyanin replaces hemoglobin under the following conditions: (*a*) if the diet contains Cu instead of Fe; (*b*) where the exchange of O and $CO_2$ is low (hemoglobin having about 4 times the O-capacity of hemocyanin); (*c*) if the salt capacity of the body-fluids is such that erythrocytes can not exist; (*d*) if a large liver retains the iron. One or more of these conditions may be present, but the appearance of one does not necessarily imply that one or the other of these coloring matters is present. Alkalescence and the amount of coloring matter he found to be parallel.

The distribution of hemoglobin throughout the vertebrates is universal, with the exception of *Leptocephalus* and possibly *Amphioxus*, and it is invariably confined under normal conditions to the erythrocytes and the structures in which these corpuscles are formed or destroyed. In the body tissues it appears chiefly in the form of myohematins. A number of compounds and derivatives, normal or abnormal, may be present.

A large number of coloring matters related and unrelated to hemoglobin have been found in both invertebrates and vertebrates, but a further consideration is not possible within the necessarily limited compass of this memoir.

### THE SOURCE OF HEMOGLOBIN PROBABLY IN CHLOROPHYL.

The close chemical relationship of chlorophyl and hemoglobin suggests either that both have sprung from a common source or that hemoglobin has had its source in chlorophyl, there probably occurring in the latter case a gradual synthesis during the progress of evolution. We find in certain of the lowest organisms a modified form of chlorophyl or chlorophylloid pigment; in others chlorophyl; in other forms of life occur coloring matters which are identical or nearly identical with certain hemoglobin derivatives; later, bodies in the form of histohematins and myohematins; and ultimately, hemerythrin, chlorocruorin, hemoglobin, etc.

That hemoglobin may have its source in chlorophyl has been shown by MacMunn, who found in *Helix pomatia* substances intermediate between chlorophyl and hemoglobin. That substances like hemoglobin derivatives found as constant constituents of certain of the lower organisms do not represent degradation products seems likely, inasmuch as it is hardly conceivable that a molecule so exceedingly complex as hemoglobin should have appeared suddenly. Unfortunately, our data are so insufficient that we can not trace this probable synthesis or possible degradation step by step, and for the same reason we can not show the causes of the extraordinary gaps in the distribution of either hemoglobin or its derivative-like bodies in the invertebrates.

## CHEMICAL NATURE OF TYPICAL RESPIRATORY SUBSTANCES, ETC.

We will now consider the functional properties of protein and other components, with especial reference to the probable misconception of the specificity of the rôle of the iron in the respiratory phenomenon.

Specific respiratory substances are doubtless essential constituents of all living organisms, except probably only certain of the very lowest forms of plant and animal life. They may be divided primarily into two groups, metal-bearing and metal-free. The former may contain manganese, copper, or iron, and they are normally, as far as known, colored; the latter are colorless, as the achroglobulins of Griffiths. That undiscovered colorless metal-bearing respiratory substances may exist in some of the lower organisms, and even in the higher forms, seems more than probable. That such bodies do exist as abnormal substances (for instance, in the form of decolorized hemoglobins or some close modification) has been ascertained by a number of investigators. It has been recorded, for instance, that hemoglobin may be crystallized and the crystals completely decolorized without change of form or elementary composition, and that the decolorized substance can even be recrystallized without alteration. Hemocyanin (reduced) is colorless. The chlorocruorins bear, as has been shown, a striking likeness to hemoglobin, spectroscopically and chemically, yet these substances may appear green, yellow, carmine, red, brown, etc. From the foregoing it is obvious that it is not the mere presence of the metal, nor the kind of metal, *per se*, that gives to the molecule its coloration, but the peculiar arrangement of the atoms or groups of the molecule. That colorless non-metal-bearing respiratory substances do exist has been shown by Griffiths. Entirely apart from this, it must be admitted that the existence of a large number of absolutely or practically colorless organisms, invertebrate and even vertebrate, in which the interchange of O and $CO_2$ in the blood goes on quite actively, clearly indicates that colorless (metal-bearing or non-metal-bearing) respiratory substances must have a wide distribution in animal life. Moreover, it is suspected by the physiological botanist that there may exist colorless plastids which are actively photosynthetic, like the chlorophyllous plastid.

The discovery by Griffiths of colorless metal-free respiratory substances in *Patella, Chiton, Tunicata,* and *Doris* (which have respiratory capacities varying from 1.2 to 1.49 c.c. per gram, or a mean of 1.31, which practically is identical with that of hemoglobin, 1.34) certainly throws grave doubts upon the universally accepted fundamental importance that is attached to the metal (iron, copper, manganese) of echinochrome, hemoglobin, chlorocruorin, hemocyanin, pinnaglobulin, etc. While the work of Griffiths has not, as far as we have been able to learn, been confirmed or disproved, it certainly has substantial support in a number of facts, especially the existence of absolutely or practically colorless invertebrates and vertebrates, in which we must from analogy admit the existence of specialized respiratory circulatory fluids, and also in the known differences in the behavior of the stromata, globin, and proteins generally, on the one hand, and of hematin, on the other, towards O and $CO_2$.

Since it is admitted that all living protoplasmic structures are respiratory, it seems but a short step in evolution to the differentiation of specialized colorless respiratory substances. While it is not improbable that in some of the lowest forms of life simple forms of respiratory substances exist, it seems that (since chloroplastids, histohematins, hemoglobin, hemocyanin, or similar compound bodies are found in very low organisms and throughout all gradations of higher animal and plant life, and from their chemistry) we should look upon a typical respiratory substance as being a compound body which consists essentially of a protein base to which is coupled an acid radical or its analogue, the former being the active respiratory component and the latter serving as a go-between and probably in the nature of an energizer.

Three types of O and $CO_2$ exchange have been observed:

(1) The analytic exchange that is characterized by the absorption of O and its utilization in the living processes in the breaking down of complex substances and the consequent formation and giving off of $CO_2$ as an effete product, a form of respiration which involves intrinsic changes: This in all likelihood is common to all forms of living matter (for even anaerobes absorb the last traces of oxygen, and even in plants this type of exchange is directly but little influenced by light).

(2) The synthetic exchange, photosynthetic and chemosynthetic, the first of which is manifested actively solely through the agency of chlorophyl and light; which is characterized by the absorption of $CO_2$ and the giving off of O; which involves intrinsic changes; and whose intensity is in direct relation to the intensity of light up to the optimal light: This exchange of $CO_2$ and O is believed to take place solely in the chloroplastid, or in some primitive non-cellular form of protein-chlorophyl combination, as in certain phanerogams in which the chlorophyl is normally found in diffused form. In the chemosynthetic exchange, light energy is replaced by energy in chemico-potential form, and the process has no necessary association with chlorophyl.

(3) The physico-mechanical exchange that goes on in the aeriferous system, intercellular air-spaces, etc., of plants, and in the erythrocytes, the blood plasma, and lymph, etc., of animals: This is dependent essentially upon differences in partial pressures and tensions of these gases and upon

mass actions, does not involve intrinsic changes, and is in effect a passive physico-mechanical function or a means of transport and storage.

The first type must be considered as representing a property that is possessed in common by all parts of the protoplasmic mass, at least until our knowledge is different from that of the present. As to the second type, the photosynthetic exchange is almost wholly confined to chlorophyllous substances, while the chemosynthetic exchange occurs chiefly in the entire absence of chlorophyl. The view held by many that chlorophyl is *per se* a primary respiratory photosynthetic substance was long ago shown to be untenable. Isolated chlorophyl has been proved to be absolutely inert in the exchange of $CO_2$ and O, and the cytoplasm of the chloroplastid has been found to be functionless in photosynthesis in the absence of chlorophyl, as in etiolated plants, in which what feeble photosynthesis may be present is attributed to the etiolin. In fact, the chloroplastid is a vital mechanism which consists, broadly speaking, of cytoplasm in some form of union with chlorophyl and etiolin, which is capable of exercising its normal photosynthetic functions only when all of the essential structural and physiological units are intact, and whose peculiar photosynthetic properties, therefore, are dependent upon some coöperative and inseparable relationship between the cytoplasm and the pigments. The exact structural relations of the cytoplasm and chlorophyl are unknown, but the chlorophyl appears to be held in a cytoplasmic stroma in vacuolar form. Moreover, normal functional activity can be maintained only so long as normal relations exist between the chloroplastid and its habitat. The isolated chloroplastid soon becomes functionless; and notwithstanding the very close resemblance of the chloroplastids of the higher forms of plant life, it is questionable if, like the erythrocyte, these structures of one genus could continue to exist as living units in an individual of another genus.

Whether or not chlorophyl is to be looked upon as being in the nature of an *energizer* or *sensitizing agent* in relation to the cytoplasm is yet a matter of speculation among physiological botanists. It is of particular interest in this connection to note that chlorophyl itself may be energized, for it has been shown in *Floridæ* that phycoerythrin, which is not assimilative and which apparently does not enter into either physical or chemical union with chlorophyl, markedly modifies the assimilatory curve of chlorophyl in relation to the spectral colors, and so greatly increases the energy of chlorophyl that a very small quantity of chlorophyl will give rise to energetic photosynthesis. While iron seems to be essential in the formation of chlorophyl, the latter is nevertheless iron-free, and the suggestion that the reducing action of the chloroplastid may be due to iron is regarded by the botanist as being absolutely untenable. In fact, apart from the necessity of iron in the formation of chlorophyl, this metal does not seem to be of more importance as a constituent of the chloroplastid in photosynthesis, or in respiration, than potassium, or magnesium, or certain other inorganic constituents.

The foregoing facts are, as a whole, strikingly paralleled in hemoglobin and the erythrocyte, the chief difference being found in the ways in which the corpuscle and pigment have been morphologically, chemically, and functionally specialized.

The erythrocyte, in common with all living structures, must be conceded to be a respiratory structure of the first type by virtue of its protoplasm, but in addition to this there exists the third type of exchange which is manifested in the continual alternating give-and-take in external and internal respiration, respectively, which is rendered possible through the feeble combinations of O and $CO_2$ in the erythrocyte in association with the differences in partial pressures and tensions of these gases in the proximal and distal portions of the vascular system, and with mass actions, in which operations the corpuscles act as a carrier and store-house for both gases. This respiratory phenomenon is to be attributed essentially to the agency of hemoglobin, and it will be noticed that it differs materially from the first and second types of exchange, which involve intrinsic changes, and which are manifestations of the activity of energy-transforming mechanisms. While, therefore, as has been shown in previous pages, hemoglobin and chlorophyl are intimately related chemically, and are the most important bodies in plant and animal life, respectively, in the exchange of O and $CO_2$, it is obvious that they have become so specialized in the character of their work that the mechanisms concerned in the exchange are totally different in character and object: we observe a phenomenon which in the first instance is manifested essentially through a passive vehicle; in the second, through the operations of an energy transformer.

The third type of respiratory activity, which is the preëminent property of the erythrocyte, is a property that belongs to the cell as a whole as an individual vital mechanism. Hemoglobin in solution in the plasma of the vertebrate blood has been shown to be in the nature of a foreign body; as a component of the erythrocyte it is an energetic respiratory substance, under which condition its dissociable O is more readily removed than when it is in solution; in the erythrocyte it behaves as though it were in colloidal form. Isolated hematin is absolutely inert in relation to both O and $CO_2$, and isolated stromata and isolated globin have not been found to have respiratory energy in O and $CO_2$ absorption and elimination greater than protein substances generally under comparable conditions. It seems therefore obvious that it is not the hemin, globin, or stroma, or the hemoglobin *per se*, that is the normal functionating substance, but a hemoglobin-stroma combination; and that from analogy, when the hemoglobin is normally in non-corpuscular form, as in certain of the invertebrates, it is probably in a primitive hemoglobin-protein combination similar to the assumed primitive non-corpuscular chlorophyl-protein combination noted in certain phanerogams.

What chemical and functional relationships the hemoglobin bears to the stroma, and hematin to the globin, are not known. But from the facts that the exchange of O and $CO_2$ goes on quite rapidly in all forms of active protoplasm, that isolated hematin, like chlorophyl, is absolutely inert in relation to these gases, and that chlorophyl behaves in the nature of an energizer in relation to the cytoplasm, it seems likely that hematin is of a like nature in relation to the globin and stroma. It has, moreover, been suspected, and even asserted, that the respiratory property of the erythrocyte, in so far as the give-and-take of O and $CO_2$ is concerned, is intensified by or even dependent upon the coöperation of an oxidase in the blood corpuscles or blood plasma. Here we have a parallel to the influence of phycoerythrin upon the energy of chlorophyl.

The absorptive and carrying property of hemoglobin in relation to O has been and continues to be attributed to the atom of iron in the molecule, and this assumption has been applied to the metal components of other respiratory substances, but upon very limited and inconclusive although seemingly plausible data, which in a word are virtually the O affinity and capacity of iron.

The absorption coefficient of hemoglobin of the bullock for oxygen has been determined experimentally by Hüfner to be 1.34 c.c. at 0° C. and 760 mm. pressure, which figure is practically identical with the capacity as estimated by the percentage of iron (0.336) present; hence, the natural conclusion and the universally accepted view that the property and capacity of hemoglobin in relation to O is specific to the atom of iron. The fact, however, should not be lost sight of in this connection that the absorptive capacities, as determined by different investigators, are by no means in accord. Thus, reducing all figures to 0° and 760 mm., the values are, according to Dybkowsky, 1.57; Hoppe-Seyler, for moist crystals 1.09, for crystals dried at room temperature 0.77, and for crystals dried at 0° and powdered 0.54; Strassburg, 0.45 to 3.88; Preyer, 1.72 and 1.8; Worm-Müller, 1.38; and Hüfner, 1.59 and 1.34 (latest). Even Hüfner's latest figures vary as much as 10 per cent, and his corrections are not beyond reasonable question. It is, moreover, doubtful if the property thus attributed to the metal, or even to the hematin, is justified, for while the quantitative relation of the oxygen capacity to the quantity of iron seems convincing as an isolated fact, the deduction is not borne out by our knowledge of this and other respiratory bodies and by other facts. After all, the absorption capacity is merely the maximal capacity for oxygen that can be observed under given conditions of pressure and temperature, other things being equal; moreover, this assumed specificity of the metal of the molecule is scarcely reconcilable with the fact of the existence of *metal-free respiratory substances (achroglobulins) which have practically identically the same absorption capacities as hemoglobin.* This quantitative coincidence of the O-capacity of hemoglobin and achroglobulin is certainly remarkable, and it shows clearly either that the specificity attributed to the iron is wrong, or that we have a substitute in the achroglobulin which has the same quantitative value as an absorptive factor, but this is hardly credible. If the accepted specificity of the iron be justified, we still have to find an explanation for the practically absolutely identical O-capacity of the metal-free achroglobulins.

Then again, assuming that the metal is the specific O-absorbing agent, hemocyanin should have an absorptive capacity, based upon the percentage of copper present, of 0.66 c.c. per gram at 0° and 760 mm., while in fact, as shown by the direct experiment of Henze with the blood of *Octopus vulgaris,* it combines with only 0.4 c.c., which is less than two-thirds of what it should be theoretically. The low O-capacity had been previously determined by Dhéré in experiments with the bloods of *Helix, Homarus, Astacus, Octopus, Carcinus, Cancer,* and *Maia.* Low figures have also been recorded by others.

From the foregoing it seems obvious that iron, manganese, or copper is an incidental rather than the essential constituent of specific respiratory substances, and that if there is a special constituent in relation to the property of respiration it is as yet not definitely known. As a specific component of these substances in relation to O, the sulphur would seem to be of far more importance than any of the metals named: its property as an energetic oxidizing agent is universally recognized; it is a universal constituent of all proteins and hence of all protoplasmic structures, which structures exhibit more or less absorptive activity towards O; the stromata of the erythrocytes, globin, leucocytes, yeast-cells, etc., energetically decompose peroxide of hydrogen. Especially energetic are the stromata of the erythrocytes. The facts that about 96 per cent of the hemoglobin molecule is protein, that stroma constitutes about 65 per cent of the erythrocyte, and that the globin and stroma together represent about 95 per cent of the erythrocyte of mammalian blood, strongly indicate a greater importance of the protein than of the hematin, and especially so because the proteins show affinities for O and $CO_2$, while the hematin is absolutely inert. The astute Bunge long ago taught (Text-book of Physiological and Pathological Chemistry, trans. by Starling, 1902, 22; trans. by Wooldridge, 1890, 24) that the respiratory function of hemoglobin can not be due to the iron alone and that it may be that the sulphur of hemoglobin, as of all other protein bodies, still retains its function as an oxidizing agent. In a very recent article, Carracido (Rev. d. l. R. Acad. d. Cienc., 1906, 33; Biochem. Centralbl., 1906–07, v, 572) concludes that either the globin participates in the oxygen absorption, or that the prosthetic group of chromoprotein is not the conjectured one, since the sulphur-capacity of the hemoglobin increases with the amount of oxygen absorbed.

The assumption that the respiratory property of hemoglobin pertaining to oxygen is essentially or solely a function of the atom of iron naturally led as a corollary to a belief of an inertness or practical inertness of the globin, and Bunge even suggested that "The enormous size of the hemoglobin molecule finds a teleological explanation if we consider that iron is eight times as heavy as water. A compound of iron which would float easily along with the blood current through the vessels could only be secured by the iron being taken up by so large an organic molecule." Bunge, later in his lectures, still leans to the belief of the importance of sulphur, for he states (*loc. cit.* 239):

> If oxygen is chemically combined with hemoglobin, we would expect them to be combined in molecular proportions. It would be interesting to ascertain how many atoms of oxygen go to one atom of iron. The analyses made up to the present time are not exact enough for this purpose; they show, however, that about 2 or 3 atoms of oxygen

correspond to 1 atom of iron. The figures so far only demonstrate that there is at least four times as much oxygen taken up in the transition of hemoglobin into oxyhemoglobin as there is in the transition from suboxid to oxid of iron, or from ferrocyanid to ferricyanid of potassium. Possibly the sulphur of the hemoglobin also plays a part in the loose oxygen compound, and a similar part may be assigned to the sulphur atoms in all proteins. It is noteworthy that, according to previous analyses, the animals that require more oxygen have likewise more sulphur in their hemoglobin.

That the protein is really an active factor in relation to the displaceable or respiratory oxygen has, for instance, been clearly indicated by the differences in the behavior of hemoglobin when in the erythrocyte and after removal from it, and by the recent researches of Ham and Balean (Journal of Physiology, 1905, XXXII, 312), who write that—

> It would appear that one of the oxygen atoms in oxyhemoglobin is differently combined to the other, *i.e.*, it is more intimately attached to the iron, and further that hematin still contains oxygen linked to the iron, only half being displaced in its formation from oxyhemoglobin by the action of dilute acids. This we should expect, from the fact that it requires a strong acid to form hematoporphyrin not only from oxyhemoglobin but also from hematin, whereas a weak acid readily effects the change in the case of reduced hemoglobin (Laidlaw). The reason for this is supposed to be that iron linked to oxygen is more stable than iron not so linked; in other words, the presence of oxygen attached to iron much increases the difficulty that acids have in removing the iron and forming hematoporphyrin. Again, since one oxygen atom, and not both, is displaced by the action of dilute acids on oxyhemoglobin in the formation of acid hematin, we are justified in supposing that the particular oxygen atom which is dissociated is not linked to iron in the same way as the one which is not displaced. Now the effect of dilute acids on oxyhemoglobin is not only to set free one oxygen atom, but also to split off the globin radicle. And we have found from experiment that as more and more oxygen is liberated so more and more acid hematin is formed, and therefore more and more globin is split off. But further than this, when we have displaced half of the replaceable oxygen we find nothing but acid hematin, and therefore all the globin must have been dissociated from the oxyhemoglobin molecule. It therefore appears that this displaced oxygen atom must bear some definite relation to the globin radicle, the other oxygen atom having both its oxygen affinities satisfied by the iron.
>
> The further action of the acid is then to split the bond between the oxygen atom and the globin radicle.

The absorptive power of erythrocytes as regards O is accredited to the hemoglobin, and as regards $CO_2$ to the alkali of the phosphates and globulin and to hemoglobin. The combination of $CO_2$ with hemoglobin does not give a compound that is to be classed with CO-hemoglobin and O-hemoglobin, because the $CO_2$ and O are not interchangeable in the quantitative relationship that is observed with CO and O. Bohr and his co-workers (Bohr, Festschrift f. C. Ludwig, 1887, 164; Compt. rend. soc. biolog., 1891, CXI, 243; Zentralbl. f. Physiologie, 1904, XVII, 688, 713; Jolin, Archiv f. Anat. u. Physiologie, 1891, III, 69; Torup, Biochem. Centralbl., 1906, v, 667; Maly's Jahr. ü. d. Fort. d. Thierchemie, 1906, XXXVI, 166) have recorded that the $CO_2$-capacity of hemoglobin is markedly higher than for O (which property has been found by Griffiths to be shown by the achroglobulins); that the $CO_2$-capacity is relatively high at low pressures and low at high pressures; that $CO_2$ is more readily dissociated than O; that the O-capacity of the blood may be decidedly affected by changes in the tension of $CO_2$, but that the $CO_2$-capacity is not affected by differences in the tensions of O; and that the absorptive capacities of hemoglobin for O and $CO_2$ are higher in the hemoglobin of the dog and guinea-pig than in that of the goose. The large $CO_2$-capacity of hemoglobin is suggestive of physiological importance, yet it seems to be almost entirely ignored by the physiologist.

Bohr, Hasselbalch, and Krogh made the important biological observation that a positive relationship exists between the percentage of O in the blood in the presence of different tensions of $CO_2$. They made a large number of experiments in which the tension of O varied from 5 to 150 mm. of Hg, and in which the tension of $CO_2$ was 5, 10, 20, 40, and 80 mm. of Hg, the absorption being determined in fresh dog's blood at 38°. They found that when the O tension is high (corresponding to the pressure in the pulmonary alveoli) differences in the tension of $CO_2$ are without important influence on the quantity of O absorbed; but when the tension of O is low (as in the blood of the capillaries of the tissues generally) an increase in the tension of $CO_2$ has a very depressing effect on the absorption of O. Assuming the tension of O in venous blood to be 25 mm. of Hg and that $CO_2$ is absent or without influence, only about 24 per cent of the O of the hemoglobin would be given off, but if the tension of $CO_2$ were 40 and 80 mm. of Hg, as much as 60 and 78 per cent, respectively, would be given off without the tension of the O falling below 25 mm. of Hg. It seems from this that the high tension of the $CO_2$ in the tissues must have an important influence on the rapid dissociation of O from the oxyhemoglobin.

## THE NON-IDENTITY OF HEMOCYANINS.

Krukenberg (page 9) has found that hemocyanins from different sources show differences in their behavior towards O and $CO_2$; Howell (page 10) noted difference in hemocyanins as regards the condition of the respiratory O and the temperature of coagulability; Cuénot (page 12) refers to a hemocyanin which is colorless, which does not become blue upon exposure to the air, and which he regards as being not respiratory; and Couvreur (page 13) noted differences in the degree of stability.

## THE IDENTITY OR NON-IDENTITY OF CORRESPONDING RESPIRATORY SUBSTANCES.

As regards the identity or non-identity of corresponding respiratory substances, it seems probable, from the literature on the subject: (1) that chlorophyl (pigment), like hematin, is an identical substance from whatever source; (2) that there are several forms of achroglobulin; (3) that hemocyanin is not a uniform substance, and that the same conclusion applies to hemoglobin, echinochrome, and chlorocruorin.

# CHAPTER II.

## SPECIFICITY OF THE BLOOD OF VERTEBRATES IN RELATION TO ZOÖLOGICAL DISTINCTION.

A large number of facts bearing upon generic and allied differences of the blood are scattered throughout the voluminous literature of the biological sciences, but these with few exceptions as isolated facts seem to be of so little importance as not to attract more than passing notice. When, however, they are considered collectively and in connection with the peculiarities pointed out or suggested in the preceding chapter, and with our discoveries of the specific peculiarities of the hemoglobin crystals shown in subsequent chapters, they will be found to be so positive in their meaning as to leave no doubt that we are on the threshold of a specialization so sensitive as to justify the prediction that the blood of each family, genus, species, and individual will be found to be absolutely specific. While it has not been possible for us to make an exhaustive collection of such data, we have brought together sufficient in the following paragraphs to show clearly that we may not only generalize but also specialize, and that with a number of determinant facts and with the present progress of research we are fast approaching the time when not only genera and species but also races, and even individuals of a race or species, can with as much or with greater certainty be distinguished by the peculiarities of their bloods as by the conventional methods of the zoölogist. Moreover, we believe, from even the limited studies we have made, that the zoölogical distinctions indicated by peculiarities of the blood will be found to be paralleled by similar peculiarities of other of the more important body fluids and solids.

### THE QUANTITY OF BLOOD IN RELATION TO BODY-WEIGHT IN REFERENCE TO GENERA.

The investigations of Welcker and others show that, while the proportions of blood to body-weight in both warm-blooded and cold-blooded animals, excepting certain of the amphibia and fishes, do not as a rule vary greatly, the differences in the various orders, classes, etc., are of zoölogical significance.

The methods of estimation are not exact, so that the figures recorded are to be regarded as being approximate. The discrepancies in the records of different observers in the proportions in members of a given species are to be accounted for in a measure in this way, and in part in variations due to age, sex, general condition, and the changes that arise from various incidental normal and abnormal states. Notwithstanding the crudity of the methods generally, the records are sufficiently in agreement to indicate specific but not important zoölogical distinctions.

TABLE 6.—*Proportion of blood to body-weight in different animals, according to figures of Welcker.*

| Kind. | No. of experiments. | Proportion to body-weight. | | |
|---|---|---|---|---|
| | | Minimum. | Maximum. | Mean. |
| Mammalia: | | | | |
| Man | 3 | 1 : 13.4 | 1 : 12.4 | 1 : 13.1 |
| Dog | 5 | 1 : 15.1 | 1 : 12.6 | 1 : 13.5 |
| Cat | 1 | ....... | ....... | 1 : 15.1 |
| Bat | 2 | 1 : 13.8 | 1 : 10.4 | 1 : 12.0 |
| Goat (young) | 1 | ....... | ....... | 1 : 16.1 |
| Rabbit | 5 | 1 : 20.8 | 1 : 16.4 | 1 : 18.1 |
| Dormouse | 3 | 1 : 17.9 | 1 : 15.8 | 1 : 16.5 |
| Common mouse | 5 | 1 : 15.7 | 1 : 11.8 | 1 : 13.1 |
| Aves: | | | | |
| Small birds | 3 | 1 : 13.1 | 1 : 11.8 | 1 : 12.4 |
| Pigeon | 3 | 1 : 13.1 | 1 : 9.0 | 1 : 10.9 |
| Reptilia, Amphibia, and Pisces: | | | | |
| Lizard (Lacerta agilis) | 1 | ....... | ....... | 1 : 20.4 |
| Do | 5 | ....... | ....... | 1 : 16.8 |
| Do | 10 | 1 : 18.4 | 1 : 14.3 | 1 : 16.8 |
| Lizard (Lacerta muralis) | 4 | ....... | ....... | 1 : 15.4 |
| Coluber natrix and Anguis fragilis | 5 | 1 : 26.7 | 1 : 12.3 | 1 : 18.3 |
| Frog (temporaria) | 5 | 1 : 20.4 | 1 : 15.3 | 1 : 17.4 |
| Triton and salamander (maculata) | 4 | 1 : 17.3 | 1 : 14.1 | 1 : 15.9 |
| Osseous fish | 2 | 1 : 74 | 1 : 53 | 1 : 63 |
| Lamprey | 1 | ....... | ....... | 1 : 19.4 |
| *Comparison of different classes.* | | | | |
| Fish | 3 | 1 : 74 | 1 : 19.4 | 1 : 49 |
| "Naked" amphibians | 4 | 1 : 33 | 1 : 15.9 | 1 : 20.5 |
| "Scaly" amphibians | 4 | 1 : 20.4 | 1 : 15.4 | 1 : 17.2 |
| Birds | 4 | 1 : 13.1 | 1 : 10.9 | 1 : 12.1 |
| Mammals | 8 | 1 : 18.1 | 1 : 12.0 | 1 : 14.7 |
| Lamprey | 1 | ....... | ....... | 1 : 19.4 |

While it will be seen (tables 6, 7, and 8) that the proportions recorded by different observers in studies of a given species differ (differences inseparable from the methods of determination and the variations that occur in the quantity of blood in any given individual even under perfectly normal conditions), the mean figures may be regarded as being sufficiently approximate for purposes of comparison. The range of mean values for the different classes is between one-forty-ninth (2 per cent) for fish and one-twelfth (8.3 per cent) for birds; in other words, in proportion to body-weight fish have less than one-fourth the quantity of blood found in birds. Comparing monkey and man, the proportion in the monkey is distinctly lower. Only two monkeys were studied, one young and the other very young, the values being 1 : 11.1 (9.02 per cent) and 1 : 13.6 (7.35 per cent), respectively. Among the members of the different orders, it will be found that the figures are higher in birds than in carnivora, herbivora, and rodents; higher in herbivora, especially in the horse, than in carnivora and rodents; and higher in carnivora than in rodents. While Welcker's table, showing a comparison of different classes, leads to the assumption that, with the exception of birds, the proportion of blood is higher as the animal is higher in the scale of life, it will be seen by comparing the values in tables 7 and 8 that such relationship is open to so many exceptions as to be untenable. When, however, comparisons are restricted to an order or class, without reference to the scale of life, the values give the impression of zoölogical distinctions.

The differences between carnivora, herbivora, etc., have already been noticed, and if now we compare certain of the members of different classes it will be seen, for instance, that there is a marked difference between the dog 1 : 13.5 (7.4 per cent) and the cat 1 : 15.4 (6.5 per cent); between the bullock 1 : 13 (7.7 per cent), the sheep 1 : 12.5 (8 per cent), the goat 1 : 16.1 (6.2 per cent), and the horse 1 : 10.3 (9.7 per cent); between the rabbit 1 : 16 (6.2 per cent), the guinea-pig 1 : 16.7 (6 per cent), and the mouse 1 : 12.3 (8.1 per cent), etc. These differences are certainly of sufficient definiteness to be suggestive of importance.

Table 7.—*Proportion of blood to body-weight in different animals, according to various observers.*

| Kind. | Proportion. | Authority. |
|---|---|---|
| Primates: | | |
| Man | 1 : 13.1 | Welcker. |
| Man | 1 : 13.5 | Bischoff. |
| Man | 1 : 20.5 | Haldane and Smith. |
| Man | 1 : 11.5 to 1 : 12.6 | Kottmann. |
| Woman | 1 : 13 | Do. |
| Monkey (young) | 1 : 13.6 to 1 : 11.1 | Sherrington and Copeman. |
| Chiroptera: | | |
| Bat | 1 : 13.5 | Welcker. |
| Carnivora: | | |
| Cat | 1 : 15.1 | Welcker. |
| Cat | 1 : 21 | Ranke. |
| Cat | 1 : 12 to 1 : 10.4 | Steinberg. |
| Cat | 1 : 17 to 1 : 15 | Jolyet and Laffont. |
| Cat | 1 : 14.2 to 1 : 13.3 | Brozeit. |
| Dog | 1 : 13.5 | Welcker. |
| Dog | 1 : 18 to 1 : 12 | Heidenhain. |
| Dog | 1 : 15 to 1 : 12 | Panum. |
| Dog | 1 : 15.1 | Ranke. |
| Dog | 1 : 14 to 1 : 11.2 | Spiegelberg and Gscheidlen. |
| Dog | 1 : 12.5 to 1 : 11.2 | Steinberg. |
| Dog | 1 : 13 to 1 : 12 | Jolyet and Laffont. |
| Dog | 1 : 14.5 to 1 : 14.9 | Sherrington and Copeman. |
| Ungulata: | | |
| Bullock | 1 : 13 | Heissler. |
| Sheep | 1 : 12.5 | Do. |
| Horse | 1 : 10.3 | Do. |
| Rodentia: | | |
| Mouse (common) | 1 : 13.1 | Welcker; Brozeit. |
| Dormouse | 1 : 16.5 | Welcker. |
| Guinea-pig | 1 : 22 to 1 : 17 | Gscheidlen. |
| Guinea-pig | 1 : 17.3 | Ranke. |
| Guinea-pig | 1 : 12 to 1 : 12 | Steinberg. |
| Guinea-pig | 1 : 18 | Jolyet and Laffont. |
| Rabbit | 1 : 18.1 | Welcker. |
| Rabbit | 1 : 20 to 1 : 15 | Heidenhain. |
| Rabbit | 1 : 22 to 1 : 17 | Gscheidlen. |
| Rabbit | 1 : 21 | Ranke. |
| Rabbit | 1 : 13.3 to 1 : 12.3 | Steinberg. |
| Rabbit | 1 : 18 | Jolyet and Laffont. |
| Rabbit | 1 : 17.2 to 1 : 12.4 | Brozeit. |
| Rabbit | 1 : 15.7 to 1 : 13.4 | Sherrington and Copeman. |
| Rabbit, buck | 1 : 20.6 | Douglass. |
| Rabbit, doe | 1 : 18.8 | Do. |
| Aves: | | |
| Small birds | 1 : 10.9 | Welcker. |
| Pigeons | 1 : 18 to 1 : 11.97 | Brozeit. |

Welcker's investigations (Prager Vierteljahressch. f. d. prakt. Heilk., 1854, iv, 63; Zeit. f. rat. Medicin, Ser. 3, 1858, iv, 147) are of especial value because of the number and variety of the species and orders represented (table 6). It is of interest to note that his records for the human being were made on executed criminals—one hanged and two guillotined. Those of Bischoff (Zeit. f. wissensch. Zoologie, 1855, vii, 331; 1857, ix, 65) were also made on guillotined men. Welcker's figures show that the proportion of the blood is in relation to zoölogical classification, it being higher in warm-blooded than in cold-blooded animals; higher in birds than in mammals; higher in "scaly" amphibia than in "naked" amphibia; higher in amphibia than in fish; and very low in fish, the value being only about one-third of that in the mammals.

Table 8.—*Means of the proportions of blood to body-weight in different animals, according to the figures of Welcker and others.*

| Kind. | Proportions to body-weight. | | Authority. |
|---|---|---|---|
| Primates: | | *Per cent.* | |
| Man | 1 : 20.5 | 4.9 | Haldane and Smith. |
| Man | 1 : 13.3 | 7.5 | Welcker and Bischoff. |
| Monkey | 1 : 12.3 | 8.1 | |
| Chiroptera: | | | |
| Bat | 1 : 12 | 8.3 | |
| Carnivora: | | | |
| Cat | 1 : 15.4 | 6.5 | |
| Dog | 1 : 13.9 | 7.2 | |
| Ungulata: | | | |
| Bullock | 1 : 13 | 7.7 | |
| Sheep | 1 : 12.5 | 8.0 | |
| Goat | 1 : 16.1 | 6.2 | |
| Horse | 1 : 10.3 | 9.7 | |
| Rodentia: | | | |
| Mouse (common) | 1 : 12.3 | 8.1 | |
| Dormouse | 1 : 16.5 | 6.1 | |
| Guinea-pig | 1 : 16.7 | 6.0 | |
| Rabbit | 1 : 16 | 6.2 | |
| Aves: | | | |
| Pigeon | 1 : 12.9 | 7.8 | |
| Small birds | 1 : 12.4 | 8.0 | |

The investigations subsequent to Welcker's have added materially to our list of genera and species among warm-blooded animals (Heidenhain, Archiv f. physiolog. Heilk., N. F., Ser. 1, 1857, 507; Panum, Archiv f. path. Anat. u. Phys., 1864, xxix, 241, 481; Brozeit, Archiv f. d. ges. Physiologie, 1870, iii, 353; Ranke, Die Blutvertheilung u. d. Thätigkeitswechsel der Organe, Leipzig, 1871; Spiegelberg u. Gscheidlen, Archiv f. Gynäcologie, 1872, iv, 530; Steinberg, Archiv f. ges. Physiologie, 1873, vii, 101; Gscheidlen, Physiologische Methodik, 1877, 333; Jolyet et Laffont, Gazette médicale, 1877, 349; Heissler, Arbeiten a. d. path. Institut z. München, 1886, 322; Haldane and Smith, Journal of Physiology, 1899–1900, xxv, 331; Douglass, Journal of Physiology, 1906, xxxiii, 493; Sherrington and Copeman, Journal of Physiology, 1893, xiv, 74; Kottmann, Archiv f. exper. Path. u. Pharm., 1906, liv, 356).

It would be a natural assumption that in those animals in which the activities of the general metabolic processes are the most intense the proportion of blood would likewise be the highest; yet in fact this is true to only a limited extent, and even then probably only incidentally so. In warm-blooded animals the value is higher than in the cold-blooded, and among the former it is higher in birds than in mammals, which is in accord with what we should expect. On the other hand, it is higher in the horse than in the human being, notwithstanding the fact that the intensity of the metabolic processes, as expressed by the intensity of oxidation per kilo of body-weight, is lower in the horse than in man. Likewise do we note an inverse relationship when we compare the dog and the guinea-pig, and small and large birds. From this it is manifest that there must be some factor or factors coupled with the relative blood-volume which compensate for the discrepancies between the proportional volume and the relative degree of tissue activity. Such incongruities might in some instances be accounted for in adaptations in the speed with which the total volume of blood is forced through the vascular system, but the chief explanation is doubtless to be found in differences in the composition of the blood, especially as regards hemoglobin and other proteins.

TABLE 9.—*Specific gravities of the blood as determined by different observers.*

| Kind. | Specific gravity. | Authority. |
|---|---|---|
| Primates: | | |
| Man | 1059 | Jones. |
| Monkey | 1054.9 | Sherrington and Copeman. |
| Carnivora: | | |
| Dog | 1060 | Pflüger. |
| Dog | 1059 | Nasse. |
| Cat | 1054 | Do. |
| Cat | 1054.6 | Sherrington and Copeman. |
| Ungulata: | | |
| Bullock | 1061 | Davy. |
| Calf | 1058.3 | Sherrington and Copeman. |
| Sheep | 1042 | Nasse. |
| Goat | 1062 | Sherrington and Copeman. |
| Horse | 1062 | Nasse. |
| Ass | 1042 | Do. |
| Pig | 1060 | Do. |
| Rodentia: | | |
| Mouse | 1059 | Sherrington and Copeman. |
| Rat | 1056 | Do. |
| Guinea-pig | 1059 | Do. |
| Rabbit | 1049 | Gscheidlen. |
| Rabbit | 1053.1 | Sherrington and Copeman. |
| Aves: | | |
| Turkey | 1061 | Davy. |
| Chicken hen | 1063.6 | Sherrington and Copeman. |
| Chicken cock | 1064 | Do. |
| Blackbird hen | 1062 | Jones. |
| Blackbird cock | 1066 | Do. |
| Sparrow hen | 1063.5 | Do. |
| Sparrow cock | 1074 | Do. |
| Hedge sparrow | 1059 | Do. |
| Greenfinch | 1068 | Do. |
| Pigeon | 1067.3 | Sherrington and Copeman. |
| Reptilia, Amphibia, and Pisces: | | |
| Snake | 1055 | Do. |
| Frogs (14 winter) | 1034 min. | Jones. |
| | 1053 max. | Do. |
| | 1041 mean | Do. |
| Frogs | 1055.6 | Sherrington and Copeman. |
| Skate | 1035 to 1038 | Harris. |

If we compare the proportions of blood and the percentages of hemoglobin and plasma proteins of the bloods of man and the frog, it will be noted that the proportion of blood in the former, according to Welcker, is 7.69

per cent, and in the latter 6 per cent, so that in the human being the proportion is but little more than in an animal comparatively low in the scale of life and in which the metabolic processes in comparison with those of man are at a comparatively low level. A partial explanation of this inconsistency becomes at once obvious in the differences in the content of the bloods as regards the constituents just referred to, the blood of the former containing about 12.5 to 13.5 per cent of hemoglobin, and about 7.6 per cent of proteins in solution in the plasma, while in the latter there are about 2.5 to 3 per cent of hemoglobin and 2.54 per cent of plasma-proteins. Likewise, mammals generally have a lower proportion of blood than birds generally, but the percentages of hemoglobin and plasma-proteins are notably higher in the former. The blood of all animals having nucleated corpuscles, if not actually poorer in erythrocytes than the blood of mammals, is usually or invariably poorer in hemoglobin and proteins. The meanings of the differences in the proportions of blood in different genera, etc., are as yet undetermined; but it seems that the proportion of blood in relation to body-weight, the proportions of vital constituents of the blood in relation to body-weight, and the rapidity with which the total volume of blood is driven through the vessels, should collectively show a definite and close relationship to the individual's position in the scale of life and to the intensity of its metabolic processes.

TABLE 10.—*Mean specific gravities deduced from the records of table* 9.

| Kind. | Specific gravity. | Kind. | Specific gravity. |
|---|---|---|---|
| Primates: | | Rodentia: | |
| Man | 1059. | Rat | 1056 |
| Monkey | 1054.9 | Mouse | 1059 |
| | | Guinea-pig | 1059 |
| Carnivora: | | Rabbit | 1053.1 |
| Cat | 1054.3 | Aves: | |
| Dog | 1059.5 | Turkey | 1061 |
| | | Chicken | 1064 |
| Ungulata: | | Blackbird | 1064 |
| Bullock | 1061 | Sparrow | 1068.2 |
| Sheep | 1042 | Hedge sparrow | 1059 |
| Goat | 1062 | Greenfinch | 1068 |
| Horse | 1060 | Pigeon | 1067 |
| Ass | 1042 | Reptilia and Amphibia: | |
| Pig | 1060 | Snake | 1055 |
| | | Frog | 1055.6 |

## THE SPECIFIC GRAVITY OF THE BLOOD IN RELATION TO GENERA.

So many conditions, especially age, diet, general nutritive state, parturition, etc., may affect to even a marked extent the specific gravity of the blood, that decided variations must be expected not only among individuals of the same species, but also in any given individual from day to day and hour to hour. Notwithstanding the difficulties of obtaining accurate data under such conditions, the results of the investigations of Lloyd Jones and of Sherrington and Copeman and others (Davy, Researches Anatomical and Physiological, London; Lloyd Jones, Journal of Physiology, 1887, VIII, 874; 1891, XII, 299; Pflüger, Archiv f. ges. Physiologie, 1886, L, 75; Nasse, Wagner's Handwörterbuch, Das Blut, I, 134; Gscheidlen, Physiologische Methodik, 1877, 328; Sherrington and Copeman, Journal of Physiology, 1893, XIV, 52; Harris, Journal of Physiology, 1903, XXX, 319) show in a general way at least that differences exist in the bloods of different species which indicate zoölogical distinctions. Examining the records of tables 9 and 10 it will be seen that specific gravity is higher in warm-blooded than in cold-blooded animals; higher in birds than in mammals; higher generally in herbivora than in man and monkey, rodents, and carnivora; highest in birds, and probably lowest in carnivora. The high standard in the frog and the snake are particularly noteworthy. Among herbivora, the specific gravity of the ass and sheep are particularly low; that of the cat is distinctly lower than that of the dog; that of the rabbit decidedly lower than that of the mouse, rat, and guinea-pig.

There is not in the differences of specific gravity the quantitative demarcation between warm-blooded and cold-blooded animals that was found to exist in the proportions of blood to body-weight, nor does there appear any approach to the class distinctions there noted.

The cause or causes of the differences in the specific gravities of the bloods of different animals are of course of much more importance than the specific gravity *per se*, because, while we may in any two or more instances find the same specific gravity in related or unrelated members of an order or class, etc., for instance, the horse and pig, or the bullock and goat, or the rabbit and cat, etc., thus expressing the same percentage of solids, gravity gives no indication as to how those solids are constituted—that is, as to how they may vary in kind and proportions in the different bloods. Since hemoglobin and other proteins represent nearly the whole of the solids, we infer that differences in specific gravity express somewhat closely corresponding differences in the percentage of one or the other, or both, of these constituents. In fact, in human blood, under normal and certain abnormal conditions, the relationship between specific gravity and the percentage of hemoglobin is so constant within narrow limits that the clinician makes use of specific gravity tables which indicate quite accurately the percentage of hemoglobin present. Thus, a blood having a specific gravity of 1.059 (water taken as 1.000, as determined by the hydrometer) will be found to contain about 14 per cent of hemoglobin; at 1.056, about 11.2 per cent; at 1.052, 9.8 per cent, etc.

While there is thus an unquestionable relationship between these factors in human blood, it does not follow that if in different species we find the same specific gravity there will be the same or even nearly the same per cent of hemoglobin. Thus, while the specific gravities of the bloods of the bullock and pig are the same, the hemoglobin percentages are 10 and 14 respectively; the specific gravities of the blood of the rabbit and cat are about the same, but the percentages of hemoglobin are 12.3 and 14.3 respectively. The blood of the dog has a higher specific gravity than that of the cat, but a lower hemoglobin content; and birds have the highest specific gravity of all animals examined, yet a relatively low proportion of hemoglobin. In the bloods of different species the protein content of the plasma is far less variable quantitatively than hemoglobin, but there is no definite relationship between the increase or decrease of one and the changes in the other.

### THE ALKALINITY OF THE BLOOD IN RELATION TO GENERA.

The reaction of the blood from the standpoint of modern physicochemistry is neutral, inasmuch as the blood does not contain a larger quantity of hydroxyl ions (OH—) than water. Moreover, this neutral state is maintained with remarkable persistency, as is shown by the fact that a very much larger quantity of sodium hydroxide is required to cause a given intensity of reaction than when added to water. This peculiarity is owing, according to Friedenthal, to the acid character of the proteins. When, however, the blood is tested with litmus, lacmus or lacmoid, or by titration with a weak acid, such as tartaric or phosphoric acid, a marked degree of alkalinity will be found. In the case of the human blood, for instance, it will be noted that 100 c.c. have an alkaline equivalent of from 250 to 300 mg. of NaOH, or in other words an alkalinity corresponding to a 0.25 to 0.3 per cent aqueous solution of sodium hydroxide. The alkalescence thus expressed is the measure of the amount of bases in combination with weak acids in the form of weak basic bodies, such as certain of the proteins, disodium phosphate, and sodium carbonate.

That the degree of alkalinity must of necessity be variable, within certain limits at least, seems apparent in the fact of the unceasing chemical changes that take place within the blood, and in the continual passage of substances of varying reactions between the blood and the tissues. It has been shown that the intensity of the reaction may be affected to even a marked degree by the character of the diet, by muscular exercise, and by various other conditions, normal and abnormal; it decreases rapidly in the shed blood and during the process of coagulation, and the more markedly as the alkalinity was previously high; it is higher in the plasma than in the serum and highest in the coagulum, and it is very high in laked blood; and it varies within limits so wide in different species that the equivalent of one may be as much as or more than twice as high as in another species.

The alkalescence of human blood has been studied by a large number of investigators, chiefly clinicians, and the values are far from being in accord, the reason for which is not far to seek when one considers the crudities of some of the methods and the fact that the reaction alters within a period so short as a couple of minutes after the blood is shed. According to Strauss (Zeit. f. klin. Medicin, 1896, xxx, 327) the unavoidable errors may range as high as 30 mg. of NaOH per 100 c.c. of blood. The alkalinity of human blood, based upon a study of the records of different observers, may be taken as corresponding to 250 to 350 (mean 280) mg. of NaOH to 100 c.c. of blood.

In the lower animals, Zuntz (Hermann's Handbuch der Physiologie, 1880, IV, 2 Th., 73; Beiträge z. Physiologie des Bluts, 1868, 13; Centralblatt f. med. Wissensch., 1867, v, 801) found in a pig a value of 330 mg. of $Na_2CO_3$ and in 10 dogs values ranging from 133 to 274 mg. of $Na_2CO_3$. In one experiment on the dog, Lassar (Archiv f. ges. Physiologie, 1874, IX, 45) found a range of 164.4 to 169.7 and a mean of 166.1 mg. of NaOH. In 6 cats this same observer records a mean of 187.3 mg. of NaOH; and in 20 rabbits, 10 German and 10 French, he records mean values of 146.3 and 164.5 mg. of NaOH respectively. Loewy (Archiv f. ges. Physiologie, 1894, LVIII, 462, 507, 511) found alkalinity higher in man than in the horse, and higher in the horse than in the dog.

Zuntz has shown in his experiments with the bloods of the horse, dog, and calf not only marked differences in the alkaline equivalents of these bloods, but marked differences in the alkalinity of the serum and clot. His determinations were made by means of dilute phosphoric acid, each cubic centimeter of which neutralized 5 mg. of $Na_2CO_3$.

The accompanying statement (table 11) shows the amount of dilute acid required to neutralize 100 c.c. of serum of blood and clot of blood of certain animals.

TABLE 11.

| Serum of blood. | | Clot of blood. | |
|---|---|---|---|
| Kind. | Amount required to neutralize 100 c.c. | Kind. | Amount required to neutralize 100 c.c. |
| Dog | 17.75 c.c. | Dog | 43.75 c.c. |
| Horse | 27.7 c.c. | Horse | 46.4 c.c. |
| Calf | 38 c.c. | Calf | 64 c.c. |

From these figures it will be seen that when the alkalescence is expressed in milligrams of $Na_2CO_3$ the values for the sera of these animals are 88.75, 138.5, and 190, respectively; and for the clots, 218.75, 232, and 320, respectively; and that the mean values for the sera and clots are for the dog 153.7, for the horse 185.25, and for the calf 225. It will also be noted, as has been shown in human and other bloods, that the alkalinity of the serum is always less than that of the whole blood and of the clots; and also that the ratios between the sera and clots are not the same in the different species, these ratios being for the dog 1 : 2.47 and for the horse and the calf 1 : 1.68.

Comparisons of the values (table 12) obtained for different species show the existence of marked zoölogical distinctions. It is shown that the alkaline equivalent is decidedly the highest in omnivora (man and pig), and then in the following order: herbivora, carnivora, and rodents, the value in the last being only about half that of the omnivora. Comparing the figures for the calf and the sheep with that of the horse, it seems as though the value for ruminants would be found to be higher than for other ungulates, except those belonging to the pig class. Further zoölogical differences are suggested by the different values of the cat and dog, of the calf and sheep, and of the German and French rabbits. Whether or not the differences in the ratios of alkalinity of sera and clots are of significance is problematical, yet the correspondence between the horse (1 : 1.68) and calf (1 : 1.68) on the one hand, as contrasted with that of the dog (1 : 2.47), is suggestive that this may be worthy of inquiry. In human blood the records of Brandenburg (Zeit. f. klinische Medicin, 1898–99, XXXVI, 280) indicate that the serum has half the alkalinity of the whole blood (blood 330 to 370; serum 160 to 190). This gives human blood a lower ratio between serum and clot than in the dog, and higher than in the horse and calf.

TABLE 12.—*Alkaline equivalents of the bloods of different animals.*

| Kind. | Alkaline equivalent. | Authority. |
|---|---|---|
| Primate: | | |
| Man | 250 to 300 (280) mg. NaOH | Jaksch. |
| Carnivora: | | |
| Cat | 170.3 to 207.2 (187.3) mg. NaOH | Lassar. |
| Dog | 133 to 274 mg. of $Na_2CO_3$ | Zuntz. |
| Dog | 164.4 to 167.9 (166.1) mg. NaOH | Lassar. |
| Ungulata: | | |
| Calf | 255 mg. of $Na_2CO_3$ | Zuntz. |
| Sheep | 190.5 mg. of NaOH | Lassar. |
| Horse | 185.25 mg. of $Na_2 CO_3$ | Zuntz. |
| Pig | 300 mg. of $Na_2CO_3$ | Do. |
| Rodentia: | | |
| Rabbits | German 103.9 to 171.8 (146.3) mg. NaOH | Lassar. |
| | French 143.6 to 182 (164.5) mg. NaOH | Do. |

NOTE.—Consult Vierordt's Anatomische, physiologische u. physikalische Daten u. Tabellen, Jena, 1906, 200, 201, 253, 507, and 525, for records of a large number of investigations with human blood.

That the erythrocytes play a part in determining the degree of alkalinity is evident in several facts, showing that the reaction is due in part to substances in solution in the plasma and in part to these corpuscles. Orlowsky (Zentralbl. f. Stoff. u. Verdauungskrankh., 1902, 111, 31) found in his investigations of diseased conditions in human beings that the degree of alkalescence was proportional to the quantity of erythrocytes; that the leucocytes do not have any important influence; and that the reaction of the blood plasma in most diseases remains normal or but little lessened. As is well known, laking the blood, by which the erythrocytes are partially or completely broken down, greatly increases the degree of alkalinity. Interesting in this connection also is the work of Gautrelet (*loc. cit.*), who found in his studies of hemocyanin that the degree of alkalinity of the blood and the amount of hemocyanin are parallel. A parallelism has also been noted between the degree of alkalinity and the percentage of hemoglobin in the human being.

Further zoölogical distinction has been shown in the resistance of the blood of different species to a diminution of alkalinity when dilute mineral acids are administered by the stomach. Human blood is readily affected in this way; the blood of the rabbit is extremely sensitive; while the blood of the dog shows a positive immunity.

### THE PROPORTIONS OF SODIUM AND POTASSIUM IN THE BLOOD, SERUM, AND CORPUSCLES IN RELATION TO GENERA.

The results of the analyses of the bloods, sera, and corpuscles of man, dog, cat, bullock, sheep, goat, horse, pig, rabbit, chicken, tortoise, frog, and toad show clearly differences in the quantities and the ratios of Na and K which are of unquestionable zoölogical importance in relation to class, genus, and species (table 13). In the whole blood, in the ruminants and carnivora the percentages of Na are almost identical, but in the horse, pig, and rabbit they are distinctly lower. The quantity of K in the blood of the ruminants is about 60 per cent higher than in carnivora, and in the horse, pig, and rabbit about 10 times higher than in carnivora. It is of interest to note that, while the quantities in the bullock, sheep, and goat are practically identical (mean, 0.400), and also in the dog and cat (mean, 0.257), they differ in the horse (2.738), pig (2.309), and rabbit (2.108) so distinctly as to indicate generic distinctions.

TABLE 13.—*The percentages and ratios of Na and K in the bloods, sera, and corpuscles of different animals.*

| Kind. | Percentage of Na. | | | Percentage of K. | | | Ratios of Na to K. | | | Authority. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Bloods. | Sera. | Corpuscles. | Bloods. | Sera. | Corpuscles. | Bloods. | Sera. | Corpuscles. | |
| Primate: | | | | | | | | | | |
| Man........ | 1.847 | 3.435 | 0.815 | 1.825 | 0.194 | 3.072 | 1:0.98 | 1:0.06 | 1: 3.77 | Wanach. |
| Carnivora: | | | | | | | | | | |
| Cat........ | 3.686 | 4.439 | 2.705 | 0.260 | 0.262 | 0.258 | 1:0.07 | 1:0.06 | 1: 0.09 | Abderhalden. |
| Cat........ | .... | .... | 2.766 | .... | .... | 0.262 | ...... | ...... | 1: 0.09 | Botazzi & Capelli. |
| Dog........ | 3.66 | 4.278 | 2.838 | 0.254 | 0.241 | 0.273 | 1:0.07 | 1:0.06 | 1: 0.09 | Abderhalden. |
| Dog........ | .... | .... | 2.865 | .... | .... | 0.277 | ...... | ...... | 1: 0.09 | Botazzi & Capelli. |
| Ungulata: | | | | | | | | | | |
| Bullock .... | .... | .... | 2.094 | .... | .... | 0.747 | ...... | ...... | 1: 0.03 | Bunge. |
| Bullock..... | 3.635 | 4.312 | 2.2322 | 0.407 | 0.255 | 0.722 | 1:0.01 | 1:0.06 | 1: 0.03 | Abderhalden. |
| Bullock..... | .... | 4.25 | .... | .... | 0.23 | .... | ...... | 1:0.06 | ........ | Sertoli. |
| Sheep...... | 3.650 | 4.294 | 2.257 | 0.407 | 0.225 | 0.742 | 1:0.01 | 1:0.05 | 1: 0.03 | Abderhalden. |
| Goat....... | 3.579 | 4.326 | 2.174 | 0.396 | 0.246 | 0.679 | 1:0.01 | 1:0.05 | 1: 0.03 | Bunge. |
| Horse...... | .... | 4.430 | None | .... | 0.270 | 4.92 | ...... | 1:0.06 | 0: 4.92 | Abderbalden. |
| Horse...... | 2.691 | 4.434 | None | 2.738 | 0.263 | 4.935 | 1:1.02 | 1:0.06 | 0: 4.935 | Do. |
| Pig......... | 2.406 | 4.251 | None | 2.309 | 0.279 | 4.957 | 1:0.9 | 1:0.07 | 0: 4.957 | Do. |
| Pig......... | .... | 4.272 | None | .... | 0.273 | 5.543 | ...... | 1:0.07 | 0: 5.543 | Bunge. |
| Rodentia: | | | | | | | | | | |
| Rabbit..... | 2.785 | 4.442 | None | 2.108 | 0.259 | 5.229 | 1:0.8 | 1:0.06 | 0: 5.229 | Abderhalden. |
| Rabbit..... | .... | .... | 0.077? | .... | .... | 4.659 | ...... | ...... | 1:60.9? | Botazzi & Capelli. |
| Aves: | | | | | | | | | | |
| Chicken..... | .... | .... | 0.160 | .... | .... | 4.650 | ...... | ...... | 1:29.2 | Do. |
| Reptilia and Amphibia: | | | | | | | | | | |
| Box tortoise | .... | .... | 0.283 | .... | .... | 3.127 | ...... | ...... | 1:11.05 | Do. |
| Frog....... | .... | .... | 0.292 | .... | .... | 2.320 | ...... | ...... | 1: 7.95 | Do. |
| Toad....... | .... | .... | 0.184 | .... | .... | 3.310 | ...... | ...... | 1:18 | Do. |

In contrast with the foregoing, while we do not find such differentiations in the percentages of either Na or K in the sera, in the corpuscles they are even far more striking than those which have been noted in the whole blood. Bunge (Zeit. f. Biologie, 1876, XII, 191), in his analyses of the bloods of the bullock, horse, and pig, recorded the interesting fact that, while Na is present in the corpuscles of the bullock in the proportion of over 2 parts per 1,000, it is entirely absent from the corpuscles of the horse and pig. The subsequent researches of Wanach (Inaug. Dissert. St. Petersburg, 1888; Maly's Jahr. ü. d. Fort. d. Thierchemie, 1888, 88) and Botazzi and Capelli (Atti della R. accad. dei Lincei, 1899, Serie V, VIII, 65; Maly's Jahr. ü. d. Fort. d. Thierchemie, 1899, 176), and Abderhalden (Zeit. f. physiolog. Chemie, 1898, XXV, 67) have accentuated the importance of this observation. From a study of the records of these investigators it is evident that the representatives of the different genera, etc., fall into four distinct groups—(1) dog and cat; (2) bullock, sheep, and goat; (3) man;

(4) horse, pig, and rabbit—each group being positively distinguished from the others, each individual of each group being distinguishable not only from the members of the other groups but from each member of the same group, by the differences in the quantities and ratios of Na and K.

In the *first group* (dog and cat) the Na content of the corpuscles is about four-tenths less than the quantity in the serum, while that of K is practically identical in both serum and red corpuscles.

In the *second group* (bullock, sheep, and goat) the Na is a little over half the proportion in the serum, while the K is about 3 times greater than the quantity in the serum.

In the *third group* (man representing the primates) the Na content is only about one-fourth that of the serum, while the K is about 16 times greater than in the serum.

In the *fourth group* (horse, pig, and rabbit) Na is absent from the corpuscles, while the quantity in the serum is about the same as in the first and second classes. On the other hand, the K content of the corpuscles is about 20 times greater than in the serum. In this class are individual representatives of two classes of ungulates (horse and pig) and one class of rodents. Whether or not Na is present in the red corpuscles of the rabbit might possibly be regarded as an open question, since Botazzi and Capelli found it (only 0.077 per cent) and Abderhalden did not, but one would be inclined to accept the work of Abderhalden, whose analysis was of the blood from 12 rabbits.

In animals having nucleated red corpuscles it has been found by Botazzi and Capelli that while the corpuscles contain Na it is in very low percentage. In the chicken the Na is particularly low, but the K percentage is quite high. In the animals of this group (chicken, tortoise, frog, and toad) there is no difficulty in differentiating one genus from the others by the differences in the ratios of the Na and K: in the chicken 1 : 29, and in the frog, toad, and tortoise 1 : 7.9, 1 : 18, and 1 : 11, respectively.

From these records of the Na and K contents of the corpuscles the following conclusions may be drawn: (1) that the Na and K percentages are in inverse but not proportional relationship; (2) that carnivora, as represented by the dog and cat, are characterized by a high Na content and a very low K content, the percentage of K being practically the same as in the serum; (3) that in ruminants the Na content is about one-fifth lower than in carnivora, and the K content nearly 3 times higher; (4) that in human blood the Na content is somewhat over one-fourth the quantity in carnivora, while the K content is about 12 times higher; (5) that in the classes represented by the horse, pig, and rabbit Na is absent, and the K content reaches its highest, being about 20 times higher than in carnivora; (6) that in birds, as represented by the chicken, the Na is the lowest noted in any of the groups (excluding Botazzi and Capelli's figure for the rabbit), while the K is very high, but not so high as in the horse, pig, and rabbit; (7) that the ratios of Na to K in the sera of all animals are practically identical; (8) that the ratios of Na to K in the corpuscles are sufficiently different to be of considerable importance in zoölogical differentiation.

### THE PHOSPHORIC ACID OF THE ASH OF THE CORPUSCLES IN RELATION TO GENERA.

Abderhalden (*loc. cit.*) has shown that the proportions of $P_2O_5$ in the ash of the serum of the bloods of the cat, dog, bullock, sheep, goat, horse, pig, and rabbit are nearly the same, while in the corpuscles they are so different as to show not only class but generic distinctions.

The proportions of $P_2O_5$ in the sera of these animals range within the narrow limits of 0.0197 and 0.025 per cent (table 14). In the corpuscles the minimum and maximum are 0.699 and 2.244. The percentages in the different animals differ in such ways as to give rise to the same differentiation as was noted with the Na and K salts.

TABLE 14.—*The percentages and ratios of $P_2O_5$ in the ash of the bloods, sera, and corpuscles of different animals.*

| Kind. | Percentage of $P_2O_5$. | | | Ratios of $P_2O_5$ (Sera : Corpuscles.) |
|---|---|---|---|---|
| | Blood. | Sera. | Corpuscles. | |
| Carnivora: | | | | |
| Cat | 0.0830 | 0.0236 | 0.1605 | 1 : 6.8 |
| Dog | .0810 | .0246 | .1577 | 1 : 6.4 |
| Ungulata: | | | | |
| Bullock | .0404 | .0244 | .0734 | 1 : 3.01 |
| Sheep | .0402 | .0236 | .0768 | 1 : 3.25 |
| Goat | .0397 | .0237 | .0699 | 1 : 2.52 |
| Horse | .1120 | .024 | .1901 | 1 : 7.9 |
| Pig | .1007 | .0197 | .2058 | 1 : 10.4 |
| Rodentia: | | | | |
| Rabbit | .0986 | .0242 | .2244 | 1 : 9.2 |

Taking again the dog and cat as the basis of comparison, the ratio of $P_2O_5$ in serum to corpuscles is 1 : 6.6 (mean); that of the ruminants 1 : 2.93 (mean); that of the horse 1 : 7.9, of the pig 1 : 10.4, and of the rabbit 1 : 9.2. These differences cause the different animals to fall into the same groups as determined by the Na and K content of blood and corpuscles. This is certainly a striking parallelism.

### THE PROPORTIONS OF CHOLESTERIN IN THE SERUM AND CORPUSCLES IN RELATION TO GENERA.

The proportions of cholesterin, according to Abderhalden's analyses (*loc. cit.*), vary within a much wider range in the sera and to even a greater extent in the corpuscles than $P_2O_5$. Here again the same well-defined distinctions are noticeable, and not only in the whole bloods and in the corpuscles, but also in the sera (table 15).

Comparing the bloods of the three groups (taking the mean percentage of the carnivora as being 0.1002), the mean percentage of cholesterin in the ruminants is distinctly higher (0.1639), while in the horse, pig, and rabbit it is the reverse, being less than half as much (0.047). In the sera and corpuscles specific differences will be noted, the cholesterin content being highest in the ruminants, lower in the carnivora, still lower in the rabbit, and lowest in the horse and pig. The percentage in the corpuscles of all species is higher than in the sera, and the differences are least marked in the horse, pig, and rabbit, but we do not find the marked differences in the ratios that were described in the case of Na, K, and $P_2O_5$, although the group distinctions appear in accord with the Na, K, and $P_2O_5$ records. In the goat, horse, pig, and rabbit the ratios are relatively low; in the carnivora higher; and in the ruminants, excepting the goat, the highest. The quantitative differences in the bullock, sheep, and goat in the blood and corpuscles are striking and indicate generic distinctions.

Table 15.—*The percentages and ratios of cholesterin in the bloods, sera, and corpuscles of different animals.*

| Kind. | Percentages of cholesterin in blood, sera, and corpuscles. | | | Ratios of cholesterin in sera and corpuscles. |
|---|---|---|---|---|
| | Bloods. | Sera. | Corpuscles. | Sera : Corpuscles. |
| Carnivora: | | | | |
| Cat | 0.0895 | 0.0600 | 0.1281 | 1 : 2.13 |
| Dog | .1110 | .0683 | .1750 | 1 : 2.55 |
| Ungulata: | | | | |
| Bullock | .1935 | .1238 | .3379 | 1 : 2.73 |
| Sheep | .1685 | .1094 | .2976 | 1 : 2.72 |
| Goat | 1299 | .1070 | .1730 | 1 : 1.62 |
| Horse | .0346 | .0298 | .0388 | 1 : 1.30 |
| Pig | .0444 | .0409 | .0489 | 1 : 1.19 |
| Rodentia: | | | | |
| Rabbit | .0611 | .0547 | .0720 | 1 : 1.32 |

## THE PROTEINS OF THE SERUM IN RELATION TO GENERA.

The percentage of proteins in the sera of different species and of individuals of the same species is variable, and even in the same individual it is not constant under either normal or abnormal conditions. Notwithstanding these differences the important fact has been demonstrated by both Salvioli (Archiv f. Anat. u. Physiologie, 1881, 269) and Hoffmann (Archiv f. path. Anat. u. Physiolog., 1882, LXXXIX, 27) that, while the total percentage of proteins is variable, the "protein quotient" (percentage of serum globulins divided by the percentage of serum albumins) in any given species or individual remains quite constant. The former has also shown that in dogs this quotient for serum, chyle, and lymph is practically the same, although the percentages of albumins and globulins may vary considerably. The ratio between serum albumins and serum globulins may therefore be of zoölogical significance.

The total percentage of proteins in the sera of warm-blooded animals is, on the whole, higher than in the cold-blooded animals, in the former the mean being a little less than 7 per cent and in the latter a little over 4 per cent (table 16). In mammals the range is between 5.357 per cent in the rabbit and 8.424 per cent in the horse. Such differences as exist in the percentages of protein do not indicate any of the group differentiations that were pointed out in the case of Na, K, $P_2O_5$, and cholesterin. On the other hand, the protein quotients differ so decidedly as to indicate generic peculiarities. In other words, the proportions of globulins and albumins vary markedly in different genera.

TABLE 16.—*The percentages of proteins, globulins, and albumins, and the protein quotients of the serum of different animals.*

| Kind. | Total proteins. | Globulins. | Albumins. | Protein quotient. | Authority. |
|---|---|---|---|---|---|
| Primate: | *Per cent.* | *Per cent.* | *Per cent.* | | |
| Man | 7.6199 | 3.103 | 4.516 | 0.688 | Hammarsten.* |
| Carnivora: | | | | | |
| Cat | 5.86 | .... | .... | .... | Abderhalden. |
| Dog | 6.063 | .... | .... | .... | Do. |
| Dog | 5.82 | 2.05 | 4.77 | 0.429 | Salvioli. |
| Ungulata: | | | | | |
| Bullock | 7.449 | 4.169 | 3.329 | 1.252 | Hammarsten. |
| Bullock | 7.250 | .... | .... | .... | Abderhalden. |
| Sheep | 6.795 | .... | .... | .... | Do. |
| Goat | 7.807 | .... | .... | .... | Do. |
| Horse | 7.257 | 4.565 | 2.677 | 1.779 | Hammarsten. |
| Horse | 8.424 | .... | .... | .... | Abderhalden. |
| Pig | 6.774 | .... | .... | .... | Do. |
| Rodentia: | | | | | |
| Rabbit | 6.225 | 1.788 | 4.436 | 0.408 | Hammarsten. |
| Rabbit | 5.357 | .... | .... | .... | Abderhalden. |
| Aves: | | | | | |
| Chicken | 4.14 | 2.90 | 1.24 | 2.339 | Halliburton. |
| Pigeon | 5.01 | 1.32 | 3.69 | 0.358 | Do. |
| Reptilia, Amphibia, and Pisces: | | | | | |
| Tortoise | 4.76 | 2.82 | 1.94 | 1.454 | Halliburton. |
| Terrapin | 5.35 | 4.66 | 0.69 | 6.753 | Howell. |
| Frog | 2.54 | 2.18 | 0.36 | 6.055 | Halliburton. |
| Toad | 3.22 | 1.82 | 1.40 | 1.293 | Do. |
| Newt | 3.74 | 3.31 | 0.43 | 7.699 | Do. |
| Salamander | 2.13 | 1.07 | 1.06 | 1.009 | Do. |
| Lizard | 5.16 | 3.33 | 1.83 | 1.819 | Do. |
| Snake | 5.32 | 4.95 | 0.37 | 1.338 | Wolfenden. |
| Eel | 6.73 | 5.28 | 1.45 | 3.641 | Halliburton. |
| Dog-fish | 1.62 | 1.17 | 0.45† | 2.6 | Do. |

* Hammarsten, Archiv f. ges. Physiologie, 1878, XVII, 413. Halliburton, Journal of Physiology, 1886, VII, 319. Abderhalden, *loc. cit.* Hoffmann, *loc. cit.* Howell (quoted by Halliburton). Wolfenden (quoted by Halliburton).

† The pleuroperitoneal fluid may have been mixed with the blood.

In birds the total protein content is low and approaches that of cold-blooded animals rather than that of mammals. The marked differences in the percentage of globulins and albumins and the protein quotients of the chicken and pigeon are very striking.

The records of the protein content of the bloods of cold-blooded animals are much more variable than in the case of mammals, and it seems that further inquiry should render possible certain sharply defined generic distinctions.

Since the total percentage of proteins is a variable one under both normal and abnormal conditions, and since the protein quotient seems to be fairly constant under both normal and abnormal conditions, the latter is by far the more important factor in indicating generic differentiation. The very much higher percentage of albumins in mammals is positive, and it is higher both relatively and absolutely than in birds and in cold-blooded animals generally. In man, the rabbit, and the dog the proportion of albumins is higher than that of globulins, but in the bullock and horse it is lower. The quotients for man, dog, and rabbit are 0.688, 0.429, and 0.408 respectively, and for the horse and bullock 1.779 and 1.252 respectively. These differences are so marked, both as regards class and individuals, as to imply that in omnivora, rodents, and carnivora the per cent of albumins will be found to be in excess of the percentage of globulins, while in herbivora the reverse is the case.

As to the birds, the albumins are in excess of the globulins in the pigeon, while in the chicken it is the reverse.

Among the cold-blooded animals the percentage of albumins is invariably lower than that of the globulins, although in the salamander these percentages are practically the same. The comparatively small percentages of albumins, absolutely and relatively, in cold-blooded animals is the chief cause of the high quotients, the lowest being 1.293 in the toad, and the highest being 7.699 in the newt. The mean quotient for all of the cold-blooded animals is 3.366, while that for the mammals is 0.913. The quotients for the several individuals of the different classes are as a rule so different as to be important in generic differentiation.

Further evidence of zoölogical distinctions is found in the results of the researches of Halliburton (Journal of Physiology, 1884, v, 152; Quar. Jour. Microscop. Science, 1877–78, xxviii, 193) and Mellanby (Journal of Physiology, 1907, xxxvi, 288), and Wallerstein (Inaug. Diss., Strassburg, 1902; Maly's Jahr. ü. d. Fort. d. Thierchemie, 1903, 256). Halliburton in his studies of the kinds of serum albumins in the sera of mammals, birds, and cold-blooded animals, reports that three kinds ($\alpha$, $\beta$, and $\gamma$) are present in the sera of man, monkey, pig, and rabbit, but that the $\alpha$-albumin is absent from the sera of the bullock, sheep, and horse (table 17). The absence of $\alpha$-albumin seems therefore to be a peculiarity of the herbivora.

Table 17.—*The $\alpha$, $\beta$, and $\gamma$-albumins of Halliburton in relation to zoölogical distinction.*

| Kind. | $\alpha$-albumin. | $\beta$-albumin. | $\gamma$-albumin. | Kind. | $\alpha$-albumin. | $\beta$-albumin. | $\gamma$-albumin. |
|---|---|---|---|---|---|---|---|
| Primates: | | | | Aves: | | | |
| Man | P | P | P | Chicken | P | P | P |
| Monkey | P | P | P | Pigeon | P | P | P |
| Ungulata: | | | | Dove | P | P | P |
| Bullock | A | P | P | Amphibia and Pisces: | | | |
| Sheep | A | P | P | Tortoise | P | A | A |
| Horse | A | P | P | Frog | P | A | A |
| Pig | P | P | P | Toad | P | A | A |
| Rodentia: | | | | Newt | P | A | A |
| Squirrel | P | P | P | Salamander | P | A | A |
| Rat | P | P | P | Lizard | P | A | A |
| Mouse | P | P | P | Eel | P | P | A |
| Water vole | P | P | P | Dog-fish | A | P | A |
| Guinea-pig | P | P | P | Fish | P | A | A |
| Rabbit | P | P | P | | | | |

P = present; A = absent.

In the sera of birds he found all three albumins, but in the cold-blooded animals, with the exception of the eel, there is only a single albumin. In the frog, toad, newt, salamander, tortoise, lizard, and fish generally there is only the $\alpha$-albumin; in the dog-fish there is only the $\beta$-albumin; and in the eel there are both $\alpha$ and $\beta$-albumins.

The sera of birds containing all three albumins are more closely related to the mammals than to cold-blooded animals, while the differences in the albumins present in the sera of the warm-blooded and cold-blooded animals make a sharp line of demarcation between these zoölogical divisions.

Mellanby (*loc. cit.*), in a recent research, has confirmed Halliburton's statement of the existence of only two of the three forms of serum albumin in the blood of the horse. He found two albumins in the precipitated proteins, 85 per cent of one and 12 per cent of the other. Wallerstein (*loc. cit.*) records some differences in eu-fibrinoglobulin and pseudoglobulin in different species.

While it is probable that both serum globulin and serum albumin, especially the latter, are of a non-unit nature, their separation into globulin and albumin fractions, respectively, is still regarded as an open question.

### THE PROTEINS OF MUSCLE PLASMA AND SEEDS IN RELATION TO GENERA.

The results of the investigations of Halliburton, Mellanby, and Wallerstein have been supplemented by the researches of Przibram (Beiträge z. chem. Physiologie u. Pathologie, 1902, ii, 143), Rosenheim and Kajuira (Journal of Physiology, 1908, xxxvi, Proceedings, p. liv), and Osborne (Science, 1908, xxviii, 417; Proc. Soc. Exper. Biology and Medicine, 1907–08, v, 105). Przibram prepared and studied the proteins of muscle plasma according to the methods of Fürth, and found that certain zoölogical relationships exist between the kind and quantity of these substances in different species. He studied 28 species, including invertebrates, fish, amphibia, reptiles, birds, an embryo sheep, and a rabbit. His chief results he summarizes as follows:

| | |
|---|---|
| A. Contain no myogen | Invertebrates |
| B. Contain myogen | Vertebrates |
|   *a.* No precipitate with salicylate of sodium (myogen) | Ammocœtes (Cyclostomata?) |
|   *b.* Precipitate with salicylate of sodium | (Gnathostomata?) |
|     *a.* Soluble myogenfibrin immediately after death | Anamnia |
|       Myoprotein in increasing amount | Pisces (Selachii, Teleostei) |
|       Myoprotein only in traces | Amphibia |
|     *b.* No soluble myogenfibrin immediately after death (myoprotein absent) | Amniota (Reptilia, Aves, Mammalia) |

Rosenheim and Kajuira record an absence of an alcohol-soluble protein (gliadin) and of an alcohol-insoluble protein (glutenin) from rice. According to Osborne, gliadin has been found in seeds of all other grasses examined (wheat, rye, oats, corn, barley, and sorghum), and glutenin forms nearly 50 per cent of the gluten of wheat.

Osborne states, from a comparative study of seed proteins, that—

> No two seeds are alike in respect to their protein constituents. Similar proteins are found only in seeds that are botanically closely related. The cereals are alike in the proportion and general character of their proteins. The seeds of each of these, with the probable exception of those of rice, contain a small amount of proteose, albumin, and globulin, and relatively considerable quantities of prolamin soluble in alcohol, and of glutelin insoluble in neutral solvents. With the exception of the nearly related wheat and rye, the proteins soluble in alcohol from each of the cereals are distinct substances. Although no certain difference has yet been detected between the gliadin of wheat and of rye, their glutelins are not alike. [See Introduction, page xi.]
>
> The leguminous seeds are similar in the general character of their proteins, but marked differences exist between the proteins of the various groups. Thus *Lupinus*,

*Vicia*, and *Phaseolus* present marked differences in their proteins, whereas the proteins of the species of each genus are very much alike. The proteins of *Lupinus luteus* and of *Lupinus angustifolia* differ slightly, but in their physical properties are clearly distinguished from any of the other seed proteins. Although similar proteins are obtained from the horse-bean, lentil, pea, and vetch, these are distinctly different from the proteins obtained from other leguminous seeds. These seeds are not alike, however, in the proportion of their several proteins. The chief protein of *Phaseolus vulgaris* appears to be identical with that of *Phaseolus radiatus*, but the small amount of other protein was found to be different in properties and composition in each of these seeds.

The cow-pea (*Vigna*) and soy-bean (*Glycine*) contain distinctly different proteins which, however, are similar to but different from those of *Vicia*. The globulins of the seeds of *Corylus* and *Juglans* are much alike, but not identical, while those from *Juglans regia*, *nigra*, and *cinerea*, so far as they have been compared, show no differences. The proteins of other seeds show marked differences, but the botanical relations of these seeds are not such as to permit of further discussion of this subject.

As stated in the Introduction, it was our intention to embody in this memoir a study of the crystallography of certain classes of plant proteins, especially those of seeds and nuts. Owing to the reasons stated, we had the opportunity of examining only an extremely limited number of these substances, but from the peculiarities noted we believe that sufficient differences will be found to enable the differentiation of one from another, and hence to be of generic specificity.

### THE ZOÖPRECIPITINS AND PHYTOPRECIPITINS AND IMMUNE SERA IN RELATION TO GENERA.

Another important means of biological differentiation has been brought to light during recent years in the discovery of the zoöprecipitins and phytoprecipitins, and by various facts pertaining to the chemistry of immune sera, etc. The results of the investigations along these lines point clearly to the specificity of certain as yet obscure but closely related proteins to certain constituents of different genera. Agglutinins and hemolysins exhibit definite specificities. Reference will be found elsewhere to specificities that are shown by hemolysins.

### THE SPECIFICITY OF THE BLOOD IN ZOÖLOGICAL DIFFERENTIATION AS SHOWN BY THE PHENOMENA OF COAGULATION.

The differences in the rapidity of the coagulation of the bloods of different genera show definite generic peculiarities. Thackrah (Ellenburger's Physiologie der Haussäugethiere, 1890, 165) found that coagulation begins in the blood of the sheep, pig, and rabbit in from 0.5 to 1.5 minutes; in the chicken in 1.5 minutes; in the dog in 1 to 3 minutes; in the bullock in 5 to 12 minutes; and in the horse in 5 to 13 minutes. These records have confirmation in those of Delafond (*ibid.*), who found the following order of coagulability: Dog and sheep in 5 to 8 minutes; pig in 12 to 16 minutes; horse in 15 to 18 minutes; and bullock in from 25 to 30 minutes.

The bloods of cold-blooded animals coagulate less rapidly than those of warm-blooded animals. There are other differences, such as the density of the contracted clot and the size of the clot. Thus, the contracted clot of birds' blood is relatively large and the volume of serum small; while in the case of cold-blooded animals the opposite seems to be true. Sheep's blood yields a larger volume of serum than the bloods of most other mammals.

Then again the fibrins of the bloods of different genera are by no means identical. Fermi states that pig's fibrin is dissolved in a 0.5 per cent solution of HCl in as many hours as the fibrin of bullock's blood is in as many days. He also notes that the solubilities of the fibrins of the pig, sheep, horse, and bullock in dilute vegetable and mineral acids differ in the order given, the highest solubility being in the fibrin of the pig and lowest in that of the bullock.

Another generic distinction is shown in the fact that commercial peptone when injected into the circulation of the dog renders the blood non-coagulable, while it is without effect on the blood of the rabbit.

Finally, the studies of Leo Loeb (Archiv f. path. Anat. u. Phys., 1903, CLXXIII, 35, 113; and 1904, CLXXVI, 10; Hofmeister's Beiträge, 1904, V, 133) on the phenomena of coagulation in both warm- and cold-blooded animals have demonstrated marked zoölogical peculiarities. In these comparative studies he has found not only that the relations between the blood plasma and the tissue extracts (tissue-coagulins) are specific in so far as different fibrinogens or different tissue-coagulins are chemically different, but that tissue-coagulins and fibrinogens show a *specific adaptation* to each other. The tissue-coagulin of one class of animals causes a more rapid coagulation of the plasma of the same class than of the plasma of another class. The demonstration of this specific adaptation could be more easily accomplished in some classes of animals than in others. It is very marked in invertebrates; but even here the specificity is not absolute; it is a relative, graduated, specific adaptation. The substances of related species are active, but not so active as the substances of the same species.

In vertebrates with nucleated red blood corpuscles and stable blood plasmas, the relative specific adaptation is likewise easily demonstrable. It is present in the case of the mammalian blood and tissues, but here it can not be demonstrated as clearly. Mammalian blood can not be kept liquid outside the body as easily as that of other animals, and in mammalian blood either the number of factors causing coagulation is greater than in the case of other classes, or one single factor is preponderating to such a degree that the specific adaptation of the tissue-coagulins to the fibrinogen becomes somewhat obscured. The relations between tissue-coagulins and plasma are similar to those of the artificially produced immune bodies to their antigens. Sometimes non-specific tissue-coagulins are admixed with the specific ones. A specificity of the thrombins exists in so far as the thrombins of different classes are different (Bordet et Gengou), but a specific adaptation can not be shown to exist in their case.

### THE LEUCOCYTES OF THE BLOODS IN RELATION TO GENERA.

The leucocytes of vertebrates contain iron as a normal constituent, but Boyce and Herdman (Philosoph. Trans., 1897, LXII, 34) have found copper in place of iron in the leucocytes of the oyster. Huppert (Centralb.

f. Physiologie, 1892, VI, 394) states that the leucocytes of the dog contain a much larger amount of glycogen than the corpuscles of herbivora. The studies of the leucocytes of the horse by Hayem (Compt. rend. soc. biolog., 1899, LI, 623) have brought to light peculiarities which indicate generic distinctions. In vertebrates the leucocytes of any given variety do not appear to vary as much in size in different genera as do the erythrocytes, but in cold-blooded animals these cells are on the whole larger than in warm-blooded animals. The polynuclears in man and the guinea-pig are somewhat smaller than the large mononuclears, while in the rat and rabbit they are considerably smaller. The granules of leucocytes exhibit peculiarities in their behavior towards dyes, some staining with acid stains (eosinophiles or oxyphiles), some with acid and basic stains (amphophiles), and some with basic dyes (basophiles). Kanthack and Hardy (Journal of Physiology, 1894–95, XVII, 22) have found that the granules of the polynuclears of man, rabbit, rat, mouse, and guinea-pig show marked differences in their affinities for acid dyes, and also in their degrees of refractivity—the higher the staining reaction the higher the refractivity. Sherrington (Proc. Roy. Soc., 1894, LV, 161) states that the granules are more refractive in the cat than in the horse, and that the shape of the granules is usually spherical in the rabbit and dog, cylindroid in the cat, roughly cuboid in the horse, and that in the cat and horse many spheroid granules are often present. He also noted that the cylindroid granule of the cat is larger than the spheroid of the dog, and that the cuboid granule of the horse is much larger than the cylindroid of the cat. The ratio of leucocytes to the erythrocytes he found was distinctly lower in the cat than in the dog.

The percentages of the several varieties of leucocytes in the bloods of different species vary sufficiently to indicate positive generic differences. Eosinophiles are few in mammalian blood, but abundant in the lower vertebrates; and they are more abundant in the horse than in human blood, and the granular matter is different. Sherrington found the coarsely granular cells to be more numerous in the cat than in the dog. The researches of Carstanjen (Jahr. f. klin. u. phys. Erziehung., 1900, LII, 237, 346) and others on man and of Kanthack and Hardy (*loc. cit.*), Silverman (University of Pennsylvania Medical Bulletin, 1904–05, XVII, 22), and Lisin (Arch. int. de pharm. et de thérap., 1908, XVIII, 237) on the lower animals show certain marked differences. The eosinophiles represent a low percentage of the total leucocytes, and the differences in the proportions in different species are probably too small to be of any significance. The large mononuclears and transitional cells are in man, the guinea-pig, and the dog distinctly more numerous than the eosinophiles; while in the rat and rabbit they are of about the same proportions as the eosinophiles.

The polynuclears and the lymphocytes represent the great bulk of the leucocytes—88.27 per cent in man, 95 per cent in the rat, 86 per cent in the guinea-pig, over 90 per cent in the rabbit, and 92 per cent in the dog. In man, the guinea-pig, and the dog the polynuclears are decidedly more numerous than the lymphocytes, while in the rat and rabbit they are less numerous. The ratios differ widely: in man 2.64 : 1, in the rat 0.9 : 1, in the guinea-pig 2.59 : 1, in the rabbit 1.33 : 1, and in the dog 4.41 : 1. While undoubtedly not only the percentages but the ratios are variable under both normal and abnormal conditions, the differences are such as to imply positive zoölogical distinctions. A serious inquiry into the zoölogical peculiarities of leucocytes will probably yield important results.

TABLE 18.—*The percentages of different varieties of leucocytes in the bloods of different species.*

| Kind. | Varieties of leucocytes. | | | | |
|---|---|---|---|---|---|
| | Polynuclears. | Lymphocytes. | Large mononuclears and transitional cells. | Eosinophiles. | Authority. |
| Man (age 20 to 30 years) .... | 64 | 24.27 | 8.62 | 3.11 | Carstanjen. |
| Rat ..................... | 45 | 50.00 | 2 | 2 | Kanthack and Hardy. |
| Guinea-pig ............... | 62 | 24.00 | 11 | 2 to 3 | |
| Rabbit .................. | 20 to 30 | 70 to 80 | 2 to 6 | 1 to 2 | |
| Rabbit .................. | 32 to 45 | 37 to 60 | 2 to 5 | 1.5 to 4 | Lisin. |
| Dog ..................... | 75 | 17 | 5 | 2.5 | Silverman. |

Finally, studies of immunity against infectious diseases show clearly that natural immunities of different species are owing to specific physiological peculiarities of the individual's leucocytes. "The dominant feature of the phenomena exhibited in natural immunity against microörganisms," writes Metchnikoff (Immunity in Infective Diseases, trans. by Burnie, 1905, 206), "is represented by the phagocytic reaction observed throughout the animal series that is exercised against parasites belonging to all the microbal groups. Phagocytosis is exhibited not only by the macrophages, but also in a high degree by the microphages which stand out as the defensive cells *par excellence* against microörganisms. The action is divided into series of vital physiological acts, such as sensitiveness to the microörganisms and their products, amœboid movements which serve to ingest the microörganisms, and into chemical and physico-chemical processes, such as the destruction and digestion of the devoured organisms. The phagocytes enter into a struggle against the microörganisms and rid the animal organism of them without requiring any previous help on the part of the body-fluids. Phagocytosis exercised against living and virulent microörganisms is sufficient to insure natural immunity."

### THE PROPORTION OF CORPUSCLES TO SERUM IN RELATION TO GENERA.

According to Welcker (*loc. cit.*), the proportion of corpuscles to serum is higher in warm-blooded than in cold-blooded animals; higher in mammals than in birds; higher in birds than in the amphibia and fish; higher in the "scaly" amphibia than in the "naked" amphibia, and lowest in fish. His figures indicate that in mammals, birds, and amphibia the corpuscles represent from about one-third to one-fourth of the blood, and in fish only about one-fourteenth of the blood. His figures for mammalian blood are, in the light of subsequent investigations, too low, the mean as shown by Abderhalden's analyses (Zeit. f. physiolog. Chemie, 1897, XXIII, 521; 1898, XXV, 65) being nearly 40 per cent; in fact, it is only in certain of the ruminants that we find so low a standard as that given by Welcker (tables 19 and 20).

The researches in recent years by Arronet, Daland, Koppe, Pfeiffer, and others (Vierordt's Daten u. Tabellen, 1906, 197), with human blood, show that the mean percentage in men is approximately between 45 and 50 per cent and in women about 3 to 8 per cent less.

TABLE 19.—*The percentages of corpuscles in the blood of various classes of animals and genera.*

| Kind. | According to Welcker. | According to Abderhalden. |
|---|---|---|
| | *Per cent.* | *Per cent.* |
| Mammals | 32 | ..... |
| Birds | 28 | ..... |
| "Scaly" amphibia | 27 | ..... |
| "Naked" amphibia | 25 | ..... |
| Fish | 7 | ..... |
| Carnivora: | | |
| Cat | .... | 43.40 |
| Dog | .... | 42.01 |
| Ungulata: | | |
| Bullock | .... | 32.55 |
| Sheep | .... | 31.28 |
| Goat | .... | 34.72 |
| Horse | .... | 39.77 |
| Pig | .... | 43.59 |
| Rodentia: | | |
| Rabbit | .... | 37.21 |

Besides Abderhalden's analyses of the bloods of the cat, dog, bullock, sheep, goat, horse, pig, and rabbit, there are a few scattered records by Sacharjin, Fudakowski, Hohlbeck, Otto, Bliebtreu, and others (Sacharjin, Hoppe-Seyler's Physiologische Chemie, 1887, 447; Bunge, Zeit. f. Biologie, 1876, XII, 191; Fudakowski, Centralblatt f. med. Wissensch., 1866, IV, 705; Hohlbeck, Hoppe-Seyler's Physiologische Chemie, 1877, 447; Otto, Archiv f. ges. Physiologie, 1885, XXXV, 467; Bliebtreu, *ibid.*, 1892, LI, 151; Arronet, Inaug. Dissert., Dorpat, 1887), but for comparison the quite recent work of Abderhalden will best serve our purposes. The highest percentage is for the pig, 43.59 per cent, and then in the following order: cat 43.4 per cent, dog 42.01 per cent, horse 39.77 per cent, rabbit 37.21 per cent, goat 34.72 per cent, bullock 33.55 per cent, and sheep 31.28 per cent. Grouping the individuals in classes, the omnivora (man and pig) stand first, then the carnivora (dog and cat), then the horse and rabbit as representatives of their classes, and finally the ruminants.

## THE BLOOD PLATELETS IN RELATION TO GENERA.

Blood platelets are found in abundance (from 200,000 to 600,000 per cubic millimeter) in mammalian blood, but they are absent from the bloods of birds, amphibia, and fish, in which there exist what are probably homologous structures in the form of small nucleated spindle-shaped cells.

## THE FORM OF THE ERYTHROCYTES IN RELATION TO GENERA.

All the vertebrates are divisible primarily into two classes in accordance with the presence of non-nucleated or nucleated red corpuscles, and further divisions and subdivisions may be made through distinctions in the forms, in the number per cubic millimeter, and in the sizes of these cells. In all warm-blooded animals, except birds, the erythrocytes are non-nucleated, and they are circular, except in the *Camelidæ,* in which they are oval. In birds, reptiles, amphibia, and fish they are nucleated, and elliptical or oval, except in the *Cyclostomata* (lamprey) and *Hippocampus* (sea-horse), in which they are circular. In the perch the extremities of the long diameters are somewhat elongated.

The forms of the erythrocytes of different species have been studied by a number of investigators, especially by Gulliver (Proc. Zoölog. Soc. London, 1875, 474). (See p. 57.) Measurements of the ratios of thickness to diameters do not show any important distinctions, but the ratios of the long and short diameters of the elliptical and oval cells vary sufficiently to be of zoölogical importance. Thus, the ratio of the diameters of the humming-bird and the shrike are at a glance obviously different; the long diameters of the corpuscles of the wild pigeon and of the august amazon are practically the same, yet the difference in the short diameters is sufficient to distinguish one from the other; the differences in the ratios of the long and short diameters of the corpuscles of the rufous pigeon and the wild pigeon, entirely apart from the difference in their long diameters, positively differentiate one from the other, etc.

### THE NUMBER OF ERYTHROCYTES IN RELATION TO GENERA.

In the bloods of all vertebrates the erythrocytes are in inconceivable numbers. Franke estimates that the total number of cells in the human body is 26,500,000,000,000 and that of this number 22,500,000,000,000 are erythrocytes—in other words, over four-fifths of the body-cells are red corpuscles. Malassez estimated that there are in the human body about 341,000,000 to each gram of body-weight. The mere fact of the relatively extraordinarily large number of these cells, entirely apart from the extremely important hemoglobin content, is sufficient to prove their great importance. In certain of the invertebrates the hemoglobin is in solution in the blood plasma, but whether or not it functionates by virtue of a combination with some protein or other substance which is an analogue of the stromata of the erythrocytes has not, as far as we know, been shown.

The number of erythrocytes per cubic millimeter of blood in any individual is so variable under both normal and abnormal conditions that the figures recorded are to be regarded as being only approximate. But even from such data it is manifest that there exist well-defined generic distinctions. Malassez found variations in different species of mammals ranging from 3,500,000 to 18,000,000 per cubic millimeter; in birds from 2,300,000 to 3,400,000; in osseous fish from 1,100,000 to 2,000,000; and in cartilaginous fish from 140,000 to 230,000. Similar striking differences are shown by the records of Welcker and others (Welcker, Zeit. f. rat. med., 1863, Ser. 3, xx, 257; Sherrington and Copeman, Journal of Physiology, 1893, XIV, 58; Vierordt, Archiv f. physiolog. Heilk., 1852, XI, 26, 327, 854, and 1854, XIII, 259; Malassez, Compt. rend. Acad. d. Sciences, 1872, LXXV, 1528; Stölzing, Ueber Zahlung der Blutkörp., Inaug. Dissert., Marburg, 1856, 16;

TABLE 20.—*The number of erythrocytes per cubic millimeter in relation to genera.*

| Kind. | Number of erythrocytes. | Authority. |
|---|---|---|
| Primate: | | |
| Man (male) | 5,223,250 | Vierordt. |
| Man (female) | 4,886,720 | Do. |
| Ungulata: | | |
| Bullock | 5,073,000 | Do. |
| Bullock | 4,200,000 | Malassez. |
| Calf | 5,120,000 | Welcker. |
| Sheep | 12,090,000 | Cohnstein. |
| Goat | 9,000,000 to 10,000,000 | Vierordt. |
| Goat | 18,000,000 | Malassez. |
| Goat | 5,400,000 | Welcker. |
| Goat (8 days old) | 9,720,000 | Do. |
| Reindeer | 6,700,000 | Malassez. |
| Llama | 10,400,000 | Do. |
| Llama | 13,890,000 | Welcker. |
| Dromedary | 10,000,000 | Malassez. |
| Horse | 6,300,000 | Do. |
| Horse | 7,212,500 | Sussdorf. |
| Elephant | 2,020,000 | Welcker. |
| Pig | 5,441,000 | Vierordt. |
| Carnivora: | | |
| Dog | 4,421,500 | Do. |
| Dog | 4,092,000 to 5,644,000 | Stölzing. |
| Dog, male | 6,115,375 | Otto. |
| Dog, female | 5,799,520 | Do. |
| Dog, female | 9,638,000 | Worm-Müller. |
| Rodentia: | | |
| Dormouse | 8,410,000 | Welcker. |
| Marmot | 4,430,000 | Do. |
| Guinea-pig | 6,293,000 | Claisse and Josué. |
| Rabbit | 4,407,000 | Vierordt. |
| Rabbit | 4,866,000 | Stölzing. |
| Rabbit, male | 4,720,076 | Otto. |
| Rabbit, female | 3,605,140 | Do. |
| Rabbit | 6,426,750 | Worm-Müller. |
| Rabbit | 4,410,000 | Malassez. |
| Rabbit | 6,502,433 | Sherrington and Copeman. |
| Aves: | | |
| Chicken | 3,100,000 | Malassez. |
| Chicken | 3,860,000 | Stölzing. |
| Turkey | 2,700,000 | Malassez. |
| Ostrich | 1,600,000 | Do. |
| Spoonbill | 3,400,000 | Do. |
| Duck | 2,800,000 | Do. |
| Swan | 2,300,000 | Do. |
| Bullfinch | 2,660,000 | Welcker. |
| Pigeon | 2,010,000 | Do. |
| Amphibia: | | |
| Frog (temporaria) | 404,000 | Do. |
| Proteus | 36,000 | Do. |
| Triton cristatus (newt) | 103,000 | Do. |
| Pisces: | | |
| Osseous fishes: | | |
| Sole | 2,000,000 | Malassez. |
| Eel | 1,100,000 | Do. |
| Cartilaginous fishes: | | |
| Skate | 230,000 | Do. |
| Skate | 350,000 | Harris. |
| Torpedo | 140,000 | Malassez. |
| Lamprey | 133,000 | Welcker. |
| Reptilia: | | |
| Lizard (Lacerta agilis) | 1,420,000 | Do. |
| Lizard (Lacerta muralis) | 960,000 | Do. |
| Salamander (maculata) | 80,000 | Do. |

Worm-Müller, Transf. u. Pleth, Christiania, 1875; Otto, Archiv f. ges. Physiologie, 1884, xxxiv, 233; Vierordt, Daten u. Tabellen, 1906, 205, 206; Ellenberger, Physiologie d. Haussäugethiere, 1890, 181; Claisse et Josué, Compt. rend. soc. biologie, 1896, xlviii, 1020; and Harris, Journal of Physiology, 1903, xxx, 319). See table 20.

The erythrocytes are, except in the elephant, more numerous per cubic millimeter in mammals than in birds, amphibia, reptiles, and fish, and generally they are much more numerous. In birds they are more numerous than in cold-blooded animals, and they are least numerous in the salamander and certain of the amphibia, in which the proportion may fall to a mere fraction of the average in warm-blooded animals. Among mammals the number is highest in the camel tribe, next highest in the sheep and goat, and lowest in the elephant. There is not any numerical distinction of the ruminants from other classes of ungulates, nor does there appear to be anything definite in the way of numerical differences between the ungulates, carnivora, and rodents. Considerable addition to our data must be made before any figure can be accepted as the mean for any species, except possibly in the case of the human being. Among the cold-blooded animals the differences are in some instances so marked as to be positive in showing generic peculiarities, as, for instance, the differences in the members of the group of *Amphibia*, the differences between the osseous and cartilaginous fishes, and the differences between the lizard and salamander. The number of corpuscles is, however, probably of less importance than the percentage of hemoglobin within them.

### THE SIZE OF THE ERYTHROCYTES IN RELATION TO GENERA.

While the erythrocytes of specimens of blood from different individuals of a given species may vary as much as 40 per cent or more in either direction from the mean diameter, a very large proportion in most if not all bloods of mammals falls within narrow limits of the mean measurements, and in different individuals of the same species the mean measurements are of such uniformity as to justify their acceptance as reliable standards of comparison and differentiation. The cells of the new-born have a somewhat larger diameter than those of the adult, and they have a larger range of measurement; the range in the female is greater than in the male.

The mean diameters of the red corpuscles of different species vary within wide limits (table 21), the smallest corpuscles thus far examined being those of the musk-deer (2.1 $\mu$, Gulliver), and the largest those of the amphiuma (69.8 to 41.4 $\mu$, Gulliver), which may be seen by the unaided eye. In many instances, however, the differences may be so slight, even in species and genera far removed from one another, as to be valueless of themselves in zoölogical differentiation. Nevertheless, it is probable that in no two species are the mean diameters exactly the same, and even when they are so close as to be practically identical there may be certain peculiarities, such as the extent of the range in size, the constant occurrence of erythrocytes of unusual dimensions, obscure appearances in the cells which have been expressed by the term "individuality," etc., which may be determining.

TABLE 21.—*The sizes of the erythrocytes in different genera according to the measurements of Gulliver, Wormley, Treadwell, Formad, Welcker, and Malassez.**

| Kind. | Gulliver. | Wormley. | Treadwell. | Formad. | Welcker. | Malassez. |
|---|---|---|---|---|---|---|
| | μ | μ | μ | μ | μ | μ |
| Primates: | | | | | | |
| Man | 7.9 | 7.9 | 7.9 | 7.9 | ... | ... |
| Chimpanzee | 7.5 | ... | ... | ... | ... | ... |
| Monkey | 7.1 | 7.5 | ... | ... | ... | ... |
| Lemur | 6.4 | ... | ... | ... | ... | ... |
| Chiroptera: | | | | | | |
| Bat (common) | 5.9 | 6.4 | ... | ... | ... | ... |
| Bat (noctula) | 5.8 | ... | ... | ... | ... | ... |
| Fruit bat | 6.6 | ... | ... | ... | ... | ... |
| Carnivora: | | | | | | |
| Cat | 5.8 | 5.8 | 5.5 | ... | 6.5 | ... |
| Leopard | 5.9 | 5.8 | ... | ... | ... | ... |
| Lion | 5.9 | 6.1 | ... | ... | ... | ... |
| Ocelot | 6.0 | 6.6 | ... | ... | ... | ... |
| Hyena | 6.8 | 7.0 | ... | ... | ... | ... |
| Wolf | 7.1 | 7.5 | ... | 7.4 | ... | ... |
| Dog | 7.2 | 7.1 | 6.9 | 7.1 | 7.3 | 7.1 |
| Fox | ... | ... | 6.5 | ... | ... | ... |
| Bear | 6.9 | 7.0 | ... | ... | ... | ... |
| Raccoon | 6.4 | 6.2 | ... | ... | ... | ... |
| Ungulata: | | | | | | |
| Bullock | 5.9 | 6.0 | 5.4 | 6.0 | ... | 6.0 |
| Sheep | 4.8 | 5.2 | 4.7 | 5.1 | 8.0 | ... |
| Goat | 4.0 | 4.1 | 3.2 | 4.2 | 4.1 | 3.5 |
| Ibex | ... | 3.9 | ... | ... | ... | ... |
| Reindeer | ... | ... | ... | ... | ... | 4.5 |
| Elk | 6.5 | 5.8 | ... | ... | ... | ... |
| Giraffe | 5.6 | ... | ... | ... | ... | ... |
| Musk-deer | 2.1 | ... | ... | ... | 2.5 | ... |
| Llama long diameter | 7.6 | 7.9 | ... | ... | 8.0 | 9.0 |
| short diameter | 4.1 | 4.0 | ... | ... | 4.0 | 4.5 |
| Camel, double-hump long diameter | 8.1 | 4.8 | ... | ... | ... | 10.0 |
| short diameter | 4.3 | 4.8 | ... | ... | ... | 4.5 |
| Camel, single-hump long diameter | 7.8 | ... | ... | ... | ... | ... |
| short diameter | 3.7 | ... | ... | ... | ... | ... |
| Vicugna long diameter | 7.1 | ... | ... | ... | ... | ... |
| short diameter | 3.9 | ... | ... | ... | ... | ... |
| Alpaca long diameter | 7.6 | ... | ... | ... | ... | ... |
| short diameter | 4.0 | ... | ... | ... | ... | ... |
| Peccary | 5.7 | ... | ... | ... | ... | ... |
| Tapir | 6.4 | 6.1 | ... | ... | ... | ... |
| Hyrax | 7.7 | ... | ... | ... | ... | ... |
| Rhinoceros | 6.8 | 7.0 | ... | ... | ... | ... |
| Hippopotamus | 7.1 | 7.4 | ... | ... | ... | ... |
| Pig | 5.9 | 5.9 | 6.1 | 6.0 | ... | 5.9 |
| Horse | 5.5 | 6.0 | 5.5 | 5.9 | ... | 5.9 |
| Mule | ... | 6.8 | 5.7 | ... | ... | ... |
| Ass | 6.4 | 7.0 | 6.3 | ... | ... | 7.0 |
| Elephant (Indian) | 9.2 | 9.3 | ... | ... | 9.4 | ... |
| Cetacea: | | | | | | |
| Whale (boöps) | 8.2 | ... | ... | ... | ... | ... |
| Whale (caaing) | 7.9 | ... | ... | ... | ... | ... |
| Porpoise | 9.2 | ... | ... | ... | ... | ... |
| Rodentia: | | | | | | |
| Squirrel (red) | 6.4 | 6.1 | 6.6 | ... | ... | ... |
| Squirrel (gray) | ... | ... | 7.6 | ... | ... | ... |
| Squirrel (ground) | 7.6 | 6.0 | 6.8 | ... | ... | ... |
| Beaver | 7.6 | ... | ... | ... | ... | ... |
| Mouse | 6.7 | 6.8 | 6.0 | ... | ... | ... |
| Dormouse | ... | ... | ... | ... | 6.2 | ... |

* A few additional measurements by Schmidt (1848), Mallinin (1875), the French Medico-Legal Society (1873), Masson (1885), Schmid (1878), and Woodward (1875) are quoted by Formad (*loc cit.*).

TABLE 21—Continued.

| Kind. | | Gulliver. | Wormley. | Treadwell. | Formad. | Welcker. | Malassez. |
|---|---|---|---|---|---|---|---|
| Rodentia—Continued: | | µ | µ | µ | µ | µ | µ |
| Rat | | 6.8 | 7.0 | 6.5 | ... | ... | ... |
| Muskrat | | 7.2 | 7.7 | 7.3 | ... | ... | ... |
| Porcupine | | 7.5 | ... | ... | ... | ... | ... |
| Guinea-pig | | 7.2 | 7.9 | 7.5 | 7.5 | ... | 7.9 |
| Capybara | | 8.0 | 8.0 | ... | ... | ... | ... |
| Rabbit | | 7.0 | 7.0 | 6.4 | 6.9 | 6.9 | 7.0 |
| Marmot | | 7.3 | ... | 7.3 | ... | ... | ... |
| Edentata: | | | | | | | |
| Sloth | | 8.9 | ... | ... | ... | ... | ... |
| Armadillo | | 7.7 | ... | ... | ... | ... | ... |
| Ant-eater | | 9.2 | ... | ... | ... | ... | ... |
| Marsupialia: | | | | | | | |
| Kangaroo | | 7.4 | 7.4 | ... | ... | ... | ... |
| Opossum | | 7.1 | 8.1 | ... | ... | ... | ... |
| Wombat | | 7.3 | ... | ... | ... | ... | ... |
| Kangaroo rat | | 6.4 | ... | ... | ... | ... | ... |
| Aves: | | | | | | | |
| Chicken | long diameter | 12.1 | 12.2 | ... | ... | 12.1 | 13.0 |
| | short diameter | 7.3 | 7.3 | ... | ... | 7.2 | 6.5 |
| Turkey | long diameter | 12.4 | 13.4 | ... | ... | 13.9 | 13.0 |
| | short diameter | 7.1 | 7.4 | ... | ... | 7.0 | 6.5 |
| Duck | long diameter | 13.1 | 13.0 | ... | ... | 12.9 | 13.0 |
| | short diameter | 7.5 | 7.3 | ... | ... | 8.0 | 7.0 |
| Pigeon | long diameter | 12.9 | 13.4 | ... | ... | 14.7 | ... |
| | short diameter | 7.0 | 6.7 | ... | ... | 6.5 | ... |
| Goose | long diameter | 13.8 | ... | ... | ... | ... | ... |
| | short diameter | 6.0 | ... | ... | ... | ... | ... |
| Quail | long diameter | 10.8 | ... | ... | ... | ... | ... |
| | short diameter | 7.3 | ... | ... | ... | ... | ... |
| Dove | long diameter | 12.7 | ... | ... | ... | ... | ... |
| | short diameter | 7.5 | ... | ... | ... | ... | ... |
| Sparrow | long diameter | 11.9 | ... | ... | ... | 11.9 | ... |
| | short diameter | 7.3 | ... | ... | ... | 6.8 | ... |
| Owl | long diameter | 14.4 | ... | ... | ... | ... | ... |
| | short diameter | 6.2 | ... | ... | ... | ... | ... |
| Ostrich | long diameter | ... | ... | ... | ... | ... | 18.0 |
| | short diameter | ... | ... | ... | ... | ... | 9.0 |
| Swan | long diameter | ... | ... | ... | ... | ... | 14.0 |
| | short diameter | ... | ... | ... | ... | ... | 7.0 |
| Spoonbill | long diameter | ... | ... | ... | ... | ... | 15.0 |
| | short diameter | ... | ... | ... | ... | ... | 7.0 |
| Bullfinch | long diameter | ... | ... | ... | ... | 12.4 | ... |
| | short diameter | ... | ... | ... | ... | 7.5 | ... |
| Reptilia: | | | | | | | |
| Tortoise | long diameter | 20.3 | 20.3 | ... | ... | ... | ... |
| | short diameter | 11.5 | 11.5 | ... | ... | ... | ... |
| Turtle | long diameter | 20.6 | ... | ... | ... | ... | ... |
| | short diameter | 13.5 | ... | ... | ... | ... | ... |
| Boa | long diameter | 17.6 | 20.4 | ... | ... | ... | ... |
| | short diameter | 10.4 | 10.0 | ... | ... | ... | ... |
| Viper | long diameter | 19.9 | ... | ... | ... | ... | ... |
| | short diameter | 14.1 | ... | ... | ... | ... | ... |
| Lizard (agilis) | long diameter | 16.3 | ... | ... | ... | 15.8 | ... |
| | short diameter | 9.2 | ... | ... | ... | 9.8 | ... |
| Lizard (muralis) | long diameter | ... | ... | ... | ... | 15.4 | ... |
| | short diameter | ... | ... | ... | ... | 10.3 | ... |
| Amphibia: | | | | | | | |
| Frog | long diameter | 22.9 | 23.3 | ... | ... | 22.3 | ... |
| | short diameter | 13.9 | 14.1 | ... | ... | 15.7 | ... |
| Toad | long diameter | 24.4 | ... | ... | ... | 30.2 | ... |
| | short diameter | 12.7 | ... | ... | ... | 18.2 | ... |
| Proteus | long diameter | 63.5 | ... | ... | ... | 58.2 | ... |
| | short diameter | 35.1 | ... | ... | ... | 33.7 | ... |

TABLE 21—Concluded.

| Kind. | | Gulliver. | Wormley. | Treadwell. | Formad. | Welcker. | Malassez. |
|---|---|---|---|---|---|---|---|
| Amphibia—Continued: | | μ | μ | μ | μ | μ | μ |
| Triton | long diameter | 30.0 | ... | ... | ... | 29.3 | ... |
| | short diameter | 19.6 | ... | ... | ... | 19.5 | ... |
| Amphiuma | long diameter | 69.8 | 70.9 | ... | ... | ... | ... |
| | short diameter | 41.4 | 40.9 | ... | ... | ... | ... |
| Cryptobranchus Jap | long diameter | ... | ... | ... | ... | 51.2 | ... |
| | short diameter | ... | ... | ... | ... | 31.7 | ... |
| Salamander | long diameter | ... | ... | ... | ... | 37.8 | ... |
| | short diameter | ... | ... | ... | ... | 23.8 | ... |
| Siren | long diameter | ... | ... | ... | ... | 41.0 | ... |
| | short diameter | ... | ... | ... | ... | 29.8 | ... |
| Pisces: | | | | | | | |
| Trout | long diameter | 16.7 | ... | ... | ... | ... | ... |
| | short diameter | 10.3 | ... | ... | ... | ... | ... |
| Perch | long diameter | 12.1 | ... | ... | ... | ... | ... |
| | short diameter | 9.0 | ... | ... | ... | ... | ... |
| Pike | long diameter | 12.7 | ... | ... | ... | ... | ... |
| | short diameter | 7.1 | ... | ... | ... | ... | ... |
| Eel | long diameter | 14.6 | ... | ... | ... | ... | ... |
| | short diameter | 8.9 | ... | ... | ... | ... | ... |
| Lamprey* | | 9.0 | ... | ... | ... | 15.0 | ... |
| Sole | long diameter | ... | ... | ... | ... | ... | 12.0 |
| | short diameter | ... | ... | ... | ... | ... | 9.0 |
| Skate | long diameter | ... | ... | ... | ... | ... | 25.0 |
| | short diameter | ... | ... | ... | ... | ... | 14.0 |
| Torpedo | long diameter | ... | ... | ... | ... | ... | 27.0 |
| | short diameter | ... | ... | ... | ... | ... | 20.0 |
| Sea-horse* | | ... | ... | ... | ... | ... | 15.0 |

* Circular.

The figures recorded by different observers (Gram, Fortschr. d. Medicin, 1884, II, 33; Georgopulus, Zeit. f. klin. Medicin, 1906, LXIII, 322; White and Treadwell, Reference Handbook of the Medical Sciences, 1901, II, 84; Gulliver, Proc. Zoölog. Society, London, 1875, 474; Wormley, Microchemistry of Poisons, 2d ed., Phila., 1888; Welcker, Zeit. f. rat. Medicin, Ser. 3, 1863, XX, 257; Malassez, Compt. rend. Acad. d. Sciences, 1872, LXXV, 1528; Formad, Comparative Studies of Mammalian Blood, Phila., 1888, and Journal of Comparative Medicine and Surgery, July, 1888, etc.) in their studies of given species differ in many instances. As a rule, Wormley's figures are somewhat higher than Gulliver's, while Formad's and Treadwell's are lower. These differences are not of importance, since on the whole they are remarkably close and entirely in accord in their indications of generic peculiarities.

Human corpuscles have been more thoroughly studied than those of any other species. The extreme limits of measurements probably lie within 4 to 10 μ, but the ordinary range may be placed at about 6 to 9.5 μ. The mean is from 7.9 to 8 μ. The remarkably large proportion that measure close to the average is shown by the figures of Gram, Georgopulus, White, and others: Gram found that 82 per cent were of about the average measurements, 13 per cent larger, and 5 per cent smaller; Georgopulus records 73 per cent between 7 and 7.5 μ, 10 per cent between 8 and 8.5 μ, and 17 per cent between 6 and 6.5 μ; and White, 79.5 per cent between 7.5 and 8.5 μ, 12 per cent between 8.5 and 9.25 μ, and 8.5 per cent between 6.25 and 7.5 μ.

The diameters of the erythrocytes of different species have been made the subject of study especially by Gulliver, Wormley, Schmidt, Welcker, Malassez, Formad, and Treadwell. Gulliver's investigations extended over a period of 35 years and included studies of about 650 species. He frankly states that his tables can not pretend to absolute exactness, and are only offered for what they may be worth, and that in the estimation of their value allowance should be made for errors, whether instrumental or personal, more or less inevitable, notwithstanding the greatest care, in observations so extensive, and that the relative value of the measurements, though probably not unexceptionable, may be entitled to more confidence as fair approximation to the truth. He further states that in spite of little mistakes or of variations in the dimensions of the corpuscles of this or that species, the comparative results will appear sufficiently uniform. Gulliver's measurements are so closely in accord with those of later observers that they are to be accepted as being sufficiently accurate to serve for purposes of comparison. His investigations were made from the point of view of the biologist, and he claims that the differences in the measurements of the erythrocytes of different species constitute an important means of zoölogical distinction. Thus, he states:

> If we compare the red corpuscles of species of one order or family, *e. g., Tragulus* and other ruminants, the corpuscles of the former animals will constantly prove the smallest; so, too, in *Paradoxurus* and *Canis,* in *Hippopotamus* and *Elephas,* in *Mus* and *Hydrochœrus,* in *Dasypus villosus* and *Orycteropus capensis,* in *Rhea americana* and *Casuarius,* in *Zootica vivipara* and *Anguis fragilis,* in *Bufo viridis* and *Bufo vulgaris,* in *Osmerus eperlanus* and *Salmo salar.* And in like manner the facts are equally clear in comparison of the different orders, so that the corpuscles are smaller in the *Ruminantia* than in the *Rodentia,* in the *Marsupialia* than in the *Edentata,* in the *Graminivora* than in *Rapaces,* in *Anura* than in *Urodela,* in *Sturiones* than in *Plagiostomi.*

Notwithstanding the foregoing positive statement, there seems to be a general, if not universal, belief that the size of the corpuscles is without much zoölogical importance, which is indicated by the very infrequent, casual, and scanty references to this subject. There is no doubt that the figures show unequivocally that the mean diameters of the erythrocytes of different genera, related or unrelated, may be practically the same, as, for instance, those of the monkey, lipped bear, hyena, and rhinoceros; and again, those of man, opossum, dingo, dog, wolf, whale, armadillo, beaver, capybara, guinea-pig, muskrat, etc. Nevertheless, it is clear that even among the members of a given order, or tribe, or genus, etc., the differences may be sufficient to be positively distinctive, and at times to have some other and more special zoölogical significance. Thus, in the primates there is seemingly an increase in the size of the corpuscles as the individual is higher in the scale of life, as, for instance, man, 7.9 $\mu$; chimpanzee, 7.4 $\mu$; monkey, 7.1 $\mu$; lemur, 6.4 $\mu$. This relationship may be in relation to differences in the sizes of the species (page 58). Another interesting relationship is noted in the horse, mule, and ass, the mule being a hybrid and the corpuscles having an intermediate measurement. Then again, comparing representatives of classes of different orders, such as ruminants, felines, canines, etc., not only may each class be readily distinguished from the others by the mean sizes of the corpuscles, but even individuals belonging to each class. (Plate A.)

The nearness of the diameters of the erythrocytes of certain of the domesticated animals to those of man is a matter of considerable importance, chiefly because of its medico-legal bearing, and it is yet an open question if the corpuscles of the dog, and especially of the guinea-pig, can with positiveness be distinguished from those of man. The mean measurements found by White (expressed in $\mu$) are: man 8.01, guinea-pig 7.47, dog 6.87, pig 6.07, ox 5.44, sheep 4.75, and goat 3.69. Not only are these measurements sufficiently different to be significant, but the variations in the ranges in the sizes in the different species are peculiar. Particularly striking is the wide range in the pig and the narrow range in the goat, the limits of the former being 3.75 to 8.50 and of the latter only 3 to 4.5. 80.5 per cent of the corpuscles of man ranged between 7.5 and 8.5, 90 per cent of the dog between 5 and 7.5, 80 per cent of the pig between 5 and 7.25, 95 per cent of the ox between 4.75 and 6.25, 89 per cent of the sheep between 4 and 5.25, and 96 per cent of the goat between 3.45 and 4.25.

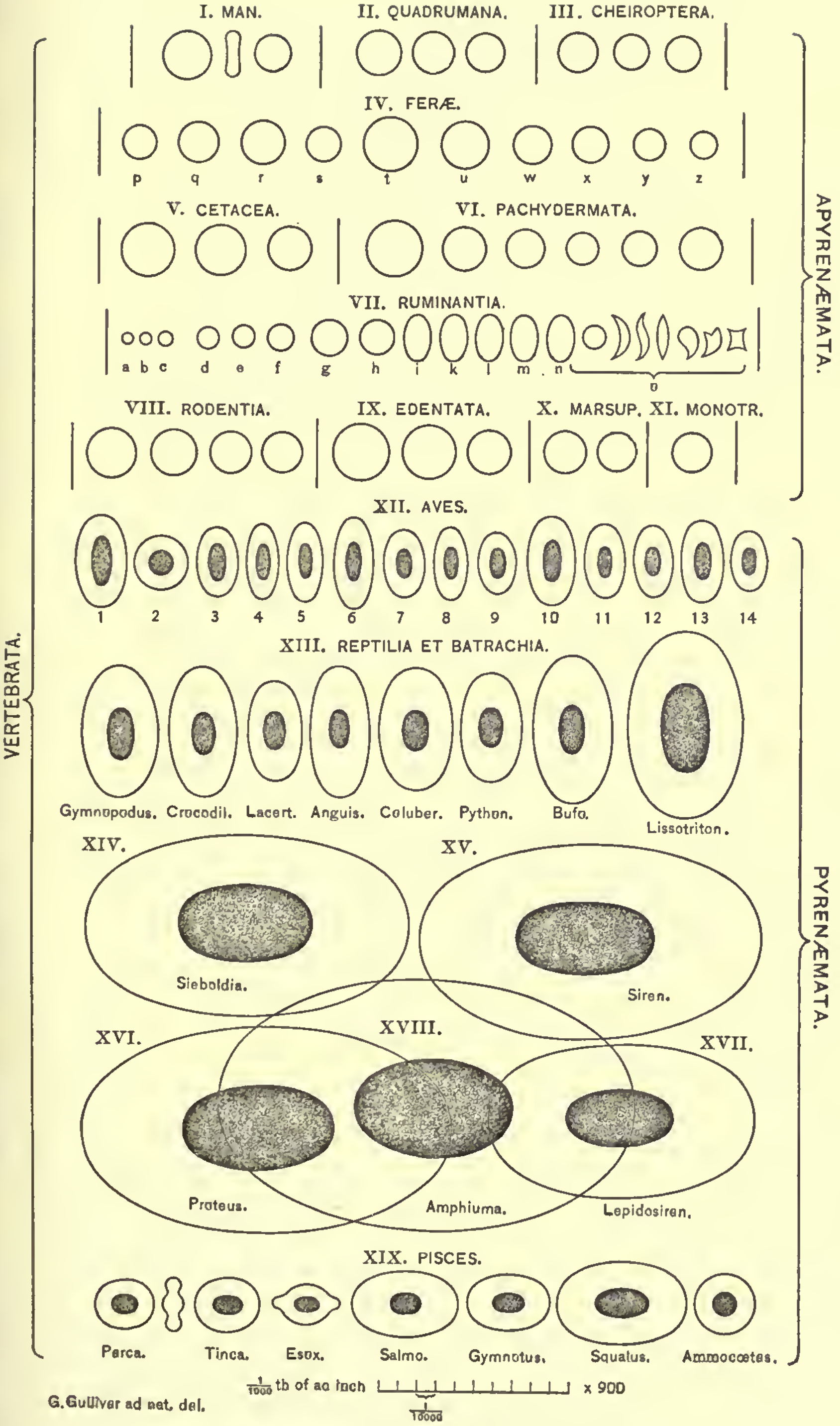
There are also certain relationships between the mean size of the corpuscles and the size of the species. Gulliver states that, if "we confine the observations to small natural groups of the class, such a relation will plainly appear in a rule that the largest corpuscles occur in the largest species and the smallest corpuscles in the small species of a single order or family. This relation is well shown in ruminants, rodents, and edentates, and even in feræ, which offer some exceptions; the largest corpuscles are found in the big seals and the smallest in the little viverras and paradoxures. In fine, though this rule is applicable only to single orders or lower sections of apyrenæmata, it extends to the whole class of birds, but neither to the reptiles, batrachians, nor fishes, except in partial instances, which seem to be rather indeterminate or accidental than regular." Attempts to trace a relationship between the number and size of the corpuscles and the speed of the animal's movements have proven negative. The very small and numerous corpuscles of the chevrotain have been associated with the fleetness of the animal; while, on the other hand, the enormously large and comparatively few corpuscles in the amphiuma have been associated with sluggishness. Such assumptions have been founded upon insufficient or erroneous data. There is, as a rule, an inverse relationship between the number of corpuscles per cubic millimeter and the mean diameter, but even in closely related genera this relationship may not exist.

### CERTAIN PROPERTIES OF THE ERYTHROCYTE IN RELATION TO GENERA.

There are certain peculiarities shown by the erythrocytes of different species which are of zoölogical significance. The well-known property of the erythrocytes of mammalian bloods to form rouleaux after the blood is shed has not been observed in the case of bloods having nucleated cells, except in the lamprey. This difference may be purely mechanical, and due to the nuclei preventing the approximation of the sides of the erythrocytes.

There are certainly differences in the specific gravities and coloration of the erythrocytes of different species.

# PLATE A



**Gulliver's micrometry of red blood corpuscles, all drawn to a uniform scale.**

Figures XIII, XIV, XV, XVI, XVII, and XVIII represent red blood corpuscles of Reptilia and Batrachia, while under figure XIX those of the fishes are given. In all these figures the names of the animals are inserted upon the plate, and do not require any comment at this place. It is evident that the blood corpuscles of the Amphiuma are so large that they can be perceived by the naked eye

### A.—VERTEBRATA APYRENÆMATA.

| | μ |
|---|---|
| I. *Homo* (*man*): | |
| 1. Corpuscles lying flat | 7.9 |
| 2. The same on edge | |
| 3. Membranous base of same after removal by water of coloring matter; it shows diminution in diameter on account of acquired spherical shape | |
| II. *Quadrumana* (*monkeys*): | |
| 4. Simia troglodytes (chimpanzee) | 7.4 |
| 5. Ateles ater (black-faced spider monkey) | 7.1 |
| 6. Lemur anguanensis | 6.4 |
| III. *Cheiroptera* (*bats*): | |
| 7. Cynonycteris collaris (fruit bat) | 6.6 |
| 8. Vespertilio noctula (large bat) | 5.8 |
| 9. Vespertilio pipistrellus (common bat) | 5.9 |
| IV. *Feræ* (*beasts of prey*): | |
| (*p*) 10. Sorex tetragonurus (shrew) | 5.6 |
| (*q*) 11. Ursus labiatus (lipped bear) | 6.8 |
| (*r*) 12. Bassaris astuta (civet cat) | 6.3 |
| (*s*) 13. Cercoleptes caudivolvulus (kinkajou) | 5.6 |
| (*t*) 14. Trichechus rosmarus (walrus) | 9.2 |
| (*u*) 15. Canis dingo (dog, Australian) | 7.5 |
| (*w*) 16. Mustela zorilla (weasel), Felis leo (lion), Felis leopardus (leopard) | 5.9 |
| (*x*) 17. Felis tigris (tiger) | 6.0 |
| (*y*) 18. Paradoxurus pallasii (Pallas paradoxure) | 4.6 |
| (*z*) 19. Paradoxurus bondar (Bondar paradoxure) | 4.5 |
| V. *Cetacea* (*whales*): | |
| 20. Balæna (boöps-whale) | 8.2 |
| 21. Delphinus globiceps (caaing-whale) | 7.9 |
| 22. Delphinus phocæna (porpoise) | 6.6 |
| VI. *Pachydermata:* | |
| 23. Elephas indicus (elephant) | 9.2 |
| 24. Rhinoceros indicus (rhinoceros) | 6.8 |
| 25. Tapirus indicus (tapir) | 6.4 |
| 26. Equus caballus (horse) | 5.5 |
| 27. Dicotyles torquatus (peccary) | 5.7 |
| 28. Hyrax capensis (Cape hyrax) | 7.7 |
| VII. *Ruminantia* (*ruminants*): | |
| (*a*) 29. Tragulus javanicus (Javan chevrotain, musk-deer) | 2.1 |
| (*b*) 30. Tragulus meminna (Indian chevrotain) | 2.1 |
| (*c*) 31. Tragulus stanleyanus (Stanleyan chevrotain) | 2.3 |
| (*d*) 32. Cervus nemorivagus (deer) | 3.6 |
| (*e*) 33. Capra caucasica (Caucasian ibex) | 3.6 |
| (*f*) 34. Capra hircus (domestic goat) | 4.0 |
| (*g*) 35. Bos urus (represented by Chillingham cattle) | 5.9 |
| (*h*) 36. Camelopardalis (giraffe) | 5.6 |
| (*i*) 37. Auchenia (vicugna) | long diameter 7.1<br>short diameter 3.9 |
| (*k*) 38. Auchenia paca (alpaca) | long diameter 7.6<br>short diameter 4.1 |
| (*l*) 39. Auchenia glama (llama) | long diameter 7.6<br>short diameter 4.1 |
| (*m*) 40. Camelus dromedarius (single-hump camel) | long diameter 7.8<br>short diameter 3.7 |
| (*n*) 41. Camelus bactrianus (double-bump camel) | long diameter 8.1<br>short diameter 4.3 |
| (o) 42. Cervus mexicanus (deer, Mexican) | 4.9 |
| VIII. *Rodentia* (*Rodents*): | |
| 43. Hydrochœrus capybara (capybara) | 8.0 |
| 44. Castor fiber (beaver) | 7.6 |
| 45. Sciurus cinereus (squirrel) | 6.4 |
| 46. Mus messorius (harvest mouse) | 5.9 |
| IX. *Edentata:* | |
| 47. Myrmecophaga jubata (ant-eater) | 9.2 |
| 48. Bradypus didactylus (sloth) | 8.9 |
| 49. Dasypus villosus (armadillo) | 7.7 |
| X. *Marsupialia:* | |
| 50. Phascolomys (wombat) | 7.3 |
| 51. Hypsiprymnus setosus (kangaroo rat) | 6.4 |
| XI. *Monotremata:* | |
| 52. Echidna histrix (echidna) | 6.6 |

### B.—VERTEBRATA PYRENÆMATA.

| | Long diam. | Short diam. |
|---|---|---|
| XII. *Aves* (*birds*): | | |
| 1. Struthio camelus (ostrich) | 15.4 | 8.5 |
| 2. Same made round and deprived of color by water. | | |
| 3. Vanga destructor (East India shrike) | 12.6 | 6.5 |
| 4. Lanius excubitor (great gray shrike) | 12.8 | 4.8 |
| 5. Bubo virginianus (horned owl) | 13.8 | 6.4 |
| 6. Syrnium nyctea (snowy owl) | 16.3 | 6.3 |
| 7. Columba rufina (rufous pigeon) | 11.0 | 7.6 |
| 8. Columba migratoria (wild pigeon) | 13.3 | 5.5 |
| 9. Dolichonyx oryzivorus (rice bird) | 10.6 | 6.1 |
| 10. Buceros rhinoceros (rhinoceros hornbill) | 15.0 | 7.9 |
| 11. Psittacus augustus (august amazon) | 12.2 | 7.0 |
| 12. Phasianus superbus (barrel-tailed pheasant) | 11.9 | 7.1 |
| 13. Pelecanus onocrotalus (white pelican) | 14.3 | 7.5 |
| 14. Trochilus sp. (humming-bird) | 9.4 | 6.6 |

Certain obscure yet obvious differences have been described in the general microscopic appearances of the erythrocytes of different species, differences which have been expressed by Johnson and by Wormley by the term "stamp of individuality."

The behavior of the erythrocytes of different species toward certain reagents shows zoölogical peculiarities. Thus, Haldane, Makgill, and Mavrogordato (Journal of Physiology, 1897, xxi, 160) record that chlorates enter the erythrocytes of man and the dog to form methemoglobin, but not those of the mouse, guinea-pig, and rabbit.

Other generic peculiarities have been pointed out by Up de Graf (The Microscope, 1883, quoted by White, *loc. cit.*), who found that the corpuscles of the dog rupture less readily than those of man when subjected to water. Differences in the osmotic properties of erythrocytes of different species have been recorded by different observers, and the differences in the osmotic pressures of erythrocytes of different species are well known.

The erythrocyte, in common with other protoplasmic structures, energetically decomposes $H_2O_2$. Bergengruen (Inaug. Dissert., Dorpat, 1888; Maly's Jahresb. ü. d. Fort. d. Thierchemie, 1888, 271) has shown that this property is due to the stroma, and, moreover, that the stromata of the corpuscles of the bullock, horse, and dog showed marked differences in their energy.

Studies of cytolysins and agglutinins show marked generic differences in the erythrocytes: Dog's serum hemolyzes the erythrocytes of the chicken, rabbit, sheep, ox, and guinea-pig. Horse serum is not hemolytic to chicken corpuscles, but chicken serum is hemolytic to horse corpuscles. Rabbit serum is not hemolytic to the corpuscles of the guinea-pig, bullock, or dog, but it is slightly hemolytic to bullock corpuscles. Bullock serum is faintly hemolytic to sheep corpuscles, and sheep serum is slightly hemolytic to dog corpuscles. Eel serum is a general strong hemolytic.

### THE PERCENTAGES OF HEMOGLOBIN IN THE DRY ERYTHROCYTES IN RELATION TO GENERA.

The dry corpuscles consist almost wholly of protein, most of which in mammals and birds is hemoglobin. The proportion of hemoglobin varies in the corpuscles of different species, the non-nucleated cells containing a much larger percentage than the nucleated cells. The very limited data at hand indicate that in mammals the percentage will range from about 85 to 95 per cent, according to Hoppe-Seyler and Jüdell (Med. chem. Unter-

TABLE 22.—*The percentages of hemoglobin in the dried erythrocytes of different genera, according to Hoppe-Seyler.*

| Kind. | Percentage of hemoglobin. |
|---|---|
| Man I | 86.79 |
| Man II | 94.30 |
| Dog | 86.50 |
| Hedgehog | 92.25 |
| Goose | 62.65 |
| Snake (Coluber natrix) | 46.70 |

such., 1868, 391; Physiologische Chemie, 1881, 401) (table 22), varying doubtless in different genera, species, individuals of the same species, etc. In birds, taking the goose as the representative, the percentage is approximately only two-thirds that in mammals, while in cold-blooded animals, as indicated by the snake, the proportion is only about half of that in mammals.

### THE PERCENTAGE OF HEMOGLOBIN IN THE MOIST ERYTHROCYTES IN RELATION TO GENERA.

The percentages of hemoglobin in the moist corpuscles probably vary, according to the records of Abderhalden and others (Schmidt, Character. d. epidem. Cholera, Leipzig, 1850; Gorup-Besanez, Physiologische Chemie, 1878, 345; Bunge, Zeit. f. Biologie, 1876, XII, 191; Biernachi, Centralb. f. inner. Med., 1894, XV, 718; Kohler, Centralb. f. inner. Med., 1897, XVIII, 724; Abderhalden, Zeit. f. physiolog. Chemie, 1898, XXV, 115, and XXVI, 65), within limits so narrow, varying as much in individuals as in species, that it seems futile to attempt any generic differentiation (table 23). In all the mammals examined the quantity of hemoglobin in reliable records approximates 32 per cent.

TABLE 23.—*The percentage of hemoglobin in the moist erythrocytes in relation to genera.*

| Kind. | Percentage of hemoglobin. | Authority. |
|---|---|---|
| Primate: | | |
| Man { I. | 15.96 | Schmidt. |
| Man { II. | 31.11 | Do. |
| Man | 29.68 | Biernachi. |
| Carnivora: | | |
| Cat | 29.8 | Kohler. |
| Cat | 32.99 | Abderhalden. |
| Dog | 32.81 | Do. |
| Ungulata: | | |
| Bullock | 31.67 | Do. |
| Bullock | 28.00 | Bunge. |
| Sheep | 31.26 | Abderhalden. |
| Goat | 32.40 | Do. |
| Horse | 31.58 | Do. |
| Pig | 26.1 | Bunge. |
| Pig | 32.68 | Abderhalden. |
| Rodentia: | | |
| Rabbit | 33.19 | Do. |

### THE PERCENTAGE OF HEMOGLOBIN IN THE WHOLE BLOOD IN RELATION TO GENERA.

The percentage of hemoglobin in the whole blood has been made the subject of study by numerous investigators by various methods, but, owing to imperfect methods and other reasons, the figures for a given species are so variable as not to permit of close comparisons of different species. Some have made their estimates by the "extinction coefficient," others by comparison by the colorimetric method, others by the percentage of iron, etc. In determinations by the "extinction coefficient," a weak solution of blood in a layer of given thickness is studied in relation to the extinction of some definite part of the hemoglobin spectrum, usually the second absorption band. These coefficients are proportional to the concentration of the hemoglobin solution. The coefficients for human blood based upon the values of Leichtenstern and others (Vierordt's Daten u. Tabellen, 1906, 220 *et seq.*) are for men 1.2359, for women 0.9559, and for children from 2 to 10 years of age 1.066. During the early weeks of life the coefficient is much higher than in the adult, but later it falls for a time to a point lower than in men, but higher than in women. Korniloff (Zeit. f. Biologie, 1876, XII, 515), in comparative studies by this method of the bloods of 110 vertebrates, including 44 species of both warm-blooded and cold-blooded animals, reports the following average figures: Mammals 0.9366, birds 0.7814, reptiles 0.4328, amphibia 0.3889, and fish 0.3564. Males, he found, have a higher hemoglobin capacity than females, and the old a higher capacity than the young.

TABLE 24.—*The percentages of hemoglobin in relation to genera.*

| Kind. | Percentage of hemoglobin. | Authority. |
|---|---|---|
| Primates: | | |
| Man | 12.09 to 15.07 | Preyer. |
| Woman | 11.57 to 13.69 | Do. |
| Man | 11.8 to 12.77 | Quinquaud. |
| Woman | 10.4 to 11.35 | Do. |
| Man | 13.00 | Müller. |
| Man | 12 to 14.5 | Henocque. |
| Man | 12.05 to 12.78 | Pelouze. |
| Man | 13.55 | Means determined from Vierordt's Daten u. Tabellen. |
| Woman | 12.67 | |
| Monkey | 5 to 14 | Henocque. |
| Carnivora: | | |
| Cat | 14.32 | Abderhalden. |
| Dog | 13.12 to 13.46 | Preyer. |
| Dog | 10.51 | Müller. |
| Dog | 14 to 14.5 | Henocque. |
| Dog | 13.34 to 14.56 | Abderhalden. |
| Dog, male | 14.16 | Otto. |
| Dog, female | 13.76 | Do. |
| Dog | 13.80 | Subbotin. |
| Dog | 16.55 to 17.4 | Fudakowski. |
| Dog | 16.83 to 18.08 | Arronet. |
| Ungulata: | | |
| Bullock | 13.33 to 13.95 | Preyer. |
| Bullock | 10.31 | Abderhalden. |
| Bullock | 10.4 to 11.35 | Quinquaud. |
| Bullock | 10.21 | Müller. |
| Bullock | 10.30 | Abderhalden. |
| Bullock | 12.10 | Subbotin. |
| Bullock | 11.43 to 13.02 | Pelouze. |
| Calf | 10.11 to 10.68 | Preyer. |
| Calf | 6.62 to 9.46 | Quinquaud. |
| Calf | 8.91 | Subbotin. |
| Sheep | 11.11 to 11.53 | Preyer. |
| Sheep | 10.93 | Müller. |
| Sheep | 9.29 to 10.28 | Abderhalden. |
| Sheep | 6.62 to 8.98 | Quinquaud. |
| Goat | 11.26 | Abderhalden. |
| Horse | 16.69 | Do. |
| Ungulata–con'd: | | |
| Horse | 12.05 to 13.19 | Pelouze. |
| Horse | 10.4 to 10.88 | Quinquaud. |
| Horse | 13 | Müller. |
| Horse | 11.34 | Arronnet. |
| Pig | 14.03 to 14.80 | Preyer. |
| Pig | 13.32 | Müller. |
| Pig | 14.22 | Abderhalden. |
| Pig | 14.09 to 14.17 | Pelouze. |
| Rodentia: | | |
| Rat | 8.85 | Do. |
| Rat | 8.5 to 9.22 | Quinquaud. |
| Rat | 8.68 to 9.12 | Preyer. |
| Guinea-pig | 14 | Henocque. |
| Rabbit | 12.35 | Abderhalden. |
| Rabbit, male | 10.51 | Otto. |
| Rabbit, female | 8.77 | Do. |
| Rabbit | 8.41 | Subbotin. |
| Aves: | | |
| Turkey | 7.93 to 8.00 | Pelouze. |
| Chicken | 8.5 | Do. |
| Chicken | 9 to 9.92 | Preyer. |
| Goose | 8.26 to 8.76 | Pelouze. |
| Duck | 8.14 to 8.19 | Do. |
| Duck | 9.16 to 9.42 | Preyer. |
| Duck | 8.2 | Quinquaud. |
| Pigeon | 9 to 11.5 | Henocque. |
| Pigeon | 7.31 to 12.56 | Subbotin. |
| Pigeon | 7.09 to 8.03 | Quinquaud. |
| Sparrow | 7.09 to 7.5 | Do. |
| Amphibia: | | |
| Sea-tortoise | 6.94 to 8.98 | Bardachzi. |
| Frog | 10.12 | Pelouze. |
| Frog | 2.35 to 3.3 | Quinquaud. |
| Lizard | 2 to 13<br>(according to season and condition) | Henocque. |
| Pisces: | | |
| Tench | 2.36 to 3.78 | Quinquaud. |
| Skate | 3.5 to 3.8 | Harris. |
| Invertebrata: | | |
| Annelids | 3.4 | Velichi. |

The estimates recorded by Preyer, Abderhalden, Henocque, and others by various methods are, broadly speaking, in accord with those of Korniloff. (Preyer, Die Blutkrystalle, 1872, 116, and Annalen d. Chemie u. Phar., 1866, CXL, 187; Müller, Archiv f. Thierheilk., 1886, XII, 96; Henocque, Compt. rend. soc. biologie, 1886, CIII, 493; Korniloff, Zeit. f. Biologie, 1876, XII, 515; Leichtenstern, Vierordt's Daten u. Tabellen, 1906, 220; Abderhalden, Zeit. f. physiolog. Chemie, 1898, XXVI, 65; Otto, Archiv f. ges. Physiologie, 1885, XXXV, 36; Subbotin, Zeit. f. Biologie, 1871, VII, 185; Pelouze, Compt. rend. soc. biologie, 1865, LX, 880; Otto *et al.*, Vierordt's Daten u. Tabellen, 1906, 220 *et seq.*; Jolyet, Gazette médicale, 1874, 383; Bardachzi, Zeit. f. physiolog. Chemie, 1906, XLIX, 465; Quinquaud, Compt. rend. soc. biologie, 1873, LXXVII, 1489, and LXXVII, 487; Velichi, Inaug. Dissert., Berlin, 1900; Centralbl. f. Physiologie, 1901, XIV, 679; Fudakowski, Centralbl. f. med. Wissensch., 1866, IV, 705; Arronet, Inaug. Dissert., Dorpat, 1887; Harris, Journal of Physiology, 1903, XXX, 319.) In mammals the percentage ranges usually between 10 and 15; in birds, between 7 and 9; and in cold-blooded animals, from 2 to 10.

Glancing over the figures for mammals (table 24), it appears that the percentages fall in the following order: pig, dog, cat, man, horse, bullock, goat, and sheep. The mean of rodents is notably lower than that of primates, carnivora, and ungulates, the percentages being in the following order: guinea-pig, rabbit, and rat. In birds the percentage is, on the whole, distinctly lower than in rodents. Among cold-blooded animals it is probably highest in the tortoise. Omitting the figures of Pelouze for the frog, which obviously are incorrect, the percentage in the frog is only about one-sixth to one-fourth of that in mammals.

## GENERAL CONSIDERATION OF THE ZOÖLOGICAL SPECIFICITIES OF THE BLOOD.

Zoölogical peculiarities of the blood or pseudo-blood are shown throughout the animal kingdom, vertebrate and invertebrate. The facts that have been brought together in this chapter show clearly not only marked zoölogical differentiations, but also what important information is to be expected from additional and detailed data along the same or related lines of inquiry. These distinctions are shown in part:

(1) By the *whole blood* in differences in the proportions of blood to body-weight, in specific gravity, in alkalinity, in the proportions of corpuscles to plasma, in the degree of coagulability and in the character of the fibrin, in the degree of decomposability, etc.

(2) In differences in the *plasma* as regards especially the percentages and kinds of proteins and in the "protein quotient," in the percentage of cholesterin, in the peculiarities of the "precipitins," agglutinins, and hemolysins, etc.

(3) In the *leucocytes* in respect to kind and relative numbers, in peculiarities of the granular matter, in specific physiological peculiarities, etc.

(4) In the *erythrocytes* in their size, structure, form, and number per cubic millimeter, in their behavior towards certain reagents, in their percentages of hemoglobin, sodium, potassium, phosphorus, and cholesterin, etc.

In subsequent pages will be found additional evidence of specificities in the differences in the stroma, in the general chemical and physical properties of the hemoglobins, and especially in the remarkable generic peculiarities of the hemoglobins as shown by their crystallographic properties.

These zoölogical differences are paralleled in the invertebrates, as will be manifest even after a most superficial inquiry, as, for instance: In the *Protozoa* and *Porifera* there is an entire absence of any fluid which we are justified in regarding as being even an analogue of the blood. In the *Hydrozoa, Actinozoa,* and *Echinodermata* the perivisceral or chyliferous fluid is the simplest expression of a rudimentary blood. In the former this fluid scarcely differs from that in which the organism lives, and it contains but few if any corpuscles, which are of a rudimentary character. In the *Actinozoa* and *Echinodermata* there is a distinct approach to a typical blood, there being both rudimentary and typical cells. The chylaqueous fluid of the *Annelida* contains typical corpuscles, and it is among the animals of the annuloid series, in *Trichoscolices* and *Annelida,* that we find the first appearance of hemoglobin, of colored corpuscles, and of a true blood—*i.e.*, a circulatory fluid which combines the functions of both circulation and respiration, and therefore that is comparable with the blood of the vertebrate. The first appearance of hemoglobin is, as far as known, in holothuridean (*Thyonella gemmata*), ophiuridean (*Ophiactis virens*), and turbellarian (*Polia sanguirubra*) echinoderms, although histohematins have been found in certain of the *Porifera* and *Actiniidæ.* The first appearance of an analogue of the erythrocyte has been noted in the *Gephyrea* (*Sipunculus nudus, S. balanorophus, S. echinorhynchus, Phascolosoma elongatum*), in which are corpuscles having a distinct cell wall which incloses a colored fluid in which a nucleus is suspended. The coloring matter of the corpuscles is allied to hemoglobin, but the corpuscles have no histological relationship to the vertebrate erythrocyte. The fluid of the pseudo-hemal system of certain *Annelida* contains a red coloring matter in the form of a histohematin which is closely allied to hemoglobin chemically and physiologically; that of others is green and colored by chlorocruorin, which also is closely allied to hemoglobin; and that of others is colored by hemoglobin, etc.

In invertebrata the blood or pseudo-blood may be colored or colorless, which differentiation is not related to the position of the organism in the scale of life; but in all of the non-generate vertebrates the blood is colored. In the invertebrates the coloring matter may be in solution in the circulatory fluid or in the corpuscles, but, as a rule, it is in the fluid, whereas in the vertebrates it is without exception in the erythrocytes. While the corpuscles of invertebrate blood are allied histologically to the leucocytes of vertebrate blood and not to the erythrocytes, when colored they may be (but usually are not) respiratory like the erythrocytes.

In the vast majority of invertebrates the coloring matter of the blood is hemocyanin, which is, as far as known, invariably in solution in the plasma, the venous blood being colorless, or nearly so, and the arterial blood of various tints of blue or violet. Various other pigments have been found, as shown in the preceding chapter. In no instance has it been recorded, as far as we are aware, that both hemocyanin and hemoglobin coexist in the same blood or even in the same individual, although in certain organisms with a hemocyanin blood histohematins and myohematins have been found in certain of the body structures. Bloods that owe their coloration to other substances than hemocyanin may exhibit a variety of colors, which colors may be properties of the plasma or corpuscles or both. Even among animals of a given subkingdom the coloring matter may differ very much. Thus, among *Annelida*, as stated, certain bloods are red owing to hemoglobin; in others the coloration tends to a reddish-brown owing to echinochrome; while in others it is green owing to chlorocruorin, etc. Among the *Arthropoda* we may find hemoglobin, hemocyanin, pinnaglobin, or other pigment present. In *Insecta* we never find hemocyanin, and rarely hemoglobin; in *Crustacea* there may be hemocyanin or hemoglobin; in *Decapoda* and *Stomatopoda* the blood coloring matter is hemocyanin; while in *Cladocera, Phyllopoda, Copepoda,* and *Ostracoda* there is hemoglobin. Among *Mollusca,* in some the blood is colorless; in others is found pinnaglobulin, which is closely related to hemoglobin, containing manganese in place of iron; in some there is hemoglobin; but in most of them the coloring matter is hemocyanin. In *Gasteropoda* and *Cephalopoda* hemocyanin is present. In the *Chætopoda* hemoglobin has been found in quite a number of species, and chlorocruorin in a few. In *Gephyrea, Nemertina,* and *Hirudinea* hemoglobin has been noted.

In *Insecta* the blood may be colorless or of various colors, but is usually colorless. The coloration is a property of the blood plasma, and except the blood of the larva of the dipterous insect *Chironomus* and *Musca domestica* there appears to be an absolute absence of hemoglobin from the bloods of these animals, although histohematins and myohematins have been found in various of the body structures of a number of them.

In all the invertebrates which have hemocyanin this substance is, as far as known, in solution in the blood plasma; but the fact that copper has been found in leucocytes of the oyster and in other structures of invertebrates, and that there are blue and violet corpuscles, leads to the belief that in certain invertebrates hemocyanin may be a component of both plasma and corpuscles, and even of other structures. In all invertebrates in which hemoglobin has been found it has been noted in solution in the blood plasma, excepting *Glycera, Capitella, Phoronis,* and *Solen,* in which it is a constituent of special blood corpuscles.

Among the invertebrates the blood corpuscles may be colorless or colored, and when the latter they may be green, red, yellow, blue, violet, purple, madder, mahogany, brown, lilac, etc.; and in certain organisms (*Spatangus*) a variety of corpuscles of different colors may be present in the same blood, such as yellow, green, brown, indigo-blue, and purple.

In addition to these exceedingly interesting peculiarities, there have been noted in the bloods of different invertebrate organisms differences in the specific gravity, in coagulability, and in the percentages of proteins, copper, and salines; and in the kinds, sizes, composition, and relative number of the corpuscles, etc.

Gaskell (The Origin of Vertebrates, London, 1908) gives evidence and arguments upon geological, anatomical, and embryological grounds that lead to the belief that vertebrates may have had their origin from palæostracans. He states (p. 65) that the evidence of geology "points directly and strongly to the origin of vertebrates from the palæostraca-arthropod forms, which were not crustacean and not arachnid, but gave origin both to the modern-day crustaceans and arachnids. The history of rocks further shows that these ancient fishes, when they first appeared, resembled in a remarkable manner members of the palæostracan group (trilobites, higher scorpion and king-crab forms), so that again paleontologists have found great difficulty in determining whether a fossil is a fish or an arthropod. Fortunately, there is still alive on this earth one member of this remarkable group—the *Limulus*, or king-crab. * * * There are no trilobites still alive, but in *Branchipus* and *Apus* we possess the nearest approach to the trilobite organization among living crustaceans."

In this connection it is of interest to note that hemocyanin has been found in the blood of *Scorpiones* (*Scorpio*), *Xiphosura* (*Limulus*), *Decapoda* (*Homarus*, *Astacus*, *Cancer*, *Carcinus*, *Nephrops*, *Eriphia*), and *Stomatopoda* (*Squilla* and *Maia*); and that hemoglobin has been found in the bloods of *Diptera* (*Chironomus*, *Musca domestica*), *Ostracoda* (*Cypris*), *Copepoda* (*Lernanthropus*), *Cladocera* (*Daphnia*), and *Phyllopoda* (*Apus* and *Branchipus*).

The great importance of hemoglobin in vertebrate life, as is indicated, for instance, in the fact of its universal presence in every living non-degenerate vertebrate, suggests that if, as Gaskell contends, vertebrates had their origin from palæostraca, it was more likely from one of the group in which hemoglobin and not hemocyanin is the respiratory pigment of the blood.

These facts, brought together as they are in so fragmentary and unsatisfactory a way, are nevertheless sufficient to be convincing that the results of detailed inquiry, which has been denied us through lack of time, will prove of the utmost importance in zoölogical differentiation.

# CHAPTER III.

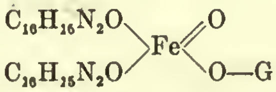
## HEMOGLOBIN; ITS GENERAL CHEMICAL AND PHYSICAL CHARACTERS, AND ITS SPECIFICITIES.

### CONSTITUENTS AND RELATIONS TO THE OTHER CONSTITUENTS OF THE ERYTHROCYTES.

Hemoglobin, $C_{54 \cdot 57}H_{7 \cdot 22}N_{16 \cdot 38}S_{0 \cdot 68}Fe_{0 \cdot 336}O_{20 \cdot 40}$ (Jacquet, Zeit. f. physiolog. Chemie, 1890, xiv, 289), is regarded as being composed of a colorless, strongly basic albuminous radical, termed globin, $C_{54 \cdot 97}H_{7 \cdot 2}N_{16 \cdot 89}S_{0 \cdot 42}O_{20 \cdot 52}$ (Schulz, Zeit. f. physiolog. Chemie, 1898, xxiv, 449), and a non-albuminous, colored radical termed hematin, $C_{34}H_{34}N_4FeO_5$ (Küster, Zeit. f. physiolog. Chemie, 1904, xl, 391). The latter constitutes 4 to 4.5 per cent of the molecule (Schulz, *loc. cit.*, and Lawrow, Zeit. f. physiolog. Chemie, 1898, xxvi, 343), and is, so far as known, probably absolutely identical in the hemoglobins of all animals; but the former is in all likelihood not identical, as is indicated by certain differences in chemical composition and constitution of the hemoglobins of different bloods; by the difference between hemoglobin and myohematin; by the fact that globin may be replaced by egg-white (Ham and Balean, Journal of Physiology, 1905, xxxii, 312), or by albumin from the blood of another species (Bertin-Sans et Moitessier, Compt. rend. soc. biologie, 1893, cxiv, 923; Bull. soc. chim., 1893, 5 Mai, 5 Sept.); and also by the fact, as stated by Schulz (*loc. cit.*), that the globins of the dog and horse are not identical with that of the goose.

Globin is a histone-like body; it has been isolated by Schulz and others; and its primary dissociation products have been studied by Fischer and Abderhalden (Zeit. f. physiolog. Chemie, 1902, xxxv, 268) and by Abderhalden (Zeit. f. physiolog. Chemie, 1903, xxxvii, 484). Only 72 per cent of these products have been accounted for—leucin 29, histidin 11, arginin 5.4, asparaginic acid 4.4, lysin 4.3, alanin 4.2, phenylalanin 4.28, prolin 2.3, glutaminic acid 1.7, tyrosin 1.3, oxyprolin 1, serin 0.6, cystin 0.3, ammonia 0.93 per cent. The sulphur is contained chiefly in the cystin, and the iron solely in the hematin.

The union between globin and hematin is very feeble, the addition of weak acid being sufficient, as has been shown by Ham and Balean (*loc. cit.*), to cause immediate dissociation. The nature of this linking is in doubt. According to Hoppe-Seyler (Archiv f. path. Anat. u. Physiolog., 1864, xxix, 233; Centralbl. f. med. Wissensch., 1864, 261, and 1865, 491), it is ester-like, while Hüfner (Archiv f. Anat. u. Physiolog., 1899, 491) and Ham and Balean (*loc. cit.*) regard it as being through the agency of oxygen. According to the hypothesis of Ham and Balean, the formula for oxyhemoglobin is:



in which G represents the globin radical (page 26). According to Zinoffsky (Zeit. f. physiolog. Chemie, 1886, x, 16), the molecule may be regarded as consisting of two molecules of globin and one molecule of hematin. Whether or not globin and hematin are thus combined, or the hematin is linked with one or several molecules of globin; whether the globin is a simple or compound body; whether the hematin may be combined with polymeric or isomeric forms of globin; whether the hematin is with certainty a uniform substance, etc., are still open questions. If, as Miescher states, the albumin molecule with its 40 atoms of carbon may have as many as a billion stereoisomers, what may be the possibilities of hemoglobin or globin molecules with their hundreds of carbon atoms? Whatever may be the chemical relations between globin and hematin, they are so peculiarly associated that undecomposed hemoglobin gives neither albuminous nor iron color reactions. It is of incidental interest to note that, except the iron in hemoglobin, nearly all of the iron of the tissue cells is contained in the nucleoproteins, and that while these substances, unlike hemoglobin, yield the protein color reactions, they, like hemoglobin, do not yield iron reactions, showing that in both the iron is in a non-ionic or "masked" state.

We are also in doubt as to the state or states in which hemoglobin exists in the erythrocytes, especially as to whether it is in a liquid, semiliquid, or solid form, and as to the nature of the compound or compounds it probably forms with other constituents of the erythrocytes. The red corpuscles consist of a stroma and hemoglobin with other substances. The former is elastic, non-contractile, seemingly homogeneous, colorless, transparent, and albuminous. According to some, the stroma is in the form of minute sacs which contain hemoglobin and other substances in solution. According to others, it is in the form of a protoplasmic mass, throughout which the hemoglobin and other substances are distributed. That the hemoglobin is not in either crystalline or amorphous form has been shown by microscopic examination with high powers; and that it is not in solution in a free state seems obvious from the fact that in the case at least of the very insoluble forms of hemoglobin, as in the guinea-pig, squirrel, rat, necturus, etc., not only are the water and the inorganic salts of the corpuscles wholly inadequate to dissolve or keep in solution the hemoglobin, but even the entire blood plasma is altogether insufficient to hold the hemoglobin in solution when freed from the corpuscles.

The assumption of Preyer that the hemoglobin is held in solution in the corpuscles by virtue of potassium salts because of the presence of a relatively high percentage of these salts in comparison with the percentage in the plasma, and because of the higher solubility of the hemoglobin in water when these salts are present, is not worthy of consideration, inasmuch as in certain bloods, for instance in those of the dog and cat, the percentage of potassium in the corpuscles is practically the same as in the plasma, and yet in the dog crystallization takes place rapidly in the plasma upon the laking of the blood. Rywosch (Centralbl. f. Physiologie, 1905, XIX, 388) believes that the hemoglobin is present in the corpuscles in a free state. He found, after destruction of the erythrocytes by grinding in sand, that by mixing the pulp with an isotonic salt solution the hemoglobin was dissolved. This he holds would not occur "if the hemoglobin was in combination with the stroma." However, his method may have been the means of breaking up a hemoglobin-stroma union. Moreover, Stewart (see below) has found, in his experiments on the influences of various agents on the osmotic properties of the erythrocytes, that the hemoglobin can not exist in the corpuscles in ordinary aqueous solution.

Hoppe-Seyler (Physiologische Chemie, 1877, 381; Zeit. f. physiolog. Chemie, 1889, XIII, 477) attempted to show, by various facts and arguments, that such differences exist between the behavior of the coloring matter of the blood as it exists in corpuscles and hemoglobin in solution that they can not be identical. Most of his deductions have, however, been found to be untenable. He distinguishes between the coloring matter of the blood, oxyhemoglobin, and reduced hemoglobin, regarding both oxyhemoglobin and reduced hemoglobin as cleavage products. He looks upon the "coloring matter" of the blood as consisting of combinations of oxyhemoglobin and hemoglobin with lecithin, forming firm chemical unions. The coloring matter of arterial blood he distinguishes as *arterin* and that of venous blood as *plebin*, the only difference between these two substances being a feebler combination of oxygen in the former. While Hoppe-Seyler's hypothesis seems to have received a tacit acceptance, it has been opposed by Gamgee (Schäfer's Text-book of Physiology, 1898, 1, 190) and questioned by others as being untenable; but it has been defended by Kobert (Das Wirbeltierblut, etc., Stuttgart, 1901, 5).

Bohr (Zentralbl. f. Physiologie, 1904, XVII, 682, 688) believes that the coloring matter of the blood, which he terms hemochrome, is not identical with hemoglobin (which he prepared without the addition of alcohol), because the latter has a lower oxygen capacity.

Recent evidence that hemoglobin exists in the corpuscles in some peculiar form of combination has been recorded by a number of investigators. Thus, Stewart (Journal of Physiology, 1899, XXIV, 211; Amer. Journal of Physiology, 1902, VIII, 103) found, in a very interesting study of the effects of laking agents, "that the relations of the hemoglobin and the electrolytes of the corpuscle to some of the other constituents of the corpuscle or to the envelop are such that under certain conditions hemoglobin may be liberated while the electrolytes are retained; while under other conditions electrolytes may pass through an envelop which refuses passage to the hemoglobin, although in general it is easier for the hemoglobin, in spite of the great size of the molecule, to escape from the corpuscles than it is for the electrolytes." He also found that, while hemoglobin may pass from the corpuscle, hemoglobin dissolved in the serum would not pass into the corpuscle. In explanation of these phenomena Stewart proposes four hypotheses as to the condition of the hemoglobin and the electrolytes in the corpuscles:

(1) A portion of the electrolytes and of the hemoglobin is in solution as such; and the rest is in solution as compounds with other substances, such compounds being unable to pass through the envelop.

(2) A portion of the electrolytes and of the hemoglobin is in solution as such, and the rest exists in a solid or semisolid form united to some constituent of the stroma.

(3) A portion of the electrolytes, but none of the hemoglobin, is in solution as such; the whole of the hemoglobin and the rest of the electrolytes being in solution in the form of such compounds as are mentioned in (1).

(4) A portion of the electrolytes, but none of the hemoglobin, is in solution as such; the rest of the electrolytes and all of the hemoglobin are united in the stroma.

The last hypothesis, he thinks, best takes account of the facts of laking.

Oxygen, it seems, serves as a connecting link not only between globin and hematin, but also between the stroma and hemoglobin. The removal of oxygen from the blood causes hemolysis. This phenomenon might, at first thought, be regarded as a mechanical effect due to the rapid discharge of O from the erythrocytes when the blood is subjected to the vacuum pump, but this is negatived by the fact that hemolysis occurs just the same when a continuous stream of $CO_2$ is passed through the blood and the O thus driven off gradually. Even the linkage between globin and hematin may be broken by $CO_2$.

That the hypothetical union between hemoglobin and the stroma must be a feeble one is evident in the readiness with which it is broken, by the removal of O from the blood, by minute quantities of foreign serum, snake venom, and certain bacterial products, by repeated freezing and thawing, etc. While it thus seems probable that the hemoglobin of the corpuscles is essentially or solely in some form or forms of union with the stroma, it is also probable, from the investigations of Hüfner (Archiv f. Anat. u. Physiologie, 1894, 135, 176), that the combination does not, in opposition to Hoppe-Seyler's statements, effect a marked alteration in the chemical nature of hemoglobin in so far as pertains to its relations to oxygen and to light, for he found that its behavior to oxygen and its spectrophotometric properties are the same as when the hemoglobin is free, provided the solution be of the same degree of concentration. On the other hand, it is positive that at least the degree of solubility in relation to the plasma and the crystallizability are lessened to a marked degree, so much so that the crystallization may occur in the plasma of partially laked blood and not in the corpuscles, even though in the latter the concentration of the hemoglobin may be greatly higher. The corpuscles of the dog contain about 33 per cent of hemoglobin, while the highest percentage that could exist in the laked blood is about half of this; but while crystallization does not occur in the corpuscles, it does occur rapidly in the laked blood. (See Chapters V and XV.)

## THE ELEMENTARY COMPOSITION OF HEMOGLOBIN.

The determinations of the centesimal composition of hemoglobin of different species of animals differ sufficiently to indicate that all hemoglobins are not alike; but these differences are not on the whole greater than those noted in the analyses of specimens of blood from individuals of the same species, and are therefore of little significance in indicating positive non-identity (table 25). In fact, the analyses, as a whole, are so discrepant that it must be admitted that hemoglobin is not a uniform substance, even in individuals of a given species, or that there are important sources of fallacy in the methods of analysis, or that the methods of preparation are so faulty as to yield either an impure or a partially decomposed substance. It seems so very improbable that the hemoglobin from normal

TABLE 25.—*The centesimal composition of hemoglobin, according to various observers.*

| Kind. | Centesimal composition. | | | | | | Authority. |
|---|---|---|---|---|---|---|---|
| | C. | H. | N. | S. | Fe. | O. | |
| Carnivora: | | | | | | | |
| Cat | 54.60 | 7.25 | 16.52 | 0.62 | 0.35 | 20.66 | Abderhalden, Physiologischen Chemie, 1906, 596. |
| Dog | 53.91 | 6.62 | 15.98 | 0.542 | 0.333 | 22.62 | Jacquet, Zeit. f. physiol. Chemie, 1888, xii, 285. |
| Dog | 54.57 | 7.22 | 16.38 | 0.568 | 0.336 | 20.93 | Jacquet, Zeit. f. physiol. Chemie, 1890, xiv, 289. |
| Dog | 53.85 | 7.32 | 16.17 | 0.39 | 0.43 | 21.84 | Hoppe-Seyler, Med. chem. Untersuch., 1868, Heft 3, 366. |
| Dog | 54.00 | 7.25 | 16.25 | 0.63 | 0.42 | 21.45 | Hüfner, Jour. f. prakt. Chemie, 1880, xxii, 362. |
| Dog | 54.15 | 7.18 | 16.33 | 0.67 | 0.43 | 21.24 | Schmidt, Preyer, Die Blutkrystalle, 1872, 65. |
| Dog | 53.64 | 7.11 | 16.19 | 0.66 | 0.43 | 20.03 | Schmidt & Böttcher, Ueber Blutkrystalle; Inaug. Dissert., Dorpat, 1862. |
| Ungulata: | | | | | | | |
| Bullock | 54.66 | 7.25 | 17.70 | 0.4 | 0.447 | 19.543 | Hüfner, Beiträge z. Physiol., C. Ludwig, Leipzig, 1887, 74. |
| Horse | 54.87 | 6.97 | 17.31 | 0.65 | 0.47 | 19.73 | Kossel, Zeit. f. physiol. Chemie, 1878–9, ii, 149. |
| Horse | 54.76 | 7.03 | 17.28 | 0.67 | 0.45 | 19.81 | Otto, Archiv. f. ges. Physiologie, 1883, xxxi, 240. |
| Horse | 54.40 | 7.20 | 17.61 | 0.65 | 0.47 | 19.67 | Hüfner & Bücheler, Zeit. f. physiol. Chemie, 1884, viii, 358. |
| Horse | 51.15 | 6.76 | 17.94 | 0.39 | 0.335 | 23.42 | Zinoffsky, Zeit. f. physiol. Chemie, 1880, x, 16. |
| Horse | 54.56 | 7.15 | 17.33 | 0.43 | ..... | ...... | Schulz, Zeit. f. physiol. Chemie, 1898, xxiv, 449. |
| Horse | 54.75 | 6.98 | 17.35 | 0.42 | 0.38 | 20.12 | Abderhalden, Zeit. f. physiol. Chemie, 1903, xxxvii, 494. |
| Horse | 54.40 | 7.25 | 17.51 | 0.45 | 0.393 | 19.85 | Jutt, Inaug. Dissert., Dorpat, 1894; Maly's Jahresbr. ü. d. Fort. d. Thierchemie, 1895, 128. |
| Pig | 54.17 | 7.38 | 16.23 | 0.66 | 0.426 | 21.634 | Otto, Zeit. f. physiol. Chemie, 1882, viii, 57. |
| Pig | 54.71 | 7.38 | 17.43 | 0.479 | 0.399 | 19.602 | Hüfner, Beiträge z. Physiol., C. Ludwig, Leipzig, 1887, 74. |
| Rodentia: | | | | | | | |
| Squirrel | 54.09 | 7.39 | 16.09 | 0.59 | 0.40 | 21.44 | Hoppe-Seyler, Med. chem. Untersuch., 1868, Heft 3, 366. |
| Guinea-pig | 54.12 | 7.36 | 16.78 | 0.58 | 0.48 | 20.68 | Hoppe-Seyler, Med. chem. Untersuch., 1868, Heft 3, 366. |
| Aves: | | | | | | | |
| Chicken* | 52.47 | 7.19 | 16.45 | 0.859 | 0.335 | 22.50 | Jacquet, Zeit. f. physiol. Chemie, 1888, xiv, 289. |
| Goose† | 54.26 | 7.10 | 16.21 | 0.54 | 0.43 | 20.69 | Hoppe-Seyler, Med. chem. Untersuch., 1868, Heft 3, 366. |
| Reptilia: | | | | | | | |
| Sea-tortoise | 54.77 | 6.99 | 17.07 | 0.38 | 0.41 | ...... | Bardachzi, Zeit. f. physiol. Chemie, 1906, xlix, 465. |
| Invertebrata: | | | | | | | |
| Earthworm I | 53.91 | 7.02 | ..... | 0.41 | ..... | ..... | Griffiths, Physiology of the Invertebrata, 1892, 147. |
| Earthworm II | 53.86 | 7.10 | ..... | 0.37 | ..... | ..... | |
| Earthworm III | ..... | .... | ..... | ..... | 0.39 | ..... | |

* $P_2O_5$=0.1973 † $P_2O_5$=0.770

individuals of a given species is not of uniform composition, that this possible source of difference need scarcely be considered. That important errors in analysis have occurred seems evident, as, for instance, in the very low C, Fe, and S percentages found by Zinoffsky in his analyses of the hemoglobin of the horse, and in the differences in the percentages and ratios of Fe and S shown by the record of different analyses of the hemoglobin from individuals of the same species (table 26). Another source of error is to be found in the different methods for determining the N content, but doubtless the most important source is in abnormalities of the substance itself which have been due to the methods of preparation. The attempts to obtain pure hemoglobin by repeated crystallization have, instead of yielding a pure product, given rise to artifacts, each recrystallization adding another step in the denaturalization and disintegration of the molecule.

TABLE 26.—*The ratios of Fe to S according to the analyses of various observers.*

| Kind. | Ratios of Fe to S. | Authority. |
|---|---|---|
| Carnivora: | | |
| Cat | 1 : 1.771 | Abderhalden. |
| Dog | 1 : 1.628 | Jacquet. |
| Dog | 1 : 1.660 | Do. |
| Dog | 1 : 0.907 | Hoppe-Seyler. |
| Dog | 1 : 1.500 | Hüfner. |
| Dog | 1 : 1.558 | Schmidt. |
| Ungulata: | | |
| Bullock | 1 : 0.853 | Hüfner. |
| Horse | 1 : 1.383 | Kossel. |
| Horse | 1 : 1.489 | Otto. |
| Horse | 1 : 1.383 | Hüfner; Bücheler |
| Horse | 1 : 1.161 | Zinoffsky. |
| Horse | 1 : 1.105 | Abderhalden. |
| Ungulata—cont'd: | | |
| Horse | 1 : 1.145 | Jutt. |
| Pig | 1 : 1.549 | Otto. |
| Pig | 1 : 1.150 | Hüfner. |
| Rodentia: | | |
| Squirrel | 1 : 1.475 | Hoppe-Seyler. |
| Guinea-pig | 1 : 1.208 | Do. |
| Aves: | | |
| Goose | 1 : 1.488 | Do. |
| Chicken | 1 : 2.564 | Jacquet. |
| Reptilia: | | |
| Sea-tortoise | 1 : 0.927 | Bardachzi. |
| Invertebrata: | | |
| Earthworm | 1 : 1 | Griffith. |

The great instability of the hemoglobin molecule has been shown in various ways, and the tenacity with which this and other proteins mechanically or chemically cling to or combine with certain substances has likewise been proved. Hoppe-Seyler (Archiv f. path. Anat. u. Physiol., 1864, XXIX, 223) in his earliest researches on hemoglobin found that neither concentrated solutions nor crystals of hemoglobin remain unchanged for even 24 hours at ordinary temperature; that hemoglobin undergoes partial decomposition when dried by the aid of an air-pump and sulphuric acid; and that with each recrystallization there is formed an insoluble residue in the form of a derivative. Halliburton (Chemical Physiology and Pathology, 1891, 287) states that, even when hemoglobin is dried in a Torricellian vacuum at 40°, not only is hematin and an insoluble protein formed but some of the water of crystallization is driven off. He also found that repeated crystallization of the hemoglobin of the squirrel ultimately changes the form of the crystals from hexagonal plates to rhombic prisms, or a mixture of these with rhombic tetrahedra. Moreover, the crystals of hemoglobin that have been analyzed have been prepared by the "alcohol method," and presumably purified by repeated recrystallization, a method which of itself makes it practically absolutely impossible to obtain a normal hemoglobin. Alcohol denaturalizes hemoglobin, as it does other proteins.

Dr. S. Weir Mitchell (Proc. Acad. Nat. Sciences, Philadelphia, 1858, x, Biolog. Dept. 2) found that the color of hemoglobin crystals could be washed out with alcohol and water without injury to their form, and that the crystals may even be redissolved in water and again obtained devoid of color but without change in crystalline type. Preyer (Archiv f. ges. Physiologie, 1868, I, 395) records that hemoglobin crystals are rendered less soluble after standing in dilute alcohol, and that they are converted into pseudomorphs when dried or when in alcohol; Nencki (Archiv f. exp. Path. u. Phar., 1885, xx, 332) states that hemoglobin crystals through the influence of alcohol are converted into an insoluble "parahemoglobin," which has the same elementary composition as hemoglobin; Struve (Berichte d. d. chem. Gesel., 1881, XIV, 930) completely deprived hemoglobin crystals of their color by treating and rendering them insoluble with alcohol and water, and without changing their form, and he also found (Jour. f. prakt. Chemie, N. F., 1884, XXIX, 304) that fresh blood crystals in strong alcohol became completely insoluble in dilute alcohol.

Loewy (Zentralb. f. Physiologie, 1899, XIII, 449) and Hüfner (Archiv f. Anat. u. Phys., 1901, Supplement, 187) both have determined that alcohol so alters the hemoglobin molecule as to render the readily dissociable O less readily removed, and therefore render it like methemoglobin. In fact, Hüfner has in recent years insisted upon the importance of avoiding the use of alcohol in the preparation of hemoglobin crystals. Kupffer (Inaug. Dissert., Dorpat, 1884), in experiments with the hemoglobin from the dog, and Krüger (Zeit. f. Biologie, 1887, XXIV, 47), with crystals from the blood of the horse, have found that with each crystallization the absorption coefficient in relation to the spectrum is altered, the absorptive ratio becoming higher and higher, which is the opposite to that which should be expected if recrystallization means merely purification.

The foregoing facts, together with others which will be found in subsequent pages, show clearly not only that alcohol is injurious but also that each step in recrystallization means probably the stripping off of extremely unstable or feebly combined radicals which are normal constituents of the molecule and which contribute in giving the molecule its distinctive properties.

The tenacity with which protein molecules hold impurities has been convincingly shown by Schulz and Zigmondy (Beiträge z. chem. Phys. u. Path., 1902, III, 137), who experienced much difficulty in obtaining egg-albumin free from colloidal substances, and that recrystallization from 5 to 7 times was often necessary to obtain a pure substance. While such recrystallization does not affect this protein, according to these observers, it without doubt, as stated, markedly affects the hemoglobin. Abderhalden (Zeit. f. phys. Chemie, 1903, XXVII, 484) states that the hemoglobin of the horse once crystallized may yield as much as 0.62 per cent of glycocoll, the presence of which he attributes to contamination with serum globulin. Since serum globulin yields a little over 3 per cent of glycocoll, there would therefore be about 15 per cent of serum globulin present. He did not find glycocoll after the second crystallization. Even after purification, protein crystals may mechanically take up foreign substances from solution, as has been shown by Wichmann (Zeit. f. physiolog. Chemie, 1899, XXVII, 575), who compares protein crystals to a sponge. Moreover, in the preparation of crystals of albumin, globulin, phycoerythrin, phycocyanin, hemocyanin, and hemoglobin by the "salting-out" process, in the case of all excepting possibly hemoglobin the crystals are not free substances, but some form of combination.

Phosphorus according to some observers is a contamination, but according to others it may be or is a normal constituent. While it can be removed from the hemoglobin of mammalian bloods by repeated crystallization, this was not found possible by Hoppe-Seyler and Jacquet in the case of the hemoglobins of bloods that contain nucleated erythrocytes, yet Bardachzi (Zeit. f. physiolog. Chemie, 1906, XLIX, 465) has obtained from the sea-tortoise crystals of hemoglobin that were free from phosphorus. The fact, however, that phosphorus has been removed from mammalian hemoglobins is not proof of its being a contamination, because it may have been stripped from the molecule. Inoko (Zeit. f. phys. Chemie, 1894, XVIII, 57) regards the phosphorus as a normal constituent existing in the form of nucleic acid which is in combination with hemoglobin. Jacquet (Zeit. f. phys. Chemie, 1888, XII, 285) also regards it a normal constituent, but Gscheidlen (Archiv f. ges. Physiologie, 1878, XVII, 421) and Gamgee (Schäfer's Text-book of Physiology, 1898, 1, 206) look upon it, doubtless correctly, as a contamination.

[Since the foregoing was put in type Abderhalden and Medigreceanu (Zeit. f. physiolog. Chemie, 1909, LIX, 165) have shown, by their analyses of the hemoglobin of the goose, that phosphorus is an impurity.]

### THE MOLECULAR FORMULA AND WEIGHT OF HEMOGLOBIN.

The molecular formulas and molecular weights of proteins, especially of the coagulable proteins, are admittedly high, and are particularly high in the chromoproteins. Owing, however, to the difficulty of obtaining these substances of uniform purity, the estimates must be regarded as being purely tentative. Vaubel (Jour. f. prakt. Chemie, 1899, LX, 55) gives the following figures which he compiled from the records of different investigators, which records were obtained by various methods of determination: Egg albumin 4618 to 6542; serum albumin 4572 to 5135; myoalbumin 4572 to 5135; casein 6500 to 6542; plant albumin 5050 to 6690; plant globulins 5257 to 8848; globin 15000 to 16086; and hemoglobin 15000 to 16730.

Inasmuch as we have not a rational formula for hemoglobin, the empirical formulas and weights must be regarded *sub judice*. The calculation of the molecular formula of the hemoglobin of the horse by Schulz was based upon the sulphur content (table 27). Preyer (Die Blutkrystalle, 1871, 65), Hüfner (Jour. f. prakt. Chemie, 1880, XXII, 362; Zeit. f. physiol. Chemie, 1884, VIII, 361), Zinoffsky (Zeit. f. physiol. Chemie, 1885, X, 16), and Jacquet (Zeit. f. physiol. Chemie, 1889, XIV, 289) made determinations based upon the percentages of Fe; and Hüfner verified Jacquet's figures for the hemoglobin of the dog by determinations of the combining properties of hemoglobin with O and CO. Hüfner and Ganser (Archiv f. Anat. u.

Physiologie, Phys. Abth., 1907, 209) have determined the molecular weights by means of osmotic pressures. Jutt's (Inaug. Dissert., Dorpat, 1894; Maly's Jahr. ü. d. Fort. d. Thierchemie, 1895, 128) estimates were founded upon the combinations of hemoglobin with heavy metals. Külz (Zeit. f. physiolog. Chemie, 1883, VII, 384) based his calculation upon the combining power of hemoglobin with CO. Hüfner and Ganser made use of the hemoglobin of the horse and bullock, freshly produced, free from alcohol, and crystallized three times without alcohol. The mean value of the hemoglobin of the bullock they found to be 16321, and of that of the horse 15115. They state it is doubtful whether the molecular weights of hemoglobins of the bullock and horse are the same or different; that the results of these latest experiments agree with the mean values formerly obtained; and that the molecule of oxyhemoglobin is composed of O and of reduced hemoglobin.

TABLE 27.—*The molecular formulas and weights of hemoglobin, according to various observers.*

| Kind. | Molecular formula. | Molecular weight. | Authority. |
|---|---|---|---|
| Dog.............. | $C_{600}H_{960}N_{154}S_3FeO_{179}$ | 13,332 | Preyer. |
| Dog.............. | $C_{758}H_{1203}N_{195}S_3FeO_{218}$ | 16,669 | Jacquet. |
| Dog.............. | $C_{636}H_{1025}N_{164}S_3FeO_{189}$ | 14,129 | Hüfner. |
| Bullock.......... | $C_{759}H_{1208}N_{210}S_2FeO_{204}$ | 16,640 | Gamgee. |
| Bullock.......... | ...................... | 16,321 | Hüfner and Ganser. |
| Horse............ | $C_{550}H_{852}N_{149}S_2FeO_{149}$ | 12,042 | Hüfner and Bücheler. |
| Horse............ | $C_{712}H_{1136}N_{214}S_2FeO_{245}$ | 16,730 | Zinoffsky. |
| Horse............ | $C_{648}H_{1040}N_{178}S_2FeO_{177}$ | 15,260 | Jutt. |
| Horse............ | ...................... | 15,115 | Hüfner and Ganser. |
| Pig.............. | $C_{609}H_{1005}N_{156}S_3FeO_{179}$ | 13,513 | Külz. |

While the formulas given vary materially, the most striking difference will be noted to be in the ratios of the percentages of Fe to S (table 26). Otto (Zeit. f. physiolog. Chemie, 1882, VII, 65) found that the hemoglobins of the dog and pig are practically identical as regards elementary composition and their coefficient of extinction, and that a close if not complete identity exists in the combining power with O. These hemoglobins, he calculates, each contain 1 atom of Fe to 3 of S. Külz estimated the same ratio for the hemoglobin of the pig, and Preyer the same ratio in the hemoglobin of the dog. But in the case of the horse and bullock the ratio is 1 of Fe to 2 of S.

## THE SOLUBILITY OF HEMOGLOBIN.

The most marked differences noted in the hemoglobins of different species have been in the degree of solubility and in the quantity of water of crystallization. While the determinations of solubilities are extremely limited and far from satisfactory, because of obvious impurities of the substances experimented with and the failure at times to record temperatures, they nevertheless show clearly very wide differences. Crystals of bullock's and pig's blood, for instance, are soluble in their water of crystallization at ordinary room temperature, while the crystals of raven's blood are practically insoluble in cold water, and between these extremes there are all gradations.

Hoppe-Seyler (Archiv f. pathol. Anat. u. Physiolog., 1864, XXIX, 233) states that the dry hemoglobin crystals of the dog are soluble in the proportion of 2 per cent at 5°. Schmidt and Böttcher (Preyer, Blutkrystalle, *loc. cit.*) with an impure preparation found that water-free hemoglobin of the dog was soluble in 12.2 parts per 100 at 18°, and the dry crystals in the proportion of 15.59 parts per 100 at 18°. Lehmann (quoted by Preyer) records that the impure dry hemoglobin of the dog is soluble in the proportion of 0.4 to 3.1 per cent, and the solubility of guinea-pig crystals 1 part in 597, or 0.167 per cent. With the crystals of the horse Otto (Archiv f. ges. Physiologie, 1883, XXXI, 240) was unable to obtain concordant results, but Hüfner and Bücheler (Zeit. f. phys. Chemie, 1884, VIII, 358) found a solubility of 2.614 per cent at 1° and 14.375 per cent at 20°. Standing in dilute alcohol renders the crystals less soluble (Preyer and others).

### THE QUANTITY OF WATER OF CRYSTALLIZATION.

The percentages of water of crystallization given by different investigators, and even those noted by a given investigator with hemoglobin of a given species, show marked discrepancies. These differences are owing chiefly to the use of impure substances, to differences in methods of drying, and to certain difficulties incidental to accurate determinations. Schmidt and Böttcher (*loc. cit.*) found 13.49 per cent of water of crystallization in the crystals of dog's blood that had been dried by standing many days over sulphuric acid. Lehmann (*loc. cit.*) found in air-dried crystals of guinea-pig in two instances 19.9 per cent, and in others 15 per cent and 16 per cent. Preyer (*loc. cit.*) notes that after the crystals of the dog's hemoglobin were dried in the usual way, and then powdered and subjected to a temperature of 100° C., they lost 4.17 per cent. This powder upon standing in a glass case (not air-tight) for 3 days increased in weight 10.93 per cent, and when dried again at 100° C. decreased in weight 10.71 per cent. Lehmann recorded figures for dry guinea-pig crystals which agree with these. At 15° they absorbed on an average 11.19 per cent of water in five experiments, according to which the air-dried guinea-pig hemoglobin would still contain 10.06 per cent of hygroscopic water, while the air-dry dog hemoglobin contains, according to Preyer's experiments, 9.67 per cent. Later Lehmann found that the air-dry dog crystals (which, however, were not pure) lost in weight in vacuo 9.79 per cent, and that the crystals dried in vacuum at 15° absorbed in 14 days 9.54 per cent of water, so that the air-dried substance would contain 8.71 per cent of water. They lost 9.09 per cent in weight at 120° C. Preyer states that his and Lehmann's figures agree very well when one considers that Lehmann worked with impure material and that he (Preyer) used a pure recrystallized substance.

Hoppe-Seyler (Med. chem. Untersuch., 1868, Heft 3, 366; Chemischen Analyse, 1883, 292) noted the following percentages of water in crystals dried at 100° C. with the aid of an air-pump: Dog 3 to 4, goose 7, guinea-pig 6, and squirrel 9.4 per cent. In another publication (Physiologische Chemie, 1877, 377) his figures for guinea-pig and goose hemoglobins are 7 and 9.4 per cent, respectively. Otto (Zeit. f. physiolog. Chemie, 1882, VII, 57) gives the percentage for pig's hemoglobin crystals dried over sulphuric acid, then at 115°, as 5.9, and for the dog 4. He also reports (Archiv f. ges. Physiologie, 1883, XXXI, 240) that he did not obtain concordant results with the crystals of horse's blood. Hüfner and Bücheler (Zeit. f. physiolog. Chemie, 1884, VIII, 358) dried horse crystals at 0° over sulphuric acid and anhydrous phosphoric acid, and then found them to contain 3.94 per cent of water. Jacquet (Zeit. f. phys. Chemie, 1889, XIV, 289) records that air-dried crystals of the dog lost 11.39 per cent of water at 115°, and those of the chicken 9.333 per cent. Hüfner (Archiv f. ges. Physiologie, 1894, 130) gives the water of crystallization of bullock's blood as being 9.98 per cent. Bohr (Exper. Untersuch. u. d. Sauerstoffaufnahme d. Blutfarbstoffes, Copenhagen, 1885) found that the percentage in bullock's blood varies from 1.2 to 6.3 per cent, which variations may be due, in part at least, to impurities of his preparations.

### THE EXTINCTION COEFFICIENTS AND QUOTIENTS.

Vierordt, Hüfner, and others have found that reliable extinction coefficients can not be obtained by measurements of a single spectral field unless the solution contains but a single coloring matter; because, while solutions of a single coloring matter affect the light intensities of the different regions of the spectrum in constant relationship to each other, irrespective of the strength of the solution, the presence of a second coloring matter alters or destroys this relationship. Therefore, two fields must be measured, and the fields to be selected should be those which are most readily influenced by the differences in the strength of the solution. Moreover, the two coefficients thus obtained serve as mutual checks. The quotient obtained from these coefficients, as shown by Hüfner (Archiv f. Anat. u. Physiologie, Physiolog. Abth., 1894, 130, and 1900, 39) in his studies of oxyhemoglobin, reduced hemoglobin, methemoglobin, and CO-hemoglobin, is absolutely constant and distinctive for each substance, and, therefore, departures in extinction coefficients show, according to him, not only the presence of impurities but also the quantity of each coloring matter present.

Hüfner measured the extinction coefficients of these substances for the mid-region between A and B absorption bands (the interval between the wave lengths 554 and 565 $\mu\mu$), and for the darkest portion of band B (the interval between 531.5 and 542.5 $\mu\mu$). In such determinations with fresh bullock's blood and solutions of the crystals of bullock's oxyhemoglobin he found constant results. The quotient for oxyhemoglobin was 1.578, for reduced hemoglobin 0.7617, for CO-hemoglobin 1.095, and for fresh rabbit's blood 1.579. Zeynek (Archiv f. Anat. u. Physiologie, Phys. Abth., 1899, 460) by the same means determined for methemoglobins of the horse 1.187, and of the pig 1.183. Hüfner in the later article states that the oxyhemoglobin quotient (1.578) and the reduced hemoglobin quotient (0.762) have each the same value independent of the degree of concentration of the solution and the species of blood.

Von Noorden (Zeit. f. phys. Chemie, 1880, IV, 9) found the mean quotient for pure oxyhemoglobin of the dog to be 1.324, for the guinea-pig

1.357, and for the rat 1.337. He also found the like value in the case of man, the cat, and the owl.

Otto (Zeit. f. physiolog. Chemie, 1882, VII, 57) noted with solutions of the oxyhemoglobin of the dog and pig a quotient of 1.33; and in a later research, with an improved form of spectrophotometer, obtained a quotient of 1.352 for horse oxyhemoglobin and 1.34 for that of the dog. Sczelkow (Archiv f. ges. Physiologie, 1887, XLI, 373) calculated, by means of Hüfner's spectrophotometer, a quotient of 1.336 for horse hemoglobin. For dog's hemoglobin he recorded 1.305, which he looks upon as not being correct, and he remarks that coefficients obtained by him differed from each other and from the mean value much more than did Otto's, which differences he explains upon the assumption that the concentration of the solutions used by him differed more than those employed by Otto.

Hüfner, in the more recent investigation referred to, found these quotients so constant and specific that he formulated tables by aid of which the quantity of oxyhemoglobin, reduced hemoglobin, methemoglobin, or CO-hemoglobin, or the quantities in mixtures of oxyhemoglobin and reduced hemoglobin, or of methemoglobin and CO-hemoglobin may be determined. Moreover, he states that these quotients, as well as the O, CO, and the iron capacities of the coloring matter of the blood, are not only the same in related but also in unrelated species, and that when this coloring matter is freed from water it has in all of the higher animals the same molecular weight and the same capacity for O and CO.

In opposition to Hüfner's assertion of the constancy of the extinction coefficients and quotients of the same and of different bloods, we find evidence in the results of a number of investigations. The quotients given by von Noorden, Otto, and Sczelkow already noted are far off. Korniloff (Zeit. f. Biologie, 1876, XII, 513) determined the extinction coefficients in relation to the second absorption band (B) of the coloring matter of the blood of 110 vertebrates, comprising 44 species. He made determinations by other regions of the spectrum, but these values deviated considerably from those obtained from the second band. Inasmuch as he did not make his determinations by the effects on two bands, which is necessary to obtain accurate results, and as the bloods doubtless contained variable proportions of oxyhemoglobin and reduced hemoglobin, and in some instances probably methemoglobin if not also other coloring matters, his figures must be looked upon as representing only approximate values. Accepting them as approximations, they differ so much as to indicate that the coefficients in at least different orders of animals are very far from being identical, as will be seen by the figures in table 28.

Krüger (Zeit. f. phys. Chemie, 1898, XXV, 256) found the quotient of cat's oxyhemoglobin to be 0.128, and for that of the dog 0.137. Velichi (Inaug. Dissert. Berlin, 1900; Centralbl. f. Physiologie, 1900, XIV, 679; Deutsch. med. Wochenschr., 1900, XXVI, 148) found such differences in the extinction coefficients that he states that the hemoglobins of all classes of animals are not identical. Dreser (*loc. cit.*) gives 1.557 as the quotient for human oxyhemoglobin, and Saint Martin (Compt. rend. soc. biologie, 1901, LIII, 302) obtained the following quotients: Human 1.60, bullock 1.62, dog

1.61 and 1.63. Müller (Archiv f. ges. Physiologie, 1904, CIII, 541) in studies of freshly drawn blood, after three years' experience with the spectrophotometer, has cast doubt upon certain of Hüfner's teachings regarding extinction coefficients, etc. He found that the relations of the extinction coefficients of the blood taken directly from the animal are not as constant as Hüfner states, and in opposition to Hüfner he holds that values which differ from 1.56 are not necessarily wrong. He goes on to state that Hüfner looks upon all values which differ materially from 1.56 as wrong, which Hüfner explains by the formation of methemoglobin. This explanation seems to Müller to be untenable, since he found in examinations of freshly drawn blood from the ears of a dog a value of 1.47 to 1.49, which figure Hüfner would designate as incorrect, and yet it was fresh blood taken from the healthy animal, so that we must either accept the presence of methemoglobin in the apparently normal animal, or else material individual differences in the optical constants of oxyhemoglobin. The second probability seems to Müller more likely, and he goes on to state that it should not be left unmentioned that Torup observed by means of the Glan photometer, after the addition of a little sodium bicarbonate to the diluted hemoglobin solutions, a shifting of the point of strongest absorption; and also, as Bohr states, an insignificant change of the hemoglobin, which has no influence whatever either upon the molecular weight or the amount of absorbed oxygen, may give an entirely different value in light absorption. Changing alkalinity of the blood seems therefore to have a disturbing influence, as has been found by others.

TABLE 28.—*Extinction coefficients in different orders of animals.*

| Orders. | No. of animals. | Extinction coefficients. |
|---|---|---|
| Fishes.............. | 16 | 0.3564 |
| Amphibia........... | 13 | 0.3889 |
| Reptiles............ | 13 | 0.4328 |
| Birds............... | 17 | 0.7814 |
| Mammals........... | 22 | 0.9366 |

In a more recent inquiry with *fresh blood,* Aron, Hans, and Müller (Archiv f. Anat. u. Physiologie, 1906, Suppl. Bd., 109) throw even more serious doubts upon Hüfner's assertions as to the constancy of the extinction coefficients. The average quotient they found to be about the same in different species (dog, horse, cat, ox, and rabbit), but 55 out of 142 calculations differ much more from the average value than can be explained by the greatest possible errors that can be accounted for in errors of method or by variations in the strength of solution. Moreover, the average value was found to be 1.47, whereas Hüfner's is 1.578. Light absorption they found to be in direct relation to the quantity of iron, and approximately the same for the blood of the rabbit, ox, and dog, but varying somewhat for the blood of the horse. They suggest that methemoglobin normally exists in the blood, which may account for differences in Hüfner's and Bohr's results in their studies of oxyhemoglobins. They also point out that by the regular method of defibrinating the blood there occurs a loss of hemoglobin which does not occur if the defibrinization be effected by agitation in a closed vessel.

Bardachzi (Zeit. f. phys. Chemie, 1906, XLIX, 465) determined the mean quotient for the fresh blood of the sea-tortoise (*Thalassochelys corticata*) to be 1.561, and for the oxyhemoglobin crystals in solution 1.569. The mean quotient for methemoglobin he records as 1.184.

The injury to the hemoglobin molecule caused by the methods of preparation has been referred to a number of times in preceding pages. Interesting in this connection are the results of the investigations of Kupffer (Inaug. Dissert., Dorpat, 1884) and Krüger (Zeit. f. Biologie, 1887, XXIV, 47), both of whom have found that recrystallization notably and injuriously affects the extinction coefficients. Kupffer determined that the oxyhemoglobin of the horse and the dog showed a higher extinction coefficient when crystallized three times than when crystallized twice; while Krüger, using the Hüfner spectrophotometer, found that even a single crystallization gives rise to a higher absorption ratio. Inasmuch as this is the opposite effect to that which should be expected if recrystallization means merely purification, it is clear that the molecule has been altered. He also calls attention to the fact that the addition of ammonia increases the solubility of the crystals about twice. The accompanying table (table 29) from Krüger is of interest:

TABLE 29.—*The effects of recrystallization and alkali upon the extinction coefficient of hemoglobin, according to Krüger.*

| Oxyhemoglobin crystals— | Number of times crystallized. | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| From defibrinated horse blood with $NH_3$ | ...... | 0.1297 | 0.1372 | ...... |
| Do. | 0.1266 | .1321 | .1372 | ...... |
| From uncoagulated horse blood with $NH_3$ | ...... | .1259 | .1317 | ...... |
| From dog blood without $NH_3$ | .1337 | .1417 | .1435 | 0.1498 |
| From dog blood with $NH_3$ | .1401 | .1429 | .1453 | .1452 |

Hoppe-Seyler in his earlier investigations (Med. chem. Untersuchungen, 1868, Heft 3, 366) noted that the intensity with which hemoglobin absorbs the light of definite portions of the spectrum is not alike for different species. Thus, to have solutions of like absorptive intensity, the following quantities were necessary in a liter of solution: 1.641 grams of goose hemoglobin, 1.682 grams of dog hemoglobin, 1.703 grams of guinea-pig hemoglobin. This has received confirmation in the researches of Abderhalden (Zeit. f. physiolog. Chemie, 1898, XXIV, 545), who found that in order to obtain the same colorimetric value 10 c.c. of a standard solution of dog hemoglobin must be diluted with 8 c.c. of water to have the same intensity as the same standard solution of cat hemoglobin.

## THE DIFFERENCES IN THE DECOMPOSABILITY OF THE HEMOGLOBIN OF DIFFERENT SPECIES.

While hemoglobin is, in comparison with proteins generally, extraordinarily resistant to the putrefactive organisms, excepting practically in so far as concerns the conversion of oxyhemoglobin into methemoglobin and reduced hemoglobin, it readily undergoes decomposition, especially so in the presence of any reagent which causes a separation of the globin and hematin, or which converts the hemoglobin into methemoglobin or reduced hemoglobin. The degree of decomposability varies in relation to different species, and in individuals of the same species under abnormal conditions. Körber (Ueber Differenzen des Blutstoffes, Inaug. Dissert., Dorpat, 1886; Centralblatt f. med. Wissensch., 1867, v, 117) found by the aid of the spectroscope in a study of the bloods of 11 species of warm-blooded animals (man, horse, bullock, sheep, pig, dog, cat, hare, goose, chicken, and crow) and 3 species of cold-blooded animals (frog, pike, and lote) interesting differences in the behavior towards certain reagents. Normal human hemoglobin was found to be more readily decomposed by acetic acid than by soda, but the opposite was noted under pathological conditions. In febrile states in the human being and in inanition in the dog decomposability was increased. The hemoglobin of typhus blood decomposed 18 times sooner than normal blood. Under given conditions, the hemoglobin of the chicken was decomposed 150 times more quickly than that of man; pig's was decomposed 780 times more slowly than that of the bullock and 350 times more slowly than that of the dog, etc. Rabbit's hemoglobin was decomposed 150 times more quickly through acetic acid than by soda, but the hemoglobin of the pike was affected much more readily by soda than by acetic acid. With a proportion of 0.5 gram of sodium hydrate to 20 c.c. of a 1 per cent solution of blood, he found that the decomposition of hemoglobin began at time intervals shown in table 30.

TABLE 30.—*Time intervals of beginning of decomposition of hemoglobin.*

| Kind. | Time interval. | Kind. | Time interval. |
|---|---|---|---|
| Typhus | 5 secs. | Sheep | 36 mins. |
| Dog | 12 secs. | Horse | 51 mins. |
| Lote | 25 secs. | Hare | 60 mins. |
| Pike | 45 secs. | Chicken | 65 mins. |
| Cat | 1 min. | Pig | 3 hours. |
| Man | 1.5 mins. | Bullock | Over 3 but less than 16 hours |
| Goose | 4.5 mins. | | |

Krüger (Zeit. f. physiolog. Chemie, 1888, XXIV, 318), in similar spectral examinations with the bloods of the dog and horse, found support to Körber's results. He states that the resistances of the hemoglobins of the dog and horse against acetic acid and sodium hydrate differ, that they vary considerably, and that the variability is due to the chemical condition of the hemoglobin itself; that the difference in the decomposability increases with the quantity of decomposing agent; and that sodium hydrate is more effective than the acid. The results of the foregoing investigations have received support in the comparatively recent investigations of Magnanimi (Bull. d. soc. Lancisiana d. osped. di Roma, 1898; Jahr. ü. d. Fort. d. Thierchemie, 1898, XXVIII, 144) and Ziemke (Vierteljahresschr. Med., 1901, XXII, 77). Magnanimi by the aid of a Krüss spectrophotometer examined the bloods of 4 men and 1 woman, and also the bloods of the dog, horse, calf, pig, wether, and lamb. He found upon the addition of sodium hydrate that the bands of human blood vanish in 38 minutes, of the dog after 110 minutes, and in other animals only after 3 hours. In the case of blood stains 6 to 60 days old, the older the stains the less resistance of the hemoglobin, but the relationship between the different bloods remains—that is, the bands of human blood disappear sooner than those of dog's blood, and those of dog's blood sooner than those of the others. Ziemke states that in order to show differences in resistance to alkali the use of the spectrophotometer is not necessary, but that by colorimetric measurements dried blood and blood stains of human blood can be distinguished from those of domesticated animals, even though methemoglobin has been formed.

### IS THE OXYHEMOGLOBIN OF THE BLOOD OF ANY INDIVIDUAL A SINGLE SUBSTANCE?

That there is always in the normal blood a mixture of oxyhemoglobin and reduced hemoglobin in varying proportions has long been established, but to what extent and constancy, if any, such substances as methemoglobin, $CO_2$-hemoglobin, and CO-hemoglobin may be present has not been determined; nor has it been shown that either oxyhemoglobin or reduced hemoglobin is a single homogeneous substance in any species of blood. Hoppe-Seyler (Zeit. f. physiol. Chemie, 1878, II, 139) found that the oxyhemoglobin of the horse appears in the form of minute needles and prisms which differ in solubility, which difference he believes may be due to different amounts of water of crystallization. Otto (Archiv f. ges. Physiologie, 1883, XXXI, 240), in repeating Hoppe-Seyler's experiments, also noted these two forms, but he found that one form could not be separated from the other by washing with dilute alcohol, as stated by Hoppe-Seyler.

Bohr (Compt. rend. soc. biologie, 1891, CXI, 243), in his studies of the homogeneity of hemoglobin, found that the portion of hemoglobin in the mother-liquor after crystallization showed a lower combining power with oxygen than that which crystallized out; and in a previous article (Centralblatt f. Physiologie, 1890, IV, 242) he reported from the results of his experiments that the oxyhemoglobin of the blood is not a simple substance, but a mixture of oxyhemoglobins, and that there may exist in the blood four forms, all giving the same oxyhemoglobin spectrum, but differing in their absorption coefficients, in their percentages of iron (0.35 to 0.46), and in their combining with O (0.4, 0.8, 1, and 2.7 c.c. O per gram). He believes that there are also several forms of $CO_2$-hemoglobin.

Hüfner (Archiv f. Anat. u. Physiologie, 1894, 130) seems, however, to have demonstrated that Bohr's several forms of oxyhemoglobin were mixtures of oxyhemoglobin with varying amounts of methemoglobin and other decomposition products which resulted from his methods of preparation. Hüfner's suggestions are borne out by the investigations of Marchand (Archiv f. path. Anat. u. Physiol., 1879, LXXVII, 488), and even by the early work of Hoppe-Seyler and by the researches of others. In the blood of cattle, Hüfner states, there is but one oxyhemoglobin, and likewise in all the higher vertebrates. By the crystallographic method, however, we have found several kinds of oxyhemoglobin in certain bloods.

# CHAPTER IV.

## THE PREPARATION AND STUDY OF HEMOGLOBIN CRYSTALS PREVIOUS TO THE INVESTIGATIONS OF PREYER.

Crystals of hemoglobin were first discovered by Hünefeld (Die Chemismus in der thierischen Organization, Leipzig, 1840, 160), who found, upon exposing the blood of the earthworm between plates of glass, that there were deposited bright red table-form crystals having sharp borders. He also refers to crystals from the blood of man and of the pig. While Hünefeld's article is the first on record, the real foundation of our knowledge of hemoglobin was laid by the discovery of K. E. Reichert (Müller's Archiv f. Anat. u. Physiologie u. wissensch. Medicin, 1849, 198), during the summer of 1847, of tetrahedral crystals of hemoglobin in the fetal membranes and in the mucous membrane of the uterus of a guinea-pig which had suddenly died, and which was examined 6 hours after death. The uterus contained 4 fetuses, and in all four placentas the crystals were found. The crystals were regular tetrahedra of various sizes. The inclination of the planes towards each other amounted to 70° 31′ 43″, and that of the planes towards the edge, 54° 44′ 8.5″. He noted truncation in rare cases, which he thought might be due to outside mechanical force. Reichert undoubtedly recognized from the studies of the chemical reactions that these crystals were albuminous. This contribution appears to have at once aroused interest in the study of the blood crystals, as is indicated by the appearance of a number of contributions during the next few years.

Leydig (Zeit. f. wissensch. Zoologie, 1849, I, 116) found crystals of the blood of *Nephelis* in the stomach of *Clepsine*. The corpuscles, he states, became decolorized and disappeared, and in the plasma were found red tabular leaflets and rods and columns, small and large, single and aggregate. He also noted that if water entered the stomach the crystals dissolved.

Kölliker (Zeit. f. wissensch. Zoologie, 1849, I, 266), in the records of his histological studies of the blood corpuscles, describes red crystals in the blood of the dog, river perch, and python, and states that the crystals were within the corpuscles and also in the plasma of the blood of the spleen and liver.

Crystals of human blood were observed by Budge (Sitz. d. Niederrh. Gesellsch. f. Natur- u. Heilkunde, 12 Dec., 1850, and Köln. Zeitung, No. 300, 1850; quoted by Preyer, *loc. cit.*) in the stomach of leech.

Shortly after this appeared the first article by Funke (Zeit. f. rat. Medicin, 1851, N. F., 1, 185), which was almost immediately followed by his second and third contributions (*ibid.*, 1852, N. F., 2, 198, 288). To Funke is due the credit of being the first to devise methods for *preparing* blood crystals, which crystals had heretofore been obtained solely by accident. His chief method, still in use and known as "Funke's method," is most simple and very satisfactory for bloods that are readily crystallizable. Funke prepared crystals from the blood of man, the horse, bullock, dog, fish, cat, pig, and pigeon. He states that if some water is added to a drop of blood (which in consequence of free evaporation has already begun to dry) spread out on an object-glass, and the edges of the preparations are observed, it can be seen that the corpuscles suddenly change. While some of the blood corpuscles vanish the others acquire dark, thick outlines, become angular, and develop into small, sharply defined rods. In this way are formed an enormous number of crystals which are too small to have their form accurately ascertained. These crystals quickly increase in length, while their diameter remains unchanged or increases only slightly, and finally the whole field is a thick network of needle-shaped crystals crossing in all directions. This process goes on so extraordinarily quickly that it is difficult to follow with the eye the first formation, as well as the steps of gradual development, on which account, he states, he could not convince himself that the crystals really arise from the corpuscles themselves. The whole phenomenon, he writes, can be most beautifully observed if the cover-glass is shifted after water has been added to the concentrated drop of blood, and then those places observed where before, on the edges of the cover-glass, thicker layers of blood were in the process of drying.

Occasionally crystals form in clots of splenic venous blood upon evaporation, but in this way there arise very incomplete crystalline formations, ordinarily a small row of pale-red leaflets or rods arranged palisade-like, without any recognizable crystal form.

When a drop of blood is mixed with ether it changes almost at once into an entangled heap of scale-shaped and leaf-shaped crystals, which are suspended in a homogeneous fluid. By the addition of alcohol Funke succeeded in producing crystals of so enormous a size that they could be recognized by the naked eye, although for the most part the crystals were badly formed. During the first minute, he states, the alcohol coagulated the blood to thick red clots. After evaporation, however, there appeared in isolated spots long, broad, sword-shaped leaves of intense red color, with irregular, often saw-shaped and indented, splintered ends. Only a few of the crystals were 4-sided prisms.

Funke made measurements of the angles of the crystals by the aid of a goniometer; but upon insufficient data he accredits the crystals to certain systems. He noted that the forms and solubilities of the crystals of different species are not alike, and therefore that species may thus be differentiated. (See Chapter VII.) He also noted that, in the bloods of the three species of fish examined, all of the corpuscles changed into crystalline form, and that upon the addition of water a great part of them were changed back to corpuscles under the eye of the observer.

The foregoing investigations, especially those of Funke, because of his being the first to *prepare* blood crystals, may justly be regarded as constituting the foundation for the rational study of hemoglobin. At that time (1851–2) the precise nature of the substance of the crystals was unknown.

Reichert looked upon the crystals as being albuminous; Kölliker refers to them as "globulin" crystals; and Funke states that the crystalline substance is the chief constituent of the blood corpuscles, which substance he regards as being a combination of globulin and hematin. Since then various terms have been suggested, such as hematoglobulin, hematocrystallin, cruorin, hemochrome, hemoglobin, etc., but the last, suggested by Hoppe-Seyler, is the universally accepted term at the present day.

Immediately following Funke, Kunde (Zeit. f. rat. Medicin, 1852, N. F., 2, 271) obtained, by a slight modification of Funke's process, crystals from the blood of the bullock, horse, dog, guinea-pig, squirrel, rat, mouse, bat, rabbit, pigeon, and tortoise. He also found crystals of human blood in the stomach of leech. He observed that the crystals from different species are not identical, from which he concludes that the form of the crystals is peculiar to each species. Remak (Archiv f. Anat. u. Physiologie, 1852, 115) found crystals in blood of the tench, perch, and roach 24 to 48 hours after death. Parkes (Medical Times and Gazette, 1852, XXVI, 103) accidentally found crystals in human blood that had putrefied. Lehmann (Berichte königl. sächs. Gesellsch. d. Wissensch. in Leipzig, math.-phys. Klasse, 1852, 23, 78; 1853, 101; Chem. pharmac. Centralblatt, 1853, 98) was the first to make an elementary analysis of the blood crystals. He diluted the blood with 1 to 1.5 volumes of water and prepared crystals from the bloods of the guinea-pig, squirrel, and hedgehog. He gives the following figures from his analyses:

$$C_{53 \cdot 4-54 \cdot 1}H_{7-7 \cdot 3}N_{15 \cdot 5-16 \cdot 2}S_{1 \cdot 2}$$

Teichmann (Zeit. f. rat. Medicin, 1853, N. F., 3, 375) obtained crystals from the blood of man and from that of dog, bullock, pig, rabbit, pigeon, and fish. He observed also decolorized crystals. He opposes the conclusion of Funke and Kunde that differences in the forms of the crystals are peculiar to species, for he found that even from the same blood various crystalline forms may be obtained, from which he concludes that the form of the crystal is accidental and due to exterior conditions. Berlin (Nederlandsch. Lancet, 1853, III, 16, and 1855–56, V, 734; Archiv f. d. Holländ. Beiträge z. Natur- u. Heilkunde, 1858, 1, 75) describes crystals of the lion and python, and he also found crystals of human blood in the leech. Robin and Verdeil (Traité de chim., anatom. et physiol., Paris, 1853, II, 335) doubted the albuminous nature of the blood crystals, which they thought were phosphates rendered impure by contaminating albuminous substances. Kölliker (Microscop. Anat., 1854, II, 2 Aufl., 280) again reported instances of intraglobular crystallization, and also of his having prepared crystals from the bloods of several species already reported. Bissegger and Bruch (Verhandlungen d. Baseler Naturforschenden Gesellschaft, 1857, I, 174) isolated crystals of the rat, and they also found crystals within some of the corpuscles. Meckel (Archiv f. d. Holländ. Beiträge, 1858, I, 90) obtained crystals from the blood of man and the pig.

Dr. S. Weir Mitchell (Proc. Acad. Natural Sciences, Philadelphia, 1858–59; Proc. Biolog. Dept., 2), who was the first American to report studies of hemoglobin crystals, examined the crystals of the sturgeon, guinea-pig, and man. He obtained crystals by Funke's method, and also by allowing the blood to stand in an open vessel exposed to light at a temperature of 60° to 70° F. to putrefy. He found that at any time after 48 hours a drop of this blood would yield by slight evaporation, without added water, the most beautiful crystals. He states that those of sturgeon's blood are hexagonal columns and tablets; that their color may be washed out with alcohol and water without injury to their form, and that the decolorized crystals may be dissolved in water and again obtained devoid of color, but unchanged in crystalline shape. Crystals in the form of hexagonal plates were frequently seen "within the envelope of the corpuscles." When a glass slide containing a group of crystals was kept for some months, the crystals were altered in their color so as to exhibit beautiful tints, such as yellow, orange, purple, and various shades of green, recalling "very strikingly the alterations of tint undergone by the leaf in the autumn." Dr. Mitchell found that the crystals are of the same form from whatever part of the body they are obtained; but he also makes a note of the fact that Dr. Johnson obtained tetrahedral forms from the splenic blood of the opossum but rhombic crystals from the blood of other vessels. He also observed that human blood of the male, the female, the fetus, and the placenta, and the blood in many diseased conditions, such as dysentery, measles, cholera, typhoid fever, yellow fever, pneumonia, etc., give in each case the same form of blood crystal.

Böttcher (Preyer, Die Blutkrystalle, Dorpat, 1871, 14; Archiv f. path. Anat. u. Physiologie, 1863, xxvii, 465) prepared crystals from the blood of dogs, cats, and other animals by anesthetizing the animals, then injecting cold water into the veins, and finally killing with chloroform. The blood was diluted with an equal volume of water and subjected to a temperature kept down to freezing-point for two days.

Schmidt (Archiv f. path. Anat. und Physiologie, 1863, xxvii, 465) analyzed crystals of dog's blood that had been dried at 110° C. He gives the following figures:

$$C_{53\cdot64}H_{7\cdot11}N_{16\cdot19}S_{0\cdot66}O_{20\cdot03}Fe_{0\cdot43}$$

also alkali and alkaline earth (0.04 per cent) and phosphoric acid (0.91 per cent), which latter shows that he had a very impure preparation.

Rollett (Versuche u. Beobachtungen am Blut, etc., Wien, 1862; Sitzungsberichte d. math.-natur. Classe d. Kaiser. Akad. d. Wissensch., Wien, 1862, xlvi, Abth. 2, 85) studied, with the help of von Lang, the crystallographic and optical characters of the blood crystals. Rollett prepared crystals from the bloods of the guinea-pig, squirrel, cat, man, rabbit, pig, and frog. Crystals from man, the rabbit, dog, cat, and guinea-pig they describe as belonging to the rhombic system, while those of the squirrel they assign to the hexagonal system. Rollett made use of several means of laking the blood to facilitate crystallization, which will be found referred to in other pages. Von Wittich (Königsberger medicinische Jahrbücher, 1862, iii, 332) obtained crystals from the blood of the rat, guinea-pig, and dog by breaking down the corpuscles with ether, but he did not succeed with the blood of man, rabbit, chicken, or frog.

Bursy (Inaug. Dissert., Dorpat, 1863; Ber. ü. d. Fort. d. Anatomie u. Physiologie, 1862, 293) studied the influences of various salts upon the crystallizability of hemoglobin. He found that sodium sulphate, sodium phosphate, sodium acetate, magnesium sulphate, and potassium sulphate favor crystallization; that potassium carbonate, potassium sulphate, sodium borate, barium nitrate, and sal ammoniac have little favorable effect; that sodium chloride, ammonium nitrate, calcium chloride, and alum were without influence; and that sodium nitrate appeared to hinder crystallization.

Ankersmit (Inaug. Dissert., Groningen, 1863; Ber. ü. d. Fort. d. Anat. u. Physiologie, 1863, 268) prepared crystals from human venous blood, which crystals he found became decolorized under certain conditions, and he therefore believed, as did Lehmann and others before him, that the crystals are only mechanically colored or stained. Klebs (Centralblatt f. med. Wissensch., 1863, I, 268) reports having found crystals in the corpuscles of the guinea-pig, rabbit, pig, sheep, and bullock, and also in man. Kühne (Centralblatt f. med. Wissensch., 1863, I, 851) recorded a process for preparing hemoglobin crystals by the addition to the blood of bile salts. He prepared in this way crystals from the blood of the horse and dog. In a later article (Archiv f. path. Anat. u. Physiologie, 1865, XLIII, 423) he states that he prepared crystals of reduced hemoglobin from the blood of the dog. He also noted the fact that the alkaline serum hinders the crystallization of hemoglobin. Valentine (Untersuch. z. Naturlehre, etc., 1863, IX, 129) obtained crystals from the blood of a marmot that had been hibernating for a long time.

At this time (1863) there still existed much difference of opinion as to the exact nature of the blood crystals, and Bojanowski (Zeit. f. wissensch. Zoologie, 1863, XII, 312), in going over certain unsettled points, concluded that only the contents of the corpuscles participate in the formation of the crystals, that the crystals are merely stained, and, therefore, that the name given by Kölliker, "globulin crystals," is entirely justifiable. His reasons for reaching this last statement were based partly on the reports of Lehmann, Teichmann, and others of having obtained decolorized crystals, and partly from his own experience. He states that if the blood crystals are allowed to stand for a time in the air they always retain their form, but become clearer and clearer, and finally completely colorless and transparent. The same is observed if to the crystals is added a strong sugar or gum solution.

Bojanowski prepared crystals from the blood of man, and from the rabbit, mouse, dog, cat, hedgehog, river bream, pike, horn-fish, herring, lark, raven, and pigeon. Crystals of human blood he obtained from the stomach of the leech, and also from venous blood. The latter was 36 hours old, and crystallization was completed within 3 to 4 hours without anything being added. The addition of water causes crystallization, but more sparingly and irregularly; but on the addition of alcohol and ether he failed in 15 experiments to obtain crystals. Crystals of the river bream he obtained without any treatment of the blood, and he states that this blood crystallizes with extraordinary rapidity. To the blood of a mouse taken from the animal 20 hours after death he added a mixture of equal volumes of alcohol and ether, and obtained, within a few minutes, numerous irregular six-sided plates and also rod-shaped crystals, occasionally in star-shaped groups. From the blood of the dog he always obtained crystals within 15 to 20 minutes after the addition of the mixture of alcohol and ether. From the cat he obtained three-sided prisms, which in most cases appeared a bright red, occasionally a bright yellow, and at times completely colorless. He also obtained crystals after the addition of water, and also after putrefaction had set in. The blood of a hedgehog that had been chloroformed 24 hours previously yielded crystals without treatment, but the crystals were much better when a mixture of alcohol and ether (1:4) had been added to the blood. Crystals of the lark he secured by the addition of a mixture of alcohol and water (1:1). Crystals from the horn-fish formed in the blood with or without the addition of water, and in the same way he obtained similar crystals from the blood of the pike. The blood of the herring he states crystallizes extraordinarily quickly, and the crystals almost always appeared colorless and possessed a shimmer similar to mother-of-pearl. From the blood of the raven he obtained crystals only after the blood had stood for 8 days in a cool place, and by the addition of chloroform and ether (1:3); but he failed to obtain crystals by the addition of distilled water, alcohol, gum solution, or sugar solution. The crystals he describes as partly colored bright yellow, partly completely colorless. Similar crystals were obtained from the pigeon by the addition of distilled water. Bojanowski notes from his investigations that the crystals of different species have something specific and characteristic about them, so that occasionally he could designate the species from which the crystals were derived.

The characteristic absorption spectrum of hemoglobin was discovered by Hoppe (Archiv f. path. Anat. u. Physiologie, 1862, XXIII, 446; Hoppe-Seyler was known as Hoppe previous to 1864), who states his belief that it is the same for the bloods of all vertebrates. He showed that hematin, which until then had been almost universally regarded as the coloring matter of the blood, is an abnormal constituent, and a product of decomposition of hemoglobin. He identified hemoglobin with the blood crystals described by Hünefeld, Reichert, Funke, and others, and he showed that while certain reagents were without effect on hemoglobin, others gave rise to a decomposition into an albuminous substance and hematin. No difference was noted in the spectra of arterial and venous blood, which was doubtless owing to the rapid oxidation of his preparations of venous blood, as he did not know, until some time later, of the difference in the coloring matter of arterial and venous blood and of the rapid oxidation of reduced hemoglobin when exposed to the air.

In Hoppe-Seyler's second contribution (Archiv f. path. Anat. u. Physiologie, 1864, XXIX, 233, 567; Centralblatt f. d. med. Wissensch., 1864, II, April 16, 261, 817, 834) he proposed the terms "hemoglobin" and "hematoglobin" to distinguish the coloring matter of the blood. This substance, he states, constitutes, excepting a few traces of other matters, the only constituent of the red corpuscles in man and the dog, while in birds and several mammals considerable quantities of albuminous substances are present in the corpuscles. Hemoglobin crystals of bloods of man, the dog, ox, sheep, guinea-pig, rat, mole, hedgehog, mouse, goose, pigeon, hen, frog, adder, and turtle, and probably of the bloods of all vertebrates, he states, contain no other substance than hemoglobin, and particularly is no hematin present in them.

While Hoppe-Seyler failed to note in his previous research any difference in the spectra of arterial and venous blood, he records that if the solution of hemoglobin is freed from O by a current of $CO_2$, or by decomposition, it shows a spectrum that is somewhat different from that of a solution that has been shaken with air. He called particular attention to the readiness with which hemoglobin in crystalline form, or in solution, undergoes decomposition. A feeble alkaline reaction of the solution, the presence of albuminous bodies, and a temperature at or below 0° preserve the hemoglobin; but the higher the temperature the quicker the decomposition. No concentrated solution remains undecomposed for 24 hours at ordinary temperature, and, as the spectrum shows, the crystals also become decomposed with like quickness. Dilute solutions are somewhat less readily decomposed than strong solutions. Even dry crystals can not be kept undecomposed, and in the presence of albumin decomposition goes on quickly. In every instance the decomposition of the hemoglobin still present in concentrated solution takes place at first very quickly, and gradually less and less rapidly. Therefore, in concentrated solutions, even after many weeks and months, some undecomposed hemoglobin remains.

In his third contribution (Archiv f. path. Anat. u. Physiologie, 1864, XXIX, 597; Centralblatt f. med. Wissensch., 1865, III, 38) Hoppe-Seyler calls attention to the loose combination of O with hemoglobin, to the combination of CO with hemoglobin, and to his centesimal analyses of hemoglobin and hematin. His mean figures for the dry hemoglobin of the dog and goose are:

$$C_{54\cdot2}H_{7\cdot2}N_{16}Fe_{0\cdot42}$$

He did not record phosphoric acid and other inorganic constituents, but in later analyses he found phosphoric acid in goose hemoglobin, but not in the hemoglobin of mammals.

In later publications (Medicinisch-chemische Untersuchungen, 1866, Heft 1, 151, 1867, and Heft 2, 293; 1868, Heft 3, 366, 386) he gives a process for preparing hemoglobin, together with much matter pertaining to the chemistry and to other properties of hemoglobin. His process is as follows: In order to obtain crystals of pure hemoglobin, the blood is defibrinated and mixed with 10 volumes of salt solution, which consists of 1 part of saturated solution of chloride of sodium and 9 parts of water, and set aside at 0° until the corpuscles have sunk to the bottom. The supernatant fluid is then drawn off and the corpuscles are washed as often as four times in this way. To the washed corpuscles are added merely enough water to dissolve the hemoglobin and afterwards an equal volume of ether, the whole is shaken, and then the excess of ether is poured off. The solution is quickly filtered. The solution of dog, guinea-pig, squirrel, and rat blood corpuscles crystallizes without further treatment. With bloods that do not crystallize readily, the filtrate is cooled to 0° and mixed with one-fourth of its volume of 80 per cent alcohol which has also been cooled to 0°, and the mixture subjected to a temperature of —5° to —10°. After crystallization, the crystals are collected on a filter paper at a temperature near 0°, and washed in the cold (at 0°) with a cold mixture (at 0°) consisting of 1 volume of alcohol to 4 volumes of water. The crystals are dried between filter paper by slight pressure. To recrystallize, the crystals are dissolved in 3 volumes of distilled water by heating to 30° to 40° C., the solution is filtered and cooled to 0°, one-fourth volume of absolute alcohol cooled to 0° is added, and the mixture subjected to —5° to —10°, as before. In this way crystals from the blood of man, of the pig, bullock, sheep, rabbit, duck, pigeon, and goose were obtained.

Appearing shortly after Hoppe-Seyler's second contribution, an article of epochal importance in physiology was published by Stokes (Proceedings of the Royal Society, London, 1864, XIII, 355, June and November), to whom is due the credit of the discovery of the "respiratory function" of the coloring matter of the blood, and also the specific differences in the spectra of oxyhemoglobin and reduced hemoglobin. Stokes writes that it was to him "a point of special interest to inquire whether we could imitate the change of color of arterial into that of venous blood, on the supposition that it arises in reduction. He found upon adding to a solution of blood a reducing agent ["Stokes's reagent"] the color almost immediately changed to a much more purple red as seen in small thicknesses, and a much darker red than before as seen in greater thickness. The change of color, which recalls the differences between arterial and venous blood, is striking enough, but the change in absorption spectrum is far more decisive." When the purple solution was exposed to air in a shallow vessel it changed immediately into its original condition. He states that the addition of a reducing agent caused reduction as before and exposure to air a return to the original condition, and that these phenomena could be repeated a number of times. From such facts he inferred that the coloring matter of the blood, like indigo, is capable of existing in two states of oxidation, distinguishable by a difference in color and a fundamental difference in the action on the spectrum. Hematin having been shown by Hoppe-Seyler in his first communication to be a decomposition product, and Stokes being obviously unaware of this communication (published in Archiv f. path. Anat. u. Physiologie and elsewhere, *loc. cit.*, several months previously), and therefore not knowing that Hoppe-Seyler had proposed terms for the coloring matter of the blood, proposed, at the suggestion of Dr. Sharpey, the term cruorin; and in order to differentiate the two states of oxidation he suggested the terms scarlet cruorin and purple cruorin. Stokes observed that the change in color from arterial to venous blood is in the direction of a change from scarlet to purple cruorin, and that the blood is reoxidized in passing through the lungs and deoxidized while passing through the tissues generally. He also noted independently of Hoppe-Seyler the feeble state of the combination of the oxygen, for he notes that shaking the blood with $CO_2$ removes the O, and he states that if, as we have reason to believe, this oxygen is for the most part chemically combined, it follows that carbonic acid acts as a reducing agent, and that we are led to regard the change of color not as a direct effect of the presence of carbonic acid, but a consequence of the oxygen. He records certain differences between effects of carbon dioxide and the "real" reducing agents, and he notes that while the former no longer acts on a dilute and comparatively pure solution of scarlet cruorin, the latter acts just as before. He infers that scarlet cruorin is not merely a greedy absorber and carrier of oxygen, but also an oxidizing agent, and he states that "as the purple cruorin in the solution was oxidized almost instantly on being presented with free oxygen by shaking with air, while the tin solution remained in an unoxidized state, so the purple cruorin of the veins is oxidized during the time, brief though it be, during which it is exposed in the lungs, while the substance derived from the blood may have little disposition to combine with free oxygen. As the scarlet cruorin is gradually reduced, oxidizing thereby a portion of the tin salt, so part of the scarlet cruorin is gradually reduced in the course of the circulation, oxidizing a portion of the substances derived from the food or of the tissues; the purplish color now assumed by the solution represents the tinge of venous blood, and a fresh shake represents a fresh passage through the lungs."

Immediately following Stokes's article a contribution by Hoppe-Seyler appeared (Centralblatt f. med. Wissensch., 1864, II, 817, 834) in which he refers to the work of Stokes as follows: "The observation of Stokes coincided fully with my observation earlier, but in addition there are phenomena described by him with which I was already familiar, but only showed in my lectures." The author then makes reference to various experiments he had carried out in this particular direction of inquiry, and also in connection with hematin.

Kühne (Archiv f. path. Anat. u. Physiologie, 1865, XXXIII, 79) identified the spectrum of the coloring matter of muscles with that of the blood described by Hoppe-Seyler. In a later article (*ibid.*, XXXIV, 423) he reports having crystallized reduced hemoglobin. He made a concentrated solution of crystals of dog's blood in very weak ammonia, and then subjected the solution in a gas chamber to pure dry hydrogen. Crystallization occurred as evaporation proceeded. Oxyhemoglobin of the dog, he notes, is very insoluble, while the reduced hemoglobin is very soluble, and he points out that the difficulty experienced in preparing reduced hemoglobin crystals is owing to their great solubility. He also noticed intraglobular crystallization. Rollett (Sitzungsb. d. k. Akad. d. Wissensch., Wien, 1866, LII, 2 Abth., 246) shortly afterward prepared reduced hemoglobin crystals by agitation with reduced iron. Schultz had (Archiv f. mikros. Anat., 1865, XXXI, 1) the year before reported crystals of monkey's blood which he obtained by the addition of water and alcohol to the blood, and which were doubtless reduced hemoglobin.

Schmidt (Preyer, De hæmoglobino observationes et experimenta, Bonn, 1866) gives the following analysis and molecular formula for the hemoglobin of the dog:

$$C_{54\cdot 15}H_{7\cdot 18}N_{16\cdot 33}Fe_{0\cdot 43}S_{0\cdot 67}O_{21\cdot 24} \quad \text{and} \quad C_{600}H_{960}N_{154}FeS_3O_{179}$$

During this same year Moleschott (Pathologie u. Physiologie, 1866, 42) made note of the occurrence of six-sided plates in guinea-pig blood.

Nawrocki (Centralblatt f. med. Wissensch., 1867, xv, 195) and shortly after Ray Lankester (Jour. Anat. and Phys., 1868, II, 114) established the identity of the coloring matters of the bloods of the worm and vertebrates.

Hoppe-Seyler (Medicinisch-chemische Untersuchungen, 1867, Heft 2, 1, 215, 293; Heft 3, 366, 394) at about that time prepared crystals from a number of bloods of different species, and made elementary analyses and observations in various directions. His elementary analyses of the hemoglobins of the dog, goose, guinea-pig, and squirrel will be found in the table on page 71. Kühne (Lehrb. d. physiol. Chemie, 1868, 198) recorded, among other kinds of hemoglobin crystals already obtained, that of the polecat. At this time Ray Lankester began the publication of a series of articles on the distribution of hemoglobin in the animal kingdom, which articles have been referred to in Chapter I.

In a research on the cellular structure of the red blood corpuscles, Richardson (Trans. American Med. Association, 1870, XXI, 261) studied intracorpuscular crystallization in the menobranchus. The corpuscles being very large and the hemoglobin readily crystallizable lend conditions extraordinarily favorable to such observations. He deposited a drop of blood upon a slide, allowing it to remain uncovered about 10 minutes, or until a mere line of desiccation appeared at the margin, and then covering it with a thin glass. On examination with a power of 200 diameters, he found that numerous corpuscles along the edge of the drop, where the liquor sanguinis had become concentrated, contained one, two, or more crystals; and under favorable conditions this process of crystallization went on until the contents of every corpuscle assumed a crystalline form, either wholly or in part. The crystals were frequently irregular, but their typical form appeared to be that of a quadrangular prism with dihedral summits, the angles being sometimes truncated.

# CHAPTER V.

## THE INVESTIGATIONS OF PREYER ON THE CRYSTALLOGRAPHY OF HEMOGLOBIN.

The monograph of Preyer (Die Blutkrystalle, Jena, 1871, 263 pages, 3 plates), which continues to the present day to be the leading authority on the crystallography of hemoglobin, had for its foundation several contributions that were published a few years previously, and which were chiefly with reference to the optical and chemical properties of hemoglobin. His first serious contribution to the crystallography of hemoglobin appeared in 1868 (Archiv f. ges. Physiologie, 1868, I, 395), in which he expresses his surprise that so little is known of hemoglobin. He writes that while blood crystals have been obtained from 47 vertebrates (23 mammals, 7 birds, 4 reptiles, 1 amphibian, and 12 fishes), in only 10 of these was the crystal system ascertained. The crystals of the human being, the dog, the guinea-pig, and the rabbit were found by von Lang to be rhombic; those of the cat according to Rollett, those of the horse according to Funke, and those of the lion, jaguar, and marbled cat according to Preyer, are also rhombic; while those of the squirrel, according to von Lang, are hexagonal. Preyer goes on to state that in addition to those mentioned the following are probably rhombic: those of the monkey, bat, hedgehog, sheep, pig, harfang (owl), and frog. The blood of the monkey yielded rhombic plates which crystallized with difficulty and which were readily soluble, even in the cold. The blood of the bat crystallized in thin plates with very pointed angles. Hedgehog blood produced right-angled elongated prisms, which even in the cold are readily soluble. Sheep's blood crystals were obtained only once in gas-free blood, and they were prisms. Pig's blood crystallized with extraordinary difficulty, mostly intraglobular (in every corpuscle a prism). The blood of the harfang (*Strix noctua*) crystallized readily, but that of the frog with difficulty. The former yielded 4-sided plates; the latter, thin prisms, which appeared to be 4-sided. Of the other blood crystals previously seen, those of the mouse and the hamster, he states, might be hexagonal. Preyer obtained from the blood from the heart of a mouse only fine needles. The hemoglobin crystals of the fox, polecat, mole, marmot, ox, raven, sparrow, pigeon, goose, duck, lark, rat, and fish were produced, but their crystallographic investigation gave little satisfaction; most of them appeared to be rhombic.

Preyer, in his review, writes that Lehmann's statement that occasionally regular octahedrons are found in guinea-pig blood is incorrect; and also that Hoppe-Seyler's assertion that guinea-pig blood crystals are tetragonal is wrong. He also states that the opinion of Funke that human and cat hemoglobins crystallize in the monoclinic system has long been refuted. Accordingly, as Preyer writes, all the hemoglobins accurately investigated crystallographically up to the present time are rhombic, with the single exception of that of the squirrel.

The remaining portion of Preyer's article, which is very largely a record of his own researches on the physical, chemical, and optical properties of hemoglobin and methemoglobin, is included practically in full in his monograph, which was published three years later. Owing to the authoritative character of this publication, its indispensable value to the physiological chemist, the practical impossibility of obtaining copies, and the rareness of the work in the libraries of this country, we have deemed it advisable to embody in this memoir a rather full and free translation of his chapters on the methods of preparation and on the descriptions of the crystals. This extract constitutes the remainder of this chapter.

### PROCESSES USED BY PREYER FOR OBTAINING CRYSTALS IN LARGE QUANTITIES.

(I) Lehmann's process has the advantage over the others which follow in that a very low temperature is not necessary. The solvent of the coloring matter of the corpuscles is water. The fresh blood is allowed to coagulate and the coagulum to shrink, the serum is drawn off, and the coagulum is ground up. The fluid is separated from the clot by straining through linen, and to this fluid are added from 1 to 1.5 volumes of water. O is then passed through this diluted extract of the clot for about half an hour, and then $CO_2$ for 10 or 15 minutes. The formation of crystals begins within a few minutes and a rich mass of crystals has separated after 2 hours. By this method crystals were obtained only from the blood of the guinea-pig, rat, and mouse. In order to prepare crystals from dog's blood, and from other blood that is not readily crystallizable, small quantities of alcohol are added to the blood before and during the passage of the gases. The solution very quickly becomes cloudy with crystals and congeals to a crystal pulp. Instead of alcohol, ether can be used in part, but it does not suffice alone. The crystals obtained in this way are, however, not pure. Since recrystallization is necessary in order to obtain pure crystals, and as this can not be done except at low temperatures, there is no advantage in this method if it is necessary to secure a pure product. Preyer found, moreover, that it is only necessary to pass for many hours dry or moist atmospheric air free from carbonic acid through the defibrinated blood of the dog in order to cause crystallization, which occurred abundantly at room temperature, or at about 35° to 38° C.

(II) As a solvent for the coloring matter of the corpuscles Rollett made use of freezing. He placed a platinum vessel containing defibrinated blood in a freezing mixture, and after about half an hour the blood was allowed to thaw slowly. It was then poured into shallow vessels to a depth of about 15 mm. These vessels were placed in the cold at even temperature to crystallize. Within an hour or so a deposit of crystals had formed. In this way guinea-pig and squirrel blood quickly yielded well-formed crystals, but cat's blood only after a longer time. With dog's blood crystallization proceeds from the surface. The crystalline mass may be lifted off, then a new one forms, and so on. Very much more time is required for human and rabbit's blood. Pig's and frog's blood gave no crystals, yet the hemoglobins of these bloods are capable of being crystallized. By repeated freezing and thawing of the blood all the blood corpuscles could be completely decolorized, but this requires larger quantities of blood, repeated freezing and thawing, and much time. Moreover, it is necessary to concentrate this laked blood by evaporating at a low temperature. It is immaterial in this process whether or not the blood be exposed to the air during the process. This method, which is particularly convenient in winter, is worthy of recommendation only where absolute purity is not necessary, as in making comparative crystallographic and optical investigations of the hemoglobins of different animals.

(III) Into the animal from whose blood the crystals are to be produced, Böttcher injected intravenously a quantity of cold water during chloroform narcosis, and then the chloroform was administered until death occurred. The blood is obtained immediately after death from the heart and vessels, and it is readily crystallizable. If mixed with an equal volume of water and alcohol, and the mixture placed in the cold, a magma of crystals will form. This method is not recommended, because of the difficulty of obtaining sufficient blood from the dead animal.

(IV) The solvent for the hemoglobin which Kühne recommends is the taurocholate and glycocholate of sodium. Thiry also employed bile salts to obtain hemoglobin crystals from bloods that crystallize with difficulty.

600 c.c. of horse blood are collected in a cylindrical vessel and cooled. As soon as the plasma has separated from the corpuscles, it is removed, together with the layer of white corpuscles, and the remaining mass of red corpuscles is mixed with 0.5 per cent solution of crystallized ox bile. The corpuscular mass is then allowed to coagulate. The fibrin which has formed has entangled the decolorized corpuscles which have not dissolved, so that the deep-red lake-colored solution, which may be drained off, contains none of the corpuscles. To this solution is added, during continual stirring, and as long as the precipitate that forms is dissolved, 90 per cent alcohol which contains a very little acetic acid. After several hours the preparation is converted into a crystal pulp, which may be collected on a filter, then washed with dilute alcohol, and subsequently with iced water.

Or 100 c.c. of dog's blood are allowed to coagulate in a shallow vessel; the coagulum is then detached from the walls of the vessel and allowed to stand in a cool place for 24 hours, until the serum has separated as much as possible. The serum is removed; the coagulum is washed with water and broken up in 50 c.c. of water by means of a syringe. After 24 hours it is strained through linen and the clot is washed out with 10 c.c. of water. To the mixture of the washing from the clot and the fluid obtained by straining are added 2 c.c. of a solution consisting of 1 part of crystallized bile and 3 parts of water. After 24 hours the solution is filtered through many thicknesses of filter-paper. On the addition of 20 c.c. of 90 per cent alcohol to 100 c.c. of the filtrate a firm crystalline pulp is soon formed, which is collected on a filter and washed, at first with a mixture of 4 parts of water and 1 part of alcohol and then with iced water. According to this method fully 5 grams of pure recrystallized dry hemoglobin are obtained. Recrystallization yields, according to Kühne, a pure preparation only if the first crystallization contained no corpuscles. This method is somewhat troublesome. The addition of crystallized bile, as well as of acetic acid, Preyer believes, may give rise to decompositions of the hemoglobin.

(V) Defibrinated dog's blood is mixed with about its own volume of distilled water, and to every 4 volumes of the blood solution there is added 1 volume of alcohol. The mixture is left for 24 hours at a temperature of 0°, or lower. The crystals that have separated are collected on a filter, pressed, dissolved in the smallest amount of water at 25° to 30°, cooled to 0°, the solution mixed with one-fourth its volume of alcohol and left standing for 24 hours at 0°, or better at —10° to —20°. The entire fluid becomes converted into a crystalline mass without freezing. This recrystallization can often be repeated.

From the bloods of several rodents, for example the guinea-pig and the rat, blood crystals were obtained on the addition of pure water after defibrinization, because the crystals are not soluble in cold water, yet they can also be recrystallized by dissolving in water at 30° and cooling, or evaporating over sulphuric acid in a rarefied atmosphere, and they can be dried at 0° without decomposition. (Method of Hoppe-Seyler.)

Preyer occasionally observed that fresh defibrinated blood of the dog, after dilution with pure water, yields the most beautiful crystals upon evaporation. Once he mixed 5 c.c. of blood with 4 c.c. of distilled water, poured the solution into a shallow porcelain vessel, and let it stand over night at a temperature between 19° and 20°. On the dried marginal portions of the solution, after about 15 hours, exceptionally beautiful crystals, 5 to 6 mm. in length and intensely red, were formed. Yet he did not always succeed in producing blood crystals from dog's blood in this way.

Of the five methods described, the last, according to Preyer, is decidedly the best. Yet, he states, it also needs improving. He proceeded, therefore, in the production of pure hemoglobin crystals on a large scale from any blood selected, in the following way:

(VI) The blood is collected in a vessel and allowed to coagulate and to stand for several hours (or, better, for a day) in a cool place. Then the serum with the white corpuscles and the fat which has collected on top are removed and the coagulum washed with distilled water and then cut into very small pieces, and these pieces in turn are repeatedly washed with cold distilled water. Then the clot is comminuted, best by freezing and reducing the frozen mass to powder. This powder is placed on a filter-paper and washed with cold distilled water until the filtrate no longer gives a very strong precipitate with bichloride of mercury. The coagula are extracted by water heated to 30° to 40°, and filtered, and the filtrate is collected in a large cylindrical vessel standing in ice. A small measured portion of the red solution thus obtained is gradually mixed during constant agitation with small quantities of alcohol until a slight precipitate forms. This determines how much alcohol may be added to the whole solution without a precipitate appearing. A slightly smaller proportion of alcohol is now added to the remaining filtrate and the mixture is placed in a cooling medium. Even after a few hours the crystals separate in great abundance. The crystals are, owing to the volume of water used, very easily filtered off in the cold. They are then washed with cold water containing a little alcohol until the filtrate yields only an insignificant cloudiness upon the addition of acetate of lead or corrosive sublimate. The product yielded is a very large one. The crystals may be purified by repeated washing by decantation until the wash-water does not become cloudy with bichloride of mercury, acetate of lead, or silver nitrate. They are then nearly pure, and the ash is free of phosphoric acid and consists of pure iron oxide. If this is not the case, then they must be dissolved in warm water and recrystallized as directed. At a temperature of less than 0° the crystals can be dried in the air without becoming decomposed.

This method differs essentially from (V) only in that instead of defibrinated blood a diluted extract of the clot is used. But in this there is a great advantage, because the crystals can be obtained pure very much more easily and quickly owing to the very small quantities of serum albumin that can adhere to them. Furthermore, the fluid, because of the lack of serum albumin, filters more quickly. It will therefore be noticed that by the coagulation of the blood, by the treatment of the clot with water, by the freezing of the same, and by the longer interval from the time of bleeding to the mixing with alcohol, the degree of crystallizability increases. Preyer obtained larger crystals by this method than by any of the others.

Of all kinds of blood, that of the horse, he states, is best adapted for the production of very large quantities of pure hemoglobin. It is defibrinated and the corpuscles are allowed to settle in a high cylindrical vessel, the serum is drawn off, and the corpuscles are frozen, etc.

If crystallized hemoglobin is to be produced quickly from the defibrinated blood of the dog, it is best to mix the blood with its own volume of distilled water, add 1.5 volumes of absolute alcohol to 4 volumes of the mixture, and then place the solution in a cylinder in a cooling mixture. After a few hours the fluid has changed to a crystal pulp. By frequent washing, by decantation, or by centrifugalization with diluted alcohol (4 volumes of water to 1 volume of absolute alcohol), crystals are obtained pure, but of course with great loss. This very convenient method has the disadvantage that the corpuscles by their remaining longer in dilute alcohol become difficult of solution in water. If the preservation of the normal solubility is disregarded an abundant crystallization can be obtained at 8° to 10° from dog's blood by mixing 1 volume of fluid with an equal volume of water and a little more than one-fourth of the whole volume of absolute alcohol. The mixture in one case had formed a thick crystal pulp in 9 hours, so that a large amount of nearly pure crystals can be obtained by decantation with a mixture of 4 volumes of water and 1 volume of absolute alcohol. Nevertheless, this method does not always give such favorable results.

### PROCESSES GIVEN BY PREYER FOR OBTAINING CRYSTALS IN SMALL QUANTITIES.

One of the simplest processes for obtaining crystals from the blood of several animals is by heating. Max Schultze (Archiv f. mikrosk. Anat., 1865, 1, 31) found that the corpuscles were dissolved at a temperature of about 60°, forming a lake-colored blood solution. Every drop then evaporated yields crystals. Preyer observed this also in guinea-pig's blood when he gradually heated a drop on a slide to about 60°, and then allowed it to cool and to evaporate slowly; and also when a large quantity of blood was warmed in a water-bath to at least 60°. With squirrel's, calf's, and human blood crystallization did not succeed. On the other hand, Preyer writes, by no other method could there be obtained from horse's blood such well-formed and large crystals. The temperature must be at least 60°, but it should not go beyond 64°. Preyer proceeded in the following way: Horse's blood was collected in a vessel, defibrinated by agitation, and decanted. The defibrinated blood was separated after several minutes into two portions—an upper layer of serum and a lower dark-red layer of corpuscles. The serum was pipetted off and the corpuscles heated in a water-bath at 60°. This produced a lake-colored solution, of which every drop, upon being cooled and evaporated, yielded extraordinarily beautiful crystals.

This crystallization, which is brought about by warmth causing a separation of the coloring matter from the corpuscles, is not to be confounded with one earlier reported by Bojanowski (Zeit. f. wissensch. Zoologie, 1863, XII, 323), who evaporated the diluted extract of the coagulum of rabbit's blood at 50°, and who noticed in so doing that the upper surface of the blood was covered with a delicate crust composed of prismatic crystals. In this process the coloring matter had been extracted by water, so that the effect of warmth can only be looked upon as an aid to rapid evaporation. The favorable influence of slight warmth as a means of hastening evaporation has been repeatedly misunderstood and denied, and generally it has been regarded as a hindrance to crystallization.

If an extract of the coagulum of dog's blood prepared with cold distilled water is shaken with sufficient ether so that it smells of it, and then a little alcohol added, and then very gradually heated in a very shallow vessel until the margin of the fluid or the drop on the object-glass begins to dry, evaporation proceeds quickly and regularly, and crystals form as the blood cools. In this way a most beautiful preparation can be obtained in a short time. However, without artificial heating as many hours are necessary to obtain crystals as minutes are required with it.

Electric shocks have a similar effect in causing a solution of the hemoglobin from the corpuscles, as was found by Rollett. A. Schmidt had already noted that a like effect is caused by the galvanic current. By both the corpuscles are decolorized and the hemoglobin (of human, cat, dog, and guinea-pig blood) crystallized in the lake-colored solution, it being immaterial whether the oxygen from the air has been admitted or not. Yet this method is not well adapted for the production of crystals on a large scale.

An observation made by Pasteur (Compt. rend. soc. biolog., 1863, LVI, 739) is very noteworthy. He allowed dog's blood to stand in a balloon of heated air at a constant temperature of 30° C. After 4 to 6 weeks the air contained 2 to 3 per cent less of oxygen and just as much more of carbonic acid. A large mass of hemoglobin crystals was formed. After several weeks not a single corpuscle was present. The clot was colorless and very elastic, and associated with an incalculable number of crystals.

Julius Bernstein (1866) conducted atmospheric air through a small amount of chloroform into defibrinated blood. He noticed that the blood soon became lake-colored, and that he could no longer find any corpuscles in it. Every drop produced crystals when evaporated. Preyer supplemented this procedure by treating the diluted extract of the coagulum in the same way. This yielded crystals, but not in great masses. Kunde had already observed (1852) the favoring influences of chloroform, ether, and alcohol on crystallization.

Alexander Schmidt found that crystallization followed upon the addition of pure alcohol to dog's blood. He mixed fresh blood with one-half to two-thirds its volume of alcohol until albumin began to separate and then left the mixture undisturbed. After a time it became laked and crystalline.

Ether causes the very same thing. Defibrinated dog's blood is shaken with ether until the blood is laked and smells of ether. If it is allowed to stand for 24 hours in the cold crystals can be seen microscopically in every drop.

Later A. Schmidt saw that dog's blood and horse's blood became lake-colored when the fresh blood was shaken with a definite amount of turpentine containing ozone, each time ascertaining by testing the amount necessary. He could then cause crystallization with alcohol (only in the dog), or ether, or sodium sulphate, or by water extraction in a vacuum.

Several times it has been observed, continues Preyer, that an addition of certain neutral salts to bloods which can be crystallized hastens crystallization. According to Bursy the salts favoring crystallization are in the order of their value as follows: Sodium sulphate, sodium phosphate, sodium acetate, potassium acetate, magnesium sulphate, and potassium nitrate. Less energetic are potassium carbonate, potassium sulphate, sodium borate, barium nitrate, and sal ammoniac. Sodium nitrate appeared to hinder crystallization when the blood was alternately frozen and thawed. Sodium chloride, ammonium nitrate, calcium chloride, and alum were without effect. For the production of crystals on a large scale the addition of salts is not to be recommended, because of the introduction of such foreign substances. Only chloride of sodium seems to be of value in the isolation of the corpuscles according to Hoppe-Seyler's method.

The crystals thus obtained can be recrystallized 5 or 6 times, or as long as the hemoglobin remains undecomposed. This plan, nevertheless, has reference only to the kinds of blood which crystallize easily. These crystals, even after several recrystallizations, show the bright-red color of arterial blood.

Another means by which the blood can be crystallized is by extracting the gases. In dog's blood from which the gases had been extracted, Rollett found that the blood was lake-colored, very dark, and that it produced hemoglobin crystals immediately. Preyer states that he found that dog's blood and sheep's blood freed from gases crystallize by evaporating a drop on an object-glass, the colorless stromata of the corpuscles still being visible. The addition of a little dilute solution of oxalic acid aided crystallization.

Preyer then tried to decide experimentally if all of the three blood-gases (carbonic acid, oxygen, and nitrogen) were necessary for the preservation of the normal condition of the blood corpuscles in the circulating blood. He investigated the blood of asphyxiated animals and found microscopically that there was some disintegration of the corpuscles. Previous to this experiment he had found that blood made rich in carbonic acid and freed of oxygen by a continuous stream of carbonic acid crystallized very slightly, and that therefore the absence of oxygen alone (without taking into account the nitrogen) is sufficient to cause a partial decomposition of the blood corpuscles into colorless stromata and hemoglobin. He then made the following experiment: The A. carotis dextra, the V. jugularis externa sinistra, and the trachea of a little dog were laid bare, and into the vessels glass cannulæ were tied. The trachea was then clamped so as to prevent the entrance of air to the lungs. The moment the conjunctiva became insensible to the touch the ligatures on the vessels were loosened and the blood drawn into separate receptacles. The blood was dark red, and that of the artery could not be distinguished from that of the vein. A drop of each kind of blood showed under the microscope a rich crystal formation within the first minute after it was caught. Under the eye of the observer the crystals increased in thickness, length, and number as long as the evaporation of the drop on the object-glass lasted, but more slowly if the drop had been covered with a cover-glass. By gently shaking in the air the blood became bright red again. All the manipulations mentioned here by which crystals can be obtained are somewhat troublesome, and are not used for purposes of microscopic preparations.

C. Bojanowski placed a drop of blood on an object-glass, exposed it to the air several minutes, breathed upon the preparation several times, then covered it with a cover-glass, and allowed it to evaporate slowly. He found that a small addition of alcohol or ether is occasionally necessary. Bojanowski obtained microscopic crystals without adding anything to the blood by merely allowing it (as it comes from the veins, or, better yet, as it is found in them after death) to stand in a vessel 2 to 4 days in a cool place. The blood coagulum partly dissolves and the blood becomes thick and dark red. A drop of the same is allowed to stand several hours between the cover-glass and the object-glass without being heated. If the blood is too thick some distilled water is added to it.

In order to produce hemoglobin crystals in a short time from fresh blood chosen for microscopic purposes, the following method is best: Several cubic centimeters of defibrinated blood are mixed with just enough water to yield a clear solution. A drop of the mixture covered with a cover-glass crystallizes on being subjected to cold. If this is not the case, about one-fourth the volume of alcohol is added to the solution and the mixture is placed in a platinum or silver vessel in a cooling mixture. Crystals are always obtained. Almost all kinds of blood yield crystals by merely allowing the blood to freeze, even ox blood.

## THE FORMS AND SYSTEMS OF CRYSTALLIZATION OF HEMOGLOBINS.

Upon this subject Preyer writes: Of the six crystal systems only five are to be taken into consideration in the classification of crystals of hemoglobins, namely, the regular (tesseral), the tetragonal, the rhombic, the monoclinic (clinorhombic, monoclinohedric), and the hexagonal. Crystals belonging to the triclinic system have not been claimed to have been found by anyone. Of the remaining five systems the regular and the tetragonal are to be eliminated—the regular because all hemoglobin crystals are doubly refractive, and the tetragonal because the only crystals assigned to this system were those of the guinea-pig by Hoppe-Seyler. Hoppe-Seyler's statement is without facts to justify it, and it has been disproved by the accurate investigations of guinea-pig crystals by Victor von Lang and others. There remain then three systems—rhombic, hexagonal, and monoclinic. The last named is also to be eliminated because Funke is the only scientist who asserts that hemoglobin crystallizes in this system, and he does not support his statement. He only asserts that human and cat hemoglobins crystallize in the monoclinic system, yet in another place he himself calls human blood crystals rhombic, and as a matter of fact so are those of the cat. Monoclinic crystals have not, then, up to this time been shown to exist. On the other hand, crystals belonging to the rhombic and hexagonal systems have been shown to exist beyond any question. The fact that crystals of different species are assigned to two systems is a matter that is not to be ignored. It is firmly settled that squirrel's blood yields crystals that belong to the hexagonal system, while dog's blood yields crystals that with as little doubt belong to the rhombic system.

The observations of V. von Lang are:

> Squirrel's, 6-sided plates formed from a 6-sided prism, showing the basal surface. These crystals belong undoubtedly to the hexagonal system, because when observed through the basal surface between crossed nicols they remained dark in all azimuths. In agreement with this, the crystals when observed through the prism surface showed double refraction. All the rays, then, are not of like intensity. The vibrations parallel to the optical and crystallographic axes are less absorbed than those which are perpendicular.

Preyer states that he confirms von Lang's observations: Between nicol prisms squirrel's blood crystals only show color when their optic axis does not lie parallel to the direction of the polarized light ray. Preyer goes on to state that if future investigations should show that all blood crystals are either rhombic or hexagonal, which is probably true, then it could not be maintained that the difference in the forms of the crystals is a case of polymorphism or dimorphism, because chemical identity is lacking.

The hexagonal system, as A. Schrauf (Jahrbuch f. Mineralogie, 1865, 46) has shown, is conceived of as being a peculiar combination of the rhombic ($P.\bar{P}\infty$, with the single condition that $\infty P : \infty P = 60^\circ$), therefore the mistake could be made of looking upon the crystallographic distinction as being a material one. The statement of Schrauf does not, however, bear the test, because even if the hexagonal forms are still the simple combination of the rhombic, the fundamental optical distinction of both systems can not be denied. Optically the rhombic crystals are biaxial and the hexagonal are uniaxial.

There exist other distinctions outside of the crystallographic systems. The peculiar crystalline form in relation to each kind of animal (of the guinea-pig, the sphenoidal; of the dog, 4-sided prisms; of man, these and rhombic plates, and so forth) is so constant and definite that only these forms could be obtained from the blood referred to. After recrystallization repeated ever so often the same form always appears, which is peculiar to each kind of animal, and which can not be changed to another (see Chapter VII). The same applies to solutions of hemoglobin. Yet little importance is to be attached to statements on the crystallographic differences of the hemoglobin of different animals, because neither is the same method of crystallization always used, nor is the blood always capable of being compared, nor has a measure of the crystallizability of any optional substance been found.

It is the same with decomposability as with the crystallizability of hemoglobin. Both vary according to the species of animal, but the investigations undertaken in this direction suffer from so many and such large errors that they prove nothing beyond what has long been known—the different quantitative determinations of the blood-coloring matter in various animals and individuals. Such matters lead to the desire to follow up through a series of animals the several properties of hemoglobin—for example, the coagulation-point, the crystalline form, and the capacity of water of crystallization—in order that the question concerning their great differences might be more closely approached: whether the various hemoglobins are present as so many different substances which only agree in certain characteristics, or whether they are entirely identical in derivation and their differences come about solely because of combining with other substances, or are properties of the crystals. Perhaps an explanation could be obtained by transfusion, as, for example, of squirrel's blood to guinea-pig, or *vice versa*.

The following table (table 31) is from Preyer, and is an excellent summary of our knowledge of the crystallography of hemoglobin up to the time of the publication of his memoir.

TABLE 31.—*Preyer's table showing the source of hemoglobin crystals, crystalline form, crystalline system, etc.*

| Name of kind. | Crystal form. | Crystal system. | Appearance. | Solubility in water. | Crystallizability. | Remarks. |
|---|---|---|---|---|---|---|
| Man............... | Elongated rectangles, rhombi and 4-sided prisms. Acute angles of the rhombi 54° 6′ (von Lang) | Rhombic (Funke, von Lang) | In blood sucked by the leech 6 to 8 weeks previously (Budge, Bojanowski); extraglobular in venous blood (Funke); intraglobular (H. Meckel) | Fresh from venous blood extraordinarily easily dissolved; that from the leech, in the cold quite difficultly, in warmth very readily soluble | Crystallizes with difficulty | Illustrations in Funke's Atlas, x, 1 and 2. Compare (1) Funke, Journ. f. prakt. Chemie, 1852, p. 384, and Zeitschr. f. rat. Med., 1852, 205. (2) V. Lang, Sitzungsber. d. Wiener Akad., xlvi, 1 Ahth., 1862, with illustrations of the crystals of Rollett's own treatise. (3) Bojanowski, Zeitschr. f. wiss. Zool., xii, 332, taf. 30, 1 and 3. (4) Kunde, Zeitschr. f. rat. Med., 1852, taf. ix, 1. Funke found the angles 73°0′ to 73° 35′ and on the almost right-angled plates $\frac{88^\circ 30'}{91^\circ 30'}$.<br>Budge, Verhandlgn. d. naturhist. Vereins der Rheinl. u. Westph., 1850. (Köln. Zeitung, No. 300, Ankersmits Diss., p. 5) Also, Berlin (Nederlandsch. Lancet, 1853 and 1854, 3 ser., 16–34) investigated the formation of the crystals in leeches. Meckel, Archiv f. d. Holländ. Beitr. zur Natur- u. Heil-Kunde, 1, 90, 1858. |
| Monkey (*Cynocephalus babuin*) | Small rhombic plates (Preyer) | ............... | Extraglobular.... | The fresh crystals readily soluble in cold (Preyer) | Crystallizes with difficulty (Preyer) | 1 produced the crystals by the addition of water and alcohol to the blood of a monkey poisoned with santonin. (Max Schultze in his Archiv, 1866, p. 195. Illust., plate iii.) |
| Bat............... | Thin plates with very acute angles | ............... | Do............ | ............... | ............... | I have only once seen a poor preparation. Kunde first produced the crystals (needles). Zeitschr. f. rat. Med., 1852, p. 285. |
| Hedgehog (*Erinaceus europæus*) | Right-angled elongated prisms | Probably rhombic (Preyer) | Do............ | Extraordinarily readily soluble in cold water (Bojanowski) | Crystallizes readily from the blood of the chloroformed animal | Illustration, Zeitschr. f. wiss. Zool., xii, plate xxx. fig. 8; Lehmann saw the hedgehog crystals 1853. I obtained prismatic crystals from the blood of a chloroformed hedgehog. |
| Mole (*Talpa europæa*) | ............... | ............... | ............... | ............... | ............... | F. Hoppe-Seyler, Handb. d. physiol. u. pathol.-chem. Analyse, 2 Aufl., Berlin, 1865, p. 201. |
| Cat (*Felis domestica*) | 4-sided prisms truncated by 1 or 2 obliquely placed planes | Rhombic (Rollett) | Extraglobular ... | In cold water not readily soluble; in warm very readily (Bojanowski) | Crystallizes readily | Illustration, Zeitschr. f. wiss. Zool., xii, plate xxx, fig. 7, and Funke's Atlas, x, 3. Compare Funke, Journ. f. prakt. Ch., 1852, lvi, 195, and Rollett, Sitzungsber. d. Wien. Akad., 1862. Funke's statement that the crystals are clinorhombic is incorrect (Zeitschr. f. rat. Med., 1852, 291). |
| Lion (*Felis leo*)...... | 4-sided prisms which terminate in 2 obliquely placed truncating planes (Preyer) | Rhombic (Preyer) | Do............ | ............... | ............... | The crystals were produced in 1866 by Theodor Deecke in Lübeck. Berlin already saw lion crystals in 1856 (Nederlandsch. Lancet, v, 734). I investigated the very beautiful Deecke preparations, which, however, after 4 months became completely useless. Instead of the crystals only fine grains appeared and the spectrum was that of oxygen-freed hemoglobin. The crystals were kept at room temperature instead of in the cold. |
| Cat (*Felis marmorata*) | Prisms as in the lion (Preyer) | Do............ | Do............ | ............... | ............... | Produced 1866 by Deecke. |
| Cougar (*Felis puma*) | Do............ | Do............ | Do............ | ............... | ............... | The same. |
| Fox............... | ............... | ............... | ............... | ............... | ............... | Hoppe-Seyler, Med. chem. Unters., ii, p. 182, 1867. |
| Polecat............ | ............... | ............... | ............... | ............... | ............... | Kühne, Lehrb. d. physiol. Chemie, p. 198, 1868. |
| Dog (*Canis familiaris*) | 4-sided prisms bounded by a perpendicularly or an obliquely placed plane (Preyer) | Rhombic........ | Intraglobular and extraglobular | In cold water not readily soluble, in warm very readily soluble | Crystallizes easily | Illustration of the crystals apparently colorless, but in reality not appearing red because of their thinness, in Funke's Atlas, ix, 5. Funke saw also rhombic plates (in venous splenic blood) with $\frac{60^\circ}{120^\circ}$ (Zeitschr. f. rat. Med., 1851, p. 190). Compare Kunde in Zeitschr. f. rat. Med., 1852, p. 271. Kölliker shows intraglobular crystals (Mikroskop. Anat., 1854, ii, 2 Hälfte, fig. 271, p. 280). |

TABLE 31.—*Preyer's table showing the source of hemoglobin crystals, etc.*—Continued.

| Name of kind. | Crystal form. | Crystal system. | Appearance. | Solubility in water. | Crystallizability. | Remarks. |
|---|---|---|---|---|---|---|
| Guinea-pig (*Cavia cobaya*) | Tetrahedral (sphenoids) only apparently regular because the angles deviate slightly from 60° (von Lang) | Rhombic (v. Lang) | Intraglobular and extraglobular. Illustrations made by Beal (Quart. Journ. of Microscopic Sc., 1864, 32–43. The crystals have a tendency to lie beside each other in saw-tooth form | Very difficultly soluble | Crystallizes very readily | Lehmann's statement (Chem.-pharm. Centralbl., 1853, p. 98) that occasionally regular octahedrons are also found rests on a gross error; so also is Hoppe's statement that the crystals are tetragonal incorrect. Moleschott's information (Pathologie u. Physiologie, Giessen, 1866, p. 42) that he also obtained 6-sided plates from guinea-pig blood can be explained in this way, that the tetrahedrons are so abundantly packed together that they apparently result in planes bounded by 6 sides. Illustration in Funke's Atlas, X, 4. Reichert, Müller's Archiv, 1849, taf. II, fig. 6. Kunde, Zeitschr. f. rat. Med., taf. IX, fig 2 (1852). |
| Squirrel (*Sciurus vulgaris*) | 6-sided plates and 6-sided prisms, often grouped rosette-like | Hexagonal (von Lang, Rollett, Kunde, Preyer) | Extraglobular ... | Very difficultly soluble | Crystallizes easily | Illustration in Funke's Atlas, X, 5. Lehmann's statement, that the crystals do not belong to the hexagonal system, is incorrect. Kunde gives an illustration in Zeitschr. f. rat. Med., taf. IX, fig. 3 (1852); also Kühne, Lehrb., p. 200. |
| Mouse (*Mus musculus*) | 6-sided plates (Bojanowski).<br>Tetrahedrons(?) (Lehmann).<br>Fine needles (Kunde) | Hexagonal ...... | Do............. | Very readily soluble (Bojanowski).<br>Very difficultly soluble (Lehmann) | Crystallizes easily (Bojanowski and Lehmann) | Illustration, Zeitschr. f. wiss. Zool., XII, taf. XXX, fig. 5. From the heart blood of the mouse I obtained only small prismatic crystals. Kunde (Zeitschr. f. rat. Med., N. F., II, 1852, 285) obtained with water, without any additional mixture, needles and "prismatic plates." |
| Rat (*Mus rattus, Mus decumanus*) | Tetrahedrons (Kunde, 1852).<br>Tetrahedrons (Lehmann, 1853).<br>Prisms (Bisegger, 1852) | ................ | Intraglobular .... | Very difficultly soluble (Lehmann) | Crystallizes very easily (Lehmann) | Hoppe-Seyler (Handbuch, 1865, p. 202) obtained crystals by simply diluting the blood with water. Compare Kunde in Zeitschr. f. rat. Med., 1852, N. F., II, 276, Bisegger and Brach found the crystals "prismatic." (Verhandl. d. naturforsch. Ges. zu Basel, 1, 1857, 174.) |
| Rabbit (*Lepus cuniculus*) | Right-angled, elongated rhombi, prisms | Rhombic (v. Lang) | Extraglobular.... | Extraordinarily readily soluble (Bojanowski) | Crystallizes rather difficultly | Illustration in Zeitschr. f. wiss. Zool., XII, taf. 30, 2, and in Rollett, Vers. u. Beob. am Blute, Wien, 1862. Compare the same, p. 25. Kunde (Zeitschr. f. rat. Med., 1882, p. 284) obtained the crystals simply by addition of water, as also did Teichmann (same place, 1853, 376). Budge, Spec. Physiol. 8 Aufl., p. 250. |
| Hamster (*Cricetus vulgaris*) | Rhombohedrons and 6-sided plates (Lehmann). Angle: $\frac{60^\circ}{120^\circ}$ (Lehmann, 1853) | Hexagonal (?) ... | Do............. | ................ | ................ | Illustration in Funke's Atlas, IX, 6. Kunde also saw the crystals. |
| Marmot (*Arctomys marmotta*) | Column-shaped crystals (Valentin) | ................ | ................ | ................ | Crystallizes not easily | Valentin, in Moleschott's Unters. z. Naturl., 1863, IX, 131. |
| Horse............. | 4-sided prisms and rhombic plates | Rhombic (Funke) 1851 | Extraglobular.... | Readily soluble.. | Crystallizes easily | W. Kühne, Med. Centralbl., 1863, No. 53, p. 833. Illustration in the Zeitschr. f. rat. Med., N. F., 1 Bd., 1851, taf. 1, figs. 4, 5, 6. Funke obtained the crystals from diluted venous splenic blood, Kunde (same, 2 Bd., 1852, p. 285) from jugular venous blood. Funke found the angle 60° 9′ and 119° 32′. |
| Sheep............. | Prisms......... | ................ | Do............. | ................ | Crystallizes with difficulty | I have seen prismatic crystals in the wether's blood after removal of the gases. But it is very difficult to bring the blood to crystallization in any other way. |
| Ox............... | Little columns placed beside each other in palisade fashion (A. Schmidt).<br>Needles with double end planes (Kunde, 1852).<br>Prisms (Preyer) | Most probably rhombic (Preyer) | Do............. | Very readily soluble in cold water | Crystallizes with extraordinary difficulty | See A. Schmidt, in Virchow's Arch., XXIX, p. 1, 1864. Funke also saw the crystals. Kunde obtained them by means of ether (Zeitschr. f. rat. Med., 1852, p. 284), Teichmann (ibid., 1853, 376) by allowing the blood to evaporate after dilution with 4 to 5 times its volume of water. |

TABLE 31.—*Preyer's table showing the source of hemoglobin crystals, etc.*—Continued.

| Name of kind. | Crystal form. | Crystal system. | Appearance. | Solubility in water. | Crystallizability. | Remarks. |
|---|---|---|---|---|---|---|
| Pig (*Sus scrofa domestica*) | Prisms (Preyer) | | Intraglobular | | Crystallizes with extraordinary difficulty | Compare Funke, Journ. f. prakt. Ch., LVI, 195, and Zeitschr. f. rat. Med., 1852, 201, and Klebs, Med. Centralbl., 1863, No. 54, p. 852. I also saw the crystals in every blood corpuscle. Funke refers to nets of crystal rods in the compounds. Meckel (Archiv f. d. Holl. Beitr. z. Nat.- u. Heilkunde) saw the intraglobular crystals also. Teichmann obtained them by the evaporation of diluted blood (Zeitschr. f. rat. Med., 1853, 376). |
| Owl (*Strix noctua*) | 4-sided plates (Preyer) | Most probably rhombic (Preyer) | Extraglobular | | Crystallizes easily (Preyer) | I obtained owl-blood crystals by letting a drop of blood, 2 days old, stand between the object-glass and cover-glass at room temperature. |
| Raven (*Corvus*) | Sphenoids | Very probably rhombic | Do | Very difficultly soluble in cold, not readily soluble in warm water (Bojanowski) | Crystallizes with difficulty | Illustration in Bojanowski, Zeitschr. f. wiss. Zool., XII, taf. 30, fig. 12. |
| Crow (*Corvus corone*) | Rhombic plates and comb-shaped and fan-shaped grouped prisms (Preyer) | Same as above | Do | | Crystallizes easily | I obtained very large crystals from frozen heart blood. |
| Lark (*Alauda cristata*) | Needle-shaped crystals ending very pointedly | | Do | Very difficultly soluble in cold, very readily soluble in warm water (Bojanowski) | | The crystal form is not clearly recognizable from the illustration in Bojanowski, Zeitschr. f. wiss. Zool., XII, taf. 30, fig. 9. |
| Sparrow | Like the lark crystals | | | | | The crystals were produced by Bojanowski, Zeitschr. f. wiss. Zool., XII, 334. |
| Pigeon | Sphenoids | | | | | Bojanowski found the pigeon-blood crystals similar to the raven-blood crystals, *loc. cit.*, p. 335. Hoppe-Seyler finds that dove-blood crystals are more easily produced pure than dog-blood crystals. Kunde, Zeitschr. f. rat. Med., N. F., II, 285, and Teichmann (in the same place, 1853, 376). |
| Domestic goose | Large 4 or 6-sided rhombic plates | Rhombic (?) | | | | Hoppe-Seyler finds that goose-blood crystals, according to his method, can be more easily produced pure than dog-blood crystals. |
| Lacerta | Prisms | | Intraglobular | | | According to Kölliker. |
| Turtle (*Testudo græca*) | Needles and plates | | | | | Kunde, Zeitschr. f. rat. Med., N. F., II, p. 285. |
| Python (*Python schneideri*) | Prisms and plates | | Extraglobular | | | Berlin found, 1856, the blood of python crystallizable. He also saw crystals in the stomach of the *Amblyomma exarnatum*, a blood-sucking parasite which the snake had brought with it from Senegal to Europe (Nederlandsch. Lancet, 3 serie, 5 Jaargang, 1855–56, p. 739). |
| Python (*Python bivittatus*) | | | Intraglobular and extraglobular | | | Zeitschr. f. wiss. Zool., 1849, I, 266 (Kölliker). |
| Frog (*Rana esculenta*) | Prisms | | Intraglobular | | Crystallizes with much difficulty | Illustration in Virchow's Archiv, XXX, taf. 15, fig. 4, and Bullet. de l'Acad. de St. Pétersbourg, VIII, 561–572. Teichmann (Zeitschr. f. rat. Med., 1855, p. 379) obtained the crystals by mixing the defibrinated blood with very much water and allowing it to evaporate at a low temperature, but since he obtained them *colorless*, it is doubtful if they consisted of hemoglobin. I saw the crystals in extravasated blood in the lymph-sac. |
| White-fish (*Leuciscus dobula*) | Do | | Intraglobular and extraglobular | | Crystallizes very easily | Illustration in Funke's Atlas, taf. x, fig. 6. Funke saw the direct change of the blood corpuscles to crystals, and on the addition of water blood corpuscles were again formed. |

TABLE 31.—*Preyer's table showing the source of hemoglobin crystals, etc.*—Concluded.

| Name of kind. | Crystal form. | Crystal system. | Appearance. | Solubility in water. | Crystallizability. | Remarks. |
|---|---|---|---|---|---|---|
| Carp (*Cyprinus carpio*) | Scaly-shaped crystals (Funke) | | | | Crystallizes very easily on the addition of water | Funke, in Zeitschr. f. rat. Med., 1851, 191. Kunde, ibid., 1852, p. 286. |
| Red-eyed roach (*Cyprinus erythrophthalmus*) | Prisms | | Extraglobular (Remak) and intraglobular (Funke) | Even more readily soluble than the tench-blood crystals (Remak) | Crystallizes very easily | Remak (Müller's Arch., 1852, 121) found the crystals 2 hours after death in the blood-vessels. Funke (Zeitschr. f. rat. Med., 1852, 200) saw the change of the crystal containing blood corpuscles into the ordinary ones on the addition of water. |
| Barbel (*Barbus fluviatilis*) | Spindle- and needle-shaped crystals | | Extraglobular | | | Kölliker, Mikroskop. Anat., 1854, II, 2 Hälfte, p. 281. |
| White bream (*Abramis blicca*) | Prisms | | Intraglobular and extraglobular (Funke) | | Crystallizes very readily | Funke saw the change of the blood corpuscles into crystals and the rechanging of the same on the addition of water, and could recrystallize them 3 to 4 times on the object-glass. |
| Tench (*Tinca chrysitis*) | Small, thin plates tapering at both ends | | Extraglobular | Crystals dissolve very easily in water (Remak) | Do | Remak (Müller's Archiv, 1852, 121) saw the crystals always 24 hours after the death of the animal in thick bundles in the vessels and in the heart. His statement that they are readily soluble in ether and alcohol rests on delusion (see Zeitschr. f. rat. Med., 1852, 213). The crystals can be recrystallized on the object-glass. |
| River bream (*Cyprinus brama*) | Prisms | | Do | Easily soluble | | Bojanowski, illustration in Zeitschr. f. wiss. Zool., XII, t. 50, 4. Kölliker saw the crystals, 1849. |
| River perch (*Perca fluviatilis*) | Needles | | Extraglobular and intraglobular | As in the red-eyed roach (Remak) | Crystallizes very easily | Kölliker saw the crystals first (Todd's Cyclop. of Anatomy and Physiology, 1849, pt. 36, Lond., p. 792, "Spleen"). Remak found them 2 hrs. after death in the blood-vessels (Müller's Archiv, 1852, 121). Illust. in Kölliker's Handbuch der Gewebelehre, 1863, 4 Aufl., p. 627). |
| Herring (*Clupea harengus*) | Plates and rods (Bojanowski) | Very probably rhombic | Extraglobular | Very readily soluble | Crystallizes with extraordinary difficulty | Illustration in Zeitschr. f. wiss. Zool., XII, taf. 30, 11 (Bojanowski). |
| Sole (*Platessa vulgaris*) | | | Intraglobular | | | Ankersmit, Diss., p. 53. |
| Pike (*Esox lucius*) | 4-sided prisms | Probably rhombio | Extraglobular | | | Illustration in Zeitschr. f. wiss. Zool., XII, 334, fig. 10 (Bojanowski). |
| Horn-fish (*Belone rostrata*) | Do | Do | Do | Very readily soluble | Crystallizes easily | Illustration in Zeitschr. f. wiss. Zool., XII, taf. 30, 10 (Bojanowski). |
| Earthworm (*Lumbricus terrestris*) | Very delicate needle-shaped crystals (Preyer) | | Do | | | The crystals are formed if a drop of earthworm blood is allowed to evaporate slowly. |
| Horse leech (?) (*Nephelis*) | Small tabular plates, little rods and columns (Leydig) | | In the stomach of Clepsine. | Readily soluble | | Zeitschr. f. wiss. Zool., I, 1849, p. 116. Illustration in the same. Leydig, Lehrb. d. Histologie, 1857, 446, taf. 8, fig. 34 B. |

# CHAPTER VI.

## THE PREPARATION AND CRYSTALLOGRAPHY OF HEMOGLOBINS SINCE PREYER'S INVESTIGATIONS.

Since the appearance of Preyer's monograph very little progress has been made either in the methods of preparing the blood crystals or in the study of their crystalline characters, although much has been added to our knowledge of hemoglobin in certain other directions. Such simple methods as have been described for preparing crystals in small quantities, together with Hoppe-Seyler's method for preparing them in large quantities, have proved so satisfactory for general laboratory purposes that there has been little encouragement to seek new processes; while Preyer's long list of crystals from different species, and the assignment of all of them to the rhombic system, except those of the squirrel, seem to have discouraged research along the lines of crystallography. In fact, with rare exceptions when crystals from a new species have been isolated, the observer has been content without further inquiry to record them as being rhombic. We are therefore now, so far as the crystallography of hemoglobin is concerned, virtually where we were when Preyer's monograph was published (1871).

The method of furthering crystallization by the putrefactive process, already pursued by a number of observers, was adopted by Gscheidlen (Archiv f. ges. Physiologie, 1878, XVI, 421), who placed defibrinated blood in a glass vessel with little air, and kept it in an incubator until the absorption spectrum showed the absence of oxyhemoglobin. When a drop of this blood was placed on an object-glass, allowed to evaporate slightly, and then covered with a cover-glass, crystals appeared under the eye of the operator. From dog's blood which had stood for several days in the incubator he obtained crystals from 3 to 4 mm. long. The rapid crystallization of the blood thus prepared he found to be due to putridity, since blood kept in sterilized vessels under the same conditions showed far less power of crystallization. In guinea-pig's blood kept in the incubator with the admission of air he found not only large tetrahedra, but also rhombic plates and prisms. By this method Gscheidlen prepared crystals from the blood of the dog, guinea-pig, sheep, bullock, rabbit, and goose. He also noted that blood kept in hermetically sealed tubes for several years crystallized in a short time upon exposure to the air. The readiness with which putrid blood crystallizes had already been noted by Schmidt, Böttcher, Blebs, and others.

A method by Kühne and Gamgee (Gamgee's Physiological Chemistry, 1880, 87) is as follows: 500 c.c. of defibrinated dog's blood are treated with 31 c.c. of ether and the mixture shaken for some minutes. It is then set aside in a cool place. After a period varying from 24 hours to 3 days the liquid becomes converted into a thick magma of crystals. The crystals may be separated by placing the mixture in tubes and using the centrifugal apparatus. The cakes of crystals thus obtained are mixed with water holding one-fourth its volume of alcohol, and again centrifugalized. By repeating this process, the crystals are said to be obtained free from serum-albumin. If requisite, the crystals are recrystallized by dissolving them in as small a quantity of water as possible at 25° to 30°, cooling the solution to 0°, and adding a fourth of its volume of alcohol. It is better to place the fluid in a freezing mixture at a temperature of —10° to —20° for 24 hours.

Crystals of reduced hemoglobin were prepared by Hüfner (Zeit. f. physiol. Chemie, 1880, IV) from human blood, diluted or not, by placing the blood in tubes from which air is excluded. After standing for a month or two at summer temperature the blood became of a beautiful purple color, and in many spots on the inner wall of the tubes there could be seen whole layers of purple-red crystals, which upon spectroscopic examination were found to give the characteristic bands of reduced hemoglobin. Wedl (Archiv f. path. Anat. u. Physiologie, 1880, LXXX, 172) obtained reduced hemoglobin crystals expeditiously by subjecting a solution of fresh or dried blood in a confined atmosphere in the presence of a solution of pyrogallic acid. The acid absorbs the oxygen and thus reduces the hemoglobin. In this way crystals of reduced hemoglobin were prepared within 24 hours from the blood of man, the rabbit, hare, deer, pig, and sheep.

Crystals of reduced hemoglobin were prepared in large quantities by Nencki and Sieber (Berichte d. d. chem. Ges., 1886, XIX, 128, 410), who, however, make the erroneous statement that no one had up to that time prepared crystals of reduced hemoglobin. Kühne (Archiv f. path. Anat. u. Physiol., 1865, XXXIV, 423), and shortly after Rollett (Sitzungsb. d. k. Akad. d. Wissensch., Wien, 1866, LII, 246), obtained crystals of reduced hemoglobin by reduction of concentrated solutions of oxyhemoglobin. Kühne used a very concentrated solution of oxyhemoglobin in very dilute ammonia, which he subjected to a stream of pure dry hydrogen in a glass chamber. As evaporation proceeded crystals formed. Rollett (*loc. cit.*) prepared reduced hemoglobin by the aid of iron filings. Gscheidlen in 1878 (*loc. cit.*) and Hüfner (*loc. cit.*) and Wedl (*loc. cit.*) in 1880 also prepared crystals of reduced hemoglobin.

Nencki and Sieber proceed in this way: Pure oxyhemoglobin crystals from the blood of the horse are dissolved in lukewarm water; the solution is then mixed with several cubic centimeters of decaying blood in a flask that is provided with an india-rubber stopper having two perforations for tubes leading to and from the flask. The mixture is then freed from air by the passage of a stream of hydrogen, after which the two tubes are sealed by heat, and then the flask is set aside at a temperature of 20° to 25° for 8 to 14 days. After a time every trace of oxygen has disappeared, the fluid is of a beautiful violet-red color, and contains only reduced hemoglobin. The solution is now cooled to 0°, an india-rubber tube is for some distance slipped over the outlet tube of the flask, and the other end of the tube is dipped in cold absolute alcohol. The flask is gently heated by immersing in lukewarm water, the end of the glass tube within the rubber tube is broken off, and by alternate cooling and heating of the flask sufficient alcohol is introduced so that the solution contains about 25 per cent of alcohol. The free end of the rubber tube is now closed by a screw clip and glass stopper and the solution is subjected to a temperature of 5° to 10°. After 12 to 24 hours the reduced hemoglobin has crystallized into glittering plates and prisms. When examined under the microscope at 0° in the mother-liquor, the crystals for the most part appear as 6-sided plates, of which some were from 2 to 3 mm. in diameter. In the microspectroscope every crystal showed only the one band of reduced hemoglobin. The prismatic crystals are doubly refracting. The color of the larger plates is a beautiful violet red; the smaller thin plates appeared greenish in transmitted light. The crystals were very sensitive to oxygen and warmth. At room temperature they quickly melt, and as quickly they lose their violet color and show by the microspectroscope the bands of oxyhemoglobin. In absolute alcohol they remain unchanged, at least in so far as their form is concerned. If the hemoglobin solution is mixed too soon with alcohol, before the bacteria have taken up the last traces of oxygen, both reduced-hemoglobin and oxyhemoglobin crystals are formed.

Besides the differences they describe in the color and spectroscopic behavior Nencki and Sieber also make note of differences in the forms of oxyhemoglobin and reduced hemoglobin. From horse's blood they obtained oxyhemoglobin in long 4-sided columns, and the reduced hemoglobin in thin 6-sided plates which are more soluble in water than the oxyhemoglobin. In horse's blood which has decomposed in well-closed or sealed vessels, the reduced hemoglobin separates as a thick crystal pulp on the addition of alcohol after standing several hours at a temperature under 0°.

Gamgee (Schäfer's Text-book of Physiology, 1898, 1, 232) gives a method for preparing reduced hemoglobin which he states he employed 20 years previously, and which seems to him to possess some advantages: A magma of pure oxyhemoglobin crystals and a small quantity of the mother-liquor are placed in a glass tube so as nearly to fill it, and the tube sealed and heated for some days in an incubator at about 35° and then set aside in a cool place. After some weeks of exposure at winter temperature crystals of reduced hemoglobin will be found. Crystals of reduced hemoglobin have also been prepared by Ewald, Frey, Uhlik, Copemann, Donogány, and others, as will be shown by subsequent references.

The changes in solubility of crystals of hemoglobin that are caused by alcohol were studied by Struve (Ber. d. d. chem. Ges., 1881, XIV, 930; Jour. f. prakt. Chemie, 1884, XXIX, 304), who found that fresh crystals placed in strong alcohol immediately became darker, without change of form, and insoluble in water. Upon treating crystals with dilute alcohol, they became faintly yellowish or completely decolorized. These and other phenomena led Struve to resurrect the long since abandoned view that the blood crystals are composed of a colorless albuminous substance which is stained or colored mechanically.

After leeches have sucked blood and crystallization has begun, specimens of hemoglobin crystals may be obtained from time to time, as shown by Stirling and Brito (Jour. Anat. and Physiology, 1882, XVI, 446), by causing the leeches to disgorge. To do this they applied pressure, an 8 per cent salt solution, weak to strong acetic acid, 2 to 1000 solution of sulphuric acid, or galvanic or faradic shocks. Within 20 days, hemoglobin crystals appeared, which was much earlier, they state, than was noted by Budge and Bojanowski; but no hemin crystals were found, as were thought by Bojanowski to be present at times. Even after a year and a half they found dusky-red purplish crystals of reduced hemoglobin of human blood in the form of 4-sided prisms, some of them nearly equal-sided, while others were oblong. From the stomach of the leech they obtained crystals from the blood of the common gold-fish, and also obtained crystals from sealed microscopic preparations of the diluted fish blood. From the blood of the frog they secured both colored and colorless crystals of exactly the same form. The former they describe as being very variable in size, highly refractive, acicular, and pointed at one extremity like the point of a pen. Stirling and Brito note that colorless crystals of frog's blood had also been discovered by Teichmann (*loc. cit.*), who mixed the defibrinated blood with water and evaporated at low temperature. Besides obtaining these crystals from the blood of the stomach of the leech they also prepared them by mixing 5 or 6 drops of the freshly drawn blood from the heart with one or two drops of distilled water, and then sealing up the preparations with gold size. They state, however, that exposure to the air favors the formation of the crystals, which first form around and in the neighborhood of coagula. In the case of one of the leeches, on exposing some of the blood on the fourth day, they obtained blood which, when sealed up and allowed to stand, developed beautiful colored crystals of exactly the same shape as those which are colorless. The sole difference, they state, was in the color, and they therefore were inclined to regard the latter as being closely related to hemoglobin, if not identical with it. They did not find any crystals from the blood of the newt that had been ejected by the leech.

Studies were also made of the influences of certain reagents on the crystallization of rat's blood. Stirling and Brito found that common salt and urine prevented the diffusion of hemoglobin from the corpuscles, and therefore prevented crystallization; but a weak solution of pure urea behaved exactly like water, liberating the hemoglobin and thus permitting of crystallization. From this they conclude that the presence of common salt in the urine is sufficient to neutralize the effect of the urea. The crystals found in the solution were exactly the same as those formed after the addition of water. Crystals appeared in a few minutes when chloroform was freely mixed with a drop of rat's blood on a slide and covered and examined in the usual way, but the ordinary flattened prisms with beveled ends were shortened so as to be hexagonal. They also made the interesting observation that the passage of a galvanic current causes a deposition of crystals equally well at both negative and positive poles, but that the induced current was without effect.

The use of chinolin to increase crystallizability was reported by Otto (Zeit. f. physiolog. Chemie, 1882, VII, 57). He employed an alcoholic solution or an aqueous solution of the hydrochlorate of chinolin, and by its aid prepared crystals of pig's blood. He notes that Hüfner previously found that the blood of the pig mixed immediately with one-third of its volume of a 1 per cent alcoholic solution of chinolin crystallizes beautifully when subjected to cold, the mixture after several days containing a mass of needles and plates which liquefied within an extremely short time when exposed at room temperature under the microscope. Otto used chinolin solutions and blood in varying proportions, as follows: (*a*) 100 c.c. of blood, 40 c.c. of 1 per cent chinolin hydrochlorate solution, and 30 c.c. of alcohol; (*b*) 100 c.c. of blood, 30 c.c. of chinolin solution, and 30 c.c. of alcohol; (*c*) 100 c.c. of blood, 25 c.c. of chinolin solution, and 25 c.c. of alcohol. The mass of crystals which had collected during 8 days was washed on a filter-paper with alcohol (diluted 4 times) and then dissolved in a small quantity of water. Adding to this solution one-eighth its volume of alcohol, the mixture was again placed in the cold, whereupon crystals sometimes separated within a few days. As a rule, the second crystallization failed to occur, and instead a mass separated out in from 8 to 14 days, which was found to be methemoglobin.

The unsatisfactory results of this method led Otto to adopt what is practically the Hoppe-Seyler method: The blood was diluted with salt solution and stood in a cylindrical vessel for two days. The corpuscles were collected and dissolved in the smallest possible quantity of water at 50°, 300 c.c. of water being sufficient for the solution of the corpuscles from 1 liter of blood. Owing to the unusual solubility of the crystals of pig's blood, which liquefy at room temperature, it is very important, as Otto states, to avoid an excess of water in dissolving the corpuscles. The solution is filtered, cooled, mixed with cold absolute alcohol in the usual proportion of 4 : 1, and then subjected to cold. As a rule, after only one day a thick mass of fine, bright-red needles was found at the bottom of the cylinder. For the purpose of recrystallization, the crystals were collected upon a folded filter-paper, washed, and crystallized 3 times with dilute alcohol in the ice-chest. He prepared dog's crystals in the same way. The crystals were finally spread upon plates and dried under a bell-jar over sulphuric acid in the cold. The crystals of pig's blood thus prepared were then powdered, heated to 115°, and subjected to a stream of hydrogen, when they gave off 5.9 per cent of water. Those of the dog similarly treated lost only 4 per cent of water. Both kinds of crystals were subjected to elementary analyses (page 71).

Otto also analyzed the methemoglobin of the pig. Studies were also made of the extinction coefficients (page 77) and of the oxygen capacities. The crystals were determined by the spectroscope to be oxyhemoglobin. In a later research (Archiv f. ges. Physiologie, 1883, XXXI, 240) Otto prepared crystals of horse's blood, which he also subjected to elementary analysis and spectroscopic examination. In his former investigation he determined that the extinction coefficients of the oxyhemoglobin of the pig and dog are the same (1.33), and in this inquiry the extinction coefficient of horse oxyhemoglobin was found to be 1.352. His elementary analyses are given on page 71. He also noted the observation of Hoppe-Seyler

(Zeit. f. phys. Chemie, 1878, 11, 149) that the crystals of horse hemoglobin appear to be of two kinds (needles and prisms) which differ in solubility—a difference which Hoppe-Seyler thought likely to be due to differences in the amount of water of crystallization. Otto states that there continually appeared, besides little needles which are in relatively greater abundance in recrystallization, long, thick prisms which prevail in the first crystallization. He endeavored, but failed, to separate the needles from the prisms by washing with dilute alcohol, as Hoppe-Seyler states could be done. He also tried to determine differences in the water of crystallization, but he failed to obtain concordant results.

Crystals from horse's blood were prepared by Hüfner and Bücheler (Zeit. f. phys. Chemie, 1884, VIII, 355) by the ordinary alcoholic method and recrystallized three times in a refrigerator. Generally needles were obtained from 2 to 3 mm. long and 0.5 mm. wide. Once they found hexagonal tablets of reduced hemoglobin, which changed quickly upon coming in contact with the air. Dried at 0° over sulphuric acid and anhydrous phosphoric acid, the crystals retained 3.94 per cent of water, which came off when the crystals were subjected to a stream of hydrogen at 115°. They made elementary analyses (page 71), calculated the molecular weight and formula (page 75), and determined the oxygen capacity.

A new method for preparing hemoglobin crystals was reported by von Stein (Centralblatt f. med. Wissensch., 1884, XXII, 404; Archiv f. path. Anat. u. Physiologie, 1884, XCII, 483), which is applicable to small quantities of blood that are readily crystallizable. A drop of defibrinated blood or blood squeezed from a clot was placed on an object-glass and exposed to the air until it began to dry up at the margins. Canada balsam was then applied, first around the drop of blood, in order to prevent any possible escape, and then the remaining space above it was filled. It is to be observed, von Stein states, that the center of the drop of blood is pushed off to the periphery. In this way a clear space is made for crystallization, otherwise the crystals are so small that their outlines can not be made out. Too thick a layer of blood is to be avoided, because the balsam does not penetrate to the deeply lying portions. Von Stein proceeded in another way, by not allowing the blood to evaporate, and by treating it immediately with the reagent and covering the mixture with a cover-glass. Canada balsam is best when it appears yellow and not entirely clear. In liquid balsam the crystals form more quickly, and sometimes have larger dimensions, but they soon become brown (in one or two days), then dull and black, and in a short time are fissured to small pieces. Preparations can be made which retain their form and color for years if the balsam has been exposed to the air for a long time, or is evaporated to such a consistence that it can be drawn out into transparent but not milky threads when lifted with a glass rod. Whichever method is used, it is important that the preparation be left uncovered in the air until the crystallization has been completed, and until the odor of the balsam has completely disappeared, which lasts ordinarily a few days. Then with a knife immersed in ether, turpentine, or oil of cloves (little should be used of either), the upper portion of the balsam is removed, and the whole covered with a cover-glass and sealed with asphalt or balsam. Crystals from human, horse, guinea-pig, and rat blood were obtained by the above methods.

Von Stein's methods were extended by Smreker and Zoth (Sitzungsber. d. Wiener Acad., 1886, XCIII, Abth. III; Maly's Jahr. ü. d. Fort. d. Thierchemie, 1886, XVI, 102), who used Canada balsam, turpentine, Peru and other balsams; solutions of colophony, damar, and mastic dissolved in xylol; fixed oils; xylol solutions of rosin; fatty acids, etc.

The doubt as to whether or not hemoglobin is a chemical individual, together with the fact of the discrepancies in the centesimal analyses of hemoglobin, led Zinoffsky (Zeit. f. physiol. Chemie, 1886, x, 16) to prepare crystals of hemoglobin in several ways and to make careful determinations of the iron and sulphur contents. In preliminary experiments he found that the washing of the corpuscles by common salt solution, according to the directions of Hoppe-Seyler, is not only superfluous, but also undesirable, because the washing introduces the danger of decomposition, owing to the fact that from 3 to 5 days are required in the process, and because it is not of importance in removing the small quantity of protein in solution. In experiments in relation to the separation of the hemoglobin from the stromata he found that, when the corpuscle pulp is heated to 35° with 3 volumes of distilled water, the hemoglobin dissolves and crystallizes and that the stromata remain undissolved and cling so tenaciously to the hemoglobin crystals that they can not be removed by filtration. They must, therefore, be dissolved before the crystallization of the hemoglobin, either (1) by the addition of very little ammonia to the fluid heated to 35°, which must then be carefully neutralized with dilute hydrochloric acid (according to the direction of Schmidt), or (2) by the addition of ether (30 c.c. of ether being sufficient for 9 liters of blood). To crystallize the hemoglobin the solution was cooled to 0°, mixed by titration with one-fourth its volume of absolute alcohol, and left standing for 72 hours. The crystals were washed by decantation with a mixture of 1 part of alcohol to 4 parts of water cooled to 0°. To obtain pure crystals, the crystals were dissolved in 3 volumes of distilled water at 35°, the solution was filtered, and the filtrate was mixed with dilute alcohol as before. Tests showed that two recrystallizations of the first product sufficed to obtain pure crystals.

Zinoffsky also makes note of the fact that the drying of the crystals *in vacuo* at 0°, according to the directions of Hoppe-Seyler, is an exceptionally lengthy process, and that the crystals can be dried in about 8 hours at 18° to 20° without being placed in a vacuum. After these preliminary investigations he prepared crystals from horse blood by three methods, viz:

*First method:* 20 liters of horse's blood were defibrinated; the blood-corpuscle pulp, which after 3 hours' standing in the cold had been deposited, was separated from the serum and mixed with 8 volumes of a 2 per cent solution of common salt. After 3 days the corpuscles were collected and placed in 3 volumes of distilled water at 35°, to which were then added 16 c.c. of one-tenth normal ammonia solution. After 5 minutes the ammonia was neutralized by titration with a very dilute hydrochloric acid. The mixture was quickly cooled to 0°, to which was then added 1 volume of absolute alcohol at 0° to every 4 volumes of the solution. After 3 days' standing in an ice-and-salt mixture, the crystals were collected and washed twice with alcohol and water (1 : 4) at 0°, and then, in the way stated, recrystallized twice and finally dried by the aid of the air-pump. This preparation yielded 200 grams.

*Second method:* The blood-corpuscle pulp obtained by decantation from 10 liters of blood, and without washing with salt solution, was dissolved in 3 volumes of water at 35°, and then treated as in the first method. The yield was 520 grams. The very much larger quantity thus obtained led Zinoffsky to believe that washing with salt solution reduces the yield.

*Third method:* The blood-corpuscle pulp from 9 liters of blood was dissolved immediately in 3 volumes of distilled water at 35°, then cooled; 30 c.c. of ether were added instead of ammonia, as in the first method, and then the solution was treated as in the first method.

The product by the third process was the purest, the ash containing but a trace of chlorine, no alkalies, and only imponderable quantities of phosphorus, lime, and magnesia. The product by the first process contained 0.0235 per cent of phosphoric acid. The second preparation was the least pure. It contained 0.0401 per cent of phosphoric acid, 0.0097 per cent of CaO, and 0.0131 per cent of MgO.

TABLE 32.—*Table from Zinoffsky, showing the percentages of sulphur and iron, the number of atoms of sulphur to each atom of iron, and the amount of sulphur and iron in the ash of hemoglobin.*

| Material and author. | S. | Fe. | Atoms of S to each atom of Fe. | Amounts of S and Fe in the ash of hemoglobin. S. | Amounts of S and Fe in the ash of hemoglobin. Fe. |
|---|---|---|---|---|---|
| Dog's blood: | *Per cent.* | *Per cent.* | | | |
| Schmidt | 0.66 | 0.43 | 2.686 | 1.6637 | 1.6637 |
| Hoppe-Seyler | .375 | .45 | 1.60 | 1.9972 | 1.7062 |
| Do | .448 | .42 | ..... | 1.4033 | 2.5122 |
| Do | .359 | .42 | ..... | 1.8915 | 4.7855 |
| Horse's blood: | | | | | |
| Bücheler | .6532 | .46370 | ..... | 1.8481 | 0.9439 |
| Do | .6443 | .47238 | 2.427 | 1.8132 | |
| Do | ...... | .46720 | ..... | ...... | ...... |
| Kossel | .65 | .47 | 2.42 | ...... | ...... |
| Otto | .67 | .45 | 2.60 | 2.2876 | 4.0866 |

Zinoffsky in the earlier part of his article shows (table 32) the marked discrepancies in the results of the analyses by different observers of specimens of bloods from different individuals of the same species. They are also of particular interest in connection with the figures obtained by Zinoffsky in this research.

The two sulphur determinations of the first preparation were 0.3902 and 0.3916 per cent; of the second preparation, 0.3583 and 0.3658 per cent; and of the third preparation 0.3899 and 0.3881 per cent. In the determinations of iron he found in the first preparation 0.325 to 0.327 per cent and in the third preparation 0.334 to 0.338 per cent. These results show, he states, that there are 2 atoms of sulphur to 1 atom of iron. The mean of his elementary analyses is

$$C_{51\cdot15}H_{6\cdot76}N_{17\cdot94}S_{0\cdot3899}Fe_{0\cdot335}O_{23\cdot4251}$$

and the molecular formula

$$C_{712}H_{1130}N_{214}S_2FeO_{245}$$

Comparing Zinoffsky's percentages with those of other analysts (see page 71), it seems as though there must be errors in his carbon and hydrogen estimations. Moreover, his iron and sulphur determinations differ materially from those of others, yet his analyses were conducted in such a way as to warrant confidence in these figures. The low C content is certainly suggestive of imperfect combustion, or, according to Hüfner, of contamination with stromata. Zinoffsky's work has been reviewed and supplemented by Hüfner (see later).

The optical properties of oxyhemoglobin, reduced hemoglobin, methemoglobin, hemin, and CO-hemoglobin were studied by Ewald (Zeit. f. Biologie, 1886, xxii, 459). He laked the blood by repeated freezing and thawing, and then spread layers of varying thickness upon microscopic slides. The margins of the preparations soon dry, and then a cover-glass is placed directly on the blood or supported by a wedge of glass. If the preparations are examined immediately, only oxyhemoglobin crystals will be found; but after several days violet-purple spots appear which consist of reduced hemoglobin, but which soon pass into solution. He also obtained crystals of reduced hemoglobin by letting the blood stand in tubes for several days; in the deeper layers the oxygen disappears and crystals of reduced hemoglobin form. He found the crystals of oxyhemoglobin and reduced hemoglobin to be doubly refracting and pleochroic, and that the pleochroism is much more marked in reduced hemoglobin.

In a research to determine whether or not the 6-sided crystals of certain rodents really belong to the hexagonal system, and to find an explanation of the difference in crystalline form that hemoglobin presents in different animals, Halliburton (Jour. of Physiology, 1886, vii, Proc. Physiol. Soc. No. 1; Jour. Microscop. Science, 1887–88, xxviii, 181) carried out a series of observations chiefly with the bloods of the rat, guinea-pig, and squirrel. The rat was taken as a type of animals whose crystals are rhombic; the guinea-pig, of those whose crystals are tetragonal; and the squirrel, of those whose crystals are hexagonal. Halliburton notes that Lehmann states, without giving any reason, that although the crystals of the squirrel are hexagonal in form they do not belong to the hexagonal system, and that von Lang, Kunde, and Preyer state that they do. In examinations of squirrel's crystals by polarized light he found evidence, as he believes, of their being true hexagons instead of their being, according to Lehmann, rhombic plates with an "hexagonal habit." It had already been found by von Lang that the tetrahedra of the guinea-pig belong to the rhombic system.

In experiments instituted to show whether differences in crystalline form are due to some agency extrinsic to the hemoglobin or to some property inherent in the hemoglobin, he in some instances mixed serum, or the serum and the stromata, or the blood of one species of animal with the blood of another, or solutions of hemoglobins of different species with one another.

The presence of the foreign serum, or serum and stromata, was without influence on crystalline form, and, while mixed bloods, or mixed hemoglobin solutions, did not affect crystalline form, they sometimes caused modifications in crystalline habit. Thus, in case of the bloods or solutions of hemoglobins of the rat and guinea-pig the crystals of the rat were rhombic with hexagonal habit, no needles or tetrahedra being present. (See table 33.)

TABLE 33.—*Forms of hemoglobin crystals in case of mixed bloods (from Halliburton).*

| Blood of— | Mixed with that of— | Form of hemoglobin crystals from the mixture. |
|---|---|---|
| Rat | Squirrel | Both rhombic prisms and hexagons present. |
| Rat | Guinea-pig | No rhombic prisms of the shape usually seen in rat's blood present. No tetrahedra. Crystals are all rhombic prisms with hexagonal habit. |
| Squirrel | Guinea-pig | Hexagonal plates and tetrahedra both present. Many tetrahedra imperfect. The tetrahedra were all reduced to about half the size of those prepared from the unmixed blood of the same guinea-pigs. |
| Dog | Squirrel | Fine rhombic needles and hexagonal plates both present in abundance. |
| Dog | Guinea-pig | The greater number of the crystals formed are very small tetrahedra, about a quarter the size of those prepared from the blood of the same guinea-pigs. The optical properties are, however, the same. Rhombic prisms very slender, like those of dog's blood, also seen. |

In another set of experiments Halliburton tried to break down the hexagonal constitution of the hemoglobin of squirrel's blood, first, by drawing off the water of crystallization and then adding water; second, by converting the hemoglobin into methemoglobin, and then by reducing agents to form once more hemoglobin, and to obtain crystals from this. Both attempts were unsuccessful.

In opposition to the statement of Preyer that recrystallization does not alter the form of the crystals, Halliburton found that by recrystallization of squirrel's hemoglobin, after 3 or 4 recrystallizations no 6-sided crystals were obtained, but a mixture of rhombic needles and tetrahedra, and that in some cases the latter were absent. In conclusion, the author states that the difference between the various forms of hemoglobin can not be a very deep or essential one, and that it seems to narrow itself down to this, either we have a case of polymorphism or the crystalline forms are due to the combination with varying proportions of water of crystallization.

In the second contribution referred to, Halliburton adds the following to our crystallographic data:

Opossum (*Didelphis cancrivora*).—Very large dark crystals can readily be obtained. They belong to the rhombic system.

Kangaroo (*Macropus giganteus*).—Crystals are more soluble, and so less readily obtained. They are rhombic prisms, slenderer than in the opossum.

Sugar squirrel (*Belideus breviceps*—a marsupial).—Crystals similar to those of opossum.

Seal (*Phoca vitulina*).—Rhombic prisms, many of them very short and simulating hexagons. Easily obtained.

Bear (*Ursus syriacus*).—Bunches of rhombic needles, easily obtained. They are slenderer than those obtained from dog's blood, as a rule, some being almost silken in appearance.

White-bellied beaver rat (*Hydromys leucogaster*).—Rhombic prisms.

White-whiskered swine (*Sus leucomytax*).—Rhombic prisms.

Water vole (*Arvicola aquatica*).—Crystals are obtained easily by adding water to the blood. They are of the usual rhombic shape.

The analyses of hemoglobin of horse's blood by Zinoffsky (*loc. cit.*) differed so much from those of previous observers that Hüfner (Beiträge z. Physiologie, Fest. f. Carl Ludwig, 1887, 74) was led to review and supplement Zinoffsky's work. Hüfner prepared hemoglobin crystals by a process that is a modification of Zinoffsky's to the extent essentially of separating the stromata of the corpuscles by mechanical instead of chemical means, that is by centrifugalization, so that the crystals could be freed from the stromata dissolved or undissolved and more expeditiously prepared. Crystals were obtained from the bloods of the pig and ox by centrifugalizing the corpuscles, extracting the hemoglobin from them by distilled water at 30° to 40°, cooling to 0°, centrifugalizing and treating by the usual method. After the crystals have formed they are centrifugalized in the cold to prevent their solution, and the hemoglobin is then three times crystallized by the usual method, and finally dried in an atmosphere at 0°. The ash of 10 grams of this product contained only an imponderable amount of phosphoric acid. The mean figures of his elementary analyses are as follows:

Pig's oxyhemoglobin, $C_{54 \cdot 71}H_{7 \cdot 38}N_{17 \cdot 43}S_{0 \cdot 479}Fe_{0 \cdot 339}O_{19 \cdot 602}$
Ox's hemoglobin, $C_{54 \cdot 66}H_{7 \cdot 25}N_{17 \cdot 76}S_{0 \cdot 447}Fe_{0 \cdot 40}O_{19 \cdot 543}$

In comparing these figures with those of Otto (*loc. cit.*), Hüfner states that the complete removal of the stromata in his preparations causes a higher percentage of C and N, Otto having found $C_{54 \cdot 17}$ and $N_{16 \cdot 23}$. Zinoffsky's C content (51.15) was very much lower than Hüfner's. Hüfner's analyses show the same ratio of S and Fe in both pig and dog hemoglobins, *i.e.*, 2 of sulphur to 1 of iron, the same as Zinoffsky found with horse hemoglobin.

The elementary analysis of the hemoglobin of the dog which was reported the following year by Jacquet (Zeit. f. physiol. Chemie, 1888, XII, 285) was of crystals prepared as follows: The corpuscles were centrifugalized, then mixed with 2 volumes of water warmed to 35°, then cooled and shaken with ether and treated according to the Hoppe-Seyler process. The crystals were twice recrystallized, and then analyzed according to the methods pursued by Zinoffsky, but he endeavored to eliminate certain possible fallacies in the iron determinations. His analyses gave a mean

$$C_{53 \cdot 91}H_{6 \cdot 62}N_{15 \cdot 98}Fe_{0 \cdot 333}O_{22 \cdot 62}$$

These figures do not agree with those of Zinoffsky for the hemoglobin of the horse, or with those of Hüfner for the hemoglobins of the pig and ox. The relation of Fe to S in dog's hemoglobin, Jacquet found to be 1 : 2.85; Zinoffsky found 1 : 2 for the horse; and Hüfner found 1 : 2 for the pig and ox. Jacquet believes his sulphur value to be too small, and that there is 1 atom of Fe to 3 of S. Later (Zeit. f. physiolog. Chemie, 1889, XIV, 289) he analyzed crystals of the hemoglobin of the dog, which he prepared by a modification of his previous process. The earlier method was used, except that the solution of the corpuscles after the addition of the ether was centrifugalized in a machine running at from 1,600 to 2,000 revolutions, whereby the stromata could be partly done away with. The hemoglobin 3 times crystallized contained only a trace of phosphoric acid, the quantity not being estimated. The mean of his analyses was

$$C_{54 \cdot 57}H_{7 \cdot 22}N_{16 \cdot 38}S_{0 \cdot 568}Fe_{0 \cdot 336}O_{20 \cdot 93}$$

The amount of water of crystallization was 11.39 per cent. The ratio of Fe to S was 1 : 2.96, which was higher than in his preceding investigation. From the values obtained he gives the formula $C_{758}H_{1203}N_{195}FeO_{218}$, and the molecular weight as 16669.

He also analyzed the oxyhemoglobin of the chicken. In the preparation of the crystals he made a special effort to get rid of the large amount of phosphoric acid (0.77 per cent) shown in the preparations of goose crystals by Hoppe-Seyler. He found that he could not treat the blood in precisely the same way as dog's blood, because when the corpuscles are agitated with ether a gelatinous mass is formed which could not be filtered. The corpuscles were therefore treated with an equal volume of water and one-third volume of ether. The mixture when heated to 35° formed into dark-red, gelatinous lumps which were separated from the fluid by centrifugalization. The clear fluid thus obtained was readily filtered, and by the customary treatment very soluble needles of hemoglobin were formed. By recrystallization both rhombic plates and prisms were obtained. The three times crystallized hemoglobin was found upon analysis to have the following composition:

$$C_{52 \cdot 47}H_{7 \cdot 19}N_{16 \cdot 45}S_{0 \cdot 8586}Fe_{0 \cdot 3353}O_{22 \cdot 5}P_{0 \cdot 1973}$$

The water of crystallization was 9.333 per cent, and the ratio of Fe to S 1 : 4.485. If the molecule be doubled the ratio is 2 : 9. In comparing the analyses of the oxyhemoglobin of the dog, chicken, and horse, he states that although these hemoglobins are different they have a similar iron capacity, which warrants the conclusion that the iron-containing group in the various hemoglobins is the same. Jacquet made ineffectual attempts to prepare crystals from fresh salmon blood, but succeeded when the blood was left to rot, there appearing clusters of crystals and beautiful single rhombic prisms.

Jolin (Archiv f. Anat. u. Physiologie, 1889, 265) records that the hemoglobins of the dog and guinea-pig differ from that of the goose in their absorptive rapidity in relation to O, as well as in the volume of O absorbed.

The increased crystallizability of putrid blood has been noted by a number of observers and referred to in previous pages, and Bond (London

Lancet, 1887, II, 509, 557) has added to our knowledge in this particular by showing a relationship between crystallizability and septic conditions in the body. He found that if a drop of blood were taken from the cleansed finger of a patient who is suffering severely from absorption of the products of putrefaction, and that if such drop be placed between a slide and a cover-glass and allowed to remain at room temperature (60° F.), in the course of 20 to 30 hours crystals of reduced hemoglobin of prismatic and needle form will be found, while within some corpuscles little bars and needles may plainly be seen, apparently distinct from the enveloping stroma. He also found that adding putrid blood facilitates crystallization, and that in cases of pernicious anemia crystallizability seemed to be increased.

The increased crystallizability of human hemoglobin in pernicious anemia that was pointed out by Bond (*loc. cit.*) was later noted by Copemann (Journal of Physiology, 1890, XI, 401), who found that when a drop of blood from the finger of a patient thus affected was allowed to fall on a glass slide, the edge of the drop allowed to dry, and a cover-glass placed on the blood, crystals of hemoglobin gradually formed in from 10 to 48 hours. The only exception to this was in the case of patients who had been treated with arsenic for some days, although crystals were obtained upon the discontinuance of the arsenic.

To imitate the influence of septicemia, as was also shown by Bond, Copemann treated the blood with decomposing serum. This method he found to be successful in the case of the bloods of the bullock, sheep, pig, dog, and cat, but unsuccessful for the blood of man, the monkey, rabbit, and squirrel. Except in the case of man and monkey the crystals were of oxyhemoglobin, and this notwithstanding that the decomposing serum invariably brought reduction of the oxyhemoglobin as it diffused from the corpuscles into the plasma. He states that this occurred to the greatest extent just inside of the edge of the cover-glass, but not extending to the edges where the layer is kept oxidized; and that it is in this intermediate zone of fully reduced hemoglobin that crystals are to be found in the greatest quantity, both in case of human and monkey blood and of that of the rabbit and squirrel; but in the latter the crystals are of oxyhemoglobin, while in the former they are of reduced hemoglobin. He also made the interesting observation that in specimens of squirrel's blood (species not stated) the crystals were in every instance in the form of fine needles and rhombic prisms, the needles sometimes being collected into bundles, while the usual hexagons were absolutely absent.

Copemann also prepared crystals from the blood of the horse, bullock, sheep, pig, dog, cat, squirrel, rabbit, guinea-pig, rat, mouse, and chicken by the following simple process: The blood is shaken with ether (16 : 1) and then kept under an atmosphere of ether for some time, which may be accomplished by performing the agitation of the blood with ether in a stoppered bottle and gradually allowing the air to escape as the ether is volatilized. By this means the contained air is gradually replaced by ether vapor, while at the same time the small portion of blood which is forced out around the stopper of the bottle on drying fixes it in its place and so prevents the ingress of air again. It seems also that it is better to leave the bottle in a room at ordinary temperature than to put it in a cool place, as advised by Gamgee. After a variable time, in case of most animals at least two days, a drop of blood is placed upon a slide, and when the margin of the drop is slightly dry a cover-glass is gently lowered on the surface of the drop. The formation of crystals will often be seen within an hour or so. Human blood subjected to the same process does not usually yield crystals, but when crystals do appear they invariably present the appearance of reduced hemoglobin. Copemann also obtained crystals from human blood by the use of bile—preferably, as he states, cat's bile.

Two methods for preparing hemoglobin crystals given by Mayet (Compt. rend. soc. biol., 1890, CIX, 156), and stated by him to be improvements on the method of Hoppe-Seyler, are as follows:

*First method:* The corpuscles are washed with sodium sulphate solution (1.5 per cent solution of the anhydrous salt) instead of sodium chloride solution. To wash the corpuscles, a glass vessel having the capacity of 5 liters is used, the upper part of the vessel being of cylindrical form and tapering conically, the lower part being in the form of a narrow cylinder which holds about 80 c.c. The latter part has at the bottom an opening which can be closed by a glass stopcock; a second opening, capable of being closed, is located where the upper conical and the lower cylindrical parts join. The treatment of the corpuscles with ether (one-fifth volume) is also performed in a special vessel consisting of a cylinder 3.5 mm. in diameter and 35 cm. long and extended by a conical part in the form of a narrow tube provided with a glass stopcock. To the blood solution is added one-fifth volume of absolute alcohol. This mixture is cooled at least 3 times for 12 hours at —14°. The crystals are separated and dissolved in water at 35°, the solution mixed with alcohol as before, and the hemoglobin at least 3 times crystallized by cooling to —14°. In this way crystals 1.5 mm. long were obtained from the bloods of the dog, horse, and ass.

*Second method:* The corpuscles are washed as above, the corpuscle pulp is shaken with water (1 volume) and pure benzine (one-fifth volume), and kept 24 hours at 5° to 8°. Then the solution is gradually mixed with one-fifth volume of absolute alcohol and treated in the usual way. The yield by the second process is the greater.

A study of the influences of various reagents upon the crystallization of oxyhemoglobin and reduced hemoglobin was made by Donogány (Mathematikai és természettudományi ertesitö, 1893, 11, 262; Maly's Jahr. ü. d. Fort. d. Thierchemie, 1893, XXIII, 126), who prepared crystals from the bloods of the dog, cat, pig, mouse, ox, rabbit, duck, guinea-pig, horse, and man. Donogány tested the usefulness of a number of the methods used for preparing oxyhemoglobin and reduced hemoglobin crystals, and he also made some examinations of the crystalline forms. Several of the methods were modified. To obtain oxyhemoglobin crystals from dog's blood, the "Canada balsam method" (*loc. cit.*) is recommended. A method of his own, which he believes equally as good, is as follows: A drop of blood is treated with a little ethyl bromide, methylene chloride, or ethylidene chloride.

From cat's blood, Donogány states, oxyhemoglobin crystals can be obtained by any of the usual methods except the methods of Gscheidlen, Rollett, and Wedl, by which only reduced hemoglobin can be produced. From horse's blood good results were recorded with Canada balsam, damar varnish, chloroform, amyl alcohol, pental, xylol, colophonium dissolved in amyl alcohol, pyrogallic acid, or by freezing. The crystals are doubly refracting, and they consist for the most part of oxyhemoglobin. With the methods of Gscheidlen and Wedl, crystals of reduced hemoglobin were obtained. If the Rollett method is used, combined with distilled water, a mass of reduced-hemoglobin and oxyhemoglobin crystals is formed. The blood of pigs, which is looked upon as crystallizing with difficulty, he found crystallized readily by the use of ethereal oils. The formation of crystals went on slowly, and the crystals were large and well developed. The crystals were doubly refracting and consisted of oxyhemoglobin.

From the blood of white mice crystals could not be produced by the aid of Canada balsam, distilled water, chloroform, ether, alcohol, or xylol. Ox blood did not crystallize by treatment with Canada balsam, damar varnish, ether, amyl alcohol, xylol, chloroform, pental, ethereal oils, or pyrogallic acid. By freezing and by Gscheidlen's method, combined with Canada balsam or damar varnish, only small needles could be obtained. From their light color, Donogány believes that they were probably oxyhemoglobin. They were doubly refracting. The coloring matter of the blood of rabbits also crystallized with difficulty. The addition of ether, Canada balsam, chloroform, pental, ethereal oils, and acetone gave negative results. With the method of Gscheidlen and with damar varnish, only small needles could be obtained. With Rollett's method rather large needles were formed. The best result was obtained with pyrogallic acid. The crystals formed, he states, consisted of reduced hemoglobin. The blood of the duck treated with damar varnish, xylol, ether, amyl alcohol, Canada balsam, chloroform, colophonium solution, distilled water, and by quick cooling, scarcely yielded crystals, and even in the most favorable instance only stunted ones. Gscheidlen's method, he writes, can be used with much better results, although here, too, crystallization goes on slowly. The crystals were purple-red, almost blue, needles or prisms, and consisted of reduced hemoglobin. Later these crystals, under the influence of atmospheric air, changed to flesh-colored rhombic, even 6-cornered tablets, which were doubly refracting, and consisted, perhaps, of oxyhemoglobin.

From guinea-pig blood Donogány produced crystals by means of Canada balsam. They formed quickly, and also became quite large if the Canada balsam used was not very thin and the preparation stood in a cool place. If form and size are not important good results can be obtained, he states, by means of ethylidene chloride. With damar varnish crystallization goes on somewhat slowly and at the sacrifice of sharp edges. Pyrogallic acid and valerian oil did not cause crystallization. Ether, chloroform, xylol, amyl alcohol, acetone, Canada balsam dissolved in xylol, freezing, a mixture of water and alcohol, and repeated treatment with Canada balsam gave only poor results. With ethyl bromide, after the course of an hour, the whole mass became crystalline, yet the crystals, because of their smallness, were unsuited to investigation. The most beautiful crystals could be obtained with ethylidene chloride according to the following method: A drop of blood is thoroughly mixed with an equal amount of ethylidene chloride, the cover-glass is placed on it, and the preparation set aside in a cool place. After the course of 10 to 12 hours it is entirely filled with crystals. With amyl nitrite or pyridine forms similar to these could not be obtained. All the crystals were oxyhemoglobin. Regarding the form of the oxyhemoglobin crystals of the guinea-pig, Donogány adheres, on the basis of the geometric and optical characteristics, to the view of von Lang that they are sphenoids belonging to the rhombic system. In a later article (Zeit. f. Krystallographie, 1894, XXIII, 499) Donogány publishes the results of his crystallographic studies of hemoglobin crystals, which will be referred to in later chapters of this memoir.

Crystals were easily obtained from human blood by pyrogallic acid and by the aid of putrefaction. Donogány first reduced the hemoglobin with a 10 per cent solution of sulphide of ammonium, which, however, is not necessary when using old decaying blood. After an interval of 5 to 6 hours crystals separate in the form of rather thick, flesh-colored or purple-red needles. After 12 to 24 hours the individual crystals are pretty well formed. Contrary to Wedl's assertion, Donogány observed that the crystals can not be kept, since they burst in the course of 2 to 3 months in spite of being properly sealed. He states that the crystals produced in decaying blood are of reduced hemoglobin and that they may be changed into oxyhemoglobin without change of form. He succeeded in producing only reduced hemoglobin directly from the human blood, and he believes it doubtful whether by the influence of atmospheric air these crystals can be changed to oxyhemoglobin. Oxyhemoglobin was prepared by means of Canada balsam, xylol, damar varnish, chloroform, alcohol, amyl and methyl alcohols, acetone, valerian oil, methylene chloride, and ethylene chloride. Pyrogallic acid and freezing gave only reduced hemoglobin. The crystals, he states, belong to the rhombic system. Wedl had produced reduced hemoglobin crystals by means of pyrogallic acid from dried blood 3 days old, and Donogány modified this method for the production of hemoglobin crystals from dry blood powder (1 year old). The powder was dissolved in a 5 to 10 per cent solution of sulphide of ammonium, pyrogallic acid was added, and crystals appeared after 10 to 12 hours. After the course of 24 to 48 hours crystallization had ceased. The crystals obtained from horse, cat, and rabbit blood in this way were very beautiful, and large crystals (1 cm. long) were not rare. The crystals were chiefly thin needles, broad prisms, and rhombic plates. In human blood, besides these forms, there appeared right-angled truncated prisms and forms similar to hexahedrons. Experiments with bloods of other animals gave less favorable results. The crystals were doubly refracting and consisted of reduced hemoglobin.

The sulphate of ammonium process devised by Hofmeister (Zeit. f. physiol. Chemie, 1890, XXIV, 165) for preparing crystals of egg albumin, and subsequently used by Gürber (Würzburger physiol. medizin. Ges., 1894, 113) and others for crystallizing serum albumin, has been used by Dittrich (Archiv f. exper. Path. u. Pharm., 1892, XXIX, 250) and others for preparing crystals of hemoglobin. Owing to the rapid conversion of hemoglobin into methemoglobin by this process, Dittrich used it also to prepare the latter. The blood of the horse was subjected to the Hoppe-Seyler process for preparing the blood-corpuscle pulp. The corpuscles were then dissolved in ether, the solution filtered, and then mixed with two volumes of a cold saturated solution of ammonium sulphate, filtered again, and then placed in flat vessels in the cold. Generally within 24 hours crystallization begins, but occasionally only after 2 to 3 days. The crystals could be recognized microscopically in transmitted light as glittering elongated prisms or broad plates. The crystals of the first crystallization were not pure; moreover, the mother-liquor contained, besides crystals, an amorphous precipitate which often could be separated only by repeated recrystallization. Generally a separation of "globulites" and spherocrystals preceded the formation of crystals. The most of the crystal mass of oxyhemoglobin changed on standing in the air, and through the processes of recrystallization, gradually and completely into methemoglobin. No further change, for example the formation of hematin, took place. The crystal pulp, recrystallized several times from ammonium sulphate solution, was finally pressed between absorbent paper, and when dry was saved in this condition. This method of production of methemoglobin renders superfluous the use of ferricyanide of potassium or any other agent, the ammonium sulphate in large quantities being sufficient to change the hemoglobin to methemoglobin. Finally, the crystal pulp with the contained ammonium sulphate is permanent, and its solubility is not lost. If, however, the preparation is completely dried over sulphuric acid *in vacuo*, the largest part of the methemoglobin is changed to an insoluble modification.

Schulz (Zeit. f. physiol. Chemie, 1899, XXIV, 454) used essentially the same process for preparing oxyhemoglobin for his studies of globin. Horse's blood was rendered incoagulable by ammonium oxalate, the corpuscles were collected by decantation and then diluted with 2 volumes of water. If the solution obtained in this way is mixed with a like volume of cold saturated ammonium sulphate solution, there is formed an abundant precipitate which consists essentially of fibrinogen and serum globulin. The precipitate after a time becomes so compact that it can be separated by filtration, but, since the hemoglobin begins to crystallize immediately, filtration is rendered difficult because the pores of the filter become quickly clogged. In the completely clear filtrate crystallization soon begins, but the quantity thus obtained is small because of the separation on the filter. If the hemoglobin solution and the ammonium sulphate solution are warmed to 40° before mixing, the separation of crystals takes place less quickly, so that the filtrate obtained is almost completely free from blood-coloring matter. If, on the other hand, both solutions are cooled in an ice-chest before the mixing, and the solution after the mixing is allowed to stand until the albuminous precipitate has completely settled, the crystallization of the hemoglobin is almost completely prevented before filtration. If the solution is filtered in the ice-chest, a clear, dark-red filtrate is obtained, which contains most of the coloring matter, and when brought to room temperature soon yields a rich crystal formation, which increases if a little concentrated ammonium sulphate solution is added. After several days the separation is complete—so complete that the filtrate appears almost colorless. It is purely crystalline, without amorphous admixtures. The crystals are without exception little rhombic plates, some of very considerable size, while ordinarily crystals of horse hemoglobin, produced according to Hoppe-Seyler, separate in the form of long 4-sided prisms. The precipitate is filtered on a Büchner filter by the aid of a Sprengel pump and finally freed from the mother-liquor by pressing between filter-paper, then dissolved in water, and again separated by the addition of an equal volume of a saturated solution of ammonium sulphate. In this way the hemoglobin can be recrystallized with ease several times. Ammonium sulphate effloresces on the surface of the firm cake that had been obtained by pressing, and can easily be removed. The cake when dried in the air can be crushed to a fine powder, which readily dissolves in water. The solution shows a pure oxyhemoglobin spectrum.

Schulz states that in this way the hemoglobin may be separated from other proteins. Fibrinogen and serum globulin separate completely in a half-saturated ammonium sulphate solution, while the albumin separates only by a higher concentration of the ammonium sulphate than was used here. While as mentioned the oxyhemoglobin, according to the method used by Dittrich (*loc. cit.*), changes to methemoglobin, even during the recrystallization, a pure oxyhemoglobin can also be obtained by this method. The preparation thus obtained is, however, limited in stability; in one case it contained after about one year considerable methemoglobin. The limit of the quantity of ammonium sulphate required for the precipitation of the hemoglobin in the amorphous condition, incidentally noticed, is distinctly higher than that for crystallization. An amorphous precipitate occurred only when in 10 c.c. of the solution there were 6.5 c.c. of concentrated ammonium sulphate solution. In the tests which contained 5, 5.5, and 6 c.c., respectively, of the saturated ammonium sulphate solution in 10 c.c., no amorphous separation occurred, but after longer standing crystallization gradually took place.

This method of preparation, according to Schulz, is good because of its convenience for experiments not depending on preparations free from salt.

The ammonium sulphate method was also used by Spiro (Zeit. f. physiol. Chemie, 1899, XXVIII, 182). The corpuscle pulp was obtained from oxalated horse's blood by decantation, diluted with 2 volumes of water, cooled in an ice-chest, after which the solution was agitated with ether in the proportion of 1,000 c.c. of blood-corpuscle pulp to 50 to 70 c.c. of ether. During continual stirring a saturated solution of ammonium sulphate in the proportion of 700 c.c. to 1 liter of blood corpuscles was gradually added, the ammonium sulphate solution having the same temperature as that of the blood corpuscles. After 5 to 10 minutes the voluminous precipitate which has formed begins to rise; but if this does not occur more ether must be added, care being exercised to avoid a great excess, since hemoglobin may be precipitated by it. Within several hours a light-red deposit has formed on the surface, while the fluid below appears clear and a dark granite-red. The mixture is filtered, and the filtrate is kept in an ice-chest. After 2 days only an insignificant quantity of crystals has formed. These crystals are suspended on the top of the mixture and are filtered off. The filtrate, which contains almost all of the hemoglobin, is poured into large porcelain vessels and set aside at room temperature. The hemoglobin separates at first as red and later as brownish crystals. After 3 days almost all of the hemoglobin has crystallized, so that the filtrate appears to be colored only slightly brownish. Microscopically investigated, the crystals contain only slight impurities which can eventually be eliminated by recrystallization. The hemoglobin is best drained on Büchner filters until the formation of firm cakes. To recrystallize, the crystals are dissolved in the least possible amount of water and mixed with ammonium sulphate (100 c.c. of the hemoglobin solution to 80 c.c. of saturated solution of ammonium sulphate). The yield from 5 liters of horse's blood was 1,500 grams.

Fluoride of sodium was added by Arthus and Huber (Compt. rend. soc. biolog., 1893, XLV, 970) to the list of inorganic salts that favor the crystallization of hemoglobin. They found that when to normal or defibrinated blood there was added an equal volume of a 2 per cent solution of fluoride of sodium, and the solution allowed to stand at room temperature, crystals of oxyhemoglobin could be obtained within a few days. They also state that crystallization is accelerated by the addition of 0.1 to 0.5 per cent of hydrochloric acid and by increasing the temperature to 40°. Crystals were prepared from the bloods of the dog, horse, cat, and guinea-pig. Guelfi (Rif. med., 1897, No. 10; Maly's Jahr. ü. d. Fort. d. Thierchemie, 1897, XXVII, 149) also reports success with fluoride of sodium. He obtained crystals from the bloods of the dog and guinea-pig by the addition of an equal volume of a 2 per cent solution of this salt and maintaining the mixture at a temperature of 40°. This method, he states, failed in the case of both arterial and venous human blood.

The statement by Bohr (*loc. cit.*) of his belief that oxyhemoglobin is not a homogeneous substance, and that it consists of a mixture of oxyhemoglobins which differ in elementary composition, molecular weight, and combining capacity with O, has been shown by Hüfner (Archiv f. Anat. u. Physiol., 1894, 130) to be untenable. Hüfner's researches proved that Bohr's methods for producing the several forms of oxyhemoglobin gave rise to mixtures of oxyhemoglobin with variable amounts of decomposition products. Hüfner made new studies of the photometric constants of oxyhemoglobin, reduced hemoglobin, and carbon-monoxide hemoglobin, and determined the absorption coefficients for O and CO. He concluded, from the constancy of the extinction coefficients, the O and CO capacities, and the percentage of iron, that in healthy fresh bullock's blood there is only one hemoglobin present, and that the blood-coloring matters of the higher animals have all, when freed from water, the same molecular weight and with it the same capacity for carbonic oxide and oxygen. Hüfner also noted that when horse's blood is crystallized in closed cylinders there appear in great abundance dark-red 6-sided plates, together with the well-known prisms. He states that if one observes under the microscope a drop of the fluid in which the crystals are suspended, before it is covered with the cover-glass, it will be seen that these hexagonal plates quickly melt, and that where they dissolve bundles of fine, bright-red, prismatic crystals suddenly shoot out. The dark-red hexagonal plates are crystals of reduced hemoglobin, as Nencki showed several years ago, while the bright-red prisms are of oxyhemoglobin. He found that horse's blood is particularly inclined to give crystals of reduced hemoglobin, and that in preparing crystals of horse's hemoglobin by the regular method, without particular exclusion of air, both forms appear at the same time.

Hemoglobin crystals from the bloods of the horse, ox, pig, and dog were prepared by Frey (Inaug. Dissert., Würzburg, 1894; Jahr. ü. d. Fort. d. Thierchemie, 1895, xxv, 108) by means of the dialyzing method of Gürber. The corpuscles were separated from the defibrinated blood by centrifugalization, mixed with 2 volumes of water, and placed in a dialyzer which was suspended in 30 to 70 per cent alcohol. Beautiful crystals were obtained after 3 to 24 hours. If a drop of blood be placed on a slide under a cover-glass, crystals form (primary crop) which dissolve as the blood becomes fully laked, when occasionally a second crop forms. By reduction the blood became yellowish, and after 3 or 4 hours it was violet-red and venous, and at the same time granules appear which finally separate towards the margin as distinct crystals. These crystals, Frey states, are of reduced hemoglobin and in addition to these are clusters of colorless crystals. Horse's blood crystallized most readily, and then that of the dog, and finally that of the pig with difficulty.

Kobert (Das Wirbelthierblut in mikrokristallographischer Hinsicht, 1901, 25) used the Gürber method to prepare crystals from the bloods of the dog and cat. Arthus (Compt. rend. soc. biolog., 1895, XLVII, 686) employed a similar method to obtain crystals of the horse and dog. The blood was prevented from coagulating by the addition of oxalate, and after the corpuscles had been separated by decantation they were mixed with 2 volumes of water and placed in a Kühne membrane dialyzer suspended in 90 per cent alcohol. Large masses of crystals were formed. Arthus in a later research (Zeit. f. Biolog., 1897, XXXIV, 444) modified his previous method: The corpuscles from oxalated blood were dissolved in 2 volumes of water and filtered, and the filtrate was placed in a parchment-paper tube which was suspended in 17 to 33 per cent alcohol. At room temperature oxyhemoglobin crystals 7 to 8 mm. long with sharp edges were formed. When stronger alcohol was used the crystals were impure owing to an amorphous precipitate.

Studies of the crystallographic characters of crystals from blood of the silkworm were made by Panebianco (Zeit. f. Krystallographie, 1897, XXVIII, 198). These crystals were colorless and it is doubtful if they were hemoglobin.

Crystals from horse, dog, and cat were prepared by Abderhalden (Zeit. f. physiol. Chemie, 1898, XXIV, 545). Success in the production of crystals, he states, depends upon using the least possible amount of water necessary to dissolve the blood corpuscles which have been freed as much as possible from serum. To horse's corpuscles he added 3 volumes of water; to dog's corpuscles, 2 volumes; and to cat's corpuscles, 1 volume. On the addition of alcohol in the cold, etc., according to the Hoppe-Seyler method, and twice recrystallization, he obtained, he states, absolutely pure crystals. His elementary analysis of cat oxyhemoglobin will be found on page 71.

The very simple and satisfactory "Canada balsam" method of von Stein (*loc. cit.*) for preparing small quantities of crystals from readily crystallizable bloods was again used by him in a later research (Archiv f. path. Anat. u. Phys., 1900, CLXII, 477) in a determination of the effects of certain reagents on crystallization of guinea-pig's blood. The addition of sodium chloride up to 2 per cent aided crystallization, while quantities beyond this hindered; calcium chloride, sulphureted hydrogen, and nitrous oxide hindered crystallization. Von Stein also noted variations in crystalline form from the typical tetrahedra to forms ranging from truncated tetrahedra to 6-sided plates.

A painstaking study of the crystallography of the crystals of pigeon's blood was made by Schwantke (Zeit. f. physiolog. Chemie, 1900, XXIX, 486). His results will be referred to fully in subsequent chapters.

A new method of getting rid of the stromata, which whether in suspension or in solution hinder the crystallization of hemoglobin, was devised by Schuurmanns-Stekhoven (Zeit. f. physiolog. Chemie, 1901, XXXIII, 296). The blood corpuscles are washed with 1 per cent salt solution by centrifugalization, and then shaken violently for 2 hours with asbestos wool. The blood-coloring matter passes into solution, while the stromata for the most part cling to the asbestos and are removed by filtration. By this method the hemoglobin is not brought in contact with ether.

The hemoglobin solution is placed in a parchment-paper dialyzer, which is suspended in 45 per cent alcohol, and put in an ice-chest. As soon as crystals begin to form on the wall of the dialyzer (after 24 to 48 hours) the contents of the dialyzer are placed in a cylindrical vessel and then set in an ice-chest until crystallization has been completed. The hemoglobin is not brought in contact with any more alcohol than is necessary for the crystallization. The crystal pulp is as far as possible freed by pressure from the mother-liquor, after which the crystals are dissolved in the smallest possible amount of water at 37°. This solution is again dissolved and placed in the dialyzer in 45 per cent alcohol. Crystallization begins much more quickly than the first time. After crystallization has been completed the crystals are separated from the mother-liquor and dried, first on porous plates and then in a porcelain bowl over chloride of calcium at room temperature.

In the monographs by Schulz (Krystallization von Eiweisstoffen und ihre Bedeutung für die Eiweisschemie, Jena, 1901) and Kobert (*loc. cit.*) much of the literature on the processes for preparing crystals of hemoglobin is referred to. The latter gives an account of blood crystals which he prepared from various species, and he attempts the support of the hypothesis of Hoppe-Seyler regarding the existence of the blood-coloring matter in the form of "arterin" and "phlebin."

Stewart (American Journal of Physiology, 1903, VIII, 102), in his studies of the actions of laking agents on the blood, found that intraglobular crystallization of necturus blood is very readily obtained by the action of various hemolytic agents.

A quick method for preparing crystals of oxyhemoglobin was reported by one of us (Reichert, American Journal of Physiology, 1903, IX, 97), who also made studies of the effects on crystallization by mixing the bloods of different species, etc. It was found that if to the blood of the dog there be added, before or after laking, from 1 to 5 per cent of ammonium oxalate, crystallization invariably begins immediately, and that any quantity of crystals can be obtained within a few hours at ordinary room temperature. If a drop of this blood be placed under the microscope, crystals will be seen to form at once near the margin of the drop, and to be deposited so rapidly that a solid mass is formed in a few minutes.

The blood of the horse does not yield quite so readily to this treatment. If a drop of blood so prepared be examined under the microscope, it will be found that crystallization will not begin usually at room temperature until after from 15 to 20 minutes or more, and that it will proceed slowly. Better results can be obtained if the blood be oxalated and centrifugalized, or set aside for the corpuscles to subside. The supernatant liquid is then poured off, and the remaining corpuscles are laked with ether.

Defibrinated blood of the rat, laked with water on a slide, and covered with a cover-glass after the margin of the drop has become dried, usually crystallizes very readily, as is well known. Quicker results can be obtained if the blood be oxalated before or after laking, and even more rapid crystallization occurs if the blood be laked with ether instead of water. Crystals form so rapidly in the oxalate-ether blood that a magma is formed in the test-tube within a few minutes.

The oxalate-ether process applied to the blood of the guinea-pig gives most satisfactory results. Crystallization does not proceed quite so rapidly as in rat's blood, yet within a minute or two innumerable tetrahedra appear, and practically complete crystallization can be obtained within a couple of hours. The blood of the necturus crystallizes readily when so treated. The crystals resemble in form those of the triple phosphates.

The rapidity with which crystallization begins and proceeds was found to be influenced decidedly both by the method of laking and the percentage of oxalate. Ethyl ether is a much better laking agent than water, and acetic ether is stronger than ethyl ether. The presence of any quantity of oxalate up to saturation increases crystallizability, but he found from 1 to 5 per cent to be the best; the larger the quantity the more is crystallization hastened. When more than 5 per cent is used, the oxalate also tends to crystallize upon the slide. If the blood be prevented from drying, as in the test-tube, the oxalate remains in solution. Asphyxial blood yields crystals more readily than normal blood.

If to the blood of one species, the blood, plasma, or serum of another species be added, the laking of the blood may be retarded, accelerated, or unaffected, according to the character of the mixture. The period required for laking may be prolonged 5 minutes or more. The crystallization of the oxyhemoglobin may be hindered or prevented in such mixtures. Thus, by varying the proportions of a mixture of the bloods of the dog and guinea-pig crystals from one or both may appear, but the process is invariably retarded, sometimes to a marked degree. If crystals of both kinds of oxyhemoglobin are deposited, those of one usually begin forming some time before those of the other, and the crystallization of one may be seemingly complete before crystals of the other are seen.

The interesting observation was also made that the typical forms of the crystals of certain kinds of oxyhemoglobin may be modified or completely changed when the bloods of two species are mixed. Thus, if to the blood of the rat there be added a definite percentage of the blood of the guinea-pig, crystals of the rat's oxyhemoglobin may appear in unaltered form, but most, if not all, of those from the guinea-pig's blood are changed; in fact, if any perfect tetrahedra are found, they will have been formed at the very end of the crystallization. If the proportions of the mixture be properly modified, not a single crystal of what can be identified as rat's oxyhemoglobin will appear, and all the crystals will be modified tetrahedra, spindles, and transitional forms between these. The spindles resemble Charcot's crystals in form, but not in color; they vary in size, some being very large, and some may have small spindles attached to them; they can be obtained having sharp edges if crystallization has not been too rapid.

This complete change in the form of the crystals of oxyhemoglobin when the bloods of two species are mixed, and the spindle-shaped form of the crystals, are, he believes, unique facts in the crystallography of this most important substance. (See Halliburton, page 115.)

Moser (Vierteljahresschr. f. gerichtl. Medizin, 1901, XXII, 44) asserts that differences in crystalline form afford a positive means of recognition of the origin of the blood, and that positive distinction can be made between human and animal blood. He examined blood stains of the fresh blood of man and 10 species of mammals and fish, and gives drawings of their appearance under the microscope. From the differences in shapes he infers differences in crystallization, which he reasons indicate differences in chemical constitution. No descriptions of the crystallographic or optical characters are given, and, as differences in the shapes of the crystals do not necessarily imply differences in crystal system, his conclusions are based upon insufficient data. Bonnel (Thèse de Paris, 1903; Jahr. ü. d. Fort. d. Thierchemie, 1903, XXIII, 182) showed, however, that the method of Moser is not worthy of recommendation because by this method he obtained from human blood crystals of different shapes. Friboes (Archiv f. ges. Physiologie, 1903, XCVIII, 434) also found that human blood treated in the manner described by Moser crystallizes in various forms. He notes that the bloods of certain animals show crystalline shapes which, with the exception of the bat and the goat, are distinguishable from human blood. He describes the different forms of the crystals he observed, but gives no definite crystallographic data by which they may be recognized. (See Chapter VII.)

In experiments with the blood of the horse, Uhlik (Archiv f. ges. Physiologie, 1904, CIV, 64) found that as putrefaction progresses the usual rhombic crystals of oxyhemoglobin are replaced by hexagonal, holohedral crystals of reduced hemoglobin. Table 34 indicates the influences of the condition of the blood and temperature upon crystallization.

TABLE 34.—*Effects of the condition of the blood and temperature upon crystallization according to Uhlik.*

| Condition of the blood. | Temperature and crystal systems. | | | | |
|---|---|---|---|---|---|
| | 0° | 5° | 10° | 15° | 20° |
| Fresh | Rhombic, abundant | Rhombic... | Rhombic, scarce | ............ | |
| Partly reduced; not any decomposition | Rhombic, scarce or none | ........... | ........... | ............ | |
| Reduced; beginning to decompose | Hexagonal, scarce or none | ........... | ........... | ............ | |
| Decomposed; inspissating | Hexagonal, abundant | Hexagonal, abundant | Hexagonal and rhombic | ............ | Rhombic. |
| Old, decomposed, and thick | Do...... | Do...... | Hexagonal, abundant | Hexagonal | Hexagonal and rhombic. |

Uhlik also notes that Pregl found that a thrice-crystallized hemoglobin appeared as hexagonal crystals. Crystals of reduced hemoglobin have been prepared and described by a number of investigators, as stated in previous pages.

The last hemoglobin to be obtained in crystalline form, excluding our own preparations, was prepared by Bardachzi (Zeit. f. physiolog. Chemie, 1906, XLIX, 465) from the blood of the sea-tortoise (*Thalassochelys corticata*). The blood was centrifugalized, the corpuscles mixed with water, and then set aside for several hours at 50°. The solution was then filtered, one-fifth volume of alcohol added to the filtrate, and the mixture placed in an ice-chest. Crystallization occurred quickly and abundantly in the form of plates. The crystals were soluble with difficulty in cold water.

For the purpose of analysis the crystals were dissolved in water at 40°, and after cooling one-seventh volume of alcohol was added, and crystallization obtained as before. The crystals were then centrifugalized off and dried in vacuum. The mean values of the elementary analyses were

$$C_{54\cdot77}H_{6\cdot99}N_{17\cdot07}S_{0\cdot38}Fe_{0\cdot41}$$

The absence of phosphorus is striking, since previous observers failed to obtain hemoglobin free from phosphorus from bloods that contain nucleated erythrocytes. The optical investigation by means of the Hüfner spectrophotometer showed decided agreement with the blood-coloring matter of such other animals as have been closely investigated up to this time. The average quotient was $e' : e = 1.561$, while Hüfner found the quotient to be 1.578. The calculation of the extinction coefficients and quotients of hemoglobin and methemoglobin agreed, he states, with those of other oxyhemoglobins and methemoglobins, so that the coloring matter of the blood of the tortoise, Bardachzi holds, is identical with that of mammals.

Abderhalden and Medigreceanu (Zeit. f. physiol. Chemie, 1909, LXIX, 165) report their preparation of crystals of goose hemoglobin free from phosphorus.

# CHAPTER VII.

## CRYSTALLOGRAPHY OF HEMOGLOBIN IN RELATION TO SPECIES, ACCORDING TO PREVIOUS INVESTIGATORS, WITH EXPLANATIONS OF VARIOUS CONTRADICTORY STATEMENTS, ETC.

As early as 1852 Kunde (Zeit. f. rat. Medicin, 1852, N. F., II, 271) and Funke (*ibid.*, 288) in coincident articles stated that the hemoglobin crystals of different species are different. Kunde prepared crystals from the bloods of a number of species, including the bat, dog, ox, horse, guinea-pig, squirrel, rat, mouse, rabbit, pigeon, and tortoise, and published some figures illustrating the shapes of the crystals. From these differences in the shape and from the differences in solubility he concluded that the blood crystals obtained from different species are not identical, but distinct and characteristic of the species. Funke was led to the same conclusion from the examination of the crystals from the blood of the horse, ox, pig, dog, cat, and several species of fish. While making no attempt to give an exact crystallographic description, Funke records a number of angles observed in two of the species examined. These contributions were almost immediately followed by an article by Teichmann (*ibid.*, 1853, III, 375), who states that from the same blood, and even in the same preparation, crystals of various forms may be obtained, from which and for other reasons he concludes that the differences are not in relationship to species, but accidental and due to exterior conditions.

Teichmann's statement seems to have arrested further interest in this subject until 10 years later, when it was taken up by Rollett (Sitzungsb. Math.-nat. Klasse d. k. k. Akad., Wien, 1862, XLVI, Abth. II, 85), and shortly after by Bojanowski (Zeit. f. wiss. Zoologie, 1863, XII, 312). Rollett prepared crystals from the bloods of man, the guinea-pig, dog, rabbit, squirrel, and cat, all of which preparations, with the exception of the last, he submitted to von Lang, a crystallographer, for crystallographic investigation. Von Lang's examinations were made with the microscope, and in some cases the optical characters were examined and a few angles recorded. Von Lang determined the crystal system in each case, and from his data Rollett concluded that while the crystals from different species are different they may all be included in two crystal systems, the orthorhombic and the hexagonal. The descriptions of von Lang are very brief, and no attempt at giving all of the crystallographic constants is made, but these are the first definite determinations on record of the systems of crystallization of hemoglobin.

Bojanowski reviewed the literature of hemoglobin crystals and prepared crystals from the blood of rabbit, mouse, dog, cat, hedgehog, river bream, pike, horn-fish, herring, lark, raven, and pigeon, and of man. He records that hemoglobin of various animals crystallizes in various forms and systems, and that he always obtained rhombic plates from the blood of man and many species of lower animals, regular 6-sided plates from the blood of the mouse and squirrel, tetrahedra from the blood of the guinea-pig, and prismatic crystals from the blood of the rabbit. Crystals from various kinds of blood which appear to possess a similar form still showed unmistakable differences in the sizes of the angles. From his investigations he reached the conclusion that the bloods of individual species have something specific and characteristic about them, so that it is *occasionally* even possible to determine the species of animal from whose blood the crystals were derived. Where, as in the case of human blood, as described by Funke, there appear to be two or more kinds of crystals in the same blood, Bojanowski considers that one of them is the characteristic form and the others undeveloped crystals. Thus, in human blood what he describes as the "right-angled plate" is, he believes, the characteristic form, while the "prisms and rhombic plates" are regarded as undeveloped forms of the right-angled plate. The descriptions given are very brief and incomplete: thus, the crystals from the dog are described as "rod-like crystals forming closely woven nets," and from the cat as "very regular *three*-sided rods," etc. The description of the crystals of dog's blood would apply equally well to any species whose hemoglobin crystals are rather insoluble, if the hemoglobin crystallized in prisms, for such hemoglobins form felted masses of capillary or long prismatic crystals. The prisms of reduced hemoglobin of the cat are not 3-sided, but nearly rectangular in section.

After a latent period in the study of the crystallography of hemoglobins for the 5 succeeding years the first contribution by Preyer appeared (Archiv f. ges. Physiologie, 1868, I, 395), which was shortly followed by his now classic and authoritative memoir (Die Blutkrystalle, Jena, 1871). When the former contribution was published blood crystals from 47 species of vertebrates had been recorded, and of these in only 10 cases had the crystal system been recorded. In his memoir these 47 species are enumerated and the data concerning them are given. Preyer evidently regarded the crystals obtained from different species as differing from one another, but he concluded with Rollett that they may all be included in the two crystal systems, the orthorhombic and the hexagonal. He states that "besides the crystal system there are other distinctions, as, for instance, the sphenoidal crystal of the guinea-pig, the 4-sided prisms of the dog, the 4-sided prisms and rhombic plates of man. These peculiar morphological shapes are obtained only from each animal, even after repeated recrystallizations; a definite form is peculiar to each animal and can not be changed to another form. The same holds good with solutions of hemoglobin. Yet little importance is to be attached to statements on the crystallographic differences of the hemoglobin of different animals, because neither is the same method of crystallization always used, nor is the blood always capable of being compared, nor has the measure of the crystallizability of any optional substance been found. It is the same with decomposability as with crystallizability—both vary according to the species of animal; but the investigations undertaken in this direction suffer from so many and such large errors that they prove nothing beyond what has long been known, that is, the different species and individuals." Preyer's statement that the form of the crystals can not be altered by repeated recrystallization, and that there is a constant and peculiar form in relation to each kind of animal, has been shown to be wrong by the records of Halliburton (page 115), Copemann (page 119), von Stein (page 127), Bonnel (page 129), Friboes (page 129), Moser (page 129), and Pregl (page 130).

The work of Preyer was so painstaking and exhaustive that his conclusions seem to have been accepted without question, and his dictum that all hemoglobins crystallize in the orthorhombic system with an exception which crystallizes in the hexagonal system seems to have absolutely discouraged investigation in the crystallography of hemoglobin, and such studies as have since been made have been chiefly with the view of distinguishing human blood from that of domesticated animals, for medico-legal purposes.

Of the papers treating of the crystallography of hemoglobin in relation to species from this standpoint, those of Guelfi (Giornal di Med. Legale, 1898; Maly's Jahr. ü. d. Fort. d. Thierchemie, 1898, 145) and Moser (Vierteljahr. ger. Med., 1901, XXII, 44) may here be noticed. Guelfi obtained "tetrahedral crystals" from guinea-pig's blood and "prismatic crystals" from dog's blood, using both fresh and dried blood in each case. Comparing these with crystals obtained from partly dried human blood, which crystals he describes as "needle-shaped," he states that they can be distinguished from each other so that "it can be definitely stated that neither the tetrahedra from the guinea-pig blood nor the prisms from the dog blood were from human blood."

Moser describes crystals obtained from the blood of about a dozen species of vertebrates including mammals and fish. His article is illustrated with drawings made from the appearances of the crystals under the microscope, but these are not accompanied by any exact crystallographic descriptions. The differences in the shapes of the crystals led him to the conclusion that differences in the forms of the crystals afford a positive means of recognition of the origin of the blood, and that in this way positive distinction can be made between human blood and the blood of other animals. The descriptions of the crystals are very brief and relate to their general morphology; this is true also of the drawings. No correlation of the different shapes of crystals found in the same species is attempted, and what are evidently different views of the same crystal are shown as different forms. It is obvious that he is distinguishing the different crystals merely and hazardously by their morphology. Moser's article has been the subject of adverse criticism, as will be pointed out.

Various observers have studied the shapes of the crystals obtained from the bloods of different species, and in a few instances the crystal system has been determined by crystallographic study, and from these data they have arrived at the conclusion that the bloods of different species may be distinguished by an examination of the hemoglobin crystals. On the other hand, this conclusion has been contradicted by many observers. Teichmann, for instance, as already stated, asserts that from the same blood, and even from the same preparation, he has obtained various crystal forms, and that still other forms may be produced by varying the method of preparation, from which he naturally concludes that the form of the crystal is something entirely accidental and dependent upon exterior conditions and not an essential character of hemoglobin. Others have made the observation that in the same blood several forms of crystals may be found. It has also been pointed out that crystals from the blood of a given species, as recorded by different investigators, are of different forms. Thus, Lehmann described the crystals from the guinea-pig as isometric tetrahedra, he also describes them as isometric octahedra; Moleschott states that they are 6-sided plates. Von Lang writes that they are only seemingly isometric, and that, while the angle of the triangular face is so near 60° that they can not be distinguished from isometric tetrahedra, the optical characters make them orthorhombic. Donogány measured the three angles of the triangular face of these crystals and records them as 64°11′, 60°50′, and 55°45′, which three angles it will be noted do not add up to 180°. Of course the explanation of the record of tetrahedra in the one instance, of octahedra in another, and of 6-sided plates in a third is very simple. All of these observers were examining crystals of the same substance, and all were, as von Lang and Donogány state, orthorhombic sphenoidal: in the case of the simple "tetrahedra" the right or left sphenoid only was observed; in the case of the "octahedra" the crystal was the combination of the right and left-handed sphenoids in approximate equilibrium; and in the last instance, of the 6-sided plates, the form seen was this combination observed normal to a sphenoid face upon which the crystal is flattened, causing the outline to be hexagonal. The outline of an octahedron looked at as it lies on one of its faces is hexagonal, but if it become flattened parallel to the face upon which it lies it appears at a casual glance to be a hexagonal plate.

Many such cases as that of the guinea-pig crystals have been noted, where the blood of the same species by varying the treatment, or even according to different observers, furnished crystals of diverse form; and many observers have been led to the conclusion that was reached by Teichmann, that the forms of hemoglobin crystals are variable in the same species, are perhaps even identical in different species, and that the differences are not to be relied upon for distinguishing the source of the blood in any given case.

When the article by Moser appeared it apparently revived interest in the subject of the differentiation of the crystals of different species, but his results were soon attacked by Bonnel (Thèse de Paris, 1903; Maly's Jahr. ü. d. Fort. d. Thierchemie, 1903, XXIII, 182) and by Friboes (Archiv f. ges. Physiologie, 1903, XCVIII, 434). Bonnel argued that because human blood treated in the way described by Moser crystallizes in different shapes the method is of no value. He points out that the method is not to be recommended for the purpose of distinguishing human and animal blood (although differences between these are to be detected), because it is only applicable to fresh blood and can not be applied to blood stains, at least if they are more than two weeks old. Friboes also attacks Moser's conclusion that the crystals serve to distinguish between human and animal blood. He states that normal human blood treated in the way described by Moser crystallizes in various forms, and that the crystals from dried human blood are different from any of these. Human blood obtained from the splenic vein and the umbilical vessels is again different from these, so that a uniform crystal shape for human blood does not exist. Thus, from fresh human blood are obtained 4-sided doubly refracting prisms, also sharp-angled rods split into brush-like forms at the end, and very characteristic rectangular plates arranged in step-like aggregates. From the blood of a young child he obtained long rectangular plates which he regards as still different. From the splenic vein he found crystals showing composite aggregates of the step-like arrangement of the rectangular plates. From the blood of the umbilical vessels he prepared rosette aggregates of ray-like crystals, and in this same blood he also noticed sheaf-like bundles of crystals and also isolated irregular crystals. The blood of other animals showed still other forms, which, however, are usually distinguishable from the crystals obtained from normal human blood, with the exception of those from the blood of the bat and goat. The distinction from human blood depends, he states, upon having a sufficient supply of blood and in obtaining it before it becomes dry.

The article by Friboes is illustrated by excellent photomicrographic reproductions of some of the blood crystals examined, but his descriptions of the crystals are very brief and in many cases incorrect. Thus, in the description of the crystals from the cat he enumerates three kinds of crystals and illustrates them by two photomicrographs. These three types are (1) long, 3-sided prismatic rods, single or in bundles; (2) 4-sided prisms, rhombic; (3) fine needles. He points out in the photomicrographs what he designates the "3-sided rods," which are evidently only an edge view of what he reports as "4-sided prisms." The fine needles are simply the same crystals in capillary form. All of these belong to the long prismatic type of crystal of cat reduced hemoglobin, and he appears not to have observed the short prismatic type nor the parallel growth aggregates that are usual in the preparation from cat's blood. His "fine needles" are generally the first crystals to appear, and his other two types, which he regards as distinct (one trigonal, the other rhombic), are but two views of the same crystal. The foregoing is simply an example of how an expert microscopist who is not a crystallographer may be misled by different appearances that he is unable to reconcile.

The objections recorded in opposition to the conclusion of Kunde and others that the blood crystals from different species are not identical and that they are characteristic of the species may be summarized briefly as follows: The form of the crystal of any species may be entirely accidental and dependent upon exterior conditions, and hence can not be characteristic of the species. In the same species different forms of crystals may be seen even in the same preparation, and by varying the method of preparation many forms of crystals may be obtained from a given species. Different forms of crystals have been obtained from the blood of different vessels of the same species or the same individual. Different observers have produced quite different crystals from the blood of a given species, some of these closely resembling or seemingly identical with those obtained from the blood of other species. There can not, therefore, be any one form of crystal that is characteristic of a given species. Preyer himself, while recognizing that crystallographic differences exist between the hemoglobins of different species, states that little importance is to be attached to statements on the crystallographic dissimilarities of the hemoglobin of different species, because neither is the same method of crystallization used nor is the blood always capable of being compared. He might have added, that in very few cases have the crystallographic descriptions been at all adequate or even accurate, but this he probably failed to recognize. His statement that all hemoglobins crystallized in the orthorhombic system excepting that of the squirrel was doubtless taken by many as an argument in favor of the assumption of the identity of the blood crystals obtained from different species.

We thus see that equally expert observers, working with the same data, have arrived at very diverse conclusions. Before attempting to reconcile these conflicting conclusions it will be of advantage to examine certain other observations that have been made on hemoglobin crystals.

A number of the earlier investigators, including Lehmann, Teichmann, Weir Mitchell, and Bojanowski, and several of the later ones, such as Struve, and Stirling and Brito, have noted that the crystals obtained from the blood may be nearly or quite colorless, or may become so on standing; or, according to several of them, the deep-red crystals may be decolorized by washing them with alcohol, or with alcohol and water, or with other reagents. Thus, Bojanowski states that the blood crystals exposed to the air retain their form, but become paler and paler and finally completely colorless. The addition of sugar or gum produces the same result. Teichmann had made similar observations on the loss of color of the deep-red crystals. Bojanowski's statement is a fairly accurate description of the paramorphous change of crystals of oxyhemoglobin to metoxyhemoglobin, many examples of which will be found in the records of this research. The color of the crystals of metoxyhemoglobin is very pale as compared with that of oxyhemoglobin, and when the crystals are thin they appear almost colorless. The very strong pleochroism of metoxyhemoglobin makes the crystals appear quite colorless in some positions. Weir Mitchell made similar observations on oxyhemoglobin crystals exposed to the air. The "colorless" crystals retain the form of the original oxyhemoglobin crystals, but after the change they are a different substance, and are in fact *pseudomorphs* of the original oxyhemoglobin crystals, and if dissolved and recrystallized the form would probably be altered only slightly, not sufficiently to be noticed by casual observation.

From the blood of the raven that had stood exposed to the air for 8 days, Bojanowski obtained "crystals which were partly bright yellow and partly colorless." This is a description of the method of producing crystals of metoxyhemoglobin, and the colors described are such as would be found in metoxyhemoglobin crystals that were rather insoluble, as these are described as being. He made a similar observation upon the crystals from the cat.

Weir Mitchell describes the production of crystals of oxyhemoglobin from the blood of the sturgeon, and states that their color may be completely removed by alcohol and water without injury to the form, and that these decolorized crystals may be dissolved in water and recrystallized in the original form.

Struve (Ber. d. d. chem. Ges., 1881, XIV, 930) decolorized blood crystals by treating them with dilute alcohol, but without causing any change of form. In a later communication (Jour. f. prakt. Chem., N. F., 1884, XXIX, 304) he gives a more detailed description of his observations: Fresh blood crystals placed in an excess of alcohol change their color to a darker tint, without change of form, and become insoluble in water and alcohol. This, he states, is due to a loss of water of crystallization and going over into an amorphous condition. These altered crystals by treatment with ammoniacal alcohol, by glacial acetic acid, or by concentrated sulphuric acid are decolorized without change of form. Struve did *not* dissolve and recrystallize them. The color extracted he regards as a hematin derivative, which he names hematin acid. His conclusion is that hemoglobin crystals are a colorless albuminous substance, mechanically mixed with a coloring matter.

On reading the descriptions of Struve it seems evident that the treatment with alcohol changes the crystals of hemoglobin by hardening them, an effect of alcohol upon albuminous substances generally; and if he started with oxyhemoglobin the darkened crystal treated with alcohol was already a different substance, a pseudomorph in fact. Such a pseudomorph might retain its form even though the substance of which it was composed should be the original material decomposed. In inorganic substances we find for instance crystals of pyrite, $FeS_2$, changed by pseudomorphism into limonite, $Fe_4O_3(OH)_6$ without the slightest change in outward form; fluorite, $CaF_2$, in this way is changed to quartz, $SiO_2$. The colorless crystals obtained by treatment of the alcoholized crystals with the agents mentioned above are but skeletons of the original oxyhemoglobin crystals, and may have quite a different composition. As Struve states, they are amorphous and not really crystals at all. But Weir Mitchell's *recrystallized* colorless crystals are not of this kind, and are not to be explained in the light of our present knowledge.

Colorless blood crystals are (with the exception of the recrystallized colorless forms described by Weir Mitchell) to be accounted for by a change of oxyhemoglobin to metoxyhemoglobin, by pleochroism, or by pseudomorphism in case of chemically treated crystals.

Besides colorless and slightly colored crystals, other variations from the typically colored oxyhemoglobin crystals have been observed. Thus, we find records of "bluish," "purple," and "pink" crystals that are evidently reduced hemoglobin; and "yellowish" and "brownish" crystals that may be methemoglobin. The failure to distinguish between methemoglobin and metoxyhemoglobin has given rise to much confusion. It is clear that different observers of blood crystals have examined crystals of oxyhemoglobin, reduced hemoglobin, metoxyhemoglobin, and methemoglobin in many instances without making any distinction between them. Since these substances in a given blood may form quite different crystals, a source of the variations in the recorded crystals of a given species is obvious.

As has been shown, equally expert observers working with bloods of the same species have arrived at very different conclusions as to the specificity or non-specificity of hemoglobin crystals in relation to species, some claiming that the crystals are occasionally specific, others that they are always specific, and others that they are not specific because the same blood may yield crystals of very different forms and that the differences are probably accidental. Crystals of various colors and varying forms have been obtained from the same blood. It has been held in favor of specificity that recrystallization, even when frequently repeated, does not effect any change in form; but this has been contradicted by observers who point to final evidence to the contrary. How are these diverse conclusions to be reconciled?

In the first place, it is evident that the substance under investigation was not always the same: sometimes it was oxyhemoglobin, or reduced hemoglobin, or metoxyhemoglobin, or methemoglobin, etc. Any one of these substances may appear in several forms of crystallization in the same blood, often as many as three of them in the blood of a given species; and it is even probable that there are other forms of hemoglobins present which have not yet been isolated. But much more important even than these sources of variation in the crystals was the failure of the observer to interpret correctly his observations. The same crystal viewed in different aspects presents different appearances, and the same crystal combination may exist in different shapes due to the variation in crystal habit. The expert microscopist might learn to interpret the different aspects presented by a single crystal, but no one who is not a crystallographer would be likely to suspect that a long rod-like crystal and a thin tabular crystal might be the same combination of crystal forms. It was such failure to interpret the forms observed that has caused the confusion between the apparent octahedrons and the apparent 6-sided plates of the guinea-pig oxyhemoglobin crystals that have been mentioned. An octahedron lying on one of its faces and observed normal to this face has a hexagonal outline, and if it grows lying on this face it will develop into a 6-sided (or a 3-sided) plate, because it grows twice as fast parallel to the plane on which it lies as it does normal to that plane, since it can not grow at all on the bottom plane. A tabular crystal seen on edge looks like a rod or prism, and has been so described by many observers.

Actual errors in observation are very common. For instance, Bojanowski, owing to the nearly square prisms of the cat hemoglobin when seen on edge, looks upon them as being 3-sided prisms; and Friboes falls into the same error, and even shows photomicrographs of the nearly square orthorhombic prisms of the same substance, and refers to them as "3-sided."

Kunde and Lehmann observed "tetrahedra" in the "hemoglobin" of the black rat. These were doubtless the $\beta$-oxyhemoglobin crystals, which are isometric, and appear as the three-sided plates that develop from the flattening of the octahedron. Such a crystal seen on edge would be described as a prism.

When the different habits that the same crystal combination may assume are considered, the difficulty of interpreting the observations increases enormously. Thus, crystallization may begin with the formation of needle-like or capillary crystals, and these may later become short prisms. Friboes describes these two forms of the same crystal as two kinds of crystals in the case of cat hemoglobin; and, as has been stated, by looking at the same crystal in two aspects at 45° to each other, he sees two kinds of prisms, thus making three kinds of crystals of the same identical crystal combination. In certain species of the cats the hemoglobin occurs in all of these variations of the prismatic type of crystal and also in the tabular form, yet the crystal forms shown may be the same in prism and plate. Under less pressure the crystals form as prisms; under greater pressure they form as plates.

Crystals from the blood of the black rat have been described as tetrahedra, prisms, elongated plates, and hexagonal plates. The tetrahedra have already been referred to, and they are evidently, as stated, $\beta$-oxyhemoglobin. The prisms, elongated plates, and hexagonal plates are all the same combination of crystal forms, the prism and macrodome, and are our $\alpha$-oxyhemoglobin. When symmetrically developed the crystal is the squarish prism terminated by the dome. Flattening of the crystal on two opposite prism faces produces the "elongated plates" of Hoppe-Seyler, and shortening of this flattened prism produces the apparently hexagonal plate. Careful focusing would show at once that this plate is not bounded by vertical sides and that the angles are not hexagonal angles. All of these forms we have observed in the crystals from the blood of the common rat.

The crystals are frequently interfered with by the slide and cover producing false planes, so that a tabular crystal on edge, thus confined, becomes a "prism." Many examples of crystals with such false planes have been figured, even as late as the work of Moser (1901).

When it comes to the determination of the crystal system, we find that most of the observers make no attempt at it. Preyer states that in his table (page 103) five of the six crystal systems are recorded, the triclinic being the only one not included. The isometric, he writes, may be ruled out because all hemoglobin crystals are doubly refracting and because isometric crystals can not be doubly refracting. Crystallographers now recognize that the tetartohedral class of the isometric system is doubly refracting, and, as will be shown later, we have found singly refracting isometric crystals of hemoglobins. The tetragonal system he eliminates because the statements of Hoppe-Seyler in regard to the tetragonal character of the guinea-pig crystals were disproved by von Lang. Similarly he excludes the monoclinic because he states that Funke, who claims to have observed monoclinic crystals in the case of the cat and man, "supports his statement by nothing." This leaves only the orthorhombic and the hexagonal.

Preyer also states that of the 47 species examined and recorded the system of crystallization is known in 10 instances, in only one of which are the crystals accredited to the hexagonal system. In fact, von Lang seems to have been the only professional crystallographer who examined blood crystals up to the time of Preyer, and his descriptions, as has been stated, are very brief. Since von Lang found only two crystal systems, so Preyer concludes there can be but two crystal systems to which the hemoglobin crystals belong. Nevertheless the five crystal systems mentioned by Preyer as having been recorded by various observers, of which he rejects three, are all represented by us in the hemoglobins included in this research. When we try to find how these investigators arrived at their conclusions as to the crystal system we are met by short, very incomplete descriptions, and we are led to the conclusion reached by Preyer in the case of Funke's monoclinic crystals, that "they support their statements by nothing." The work of von Lang was evidently accurate, although his crystallographic notes are brief; Donogány confirmed von Lang's findings in the case of guinea-pig's crystals, but, as we have already pointed out, he records three angles of a triangle which sum up to 180° 46′. The only contribution that has appeared giving the crystallographic constants and an accurate description of hemoglobin crystals is that of Schwantka (Zeit. f. physiol. Chemie, 1900, XXIX, 486) on the oxyhemoglobin of the pigeon, which will be found referred to at length under that species in a later chapter.

The foregoing is in effect a brief statement of the status of the crystallography of hemoglobins at the inception of this research and up to the present time.

# CHAPTER VIII.

## METHODS FOR PREPARING, EXAMINING, AND MEASURING CRYSTALS OF THE HEMOGLOBINS EMPLOYED IN THIS RESEARCH.

### METHODS FOR PREPARING CRYSTALS OF HEMOGLOBIN.

The necessarily limited quantities of blood that have been furnished us led, as a consequence, to the study of only such methods as are especially applicable to very small supplies, such for instance as 1 to 5 c.c. of fluid or clotted blood, although several of our processes may be used to advantage in the preparation of very large quantities if a method be selected that is suited to the species and to the condition of the blood. In only a few instances were we unsuccessful in obtaining crystals, and when we failed it was owing to an inadvertent selection of a wrong method or to attendant conditions over which we had no control. Our difficulty was not so much in the way of securing crystals as it was in the preparation of specimens that were adapted to the peculiar requirements of our investigation. We found, as we gained experience with the bloods of different species, that, while the blood of each species must be treated as an individual, we could nevertheless depend with some confidence upon the guidance of certain generalizations in the selection of the best method to be pursued. Thus, we found that usually the hemoglobins of *Rodentia* and *Canidæ* crystallize with great readiness, those of *Marsupialia* very readily, those of *Felidæ* readily, those of *Ungulata* not readily, those of *Aves* with difficulty, etc.; but there were so many unexpected exceptions that we were often misled, and, as a consequence, obtained inferior results, as a number of our photomicrographic reproductions show.

Even in the case of species closely related, as, for instance, certain of the rats, we found striking exceptions: The blood of the common albino or white rat (*Mus norvegicus* var. *albus*)* and that of *Mus decumanus* Pall. (*Mus norvegicus* Erxleben—brown rat) crystallize with such readiness that we found it desirable to use a restrainer to obtain crystals of desirable size for study; on the other hand, the bloods of *Mus rattus* (black rat) and *Mus alexandrinus* (alexandrine rat) crystallize much less readily, and hence should be treated in an entirely different way.

We absolutely avoided the use of alcohol, because, notwithstanding the fact that it has proven one of the most widely used and most valuable agents in the preparation of hemoglobin crystals, it so deleteriously affects the hemoglobin molecule that even when present in dilute solution it lessens

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* Hatai (Biological Bulletin, Wistar Institute of Anatomy and Biology, Philadelphia, 1907, XII, 266) states, upon morphological grounds, that the albino rats of Chicago and Philadelphia are a variety of *Mus norvegicus*.

solubility, alters the extinction coefficient, gradually decolorizes the crystals, and doubtless affects the water of crystallization. Alkalies and mineral acids have likewise been avoided, because of their pernicious influences. Hemoglobin, whether in crystalline form or in solution, especially when in concentrated solution, undergoes rapid alteration; we therefore made our studies as soon as possible after we obtained satisfactory crystals, usually within a few hours. In none of our examinations have we used recrystallized hemoglobin. Our specimens have been too small in quantity to permit of satisfactory recrystallization, and, moreover, the disadvantages of recrystallization, especially in so far as the methods of our investigation are concerned, quite outweigh the advantages. The injurious effects of recrystallization have been fully referred to in previous pages.

At the inception of our research it seemed to us that the best results, on the whole, were to be obtained by the use of fluid blood, either defibrinated or rendered incoagulable by oxalate, fluoride, or other anticoagulant, so that in the case of bloods which do not crystallize readily the corpuscles could be collected from the serum or plasma by centrifugalization, and thus eliminate certain substances in these fluids which retard crystallization and at the same time obtain a concentrated solution of hemoglobin. Since it seemed impracticable to obtain defibrinated blood, owing to the circumstances under which our specimens were to be collected, and since one of us (Reichert, page 128) had already found that the presence of an anticoagulant, such as neutral oxalate, was not only not injurious but actually beneficial, we made use of oxalate of ammonium in all of our preparations except in a very few instances, when for some special reason its absence was desirable or necessary. The addition of oxalate, in the proportion of 1 to 5 per cent of the dried powder, it was found, very much favors crystallization; the larger the quantity up to the point of saturation the better the effect, saturation not being a disadvantage beyond the appearance of crystals of oxalate, which, however, are readily distinguishable from those of hemoglobin. In fact, in several instances these crystals appeared to be of advantage, because hemoglobin crystals formed on them, but not in other parts of the preparations. When we had defibrinated blood or clots to work with, oxalate was added at the proper time during our procedures of preparation.

Since the presence of foreign bodies may, as is well known, not only augment or hinder crystallization, but also affect crystallization in other and even more important ways, we made appropriate tests to determine especially if the presence of the oxalate in any particular quantity affected either the type of the crystals or the optical properties of hemoglobin. The optical properties were not in any way appreciably affected. The habit of crystallization, as in the case of *Necturus*, seemed to be affected in the direction of causing the crystals to be shorter and thicker. The only important influence of the oxalate, apart from the accelerating effect, we found in our experiments with the bloods of the horse and mule, in which we discovered that by modifications in the quantity of oxalate we could obtain a relative abundance of one or the other or of both kinds of oxyhemoglobins that are normally present in these bloods. In no instance did we find any evidence of any influence on the type of crystals that is peculiar to the species. We did not make any investigations of the possible influences of diseased conditions upon the form of crystallization, because, in the first place, of an insufficiency in our supplies, and, secondly, because Dr. S. Weir Mitchell and others have found, as far as their studies have gone, that disease is without influence on the type of crystals. Assuming, however, that the presence of ammonium oxalate might have some undiscovered effect upon the morphological or optical properties of the crystals, we, with the few exceptions indicated, always introduced the oxalate, and as nearly as possible in the same proportion.

Most of our specimens were in various stages of putrefaction. Fortunately, hemoglobin, in comparison with other proteins, is remarkably resistant to putrefaction, so that even when the plasma proteins are in an advanced state of decomposition the hemoglobin may have merely suffered a partial alteration to reduced hemoglobin and metoxyhemoglobin. Upon exposure of the blood to the air, especially shaking with the air or with an atmosphere of pure oxygen, a rapid restoration of oxyhemoglobin is readily brought about, and unless the blood is excessively putrid the exposure of the drops of the prepared blood upon the slides antecedent to the covering with cover-glasses is sufficient to yield good preparations of oxyhemoglobin.

Each of our processes is characterized by three major procedures, which are accompanied by such accessory procedures as conditions indicated: (1) the addition of oxalate; (2) laking with ethyl ether (Squibb's); (3) centrifugalization; (4) occasional accessory procedures, such as variations in temperature, the addition of asbestos wool, alumina, etc., to aid in the separation of the stromata or the nuclei of erythrocytes, keeping the preparations in a moist chamber, etc. Oxalate was added (*a*) to prevent coagulation, (*b*) to increase crystallizability, or (*c*) to obtain one or another form of oxyhemoglobin present in the same blood. In the process of laking, the ether was usually added in three or four portions, the mixture being shaken vigorously after each addition, and sufficient ether being added to cause marked pressure within the test-tube when the opening of the tube is closed by the finger. When the blood is very putrid no more ether should be used than is absolutely necessary to cause complete laking, otherwise the hemoglobin is likely to be thrown down in the form of an insoluble precipitate. Caution must also be practised when working with bloods, oxalated or not, which contain nucleated erythrocytes. An excess of ether is likely to cause coagulation, and especially so the larger the quantity of oxalate present. One of us (Reichert, Journal of Experimental Medicine, April, 1905) has found that even oxalated *defibrinated* blood may be converted into a gelatinous mass by the addition of ether. Centrifugalization was practised, (*a*) to collect the corpuscles, and thus get rid of substances which hinder crystallization, and at the same time to secure a concentrated solution of hemoglobin; and (*b*) to clear the laked preparation of the stromata and other bodies in suspension. Occasionally our specimens were too small to centrifugalize.

Our methods are briefly as follows:

*First method:* The whole blood is laked and centrifugalized. If the blood had been defibrinated, oxalate was added before centrifugalization.

*Second method:* The corpuscles are separated by centrifugalization and then laked, oxalate added to the solution, and the solution centrifugalized.

*Third method:* The blood-clot is ground in sand, or the frozen clot comminuted to liquefaction; the fluid thus obtained is laked, oxalate is added, and then centrifugalized.

*Fourth method:* In order to retard crystallization there may be added to the blood, before or after laking, such inert substances as plasma, serum, egg-white, water, glucose, gum, etc. The best results we have obtained by the use of plasma, serum, or a 50 per cent solution of egg-white. This latter is prepared by adding to the white of egg an equal volume of distilled water, shaking violently in a flask for a few moments, and then straining through linen. From 0.5 to 2 or more volumes may be added to the blood in accordance with the effect required. The mixture is then centrifugalized until a clear preparation is obtained. Occasionally the solution of egg-white was clarified by agitation with ether and then by centrifugalization before it was added to the already centrifugalized solutions of hemoglobin. Blood very readily crystallizable will often be changed within a few minutes into a magma of crystals, in which case excellent crystals can usually be obtained by using the mother-liquor which has been separated by centrifugalization.

When the blood is badly decomposed it is better to complete the laking by repeated alternate freezing and thawing than by the addition of ether.

The clear preparation obtained by these methods is placed upon slides, and after the margins of the drops have become sufficiently dried cover-glasses are put on, and in the course of an hour or two the covers sealed with Canada balsam.

The first method is especially adapted to bloods that crystallize readily, such as those of *Pisces* and *Marsupialia;* the second, to those which do not crystallize so readily, as those of *Felidæ* and *Ungulata;* the third, to those which crystallize with more or less difficulty, as those of *Primates, Aves,* and *Reptilia;* and the fourth, to those which tend to crystallize so rapidly as to yield crystals of too minute size, as those of *Rodentia* and *Canidæ.* Various accessory incidental procedures will be referred to at the proper places.

## THE VALUE OF THE CRYSTALLOGRAPHIC METHOD OF INVESTIGATION.

When a chemical compound solidifies from fusion, solution, or vapor under conditions which are favorable to the development of individuals, its particles tend to arrange themselves in regular order, so that a definite structure is produced. The external form of the individuals is also regular, being bounded by planes in definite relation to each other so that polyhedral solids are produced which are called crystals. The regular arrangement of the atoms among themselves, and of the molecules which they build up, is so characteristic of substances of definite composition that the crystalline condition of dead matter is the normal condition. Differences of chemical constitution are accompanied by differences of physical structure, and the crystallographic test of differences of chemical constitution is recognized as the most delicate test of such differences. For instance, in the case of isomerides, the chemical differences between such substances consist in the differences in the arrangement of their constituent atoms, the position of a replaced hydrogen atom in a group (in which several such replacements are possible) altering the structure. Hence the following is true: "*Isomeric substances possess different crystal structure.*" (Groth, Chemical Crystallography, trans. by Marshall, 1906, 63.) Such differences in crystal structure can generally be readily recognized, but to detect chemical differences between isomerides by any centesimal chemical analysis is obviously impossible. Chemical differences between such substances are detected by differences in solubility, in melting-point, in rotatory power, in reactions in which the substance is altered or decomposed; but when large numbers of isomerides are possible, as in the enormous molecules of the proteins, the detection of differences between them by purely chemical processes has thus far, except in rare instances, been found impossible.

The crystallographic method is, of course, adapted to detecting differences between substances that show differences in centesimal composition even better than between isomerides, for here the differences in structure may be more profound than in the case of the isomerides; and differences in centesimal composition must of necessity imply differences in structure. Hence the general law may be enunciated: *Substances that show differences in crystallographic structure are different chemical substances.*

### THE PETROGRAPHICAL MICROSCOPE AND ITS USE.

The necessity of studying small crystals, especially sections of such crystals as are met with in rock sections, has resulted in the evolution of a form of microscope which is at once a goniometer, a polariscope, and an instrument for measuring optic axial angles—in short, for determining the physical crystallographic constants of small crystals. Of necessity, in some of its measurements it is not so exact as other instruments that may be employed for the same purpose, for its parts must be light, and its circles can not be read to the same degree of accuracy as those of the reflecting goniometers and spectrometers. The determination of the angle of a crystal by this instrument is, under favorable conditions, not accurate to less than 10′ of arc. But when it is remembered that carefully made measurements on the reflecting goniometer often vary as much as this in different crystals of the same substance it is seen that data of value may be procured with the aid of such a microscope.

The polariscope portion of the petrographical microscope enables the observer to determine the position and relative value of the elasticity axes of crystals, to observe the position of the optic axes, and to determine their inclination to each other and to the elasticity axes. From these data the optical character of the crystal is determined. These *optical reactions* may be studied by this instrument with as much ease, and in general with as much accuracy, as with the larger and better graduated polariscope; and the data thus obtained are quite as accurate in most cases as those obtained by the use of the larger instruments. The use of the special eyepieces arranged with artificial twins of calcite or quartz enables the observer to determine the extinction angles of the crystals with as much accuracy as can be done with any form of polariscope.

From such observations, made with the aid of this form of microscope, the following constants may be determined:

(1) The plane angles of the crystals, in most cases the interfacial angles, giving the data from which the axial ratios are computed—in other words, the morphological constants of the single crystals.

(2) The relation of the parts of the composite crystals or twins to each other, their angles, and the position of the twin plane, twin axis, composition plane, and other constants of the twin crystals.

(3) The pleochroism of the crystals, the character of the colors of the light vibrating parallel to the elasticity axes in the crystal. This is effected by the use of the single polarizing prism below the stage. By analyzing this light with the microspectroscope the differences of tint and color may be given quantitative values in wave lengths.

(4) The position and relative values of the light elasticity axes in the crystals, upon which depend the angles of extinction of the crystals, measured from certain crystallographic axes or planes or edges. In uniaxial crystals (tetragonal and hexagonal systems) there are two such elasticity axes—the ordinary ray, designated as $\omega$, and the extraordinary ray, designated as $\varepsilon$. Either one of these may be the axis of greater or less elasticity, and according as the extraordinary ray is the axis of less elasticity or of greater elasticity the crystal is called optically *positive* or optically *negative.* In biaxial crystals (orthorhombic, monoclinic, and triclinic systems) there are three elasticity axes at right angles to each other, and these are designated as $\mathfrak{a}$, the axis of greatest elasticity; $\mathfrak{b}$, the axis of mean elasticity; and $\mathfrak{c}$, the axis of least elasticity.

(5) The position and angle of inclination of the optic axes or lines of single refraction through the crystals. These always lie in the plane of the elasticity axes $\mathfrak{a}$ and $\mathfrak{c}$ and the angles between the optic axes are bisected by the axes $\mathfrak{a}$ and $\mathfrak{c}$. According as to whether $\mathfrak{c}$ or $\mathfrak{a}$ is the axis bisecting the acute angle, the *acute bisectrix*, $Bx_a$, the crystal is called optically *positive* or *negative.* Thus if $Bx_a = \mathfrak{c}$, the optical character is *positive.* The apparent angle between the optic axes is determined by means of an eyepiece micrometer in an observation of the interference figure, looking along the acute bisectrix of the optic axes, and this angle is designated as $2E$. The character of the double refraction may be determined by this angle.

When good crystals were available for examination the physical data above enumerated could all be determined. Other data were recorded, such as the morphological habit, the character of the heterogeneous aggregates formed by the crystals, their relative dimensions, general color, etc. The character of the material under investigation was determined by the use of a Zeiss microspectroscope. As a general rule, the crystals were kept under examination until they ceased to change or until they were destroyed by bacterial decomposition. In this way the changes that the crystals went through, the formation of successive crops of oxyhemoglobin and also reduced hemoglobin, methemoglobin, and metoxyhemoglobin were observed. Some crystals kept well for weeks, some altered inside of a day or two. In general the best results were obtained with crystals that formed at room temperature and did not dissolve on slight increase of temperature, but in many cases all the observations had to be made near freezing temperatures. This was done by working in a cold room, at a temperature near 0° C. Even at such a temperature the heat of the body or breath often produced partial solution of some of the crystals, so that the measurements had to be made rapidly. Having usually a number of slides at hand, this could generally be done conveniently, as, when the crystals began to lose shape, the slide under investigation could be replaced by another from the supply kept at a temperature below freezing, and the crystals of the first slide would usually soon regain their form. When the blood crystals were not very soluble it was always found more advantageous to keep them at a temperature much above freezing rather than near the freezing-point; they could then be examined and photographed without fear of solution because of an increase of temperature.

# CHAPTER IX.

## CRYSTALLOGRAPHY OF THE HEMOGLOBINS OF PISCES, BATRACHIA, AND REPTILIA.

While this research is mainly upon the hemoglobins of mammals, certain species of fish, batrachians, reptiles, and birds were investigated, in order that it might be seen whether the results obtained for mammals extended to these classes also. Only a very limited number of species were examined, including 4 fish, 1 batrachian, 1 reptile, and 10 birds; but the results are entirely in conformity with those obtained from the examination of mammalian blood, as indicating the specific character of the properties of the hemoglobin in each species. In most cases the species were too far separated from each other to show similarities due to their zoölogical affinities, such as are shown in the hemoglobins of mammalia, but in the case of the birds the relationship was sufficiently close to bring out family and generic characteristics.

### PISCES.

#### Barndoor Skate, *Raia lævis.* Plate 1.

One species of the selachians or cartilaginous fishes, the barndoor skate, *Raia lævis,* was examined. This skate was sent to the laboratory from the New York Aquarium, and the blood was extracted by the dissection of the dead fish. The blood was oxalated and centrifugalized, the corpuscles were ether-laked, a little water was added, and the mixture again centrifugalized. From the clear solution thus obtained the slide preparations were made. The crystals developed readily and were oxyhemoglobin, as determined by spectroscopic examination. They occur in single crystals and in sheaf-like or branching aggregates of these crystals. The individual crystals are rhombic plates.

The crystallographic description is as follows:

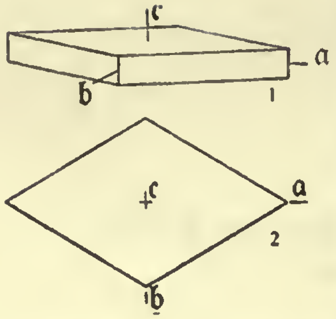
*Oxyhemoglobin of Raia lævis.*

Orthorhombic: Axial ratio $a : b : \dot{c} = 0.6008 : 1 : 0.4024$.

Forms observed: Prism (110), base (001), and from twins the unit pyramid (111) and a macrodome (401).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 62°$; prism to base $110 \wedge 001 = 90°$; prism to pyramid $110 \wedge 111 = 38°$; macrodome to base $401 \wedge 001 = 69°$ (calculated 69° 33′).

Habit thin tabular on the base (001), the crystals being a very short prism (text figures 1 and 2). Twinning is on the pyramid (111); this form was only observed in twins; or on the macrodome (401), a form also only observed in twins. Also in crystals grown together on the base into groups, which, seen on the edge aspect of the plate,



Figs. 1, 2. *Raia lævis* Oxyhemoglobin.
Fig. 3. *Acipenser sturio* Oxyhemoglobin.

produce sheaf-shaped and fan-shaped groups, and on the flat show the irregularly overlapping plates (see plate 1, figs. 1, 2, 3). Frequently the orientation of the crystals in a group is such that the entire group extinguishes simultaneously in polarized light.

Extinction is symmetrical when the plate is viewed on the base (001), and parallel when viewed on edge (orthorhombic). The interference figure was not observed, but, from the pleochroism and the elasticities of the axes as observed, the orientation of the elasticity axes with reference to the crystal axes is as follows (text figure 2): $\mathfrak{a}=b$, $\mathfrak{b}=a$, $\mathfrak{c}=\acute{c}$. From the fact that the figure could not be seen when looking along $a$ and $\acute{c}$, and from the elasticities and pleochroism, the acute bisectrix of the optic axes $Bx_a=\mathfrak{a}$. The optical character is hence *negative.*

Three species of the *Teleostomi* or Bony Fishes were examined. In the order *Actinopterygii* the common sturgeon, *Acipenser sturio,* was examined.

### Sturgeon, *Acipenser sturio.* Plates 1 and 2.

The blood was obtained from the living fish, oxalated, ether-laked, and centrifugalized, giving a clear solution, from which the preparations were made in the usual manner. The slides were prepared inside of 4 hours from the time of the bleeding of the fish. The crystals are deposited rapidly from the solution, and inside of a few minutes after the blood is placed upon the slide an abundant crop of small lath-shaped crystals has formed. After 1 hour the entire slide is filled with a felt of these crystals, and the solution is colorless. No particular change in the slides was noted after this condition was reached, showing that the crystals were quite insoluble in the plasma.

In order to retard the rate of formation of the crystals a portion of the oxalated normal blood, which was found to be filled with crystals after standing 24 hours, was diluted with 5 volumes of water and centrifugalized. The clear supernatant liquid was then used to make slide preparations, but while it readily crystallized, the crystals were not so perfect as those made from the whole blood. The crystals from the whole blood were therefore used for the examination.

The color of the crystals, a brownish-red, indicated that they were oxyhemoglobin mixed with some methemoglobin, which latter was observed in the normal blood of other fish when obtained fresh from the living animal. The blood was not examined by the spectroscope.

The crystallographic description of the crystals is as follows:

#### *Oxyhemoglobin of Acipenser sturio.*

Orthorhombic: Axial ratio not determined, only one fundamental angle being observed, and that a doubtful one.

Forms observed: Macropinacoid (100), brachypinacoid (010), brachydome (011).

Habit of the crystals long lath-shaped (text figure 3), the length 20 to 30 times the breadth, which latter is about 4 times the thickness. The crystal appears to consist of the two pinacoids, terminated by a flat dome. This is probably the brachydome. Its interfacial angle was measured very roughly as 110°, giving the angle between face normals as 70°. The large plane in the prismatic zone is then taken as the brachypinacoid (010), and the smaller plane, visible when the crystal is on edge, is hence the macropinacoid. On the flat, the long lath-shaped crystal has square ends, measured as 90° with the sides; and on edge the flat brachydome terminates the crystal.

Pleochroism is rather strong; for 𝔞, the direction of greatest elasticity, which is parallel to the length, the color is pale yellowish; 𝔟 and 𝔠 are nearly equal, and the color is reddish-brown. Extinction is straight in both the edge view and on the flat or side view. The polarization and pleochroic colors are not interfered with by the color of the plasma, as practically all of the hemoglobin crystallizes. Between crossed nicols, the colors seen on the flat ranged from blue-slate of the first order up to straw-yellow and orange of the first order in the different thicknesses of crystals, indicating a moderately strong double refraction. No interference figure was observed.

### SHAD, *Alosa sapidissima*. Plates 2, 3, and 4.

Shad blood was obtained by bleeding the living fish, and also by obtaining blood from dead fish purchased in the market. In the former case it was either oxalated or allowed to clot; in the latter it was obtained in the form of clots from the larger vessels, etc. The clotted blood was treated by oxalating and ether-laking, and also by grinding the clot with sand and ether-laking, centrifugalizing, and oxalating the clear blood. The fresh oxalated blood, either laked by ether and centrifugalized, or the corpuscles broken down by repeatedly freezing and thawing the blood, always showed a combination of methemoglobin and oxyhemoglobin, the material often described as "methemoglobin." Much difficulty was experienced in getting rid of the nuclei of the corpuscles by centrifugalizing, but this was partly overcome by using thoroughly clotted blood and breaking up the clots by grinding in sand. On allowing the blood to stand in a corked tube in the refrigerator, the blood usually passed largely into reduced hemoglobin. The blood that was freely exposed to the air and had been kept for some days, or the deoxidized blood exposed to the air, and, finally, the clotted blood obtained from fish that had died in the air and had been dead a few days, as obtained in market, always contained some methemoglobin which would crystallize as such and not as metoxyhemoglobin.

It would seem, therefore, that the blood of the shad during the spawning season, in which it is obtained in our rivers, contains a large proportion of methemoglobin in combination with oxyhemoglobin, or a substance intermediate between oxyhemoglobin, methemoglobin, and metoxyhemoglobin; and that further exposure to air changes this to pure methemoglobin in part, leaving a residue of pure oxyhemoglobin, which two substances may be crystallized simultaneously from the blood in distinct crystals of pure methemoglobin and pure oxyhemoglobin, which substances do not form in the freshly drawn, slightly oxidized blood. This occurrence of metoxyhemoglobin in the freshly drawn blood may be due to the state of inactivity that the digestive organs of the animal are in during the spawning season, as the fish do not feed during this time. The same condition in the blood is noted in the case of bears, for example, during the hibernating period.

From the blood of the shad, therefore, crystals of the following substances were obtained: (1) Oxyhemoglobin, from blood that had been exposed to the air. (2) Metoxyhemoglobin from the fresh blood. (3) Methemoglobin from blood that had been exposed to the air, with (1) or from deoxidized blood, with (4), but not with (2). (4) Reduced hemoglobin * from stale blood that had not been exposed to the air. This was also formed by reduction of (2).

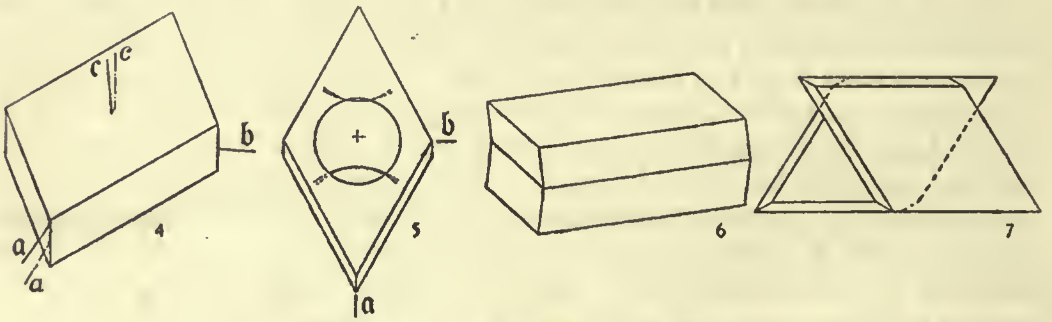
### (1) *Oxyhemoglobin of Alosa sapidissima.*

Monoclinic: Axial ratio $a : b : \dot{c} = 1.804 : 1 : \dot{c}$; $\beta = 68°$.

Forms observed: Prism (110) and base (001).

Angles: On base (001) edges $110\text{–}001 \wedge 1\bar{1}0\text{–}001 = 58°$; edge of $(110\text{–}1\bar{1}0 \wedge 001) = \beta = 68°$.

The angles of the plates varied, the acute angle being often not the supplement of the obtuse angle in the same plate, this difference being apparently due to some form of twinning. Thus, the acute angle often ranged up to 62°, and some were exactly 60°. The obtuse angle was always 120° or over, up to 124° and even 125° in a few cases. But in simple, untwinned crystals the angles of 58° and 122° seemed to be the average.



Figs. 4, 5, 6, 7. *Alosa sapidissima* Oxyhemoglobin.

Habit of the crystals (text figures 4 and 5) tabular on the base (001), the prism very short, making the crystal a rhomboidal plate with the plane of symmetry including the long diagonal of the plate. Twins with the base (001) as composition face, Manebach type (text figure 6), also in "homogeneous regular growth" like a twin on the pyramid, but with the two parts uniting on the base (001) and the prism edges (110) and ($\bar{1}$10) in juxtaposition (text figure 7). This twin, very common in all these tabular crystals of the monoclinic system, is called the "horse-type" of twin, and is fully described under Horse (p. 192). Regular growths with methemoglobin formed (heterogeneous regular growths) the methemoglobin crystals, which are hexagonal, forming in symmetrical position on the monoclinic oxyhemoglobin (text figure 14; see also plate 4, figs. 21, 22, and 23). The twinned crystals of the oxyhemoglobin with angles of 60° were usually found so overgrown; but this was not always the case, as may be seen by reference to the figures, see plate 4, fig. 24.

Crystals are strongly pleochroic; $\mathfrak{a}$ pale yellowish-red, $\mathfrak{b}$ deep red, $\mathfrak{c}$ deep red; the colors for light vibrating along $\mathfrak{b}$ and $\mathfrak{c}$ are about alike. Axial plane ⊥ to axis $\mathfrak{b}$ or in the plane of symmetry. Orientation of the elasticity axes is as follows: $\mathfrak{b} = b$, $\mathfrak{a} \wedge a = 6°$ in the obtuse angle, $\mathfrak{c} \wedge \dot{c} = 16°$ in the obtuse angle; the extinction angle looking along $\mathfrak{b}$ is hence 6° from the trace of (001), which is the extinction angle in edge view of the plate, with the long diagonal of the plate normal to the line of sight. On the base, traces

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* The term "reduced hemoglobin," while a misnomer, is nevertheless conventional to express a specific substance. The indiscriminate use of the term "hemoglobin" to indicate several entirely different bodies necessitates the continued use of the term "reduced hemoglobin" or the adoption of some equally specific substitute.

of two interference brushes are seen in convergent light; they are widely separated, indicating that the axial angle is large. They are nearly symmetrical, owing to the small angle of extinction. The acute bisectrix $Bx_a$ appears to be 𝔠, making the optical character *positive.*

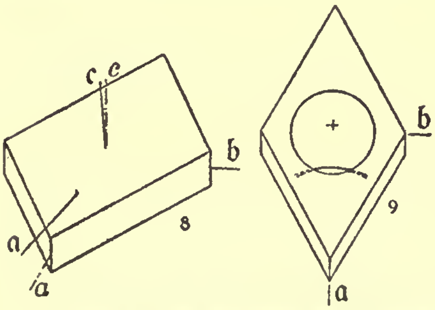
These oxyhemoglobin crystals were only obtained in quantity from the blood of a shad that was purchased in the market, and that had been dead some days and exposed to the air. The blood was extracted from the heart and larger blood vessels in the form of soft clots, oxalate was added, and the blood ether-laked and centrifugalized. Both blood and preparations were full of gas bubbles. At first only the one type of crystals, as described above, developed, but after some hours hexagonal plates of methemoglobin began to appear in quantity, and these grew isolated or attached to the oxyhemoglobin in regular growth, as described under methemoglobin below (see text figure 12).

### (2) *Metoxyhemoglobin of Alosa sapidissima.*

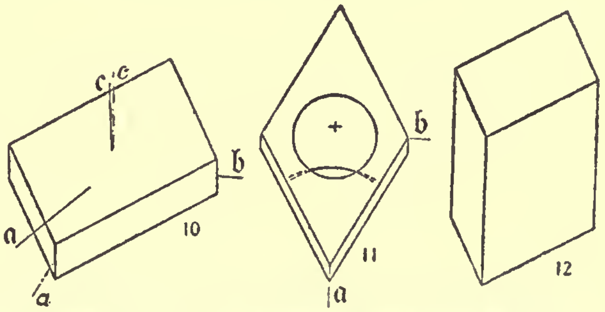
Monoclinic: Axial ratio $a : b : \dot{c} = 1.786 : 1 : \dot{c}$; $\beta = 70°$.

Forms observed: Prism (110), base (001).

Angles: On base (001) edges 110–001 ∧ 1$\bar{1}$0–001 = 58° 30′ on perfect and untwinned crystals; but on the larger and twinned crystals this angle runs often over 60° and averages about 60° 40′, giving $a = 1.709$; angle $\beta$ = edge of 110–1$\bar{1}$0 ∧ 001 = 70°.



FIGS. 8, 9. *Alosa sapidissima* Metoxyhemoglobin.



FIGS. 10, 11, 12. *Alosa sapidissima* Reduced Hemoglobin.

Habit of the crystals tabular on the base (001), with short prism (110) as in the shad oxyhemoglobin, the long diagonal of the plate being in the plane of symmetry as above (text figures 8 and 9). The forms of twinning are exactly similar to those of the oxyhemoglobin, but in the second kind of twinning (horse-type) the crystals often elongate in the direction of the common prism-base edge (plate 3, fig. 15), and a very similar looking twin is formed by twinning on a pyramid (111). The angle of the plates in this twin (which is an interpenetrant twin) was not determined. Examples of it may be seen on plate 3, fig. 15. Cleavage was readily obtained by crushing the crystals under the cover, showing them to be brittle; the cleavage is parallel to the prism (110) and is perfect.

Pleochroism is strong; 𝔞 pale yellow to nearly colorless, 𝔟 reddish-brown, 𝔠 deeper reddish-brown. The spectra for light vibrating parallel to 𝔞 and 𝔟 were carefully observed, the chief difference being in the very much stronger absorption of the blue end of the spectrum, up to the green in case of 𝔟. The absorption bands are: for 𝔞 a band from 635 $\mu\mu$ to 625 $\mu\mu$, one from 580 $\mu\mu$ to 565 $\mu\mu$, and one from 548 $\mu\mu$ to 530 $\mu\mu$. For 𝔟 the bands are shifted somewhat, 640 $\mu\mu$ to 620 $\mu\mu$, and very faint beyond 585 $\mu\mu$, when a band begins that extends to 565 $\mu\mu$; another in the almost absorbed spectrum runs from 550 $\mu\mu$ to 530 $\mu\mu$. The first band (635 $\mu\mu$ to 625 $\mu\mu$, etc.) is the methemoglobin band in the red, the other two mark the position of the two oxyhemoglobin bands somewhat displaced, and the methemoglobin band in the blue-green is not visible, owing to the absorption of this end of the spectrum. But it will readily be seen that this is quite different from the spectrum of pure methemoglobin, which contains a band in the red (640 $\mu\mu$ to 620 $\mu\mu$) and one in the blue-green (513 $\mu\mu$ to 488 $\mu\mu$) only.

The axial plane lies in the plane of symmetry, and the orientation of the optic axes is as follows: $\mathfrak{b}=b$, $\mathfrak{a} \wedge a=10°$ in the obtuse angle, $\mathfrak{c} \wedge \acute{c}=10°$ in the obtuse angle; the extinction angle, looking at the crystals in edge view along the ortho-axis $b$, is 10° from the trace of (001) or from the edge of (110). On the flat view, the basal pinacoid (001), the crystal examined in convergent light shows one brush of the interference figure, with the other appearing on rotation of the crystal, the plane of the optic axes being the plane of symmetry, and their position indicates an angle between the optic axes, $2E=100°$ or more. Seen in this aspect, the extinction is, of course, symmetrical with the rhombic section of the crystal. The optical character is *positive*, $Bx_a=\mathfrak{c}$.

When crystals of this material are allowed to dry on the slide, shearing cracks develop that are normal to the plane of symmetry, as was also noted in the case of the reduced hemoglobin crystals.

The shad from whose blood these crystals were prepared was taken alive from the seine and immediately bled into a tube; the blood was not oxalated, but allowed to clot. It was hence not exposed to the oxidation that occurs in fish that die in the air, when the blood becomes fully oxygenated, as with a fish that was purchased in the market. This freshly drawn, clotted blood was then ground in sand, ether-laked, and centrifugalized; and after it became clear it was oxalated. Slides were prepared in the usual way, and crystals formed very soon after the preparations were covered. The negatives from which the photographic illustrations were taken were made on the following day.

### (3) *Reduced Hemoglobin of Alosa sapidissima.*

Monoclinic: Axial ratio, $a : b : \acute{c}=1.786 : 1 : \acute{c}$; $\beta=70°$ as in metoxyhemoglobin, but the ratio in some crystals becomes $a : b=1.732 : 1$.

Forms observed: Prism (110), base (001).

Angles: On the base (001) edges $110 \wedge 1\bar{1}0=58° 30'$ for the smaller and more perfect crystals, but in reduced hemoglobin, recrystallized from solution of the metoxyhemoglobin after reduction of the solution by a reducing agent, the crystals had angles of 60° almost exactly. The angle of 60° was seen in crystals that were evidently twinned, as is the case in the metoxyhemoglobin. In fact, the form is so nearly the same in the two that the hemoglobin might be a paramorph of the metoxyhemoglobin. In some of the preparations, however, the habit in the reduced hemoglobin is quite different from that in the metoxyhemoglobin. The angle $\beta$ was 70° or a little over.

Habit of the crystals usually tabular, flattened on the base (001) (text figures 10 and 11), or short prismatic, the prism length usually falling between the longer and shorter diagonals of its section. In crystals of this latter habit (text figure 12), the side view is commonly seen, and in some aspects the crystal looks like a rhombohedron. Twins are very common, the forms being the same as those described under oxyhemoglobin, especially the "horse-type"—this twin, having the parts grown together on the base and two prism-base edges matched, is the normal form. Three crystals growing together in this way, interpenetrating with the composition face normal to the base, and including the prism-base edge, produce a six-pointed star, which has the orientation of the opposite points the same. Text figure 15 shows an optical section of the homogeneous regular growth, the first kind, the orientation being such that for two parts of the twin the extinction is straight, looking nearly along *a;* while for the other parts the extinction is about 14°, indicating that the crystal is being examined along $b$. The cleavage is perfect, parallel to the prism, and was readily obtained by crushing the crystals. It develops also from the pressure of the cover upon the crystals, due to evaporation. On allowing the crystals to dry, they develop cracks normal to the plane of symmetry, as in the case of the metoxyhemoglobin crystals. These cracks are due to tension, and are at 60° to the cleavage.

Pleochroism strong; $\mathfrak{a}$ nearly colorless, $\mathfrak{b}$ rose-pink, $\mathfrak{c}$ deep red, as is usual in reduced hemoglobin. The spectrum is the normal reduced hemoglobin spectrum, with a strong absorption band between $D$ and $E$ and the blue end absorbed up to near 450 $\mu\mu$.

The pleochroism is very noticeable in the twinned plates where the twinning is the contact twin of the "horse-type." The part where one crystal overlaps the other may be of a brownish-yellow color when the two parts of the single crystals projecting from the common part are respectively deep red ($\mathfrak{c}$) and pale lilac (near $\mathfrak{a}$). All parts show the reduced-hemoglobin spectrum, however, the absorption band varying in width and the limit of absorption varying with the different colors, as seen in the different parts of the composite crystal. The axial plane lies in the plane of symmetry, and the orientation of the optic axes is similar to that of the oxyhemoglobin, etc. The mean elasticity axis $\mathfrak{b}=b$, $\mathfrak{a} \wedge a=14°$; $\mathfrak{c} \wedge \acute{c}=6°$, both in the obtuse angle. Extinction is straight on the base, and on edge view, looking along $\mathfrak{b}$, the extinction is 14° from the trace of (001) and 6° from the edge of (110). From these maxima the extinction varies down to parallel in other edge views; this is readily observed in twins. In the star-shaped twin the opposite sectors extinguish simultaneously. Upon the basal aspect the interference figure is seen with one brush in the field and the other outside of it. The axial angle is large as in the former cases. Optical character positive, $Bx_a=\mathfrak{c}$.

Reduced hemoglobin crystals developed in all bloods that had been kept for some days, either at ordinary temperature or in a refrigerator. They usually developed in blood that had contained oxyhemoglobin, or had been exposed to the air before the reduction to hemoglobin began. In short, the two forms above described seem to pass into reduced hemoglobin when in solution and even when in the form of crystals, the latter by paramorphic change. But evidently most of the crystals of reduced hemoglobin examined crystallized from solution as such.

These reduced-hemoglobin crystals were often associated with the pure methemoglobin crystals, in regular growths, in the same way that was noted in the case of oxyhemoglobin, text figures 14 and 15.

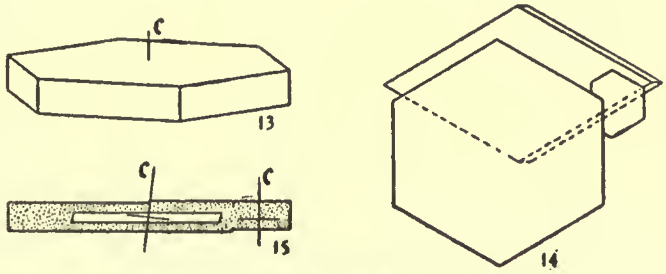
### (4) *Methemoglobin of Alosa sapidissima.*

Hexagonal: Axial ratio not determined.

Forms observed: Prism (10$\bar{1}$0), base (0001).

Angles: Prism 120° (60°); prism to base 90°.

Habit tabular, in hexagonal plates, by strong development of (0001) (text figure 13). The plates grew at first singly, but afterwards developed upon the crystals of oxyhemoglobin and of reduced hemoglobin, when these latter by twinning and adjustment had developed angles of 60° and 120°, and these regular growths are symmetrical, the methemoglobin growing on the sides and angles of the monoclinic crystals, with the basal surfaces parallel in the two substances (text figures 14 and 15). This brings the axes of least elasticity almost parallel in the two. The methemoglobin often completely incloses the monoclinic crystal, which can be seen through the enveloping layer, owing to its strong pleochroism and double refraction.



FIGS. 13, 14, 15. *Alosa sapidissima* Oxyhemoglobin.

Pleochroism is rather weak, seen only on edge view; the colors are $\varepsilon$ deep brownish-red, $\omega$ paler, but differing only in shade. In convergent light the uniaxial figure is seen on the basal aspect; in parallel light the crystal is singly refracting in this aspect. Examined on edge, $\varepsilon$ has less elasticity than $\omega$. Hence $\varepsilon > \omega$ and the crystal is *positive.*

From the characters of these methemoglobin crystals, it is very likely that the substance is really a mimetic twin and only pseudohexagonal. It is not very permanent, but decomposes and produces a granular brownish precipitate, leaving the monoclinic crystals (usually now changed to reduced hemoglobin) unaltered.

These methemoglobin crystals were found in blood of shad that had died in the air, and in freshly drawn blood that had been exposed to the air, and seem to be due to a separation of the metoxyhemoglobin into methemoglobin and oxyhemoglobin, which latter may be afterwards changed to reduced hemoglobin before the methemoglobin disappears. The formation of the pure methemoglobin, which crystallizes in these hexagonal plates, is probably due to the further oxidation.

### CARP, *Cyprinus carpio.* Plates 5 and 6.

Blood of the carp was obtained from live fish caught at Gloucester, New Jersey. It was oxalated, ether-laked, and slides prepared within a few hours after it was collected. The blood had a brownish color, and was probably the metoxyhemoglobin mixture. After standing in a test-tube for 24 hours it was practically all converted into reduced hemoglobin. Preparations of this were also made and examined. Both the metoxyhemoglobin and the reduced hemoglobin crystallized readily, but without any separation of pure methemoglobin. They are isomorphous, having apparently almost the same axial ratio, and perhaps the axial ratios are actually identical.

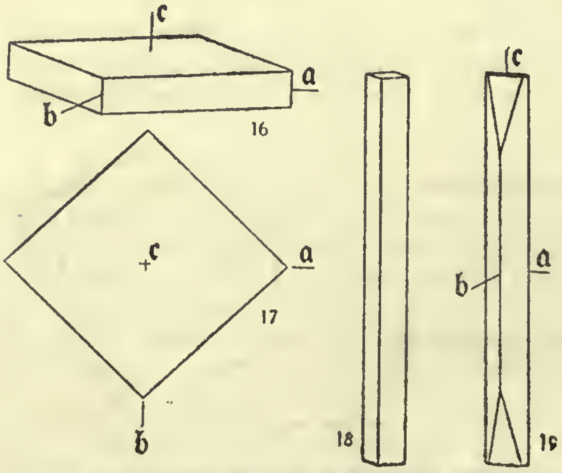
#### (1) *Metoxyhemoglobin of Cyprinus carpio.*

Orthorhombic: Axial ratio, $a : b : \acute{c} = 0.949 : 1 : 1.03$.

Forms observed: Prism (110), base (001), and, from twins on the macrodomes, also macrodomes (301), (201), (302).

Angles: $110 \wedge 1\bar{1}0 = 93°$ (87° normals). $110 \wedge 001 = 90°$; from twins $302 - \bar{3}02 = 68° 30'$; $201 \wedge \bar{2}01 = 55° 15'$; $301 \wedge \bar{3}01 = 37° 30'$.

Habit generally tabular, nearly square plates, formed by flattening on the base (001) in combination with the prism (110) (text figures 16 and 17); also long prismatic, formed by development of the prism in the same combination (text figure 18). The prismatic crystals are the first to appear; these are gradually absorbed as the plates develop. Twins are common in the prismatic habit, apparently on the macrodomes noted above; they are not so common in the tabular habit, but apparently the twin on (302) occurs. Parallel growths are common in the tabular form; perhaps also homogeneous regular growths occur. This parallel growth produces a piling up of the plates, and composite crystals and groups result.



FIGS. 16, 17, 18. *Cyprinus carpio* Metoxyhemoglobin.
FIG. 19. *Cyprinus carpio* Reduced Hemoglobin.

Pleochroism is marked; the colors are shades of brownish-red. Orientation of the elasticity axes was made out as follows: $\mathfrak{a} = b$, $\mathfrak{b} = a$, $\mathfrak{c} = \acute{c}$. The interference figure was not observed. Extinction is symmetrical on the plates when examined on the base (001) and straight when examined on edge.

#### (2) *Reduced Hemoglobin of Cyprinus carpio.*

Orthorhombic: Axial ratio, $a : b : \acute{c} = 0.949 : 1 : 1.098$.

Forms observed: Prism (110), base (001), macrodome (401).

Angles: $110 \wedge 1\bar{1}0 = 93°$ (87° normals); $110 \wedge 001 = 90°$; $401 \wedge \bar{4}01 = 27°$.

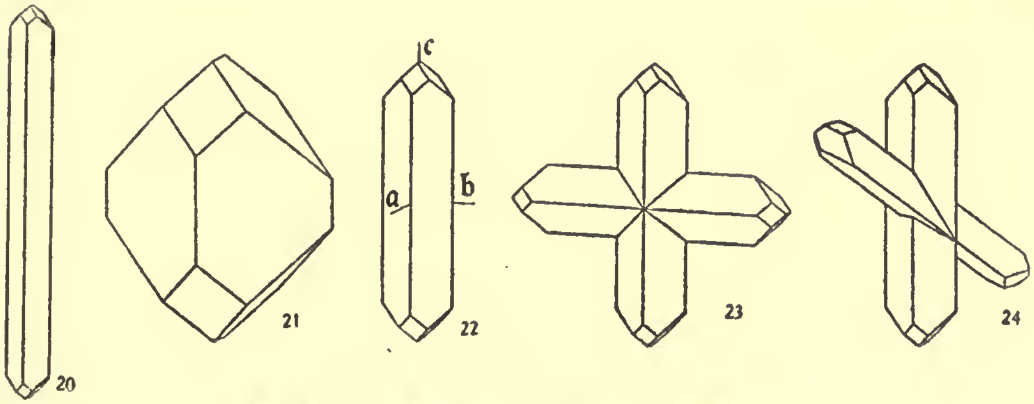
Habit at first prismatic, long lath-shaped crystals consisting of the prism (110) and the base (001); these later develop the acute macrodome (401), showing then the combination (110) and (401) with sometimes the base also (text figure 19). They gradually give place to the tabular form, analogous to the second form of the metoxyhemoglobin. The tabular form consists of the prism (110) and the base only, beginning as very thin plates, but gradually becoming thick tables or blocks, due to the elongation of the prism. The rods grow into sheaf-like tufts, but do not appear to twin on the macrodome as in the metoxyhemoglobin form. The tabular crystals also aggregate into groups by parallel growth on the base.

Pleochroism is rather strong; $\mathfrak{a}$ rose-pink, $\mathfrak{b}$ rose-red, $\mathfrak{c}$ deep red. Orientation of the elasticity axes is as in the metoxyhemoglobin, $\mathfrak{a}=b$, $\mathfrak{b}=a$, $\mathfrak{c}=\acute{c}$; this orientation is exactly the same in the prismatic and in the tabular habits. Extinction is symmetrical on the basal aspect of the plates and straight in all other aspects; the interference figure was not observed.

## BATRACHIA.

### NECTURUS, *Necturus maculatus*. Plates 6 and 7.

The blood of *Necturus* was examined on a number of different occasions, and was always freshly obtained by killing and bleeding the animal. Preparations were made by various methods: in some the usual method of oxalating and ether-laking was followed; in some the blood was defibrinated by beating it, and then it was ether-laked without the addition of oxalate; to the defibrinated blood oxalate was added in the normal amount, and the crystals from the oxalated and non-oxalated bloods compared. Beyond differences in habit, no change in the crystallization was produced by the variations in treatment. Both oxyhemoglobin and reduced hemoglobin were obtained, but they seemed to crystallize in exactly the same form. Many of the crystals were quite brownish, but the spectroscope failed to reveal any methemoglobin.



FIGS. 20, 21, 22, 23, 24. *Necturus maculatus* Oxyhemoglobin.

### *Oxyhemoglobin of Necturus maculatus.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.6494 : 1 : 1$.

Forms observed: Prism (110), macrodome (110), brachydome (011); and in twins (111).

Angles: $110 \wedge 1\bar{1}0 = 66^\circ$; $011 \wedge 0\bar{1}1 = 90^\circ$; $101 \wedge \bar{1}01 = 66^\circ$; from twin, angle of pyramid $111 \wedge \bar{1}\bar{1}1 = 58^\circ$ (calculated 57° 8′).

Habit prismatic, long or short prisms, terminated by one or both domes, the habit varying with the method of preparation. In crystals prepared by defibrinating and ether-laking without addition of oxalate the crystals are very long prismatic, 20 to 30 times as long as they are thick (text figure 20); those prepared with an excess of oxalate are very short prismatic, nearly equidimensional (text figure 21); when the normal amount of oxalate is used the crystals are intermediate in form, the ratio of thickness to length being about 5 : 1 (text figure 22). Twins are common, cross-shaped, as in staurolite, one on the brachydome (011) making a square cross (text figure 23), and one on the pyramid (111) making an oblique cross (text figure 24), being the usual forms. A twin on what appears to be (032) was also observed, and another on (043); these were somewhat doubtful. No cleavage was observed, but the crystals are brittle.

Pleochroism is very weak, the colors much more brownish than is usual with oxyhemoglobin, but with the spectroscope the double absorption band of oxyhemoglobin is shown in most specimens; in one case, when excess of oxalate was used, but one faint dusky band appeared at 590 *μμ* to 570 *μμ*. The colors are pale brownish-red; 𝔠 and 𝔟 are nearly equal and darker than 𝔞. Orientation of the elasticity axes is 𝔞 = *a*, 𝔟 = *b*, 𝔠 = *ć*. The plane of the optic axes is the brachypinacoid, and the interference figure appears when looking along 𝔞, which is the acute bisectrix. Hence, the optical character is *negative.* But 𝔟 and 𝔠 are nearly equal, and in some cases a figure was seen with 𝔠 = *b* and 𝔟 = *ć*, the basal pinacoid being the plane of the optic axes. The axial ratio shows the crystal axes *b* and *ć* equal. Pleochroism is hence scarcely noticeable when looking along 𝔞, but becomes distinct when looking along 𝔟. The axial angle is about $2E$ = 35°, the figure being rather dusky.

### *Reduced Hemoglobin of Necturus maculatus.*

The crystals of reduced hemoglobin were detected in only one preparation. These gave the spectrum of reduced hemoglobin distinctly. They were formed in a preparation in which oxalate was not used, and were mixed with crystals of oxyhemoglobin. In form and angles, and in optical characters, they were not distinguished from the crystals of oxyhemoglobin above described. They may have been paramorphous alterations from the oxyhemoglobin crystals.

## REPTILIA.

### INDIAN PYTHON, *Python molurus.* Plates 7 and 8.

Blood from two specimens was received, one from the Philadelphia Zoölogical Gardens, and one from the National Zoölogical Park at Washington. The Philadelphia specimen was fresh blood, that from Washington was received in a somewhat putrid condition. Both developed oxyhemoglobin crystals; but in the specimen from Washington another type of crystal developed besides the normal α-oxyhemoglobin crystal. The Philadelphia specimen was prepared in two ways, (1) oxalated, ether-laked, and centrifugalized; and (2) frozen, and after thawing centrifugalized. The monoclinic crystals of habit (a) developed in both, and with a few of the plates of habit (b) in both preparations. The specimen from Washington was in a firm clot, and this was broken up by rubbing with sand; ether was added, and the blood diluted with a little neutral saline so that it could be readily centrifugalized. The monoclinic crystals of habit (b) developed in these slides; and in a few slides the tetragonal type of crystal (β-oxyhemoglobin) occurred rather plentifully. They developed after the monoclinic crystals of α-oxyhemoglobin.

### *a-Oxyhemoglobin of Python molurus.*

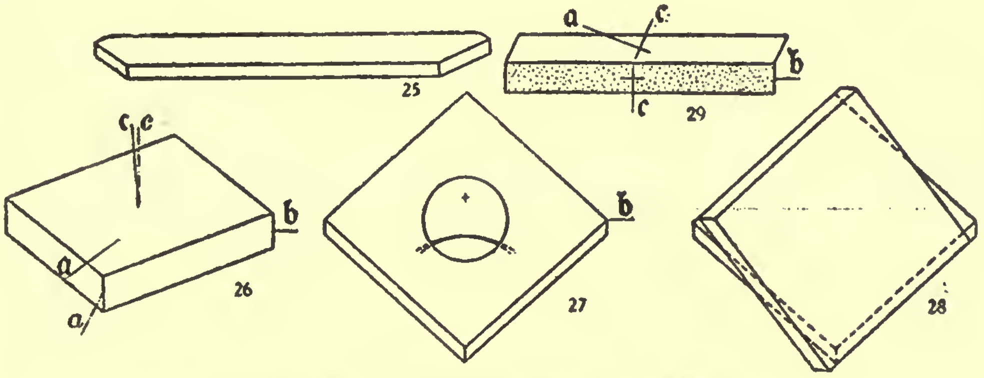
Monoclinic: Axial ratio $a : b : \dot{c} = 0.900 : 1 : \dot{c}$; $\beta = 65°$ (about).

Forms observed: Prism (110), base (001), orthopinacoid (100).

Angles: Traces of $110 \wedge 1\bar{1}0$ on base $= 84°$ (normals); $100 \wedge 001 = 65° = \beta$. The angle of the prism varied from 81° to 86°, but the best measurements ran very close to 84°.

Habit (a), long lath-shaped crystals with oblique ends, due to elongation on the ortho-axis, with the combination (001) (100), and two faces of the prism 110 and $1\bar{1}0$ (text figure 25); these appeared first in the fresh blood. Habit (b) tabular by development on (001) with a short prism (110) (text figures 26, 27; also plate 7, figures 39 to 42); these appeared later in the fresh blood and were the common type in the stale blood. Habit (b), the tabular, crystals were also seen with the combination of habit (a), but distinctly tabular. The crystals of habit (a) and these occasional crystals of habit (b), which show the same planes, are distinctly clinohedral or domatic in form, that is, hemimorphic on an axis normal to the ortho-axis. But habit (b), which appears to be the normal form of crystal of the oxyhemoglobin, is only occasionally seen in these clinohedral crystals.

Homogeneous regular growths occur, the crystals having a common prism-basal-pinacoid edge and uniting on the base (text figure 28). This closely resembles the mica twin. It appears, sometimes, to be repeated in irregular order and probably accounts for anomalous extinction seen in a few large, apparently composite, crystals. Seen in edge view the twin is readily recognized by the different extinction angles in the several parts of the composite crystal (text figure 29), which shows the twin in section, examined along the axis $a$.



FIGS. 25, 26, 27, 28, 29. *Python molurus* a-Oxyhemoglobin.

The color of the crystals is deep oxyhemoglobin red. Pleochroism is rather strong in both habits of crystals when examined on the base, but not so noticeable on the edge view, when the plates are examined, on account of the strong color in this aspect. The pleochroism is best seen in the plates, because the dimensions of the rods are such that the depth of color is influenced by the greater thickness of the crystals along $a$ than along $\dot{c}$.

The pleochroic colors are: 𝔞 yellowish-red, 𝔟 deeper and somewhat blood-red, 𝔠 deep blood-red. Orientation of the elasticity axes is $\mathfrak{a} \wedge a = 21°$ in the obtuse angle, $\mathfrak{b} = b$, $\mathfrak{c} \wedge \dot{c} = 4°$ in the obtuse angle. The axial plane lies in the plane of symmetry, and $\mathfrak{c} = Bx_a$, the acute bisectrix. The optical character is hence *positive*. The interference figure is seen on the base in the tabular crystals and shows the symmetrical extinction, with a single brush of the figure in the field. The angle $2E$ could not be measured, but was large; the other brush is out of the field.

### *β-Oxyhemoglobin of Python molurus.*

Tetragonal: Axial ratio $a : \dot{c} = 1 : 0.537$.
Forms: The tetragonal bipyramid (111) was the only form seen.
Angles: Profile views were obtained, giving the angle of the pyramid edges as 56½°.

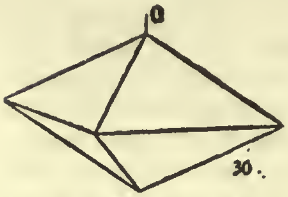


Fig. 30. *Python molurus* β-Oxyhemoglobin.

Habit pyramidal; small, very symmetrical, tetragonal pyramids, occurring singly, without producing twins or other aggregates (text figure 30).

Color, the normal oxyhemoglobin red; pleochroism noticeable even in the small crystals; $\omega$ deeper red than $\varepsilon$. $\varepsilon$ is the axis of greater elasticity, and hence the optical character is *negative*.

These small crystals of β-oxyhemoglobin appeared in the blood received from Washington several days after the slides had been prepared, this being usual with bloods developing several forms of oxyhemoglobin.

Table 35.—*Characters of crystals of reduced hemoglobin, oxyhemoglobin, etc., of the Pisces, Batrachia, and Reptilia examined.*

| Name of species. | Axial ratio. | Angle β. | Prism angle. | Extinction angle. | Optical character. | System. | Substance. |
|---|---|---|---|---|---|---|---|
| | | ° | ° ′ | | | | |
| Pisces: | | | | | | | |
| Raia lævis | 0.6008 : 1 : 4024 | 90 | 62 0 | 0° | Negative | Orthorhombic | OHb. |
| Acipenser sturio | .............. | 90 | ..... | 0° | ........ | Do. | OHb. |
| Alosa sapidissima | 1.804 : 1 : $\dot{c}$ | 68 | 58 0 | $\mathrm{a} \wedge a = 6^\circ$ | Positive | Monoclinic | OHb. |
| Do | 1.786 : 1 : $\dot{c}$ | 70 | 58 30 | $\mathrm{a} \wedge a = 10^\circ$ | Do. | Do. | MOHb. |
| Do | 1.786 : 1 : $\dot{c}$ | 70 | 58 30 | $\mathrm{a} \wedge a = 14^\circ$ | Do. | Do. | Hb. |
| Do | .............. | 90 | 60 0 | 0° | Do. | Hexagonal | MHb. |
| Cyprinus carpio | 0.949 : 1 : 1.03 | 90 | 87 0 | 0° | ........ | Orthorhombic | MOHb. |
| Do | 0.949 : 1 : 1.098 | 90 | 87 0 | 0° | ........ | Do. | Hb. |
| Batrachia: | | | | | | | |
| Necturus maculatus | 0.6494 : 1 : 1 | 90 | 66 0 | 0° | Negative | Do. | OHb. |
| Reptilia: | | | | | | | |
| Python molurus | 0.900 : 1 : $\dot{c}$ | 65 | 84 0 | $\mathrm{a} \wedge a = 21^\circ$ | Positive | Monoclinic | α-OHb. |
| Do | 1 : 0.537 | 90 | 90 0 | 0° | Negative | Tetragonal | β-OHb. |

# CHAPTER X.

## CRYSTALLOGRAPHY OF THE HEMOGLOBINS OF AVES.

The hemoglobin crystals of 10 species of birds were examined, 2 species belonging to the subclass *Ratitæ* or flightless birds, and the remainder to the subclass *Carinatæ* or flying birds. The *Ratitæ* examined were the African ostrich, a representative of the *Struthionidæ;* and the cassowary, a member of the *Casuariidæ.* The carinate birds represented 4 orders, and were distributed as follows: *Anseres,* 3 species, the goose, trumpeter swan, and whistling swan; *Gallinæ,* 3 species, chicken, Virginia quail, and guinea-fowl; *Columbæ,* 1 species, the carrier pigeon; and *Passeres,* 1 species, the crow.

It will be noticed that this is not a representative list of birds, but it includes examples from the two principal subclasses, the *Ratitæ* and the *Carinatæ.* Of the 23 orders of living birds ordinarily recognized but 5 are represented in this list. In the case of the *Anseres* the 3 species are closely related and 2 belong to the same genus, thus permitting of close comparison. In the *Gallinæ,* too, are 3 species usually regarded as closely related. Two of these, the chicken and quail, will be seen to resemble each other closely, but the third, the guinea-fowl, is quite far removed from them as shown by its hemoglobin crystals. Indeed, the crystals of the guinea-fowl show closer resemblance to those of the African ostrich, one of the *Ratitæ,* which in its zoölogical relations is generally regarded as far removed from the *Gallinæ.* The chicken and quail crystals, however, show some resemblance to those of the *Anseres,* and even to the *Columbæ.* The one passerine bird studied gave crystals that were quite different from any of the others examined.

The table given at the end of the chapter shows some of the characters of the crystals of the oxyhemoglobin of the birds examined, and it will be noticed that, with two exceptions, they are either orthorhombic or tetragonal. In the detailed descriptions which follow it will be shown that the orthorhombic crystals have a tendency to become pseudo-tetragonal (or not distinguishable from tetragonal) by mimetic twinning; so that it seems very likely that the two species recorded as tetragonal may in reality be only pseudo-tetragonal.

### AVES.

#### African Ostrich, *Struthio camelus.* Plate 8.

The sample of blood was received from the National Zoölogical Park at Washington in an exceedingly putrid condition, and clotted. The clot was rubbed in sand, with addition of ether, and the mixture centrifugalized. But a small quantity of the clear liquid was obtained, from which slides were made. After 48 hours at near a freezing temperature crystals appeared in one of the slides; they appeared to be reduced hemoglobin, but the deep-colored solution prevented spectroscopic determination. Later they improved somewhat and then appeared to be methemoglobin. They were very small—too small for obtaining an interference figure. They did not appear to dissolve readily when once formed, although they formed very slowly.

*Methemoglobin (?) of Struthio camelus.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.5658 : 1 : \acute{c}$.

Forms observed: Prism (110), base (001).

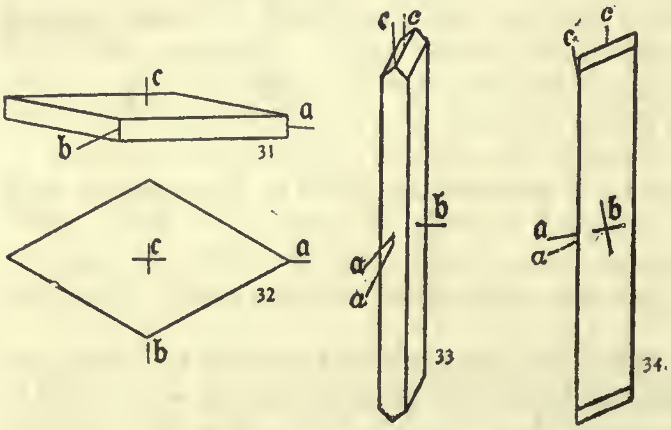
Angles: $110 \wedge 1\bar{1}0 = 59°$ (normals); $110 \wedge 001 = 90°$.

Habit in small rhombic tables, very thin (text figures 31 and 32); occurring singly and not twinned, as is common in these rhombic plates.

Pleochroism rather strong; a colorless, b yellowish-red, c deep red. Extinction was straight on all edge views and symmetrical on the base. Orientation of the elasticity axes is $a = b$, $b = a$, $c = \acute{c}$; plane of the optic axes is the macropinacoid. No interference figure could be obseved.

### Cassowary, *Casuarius galeatus*. Plate 8.

The specimen was received from the National Zoölogical Park at Washington, District of Columbia. The blood was in the form of a small clot and was somewhat decomposed. Owing to the small quantity, it was only treated with ether to lake it, but it was at once converted into a jelly. After being exposed to temperatures ranging above and below the freezing-point for 24 hours, some fluid was obtained and 5 slides were prepared. The crystals formed gradually, but were very poor. They did not appear to be very soluble. The spectroscope showed a mixture of oxyhemoglobin and methemoglobin in the serum, and the crystals were rather brownish-red, but probably were oxyhemoglobin. The serum was highly colored, and photographs were obtained with some difficulty. The crystals formed mainly along the edge of the cover-glass.



Figs. 31, 32. *Struthio camelus* Methemoglobin.
Figs. 33, 34. *Casuarius galeatus* Oxyhemoglobin.

*Oxyhemoglobin of Casuarius galeatus.*

Monoclinic: Axial ratio not determined, $\beta = 64° 30'$.

Forms observed: Clinopinacoid (010), orthopinacoid (100), and clinodome (011).

Angles: $010 \wedge 100 = 90°$; $100 \wedge$ edge of $011 = a \wedge \acute{c} = \beta = 64° 30'$.

The angle of the dome was not satisfactorily measured.

Habit prismatic by development of the two pinacoids (100) and (010) in the zone of $\acute{c}$; the termination is oblique and is produced by the clinodome (011), text figures 33 and 34, usually only one end of the crystal being seen.

Pleochroism rather strong; $\mathfrak{a}$ pale yellowish, $\mathfrak{b}$ deep red, $\mathfrak{c}$ deep red. Extinction on (100) is straight; on (010) it is oblique with the angle $\mathfrak{c} \wedge \acute{c} = 11°$. Orientation of the elasticity axes is hence $\mathfrak{a} \wedge a = 14\frac{1}{2}°$, $\mathfrak{c} \wedge \acute{c} = 11°$, $\mathfrak{b} = b$. The plane of the optic axes is the plane of symmetry, and from the character of the double refraction $\mathfrak{b}$ is nearly equal to $\mathfrak{c}$; the acute bisectrix $Bx_a = \mathfrak{a}$, hence the optical character is *negative.*

### Goose, *Anser anser.* Plate 9.

Blood of the domestic goose was obtained by killing and bleeding the bird. Repeated attempts were made to crystallize it. Finally, after repeatedly freezing and thawing the blood, and then centrifugalizing and adding ether, and (in preparing the slides) allowing the drop to become very concentrated before covering, crystals were obtained by keeping the slides for several hours at about the freezing-point. On bringing the preparations into a warm room the crystals dissolve readily, and all examinations were made and photographing done at a room temperature of about 0° C. The crystals were normal oxyhemoglobin.

### *Oxyhemoglobin of Anser anser.*

Tetragonal or pseudo-tetragonal: No axial ratio could be determined, as no pyramidal planes were developed.

Forms observed: Prism (110) and base (001).

Angles: $110 \wedge 1\bar{1}0 = 90°$; $110 \wedge 001 = 90°$.

Habit thick tabular, the thickness one-third to one-fourth of the width of the plate (text figure 35); some also quite thin. The plates grow in clusters, as though twinned on a pyramid (101), also in irregular aggregates; sometimes arborescent by the overlapping of the plates and extension along a crystal axis. Smaller crystals, developing later than the normal plates, are nearly equidimensional and closely resemble cubes.

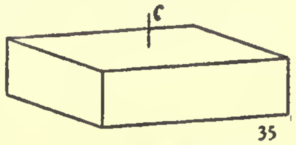


Fig. 35. *Anser anser* Oxyhemoglobin.

Pleochroism was not noticeable in most cases, perhaps due to the very deep color of the plasma; some large thick crystals showed a slight pleochroism. On the basal pinacoid the crystals are singly refracting when examined in parallel polarized light; in convergent light, the uniaxial interference figure is seen as a very dusky cross. Examined on edge, the vertical axis (the extraordinary ray) was seen to be the direction of less elasticity; hence $\varepsilon > \omega$ and the optical character is *positive.*

When examined on edge, the crystals polarized as a whole and did not indicate any appearance of twinning, but from the fact that similar tetragonal characters are produced by what appears to be homogeneous regular growth in the case of the whistling-swan blood—*Olor columbianus*—which is distinctly orthorhombic, it is quite possible that these blood crystals are only pseudo-tetragonal.

### Trumpeter Swan, *Olor buccinator.* Plates 9 and 10.

This specimen of blood was received from the Philadelphia Zoölogical Gardens. The blood was oxalated, ether-laked, and centrifugalized, and preparations made as usual. The slides were kept at a temperature near the freezing-point, but no crystals developed until after 24 hours. Even then only scattered crystals appeared, excepting in two slides. The crystals were very soluble and had to be kept at about 0° C. during the examination and photographing. Several other trials were subsequently made with the same blood, but they failed to yield crystals. The crystals obtained were found to be oxyhemoglobin by spectroscopic examination.

### *Oxyhemoglobin of Olor buccinator.*

Tetragonal or pseudo-tetragonal: No axial ratio determined, as no pyramidal planes developed.

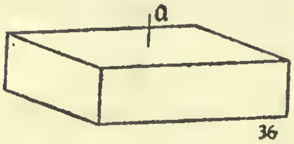
Forms observed: Prism (110) and base (001).

Angles: $110 \wedge 1\bar{1}0 = 90°$; $110 \wedge 001 = 90°$.

Habit thin to thick tabular, by development of (001) and (110) (text figure 36); in single crystals and in groups, often arborescent by growing together on the base or on a pyramid; also in clusters, but without definite appearance of twinning. Some of the large crystals were evidently composite, but did not show any appearance of twinning when examined on edge in polarized light.

Pleochroism faint, apparently abnormal; the absorption for the direction of greater elasticity appears to be slightly greater than for the direction of less elasticity. Colors are deep oxyhemoglobin red; somewhat paler for $\omega$.

Uniaxial, singly refracting on the base in parallel polarized light, and showing a faint dusky cross in convergent light. Seen on edge the double refraction is very weak, but is observable with the aid of the quartz wedge, etc.; when it is seen that $\omega$ is the direction of less elasticity and hence $\omega > \epsilon$ and the optical character is *negative.* The fact that the double refraction is so weak would favor the suspicion that the crystals are composite and really only pseudo-tetragonal, as is the case with some crystals in the next species, *Olor columbianus.*



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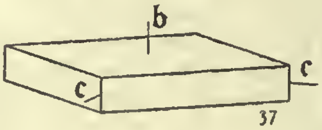

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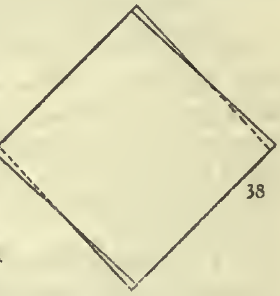

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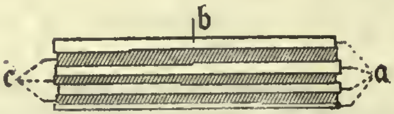

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Fig. 36. *Olor buccinator* Oxyhemoglobin. Figs. 37, 38, 39. *Olor columbianus* Oxyhemoglobin.

### Whistling Swan, *Olor columbianus.* Plate 10.

The specimen was received from the Zoölogical Gardens at Washington and was clotted and in a very putrid condition. The clot was ground up with sand, etherized, and the liquid obtained oxidized by exposing it to the action of pure oxygen. It was then centrifugalized, and slides prepared as usual, the drops being allowed to become very concentrated before covering. In only two slides out of some two dozen prepared did crystals appear. The crystals were rather dark, but were oxyhemoglobin.

### *Oxyhemoglobin of Olor columbianus.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.9657 : 1 : \acute{c}$; also pseudo-tetragonal by homogeneous regular growth.

Forms observed: Prism (110), base (001).

Angles: $110 \wedge 1\bar{1}0 = 88°$ (normals); $110 \wedge 001 = 90°$.

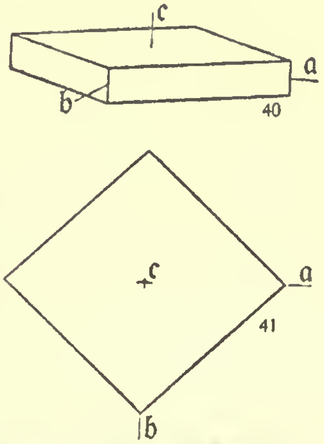
Habit thin tabular in simple crystals (text figure 37), becoming thicker by homogeneous regular growth, in which the prism-base edge of one member of the group is in line with prism-base edge of the next following layer (text figure 38); the successive layers arranged in polysynthetic order (text figure 39). Composite crystals are produced by this piling up of the successive individuals of the group, that finally develop angles of 90° for the plate, by averaging of the angles 88° and 92°; the result not being distinguishable from a tetragonal crystal when examined on the flat, and even giving a dusky uniaxial cross in convergent light. This kind of crystal shows the composite character by being less regularly developed than the simple crystal. On edge the separate individuals in the group are at once distinguished by pleochroism, using one nicol, and with crossed nicols by the difference of double refraction. Some were seen consisting of two or three individuals, but many consisted of a much larger number in the polysynthetic arrangement. The crystals were large, but did not grow into arborescent groups as in the case of those from the blood of *Olor buccinator.*

Pleochroism is rather marked on the flat view, less so on the edge; the absorption is $\mathfrak{c} > \mathfrak{b} > \mathfrak{a}$. The colors are shades of the oxyhemoglobin red, $\mathfrak{a}$ being yellowish-red. The orientation of the elasticity axes is $\mathfrak{a} = a$, $\mathfrak{b} = \acute{c}$, $\mathfrak{c} = b$; the optic axes being in the plane of the basal pinacoid. No interference figure appears therefore on the flat view (001); but on edge, when looking nearly along $\mathfrak{a}$, one brush of the figure is seen. In the composite crystals this also is visible, the arrangement of their elasticities being as shown in text figure 39. From these figures it is seen that the axis $\mathfrak{b}$ keeps its position in the regular growth, and the axes $\mathfrak{a}$ and $\mathfrak{c}$ alternate in the successive layers. In case of very thin layers in the composite crystals, so that the layers become too thin to show by the microscopic examination, this averaging of $\mathfrak{a}$ and $\mathfrak{c}$ would greatly reduce the amount of the double refraction, so that it might become almost zero, which is the condition in the species of swan examined, and leads to the suspicion that in the blood of *Olor buccinator* the crystals are only pseudo-tetragonal. From the position of the brush of the interference figure it is evident that the acute bisectrix $Bx_a = \mathfrak{a}$, and the optical character is *negative.*

The mimetic crystals produced by the homogeneous regular growth are singly refracting when examined on (001) and are not strongly doubly refracting when examined on edge view, especially when the individual layers are thin and not of the same thickness throughout; they are hence in some cases truly pseudo-tetragonal. The averaging of the angles of 88° and 92° to 90° makes them strictly tetragonal in form.

### CHICKEN, *Gallus domestica.* Plate 11.

Blood was obtained from the living chicken, oxalated and centrifugalized, and only the corpuscles used. The corpuscles were treated by the usual method and crystallized at a temperature near the freezing-point. All examinations had to be made at the same low temperature, the room being kept at about the freezing-point or below. Very few slides showed crystals, and they were usually isolated or in small groups. Blood from two different birds was examined at different times, but the habit was about the same in both cases. The crystals were oxyhemoglobin.



FIGS. 40, 41. *Gallus domestica* Oxyhemoglobin.

*Oxyhemoglobin of Gallus domestica.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.949 : 1 : \acute{c}$.
Forms observed: Prism (110), base (001).
Angles: $110 \wedge 1\bar{1}0 = 87°$ (normals); $110 \wedge 001 = 90°$.

Habit tabular, the square tables (text figures 40 and 41) aggregated into groups by piling up of the plates or perhaps by twinning on an axis normal to the edge (110-001); also by what appears to be twinning on a dome; the crystals usually occur in isolated clusters. Sometimes skeleton crystals are seen that look tetragonal, but are orthorhombic according to their optical characters.

Pleochroism is not very marked, hardly noticeable on the flat view, but stronger on the edge view, especially when looking along b. Orientation of the elasticity axes, a=b, b=a, c=ć. The plane of the optic axes is the macropinacoid; $Bx_a$=a, hence the crystal is optically negative. Absorption c>b>a. On looking along a in convergent light the interference figure is seen with the brushes rather widely separated.

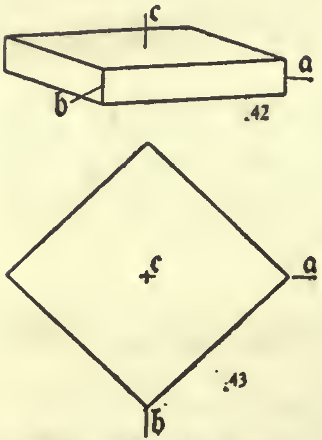
### QUAIL, *Colinus virginianus*. Plate 12.

The blood was obtained from the living bird, and prepared in the usual manner. Corpuscles were used for extraction of the oxyhemoglobin which was tested by the spectroscope. The crystals form sparingly and melt readily at a little above 0° C.

#### *Oxyhemoglobin of Colinus virginianus.*

Orthorhombic: *a* : *b* : *ć* =0.9657 : 1 : *ć*.
Angles: 110 ∧ 1$\bar{1}$0=88° (normals); 110 ∧ 001=90°.
Forms observed: Prism (110), base (001).

Habit thin to thick square tabular; the tables consisting of the above combination and varying in thickness from one-fourth to one-half of the width of the plate (text figures 42 and 43); the crystals grew singly and in groups, but did not grow in the radiating form of the chicken oxyhemoglobin. Perhaps they twin on the axis normal to the prism-base edge, as the plates pile up on the base and overlap somewhat irregularly.



FIGS. 42, 43. *Colinus virginianus* Oxyhemoglobin.

Examined on (001), the crystals show no perceptible pleochroism; on edge the pleochroism is weak, but noticeable. The angle of the prism is so near 90° and the plates so irregular, due to overlapping, etc., that it is difficult to determine the exact orientation of the optic axes; the extinction is straight on the edge view and symmetrical on the (001) view. One of the diagonals is readily made out by the quartz-wedge to be an axis of greater elasticity than the other, but on edge views it is seen that c=ć. The plane of the optic axes is probably the macropinacoid, and when looking along a (in edge view) the interference figure is seen, showing $Bx_a$=a, and the optical character is hence *negative*. Absorption is c=b (nearly) > a. Pleochroism: c and b deep red, a paler red.

### GUINEA-FOWL, *Numida meleagris*. Plate 12.

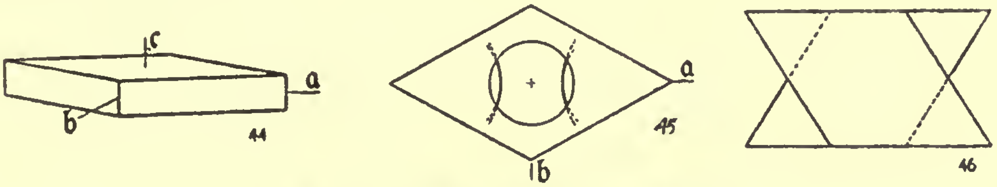
The blood was obtained by bleeding the living bird and was oxalated and prepared in the usual manner. Crystals formed readily and did not appear to be very soluble, as they remained in perfect condition at room temperature. The oxyhemoglobin crystallizes readily at ordinary temperature in well-formed crystals, in contrast to the crystals obtained from the bloods of most of the birds examined.

### *Oxyhemoglobin of Numida meleagris.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.554 : 1 : \acute{c}$.

Forms observed: Prism (110), base (001); and, in twins, unit pyramid (111).

Angles: $110 \wedge 1\bar{1}0 = 58°$ (normals); $110 \wedge 001 = 90°$. The angle of the prism sometimes appears to approximate 60°, but ran down as low as 57°, and 58° seems to be the best measurement.



FIGS. 44, 45, 46. *Numida meleagris* Oxyhemoglobin.

Habit thin to thick tabular; the rhombic plates varying in thickness from one-tenth to one-fourth of the long diagonal of the prism; the crystals consist simply of the short prism (110) and the base (001) (text figures 44 and 45). Twinning on the normal to the prism-base edge as twin axis (horse-type) common (text figure 46); also twins on the pyramid and perhaps a macrodome were observed. The crystals usually occur singly or in twins, not in more complicated groupings.

Pleochroism strong; $\mathfrak{a}$ very pale yellow-red, $\mathfrak{b}$ moderately deep scarlet-red, $\mathfrak{c}$ very deep red. Extinction on the base is symmetrical and on edge is straight in all positions. The plane of the optic axes is the macropinacoid; the orientation of the elasticity axes is $\mathfrak{a} = b$, $\mathfrak{b} = a$, $\mathfrak{c} = \acute{c}$. On the base, in convergent light, the biaxial interference figure is seen with the axes rather widely separated; hence $Bx_a = \mathfrak{c}$ and the optical character is *positive.*

### CARRIER PIGEON, *Columba livia var.* Plate 13.

The bird was killed and bled and the blood allowed to form a clot. This clot was then ground in sand, with excess of ether, and the mixture centrifugalized; afterwards 2 per cent of ammonium oxalate was added and the preparations made as usual. Crystals began to form in about 2 hours. These crystals were oxyhemoglobin, and formed only in the cold. When taken into a warm room they melted rapidly. Another preparation with and without oxalate was tried, but crystals did not form except when the oxalate was present.

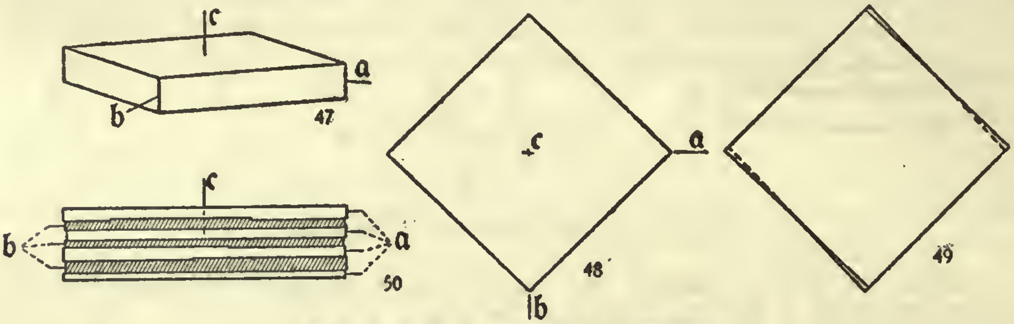
In the slides from the first preparation containing the *a*-oxyhemoglobin crystals (crystal *a*) there was formed after some days a second crop of crystals (crystal *b*) of metoxyhemoglobin and the *a*-crystals changed to methemoglobin by paramorphism. The solution meanwhile had changed to reduced hemoglobin as shown by the spectroscope, and finally needle-like crystals of reduced hemoglobin (crystal *c*) appeared in the slides. By the time that the reduced-hemoglobin crystals had formed, the *b*-crystals were converted by paramorphous change into pure methemoglobin. The oxyhemoglobin and the metoxyhemoglobin also were both converted into pure methemoglobin in the presence of an increasing amount of reduced hemoglobin.

### (*a*) *a-Oxyhemoglobin of Columba livia var.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.9856 : 1 : \acute{c}$; and by twinning this becomes pseudo-tetragonal.

Forms observed: Prism (110) and base (001).

Angles: $110 \wedge 1\bar{1}0 = 90° 50'$; $110 \wedge 001 = 90°$. The angle of the prism of 90° 50′ (89° 10′ normals) becomes by twinning exactly 90°.



FIGS. 47, 48, 49, 50. *Columba livia* *a*-Oxyhemoglobin.

Habit tabular, in square plates (text figures 47 and 48), which pile on one another by twinning; also growing into somewhat arborescent groups by parallel growth in the direction of a crystal axis. Twins on an axis normal to the prism-base edge, with the base as the composition face (text figure 49); this being repeated produces, by mimetic twinning, a composite crystal that is practically tetragonal, being isotropic on the flat and nearly so on the edge view (text figure 50).

Pleochroism is rather marked; $\mathfrak{a}$ pale yellowish-red, $\mathfrak{b}$ deeper yellowish-red, $\mathfrak{c}$ deep red. In the twinned crystals, that show little double refraction on the edge view, the absorption $\mathfrak{c} > \mathfrak{b}$ or $\mathfrak{a}$ is still noticeable. Extinction is straight on all edge views and symmetrical on the base. The orientation of the elasticity axes is $\mathfrak{a} = b$, $\mathfrak{b} = a$, $\mathfrak{c} = \acute{c}$. The axis of least elasticity $\mathfrak{c}$ appears to be the acute bisectrix; $Bx_a = \mathfrak{c}$, hence the optical character is *positive*.

These crystals were gradually converted by paramorphous change into pure methemoglobin, giving the absorption band 630 μμ to 605 μμ and extending to 680 μμ in the red; this change to pure methemoglobin appeared first in these crystals, although pure methemoglobin was later seen in the form of *b*-crystals also.

### (*b*) *Metoxyhemoglobin of Columba livia.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.4615 : 1 : \acute{c}$.

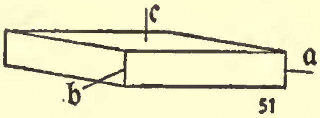
Forms observed: Prism (110), base (001).

Angles: $110 \wedge 1\bar{1}0 = 49° 33'$; $110 \wedge 001 = 90°$.

Habit tabular, in rather acute rhomboidal plates (text figures 51 and 52), usually occurring singly, elongating on the macrodiagonal by parallel growth or sometimes on the brachydiagonal; but not twinning in the way commonly seen in these tabular crystals, on an axis normal to the prism-base edge. After the crystals of this metoxyhemoglobin *b*-type had passed into the pure methemoglobin, they formed a sort of regular growth with the tufts of needles of the reduced hemoglobin, the needles being arranged in tufts growing nearly normal to the prism-base edge of the crystals (see plate 13, fig. 77). These crystals showed a laminated structure parallel to the plane of symmetry, perhaps indicating a cleavage in that direction (see plate 13, fig. 76).

The color of these crystals is reddish-brown, rather dark, and the spectrum showed absorption bands at 640 μμ to 615 μμ, rather faint (the methemoglobin red band); and stronger bands at 580 μμ to 565 μμ and 550 μμ to 530 μμ, the oxyhemoglobin bands. When they finally became converted to methemoglobin, the oxyhemoglobin absorption bands disappeared entirely.

Pleochroism is very strong; $\mathfrak{a}$ colorless or nearly so, $\mathfrak{b}$ deep brownish-red, $\mathfrak{c}$ very deep brownish-purple. The absorption for $\mathfrak{c}$ and $\mathfrak{b}$ is very strong. Double refraction strong; the extinction is straight on edge views and symmetrical on the base. Orientation of the elasticity axes is $\mathfrak{a}=b$, $\mathfrak{b}=a$, $\mathfrak{c}=\dot{c}$. On the base, traces of an interference figure are seen, but the brushes pass out of the field; looking along $\mathfrak{a}$ the complete figure is seen, showing that the acute bisectrix $Bx_a=\mathfrak{a}$, and the optical character is *negative*. This was confirmed by observations with the quartz wedge upon the interference figure.



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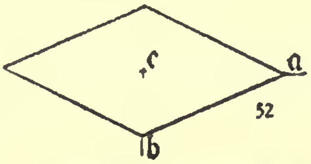

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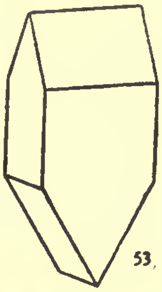
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Figs. 51, 52. *Columba livia* Metoxyhemoglobin.
Fig. 53. *Columba livia* β-Oxyhemoglobin.

*Reduced Hemoglobin of Columba livia.*

Orthorhombic (?).

In fine needle-like crystals, growing in tufts, often on the $b$-type of crystals in a sort of regular growth, also not connected with other types of crystals. The double refraction is rather strong; the extinction is straight. The length of the needles appears to be the direction of greatest elasticity; the pleochroic color of $\mathfrak{a}$ is rose-pink. The directions of less elasticity normal to this show deep purplish-red colors. The crystals were not well enough formed to make out much as to their characters.

Schwantka (Zeit. für physiolog. Chem., 1900, xxx, 486) examined crystals of oxyhemoglobin of pigeon's blood that were prepared by A. Kossel, which were sufficiently large to be measured on the reflecting goniometer. The examination was conducted at a room temperature of between 5° and 10° C. Schwantka's description furnishes the following data:

*β-Oxyhemoglobin of Columba livia.*

Tetragonal sphenoidal: Axial ratio $a : \dot{c} = 1 : 1.175$.

Forms observed: Unit prism (110), unit sphenoid (111).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 90°$; prism to sphenoid $110 \wedge 111 = 31°$; sphenoid faces over pole $111 \wedge \bar{1}\bar{1}1 = 118° 6'$; sphenoid faces $111 \wedge 1\bar{1}\bar{1} = 106° 39'$ (calculated $108° 18'$).

The crystals consisted of the unit prism and the sphenoid, with somewhat prismatic development, elongated on the vertical axis and sometimes flattened on two opposite prism faces, making the crystal somewhat tabular (text figure 53). In many crystals a face produced by contact with the vessel in which the crystals were grown was seen; this was vicinal to the prism face (110), but it did not give good reflections. The development of this accidental face caused a distorted appearance. What seems to be parallel growth was also observed.

The crystals were determined to be uniaxial and showed only a weak pleochroism, changing from a brighter to a duller red. They extinguished parallel to the vertical axis. The optical character is not recorded.

Crow, *Corvus americanus*. Plates 14 and 15.

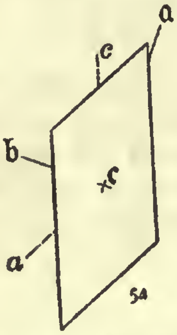
The fresh blood was obtained from the living bird and was allowed to clot. This clot was ground up with sand and with a large excess of ether and centrifugalized; afterwards the clear fluid was oxalated. The preparations were made as usual and the crystals began to form soon after the slides were covered. The slides stood for several days before they were photographed, and reduced hemoglobin developed along with the oxyhemoglobin. Other preparations were made from the clear fluid, prepared as above; and were recorded while containing only oxyhemoglobin. Upon long standing, the crystals in the slides were converted into metoxyhemoglobin. These crystals were all very soluble, but the oxyhemoglobin much more so than the others, and when removed from the cold into a warm room the crystals of oxyhemoglobin dissolved rapidly. Owing to this ready solubility the examinations had to be made in a room kept a temperature near the freezing-point. Measurements were made of the three forms observed, and the substantial identity in form of the oxyhemoglobin, reduced hemoglobin, and metoxyhemoglobin was made out.

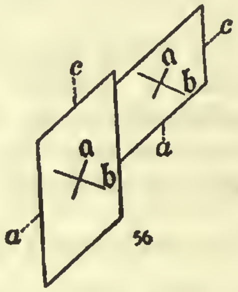
### *Oxyhemoglobin of Corvus americanus.*

Monoclinic: Axial ratio $a : b : \acute{c} = 1 : b : 1.044$; $\beta = 50°$.

Forms observed: Orthopinacoid (100), clinopinacoid (010), base (001), orthodome ($\bar{1}01$), and a prism (*mm* 0).

Angles: $100 \wedge 001 = \beta = 50°$ (130°); $100 \wedge 010 = 90°$; $001 \wedge 010 = 90°$; $\bar{1}10 \wedge 001 = 63°$. The angle of the flat prism (clinoprism) was not observed.





Figs. 54, 55, 56. *Corvus americanus* Oxyhemoglobin. Fig. 57. *Corvus americanus* Reduced Hemoglobin.

Habit thick or thin tabular by development on the plane of symmetry (010), the usual crystal showing only the three pinacoids, but elongated along the vertical axis (text figure 54) and some crystals showing a clinoprism in this zone. In a few crystals the full combination was observed, of three pinacoids, prism, and positive hemiorthodome (text figure 55), and some showed this orthodome without the prism. The direction of the vertical axis is shown by inclusions of the mother-liquor and by cracks parallel to the vertical axis. Perhaps a prismatic cleavage is indicated. "Twins" form with a twinning axis about the normal to the orthodome and with the orthopinacoid of one member in contact with the base of the other member (text figure 56).

Pleochroism strong; $\mathfrak{a}$ pale yellow, $\mathfrak{b}$ and $\mathfrak{c}$ deep red. In thick crystals the color of $\mathfrak{a}$ rises to pale yellowish-red. All show the oxyhemoglobin spectrum. Extinction on the edge view, zone of (100) and (001) is straight; on the plane of symmetry (010) the extinction is oblique, nearly bisecting the acute angle of the plate, $\mathfrak{a} \wedge \acute{c} = 22°$, the extinction angle, measured from the edge 100–010. Orientation of the elasticity axes is $\mathfrak{a} \wedge \acute{c} = 22°$ in the acute angle, $\mathfrak{b} \wedge a = 62°$ in the obtuse angle, $\mathfrak{c} = b$; the plane of the optic axes is normal to the plane of symmetry. On the clinopinacoid the two brushes of the interference figure are seen, but pass out of the field; the acute bisectrix, therefore, probably lies in the plane of symmetry and $Bx_{\mathfrak{a}} = \mathfrak{a}$, or the crystal is optically *negative*. It will be noticed that the symmetry is nearly orthorhombic, but the parting or cleavage along the axis $\acute{c}$ and the habit of the crystal, especially when the prism is developed, all indicate monoclinic symmetry.

### *Reduced Hemoglobin of Corvus americanus.*

Monoclinic: Ratio of *a* : *ć* =1 : 1.044; β=50° 30′.

Forms observed: Orthopinacoid (100), clinopinacoid (010), base (001), positive hemiorthodome (101). No prism was definitely observed.

Angles: 100 ∧ 001=β = 50° 30′ (129° 30′); 100 ∧ 010 = 90°; 001 ∧ 010=90°; 100 ∧ 001=63°.

Habit more distinctly orthorhombic than the oxyhemoglobin crystals; tabular by development on (010) and nearly equidimensional in many cases, not elongated vertically, but in nearly regular rhombic plates (text figure 57). It forms regular growths with the oxyhemoglobin (see plate 15, fig. 88).

Color the usual reduced hemoglobin purplish-red.

The optical characters were not determined for this form of crystal and perhaps it may be orthorhombic. The cleavage in the direction of the vertical axis is not noticeably stronger than parallel to *a*, hence it might be a prismatic cleavage in the orthorhombic system.

TABLE 36.—*Some of the characters of the crystals of the oxyhemoglobins of the Aves.*

| Name of species. | Axial ratio *a* : *b* : *ć*. | Angle β. | Prism angle. | Extinction angle. | Optical character. | System. |
|---|---|---|---|---|---|---|
| Ratitæ: | | ° | ° ′ | ° | | |
| Struthio camelus......... | 0.5658 : 1 : *ć* | 90 | 59 0 | 0 | ........ | Orthorhombic. |
| Casuarius galeatus ....... | .............. | .. | ..... | .. | Negative | Monoclinic. |
| Carinatæ: | | | | | | |
| Anseres: | | | | | | |
| Anser anser............ | .............. | 90 | 90 0 | 0 | Positive | Tetragonal. |
| Olor buccinator........ | .............. | 90 | 90 0 | 0 | Negative | Do. |
| Olor columbianus ...... | 0.9657 : 1 : *ć* | 90 | 88 0 | 0 | Do. | Orthorhombic and pseudo-tetragonal. |
| Gallinæ: | | | | | | |
| Gallus domestica....... | 0.949 : 1 : *ć* | 90 | 87 0 | 0 | Do. | Orthorhombic. |
| Colinus virginianus..... | 0.9657 : 1 : *ć* | 90 | 88 0 | 0 | Do. | Do. |
| Numida meleagris...... | 0.554 : 1 : *ć* | 90 | 58 0 | 0 | Positive | Do. |
| Columbæ: | | | | | | |
| Columba livia, var...... | 0.9856 : 1 : *ć* | 90 | 89 10 | 0 | Do. | Orthorhombic and pseudo-tetragonal. |
| Passeres: | | | | | | |
| Corvus americanus..... | 1 : 1 : 1.044 | 50 | ..... | *a* ∧ *ć* = 22° | Negative | Monoclinic. |

# CHAPTER XI.

## CRYSTALLOGRAPHY OF THE HEMOGLOBINS OF THE MARSUPIALIA, EDENTATA, AND SIRENIA.

Nine specimens of the *Marsupialia* were examined, including representatives of the following families:

- *Didelphyidæ*: 1 species, the common opossum, *Didelphis virginiana.*
- *Dasyuridæ*: 4 species, the Tasmanian devil, *Sarcophilus ursinus;* the spotted dasyure, *Dasyurus maculatus;* the Australian cat, *Dasyurus viverrinus;* and the Tasmanian wolf, *Thylacynus cynocephalus.*
- *Phalangeridæ*: 1 species, the vulpine phalanger, *Trichosurus vulpecula.*
- *Macropodidæ*: 3 species, the rat-kangaroo, *Æpyprymnus rufescens;* the kangaroo, *Macropus giganteus*(?); and the rock-kangaroo, *Petrogale* sp.

The normal crystals of every species of marsupial examined are monoclinic, and only in 2 of the 9 species examined were crystals of other systems observed. One of these was a $\beta$-form of oxyhemoglobin found in the opossum and the other a $\beta$-form of oxyhemoglobin which developed in the blood of the Tasmanian wolf. Comparing the crystals of the *Polyprotodontia,* to which the opossum, dasyurus, Tasmanian devil, and the Tasmanian wolf belong, a close resemblance can be traced in the form of all the species except the Tasmanian wolf, and in this species the development of the crystals was such that direct crystallographic comparison with the other species was not possible, as no axial ratio could be determined. In the species in which this constant was determined it was found to vary for the axis $a$ from 1.7856 in the opossum and Australian cat to 1.8047 in the Tasmanian devil for oxyhemoglobin, a variation that was considerably less than the difference between the $\alpha$-oxyhemoglobin and the reduced hemoglobin in the opossum (1.7856 and 1.963). The optical character of all of these species is the same, with the exception of the Tasmanian wolf.

The crystals of the *Diprotodontia* (including the phalangers and the kangaroos) showed the kangaroos (*Macropodidæ*) forming one group, and the phalanger apparently closer related to the *Didelphyidæ* and the *Dasyuridæ* in ratio, while the phalanger form recalls the Tasmanian wolf crystals of which no ratio was obtained. The kangaroos examined belonged to the genera *Macropus, Æpyprymnus,* and *Petrogale,* and they naturally showed some variation, but formed a fairly close group.

In this chapter there is also included the description of one species of the *Edentata* and one of the *Sirenia* which naturally do not show any very close resemblance to the *Marsupialia* nor to each other.

## MARSUPIALIA.

### Opossum, *Didelphis virginiana*. Plates 15–17.

The first specimens were received from the Philadelphia Zoölogical Gardens, and the blood was putrid. The blood was oxalated and ether-laked, and yielded crystals very readily, the crystals forming soon after the slides were covered. These crystals were reduced hemoglobin. Later, living animals were procured, from which fresh blood was obtained. The animal was bled into oxalate in each case, and preparations were made of the whole blood, of the corpuscles alone, and of the corpuscles with various diluents. From these, oxyhemoglobin was obtained; but in several cases reduced-hemoglobin crystals appeared in the slides soon after the oxyhemoglobin began to form, and these developed along with the crystals of the first-formed oxyhemoglobin called α-oxyhemoglobin. Later the oxyhemoglobin, and eventually even the reduced hemoglobin crystals, dissolve, and a second form of oxyhemoglobin called β-oxyhemoglobin begins to appear, but all three, α-oxyhemoglobin, reduced hemoglobin, and β-oxyhemoglobin, may be seen side by side in the same slide (see plates 16 and 17). These three forms of crystals are produced independently of each other, and there is no paramorphous change of the one into the other, as may readily be seen from the difference in angles of the crystals, and also by the difference in crystallization between the β-oxyhemoglobin and the other two forms. These three kinds of crystals were obtained in several different preparations of the fresh blood. Carbon-monoxide hemoglobin was also made from the opossum blood, and it was found to crystallize in two forms analogous to those of the oxyhemoglobin α and β. The old blood was regenerated, after it had stood for some weeks and become putrid, by shaking with oxygen, and also by addition of a diluted solution of commercial hydrogen peroxide.

The treatment with oxygen was much more satisfactory, because if the hydrogen peroxide is used in too concentrated a form the result is methemoglobin; and if it is very much diluted, the solution becomes too dilute to crystallize well. But the corpuscles, after laking, make such a thick preparation that it was usually found better to dilute somewhat with normal salt solution. The procedure of regenerating was usually carried out as follows: The brownish stale blood was oxidized by shaking in a flask with oxygen gas until the color changed to bright oxyhemoglobin red; if corpuscles alone were used, to two parts of the corpuscles laked with ether was added one part of normal salt solution and one-fourth part of ether, and the whole briskly shaken, and then centrifugalized in a small hand machine for about 2 minutes. The solution was perfectly clear after this treatment, and a thick amorphous mass rose to the top of the liquid, which carried all of the precipitate and granular matter, leaving the solution as clear as if centrifugalized for 2 hours in the ordinary process of treatment. The crystals of oxyhemoglobin thus obtained were very sharp and fine, and of exactly the same form as those prepared in the usual way.

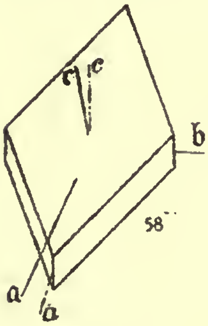
The preparations of CO-hemoglobin were made in a similar manner, the blood being first shaken with oxygen gas under slight pressure, and then laked with ether. After the ether-laking it was shaken with illuminating gas and the oxygen displaced by CO as shown by the spectroscope, after which it was centrifugalized. Most of the preparations examined were made in the usual manner of laking the oxalated blood with a few drops of ether and centrifugalizing for several hours. From the fresh blood the first crystals to form were always α-oxyhemoglobin, but from stale blood reduced hemoglobin formed. The regenerated stale blood gave only α-oxyhemoglobin at first, but later the crystals of the other hemoglobins appeared.

*α-Oxyhemoglobin of Didelphis virginiana.*

Monoclinic: Axial ratio $a : b : \grave{c} = 1.7856 : 1 : 2.6685$; $\beta = 48°$ (about).

Forms observed: Prism (110), base (001), orthopinacoid (100), pyramid (111), orthodome ($\bar{1}01$).

Angles: Prism-base edges $110\text{–}001 \wedge 1\bar{1}0\text{–}001 = 58° 30'$; $001 \wedge 010 = 90°$; $001 \wedge 100 = \beta = 48°$, or perhaps somewhat less; $\bar{1}01 \wedge 001 =$ about 90°. The angle of the orthodome to the base was not exactly determined. The angle $\beta$ was only determined approximately, and some of the crystals measured showed the angle to be apparently about 43°.



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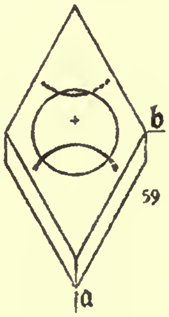

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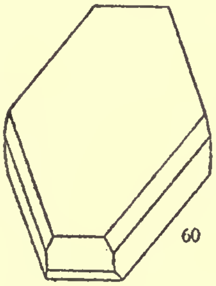
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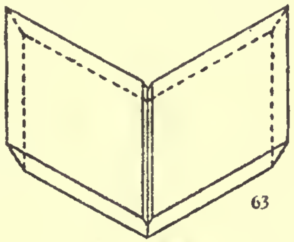
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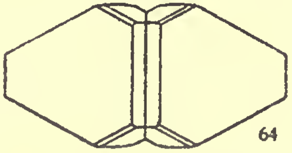
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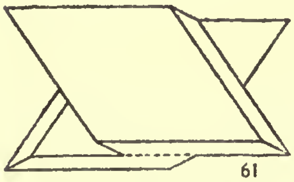
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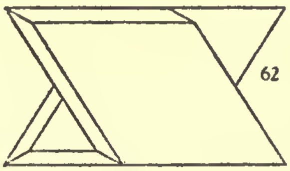
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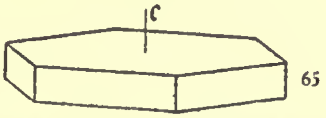


65

FIGS. 58, 59, 60, 61, 62, 63, 64. *Didelphis virginiana* α-Oxyhemoglobin. FIG. 65. *Didelphis virginiana* β-Oxyhemoglobin.

Habit tabular parallel to the base (text figures 58 and 59), the first crystals to form being very thin and very symmetrical rhombic tables; as they increase in size they develop other planes besides the simple prism and base, which is the usual crystal. The first plane to appear, besides these two, is generally the positive hemiorthodome ($\bar{1}01$) which cuts off the ends, or sometimes mainly one end, of the rhombic plate (see plate 16, fig. 91). The best crystals were obtained from the laked corpuscles and these showed in some cases the negative hemipyramid (111), in addition to the orthodome, beveling the edges of the plate in the obtuse angle (text figure 60). Composite groupings formed by parallel growth are common, especially in the direction of *b*. Twins are of several kinds, but are not so frequently observed as is usually the case in these rhombic plates of the monoclinic system. A common form is a twin with the twinning axis parallel to the prism-base edge 110–001, and the base as the composition face (text figure 61). These form the symmetrical twin shown on plate 16, fig. 92. The similar twin on the base as composition face and the twinning axis normal to prism-base edge and lying in the base, and the plane of twinning normal to the base, was also observed (text figure 63). This twin with the composition face the base is the usual type of twin in such monoclinic rhombic plates (text figure 62) (horse-type; see description of this twin under Horse). A third type is shown on plate 17, fig. 99, with the orthodome ($\bar{1}01$) as the plane of twinning and the composition face (text figure 64). This form was rather common. From an examination of such twins it would seem that this orthodome makes an angle of exactly 90° with the base. From this, if $\beta$ were accurately determined, the value of $\dot{c}$ could be easily calculated. Taking $\beta$ at 48° the value of $\dot{c}$ becomes 2.6685.

Pleochroism is very marked, $\mathfrak{a}$ pale yellowish-red, $\mathfrak{b}$ deep red, $\mathfrak{c}$ very deep red. The plane of the optic axes is the plane of symmetry; the orientation of the elasticity axes is $\mathfrak{a} \wedge a = 17°$ in the obtuse angle; $\mathfrak{b} = b$; $\mathfrak{c} \wedge \dot{c} = 25°$ in the obtuse angle. On the flat the extinction is symmetrical; on edge view, looking along $b$, the extinction angle is 17° from the edge 001–010 or from the trace of 001. On the flat the interference figure is seen, somewhat unsymmetrically arranged, and the acute bisectrix, $Bx_a = \mathfrak{c}$. The optical character is hence *positive.*

### *β-Oxyhemoglobin of Didelphis virginiana.*

Hexagonal or pseudo-hexagonal. No axial ratio determinable.

Forms observed: Prism (1010), base (0001).

Angles: Prism angle $1010 \wedge 01\bar{1}0 = 60°$ (120°), prism to base $10\bar{1}0 \wedge 0001 = 90°$.

Habit tabular, thick or thin plates consisting of prism and base, with great development of the base (text figure 65). The $\beta$-oxyhemoglobin crystals appear after the $\alpha$-oxyhemoglobin crystals, and the appearance of the former is accompanied by the resolution and disappearance of the latter. They form first in the protein ring, but later may appear anywhere in the slide. Apparently the solution of the $\alpha$-oxyhemoglobin is due to the action of bacteria, but frequently the $\beta$-oxyhemoglobin crystals appear growing on the $\alpha$-oxyhemoglobin crystals as regular growth and with the $\alpha$-crystal unaffected. The orientation of the regular growth appears to be such that the edges of the plate of the $\alpha$-crystal are approximately normal to the edges of the $\beta$-crystal in some cases, and parallel to them in others. Etching figures are seen, elongated normal to the edges of the $\beta$-oxyhemoglobin plates. Some of them appear to be composite crystals (see plate 17, fig. 98), as in the mica twins that are nearly uniaxial, and yet on edge the layers of the twin are so thin, or so intergrown, that polarized light does not seem to show any trace of composite character. No regular twins of these crystals occur, but parallel growth is common.

The color of these $\beta$-oxyhemoglobin crystals is brighter red, more scarlet than the color of the $\alpha$-oxyhemoglobin crystals, but this is evidently due to the fact that they show little or no pleochroism; and the spectroscope does not show any difference from the normal oxyhemoglobin spectrum. Pleochroism is not noticeable on the basal view, and there is practically no pleochroism or absorption on the side or edge view, looking normal to the vertical axis. There is no double refraction that can be detected on the basal view; on edge view the double refraction is easily seen and the extinction is straight. The vertical axis is the axis of least elasticity, $\varepsilon > \omega$, and the crystal is *positive.* On the base, in convergent light, the uniaxial cross is readily seen; in some cases the crystal is slightly biaxial, as in the nearly uniaxial micas, and the axis of least elasticity is normal to a prism face. The biaxial crystals are also distinctly positive. The separation of the brushes is only very slight, the angle $2E$ is very small.

While these crystals are seen in all sizes, and do not appear to be composite, there can be little doubt that they are really mimetic hexagonal only and are twins of the $\alpha$-oxyhemoglobin on the base in one of the two forms of twinning that have been described under the $\alpha$-oxyhemoglobin. If the twin laminæ were thin enough, the polarization test would not show the composite character and this would be especially true if, as is usually the case in these twins, the same layer did not run as a plane entirely across the basal surface. In looking through from side to side the different orientation of the layers would hence average, and neutralize each other. Of course, this averaging would happen on the flat view to a still greater degree, and the elasticity axes $\mathfrak{a}$ and $\mathfrak{b}$ in different orientation would completely extinguish each other, making a uniaxial effect. This may be done artificially with only three plates of mica, twinned as these $\alpha$-oxyhemoglobin crystals twin, and has been observed in the oxyhemoglobin of many species that twin in this way, where it is easily seen that the crystal is composite. If both kinds of twins formed in the same crystal, the averaging of the elasticities might be perfect. But, as has been observed in other species that form hexagonal plates (compare rats, squirrels, etc.), the growth of the composite plate by this form of twinning produces an averaging of the angles, so that prism angles that are nearly 60° (58° 30′ as in this $\alpha$-oxyhemoglobin) become exactly 60° in the twin. It might be possible that this crystal was an averaging of right- and left-handed forms, resulting in the more symmetrical mimetic twin. From the forms of twinning assumed, the elasticity axis, $\mathfrak{c}$, remains always in the same position in all of the members of the composite crystal, and hence the vertical axis, $\dot{c}$, becomes the axis of less elasticity, and the composite remains positive.

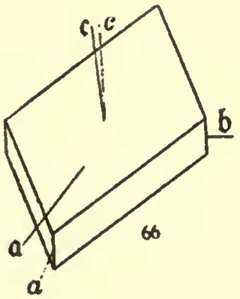
If the above view of these crystals is correct the substance of the $\alpha$-oxyhemoglobin and of the $\beta$-oxyhemoglobin may be the same unless perhaps the $\beta$-oxyhemoglobin is a union of right-handed and left-handed crystals of the $\alpha$-oxyhemoglobin.

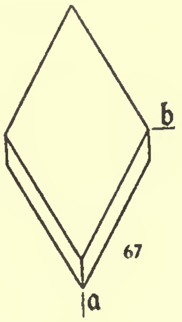
### *Reduced Hemoglobin of Didelphis virginiana.*

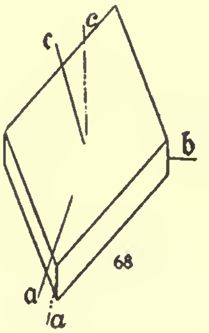
Monoclinic: Axial ratio $a : b : \dot{c} = 1.963 : 1 : \dot{c}$; $\beta = 66°$.

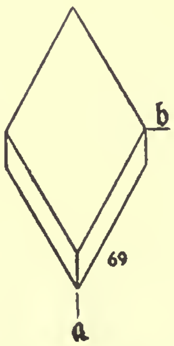
Forms observed: Prism (110), base (001).

Angles: Prism angle traces on the base of $110 \wedge 1\bar{1}0 = 54°$; prism edge to base $110\text{–}1\bar{1}0 \wedge 001 = \beta = 66°$; base to plane of symmetry or to side prism edge $001 \wedge 110\text{–}1\bar{1}0 = 90°$.









FIGS. 66, 67. *Didelphis virginiana* Reduced Hemoglobin. FIGS. 68, 69. *D. virginiana* $\alpha$-Carbon-monoxide Hemoglobin.

Habit, rhombic plates with oblique sides, composed of base and prism, the crystals generally very perfect and sharp (text figures 66 and 67). They usually occur singly, but also twin with the normal to the base as the plane of twinning and the twin axis normal to the prism-base edge, the composition face being the basal pinacoid. This type of twin, "horse-type," is seen in $\alpha$-oxyhemoglobin (text figure 51) and is the common twin on the base in all of these monoclinic reduced hemoglobins and oxyhemoglobins, especially when the prism angle is near 60°. These twins are often complex and the polysynthetic arrangement is very common.

The crystals are readily distinguished from the $\alpha$-oxyhemoglobin by their color, and by the fact that they occur singly and not in parallel growth, as is so commonly the case in the $\alpha$-oxyhemoglobin. In the photographs they appear as lighter-colored, more transparent crystals than the oxyhemoglobin crystals. Pleochroism is very strong; $\mathfrak{a}$ very pale violet, nearly colorless; $\mathfrak{b}$ deep reddish; $\mathfrak{c}$ deep claret-color to purple. Extinction is symmetrical or nearly so on the flat basal face; on edge looking along the axis $b$ it is oblique; the extinction angle is $\mathfrak{a} \wedge a = 13°$, in the obtuse angle. The orientation of the elasticity axes is as follows: The axial plane is the plane of symmetry; $\mathfrak{a} \wedge a = 13°$ in the obtuse angle, $\mathfrak{b} = b$, $\mathfrak{c} \wedge \dot{c} = 11°$ in the obtuse angle. On the base, the interference figure is readily observed, with the two brushes showing, but in somewhat unsymmetrical arrangement, due to the angle of 13° between $\mathfrak{a}$ and $a$; the brushes are widely separated. The acute bisectrix is hence $Bx_a = \mathfrak{c}$, and the optical character is *positive*.

The twinning in some cases, as seen in edge view, seems to be perhaps of the Manebach type, and the two overlapping and crossing plates as seen in the $\alpha$-oxyhemoglobin (plate 17, fig. 100) are rarely seen. But the type of twinning described above appears to be the normal type. On the flat in some cases the extinction did not seem to be quite symmetrical, but if the crystals are triclinic they must approach the monoclinic very closely, within a degree or two. The form was symmetrically monoclinic.

## CARBON-MONOXIDE HEMOGLOBIN OF OPOSSUM.

(Dimorphous: $\alpha$-CO-hemoglobin, monoclinic; $\beta$-CO-hemoglobin, hexagonal or pseudo-hexagonal.)

The stale blood was treated by regenerating with oxygen as above described, and then shaking with illuminating gas (water-gas) for 2 hours. After etherizing and centrifugalizing for 3 hours granular matter (stromata of corpuscles?) still remained suspended in the solution. The slides were prepared as usual. Two forms of crystals developed, at first the monoclinic $\alpha$-CO-hemoglobin and later the hexagonal plates of the $\beta$-CO-hemoglobin.

### *a-CO-hemoglobin of Didelphis virginiana.*

Monoclinic: Axial ratio $a : b : \dot{c} = 1.804 : 1 : \dot{c}$; $\beta = 41°$.

Forms observed: Prism (110), base (001), clinopinacoid $b$ (010), positive orthodome ($\bar{1}01$).

Angles: Prism angle, traces on the base of $110 \wedge 1\bar{1}0 = 58°$; prism edge to base, edge $110\text{–}1\bar{1}0 \wedge 001 = \beta = 41°$; base to clinopinacoid $001 \wedge 010 = 90°$.

Habit thin, tabular, rhombic plates consisting of the base (001) with a very short prism (110) (text figures 68 and 69). The crystals occur singly or twinned, but not in parallel growth as in the $\alpha$-oxyhemoglobin to any considerable extent. Twins are rare, but a type with the twin axis in the basal plane and at 45° to the crystal axes, the twinning plane being normal to the base, and the composition face being the base, was apparently observed in several cases. In this form of twin, which somewhat resembles the one in which the arrangement is similar but the twinning axis the normal to the prism-base edge, the axis of $a$ in one member of the twin falls in line with the axis of $b$ in the other member.

The crystals show the usual CO-hemoglobin color and spectrum, and they are rather strongly pleochroic. The pleochroism is as follows: $\mathfrak{a}$ pale rose, nearly colorless, $\mathfrak{b}$ deep red, $\mathfrak{c}$ very deep blood-red. The pale rose color of $\mathfrak{a}$ is in marked contrast with the usual yellow or reddish-yellow of $\mathfrak{a}$ in the oxyhemoglobin crystals. The extinction is symmetrical on the base and oblique on the clinopinacoid aspect; the extinction angle is 13°. The axial plane is the plane of symmetry and the orientation of the elasticity axes is $\mathfrak{a} \wedge a = 13°$ in the obtuse angle, extinction angle; $\mathfrak{b} = b$; $\mathfrak{c} \wedge \dot{c} = 36°$ in the obtuse angle. On the base the biaxial interference figure is seen with somewhat unsymmetrical arrangement and the brushes widely separated. The acute bisectrix emerges at an angle of 13° with the normal to the base and is the axis of least elasticity, or $Bx_a = \mathfrak{c}$, and the optical character is *positive*. On all sections in the zone of (001)–(100) the extinction is straight.

Compared with $\alpha$-oxyhemoglobin, the characters of this $\alpha$-CO-hemoglobin are shown in table 37.

TABLE 37.—*Comparison of characters of a-oxyhemoglobin and a-CO-hemoglobin of Didelphis virginiana.*

| Substance. | Axial ratio. | Angle $\beta$. | Angle $110 \wedge 110$. | Angle $\mathfrak{a} \wedge a$. | Optical character. |
|---|---|---|---|---|---|
| | | | ° ′ | | |
| $\alpha$-oxyhemoglobin......... | $a = 1.7856$ | $\beta = 48° \pm$ | 58 30 | 17° | Positive |
| $\alpha$-CO-hemoglobin......... | $a = 1.804$ | $\beta = 41°$ | 58 0 | 13° | Do. |

The correspondence is probably even more close, for the angle β of the α-oxyhemoglobin was perhaps 43°, as some measurements made it. The prism angles are practically identical. The extinction angles vary 4° and in the same direction as the angle β. The optical characters are identical.

### *β-CO-hemoglobin of Didelphis virginiana.*

Hexagonal: No axial ratio determinable.

Forms observed: Prism $(10\bar{1}0)$, base (0001).

Angles: Prism angle $10\bar{1}0 \wedge 01\bar{1}0 = 60°$ (120°); base to prism $0001 \wedge 10\bar{1}0 = 90°$.

Habit tabular, the crystals consisting of the short prism and base (text figure 70), with the base greatly developed. They occurred sparingly in a few slides, and were not common, as was the case with the β-oxyhemoglobin crystals.

The color and absorption spectrum showed them to be CO-hemoglobin, but they were darker than the α-CO-hemoglobin crystals owing to their lack of pleochroism. On the base the crystals show no double refraction or pleochroism, and on edge the pleochroism was practically nothing. On side view they show a very weak double refraction and extinguish straight. On the base in convergent light a very dusky cross of a uniaxial interference figure was observed; the vertical axis is the axis of less elasticity, or $\varepsilon > \omega$, and the optical character is *positive*.

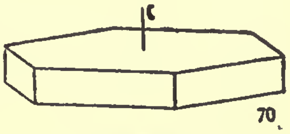


Fig. 70. *Didelphis virginiana* β-Oxyhemoglobin.

Compared with the β-oxyhemoglobin these crystals are seen to have identical characters, and there is probably no doubt that if the β-oxyhemoglobin crystals are mimetic twins these are also. The forms of twinning noted for the α-oxyhemoglobin would produce such mimetic forms if the twinning was repeated or polysynthetic. Such twins, with the twin axis lying in the basal pinacoid and normal to the prism-base edge, and the base as the composition face, were apparently observed in the α-CO-hemoglobin along with the type of twin already described.

The close resemblance of the oxyhemoglobins and the CO-hemoglobins in this species is what might be expected from the other resemblances between these compounds. It will be noted, however, that the reduced hemoglobin varies from either in the inclination of the base and in the prism angle. Table 38 shows these differences plainly.

TABLE 38.—*Differences of the oxyhemoglobins and CO-hemoglobins in Didelphis virginiana.*

| Substance. | Axial ratio. | Angle β. | Prism angle. | Extinction angle. | Optical character. | System. |
|---|---|---|---|---|---|---|
| | | ° | ° ′ | ° | | |
| α-oxyhemoglobin | $a = 1.7856$ | 48 (43?) | 58 30 | 17 | Positive | Monoclinic. |
| α-CO-hemoglobin | $a = 1.804$ | 41 | 58 30 | 13 | Do. | Do. |
| α-reduced hemoglobin | $a = 1.963$ | 66 | 54 0 | 13 | Do. | Do. |
| β-oxyhemoglobin | ........ | 90 | 60 0 | 0 | Do. | Hexagonal. |
| β-CO-hemoglobin | ........ | 90 | 60 0 | 0 | Do. | Do. |

### TASMANIAN DEVIL, *Sarcophilus ursinus*. Plate 18.

The specimen was obtained from the National Zoölogical Park at Washington, District of Columbia, and consisted of about 2 c.c. of oxalated blood preserved in our usual collecting tube. The blood was centrifugalized and the corpuscles separated and laked with ether. Preparations were made in the usual manner. The blood crystallized very readily and photographs could be taken within 2 hours of making the preparations. The blood being in good condition, the crystals were oxyhemoglobin, as determined by the spectroscope.

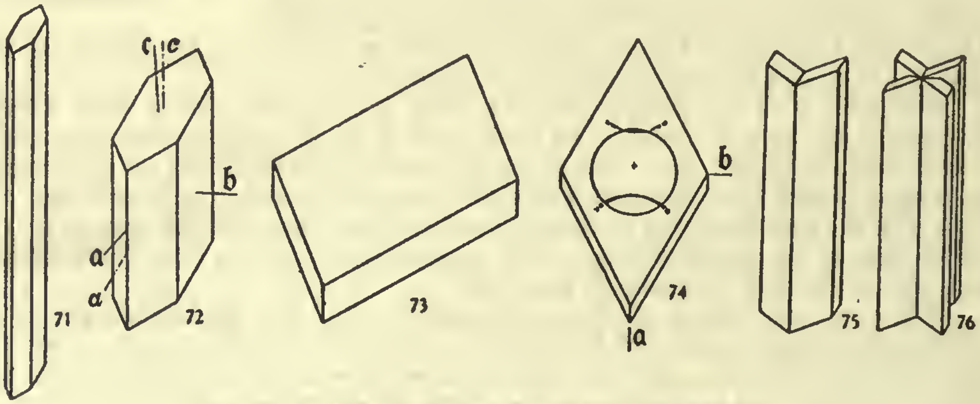
### *Oxyhemoglobin of Sarcophilus ursinus.*

Monoclinic: Axial ratio $a : b : \acute{c} = 1.804 : 1 : \acute{c}$; $\beta = 69°$ (111°).

Forms observed: Prism (110), base (001), clinopinacoid (010); also in twins $(\bar{1}11)$ a positive hemipyramid, of which the angle was not determined.

Angles: Traces of prism on the base, edge $110\text{–}001 \wedge \bar{1}10\text{–}001 = 58°$ (122°); edge of $110\text{–}1\bar{1}0 \wedge 001 = \beta = 69°$ (111°).

Habit prismatic, also tabular; the first crystals to appear are usually long thin prisms and also shorter prisms with the length varying from 20 times to 6 times the thickness (text figure 71); later nearly equidimensional blocks appear, short prisms with ratio of length to thickness varying from 2 : 1 to 1 : 2 (text figure 72); finally a few crystals become tabular on the base, with the prism reduced to perhaps one-tenth of the thickness or width of plate (text figures 73 and 74). Twinning was observed with the plane of twinning and composition plane the prism face (twin on the prism), both contact (text figure 75) and interpenetrant (text figure 76) twins; also long prisms twinned on a positive hemipyramid, of which the angle was not determined, called $(\bar{1}11)$, as the plane of twinning and the twinning axis normal to this plane; these were interpenetrant twins.



FIGS. 71, 72, 73, 74, 75, 76. *Sarcophilus ursinus* Oxyhemoglobin.

Pleochroism strong; $\mathfrak{a}$ pale yellowish, nearly colorless; $\mathfrak{b}$ red, $\mathfrak{c}$ deep red. Extinction is symmetrical on the base in the plates and all sections in zone of $\mathfrak{b}$; on side view, on (010) the extinction is oblique with an extinction angle of 10° from the prism edge, or 11° from the trace of the base (001). The axial plane is the plane of symmetry (010) and the orientation of the elasticity axes is $\mathfrak{c} \wedge \acute{c} = 10°$ lying in the obtuse angle; $\mathfrak{a} \wedge a = 11°$ in the obtuse angle; $\mathfrak{b} = b$. The interference figure is seen on this basal section, with the brushes slightly unsymmetrical and rather widely separated. $2E =$ above 80°, and the angle between $\mathfrak{c}$ and the normal of (001) is 11°. The acute bisectrix, $Bx_a = \mathfrak{c}$, and the optical character is hence *positive.*

### SPOTTED DASYURE, *Dasyurus maculatus.* Plate 18.

The blood as received was thick and dark colored, somewhat putrid. It was prepared by oxalating and freezing and thawing, then centrifugalizing. The best crystals were obtained by diluting the centrifugalized blood with an equal volume of water. The needle-like, prismatic crystals appeared soon after making the preparations. After 48 hours the crystals had developed into large plates. The crystals were all reduced hemoglobin, and even in ordinary light varied much in color, owing to the strong pleochroism.

### *Reduced Hemoglobin of Dasyurus maculatus.*

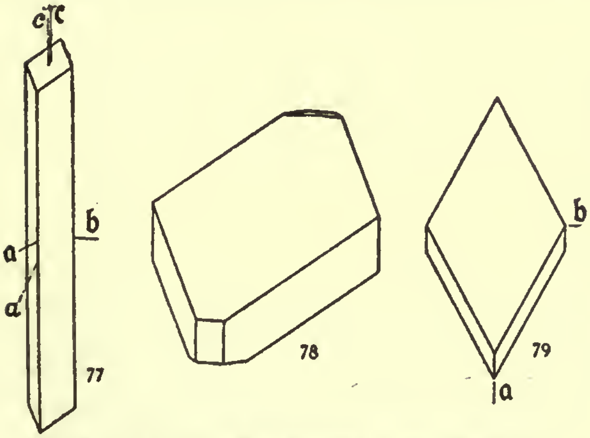
Monoclinic: Axial ratio $a : b : \acute{c} = 1.8227 : 1 : \acute{c}$; $\beta$ about 63°.

Forms observed: Prism (110), base (001), positive hemiorthodome $(\bar{1}01)$, orthopinacoid (100).

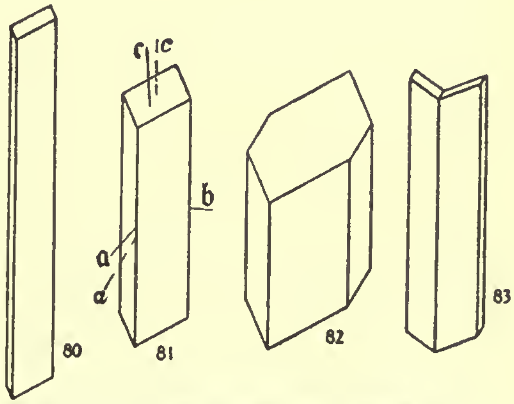
Angles: Traces of prism on the base, edge $110\text{–}001 \wedge 1\bar{1}0\text{–}001 = 57° 30'$; prism edge with base, edge $110\text{–}1\bar{1}0 \wedge 001 = 63°$ about. The angles of the hemiorthodome with base were not obtained, as it occurred only occasionally and not in good position for measurement. It was nearly normal to this prism edge.

Habit of the crystals at first long prismatic, the prism terminated obliquely by the base (text figure 77), later the prisms become thick, and finally thin tabular crystals develop by extension parallel to the basal pinacoid. These latter (text figure 78) have the usual rhombic shape of the hemoglobin crystals of the species with angles near 60°. A crystal showing the hemiorthodome and orthopinacoid is shown in text figure 79. They form parallel growths, but twinning was not observed. The prismatic crystals grow in tufts and radiating groups; the plates generally grow singly or in simple parallel growths. Some of these may be twins on an axis normal to a prism-base edge, with the base as composition face.

Pleochroism is very strong, as is commonly the case with hemoglobin; $\mathfrak{a}$ colorless, $\mathfrak{b}$ rose-red, $\mathfrak{c}$ purplish-red. Extinction measured from the prism edge is nearly straight in all cases, so that in prismatic crystals the extinction appears to be straight and only varies about one degree from parallelism with the edge of the prism when looking along the ortho-axis. But in the plates, when seen on edge, the extinction is 28° with the trace of the base, or with the $a$ axis when looking along the ortho-axis. The elasticity of $\mathfrak{b}$ and $\mathfrak{c}$ appears to be nearly equal, and looking along $\mathfrak{a}$ the double refraction is rather weak. The optic axes lie in the plane of symmetry and the orientation of the optic axes is $\mathfrak{a} \wedge a = 28°$, in the obtuse angle; $\mathfrak{b} = b$; $\mathfrak{c} \wedge \acute{c} = 1°$, in the acute angle. The small angle between $\mathfrak{c}$ and $\acute{c}$ makes the extinction appear nearly parallel with the prism edge or the axis $\acute{c}$. A brush of the interference figure was seen emerging nearly parallel to the clino-axis $a$, and this would indicate that $Bx_a$ was $\mathfrak{a}$, which the double refraction also indicates. The optical character is hence very probably *negative.*



FIGS. 77, 78, 79. *Dasyurus maculatus* Reduced Hemoglobin.



FIGS. 80, 81, 82, 83. *Dasyurus viverrinus* Oxyhemoglobin.

### AUSTRALIAN CAT, *Dasyurus viverrinus.* Plate 19.

The specimen of blood was received from the National Zoölogical Park at Washington. The blood was oxalated, frozen repeatedly, and centrifugalized without any addition of ether. Crystals formed readily at ordinary room temperature, and were very sharp. They showed no signs of dissolving with change of temperature, and they were evidently not very soluble. The spectroscope showed them to be oxyhemoglobin.

### *Oxyhemoglobin of Dasyurus viverrinus.*

Monoclinic (?): Axial ratio $a : b : \grave{c} = 1.7856 : 1 : \grave{c}$; $\beta = 69°$.

Forms observed: Prism (110), base (001), clinopinacoid (010).

Angles: Prism angle, traces of the prism on the base = 58° 30′, base to prism edge $= \beta = 69°$.

Habit long and short prismatic, the prism often flattened on two faces, producing a strongly triclinic habit (text figure 80), elongated in the direction of the $\grave{c}$ axis; also in symmetrical prisms long or short and terminated by the base (text figure 81). Some crystals show also the plane of symmetry, (010), in combination with the prism and base (text figure 82). Twins are very common, on the narrow prism face, and making an elongated geniculated twin or sort of V-shaped trough, the faces 110 and $\bar{1}\bar{1}0$ being in contact (text figure 83). Plates in which the base is the large plane do not appear to be common.

Pleochroism rather strong, the colors ranging from pale yellowish-red to deep red; $\mathfrak{a}$ pale yellowish-red, $\mathfrak{b}$ deeper red, $\mathfrak{c}$ deep red. The extinction angle was not exactly observed; $\mathfrak{c} \wedge \grave{c} =$ about 12° or more. On crystals when the plane of symmetry is presented on edge the extinction is straight. The axial plane is the plane of symmetry and the orientation of the axes, not very exactly determined, was approximately $\mathfrak{a} \wedge a =$ about 9° in the obtuse angle; $\mathfrak{b} = b$, $\mathfrak{c} \wedge \grave{c} = 12°$ about in the obtuse angle. The interference figure was not observed and the optical character was not determined. It appears to be positive, judging from the pleochroism and the elasticity.

The strong triclinic habit of these crystals as they at first appeared led to the supposition that they were really triclinic, but further examination of the later crystals and of the photographs seems to indicate that they are really monoclinic, but appear triclinic through unsymmetrical development. They do not show the development of these plates on the base as is usual in plate-like crystals, but they show only the flattening on a pair of prism faces, which is not common.

## Tasmanian Wolf, *Thylacynus cynocephalus.* Plate 20.

The blood was stale when received, it was thick and beginning to putrify. It was diluted with an equal volume of water and centrifugalized for one experiment, also ether-laked and centrifugalized for another. In all cases the preparations were made in the usual manner. The crystals were allowed to form at temperatures near 0° C. and up to 5° or 6° C. The crystals formed readily, soon after covering, and were at first small plates, which afterwards increased in size. These were $\alpha$-oxyhemoglobin. In many slides on standing for some days a second crop of crystals developed; these were isometric dodecahedra, the only isometric crystals thus far observed in the blood of any species. They were also oxyhemoglobin, and are described as $\beta$-oxyhemoglobin. All determinations of the character of the crystals were made with the Zeiss microspectroscope, and the spectra of these two varieties of oxyhemoglobin did not differ as far as could be observed. The blood after standing in a test-tube exposed to the air at near 0° C. for some days developed the crystals of $\beta$-oxyhemoglobin simultaneously with the crystals of the $\alpha$-oxyhemoglobin.

### *$\alpha$-Oxyhemoglobin of Thylacynus cynocephalus.*

Monoclinic: Axial ratio not determinable. $\beta = 77°$.

Forms observed: Clinopinacoid (010), orthopinacoid (100), base (001).

Angles: $010 \wedge 100 = 90°$; $010 \wedge 001 = 90°$; $100 \wedge 001 = \beta = 77°$.

Habit thin tabular, by development of the plane of symmetry; the plates being very thin and showing a tendency to elongate in the direction of the clino-axis (text figure 84). They frequently grow in diverging groups, the plates uniting on the plane of symmetry (see plate 20) and also into parallel growths by the same process. Etching figures show the monoclinic character of the symmetry; they appear on the clinopinacoid as pits formed of negative prism and clinodome and show the position of the vertical axis, elongating along the prism. On the orthopinacoid they also appear as pits elongated in the zone of the ortho-axis and on the base they are elongated in the zone of the clino-axis. Twins of the carlsbad type were observed (text figure 85), the twin axis being the vertical axis and the composition face the clinopinacoid, the edges of the twin matching along the (100)–(010) edge.

Double refraction is very weak and pleochroism is hardly noticeable. On edge views the extinction is straight and the axis of symmetry $b$ is always the direction of greater elasticity. On the clinopinacoid or flat view the double refraction is only observable with the aid of a quartz wedge and the axis of greater elasticity in this aspect nearly or quite bisects the obtuse angle of $a$ and $\acute{c}$. The plane of the optic axes is normal to the plane of symmetry and the orientation of the elasticity axes is $\mathfrak{a}=b$; $\mathfrak{c} \wedge \acute{c}=$about 37° in the acute angle; $\mathfrak{b} \wedge a=50°$ in the obtuse angle. No interference figure could be observed, but from the very weak double refraction when looking along $\mathfrak{a}$ it is probable that this axis is the acute bisectrix, $Bx_a=\mathfrak{a}$, and hence the optical character is probably *negative.*

### *β-Oxyhemoglobin of Thylacynus cynocephalus.*

Isometric, normal.

Forms observed: Dodecahedron (100), cube (100).

Angles: $110 \wedge 1\bar{1}0=90°$; $110 \wedge 101=120°$ (60°); $100 \wedge 010=90°$.

Habit dodecahedral, generally very symmetrical isometric dodecahedra; sometimes, in case of large crystals, in combination with the cube (text figure 86), the dodecahedron usually predominating; also in very small crystals, almost drops, with the dodecahedral axes appearing as points on the nearly spherical mass, and the crystal looking like a trapezohedron. In profile, the crystals present hexagonal outlines (looking along a trigonal axis) or squares (looking along a tetragonal axis); in case of the cube-dodecahedron combination, the profile may be octagonal. Looking along a binary axis the aspect is six-sided, but looks orthorhombic. These crystals generally formed around the edges of the slides and appeared in immense numbers, but only occasional crystals grow to large size; some of these large ones, however, attained a breadth of 1 mm. They are absolutely isotropic, showing no indication of double refraction in any aspect, even when tested by the quartz wedge, etc. Careful examination with the Zeiss microspectroscope showed no difference between their spectrum and the spectrum of ordinary oxyhemoglobin.

## Vulpine Phalanger or Cuscus, *Trichosurus vulpecula.* Plate 21.

The blood was received in a putrid condition and contained numerous globules resembling fat globules. It was prepared in the usual manner and crystallized readily after the slides were covered. The crystals showed no tendency to dissolve, but remained in perfect condition for days. The negatives were taken 24 hours after the preparations were made. It was not found possible to separate the fat or oil from the blood without sacrificing the small amount of material available, and the fat globules, therefore, show in all of the negatives prepared. The color of the solution was very deep, and the color of the crystals was hard to observe. They appeared to be oxyhemoglobin.

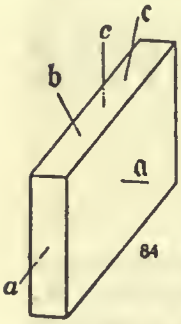
### *Oxyhemoglobin of Trichosurus vulpecula.*

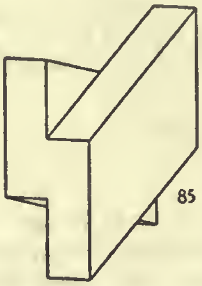
Monoclinic: Axial ratio $a : b : \acute{c} = 0.9163 : 1 : \acute{c}$; $\beta=55°$.

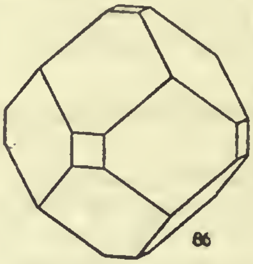
Forms observed: Unit prism (110), clinoprism (230), base (001).

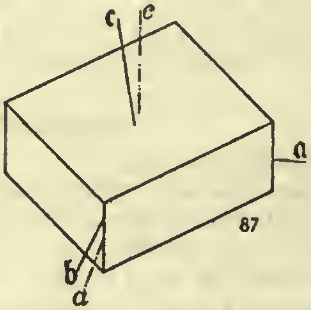
Angles: Traces of unit prism on base, edges $110\text{–}001 \wedge 110\text{–}001 = 95°$ (85°); of the clinoprism on base, edges $230\text{–}001 \wedge 2\bar{3}0\text{–}001 = 75°$ (105°); base to prism edge $(\acute{c} \wedge b)$ $001 \wedge 110\text{–}\bar{1}10 = 90°$; base to prism edge $(\acute{c} \wedge a)$ $001 \wedge 110\text{–}1\bar{1}0 = \beta = 55°$.

Habit short prismatic to tabular, the tabular crystals formed by the unit prism and base being nearly square in outline (text figure 87). The more prismatic crystals showed the unit prism and base, and varied from equidimensional blocks to prisms with a length equal to 3 to 4 times the thickness. The prismatic crystals were sometimes the clinoprism (230) (text figure 88), but combinations of this prism with the unit prism were not observed. The crystals, especially those of the prismatic type, aggregated themselves in stellate groups by growing together in the orthodome zone, but definite twins were not observed. Crystals growing along the cover edge were often elongated into long, prismatic-looking forms, growing in tufts. These were usually edge views of the plates, which also occasionally aggregated together by uniting on the base, or on faces vicinal to the base, but without definite twinning orientation.









FIGS. 84, 85. *Thylacynus cynocephalus* α-Oxyhemoglobin. FIG. 86. *Thylacynus cynocephalus* β-Oxyhemoglobin.
FIGS. 87, 88. *Trichosurus vulpecula* Oxyhemoglobin.

On account of the deep color of the plasma the pleochroic colors could not be definitely observed, but the crystals are strongly pleochroic, with absorption $\mathfrak{c} > \mathfrak{b} > \mathfrak{a}$. On the base, the crystal extinguishes symmetrically, with the axis of greater elasticity bisecting the acute angle of the plate. On the clinopinacoid sections the extinction is oblique, making an angle of 9° with the edge 010–001 or with the clino-axis, *a*, in the obtuse angle. The plane of the optic axes is normal to the plane of symmetry and $\mathfrak{a} = b$. The orientation of the other elasticity axes is, $\mathfrak{b} \wedge a = 9°$ in the obtuse angle, the extinction angle; and $\mathfrak{c} \wedge \acute{c} = 26°$, in the obtuse angle. Traces of the brushes of an interference figure show on some of the plates that are tilted, with the orientation as above for the elasticity axes. On the clinopinacoid sections the interference figure shows when looking along $\mathfrak{a}$, the brushes being only slightly separated. The acute bisectrix is hence the axis of greatest elasticity, $Bx_a = \mathfrak{a}$, and the optical character is hence *negative*.

### RAT-KANGAROO, *Æpyprymnus rufescens*. Plates 21 and 22.

The specimen was received from the National Zoölogical Park at Washington. The blood was putrid, but, being collected in oxalate, was liquid. The contents of the tube were centrifugalized. The separated corpuscles were then laked with ether and again centrifugalized, and slides prepared in the usual manner. Crystallization was rapid; the crystals formed readily at room temperature and appeared to be relatively insoluble. The color of the solution was almost discharged, showing that the crystallization was very complete. Negatives were made within 5 hours after the slides were prepared. The crystals were oxyhemoglobin as determined by the spectroscope.

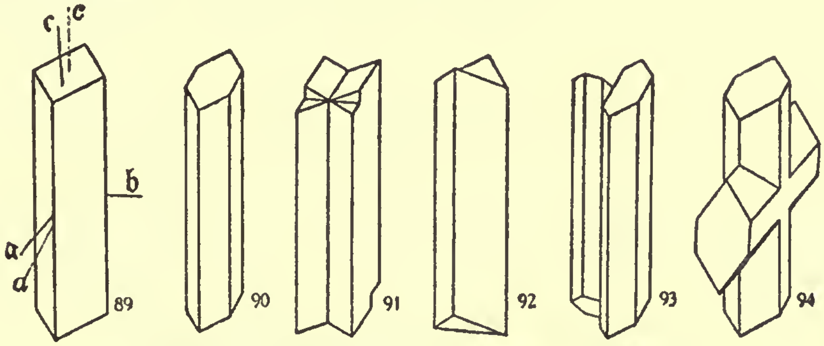
### *Oxyhemoglobin of Æpyprymnus rufescens.*

Monoclinic: Axial ratio $a : b : \acute{c} = 1.4825 : 1 : 1.338$; $\beta = 67°$.

Forms observed: Prism (110), clinopinacoid (010), base (001); and, in twins, positive hemiorthodome ($\bar{1}01$), orthopinacoid (100).

Angles: Prism angle $110 \wedge \bar{1}10 = 112°$ (68°); $001 \wedge 010 = 90°$; $100 \wedge 001 = \beta = 67°$; $100 \wedge \bar{1}01 = 51°$, from twin.

Habit prismatic, elongated on the vertical axis, with the prism (110) and base (001) alone (text figure 89) or in combination with the clinopinacoid (010) (text figure 90), and frequently flattened on the clinopinacoid; the base produces an oblique termination in all crystals. The prisms are sometimes long, but in the greater number of crystals are not more than 4 to 6 times as long as wide. The orthopinacoid is not developed as a face and only appears in twins; hence no square crystals are seen. Twinning is of several types, interpenetrant twins on the prism being common (text figure 91), see plate 22, fig. 127; twins also form on the orthopinacoid (text figure 92) (gypsum type) and on the clinopinacoid similar to the carlsbad type (text figure 93). The twin on the positive hemiorthodome ($\bar{1}01$), from which the value of $\acute{c}$ was obtained, is an interpenetrant twin, often seen in crystals with the combination (110) (010) (001). The base of one member is nearly parallel with the orthopinacoid or prism edge of the other and the acute angles are opposed with the obtuse angles pointing outward (see text figure 94). In the twins of the carlsbad type the opposed prism faces on either side of the plane of twinning appear to be developed more than the other pair in each case. The twins on the prism are not only interpenetrant, they are frequently juxtaposed, and even in this case polysynthetic (see plate 22, fig. 128).



FIGS. 89, 90, 91, 92, 93, 94. *Æpyprymnus rufescens* Oxyhemoglobin.

Pleochroism is very strong; $\mathfrak{a}$ pale yellowish-red, $\mathfrak{b}$ rather deep red, $\mathfrak{c}$ very deep red; the pleochroism is readily observed on account of the almost complete crystallization of the oxyhemoglobin, leaving a nearly colorless solution. Extinction on all of the usual aspects is oblique, the crystals generally presenting the clinopinacoid or prism face. In the twins on the orthopinacoid, prism, and orthodome the extinction is symmetrical with the plane of twinning. The plane of the optic axes is the plane of symmetry, the orientation of the elasticity axes is $\mathfrak{a} \wedge a = 11°$ in the obtuse angle; $\mathfrak{b} = b$, $\mathfrak{c} \wedge \acute{c} = 12°$ in the obtuse angle. Only brushes of the interference figure in unsymmetrical arrangement were seen on such optical sections as could be observed; the optical character appeared to be *negative*, or $Bx_a = \mathfrak{a}$.

### KANGAROO, *Macropus giganteus* (?). Plate 22.

The specimen was received from the National Zoölogical Park at Washington during the summer and was kept frozen in the original collecting tube until examined. The blood was rather putrid and contained many small clots and amorphous matter. It had been drawn into a collecting tube supplied with oxalate, hence did not clot after the specimen was placed in the tube. Ether added to lake the blood appeared to increase the precipitate of amorphous granular matter, and centrifugalizing did not entirely free the blood from this precipitate. The crystals formed readily at ordinary room temperature and did not appear to be very soluble. Photographs could be made inside of 4 hours after the slides were prepared. Examination with the spectroscope showed the crystals to be oxyhemoglobin.

*Oxyhemoglobin of Macropus giganteus.*

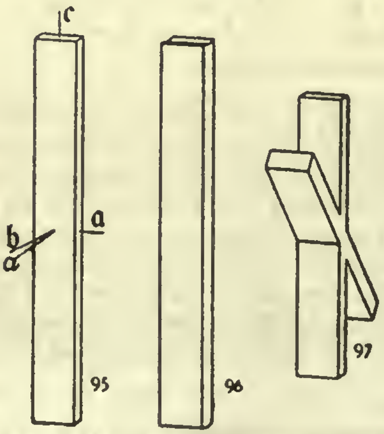
Monoclinic: Axial ratio $a : \dot{c} = 1 : 0.497$; $\beta = 87°$ (93°).

Forms observed: Orthopinacoid (100), clinopinacoid (010), base (001); also an orthodome $(\bar{1}01)$. Some crystals apparently showed a square prism (110) in place of the two vertical pinacoids; this was not clearly made out.

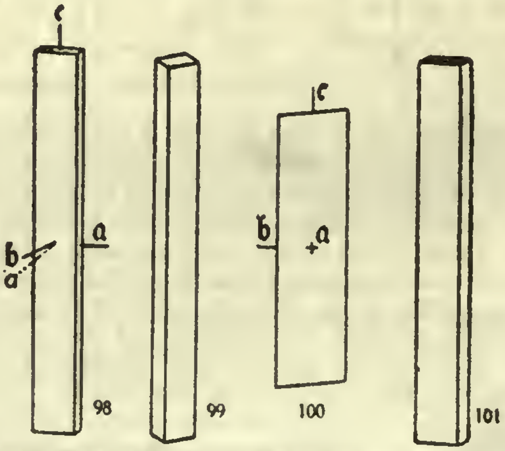
Angles: $100 \wedge 001 = \beta = 87°$ (93°); $001 \wedge 010 = 90°$; $100 \wedge 101 = 66°$. The positive hemiorthodome $(\bar{1}01)$ was also observed as a plane of twinning with angle between the $\dot{c}$ axes of the twin of 48° 30′ as measured. This gives the angle $100 \wedge \bar{1}01 = 65° 45'$.

Habit lath-shaped crystals, consisting of orthopinacoid (the principal plane) and clinopinacoid; the two forming a flattened prismatic crystal elongated along the vertical axis and terminated by the base (001) (text figure 95). They, being flattened on (100), generally present this aspect and hence appear to be terminated by a plane normal to the length, but the square end is produced by the aspect in which the crystal is usually presented, and on an edge view, looking along the $b$-axis, the end is seen to be oblique. The orthodome was seen in a few crystals only. The lath-shaped crystals aggregate into sheaf-like bundles and stellate radiating groups, and also grow singly. Twins on the orthopinacoid (100), gypsum type, were occasionally seen (text figure 96) and one distinct twin on the hemiorthodome $(\bar{1}01)$ was observed with the angle as given above (text figure 97).

Pleochroism is very pronounced; $\mathfrak{a}$ pale pink, $\mathfrak{b}$ rose-pink, $\mathfrak{c}$ deep rose-pink. Extinction is straight or nearly so in both side and edge views. The end view was not seen. The orientation of the elasticity axes is $\mathfrak{c} = \dot{c}$, $\mathfrak{a} = b$, $\mathfrak{b} \wedge a = 3°$, in the obtuse angle; the axial plane is normal to the plane of symmetry. No interference figure could be observed, but indications seemed to show that $Bx_a = \mathfrak{c}$, and the optical character is hence probably *positive.*



FIGS. 95, 96, 97. *Macropus giganteus* Oxyhemoglobin.



FIGS. 98, 99, 100, 101. *Petrogale* sp. Oxyhemoglobin.

ROCK-KANGAROO, *Petrogale* sp. Plate 23.

The specimen was received in July and kept frozen in the original collecting tube until examined. The blood was full of a granular precipitate which did not separate on centrifugalizing. The blood had been oxalated when collected and was not ether-laked. The slides were prepared in the usual manner, and crystals formed readily at room temperature, showing no signs of dissolving. They were oxyhemoglobin, as shown by the spectroscope.

### *Oxyhemoglobin of Petrogale sp.*

Monoclinic: Axial ratio not determined. $\beta =$ about 84° (96°).

Forms observed: Orthopinacoid (100), clinopinacoid (010), prism (110), base (001).

Angles: $100 \wedge 001 = \beta = 84°$; $001 \wedge 010 = 90°$.

Habit lath-shaped by development of the two vertical pinacoids (100) and (010) and terminated obliquely by the base (001) (text figure 98), also prismatic with the combination of prism (110) and base (001) (text figure 99). The crystals are capillary in some cases (probably the prism and base combination); and in other cases are long lath-shaped, with the orthopinacoid predominating, and then with square ends (text figure 98); or short lath-shaped, with the clinopinacoid predominating (text figure 100), and then with oblique ends. The prism seems to be nearly square, but its angle could not be obtained. Contact twins on the orthopinacoid (gypsum type) are common (text figure 101); twins on a pyramid of the interpenetrant type were also observed (compare text figure 97).

Pleochroism is rather strong; $\mathfrak{a}$ pale yellowish-red, $\mathfrak{b}$ red, $\mathfrak{c}$ deep cochineal-red. Extinction was apparently straight in both aspects, on (100) and on (010), the side and edge views. The orientation of the elasticity axes is $\mathfrak{a} = b$; $\mathfrak{b} \wedge a = 6°$, in the obtuse angle; $\mathfrak{c} = \acute{c}$; the axial plane is hence normal to the plane of symmetry. No interference figure was observed, but evidence of the brushes was noticed on the side and edge views, which points to $Bx_a = \mathfrak{c}$, and the optical character is hence probably *positive.*

It will be noticed that the crystals that show flattening on the ortho-axis are flattened along the axis of greatest elasticity; this is to be expected, as they developed later and hence under greater pressure, and this axis should be most sensitive to the increase of pressure.

## EDENTATA.

### ANT-EATER, *Myrmecophaga* (?). Plate 23.

The specimen of blood was received from the National Zoölogical Park at Washington. The corpuscles were separated, oxalated, ether-laked, and centrifugalized, and preparations made in the usual manner. The crystals formed readily and were kept in the cold, as they showed a tendency to dissolve when brought into the room. After 24 hours they began to dissolve, even in the cold, especially the crystals in the protein ring; the solution beginning with the ends of the crystals, which lose their sharp outlines and become rounded. By the spectroscope these crystals were determined to be oxyhemoglobin.

### *Oxyhemoglobin of Myrmecophaga* (?).

Orthorhombic: Axial ratio $a : \acute{c} = 1 : 0.4348$.

Forms observed: Brachypinacoid (010), macropinacoid (100), macrodome (101), basal pinacoid (001).

Angles: Macropinacoid to macrodome, $100 \wedge 101 = 66° 30'$; dome to dome faces $101 \wedge \bar{1}01 = 47°$ (133°); macropinacoid to base $100 \wedge 001 = 90°$.

Habit long lath-shaped, flattened on the brachypinacoid; the macropinacoid very narrow, and the crystal terminated by a macrodome or by the base (text figure 102). The crystals are capillary or even acicular and sharply pointed when first forming, but the terminations become distinct as the crystal grows. The first-formed crystals show less of the brachypinacoid (010) than those that are fully developed. In the fully developed crystals it is seen that they thin on the plane (010) towards the ends of the crystals, by development of (0m1). Cleavage is apparently basal.

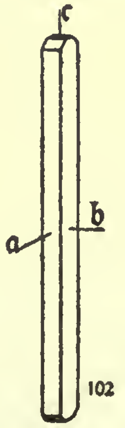


FIG. 102. *Myrmecophaga* Oxyhemoglobin.

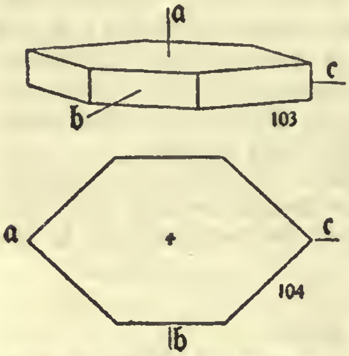
The colors are rather pale; pleochroism is quite strong; $\mathfrak{a}$ pale yellow, $\mathfrak{b}$ deep pink, $\mathfrak{c}$ deep rose-pink or rose-red; $\mathfrak{b}$ and $\mathfrak{c}$ are nearly the same tint. Absorption is in the order $\mathfrak{c} > \mathfrak{b} > \mathfrak{a}$. Extinction is straight on (100) and on (010). The orientation of the elasticity axes is $\mathfrak{a} = a$; $\mathfrak{b} = b$; $\mathfrak{c} = \acute{c}$. No interference figure was seen on $b$; but on $a$, or looking along $\mathfrak{a}$, traces of a figure were seen, the brushes passing out of the field in open position. The aspect looking along $\mathfrak{c}$ could not obtained, but there seems to be no doubt that $Bx_a = \mathfrak{c}$, or that the optical character is *positive*.

The axis of $\acute{c} = \mathfrak{c}$ is evidently the axis of least cohesion, as is shown by the cleavage and by the solution of the crystals in this direction. The axis of greatest elasticity $\mathfrak{a}$ is the direction in which the crystals develop after the full length has been attained.

## SIRENIA.

### MANATEE, *Manatus americanus.*

Oxalated blood was received from the New York Zoölogical Gardens, and was prepared as usual. The crystals are very soluble and dissolve rapidly when brought into a warm room.



FIGS. 103, 104. *Manatus americanus* Oxyhemoglobin.

*Oxyhemoglobin of Manatus americanus.*

Orthorhombic: Axial ratio 0.949 : 1 : $\acute{c}$.

Forms observed: Unit prism (110), macropinacoid (100), base (001).

Angles: Prism angle 110 ∧ $1\bar{1}0$ = 95° (85° normals); prism to base 110 ∧ 001 = 90°.

Habit tabular, elongated parallel to the macro-axis, the prism faces and the macropinacoid often in equilibrium (text figures 103 and 104).

Pleochroism was difficult to observe, owing to the high color of the plasma, but evidently the color is deeper parallel to the $b$-axis ($\mathfrak{c}$). Extinction is symmetrical on the flat views of the plates and straight on edge views. Orientation of the elasticity axes is $\mathfrak{a} = \acute{c}$, $\mathfrak{b} = a$, $\mathfrak{c} = b$. The interference figure was not observed, the crystals dissolving while under observation. The optical character was hence not determined.

TABLE 39.—*Crystallographic characters of the hemoglobins of the Marsupialia, Edentata, and Sirenia.*

| Name of species. | Axial ratio. | Angle $\beta$. | Prism angle. | Extinction angle. | Optical character. | System. | Substance. |
|---|---|---|---|---|---|---|---|
| Marsupialia: | | ° | ° ′ | | | | |
| Didelphis virginiana....... | 1.7856 : 1 : $\acute{c}$ | 48 | 58 30 | $\mathfrak{a} \wedge a = 17°$ | Positive | Monoclinic | $\alpha$-OHb. |
| Do.................. | ................. | 90 | 60 0 | 0° | Do. | Hexagonal | $\beta$-OHb. |
| Do.................. | 1.963 : 1 : $\acute{c}$ | 66 | 54 0 | $\mathfrak{a} \wedge a = 13°$ | Do. | Monoclinic | Hb. |
| Do.................. | 1.804 : 1 : $\acute{c}$ | 41 | 58 0 | $\mathfrak{a} \wedge a = 13°$ | Do. | Do. | $\alpha$-COHb. |
| Do.................. | ................. | 90 | 60 0 | 0° | Do. | Hexagonal | $\beta$-COHb. |
| Sarcophilus ursinus ....... | 1.804 : 1 : $\acute{c}$ | 69 | 58 0 | $\mathfrak{a} \wedge a = 11°$ | Do. | Monoclinic | OHb. |
| Dasyurus maculatus ...... | 1.8227 : 1 : $\acute{c}$ | 63 | 57 30 | $\mathfrak{a} \wedge a = 28°$ | Negative? | Do. | Hb. |
| Dasyurus viverrinus....... | 1.7856 : 1 : $\acute{c}$ | 69 | 58 30 | $\mathfrak{a} \wedge a = 9°$ | Positive? | Do. | OHb. |
| Thylacynus cynocephalus.. | ................. | 77 | ..... | $\mathfrak{c} \wedge \acute{c} = 37°$<br>$\mathfrak{b} \wedge a = 50°$ | Negative | Do. | $\alpha$-OHb. |
| Do.................. | 1 : 1 : 1 | 90 | 90 0 | ......... | Isotropic | Isometric | $\beta$-OHb. |
| Trichosurus vulpecula ..... | 0.9163 : 1 : $\acute{c}$ | 55 | 95 0 | $\mathfrak{b} \wedge a = 9°$ | Negative | Monoclinic | OHb. |
| Æpyprymnus rufescens.... | 1.4825 : 1 : 1.338 | 67 | 68 0 | $\mathfrak{c} \wedge \acute{c} = 12°$ | Do. | Do. | OHb. |
| Macropus giganteus ....... | $a : \acute{c} = 1 : 0.497$ | 87 | ..... | $\mathfrak{c} = \acute{c}$, 0° | Positive? | Do. | OHb. |
| Petrogale sp............... | ................. | 84 | ..... | $\mathfrak{c} = \acute{c}$, 0° | Do. | Do. | OHb. |
| Edentata: | | | | | | | |
| Myrmecophaga sp.(?) ..... | $a : \acute{c} = 1 : 0.4348$ | 90 | ..... | 0° | Positive | Orthorhombic | OHb. |
| Sirenia: | | | | | | | |
| Manatus americanus ...... | 0.949 : 1 : $\acute{c}$ | 90 | 95 0 | 0° | ........ | Do. | OHb. |

# CHAPTER XII.

## CRYSTALLOGRAPHY OF THE HEMOGLOBINS OF THE UNGULATES.

The hemoglobins from 20 species of the ungulates were studied, of which only 2 belonged to the perissodactyls and the remaining 18 species to the artiodactyls.

The perissodactyls examined were the horse, *Equus caballus,* and the mule, a cross between *Equus asinus* male and *Equus caballus* female. The crystals of these two species closely resemble each other, but those of the mule are distinguishable by measurement from the crystals of the horse. Unfortunately, the blood of *Equus asinus* was not obtained for examination, so that whether the hemoglobin of the cross is intermediate between that of the horse and the ass could not be determined.

The artiodactyls examined included the hippopotamus, 2 species of peccary, the common swine, a chevrotain, 5 species of the *Cervidæ* or deer, 4 antelopes, 2 species of sheep, and 2 species of the genus *Bos*, the ox and the buffalo, or bison. These represent the principal families of the *Artiodactyla* except the *Camelidæ* and *Giraffidæ.* Of the *Hippopotamidæ* but one species was examined, the *Hippopotamus amphibius.* Of the *Dicotylidæ,* the two species were the white-lipped peccary, *Dicotyles labiatus,* and the collared peccary, *Dicotyles tajacu.* The chevrotain was the muis deer, *Tragulus meminna,* a small deer-like animal. As will be seen, its crystals do not resemble those which were obtained from the true deer of the family *Cervidæ.* The deer included 4 species of the genus *Cervus,* the wapiti or elk, the red brocket deer (?), the Venezuela deer, and the fallow deer; also the muntjak of the genus *Cervulus, C. muntjak.*

Of the *Bovidæ,* the subfamily *Antilopinæ* is represented by 4 species, belonging to the genera *Antilope, Cervicapra, Gazella,* and *Cephalophus.* The subfamily *Ovinæ* is represented by the common sheep and the bharal or blue sheep of Thibet, both belonging to the genus *Ovis*; these two species will be found to resemble each other very closely and to differ from all of the other ungulates examined. They both show a very remarkable type of twin crystal, a fiveling that produces a pentagonal composite crystal, that is practically unique. It may be called the sheep-type of twin. The subfamily *Bovinæ* is represented by the ox, *Bos taurus,* and the buffalo, *Bos bison.*

The crystal systems represented in the hemoglobin crystals of the ungulates include the monoclinic, orthorhombic, hexagonal, and tetragonal systems, and it will be seen that the optical characters (with the exception of one case in which the character is questionably determined, and the single example of the hexagonal, in which it could not be determined) bear a certain definite relation to the crystal system. Thus the monoclinic crystals are (with the one exception above noted) all optically *positive,* while the orthorhombic and tetragonal crystals are all optically *negative.* In the case of some species of the ungulates of which we had ample supplies of blood, such for instance as the horse and mule, several kinds of oxyhemoglobin were observed; the same was seen in one of the antelopes, and in these cases the optical characters followed the above rule, and changed in the same species with the crystal system. In most of the species, however, but one form of oxyhemoglobin was noted.

Reduced-hemoglobin crystals were recorded in several cases, and sometimes they crystallized in the same system as the oxyhemoglobin, but in other cases they did not. Thus in the chevrotain, the common sheep and the blue sheep of Thibet, the oxyhemoglobin crystallizes in the monoclinic system, in each species, and the reduced hemoglobin in the orthorhombic system. In every instance, when the oxyhemoglobin and the reduced hemoglobin were observed in the same species, the two substances could readily be distinguished by the form of the crystals, even though both crystallized in the same system.

## UNGULATA.

### HORSE, *Equus caballus.* Plates 24 to 26.

Horse blood was obtained from the Veterinary Department of the University of Pennsylvania when needed, being from horses undergoing operations, etc. It was received in fresh condition and was prepared in various ways, the whole blood or the centrifugalized corpuscles being used. Experiments were made on this blood to test the influence on the crystals of various salts and substitutes for plasma, etc., and it has been probably more thoroughly studied in this research than the blood of any other species, except the mule. The usual method of preparing the blood by oxalating, laking with ether, and centrifugalizing gives in the fresh blood the monoclinic plates that are characteristic of horse blood, but the first crystals to form in this case are orthorhombic prisms; these, however, are rapidly dissolved as the monoclinic plates develop. When the oxalate is omitted the orthorhombic prisms are more permanent, and sometimes remain the principal crystals in the slide, but the monoclinic plates also appear with no difference in habit or form from those which develop in the oxalated blood. Carbon-monoxide hemoglobin was made by displacing the oxygen in oxyhemoglobin by the carbon monoxide of illuminating gas (water-gas) and this was crystallized under various conditions and compared with the oxyhemoglobin crystals. In both oxyhemoglobin and CO-hemoglobin two forms of crystals were observed, showing that both oxyhemoglobin and CO-hemoglobin are dimorphous. These varieties are distinguished as α-oxyhemoglobin, β-oxyhemoglobin, etc.

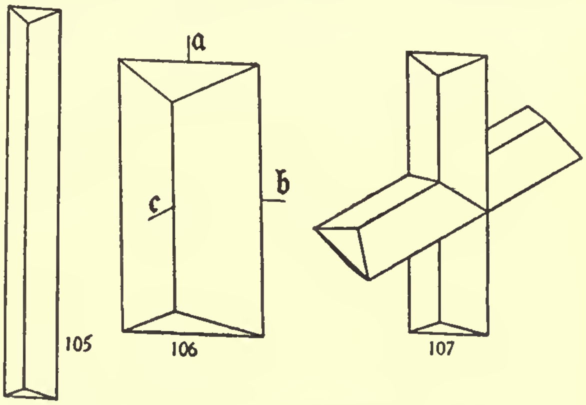
#### *a-Oxyhemoglobin of Equus caballus.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.7467 : 1 : 0.4097$.

Forms observed: Unit prism (110), macrodome (101).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 73° 30'$ (normals); macrodome angle $101 \wedge \bar{1}01 = 57° 30'$ (normals).

Habit, at first, long hair-like crystals, which soon become more or less stout prisms; consisting of the unit prism terminated by the macrodome (text figures 105 and 106); the relative development of the two forms depending upon the method of preparation, and ranging from a prism with a length double the thickness (text figure 106) to one with the length ten times the thickness or more (text figure 105). They grow at first in the dried plasma ring, almost as soon as the cover is put on the drop, and develop very rapidly, these being the long hair-like crystals that are afterwards dissolved. But they also develop throughout the slide in shorter, doubly terminated crystals. The rods in the protein ring begin to form within 3 or 4 minutes after placing the blood on the slide, and even before the cover is applied in case of blood containing oxalate; in such a blood with oxalate, the monoclinic tabular crystals of $\beta$-oxyhemoglobin appear within 10 minutes after placing the drop of blood on the slide. But to obtain good orthorhombic crystals of $\alpha$-oxyhemoglobin without the $\beta$-oxyhemoglobin the oxalate is omitted, and the preparation made as follows: The blood is defibrinated by beating and then centrifugalized to collect the corpuscles; these are separated by draining off the serum and then laked with ether, excess of ether being added to dilute the thickened blood until it will centrifugalize readily. This can be done rapidly, with the excess of ether, and in a few minutes the blood is clear enough to mount. The drops are placed on the slides and allowed to evaporate until large crystals begin to form, when the cover is applied. Such preparations rarely show the $\beta$-oxyhemoglobin plates before about 24 hours or more after the preparations are made, but the $\alpha$-oxyhemoglobin crystals are very large and fine. As the $\beta$-oxyhemoglobin forms sparingly in these slides, the $\alpha$-oxyhemoglobin crystals are quite permanent and show no tendency to dissolve. These $\alpha$-oxyhemoglobin crystals generally grow singly or in tufts and parallel groupings from the protein ring or from the cover edge; they usually occur singly through the body of the slide, and twin only rarely. What seem to be twins on a unit pyramid were observed (text figure 107).



FIGS. 105, 106, 107. *Equus caballus* $\alpha$-Oxyhemoglobin.

Pleochroism is quite marked; $\mathfrak{a}$ pale yellowish-red to flesh-pink, $\mathfrak{b}$ rose-red, $\mathfrak{c}$ deep blood-red. The extinction is straight in all aspects. The orientation of the elasticity axes is $\mathfrak{a}=\dot{c}$; $\mathfrak{b}=b$; $\mathfrak{c}=a$. The plane of the optic axes is the brachypinacoid; and on basal sections, looking along $\dot{c}$ in convergent light the biaxial figure is seen with the brushes well separated; the angle between the axes measured in white light (practically for red, owing to the color of the crystals) $2E=$about 45°. The acute bisectrix is hence the axis of greatest elasticity, $Bx_{\mathfrak{a}}=\mathfrak{a}$, and the optical character is *negative.*

### $\beta$-*Oxyhemoglobin of Equus caballus.*

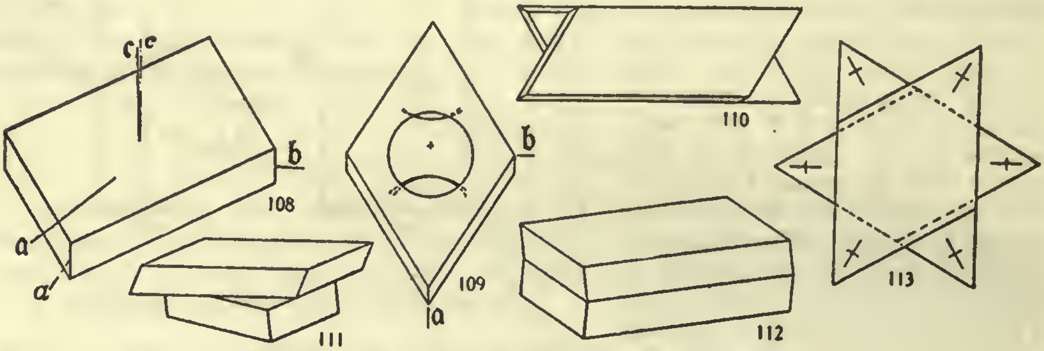
Monoclinic: Axial ratio $a : b : \dot{c} = 1.600 : 1 : \dot{c}$; $\beta = 72°$ for untwinned crystals; in those that are twinned the angles change slightly and the ratio is $a : b : \dot{c} = 1.6976 : 1 : \dot{c}$, with the same angle for $\beta$.

Forms observed: Unit prism (110), base (001), orthopinacoid (100).

Angles: Prism angle, traces of the prism on the base, edges $110\text{–}001 \wedge 1\bar{1}0\text{–}001 = 64°$ in untwinned crystals, but usually about 61° as the crystals are generally twinned; prism edge to base, edge $110\text{–}1\bar{1}0 \wedge 001 = 72° = \beta = 100 \wedge 001$.

Habit, in lozenge-shaped tables, tabular on the base (text figures 108 and 109), the crystal being the oblique section of the prism made by the basal pinacoid, and the ratio of the length of the prism to the length of the symmetry axis varying from 1 : 1 to

1 : 10, or even flatter in very large rhombic plates; rarely in prismatic development with the above ratio 2 : 1. The crystals are normally twinned "horse-type twin" (text figures 110 and 111), with the two parts united along a prism-base edge and the axis of the hemitrope twin normal to this common edge and in the basal pinacoid, which is the ordinary composition plane. In some cases this composition plane is the plane normal to the base, that includes the common edge, or is the so-called plane of twinning, normal to the twin axis. In some crystals both of these composition planes occur in the same individual, by one crystal overgrowing the other, over the common edge; and the two uniting by filling up the re-entrant angle and forming the false plane normal to the base and in the zone of the prism-base. In some cases the two parts of the twin are normally developed crystals; but usually, when the base is the composition face, the two crystals become elongated in the direction of the common edge (text figure 110), and the ratio of length to breadth of such a crystal may be 6 : 1. Such crystals show the overlapping at each end and recall the arrangement of the Carlsbad twin. When the composition face is the twin plane, normal to the base, the two crystals may slightly overlap, or this may not be noticeable and they are simply juxtaposed along this plane, recalling the common gypsum twin on the orthopinacoid; and even becoming elongated along the common edge, but not to the same extent as in the case when the base is the composition face. Another hemitrope twin of this type is possible in which the twin axis is the common prism-base edge and the composition face is the base or the normal to the base. This also appears to occur, but not so commonly. There is a third kind of twinning that was occasionally seen, of the Manebach type (text figure 112), when the base is the composition face and twinning plane, or the normal to the base is the twin axis. From the way in which these twins develop it would seem better to assume a twin axis parallel to the edge 010–001, and then the twinning plane would be the plane normal to the base in the zone of (100)–(001). This plane actually appears to occur as a composition face in these twins. In the first type of twinning (horse-type) especially, when the base is the composition plane, they twin several times on the different prism edges so that three or more may occur in a group (text figure 113), in partial polysynthetic order. In some cases this produces a six-pointed star with three individuals, or more often with four.



Figs. 108, 109, 110, 111, 112, 113. *Equus caballus* β-Oxyhemoglobin.

These monoclinic crystals are produced in great numbers in the blood to which oxalate has been added, and they increase in proportion to the amount of oxalate added, and also in inverse proportion to the number of α-oxyhemoglobin crystals. But they are produced in blood to which no oxalate has been added, although in comparatively small numbers. The oxalate does not alter the habit, form, or other characteristics of the crystals at all. They form evidently from concentrated or dense solutions of the hemoglobin, and the function of the oxalate is perhaps to increase the pressure of the solution or to make it more concentrated by taking some of the water. Slow evaporation of non-oxalated blood has the same effect of making the solution more dense. It is also possible that the oxalate helps to convert one isomer into another.

Pleochroism is strong; $\mathfrak{a}$ pale yellowish-red, $\mathfrak{b}$ rather bright red, $\mathfrak{c}$ deep blood-red. Extinction is symmetrical on the base, but oblique on the clinopinacoid sections, as well as on all prism faces. The orientation of the elasticity axes is $\mathfrak{a} \wedge a = 13°$, in the obtuse angle; $\mathfrak{b} = b$; $\mathfrak{c} \wedge \acute{c} = 5°$, in the obtuse angle; extinction on the clinopinacoid section is hence 13° from the trace of the base, and 5° from the prism edge. The plane of the optic axes is the clinopinacoid; and on the basal section one brush of the interference figure is seen, somewhat out of the center of the field, revolving as the crystal is revolved. The other brush is out of the field. The axial angle $2E$ is evidently more than 50°, perhaps about 60°. The acute bisectrix is the axis of least elasticity $Bx_a = \mathfrak{c}$, and the crystal is optically *positive*.

### CARBON-MONOXIDE HEMOGLOBIN OF HORSE.

The CO-hemoglobin was made by exposing the blood to illuminating gas (water-gas) for several hours, and in laked bloods crystals form during the time of exposure to the gas at the ordinary room temperature. The solution, from which the crystals have been separated by centrifugalizing, crystallizes very rapidly; too rapidly, in fact, when making slide preparations in the usual manner. In order to make the crystallization less rapid the blood was diluted with a 50 per cent solution of egg-white and from this the best crystals were obtained. As in the case of the oxyhemoglobin, the addition of oxalate causes the development of the monoclinic crystal, while in absence of oxalate mainly the orthorhombic crystals are formed; but the addition of egg-white only retards the formation of the crystals, and makes them finer in development, without in any way altering their characters. The heat of the hand was found to be sufficient to dissolve the crystals that had formed in the flask, during exposure to CO. To produce the orthorhombic crystals, the laked blood, after exposure for some hours to illuminating gas, was simply warmed by the hand, or by immersing the tube in water at body temperature, and, after centrifugalizing a few minutes, the preparations made. Only the orthorhombic crystals developed at first. The best crystals were made by mixing one part of the CO-blood with one part of egg-white, shaking with excess of ether and centrifugalizing a few minutes.

By addition of oxalate to either the undiluted CO-blood, or the CO-blood diluted with one part of a 50 per cent solution of egg-white, and warmed in each case to body temperature, the monoclinic form only developed. The crystals were of the same habit in preparations from the undiluted blood and from the diluted blood. All of these CO-hemoglobin preparations were made from fresh blood that had been ether-laked and centrifugalized, so that it was clear before exposure to the water-gas. There are hence two forms of the CO-hemoglobin, as was the case with the oxyhemoglobin. These have been distinguished as α-CO-hemoglobin and β-CO-hemoglobin.

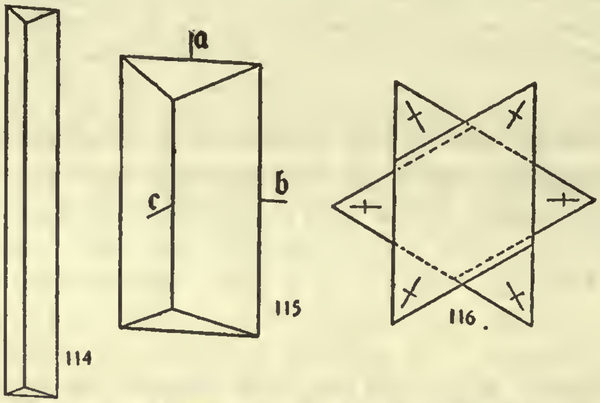
#### *α-CO-hemoglobin of Equus caballus.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.7332 : 1 : 0.4106$.

Forms observed: Prism (110), macrodome (101).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 72° 30'$ (normals); dome angle $101 \wedge \bar{1}01 = 58° 30'$ (normals).

Habit long or short prismatic (text figures 114 and 115), prisms from 2 to 10 times as long as they are thick, and terminated by the macrodome, with generally equal development of the dome faces; the prisms are often flattened on two opposite faces, but usually symmetrically developed. They frequently grow in radiating groups and in some cases appear to be twinned on a pyramid of the unit series (text figure 116) but the angle of this pyramid was not determined. Parallel growths of two crystals, side by side with a common dome edge, are frequently seen. Some of the prismatic crystals are very long, the ratio of length to thickness being 20 : 1 or more.



Figs. 114, 115, 116. *Equus caballus* α-Carbon-monoxide Hemoglobin.

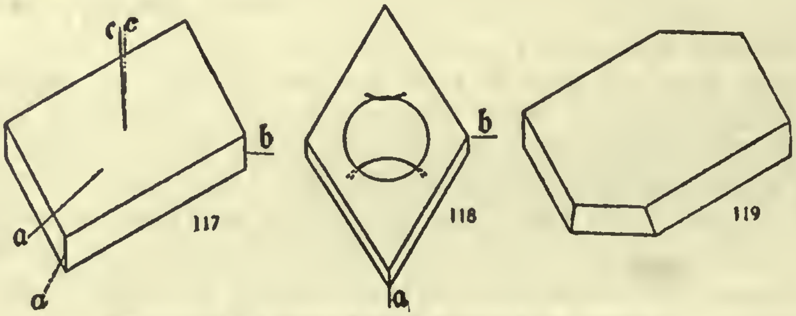
Pleochroism is not so strong as in the oxyhemoglobin, but $\mathfrak{a}$ is pale rose-pink and $\mathfrak{b}$ and $\mathfrak{c}$ nearly equal and rose-red. The spectrum was that of CO-hemoglobin as determined by the microspectroscope. The orientation of the elasticity axes is $\mathfrak{a} = \acute{c}$, $\mathfrak{b} = b$, $\mathfrak{c} = a$; the plane of the optic axes is the brachypinacoid; the optical character, judging from the pleochroism, is *negative*, and the acute bisectrix, $Bx_a = \mathfrak{a}$.

*β-CO-hemoglobin of Equus caballus.*

Monoclinic: Axial ratio $a : b : \acute{c} = 1.664 : 1 : \acute{c}$; $\beta = 68°$.

Forms: Unit prism (110), positive hemiorthodome $(\bar{1}01)$, base (001).

Angles: Prism angle, traces of the prism on the base, edges $110\text{–}001 \wedge 1\bar{1}0\text{–}001 = 62°$; angle of the hemiorthodome on the base not obtained; prism edge to base, edge $110\text{–}1\bar{1}0 \wedge 001 = 68°$.



Figs. 117, 118, 119. *Equus caballus* β-Carbon-monoxide Hemoglobin.

Habit thin tabular on the base, consisting of the base cut by the unit prism (110) (text figures 117 and 118) and in some cases by the hemiorthodome (101) also (text figure 119), often this form appearing on one end of the plate only. This hemiorthodome was also observed in some of the twins. Twinning is normal in these crystals; an untwinned crystal is exceptional. The usual twin (horse-type) is on the normal to a common prism-base edge as twin axis, the normal lying in the base and the plane of twinning, hence a plane including the prism-base edge and normal to the base as already described under β-oxyhemoglobin of the horse. The majority of these crystals consist of at least three individuals; often more, up to six. They frequently form complicated groups. The twinned crystals are generally elongated in the direction of the common edge, as was the case with the β-oxyhemoglobin.

Pleochroism rather marked; $\mathfrak{a}$ pale pink, $\mathfrak{b}$ deep rose-pink, $\mathfrak{c}$ very deep rose-red. Extinction is symmetrical on the base and straight on edge, looking along the clino-axis on edge, but looking along the ortho-axis, the extinction angle is about 15° from $a$. The orientation of the elasticity axes is $\mathfrak{a} \wedge a = 15°$, in the obtuse angle; $\mathfrak{b} = b$; $\mathfrak{c} \wedge \acute{c} = 7°$, in the obtuse angle. The plane of the optic axes is the clinopinacoid and on the base in convergent light a biaxial figure is seen, with rather widely separated brushes, showing that the acute bisectrix $Bx_a = \mathfrak{c}$, and the optical character is *positive*. The twinning produces apparent optical anomalies; the twins, consisting of three, show a nearly uniaxial figure, and, even with two, the twin sometimes shows two symmetrically placed brushes as though orthorhombic. In the more complicated groups the apparently uniaxial figure is normal, but in all of these the interference cross opens slightly upon revolution of the crystals.

### MULE, *Equus asinus* (male) × *Equus caballus* (female). Plates 27–29.

The blood was obtained fresh and was not oxalated when collected, but defibrinated by beating. Centrifugalized corpuscles were laked and centrifugalized again, preparations being made both with and without oxalate. As with horse, the crystals are dimorphous; and the non-oxalated blood crystallized principally in the orthorhombic system, while the oxalated blood crystallizes principally in the monoclinic system; but in each, oxalated and unoxalated, both kinds of crystals appeared in the slides. For example, the corpuscles were laked with a large excess of ether and centrifugalized for a few minutes; preparations from this treatment showed only the orthorhombic crystals at first, but inside of 20 hours the monoclinic plates had developed sparingly, a few to the slide, in large, well-formed crystals. In the same way, the orthorhombic prisms appeared only sparingly in the preparations with a large amount of oxalate. The crystals were all oxyhemoglobin, as determined by the microspectroscope. The two forms are distinguished as α-oxyhemoglobin and β-oxyhemoglobin.

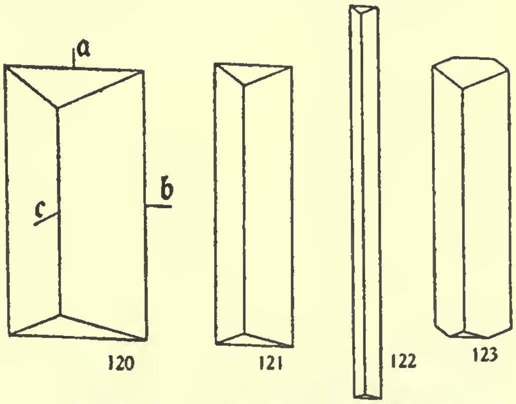
### *α-Oxyhemoglobin of Mule.*

Orthorhombic: Axial ratio 0.7813 : 1 : 0.4198.

Forms observed: Unit prism (110), macrodome (101).

Angles: Prism angle 110 ∧ $1\bar{1}0$ = 76° (normals); dome angle 101 ∧ $10\bar{1}$ = 56° 30′ (normals).

Habit prismatic, either long or short, elongated along (110) (text figures 120, 121, and 122), the ratio of the length of the prism to its thickness being about 2 : 1 in the short prisms (text figure 120) and varying up to 20 : 1 in long prisms (text figure 121); some are even almost hair-like. The prisms grow in irregular groups in the protein ring and along the cover edge, and are also commonly found scattered singly through the slides. The macrodome is usually unequally developed, one face larger than the other, giving a rather monoclinic aspect to the crystals (text figure 123). The prism faces are also sometimes unequally developed, the prism being flattened on two opposite faces. Twinning was not definitely made out.



Figs. 120, 121, 122, 123. Mule α-Oxyhemoglobin.

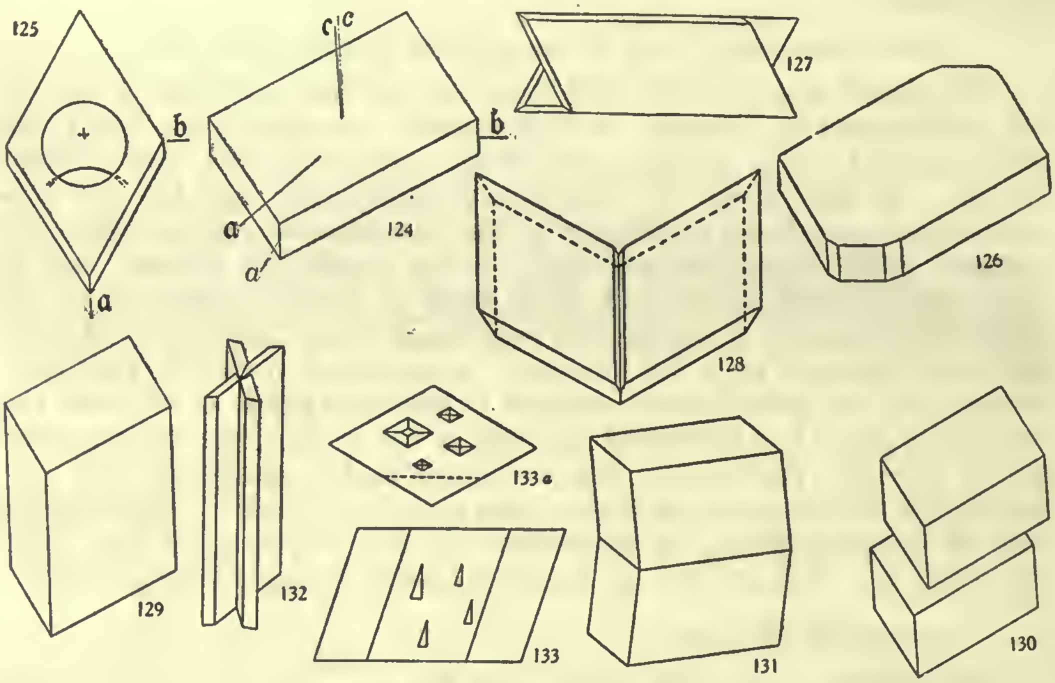
Pleochroism is readily observed; $\mathfrak{a}$ pale pinkish, $\mathfrak{b}$ rather moderately deep red, $\mathfrak{c}$ deep red, $\mathfrak{b}$ and $\mathfrak{c}$ being nearly equal. Double refraction is not very strong; extinction is straight in all aspects. The orientation of the elasticity axes is $\mathfrak{a} = \dot{c}$, $\mathfrak{b} = b$, $\mathfrak{c} = a$. Axial plane is the brachypinacoid, the acute bisectrix $Bx_a = \mathfrak{a}$; hence the optical character is *negative*. Traces of the brushes of an interference figure show on looking along $\mathfrak{c}$, and the separation of the axes is hence probably wide.

### *β-Oxyhemoglobin of Mule.*

Monoclinic: Axial ratio $a : b : \dot{c} = 1.7147 : 1 : \dot{c}$; $\beta = 72°$.

Forms observed: Unit prism (110), orthoprism (210) (?), orthopinacoid (100), base (001).

Angles: Prism angle, traces of prism on base, edges $110\text{–}001 \wedge 1\bar{1}0\text{–}001 = 60° 30'$; prism edge to base, edge $110\text{–}1\bar{1}0 \wedge 001 = 72° = \beta$.



Figs. 124–133a. Mule β-Oxyhemoglobin.

Habit tabular on the base, the crystal consisting of the basal pinacoid cut by the prism (text figures 124 and 125) and sometimes (but rarely) showing the orthopinacoid (100) and a very small prism face which is probably (210) (text figure 126). The crystals are normally twinned on an axis lying in the base and normal to a prism-base edge as in the horse, etc. In this twin (text figure 127), the composition face is usually the base, but may also be the plane normal to the base that includes the common prism-base edge (see plate 28, fig. 164). This produces a twin, which, owing to the elongation of the crystals along the common prism-base edge as an axis, closely resembles the common gypsum twin on the orthopinacoid (text figure 128). When considerable oxalate is used, the crystals that appear after the first crop are frequently developed along the vertical axis, until they become equidimensional (text figure 129), or even somewhat prismatic in habit. These crystals show the symmetry of the twins better than the simple plates. In some of these, a sort of parallel growth on the base is indicated, with the orientation of the $a$ axes of both members of the group such that both lie in the same vertical plane of symmetry (text figure 130); and also, in other cases, the group in the reverse position, forming a Manebach twin (text figure 131). But, in general, the type of twinning above described is the one that is present. Twinning on a pyramid, making an interpenetrant twin with an X-shaped cross-section, is also seen in some cases (text figure 132).

Etching figures were observed on the base in many cases when the crystals had begun to dissolve; they consisted of shallow lozenge-shaped pits, with sides parallel to the prism-edge boundaries of the plate; and, even, evidently negative pyramids (see text figure 133). On the clinopinacoid, which was developed as a plane of contact with the cover or slide, etching figures were also observed; triangular, with one side (the short side of the triangle) parallel to the clinopinacoid-base edge. They have a rather hemimorphic aspect (see text figure 133). One side of this triangle, as stated, is parallel to the $a$-axis; of the others, one is parallel to the $\acute{c}$ axis, but the third is inclined. Hence this is a negative positive hemi-pyramid, base, and probably prism, for the three planes of the triangular depression.

Pleochroism is strong; $\mathfrak{a}$ pale yellowish-red, $\mathfrak{b}$ bright cochineal-red, $\mathfrak{c}$ deep blood-red. Extinction is symmetrical on the base, and straight, looking along $a$, but oblique looking along $b$, about 12° or 13°, measured from the trace of the base. The orientation of the elasticity axes is $\mathfrak{a} \wedge a = 12°$ to 13°, in the obtuse angle; $\mathfrak{b} = b$; $\mathfrak{c} \wedge \acute{c} = 5°$ to 6°, in the obtuse angle. The plane of the optic axes is the plane of symmetry; and, looking along the normal to the base, one excentric brush of the biaxial figure is seen, but the other is out of the field. The angle $2E$ is hence probably above 85°. The acute bisectrix is, however, $\mathfrak{c}$, $Bx_a = \mathfrak{c}$, and the optical character is *positive.*

### HIPPOPOTAMUS, *Hippopotamus amphibius.* Plates 30 and 31.

The specimen of blood was received from the National Zoölogical Park at Washington, and was in good condition. The oxalated blood was laked with ether and centrifugalized and the preparations made in the usual manner. Crystals formed readily, soon after covering the slides; at first rather short prisms, later plates or tabular crystals, which showed a great tendency to form aggregates and to twin. While the crystals formed readily, they were rather soluble, and soon began to show signs of corrosion when brought into the room (20° C). The twinned crystals especially grew to large size, so that over night some of these composite crystals had increased to a couple of millimeters long. These large crystals, grown in the cold, dissolved readily upon a slight increase of temperature. The composite crystals and parallel growths formed quite complex groups and the crystallography was not very easy to make out. Examination with the spectroscope showed that the crystals were oxyhemoglobin.

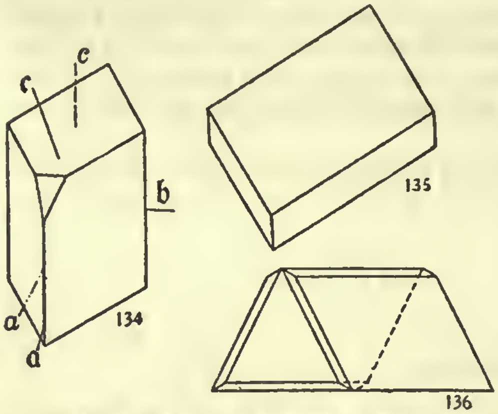
#### *Oxyhemoglobin of Hippopotamus amphibius.*

Monoclinic: Axial ratio $a : b : \acute{c} = 1.600 : 1 : \acute{c}$; $\beta = 66°$ (about).

Forms observed: Prism (110), base (001), hemiorthodome (101).

Angles: Angle of prism, traces on the base, edge 110–001 $\wedge$ $1\bar{1}0$–001 = 64°, edge 110–$1\bar{1}0$ $\wedge$ 001 = 66° = $\beta$. The angle of the orthodome to base was not accurately determined.

Habit at first rather short prismatic, the prism being two or three times as long as it is thick (text figure 134); also nearly equidimensional, resembling rhombohedrons, and the prism reduced to such an extent that the crystal becomes tabular on the base (text figure 135). Only occasionally did the hemiorthodome appear; it was in the obtuse angle (text figure 134). Twins form in the plates in the usual way (horse-type), the plane of twinning normal to the base, and parallel to a prism-base edge, which is a common direction for the two members of the twin, or the twin axis is normal to the edge 110–001 and lies in the plane of the base. In this twin the two halves continue to grow until they form a plate, with the bases in contact throughout, and bounded by two long sides of unequal length and two short sides of equal length (see text figure 136, also plate 31, fig. 182). This produces an apparently hemimorphic crystal, the axis of hemimorphism being the twin axis. The twin elongates along the normal to the twin axis or along the common prism edges. The large crystals that developed in the slides, up to 2 mm. or more long, were of this type. The composite character is evident in the large crystals, but not so much so in the smaller ones. The more complicated groups appear to be either twins of the above-described type, in polysynthetic development, or twins on a pyramid of the unit series (see plate 31, fig. 184, for both types).



Figs. 134, 135, 136. *Hippopotamus amphibius* Oxyhemoglobin.

In the crystals that were being dissolved by warming the solution, corrosion planes appeared in the zone of the negative unit pyramid. Etching figures also appeared, which seem to show monoclinic symmetry.

Pleochroism is quite strong, and is very noticeable even in the twins; $\mathfrak{a}$ nearly colorless, pale yellowish-red; $\mathfrak{b}$ moderately deep red, $\mathfrak{c}$ very deep red. On all sections parallel to axis *b* the extinction is symmetrical. On side view, looking along *b*, it is oblique, with an angle of 20° or more with the edge 010–001. The extinction in the twins described above is variable with the thickness of the members of the twin; it was not often symmetrical or parallel to the axis, but usually somewhat oblique. In convergent light, an axis is seen to emerge along the short pseudo-trigonal axis of the rhombohedron-like stumpy crystals, showing a single brush, which rotates as the crystal is rotated, and indicates the position of the axial plane. No complete interference figure was observed. The plane of the optic axes lies in the plane of symmetry and the orientation of the elasticity axes is as follows: $\mathfrak{a} \wedge a =$ about 20°, in the acute angle; $\mathfrak{b} = b$, $\mathfrak{c} \wedge \dot{c} = 44°$, in the obtuse angle. Hence, $\mathfrak{c}$ should be the acute bisectrix and the optical character is *positive.*

### PECCARY, *Dicotyles labiatus.* Plate 31.

The blood was from a young peccary, received from an unrecorded source. The slides were prepared in the usual manner, and the crystals formed soon after the slides were covered; but they began to break down and dissolve, within 4 hours after the preparation was made. They were not very perfect and appeared to be soft and porous. The crystals were oxyhemoglobin.

*Oxyhemoglobin of Dicotyles labiatus.*

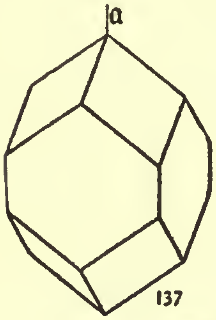
Tetragonal: Axial ratio $a : \dot{c} = 1 : 0.7133$.

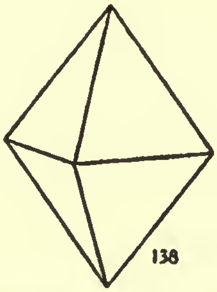
Forms observed: Unit pyramid (111), diametral prism (100).

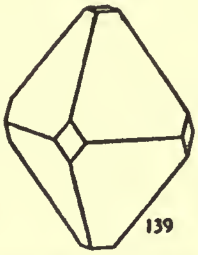
Angles: The angle of the pole edges of the pyramid was 109°. The angle of the prism faces was 90°.

Habit dodecahedral, the combination of the unit pyramid (with angles almost that of the isometric dodecahedron) and the diametral prism giving, when in equilibrium, crystals that look almost exactly like the isometric form (text figure 137). The development is usually somewhat greater in the direction of the vertical axis, and hence the habit is rather tetragonal in many crystals. But looking down what would be the trigonal axis, if it were isometric, along the normal to (201), the trigonal appearance of the crystal is very pronounced. In fact, the crystals could be taken for isometric, were it not for the polarization character.

Color deep blood-red, pleochroism weak; the shade of red is somewhat paler when the light is vibrating parallel to $\varepsilon = \dot{c}$, which is the axis of greater elasticity. Extinction is straight on all side views, normal to the $\dot{c}$ axis; and looking along $\dot{c}$ the crystal shows only single refraction; it does not polarize. The refractive index of the extraordinary ray $\varepsilon$ is less than that of the ordinary, $\varepsilon < \omega$, and hence the optical character is *negative.*







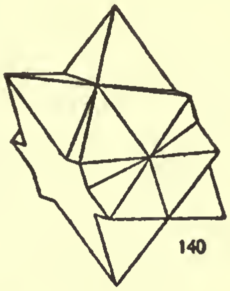


FIG. 137. *Dicotyles labiatus* Oxyhemoglobin. FIGS. 138, 139, 140. *Dicotyles tajacu* Oxyhemoglobin.

### COLLARED PECCARY, *Dicotyles tajacu.* Plate 32.

The specimen was received from the Philadelphia Zoölogical Gardens. The blood was quite putrid. It was laked with ether and centrifugalized, and the slides prepared in the usual manner. Crystallization proceeded rapidly, after the slides were covered, and they soon became filled with small octahedra, apparently of the tetragonal system. They did not keep well, and inside of 24 hours many of them were broken down. They did not seem to be dissolved, but rather to be decomposed. They appeared to be soft, as is the case with the first-formed crystals from the blood of the common swine. The spectroscope showed that these crystals were oxyhemoglobin.

*Oxyhemoglobin of Dicotyles tajacu.*

Tetragonal: Axial ratio $a : \dot{c} = 1 : 1.303$.

Forms observed: Unit pyramid (111), diametral prism (100), base (001).

Angles of the pole edges of the pyramid = 73°; $111 \wedge \bar{1}\bar{1}1 = 55°$, measured over the pole. The crystals were so small that the faces (100) and (001) could not be measured. Edges of the pyramid, in the horizontal plane, make an angle of 90° with each other.

Habit octahedral or pyramidal, usually the unit pyramid alone, very symmetrically developed (text figure 138); but some larger crystals show the base and some show the diametral prism (text figure 139); what appears to be twinning was observed in these crystals, the twinning plane a pyramid face and the twin an interpenetrant one analogous to the spinel twin (text figure 140).

Pleochroism is practically nothing, and the double refraction in aspects normal to the tetragonal axis is weak; looking along the axis, on the square sections, the crystals show no double refraction.

### PIG, DOMESTICATED VARIETY OF *Sus scrofa.* Plates 32 and 33.

Fresh blood, defibrinated by beating, was centrifugalized and the corpuscles alone used for the preparations. The corpuscles, from which the supernatant serum had been drawn off with a pipette, were etherlaked and again centrifugalized; then the clear liquid was saturated with oxalate, and the preparations made in the usual manner. The blood begins to crystallize soon after putting the covers on the slides, and the crystals continue to improve for about 2 hours, after which they begin to dissolve in the serum, becoming porous and losing their form. This breaking down of the crystals takes place first in thin preparations and is very likely due to an increase of pressure on the liquid of the preparation owing to the hardening of the balsam seal of the slide. In the thicker slides, with a thick layer of serum, the crystals do not show the same tendency to dissolve as they do in the thinner preparations. Inside of 24 hours the crystals of reduced hemoglobin begin to make their appearance, along with isolated crystals of the oxyhemoglobin, which latter are rather better formed than those observed within a couple of hours of first making the preparation.

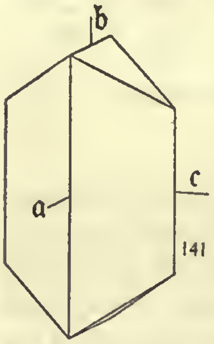


FIG. 141. Pig Oxyhemoglobin.

### *Oxyhemoglobin of Domesticated Variety of Sus scrofa.*

Orthorhombic: $a : b : \acute{c} = 0.6248 : 1 : 0.6008$.

Forms observed: Unit prism (110), unit brachydome $(0\bar{1}1)$.

Angles: $110 \wedge 1\bar{1}0 = 64°$ (normals); $011 \wedge 0\bar{1}1 = 62°$ (normals).

Habit short prismatic, nearly equidimensional or somewhat elongated vertically (text figure 141); sometimes distorted, by elongation parallel to two parallel dome faces along an axis lying in the macropinacoid, and then having a monoclinic aspect. Twinning was not observed, and the crystals usually occurred singly.

Pleochroism is hardly noticeable when looking along $\mathfrak{a}$, but in the plane of $\mathfrak{a}$ and $\mathfrak{c}$ or $\mathfrak{a}$ and $\mathfrak{b}$ it is readily observed; $\mathfrak{a}$ is very pale yellowish-red; $\mathfrak{b}$ is deep scarlet-red; $\mathfrak{c}$ is about same color. Extinction is straight or symmetrical in all aspects, but looking along $\mathfrak{a}$ the double refraction is very weak. The axial plane is the basal pinacoid and the orientation of the elasticity axes is $\mathfrak{a} = a$, $\mathfrak{b} = \acute{c}$, $\mathfrak{c} = b$; absorption $\mathfrak{c} > \mathfrak{b} > \mathfrak{a}$. From the double refraction and the pleochroism, as well as from the absorption, which show $\mathfrak{c}$ and $\mathfrak{b}$ to be nearly equal, it would seem that the acute bisectrix $Bx_a = \mathfrak{a}$, and the optical character is *negative.*

### *Reduced Hemoglobin of Sus scrofa, Domesticated Variety.*

In the slides after standing for 24 hours there always developed numerous long prismatic crystals of reduced hemoglobin, which appear at first around the margin of the cover, and later throughout the body of the slides. They show straight extinction on most aspects, but have a decidedly monoclinic habit, being terminated obliquely in many cases. Some appeared to have square ends; others, a single plane like a basal pinacoid, but oblique. They appear to be monoclinic. They grow in tufts and in sheaf-like aggregates, sometimes even in feathery groups. They appear to twin on a dome or pyramid, and also on the prism. The terminal plane is usually very imperfect, due to a fibrous character which the crystals show, the ends of the fibers making a rough plane. Smaller crystals and short stout prisms show a very monoclinic aspect.

## MUIS DEER OR CHEVROTAIN, *Tragulus meminna*. Plate 34.

The specimen was obtained from the *post mortem* of an animal that died in the Philadelphia Zoölogical Gardens. The blood was oxalated, ether-laked, and centrifugalized; the slides were prepared in the usual manner. Crystals formed readily and did not show a tendency to dissolve on bringing them into a warm room. They were oxyhemoglobin. Later, the same slides developed crystals of reduced hemoglobin, along with those of the oxyhemoglobin; these latter being relatively enormous. Both kinds of crystals were very sharp and well defined.

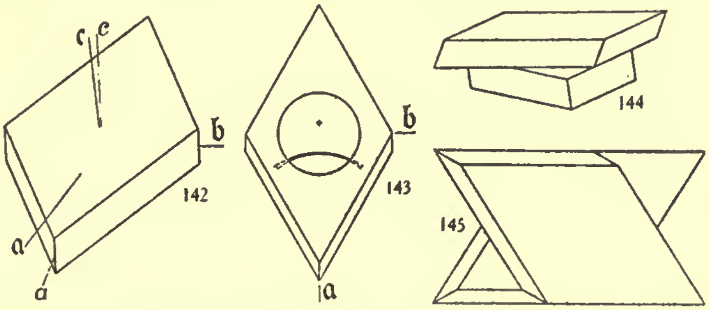
### *Oxyhemoglobin of Tragulus meminna.*

Monoclinic: Axial ratio $a : b : \acute{c} = 1.804 : 1 : \acute{c}$; $\beta = 63°$.

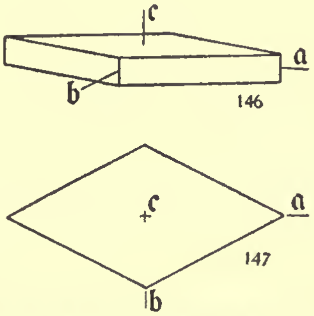
Forms observed: Unit prism (110), base (001).

Angles: Traces of prism on the base, edges $110\text{–}001 \wedge 1\bar{1}0\text{–}001 = 59°$; true angle $110 \wedge 1\bar{1}0 = 64° 50'$ (calculated); prism edge to base, edge $110\text{–}1\bar{1}0 \wedge 001 = 63°$ (normals) $= \beta$.

Habit of the single crystals tabular on the base (text figures 142, 143), the plate bounded by the prism faces, generally symmetrical or nearly so; but most of the crystals are twinned with the prism-base edge (110–001) as twin edge and a normal to this edge in the plane of the base as the twin axis (text figures 144 and 145). In these twins the composition face is the base and along one of the prism-base edges, where they unite, there is a reentrant angle, while on the opposite edge there is an ordinary dihedral angle. In these twins (horse-type), which are common in all hemoglobins with angles that approximate 60°, the compound crystal in this species is usually elongated along the common edge, and the two crystals overlap each other at the ends of this elongated crystal forming reentrant angles in the outlines of these ends. The twinning is frequently repeated in polysynthetic order; and it is often complicated by parallel growth in one or more of the members of the twin. It does not appear to tend to produce hexagonal forms by twinning on more than one pair of the prism-base edges, however, as is commonly the case in this kind of twinning. Twinning on the base as twin plane is also found apparently, but it is rare. This twinning seems to tend to make the angle of the plate nearer 60°. In some cases the opposite prism-base edges do not appear to be parallel, due perhaps to a vicinal prism face in one member of the twin; this non-parallelism would tend to average the angles to near 60°.



FIGS. 142, 143, 144, 145. *Tragulus meminna* Oxyhemoglobin.



FIGS. 146, 147. *Tragulus meminna* Reduced Hemoglobin.

Pleochroism is strong; $\mathfrak{a}$ is pale yellowish with a reddish tinge, $\mathfrak{b}$ is a blood-red and $\mathfrak{c}$ is still deeper red than $\mathfrak{b}$. Extinction seems to be symmetrical with the sides of the plate; in some cases it appeared a little oblique, but probably the plates were somewhat tilted. In twins with symmetrical extinction the angle was about 10° from the prism-base edge. Looking along the symmetry axis, it was about 15° from the trace of the base. The orientation of the elasticity axes is $\mathfrak{a} \wedge a = 15°$, in the obtuse angle, $\mathfrak{b} = b$, $\mathfrak{c} \wedge \acute{c} = 12°$ (about), in the obtuse angle. The axial plane lies in the plane of symmetry; the acute bisectrix of the optic axes is $\mathfrak{c}$, hence the optical character is *positive*. Looking at the crystal normal to the base, in convergent light, the biaxial figure is seen, with one brush constantly in the field, the other passing out on rotating the crystal. The axial angle is hence large.

The oxyhemoglobin crystals changed to metoxyhemoglobin by paramorphism, without apparent change of form, and they retained their optical orientation and all optical characters, except the pleochroism, which no longer showed any blood-reds, but dull brownish-reds. Otherwise they resembled the normal oxyhemoglobin. This change took place after the reduced-hemoglobin crystals began to appear.

### *Reduced Hemoglobin of Tragulus meminna.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.5205 : 1 : \acute{c}$.

Forms observed: Unit prism (110), base (001).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 55°$ (normals); base to prism $(001) \wedge 110 = 90°$.

Habit tabular on the base, the combination of the prism and base making the rhombic plate (text figures 146 and 147). The orientation of the axes is changed from that in the oxyhemoglobin, the symmetry axis of the monoclinic crystal becoming the macro-axis of the reduced hemoglobin crystal, so that to compare them with the oxyhemoglobin crystals, the position of these axes must be reversed. Making such reversal the axial ratio of the hemoglobin would be $a : b = 1.9209 : 1$ as against 1.804 : 1 in the oxyhemoglobin; but this difference would be still greater if the true cross-section of the oxyhemoglobin prism were taken. The difference is not only seen in the prism angles, however; the reduced hemoglobin crystals do not show the twinning so characteristic of the oxyhemoglobin.

Pleochroism is very strong; $\mathfrak{a}$ is colorless to pale pinkish; $\mathfrak{b}$ is deep rose-pink; $\mathfrak{c}$ is deep ruby-red. The extinction is symmetrical on the base and straight on edge views. Looking along the $b$ axis, in convergent light, the biaxial interference figure is seen; the brushes are rather widely separated. The orientation of the elasticity axes is $\mathfrak{a} = b$, $\mathfrak{b} = a$, $\mathfrak{c} = \acute{c}$, analogous to the arrangement of the elasticity axes in the monoclinic oxyhemoglobin. The plane of the optic axes is the macropinacoid, the acute bisectrix $Bx_a = \mathfrak{a}$; the optical character is hence *negative.*

A comparison of the characters of the monoclinic oxyhemoglobin and the orthorhombic reduced hemoglobin will show their differences at a glance. Such a comparison is given in table 40.

TABLE 40.—*Crystallographic characters of oxyhemoglobin and reduced hemoglobin of Muis deer.*

| Substance crystal system, and twinning habit. | True prism angle $110 \wedge 110$. | Axial ratio on plane normal to prism edge. | Optical character. |
|---|---|---|---|
| Oxyhemoglobin, monoclinic, normally twinned | 64° 50′ | $b : a = 0.6350 : 1, \beta = 63°$ | Positive, $Bx_a = \mathfrak{c}$ |
| Reduced hemoglobin, orthorhombic, not normally twinned | 55 | $a : b = 0.5205 : 1, \beta = 90°$ | Negative, $Bx_a = \mathfrak{a}$ |

## ELK OR WAPITI, *Cervus canadensis.* Plates 34 and 35.

Two specimens were examined, one probably from the Philadelphia Zoölogical Gardens and the other from the National Zoölogical Park at Washington. The first specimen was putrid; the last was in better condition. Both gave crystals of oxyhemoglobin. The putrid blood was prepared without the use of ether, which is likely to produce precipitation in such blood; it was simply repeatedly frozen and thawed, until the corpuscles were broken down, and then centrifugalized. The crystals formed readily after the slides were covered, and were large enough to photograph inside of a few hours.

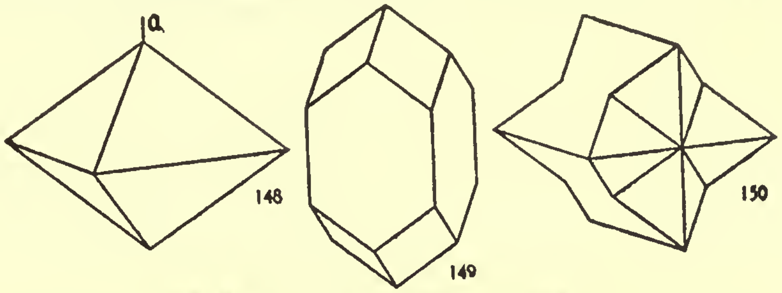
### *Oxyhemoglobin of Cervus canadensis.*

Tetragonal: Axial ratio $a : \acute{c} = 1 : 0.7133$.

Forms observed: Unit pyramid (111), diametral prism (100).

Angles: $110 \wedge 1\bar{1}0 = 90°$ from outlines of the pyramid in plane; $101 \wedge \bar{1}01 = 109°$ (or 71° normals) from outlines of pyramid in elevation; the other elevation looking along the diagonal axis gave about 56° (normals) (55° 15′ by calculation about). This angle as ordinarily presented appears to be somewhat higher, up to 60° or more.

Habit bipyramidal (text figure 148); also combinations of the diametral prism with the unit pyramid (text figure 149); these latter occur more rarely. The crystals were usually single and isolated; but in some cases formed irregular linear aggregates, straight or curved, some being evidently parallel growths. Twinning on the unit pyramid, as the plane of twinning, was observed, but was not common; the twins are penetration twins, but otherwise resemble the spinel twins somewhat (text figure 150).



Figs. 148, 149, 150. *Cervus canadensis* Oxyhemoglobin.

The color was oxyhemoglobin red and the pleochroism is not very marked; absorption for $\omega$ greater than for $\epsilon$. The double refraction is not very strong, showing better in the prismatic crystals; extinction is parallel to the vertical axis $\dot{c}$, which is the axis of greater elasticity. On the square sections, looking along the polar axis, the crystals are singly refracting and do not polarize; and in convergent light they show a uniaxial figure in the shape of a dusky cross. The optical character is *negative,* $\omega > \epsilon$.

### Red-backed Deer (Probably the Red Brocket, *Cariacus rufus*). Plate 35.

The specimen of blood was received from the Philadelphia Zoölogical Gardens, and was beginning to putrefy. It was treated by oxalating and freezing, then laked with ether and centrifugalized. The slides were prepared in the usual manner and kept at a temperature near the freezing-point. It crystallized readily, the crystals that were the first to form being long lath-shaped rods; these were followed by large rectangular plates, very thin, and evidently the same as the rods, but of a tabular habit. When the slides were brought into a warm room the plates were rapidly dissolved; the rods showed more resistance to solution, but it was found necessary to examine the slides in the cold, and all photographs were taken at a temperature near the freezing-point. The crystals gave the spectrum of reduced hemoglobin.

#### *Reduced Hemoglobin of Cariacus rufus.*

Monoclinic: Axial ratio could not be determined, as only pinacoids were seen. Angle $\beta$ appears to be about 90°.

Forms observed: Base (001), clinopinacoid (010), orthopinacoid (100).

Angles: Clinopinacoid to orthopinacoid, the outline of the plates, $010 \wedge 100 = 90^\circ$; base to orthopinacoid, angle $\beta$, about 90°, perhaps exactly 90°. The third angle was not observed, but must be 90° in this system.

Habit lath-shaped crystals elongated on the clino-axis, and flattened on the base; these, by elongation on the ortho-axis also, became rectangular plates, which are frequently as long as the lath-shaped rods and perhaps 30 times as wide. The plates appear to be produced by the piling up of narrow plates all in parallel position and this produces striation on most of these plates parallel to the $a$ axis. The rods grow in tufts, radiating or brush-like, generally united with each other on the base, or in the zone of (001)–(100). The composite plates are produced in the same way, by uniting on the base. No definite twinning was observed, but possibly the piled-up plates may be polysynthetic twins.

Pleochroism was strong, as is common in reduced hemoglobin; $\mathfrak{a}$ pale pink, nearly colorless; $\mathfrak{b}$ purplish, deeper than $\mathfrak{a}$; $\mathfrak{c}$ deep purplish-red. Absorption was in the order $\mathfrak{c} > \mathfrak{b} > \mathfrak{a}$. Extinction on the base was straight; on edge views the extinction angle was about 30° with the length of the rod-like section. The plane of the optic axes is the plane of symmetry; the orientation of the elasticity axes is $\mathfrak{a} \wedge a = 30°$, $\mathfrak{b} = b$, $\mathfrak{c} \wedge \dot{c} = 30°$. On the base, in convergent polarized light, a single brush of a biaxial interference figure is seen, the optic axis emerging at a small angle with $\mathfrak{c}$ or the normal to the plate; the acute bisectrix is hence evidently $\mathfrak{c}$, and the optical character is *positive*.

### VENEZUELA DEER, *Mazama americana savannarum* (?). Plate 36.

The specimen of blood was received from the National Zoölogical Park at Washington during the summer and was kept frozen until examined. The quantity of blood was small, and it was quite thick and putrid, and full of extraneous matter. This latter was centrifugalized off as far as possible, but the specimen was not thoroughly cleansed, owing to an accident to the centrifugal machine. The slides were prepared in the usual manner, and crystals formed readily in the cold. They were not dissolved at a temperature of 10° C. A spectroscopic examination of the plasma showed the presence of oxyhemoglobin, but only crystals of reduced hemoglobin were obtained, they being determined as such by the microspectroscope.

#### *Reduced Hemoglobin of Mazama americana savannarum.*

Monoclinic: Axial ratio not determinable as the pinacoids only are developed. The angle $\beta$ seems to be 90°.

Forms observed: Base (001), clinopinacoid (010), orthopinacoid (100).

Angles: Clinopinacoid to orthopinacoid, the outline of the plates $010 \wedge 100 = 90°$; base to orthopinacoid $001 \wedge 100 = 90° = \beta$. The third angle could not be obtained, but is necessarily 90°.

Habit broad or narrow lath-shaped, flattened on the base and elongated parallel to the clino-axis (text figure 151); the lath-shaped crystals by development along the symmetry axis $b$ become broad plates. When the plate-like habit is assumed, the tabular crystals are seen to be composite, by parallel growth and uniting on the base, producing strong striation parallel to the clino-axis. The crystals grow in tufts, radiating from a center, and the majority of the crystals are broad lath-shaped or tabular, with the length of the plate 2 to 3 times the width. On edge view they do not show the usual tendency to radiate in a brush-like manner to any very marked degree.

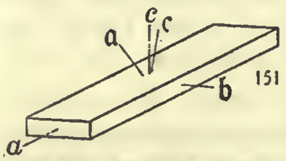


FIG. 151. *Mazama americana savannarum* Reduced Hemoglobin.

The color is reduced hemoglobin purple; the pleochroism, as usual in hemoglobin, is very strong; $\mathfrak{a}$ is pale rose-pink; $\mathfrak{b}$ is strong rose-pink; $\mathfrak{c}$ is deep rose-red. On the flat the extinction is straight, parallel to the edges of the plate or lath-shaped crystals; on edge it is oblique, about 30° measured from the length of the rod. The plane of the optic axes is the plane of symmetry; and the orientation of the elasticity axis is $\mathfrak{a} \wedge a = 30°$, the extinction angle; $\mathfrak{b} = b$; $\mathfrak{c} \wedge \dot{c} = 30°$. Traces of the interference figure were seen on the flat view, on (001), but it was not definitely observed, except as to the position of the plane of the optic axes. Either the angle between the axes is large or the axis of greatest elasticity is the acute bisectrix.

### FALLOW DEER, *Cervus dama*. Plate 36.

The specimen of blood was received from the New York Zoölogical Gardens, having been collected in a tube containing oxalate. It was clotted and somewhat putrid. The clots were ground in sand with ether and the mixture centrifugalized, and from the clear solution the slides were prepared in the usual manner. Crystallization began in the protein ring shortly after covering the slides, but soon after they formed these crystals began to dissolve; due, no doubt, to the establishment of equilibrium between the concentrated solution near the protein ring and the less concentrated solution throughout the slide. The crystals subsequently formed mainly around the edge of the cover, owing to concentration of the solution in that region, due to evaporation through the balsam seal. Crystals of oxyhemoglobin, in the form of bipyramids, and of hemoglobin, in the form of broad lath-shaped crystals, were developed side by side; but the reduced hemoglobin crystals began to appear later than those of oxyhemoglobin. Both kinds of crystals were tested by the spectroscope. The crystals formed practically at room temperature, although the slides were kept at a temperature below 10°.

### *Oxyhemoglobin of Cervus dama.*

Tetragonal: Axial ratio $a : \acute{c} = 1 : 1.200$.

Forms observed: Unit pyramid (111), traces of base (001).

Angles: $111 \wedge \bar{1}\bar{1}1 = 61°$, measured over the pole; the angle of the pole edges, measured in the same way, was edge $111\text{–}1\bar{1}1 \wedge 1\bar{1}1\text{–}\bar{1}\bar{1}1 = 79°$ (calculated 79° 36′); profile of pyramid looking along $\acute{c}$ gave 90° between the edges.

Habit pyramidal (text figure 152), the unit pyramid in very perfect development or with some faces larger, due to lying on the slide; a few crystals seemed to show the base (001). The crystals are very small, but well formed. In some cases they appeared like skeleton crystals, due to some tendency to parallel growth apparently; some formed interpenetrating groups resembling twins; in one or two cases these appeared to be twins on the pole edge as twin axis. From the optical anomalies noted in some crystals, they may be some form of a mimetic twin of the orthorhombic system, with the groups producing tetragonal symmetry. The skeleton-like crystals mentioned might be such interpenetrant orthorhombic twins.

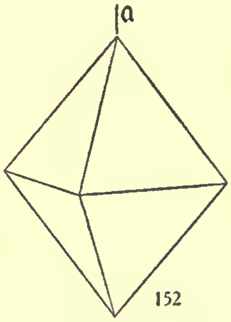


FIG. 152. *Cervus dama* Oxyhemoglobin.

Pleochroism was not very strong, but the axis of greater elasticity was the axis of less color. On the basal aspect, looking along $\acute{c}$, the crystals normally show single refraction, but the crystals presenting this aspect were too small to show an interference figure. A few of them showed double refraction, not very strong, but extinguishing along the two equal diagonals of the square section. This optical anomaly may indicate a twinned orthorhombic structure. On the side views normal to $\acute{c}$, the extinction is parallel to the vertical axis $\acute{c}$ in all aspects. The double refraction is fairly strong, the vertical axis $\acute{c} = \varepsilon$ being the axis of greater elasticity. Hence $\omega > \varepsilon$ and the optical character is *negative.*

### *Reduced Hemoglobin of Cervus dama.*

Probably orthorhombic, perhaps monoclinic; the crystals were very imperfect. They showed sometimes a roughly four-sided cross-section, with an angle of perhaps 85°, but most of them seemed to be rather lath-shaped. They were generally not terminated; a few showed square-cut ends, but the majority were merely shred-like masses, with more or less straight sides and splintery looking ends. They were also in spherulitic masses and are probably parallel or radiating groups of smaller prisms; the parallel groups forming the prism-like shreddy crystals and the radiating groups the spherulites and tufts of crystals. The parallel masses show straight extinction, the spherulites extinguish parallel to the fibers and show the usual extinction cross of spherulitic masses of crystals in polarized light. The straight groups of crystals show the length of the prisms to be the direction of greater elasticity and the direction normal to this to be the direction of less elasticity. Pleochroism is strong, parallel to the length of the fibers; $\mathfrak{a}$ is colorless, normal to the length, $\mathfrak{b}$ and $\mathfrak{c}$ are purple. In the spherulitic masses the pleochroism shows strongly, dividing the spherulite into sectors, the opposite sectors being of the same color; $\mathfrak{a}$ colorless, $\mathfrak{c}$ and $\mathfrak{b}$ purple, as in the prisms.

### MUNTJAK, *Cervulus muntjak*. Plates 36 and 37.

The specimen of blood was received from the New York Zoölogical Gardens and was in a somewhat putrid condition. The blood, containing oxalate, was laked with ether and centrifugalized and from the clear solution slides were prepared in the usual manner. The blood crystallized slowly and the crystallization was therefore carried on at temperatures near 0° C. The first crystals to form were oxyhemoglobin, short prisms with very oblique terminations; later, crystals of reduced hemoglobin formed in the shape of long square-ended rods or lath-shaped crystals, and in curving arborescent forms. The crystals of the oxyhemoglobin withstood changes of temperature fairly well, but the crystals of reduced hemoglobin were rapidly dissolved when brought into the warm room.

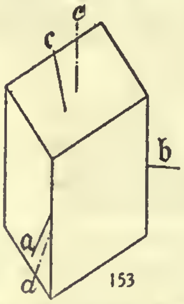


FIG. 153. *Cervulus muntjak* Oxyhemoglobin.

#### *Oxyhemoglobin of Cervulus muntjak.*

Monoclinic: Axial ratio $a : b : \dot{c} = 1.303 : 1 : \dot{c}$; $\beta = 52°$.

Forms observed: Unit prism (110), base (001).

Angles: Traces of the prism angle on the base, or plane angle of the basal section, edges 110–001 $\wedge$ 1$\bar{1}$0–001 = 75° (105° normals), base to prism edge = $\beta$ = 52°, actual prism angle 110 $\wedge$ 1$\bar{1}$0 = 91° 30′ (calculated 91° 45′).

Habit short prismatic, the unit prism terminated by the oblique basal pinacoid; sometimes in equilibrium (text figure 153), and then looking rather like a rhombohedron. The crystals grow isolated or crowded together in great numbers, and sometimes appear to twin on a pyramid of the unit series or perhaps on a hemiorthodome.

Pleochroism is rather marked; $\mathfrak{a}$ nearly colorless, somewhat yellowish; $\mathfrak{b}$ strong red, $\mathfrak{c}$ deep red. On all sections in the zone of (001)–(100) the extinction is symmetrical; looking along the symmetry axis $b$ the extinction angle is 30° with the prism edge. The plane of the optic axes is the plane of symmetry, and the orientation of the elasticity axes is as follows: $\mathfrak{a} \wedge a = 8°$, in the obtuse angle; $\mathfrak{b} = b$; $\mathfrak{c} \wedge \dot{c} = 30°$, in the obtuse angle. In convergent light, looking nearly along $\mathfrak{c}$, the biaxial interference figure was seen with the brushes rather widely separated. The optical character is hence *positive*, as the acute bisectrix is $\mathfrak{c}$.

#### *Reduced Hemoglobin of Cervulus muntjak.*

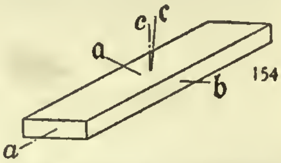


FIG. 154. *Cervulus muntjak* Reduced Hemoglobin.

Monoclinic: Axial ratio can not be determined, as only the pinacoids are developed. The angle $\beta$ seems to be 90°.

Forms observed; Base (001), orthopinacoid (100), clinopinacoid (010).

Angles of orthopinacoids and clinopinacoids, 90°; base to orthopinacoid about 90°.

Habit lath-shaped, flattened on (001) and elongated along $a$ (text figure 154), also hair-like, in feathery and arborescent groups and tufts; when broad lath-shaped, the crystals compound on the base. The crystals melt or dissolve very rapidly on being brought into a warm room. They are very much larger than the oxyhemoglobin crystals.

Pleochroism is rather marked; $\mathfrak{a}$ very pale bluish-lilac; $\mathfrak{b}$ purplish, pale but stronger than $\mathfrak{a}$; $\mathfrak{c}$ deep reddish-purple. Extinction is straight on the flat and about 18° with the length on edge. The axial plane lies in the plane of symmetry and the orientation of the elasticity axes is $\mathfrak{a} \wedge a = 18°$, $\mathfrak{b} = b$, $\mathfrak{c} \wedge \acute{c} = 18°$. The interference figure was not observed, but from the character of the pleochroism it may be judged that the crystals are optically *positive.*

### INDIAN ANTELOPE, *Antilope cervicapra.* Plate 37.

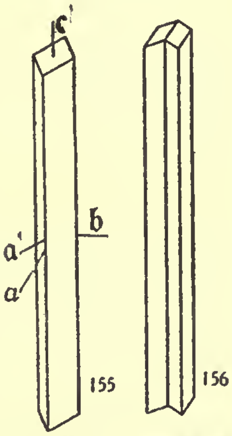
The specimen was received from the Philadelphia Zoölogical Gardens. The blood was partly in clots and somewhat putrid. It was ground with sand and ether, and centrifugalized. After it had cleared, some oxalate was added and the solution again centrifugalized. From the clear solution, the slide preparations were made in the usual manner. Crystals formed readily in the dried protein ring and along the edge of the cover. The photographs were made on the following day. Examination with the microspectroscope showed that the crystals were oxyhemoglobin. They appeared quite insoluble, showing no tendency to dissolve on bringing the slides into a warm room.

#### *Oxyhemoglobin of Antilope cervicapra.*

Monoclinic: Axial ratio $a : b : \acute{c} = 1.887 : 1 : \acute{c}$; $\beta = 71° 45'$.

Forms observed: Unit prism (110), base (001).

Angles: Prism angle on cross-section of prism $110 \wedge 1\bar{1}0 = 58° 20'$; angle of prism traces on the base, edge $110\text{–}001 \wedge 1\bar{1}0\text{–}001 = 56°$ (about); angle of prism edge to base $110\text{–}110 \wedge 001 = 71° 45'$ (normals) $= \beta$.



FIGS. 155, 156. *Antilope cervicapra* Oxyhemoglobin.

Habit prismatic, elongated on the vertical axis, the acute prism obliquely terminated by the base (text figure 155). In many crystals the prism appears to be flattened on two of the opposite faces, becoming lath-shaped. The faces of the prism are vertically striated, and there is an appearance of cleavage, parallel to the prism faces. The crystals are large, and the length is very great in proportion to the width. When seen in the aspect looking along $a$, the termination of the prism frequently appears to be square. The crystals grow in groups and slightly divergent tufts. Twins on the prism face as the twin plane were noted; they were rare (text figure 156). They seemed to be usually juxtaposed twins, but a few interpenetrant twins were seen.

The color is oxyhemoglobin red, but the pleochroism is strong and the crystals look light or dark according as the aspect presented is normal to $\mathfrak{a}$ or parallel to $\mathfrak{a}$. The colors are: $\mathfrak{a}$ pale pinkish, $\mathfrak{b}$ red, $\mathfrak{c}$ deep red. Extinction seems to be parallel to the prism edges in all positions of the prism normal to the $\acute{c}$ axis, and symmetrical on the cross-sections. The orientation of the elasticity axes is $\mathfrak{a} \wedge a = 18° 15'$, in the obtuse angle; $\mathfrak{b} = b$; $\mathfrak{c} = \acute{c}$; the plane of the optic axes is hence the plane of symmetry and the angle between $\mathfrak{c}$ and $\acute{c}$ is 0°. Looking along $\mathfrak{a}$, traces of the interference brushes are seen, but pass far out of the field; this would seem to indicate that the axis of least elasticity is the acute bisectrix, $Bx_a = \mathfrak{c}$, and the optical character is *positive.*

From the straight extinction, it looks as though this might be an orthorhombic crystal with one pair of macrodome faces developed; but such symmetry as the crystals show is plainly monoclinic.

### REDUNCA ANTELOPE, NAGOR, *Cervicapra redunca.* Plate 38.

The blood was from a specimen of the nagor that died in the New York Zoölogical Gardens, and was received from New York in good condition. It was oxalated, laked with ether, and centrifugalized; the slide preparations were made in the usual manner. The crystals formed readily, rather faster than in horse blood for example, and the first crystals to form were rather long rods. These formed at a temperature of about 24° C.; on placing the slides in the cold, a second crop of larger and stouter crystals formed, which appear to be more soluble than those of the first crop, dissolving readily when brought into a temperature much above 12° C. When placed in the cold, the corroded edges and angles that were produced by the increase of temperature are soon repaired. The crystals of the first crop do not seem to be affected by such temperature change as would cause solution of the second crop. Examination with the spectroscope failed to show any difference in the spectra of these two types of crystals; and they have identical axial ratios, although the habit is quite different. After some days the terminations of the crystals of the first crop became imperfect or even dissolved; but this occurred while they were kept in the cold. The crystals of the second crop seemed to keep very well, so long as the temperature was kept below 10° C.; at a few degrees above this temperature they began to dissolve. About two weeks after the slides were prepared they showed mainly very large crystals of prismatic habit, somewhat similar to the first crop, but with the ends corroded. From cross-sections these were evidently unit prisms, of the character of those formed in the first crop of crystals.

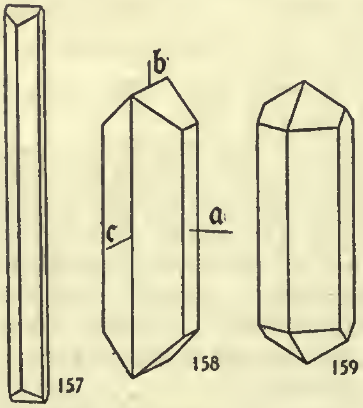
### *Oxyhemoglobin of Cervicapra redunca.*

Orthorhombic: Axial ratio $a : b : \dot{c} = 0.839 : 1 : 0.5877$.

Forms observed: Unit prism (110), brachypinacoid (010), macropinacoid (100) (?), macrodome (101), brachydome (032), unit pyramid (111).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 80°$ (normals); macrodome $101 \wedge \bar{1}01 = 69°$ (normals); brachydome $032 \wedge 0\bar{3}2 = 43°$ (normals); unit pyramid edges over the pole or unit brachydome $011 \wedge 0\bar{1}1 = 54°$. The value for $\dot{c}$, calculated from the macrodome (first crop), was 0.5870 and from the pole edge angle of the unit pyramid (second crop) was 0.5877, which are substantially identical.

Habit of the first crop crystals (text figure 157) is long prismatic, consisting of the unit prism (110) in equilibrium with the brachypinacoid (010), and terminated by the unit macrodome (101); the second crop crystals are short prismatic, becoming tabular by development of the brachypinacoid as they become larger, and consisting of the unit prism (110) and the brachypinacoid (010) in the prismatic zone, terminated by the brachydome (032) (text figure 158); or in some cases by the unit pyramid (111) (text figure 159). In some crystals the macropinacoid seemed to be developed, probably by pressure of the cover. The prismatic crystals, found in the slides that had been standing in the cold for two weeks, were of the first type; but the brachypinacoid was much reduced in size and the terminations were wanting. Small crystals developed a few days after the slides were prepared, which showed the same habit, and, even with the brachypinacoid entirely absent, these were terminated by the unit macrodome. The first crop of crystals grew from the edges of the cover and from the protein ring in irregular tufts, also in radiating stellate groups through the body of the slide; and the mass of the crystals into which the protein ring was converted were of this type. The second-crop crystals appeared near the edge of the cover, singly or in roughly



FIGS. 157, 158, 159. *Cervicapra redunca* Oxyhemoglobin.

parallel grouping with the vertical axis nearly normal to the cover edge. The relation of the first and second crops seems to be, that from the first supersaturated solution the first crop crystals developed, until the solution was in equilibrium for that temperature; and when the slides were placed in the cold a second crop developed gradually, probably under increased pressure as the balsam seal of the slides hardened, until equilibrium had been reached in the saturation of the solution for the lower temperature of about 10° or less. Of course, on bringing the slides into the warmth of a heated room, this equilibrium would be disturbed and resolution of the last-formed crystals would take place. As will be seen from reference to the data in regard to the position of the elasticity axes, the relative shortening of the crystal axes in the second-crop crystals is in the inverse order of their elasticities; $\mathfrak{a}$, the axis of greatest elasticity, shortening more than $\mathfrak{b}$, the axis of mean elasticity; while $\mathfrak{c}$, the axis of least elasticity, lengthens with respect to the other two.

The color of the crystals was the usual oxyhemoglobin red. Pleochroism is rather strong; $\mathfrak{a}$ is pale yellowish-red; $\mathfrak{b}$ is a pale red, somewhat rose-pink; $\mathfrak{c}$ is deep blood-red. Absorption is in order, $\mathfrak{c} > \mathfrak{b} > \mathfrak{a}$. Extinction is straight or symmetrical in all aspects. The plane of the optic axes is the basal pinacoid and the orientation of the elasticity axes is $\mathfrak{a} = b$, $\mathfrak{b} = \acute{c}$, $\mathfrak{c} = a$. On side views of the prism or on the vertical pinacoids traces of an interference figure are seen in convergent light; on (010) the two brushes of the biaxial figure are seen in the field, but they are widely separated. On (100) two brushes show also, but pass out of the field in the diagonal position. The acute bisectrix is hence evidently $\mathfrak{a}$, and the crystals are optically *negative.* This is indicated also by the pleochroism, for $\mathfrak{b}$ and $\mathfrak{c}$ are much nearer together than $\mathfrak{b}$ and $\mathfrak{a}$.

## DORCAS GAZELLE, *Gazella dorcas.* Plate 39.

This specimen was received from the Philadelphia Zoölogical Gardens. The blood was clotted, but in good condition. The clot was ground in sand with ether and the mixture centrifugalized; from the solution the slide preparations were made in the usual manner. Crystals formed readily in the dried protein ring, but were gradually dissolved as equilibrium was established in the solution after covering; and then they reformed along the cover edge, as they were dissolved from the protein ring, until the solution in the slides was homogeneous. When the condition of equilibrium was reached in the solution, the crystals showed no sign of being dissolved and were in good condition for days. The first crystals to form are small rectangular plates, but with them are long rods; both seem to be the same, however, and both are oxyhemoglobin, as determined by the spectroscope.

### *Oxyhemoglobin of Gazella dorcas.*

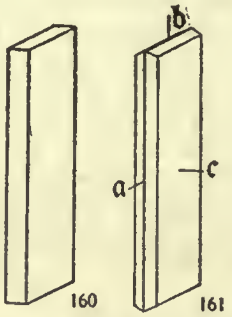
Orthorhombic: Axial ratio $a : b : \acute{c} = 0.3639 : 1 : 0.4452$.

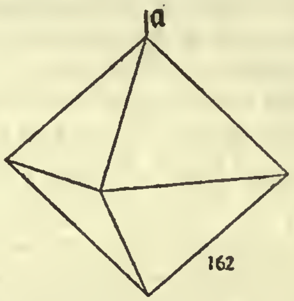
Forms observed: Brachypinacoid (010), base (001), unit prism (110), brachydome (011), and, without measurement of angles, the macrodome (101) and the macropinacoid (100).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 40°$ (normals); brachydome angle $011 \wedge 0\bar{1}1 = 48°$ (normals); outline of plates, $100 \wedge 001 = 90°$.

Habit tabular on (010), the plate bounded by the other two pinacoids (100) and (001) when the crystals begin to grow (text figure 160) or by the combination of the prism (110) and the brachydome (010) in the larger crystals (text figure 161). The macropinacoid disappears as the crystals increase in size, but the base sometimes appears to persist, although it is generally replaced by the brachydome. The crystals are usually elongated along the vertical axis, so that on the brachypinacoid aspect the length is about double the width; but some are much more elongated, and others are reduced to nearly square tabular plates. They frequently show parallel growth, uniting on the brachypinacoid or on the macropinacoid and also grow in irregular, somewhat radiating aggregates.

Pleochroism is rather strong; $\mathfrak{a}$ is pale yellowish-red; $\mathfrak{b}$ is deep blood-red, $\mathfrak{c}$ is deep red, somewhat deeper than $\mathfrak{b}$. Extinction is straight in all aspects. The orientation of the elasticity axes is $\mathfrak{a}=a$, $\mathfrak{b}=\dot{c}$, $\mathfrak{c}=b$. The plane of the optic axes is the base, and $\mathfrak{a}$ is the acute bisectrix, $Bx_a=\mathfrak{a}$. Looking along this axis of $\mathfrak{a}$, the interference figure is seen in convergent light, with the angle between the optic axes, $2E=40°$. The optical character is *negative*.





FIGS. 160, 161. *Gazella dorcas* Oxyhemoglobin. FIG. 162. *Cephalophus grimmi* α-Oxyhemoglobin.
FIG. 163. *Cephalophus grimmi* β-Oxyhemoglobin.

### DUICKERBOK, *Cephalophus grimmi*. Plate 40.

The specimen was received from the National Zoölogical Park at Washington. The blood was oxalated, laked with ether, and centrifugalized, and preparations were made in the usual manner. The slides were kept at a temperature of about 0° C., and soon were filled with the small pyramidal crystals, which showed a tendency to dissolve, and were hence examined and photographed at temperatures near the freezing-point. Later, the slides developed the second kind of crystals, the hexagonal plates. Both kinds of crystals were oxyhemoglobin.

### *a-Oxyhemoglobin of Cephalophus grimmi.*

Tetragonal: Axial ratio $a : \dot{c}=1 : 0.8687$.

Forms observed: Unit pyramid (111), also traces of (100).

Angles: Between the pyramid edges in the horizontal plane, normal to the axes $= 110 \wedge 1\bar{1}0=90°$; between the pyramid edges in the vertical axial plane (calculated) $= 101 \wedge \bar{1}01=98°$, observed angle of pyramid over the pole $=111 \wedge \bar{1}\bar{1}1=77°$.

Habit pyramidal (text figure 162), the crystals occurring singly or in irregular groups and in parallel growths. Twinning seems to occur on the pyramid as the plane of twinning.

The color is the normal oxyhemoglobin red; pleochroism is scarcely noticeable. Double refraction is weak, but the extinction is symmetrical on the aspects normal to the vertical axis. Looking along this axis, the crystals are singly refracting in parallel polarized light and do not polarize; but in convergent light they show a faint dusky uniaxial cross. By the quartz wedge it is seen that the vertical axis is the direction of greater elasticity, hence $\omega > \epsilon$ and the optical character is *negative*.

### *β-Oxyhemoglobin of Cephalòphus grimmi.*

These crystals develop after the pyramids of the *a*-oxyhemoglobin. They are in the form of very thin hexagonal plates (text figure 163), occurring both singly and also growing in groups, often with the orientation of parallel growth. They are evidently hexagonal, the angle of the plate being 120° (60° normals) and the sides square with the terminal plane. No axial ratio could be determined, as the combination of forms is simply unit prism $(10\bar{1}0)$ and base (0001). They are singly refracting when viewed on the base, but too thin to give an interference figure. On edge view they show very weak double refraction and extinguish parallel to the base. The optical character could not be determined owing to the very weak double refraction.

## SHEEP, *Ovis aries.* Plates 40–42.

The fresh blood was collected in oxalate from the abattoir, and centrifugalized to throw down the corpuscles. The plasma was drained away, the corpuscles were laked with ether, oxalate added almost to saturation, and the solution centrifugalized for 2 hours. From the clear liquid the slide preparations were made as usual. The preparation crystallized at room temperature, and the crystals showed no tendency to dissolve. Some crystals were obtained within 5 hours of making the preparations. The crystals at first formed were fine needles, but soon tabular crystals began to appear. Several other preparations were made from the same blood, and in all the crystals kept well. After about a week, crystals of reduced hemoglobin began to make their appearance, along with the crystals of oxyhemoglobin, which formed in the freshly prepared slides. These crystals of reduced hemoglobin, like the oxyhemoglobin, were not dissolved on slight increase of temperature. The slides were kept cool, at about 10° C., except when under examination. Both the oxyhemoglobin and the reduced hemoglobin were identified by the spectroscope.

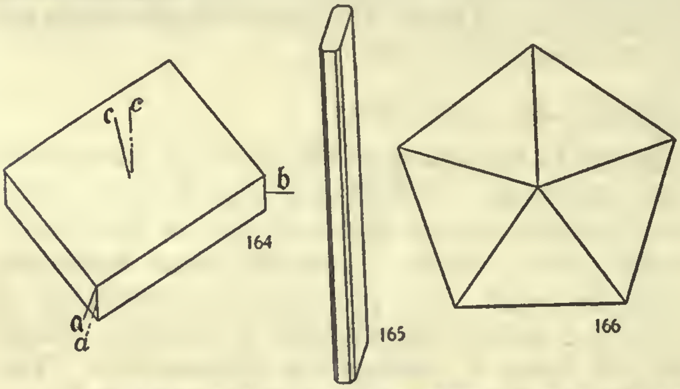
### *Oxyhemoglobin of Ovis aries.*

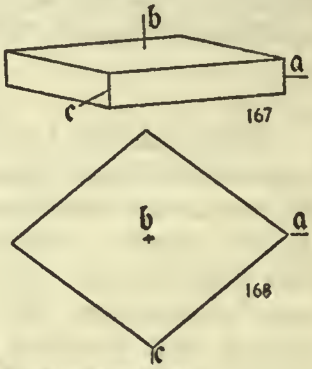
Monoclinic: Axial ratio $a : b : \dot{c} = 1.140 : 1 : 0.970$; $\beta = 54°$.

Forms observed: Unit prism (110), positive hemiorthodome $(\bar{1}01)$, base (001), clinopinacoid (010), orthopinacoid (100).

Angles: Prism angle $110 \wedge 1\bar{1}0$, traces on the base, or angle of edges $110\text{–}001 \wedge 1\bar{1}0\text{–}001 = 82° 30'$ (actual angle); orthodome to orthopinacoid $\bar{1}01 \wedge 100 = 72°$; orthopinacoid to base $100 \wedge 001 = 54° = \beta$ (normals).

Habit of the first crystals to form minute needles without definite outlines, tapering to a point at either end; with these soon appear tabular crystals consisting of the base with a very short prism, tabular on the base (text figure 164). After about a day, long prismatic crystals appear consisting of the three pinacoids, elongated parallel to the vertical axis and generally flattened on the orthopinacoid. These sometimes show the prism as a bevel on the edges (text figure 165), but more often are simply the three pinacoids. These crystals twin and form networks of rods, and frequently on the orthopinacoid faces twin growths develop, producing a cross-banded effect due to the strong pleochroism. Twins are hemitrope, on the orthopinacoid (100) and on the hemiorthodome $(10\bar{1})$, the two occurring together and making fivelings of exactly pentagonal shape (text figure 166). These little fivelings grow on the sides of the long crystals, or singly, scattered through the slides; and they grouped themselves along the crystals of oxalate that formed in some of the slides, strung like beads along the needles of the oxalate. In this occurrence they present edge views to the observer. When seen in side view they are generally more or less perfect pentagons, divided by the contact planes into five sectors meeting at a point in the center; sometimes, by irregularity of growth, 7 individuals are seen in the group. The following more detailed description will make the arrangement plain. Suppose a fiveling, the members taken in cyclic order around from 1 to 5. Nos. 1 and 2 twin on the orthopinacoid (100); 3 is twinned to 2 on the hemiorthodome (10$\bar{1}$) and to 4 on the orthopinacoid; 5 is twinned to 4 on the hemiorthodome which brings its orthopinacoid in parallel position with the hemiorthodome of 1, thus completing the cycle. In each member of the twin the base forms one of the sides of the pentagon.





FIGS. 164, 165, 166. *Ovis aries* Oxyhemoglobin. FIGS. 167, 168. *Ovis aries* Reduced Hemoglobin.

These twins are often seen terminating a long prismatic crystal and, as already noted, they sprout out of the side planes of the long crystals, producing the banded appearance seen in the photographs.

Pleochroism is very strong; $\mathfrak{a}$ nearly colorless, $\mathfrak{b}$ moderately strong red, $\mathfrak{c}$ very deep blood-red. In the plates, the extinction is symmetrical on the base, and straight on the aspect looking along $a$; on the (010) aspect, the extinction angle is 30° with the prism edge. The long prismatic crystals show the same extinction as the plates. The orientation of the elasticity axes is $\mathfrak{a} \wedge a = 6°$, $\mathfrak{b} = b$, $\mathfrak{c} \wedge \dot{c} = 30°$. The axial plane is the plane of symmetry, $\mathfrak{a}$ is probably the acute bisectrix, but the interference figure was not observed. The optical character is probably *negative.*

### *Reduced Hemoglobin of Ovis aries.*

Orthorhombic: Axial ratio $a : b : \dot{c} = 0.7813 : 1 : \dot{c}$.

Forms observed: Unit prism (110), base (001).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 76°$ (normals); prism to base $110 \wedge 001 = 99°$.

Habit tabular on the base, the crystals consisting of a very short prism and the basal pinacoid (text figures 167 and 168), the ratio of the length of the prism to the short diagonal of the base being about 1:5. The crystals occurred singly and did not appear to form twins. They appeared rather sparingly in the slides about a week after the preparations were made and seemed to show no tendency to dissolve on slight increase of temperature.

Pleochroism is strong; $\mathfrak{a}$ nearly colorless, $\mathfrak{b}$ deep red, $\mathfrak{c}$ deep purplish-red; the colors of $\mathfrak{b}$ and $\mathfrak{c}$ are nearly equal. Extinction is straight in all positions on edge and symmetrical on the base. The orientation of the elasticity axes is $\mathfrak{a} = b$, $\mathfrak{b} = \dot{c}$, $\mathfrak{c} = a$. The plane of the optic axes is the basal pinacoid and the acute bisectrix, $Bx_a = \mathfrak{a}$. The optical character is hence *negative.*

### BURRELL OR BHARAL, *Ovis nahura.* Plates 42 and 43.

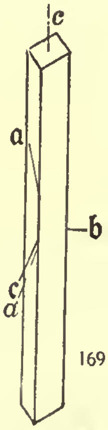
The specimen was received from the National Zoölogical Park at Washington, and consisted of a small quantity of blood in the shape of clots, with a small amount of liquid. It contained much foreign matter in suspension. The blood was oxalated, treated with ether and centrifugalized in the usual way, yielding a small amount of clear solution, from which slide preparations were made as usual. The blood began to crystallize in needles soon after the preparations were covered. These formed in the protein ring, but afterwards dissolved as the solution under the cover came to an equilibrium, while new rod-like and tabular crystals appeared throughout the slides. These crystals seemed to be not very soluble, although rise of temperature caused a loss of the planes on the ends of the long lath-shaped crystals; and with the oxyhemoglobin crystals were mixed occasional crystals of reduced hemoglobin. The absorption spectra of both kinds of crystals were examined by the microspectroscope. The photographs were made 3 days and 5 days after the preparation of the slides.

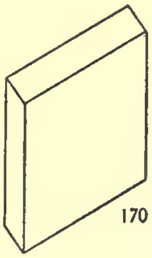
### *Oxyhemoglobin of Ovis nahura.*

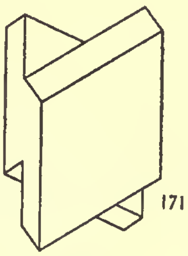
Monoclinic: Axial ratio $a : b : \acute{c} = 1.232 : 1 : \acute{c}$; $\beta = 54° 15'$.

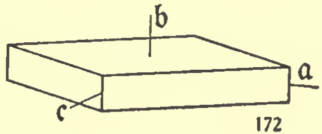
Forms observed: Unit prism (110), base (001), orthopinacoid (100), clinopinacoid (010), hemiorthodome (101).

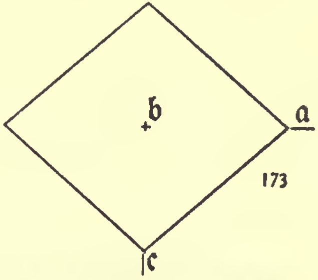
Angles: Plane angle of base on prism, edges $110\text{–}001 \wedge 1\bar{1}0\text{–}001 = 63°$ (angle of termination of the flattened prisms), orthopinacoid to base $100 \wedge 001 = \beta = 54° 15'$; angle over edges of pentagonal twin 71° 30′ (normals).











FIGS. 169, 170, 171. *Ovis nahura* Oxyhemoglobin. FIGS. 172, 173. *Ovis nahura* Reduced Hemoglobin.

Habit prismatic, elongated on the vertical axis, forming prisms with the ends terminated obliquely by the base (text figure 169), the plane angle of the trace of the base on the prism being about 83°, but not exactly determined; also in lath-shaped crystals, by flattening on two opposite prism faces, the ends then terminated by the oblique plane of the base producing a triclinic aspect (text figure 170); and in this form often with the prism so shortened that the rhombic plates appear to be triclinic tables with plane angles of 63°. Twins on the flattened prism, resembling carlsbad twins (text figure 171), rather rare; also commonly twins on the orthopinacoid and on the hemiorthodome as in sheep (text figure 166). This second kind of twinning forms both pentagonal groups, as in sheep, and also networks of rod-like crystals, this latter perhaps by twinning on the base instead of the orthopinacoid (see plate 43). The little pentagonal groups grow attached to the long prism-like crystals, as in the sheep crystals, and produce a cross-barred effect upon the orthopinacoid surfaces, due to the strong pleochroism. The crystals strongly resemble those of the sheep in these twins, but the prismatic habit is different. A third kind of twinning on a prism, producing an X-shaped interpenetrant twin, was seen, but it occurred very rarely. The pentagonal twins are much more irregular than in the sheep crystals; and they show usually a skeleton form, instead of even-sided pentagons, due to parallel growth. The description of this twin will be found under Sheep.

Pleochroism is marked; $\mathfrak{a}$ pale yellowish-red, nearly colorless, $\mathfrak{b}$ red, $\mathfrak{c}$ deep cochineal-red. Extinction on the ordinary crystals is oblique, except on the rods, which show the orthopinacoid aspect. In this aspect the extinction is straight; and in convergent light one brush of a biaxial interference figure appears in the field, somewhat eccentrically placed and revolving with the revolution of the crystal. The orientation of the elasticity axes is $\mathfrak{a} \wedge \acute{c}=30°$ about; $\mathfrak{b}=b$, $\mathfrak{c} \wedge a=5° 45'$. The extinction angle on the (010) aspect is hence 30°; on the usual oblique aspects it is 18° to 23°, measured from the prism edges. The plane of the optic axes is the clinopinacoid and the angle of the axes, $2E$, must be between 50° and 60°. The bisectrix of the optic axes is $\mathfrak{c}$, $Bx_a=\mathfrak{c}$, and the optical character is hence *positive*.

### *Reduced Hemoglobin of Ovis nahura.*

Orthorhombic: $a : b : \acute{c} = 0.885 : 1 : \acute{c}$.

Forms observed: Unit prism (110), base (001).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 83°$; prism to base $110 \wedge 001 = 90°$.

Habit thin tabular on the base, the plane bounded by the prism faces (text figures 172 and 173); and the ratio of the short diagonal on the base to the prism length is about 5 : 1. The crystals occur singly, or sometimes piled up on each other on the basal surface; and appear somewhat fan-shaped in edge view, but twinning was not observed. These crystals did not occur in large numbers in any of the slides.

Pleochroism is marked; $\mathfrak{a}$ nearly colorless, $\mathfrak{b}$ purplish-red, $\mathfrak{c}$ very deep purplish-red. Extinction is symmetrical on the base and straight on all edge views. The orientation of the elasticity axes is, $\mathfrak{a}=b$, $\mathfrak{b}=\acute{c}$, $\mathfrak{c}=a$; the plane of the optic axes is hence the basal pinacoid. Looking along the axis of greatest elasticity, $\mathfrak{a}$, the biaxial interference figure is seen; the separation of the axes $2E$ is about 50°. The acute bisectrix is hence $\mathfrak{a}$, $Bx_a=\mathfrak{a}$, and the optical character is *negative*.

## BULLOCK OR OX, *Bos taurus*. Plate 44.

Fresh blood was obtained from the abattoir, oxalated, the corpuscles settled by centrifugalizing and the plasma drained off. The corpuscles thus nearly freed of plasma were then laked with ether, and the solution saturated with ammonium oxalate and centrifugalized. The slide preparations were made as usual, the drops being allowed to evaporate so that a thick protein ring was formed. Crystals formed slowly at room temperature, and they appeared to be somewhat porous and imperfect. They were fairly permanent, however, and the photographs were made 2 days or more after the slides were prepared. The spectroscope showed only oxyhemoglobin. Several preparations were made, varying the amounts of oxalate and the treatment in laking; but the first-formed crystals were all about of the same type. As the solution became more dense in the slides, by evaporation through the seal of balsam, the crystals developed more faces and changed slightly in habit. Only crystals of oxyhemoglobin appeared.

### *Oxyhemoglobin of Bos taurus.*

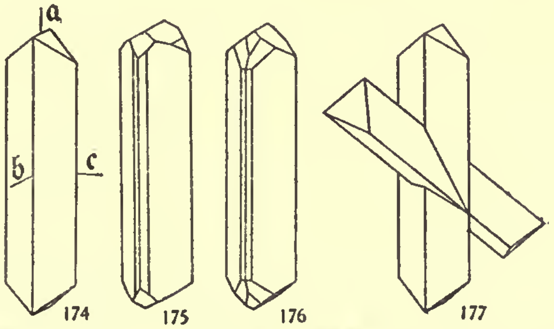
Orthorhombic: Axial ratio $a : b : \acute{c} = 0.7467 : 1 : 0.619$.

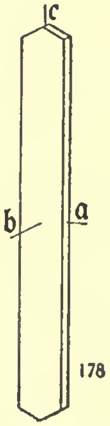
Forms observed: Unit prism (110), brachyprism (120), brachydomes (011), (054), macrodome (302), pyramid (221).

Angles: Unit prism $110 \wedge 1\bar{1}0 = 73° 30'$ (normals); brachyprism $120 \wedge 1\bar{2}0 = 112° 30'$ (normals); brachydomes $011 \wedge 0\bar{1}1 = 63° 30'$ (normals); $054 \wedge 054 = 54°$ (normals); macrodome, $302 \wedge \bar{3}02 = 75°$ roughly; edge angle of pyramid over pole, edge $221\text{–}2\bar{2}1 \wedge \bar{2}21\text{–}\bar{2}\bar{2}1 = 99°$ about.

Habit prismatic, the first crystals to form consisting of the unit prism and brachydome (054) in prisms about 4 times as long as the width on the macro-axis (see text figure 174). Later, as the solution becomes more concentrated, other planes develop and the brachyprism (120) predominates, the unit prism being reduced to a bevel on the brachyprism; in these crystals the two brachydomes (011) and (054), the former predominant, sometimes develop with a small macrodome (302) (text figure 175) or the pyramid (221) with (054) and (302) (text figure 176). The crystals from the concentrated solution are hence of two types, in both of which the prism taken as the brachyprism predominates; but both of these types occurred sparingly and in a few slides. The crystals often grow in tufts from the oxalate crystals which separate from the supersaturated oxalate solution; usually, however, they occur singly, scattered through the slides or massed in the protein ring and along the cover edge. Twinning was observed on a pyramid, which appears to be the observed pyramid (221); these are interpenetrant twins (text figure 177).

The deep color of the solution in most cases conceals the pleochroism, but in thin slides it is quite marked; $\mathfrak{a}$ is colorless to pale red; $\mathfrak{b}$ is pink to red; $\mathfrak{c}$ is deep blood-red; on sections showing $\mathfrak{b}$ and $\mathfrak{c}$ the pleochroism is not pronounced. Extinction is straight in all aspects of the crystals. The orientation of the elasticity axes is $\mathfrak{a}=\acute{c}$, $\mathfrak{b}=a$, $\mathfrak{c}=b$. The plane of the optic axes is hence the macropinacoid; and on basal sections in convergent light the biaxial interference figure is visible with the axes rather widely separated, $2E=75°$, about. The axis of greatest elasticity $\mathfrak{a}$ is hence the acute bisectrix, $Bx_a=\mathfrak{a}$ and the optical character is *negative.*





Figs. 174, 175, 176, 177. *Bos taurus* Oxyhemoglobin. Fig. 178. *Bos bison* Oxyhemoglobin.

## Buffalo, *Bos bison.* Plate 44.

The specimen was received from the Philadelphia Zoölogical Gardens in a very putrid condition. It was centrifugalized, and preparations made in the usual manner. The blood showed the spectrum of oxyhemoglobin, but with some traces of methemoglobin; the crystals, which formed readily at room temperature, showed only oxyhemoglobin. While the crystals formed readily, they were not well developed, and the determination of their characters was unsatisfactory. Their description is as follows:

### *Oxyhemoglobin of Bos bison.*

Orthorhombic: Axial ratio only approximately determined; $a:b:\acute{c}=a:1:0.5095$.

Forms observed: Macropinacoid (101), brachypinacoid (011), brachydome (011).

Angles: The angle of the brachydome was measured at about 126° actual angle. This is the angle of the brachydome of the bullock oxyhemoglobin (054) crystals, which is 126° actual angle. The measurements of the bison crystal may be 1° out of the way.

Habit very long lath-shaped, elongated on the vertical axis and flattened on the macropinacoid, which is the only plane of any size on the crystal (text figure 178); the brachypinacoid and brachydome are both very narrow. The crystals are often terminated by one plane of the brachydome only, the other being wanting or very small. Some crystals are simply acicular, with a long tapering point, as is common in imperfectly developed crystals.

Pleochroism strong, but only observed on the flat view or on the macropinacoid with any exactness. Probably this is the plane of the optic axes, as in the bullock. Assuming this to be the case, the pleochroism is: $\mathfrak{a}$ nearly colorless, $\mathfrak{c}$ deep red. The orientation of the axes on this basis would be $\mathfrak{a}=b$, $\mathfrak{b}=a$ (not exactly observed), $\mathfrak{c}=\acute{c}$. No interference figure was observed, and the optical character can not be fixed without further data. The pleochroism indicates that it is *negative*.

TABLE 41.—*Crystallographic characters of the hemoglobins of the Ungulata.*

| Name of species. | Axial ratio $a:b:\acute{c}$, &c. | Prism angle (traces on base). | Angle β. | Extinction angle. | Optical character. | System. | Substance. |
|---|---|---|---|---|---|---|---|
| Perissodactyla: | | | | | | | |
| Equidæ: | | ° ′ | ° ′ | | | | |
| Equus caballus ......... | 0.7467 : 1 : 0.4097 | 73 30 | 90 0 | 0° | Negative | Orthorhombic | α-OHb. |
| Do................ | 1.600 : 1 : ć | 64 0 | 72 0 | $\mathfrak{a}\wedge a=13°$ | Positive | Monoclinic | β-OHb. untwinned. |
| Do................ | 1.6976 : 1 : ć | 61 0 | 72 0 | $\mathfrak{a}\wedge a=13°$ | Do. | Do. | β-OHb. twinned. |
| Do................ | 0.7332 : 1 : 0.4106 | 72 30 | 90 0 | 0° | Negative | Orthorhombic | α-COHb. |
| Do................ | 1.664 : 1 : ć | 62 0 | 68 0 | $\mathfrak{a}\wedge a=15°$ | Positive | Monoclinic | β-COHb. |
| Equus asinus × Equus caballus .......... | 0.7813 : 1 : 0.4198 | 76 0 | 90 0 | 0° | Negative | Orthorhombic | α-OHb. |
| Do................ | 1.7147 : 1 : ć | 60 30 | 72 0 | $\mathfrak{a}\wedge a=12°$ to 13° | Positive | Monoclinic | β-OHb. |
| Artiodactyla: | | | | | | | |
| Hippopotamidæ: | | | | | | | |
| Hippopotamus amphibius | 1.600 : 1 : ć | 64 0 | 66 0 | $\mathfrak{a}\wedge a=20°$ | Do. | Do. | OHb. |
| Dicotylidæ: | | | | | | | |
| Dicotyles labiatus....... | a : ć = 1 : 0.7133 | 90 0 | 90 0 | 0° | Negative | Tetragonal | OHb. |
| Dicotyles tajacu ........ | a : ć = 1 : 1.303 | 90 0 | 90 0 | 0° | ........ | Do. | OHb. |
| Suidæ: | | | | | | | |
| Domesticated var. of Sus scrofa........ | 0.6248 : 1 : 0.6008 | 64 0 | 90 0 | 0° | Negative | Orthorhombic | OHb. |
| Do................ | ................ | ..... | ..... | ......... | ........ | Monoclinic? | Hb. |
| Tragulidæ: | | | | | | | |
| Tragulus meminna ...... | 1.804 : 1 : ć | 59 0 | 63 0 | $\mathfrak{a}\wedge a=15°$ | Positive | Monoclinic | OHb. |
| Do................ | 0.5205 : 1 : ć | 55 0 | 90 0 | 0° | Negative | Orthorhombic | Hb. |
| Cervidæ: | | | | | | | |
| Cervus canadensis....... | a : ć = 1 : 0.7133 | 90 0 | 90 0 | 0° | Do. | Tetragonal | OHb. |
| Cervus dama ........... | a : ć = 1 : 1.200 | 90 0 | 90 0 | 0° | Do. | Do. | OHb. |
| Cariacus rufus .......... | ................ | ..... | ±90 0 | $\mathfrak{c}\wedge\acute{c}=30°$ | Positive | Monoclinic | Hb. |
| Mazama americana savannarum (?).......... | ................ | ..... | ±90 0 | $\mathfrak{c}\wedge\acute{c}=30°$ | Negative? | Do. | Hb. |
| Cervulus muntjak....... | 1.303 : 1 : ć | 75 0 | 52 0 | $\mathfrak{a}\wedge a=8°$ | Positive | Do. | OHb. |
| Do................ | ................ | ..... | ±90 0 | $\mathfrak{c}\wedge\acute{c}=18°$ | Do. | Do. | Hb. |
| Bovidæ: | | | | | | | |
| Antilopinæ: | | | | | | | |
| Antilope cervicapra ... | 1.887 : 1 : ć | 58 20<br>56* | 71 45 | $\mathfrak{a}\wedge a=18°\,15'$ | Do. | Do. | OHb. |
| Cervicapra redunca ... | 0.839 : 1 : 0.5877 | 80 0 | 90 0 | 0° | Negative | Orthorhombic | OHb. |
| Gazella dorcas ........ | 0.3639 : 1 : 0.4452 | 40 0 | 90 0 | 0° | Do. | Do. | OHb. |
| Cephalophus grimmi .. | a : ć = 1 : 0.8687 | 90 0 | 90 0 | 0° | Do. | Tetragonal | α-OHb. |
| Do............ | ................ | 60 0 | 90 0 | 0° | ........ | Hexagonal | β-OHb. |
| Ovinæ: | | | | | | | |
| Ovis aries ............ | 1.140 : 1 : 0.970 | 82 30 | 54 0 | $\mathfrak{c}\wedge\acute{c}=30°$ | Negative? | Monoclinic | OHb. |
| Do............ | 0.7813 : 1 : ć | 76 0 | 90 0 | 0° | Do. | Orthorhombic | Hb. |
| Ovis nahura .......... | ................ | 63 0 | 54 15 | $\mathfrak{a}\wedge\acute{c}=30°$ | Positive | Monoclinic | OHb. |
| Do............ | 0.885 : 1 : ć | 83 0 | 90 0 | 0° | Negative | Orthorhombic | Hb. |
| Bovinæ: | | | | | | | |
| Bos taurus ........... | 0.7467 : 1 : 0.619 | 73 30 | 90 0 | 0° | Do. | Do. | OHb. |
| Bos bison ............ | ±a : 1 : 0.5095 | ..... | 90 0 | 0° | Do. | Do. | OHb. |

* True angles.

# CHAPTER XIII.

## CRYSTALLOGRAPHY OF THE HEMOGLOBINS OF THE RODENTIA.

The blood of 18 species of the rodents was examined, representing 6 of the 18 families into which the order is usually divided. Of the *Sciuridæ* 3 species of *Sciurus*, the European red squirrel, the fox-squirrel, and the gray squirrel were examined; also the flying-squirrel, *Sciuropterus*, and the ground-squirrel, *Tamias;* as well as the prairie-dog, *Cynomys*, and the ground-hog, *Marmota*. Of the *Castoridæ*, the only species examined was the beaver, *Castor canadensis*. Five members of the *Muridæ* were examined, representing the genera *Mus* and *Fiber;* the rats (genus *Mus*) were the Norway rat, the albino of the same species, the black rat, and the Alexandrine rat; while the muskrat represented the genus *Fiber*. The *Erethizontidæ* were represented by the porcupine, *Erethizon dorsatus;* the *Caviidæ* by the guinea-pig, *Cavia*, and the capybara, *Hydrochærus*. All of the above species are members of the group *Simplicidentata*, while the *Duplicidentata* were represented by 2 species of the *Leporidæ*, the common domestic rabbit, *Lepus cuniculus*, and the Belgian hare, *Lepus europæus*.

The crystals from the squirrels of the genera *Sciurus* and *Sciuropterus* form hexagonal plates, which, in probably all cases, are pseudohexagonal only, and mimetic twins of the $\beta$-oxyhemoglobin, which crystallizes in the orthorhombic system. In these hexagonal crystals no axial ratio was determinable, as the pyramidal planes were never developed. The prism angle of the hexagonal and of the orthorhombic crystals was the same, 60°, which explains the possibility of the mimetic twinning that appears to be the cause of the more symmetrical development. Similar hexagonal crystals, probably mimetic twins of the $\gamma$-oxyhemoglobin, which crystallizes in the monoclinic system, with a prism angle of 58°, were seen in the case of the ground-hog, *Marmota monax*.

The hemoglobins of all species of the *Sciuridæ* were rather insoluble, and crystallized very readily; so that usually crystallization had to be somewhat restrained to produce satisfactory crystals. In some cases, as, for instance, the prairie-dog, *Cynomys ludovicianus*, the crystals formed so rapidly that it was not possible to determine the crystallographic constants, on account of the hair-like character of the crystals. This blood was examined before we had perfected our methods for restraining the rapid formation of crystals, and no doubt we could now crystallize it satisfactorily. In the ground-hog three kinds of oxyhemoglobin crystals were observed, crystallizing in the hexagonal, orthorhombic, and monoclinic systems, and in several other cases two forms of oxyhemoglobin were noted.

The rats examined (genus *Mus*) form a distinct group, with crystals that closely resemble each other in form, but vary very much in habit. An examination of these crystals will at once show that the four kinds of rats examined may be arranged in two series, the Norway rat and the white rat being evidently the same species, the albino variety, however, varying somewhat from the typical Norway rat. On the other hand, the crystals of the blood of the black rat and the Alexandrine rat closely resemble each other, but differ markedly in habit from those of the Norway and white rats. The crystals of the Norway and white rats form rapidly, and are more insoluble than those of the black and Alexandrine rats; they are much smaller, and hence paler in color, and they show a rather stronger double refraction than those of the black and Alexandrine rats. These latter crystals are larger and more regularly formed and do not twin so constantly as those of the Norway and white rats.

In the monoclinic crystals of the beaver, the muskrat, and the porcupine considerable resemblance is to be seen, although each animal belongs to a separate family; the distinctions between the species will be readily made out on comparing the photographs of the crystals, and their descriptions, as given under each species. It will be noted that the prism angles of these three species also run near 60°, which appears to be common in the rodents.

The guinea-pig and capybara differ from all of the other rodents examined in having distinctly pyramidal, rather than prismatic or tabular crystals. The crystals of the guinea-pig are orthorhombic sphenoidal, while those of the capybara are tetragonal.

The crystals from the blood of the common rabbit and the Belgian hare closely resemble each other, and, as may be seen from the axial ratios, the extinction angles, and the prism angles of the monoclinic form, these two species are closely related. Here again the prism angle approaches 60°. The data in regard to the Belgian hare are not complete, however, so that the comparison is not perfect. In solubility, and even in the form of their crystals, these two species of *Lepus* differ very widely from the other rodents. Whereas the crystals of the rodents in general are rather insoluble, those of the rabbit and hare are very soluble, and they must be examined in cold weather or they will dissolve while under investigation.

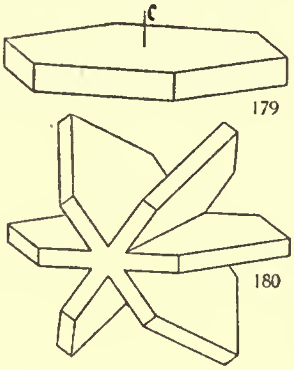
## RODENTIA.

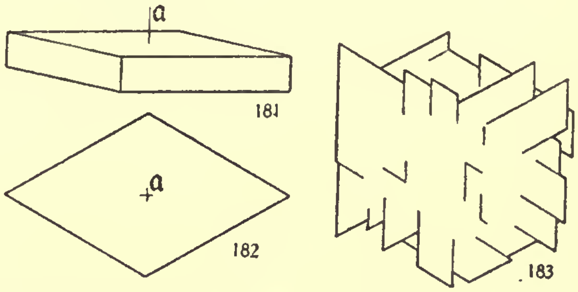
### EUROPEAN RED SQUIRREL, *Sciurus vulgaris*. Plate 45.

Two specimens of blood were examined, one from an animal purchased from a dealer and bled in the laboratory, and one from an animal that died at the Philadelphia Zoölogical Gardens. The latter specimen consisted of only a small amount of fluid blood, and was in a very putrid condition. In both cases the oxalated blood was laked and (in the case of the fresh blood) centrifugalized, and the slide preparations made as usual. Crystals formed very readily at room temperature, and they were quite insoluble, showing no tendency to dissolve. Good photomicrographs were obtained within 2 or 3 hours after making the preparations. In both cases the blood yielded crystals of oxyhemoglobin. The crystals obtained from the putrid blood were of a different type from those of the fresh blood, and they will be described separately. They are, nevertheless, probably both the same substance, but the crystals from the putrid blood probably throw light on the actual structure of the crystals in all species of *Sciurus*. The two types of crystal will be distinguished as α-oxyhemoglobin (from the fresh blood) and β-oxyhemoglobin (from the putrid blood).

### *α-Oxyhemoglobin of Sciurus vulgaris, from the Fresh Blood.*

Hexagonal (or pseudohexagonal): No axial ratio determinable.
Forms: Unit prism (10$\bar{1}$0), base (0001).
Angles: Prism angle 60°; prism to base 90°.





Figs. 179, 180. *Sciurus vulgaris* α-Oxyhemoglobin. Figs. 181, 182, 183. *Sciurus vulgaris* β-Oxyhemoglobin.

Habit tabular, in thin hexagonal plates consisting of the short prism and the base, with a ratio of breadth of plate to its thickness of about 10 : 1 (text figure 179). The plates are variable in size and are not all equally developed on all faces of the prism, but the majority are strictly hexagonal in development, as they all are in angles. Many, however, show a distinctly orthorhombic development, two opposite faces being much larger than the other four. No distinctly rhombic plates were seen, as in the crystals from the putrid blood. Many irregular crystals can be seen in the photographs, they are broken parts of twins. The plates occurred singly or piled on the basal surfaces into irregular parallel growths. Twins occurred, interpenetrant and contact; sometimes the crystals on edge showed a rough six-pointed star from three interpenetrant crystals (text figure 180). These are evidently due to twinning on a second-order pyramid, and the angle of two of the plates with one is about 54° for each. Apparently there are two second-order pyramids that become twin planes, and they would be (12$\bar{1}$2) and (12$\bar{1}$1). These are not always interpenetrant; some are simply juxtaposed. A twin on the first-order pyramid was also observed.

The color is rather pale oxyhemoglobin red, but this is due to the thin crystals, and it was of course quite strong on edge views. Pleochroism is scarcely noticeable, but the absorption seemed to be slightly stronger for $\omega$. Double refraction is very weak; on the flat the crystals are singly refracting, but on edge the weak double refraction may be observed, with difficulty, by means of the quartz wedge. They are then seen to extinguish parallel to the base. The symmetry axis is the axis of less elasticity, or $\omega > \epsilon$ and the optical character is slightly *positive*. On the basal aspect a very faint dusky cross in convergent light shows the uniaxial character of the crystals.

### *β-Oxyhemoglobin of Sciurus vulgaris, from the Putrid Blood.*

Orthorhombic: Axial ratio $a : b : \dot{c} = 0.577 : 1 : \dot{c}$.
Forms observed: Unit prism (110), base (001).
Angles: Prism angle 110 ∧ 1$\bar{1}$0 = 60°; prism to base 110 ∧ 001 = 90°.

Habit very thin tabular on the base, with the very short prism determining the outline of the rhombic plate (text figures 181 and 182). The crystals rarely occur singly, however, but in twins of the usual type on an axis in the base and normal to a prism-base edge, the composition face being the basal pinacoid. These occur on any or all of the prism-base edges, making a group that is composed of many individuals and generally of a roughly hexagonal outline (text figure 183). Hexagonal plates of normal development occur with these twins, similar to the plates of α-oxyhemoglobin; and they are probably mimetic twins of the β-oxyhemoglobin, due to the complicated groups above described becoming developed into hexagonal plates. Twins on pyramid faces, as seen in the hexagonal α-oxyhemoglobin, were not observed.

The color was the usual oxyhemoglobin red, perhaps a little darker than for the corresponding thickness in the α-crystals. Pleochroism is very slight on edge and not noticeable on the basal aspect; $\mathfrak{a}$ deep red, $\mathfrak{b}=\mathfrak{c}$ somewhat deeper red. Double refraction on the base is not noticeable, even with the quartz wedge ($\mathfrak{b}=\mathfrak{c}$); on edge the extinction is straight and the relative elasticities may be made out with the quartz wedge. On the base no interference figure of any kind could be detected in convergent light, but it is evident that the vertical axis is the axis of greatest elasticity, and that $\mathfrak{b}=\mathfrak{c}$; hence the acute bisectrix $Bx_a=\mathfrak{a}$, and the optical character is *negative.*

On comparing these two types it is evident that the characters of the β-oxyhemoglobin are such that it would readily become hexagonal by mimetic twinning, the prism angle being exactly 60°, and the double refraction of the β-modification is such that, but for the form of the crystal, it might be hexagonal. In the mimetic twins, produced by piling up of the rhombic plates to build a hexagonal composite plate, it might readily happen, with the very weak double refraction, that the crystal might become more dense in the direction in which the plates are piled, and hence the vertical axis, or normal to the plates, become the axis of greater density or less elasticity, when the pseudohexagonal crystal would become positive. It is hence entirely probable that the two modifications are really one and the same, the α-oxyhemoglobin being a mimetic twin of the β-oxyhemoglobin and only pseudohexagonal.

### FOX-SQUIRREL, *Sciurus rufiventer neglectus.* Plate 46.

The specimen was purchased from a collector at Orlando, Florida, and was bled in the laboratory, oxalated, ether-laked, centrifugalized, and the slide preparations made as usual. Crystals formed rapidly in the slides, and showed no tendency to dissolve. The blood crystallizes more readily than that of the related gray squirrel. The crystals were shown to be typical oxyhemoglobin by the spectroscope.

#### *Oxyhemoglobin of Sciurus rufiventer neglectus.*

Hexagonal: Axial ratio not determinable.

Forms: Unit prism (10$\bar{1}$0), base (0001).

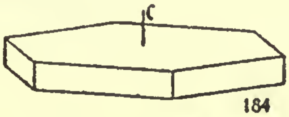
Angles: Prism angle 60°, prism to base 90°.

Habit, thin tabular on the base, very symmetrical hexagonal plates consisting of the short prism and the basal pinacoid (text figure 184). The crystals occur singly, or in parallel growths and piled groups on the base, the smaller crystals piled concentrically on a larger crystal. In single crystals the thickness of the plate is one-tenth to one-twentieth of the width, but this is very variable. Many single perfect plates are seen, but this varies in different slides. In some cases the plates elongated on two prism faces or along the diameter of the hexagon parallel to a crystal axis, becoming somewhat orthorhombic looking; most of them are almost perfect hexagons. Twins on a first-order pyramid occur, mostly contact twins (text figure 185).

The color of the plates is variable with the thickness, but pleochroism is very slight. On the base they are singly refracting, and polarize very faintly on edge; the double refraction is very weak. The elasticity for the ordinary ray, $\omega$, is somewhat greater than for the extraordinary ray or $\epsilon > \omega$ in refraction indices, and the optical character is hence weakly *positive*.

### GRAY SQUIRREL, *Sciurus carolinensis*. Plates 46 and 47.

The living animal was obtained from a collector at Newport News, Virginia, and was bled in the laboratory. The oxalated blood was laked with ether and centrifugalized, and the slide preparations made in the usual manner. Crystals formed more slowly than with the other squirrels examined; they were larger and showed more tendency to produce composite crystals than in the other species. They showed no tendency to dissolve, however, and are evidently quite difficultly soluble in the plasma. Examination with the microspectroscope shows that these crystals are typical oxyhemoglobin.



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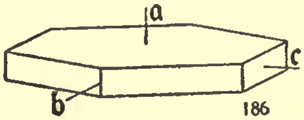

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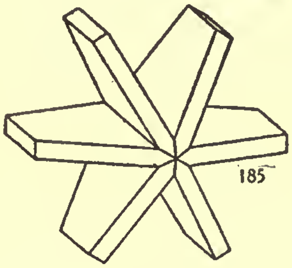

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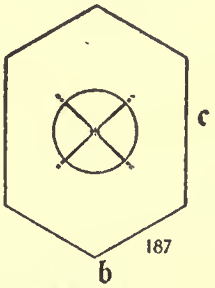

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FIGS. 184, 185. *Sciurus rufiventer neglectus* Oxyhemoglobin. FIGS. 186, 187. *Sciurus carolinensis* Oxyhemoglobin.

### *Oxyhemoglobin of Sciurus carolinensis.*

Orthorhombic; pseudohexagonal: Axial ratio $a : b : \dot{c} = 0.577 : 1 : \dot{c}$.

Forms observed: Unit prism (110), brachypinacoid (010), basal pinacoid (001); or, as pseudohexagonal, prism and base.

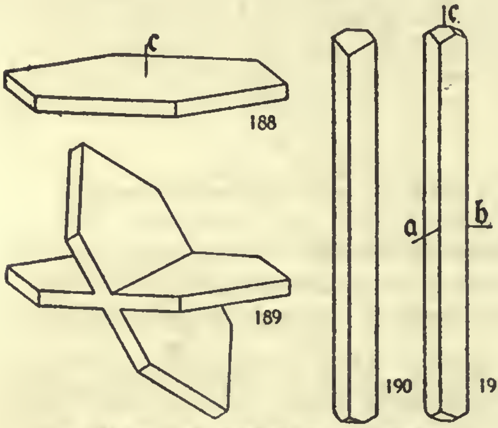
Angles: Prism angle $110 \wedge 1\bar{1}0 = 60°$ (normals); prism to brachypinacoid $110 \wedge 010 = 60°$ (normals), the two making a perfect hexagonal plate; prism to base $110 \wedge 001 = 90°$.

Habit pseudohexagonal, tabular on the base, and with the prism and brachypinacoid faces in equilibrium, so that the plate is a perfect hexagon; sometimes, however, the plate is elongated on the brachy-axis, producing a distinctly orthorhombic habit (text figures 186, 187). The plates are large and perfect hexagons, but are not often simple; they produce groups by piling up on the base, more or less concentrically, and often with curving of the crystals, producing the form of the "*eisen rose*" of hematite, (see plate 47, fig. 277). The parallel growths on the base may, however, start from several centers, and it is very common to see a small group of this kind near one side of a large plate, not central, but in perfect orientation with the large plate. Twinning seems to be on a brachydome. In the protein ring the crystals form spherulitic masses of the radiating plates, and when these are seen on edge, or interfered with by the cover-glass, they look like lath-shaped crystals. When the piled-up plates are seen on edge, in section, they present a sheaf-shaped appearance.

The color varies much with the thickness, but in the thicker crystals it shows the normal oxyhemoglobin red. Pleochroism does not show on the flat aspect, the crystal acting like a hexagonal crystal; but on edge the pleochroism is noticeable, the color being deeper normal to the plate than parallel to it. On the base the crystal is nearly singly refracting; but by use of the quartz wedge it is seen to be very weakly doubly refracting; on edge the double refraction is easily seen, and extinction is parallel with the base. In convergent light, a uniaxial cross shows in most aspects; but on revolution the brushes open slightly and the crystal is as strongly biaxial as some of the biotite micas. The orientation of the elasticity is $\mathfrak{a}=\acute{c}$, $\mathfrak{b}=a$, $\mathfrak{c}=b$. The macropinacoid is the plane of the optic axes and the acute bisectrix $Bx_{\mathfrak{a}}=\mathfrak{a}$. The optical character is hence *negative*.

### Flying-squirrel, *Sciuropterus volans*. Plate 47.

Blood was obtained from the living animal, oxalated, ether-laked, and centrifugalized, and the slide preparations made as usual. Crystallization begins as soon as the blood is laked, and proceeds with great rapidity, so that the preparations are soon full of minute scales or tabular crystals. To retard the formation of the crystals somewhat, and permit them to grow to a larger size, some preparations were made by diluting the blood with about 3 times its volume of the blood plasma; but, even with this dilution, the crystals begin to form immediately upon laking the corpuscles. They are always small, much smaller than in other species of squirrels, but otherwise resemble those formed in the squirrel bloods in general. They were oxyhemoglobin, as determined by the microspectroscope.



Figs. 188, 189. *Sciuropterus volans* Oxyhemoglobin.
Figs. 190, 191. *Tamias striatus* Oxyhemoglobin.

*Oxyhemoglobin of Sciuropterus volans.*

Hexagonal. No axial ratio determinable.

Forms observed: Unit prism (1010), base (0001).

Angles: Prism angle 60° (normals); crystals are so thin that prism to base could not be measured with any exactness, but it appears to be 90°.

Habit very thin tabular on the base (text figure 188), minute hexagonal scales or plates, with very little color, owing to their being so thin. They develop in enormous numbers, the slides becoming completely filled with them. They generally occur singly or in irregular groups, but a twin on a pyramid of the second order seems to occur, interpenetrant and of the same general form as the tridymite twin (text figure 189).

The crystals are very faintly colored, when seen on the flat, but on edge have the red color of oxyhemoglobin, and show pleochroism; $\omega$ deep red, $\epsilon$ very pale red. On the flat, the crystals are singly refracting; on edge they polarize, but not strongly. The direction of the vertical axis or of the optic axis $\epsilon$ is the direction of greater elasticity; hence $\omega > \epsilon$, and the crystals are *negative*.

### Ground-squirrel or Hackee, *Tamias striatus*. Plate 48.

The specimen was purchased from a collector in eastern Pennsylvania. The animal was bled into oxalate, the blood laked with ether and centrifugalized, and slide preparations made in the usual manner. The blood crystallized readily at a temperature of 22° C. The crystals are quite insoluble and keep well at ordinary room temperature, showing no tendency to dissolve, even in the rays of the electric arc lamp, when making the photomicrographs. They are the usual oxyhemoglobin red, and were determined as oxyhemoglobin by the spectroscope.

### *Oxyhemoglobin of Tamias striatus.*

Orthorhombic (?): Axial ratio about $a : b : \acute{c} = 0.9246 : 1 : 0.589$.

Forms observed: Unit prism (110), macrodome (101), brachydome (011).

Angles: Macrodome angle $101 \wedge \bar{1}01 = 65°$ (normals); brachydome angle $011 \wedge 0\bar{1}1 = 61°$ (normals); prism angle (calculated) $110 \wedge 1\bar{1}0 = 85° 30'$. The prism angle was not observed, but was calculated from the two dome angles which were measured, but not very satisfactorily; hence the uncertainty as to the exact axial ratio.

Habit prismatic on the vertical axis; the first prisms that develop are very long and slender; later, stouter crystals form on which some measurements of the terminal planes can be made. The common termination is the macrodome, one face much more developed than the other, giving the crystal a very monoclinic aspect (text figure 190). It may in fact be monoclinic, but the measurements of prism edge to macrodome seemed to be symmetrical in the crystals examined, and extinction is straight in all aspects. The prisms range in ratio of length to thickness from 15 : 1 to 100 : 1, and in most of them the terminal macrodome is unsymmetrically developed. In some a brachydome appears (text figure 191) and, some days after the slides were prepared, the two domes were seen in equilibrium, in a few cases. The crystals grow in radiating tufts from the protein ring and cover edge, and also scattered irregularly through the body of the slide; but they do not appear to form twins.

Pleochroism is rather pronounced; $\mathfrak{a}$ pale yellowish-red, $\mathfrak{b}$ pale rose-pink, $\mathfrak{c}$ deep red. The orientation of the elasticity axes is apparently $\mathfrak{a} = a$, $\mathfrak{b} = b$, $\mathfrak{c} = \acute{c}$; but no interference figure was made out. As stated above, the extinction is straight in all aspects of the crystals that could be examined. The optical character could not be determined, but, from the pleochroism, it should be *positive*.

## PRAIRIE-DOG, *Cynomys ludovicianus*. Plate 48.

Specimens of prairie-dogs were purchased from collectors in Ohio and in Kansas City, and the animals were bled in the laboratory. Preparations were made from the corpuscles, but not from the whole blood, which probably prevented the characteristic plate-like crystals, common in rodent blood, from developing. The corpuscles were oxalated, ether-laked, and centrifugalized and from the clear solution the slide preparations were made as usual. Only one type of crystals developed and these were not very favorable for observing the characters. They were oxyhemoglobin.

### *Oxyhemoglobin of Cynomys ludovicianus.*

Probably orthorhombic: No axial ratio determinable.

Forms observed: Evidently a unit prism, but the terminations were not perfect.

Angles: No angles of the crystals could be measured.

Habit of the crystals obtained was long prismatic, practically hair-like, and tapering gradually to an acute point; but, in the larger crystals, a high power showed that they were four-sided prisms, with a lozenge-shaped cross-section; and they probably are orthorhombic, possibly tetragonal, but certainly not hexagonal. The polarization characters showed that they must be one of these three systems. The needles grow in tufts, radiating from a center, the adjacent tufts penetrating each other and forming networks of interlacing fibers.

The needles in the dense tufts show the oxyhemoglobin color, but individual needles are very pale owing to their tenuity. Pleochroism is noticeable, the direction of the length of the needles showing more color than the normal to the length. The elasticity is greater normal to the length of the fiber and less parallel to the length. They are so thin that no characters can be made out in convergent light; but extinction is straight in all aspects; and this, with the four-sided cross-section, reduces the possible crystal systems to two, orthorhombic and tetragonal. The lozenge-shaped section indicates that the crystallization is orthorhombic. The blood was examined before we had developed our methods of retarding crystallization in order to produce better crystals, and hence this blood should be further investigated.

### Ground-hog or Woodchuck, *Marmota monax*. Plates 49 and 50.

Specimens of this animal were purchased at different times from collectors in eastern and central Pennsylvania, and were bled in the laboratory. The blood was collected in oxalate. The first preparations were made by laking the oxalated corpuscles, and centrifugalizing, and from the clear solution preparing the slides as usual. As these preparations produced mainly long needles, that did not show the crystallographic characters definitely, and as the hexagonal plates that finally appeared were so imperfect that better preparations seemed necessary, others were made, using the whole blood, the preparations being made as above described. In these preparations from the whole blood, the first crystallization in the dried protein ring is in the form of minute hexagonal plates; these soon become covered by the rapidly developing needles, and in part dissolve; so that the slides finally contain only masses of the needles. A preliminary trial of diluting with the blood plasma, and etherizing strongly before centrifugalizing, proving satisfactory in developing the plates, preparations were made by diluting the whole blood with an equal volume of the blood plasma and laking, and carrying out the preparation as above described. In this diluted blood, the plates developed readily and grew to large size, with only a slight development of the rods. The hexagonal plates kept well and passed by paramorphous change into reduced hemoglobin and also into metoxyhemoglobin. The crystals at first formed were, in all cases, oxyhemoglobin. Crystals form very readily in solutions of either the corpuscles, the whole blood, or the whole blood diluted with plasma; but much more rapidly, of course, in solutions of the corpuscles alone than in the less concentrated solutions. The development of the needles, or of the plates, can be controlled at will by the amount of dilution. The same principle applies to other bloods that develop needles or hair-like crystals from the whole blood. Unfortunately, however, the amount of blood in the samples received was rarely enough to try the experiment, or the plasma was not in good condition owing to putrescence. In rodents in general, dilution of the blood by the plasma or serum will probably be found advantageous. Two kinds of tabular crystals were observed in the blood of the ground-hog; the one, hexagonal plates, that are probably only pseudohexagonal and mimetic twins of the second kind, which latter are in the form of rhombic plates, belonging to the monoclinic system. These two will be described as $\alpha$-oxyhemoglobin and $\gamma$-oxyhemoglobin, respectively. The rods are possibly a form of the $\gamma$-oxyhemoglobin with prismatic development, but they appear to be orthorhombic, and will be called $\beta$-oxyhemoglobin. The other forms observed, which are reduced hemoglobin and metoxyhemoglobin, were simply paramorphous alterations of the normal crystals. They appeared mainly in the $\alpha$-oxyhemoglobin form, and only in slides that had been kept for some days.

### *α-Oxyhemoglobin of Marmota monax.*

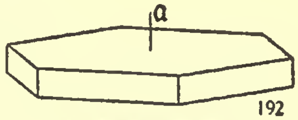


FIG. 192. *Marmota monax* α-Oxy hemoglobin.

Hexagonal or pseudohexagonal: Axial ratio not determinable, as no pyramidal forms were observed.

Forms observed: Unit prism $(10\bar{1}0)$, base (0001).

Angles: Prism angle 60°; prism to base 90°.

Habit thin tabular; in the whole blood preparations, the first crystals to appear are very minute hexagonal plates in the protein ring; these are later dissolved with development of the needles of $\beta$-oxyhemoglobin. In diluted blood, the typical $\alpha$-oxyhemoglobin plates are developed; they are large, well-formed, and very regular hexagonal plates (text figure 192), occurring singly or in complicated groups in parallel growth orientation, either piled on the base (plate 49, figs. 290, 291, and 292) or in arborescent forms (plate 50, fig. 297); also in partial orientation, which looks complete on the base, but is seen to be partial in edge view, the plates radiating from the center of the main groups as though twinned in the zone of two opposite unit-pyramid faces (plate 59, figs. 295 and 296). Interference with the slide and cover produces in these groups on edge broad lath-shaped individuals which look rather orthorhombic. Often a single large plate may have on its basal surface several small concentric groups, all in perfect parallel growth orientation with the main large crystal. Twins are on the unit pyramid, but owing to the tendency to produce radiating groups the angle of the pyramid could not be determined with any certainty.

The color of the plates varies with the thickness, but they show rather strong pleochroism; $\omega$ deep red, $\epsilon$ pale reddish to colorless. On the base, the crystal is singly refracting; on edge, the double refraction is quite strong, and the extinction is straight parallel to the base. In convergent light, a dusky cross appears on the basal aspect, showing the uniaxial character. The vertical axis is the direction of greater elasticity, $\omega > \epsilon$, and the optical character is *negative.*

### *β-Oxyhemoglobin of Marmota monax.*

Orthorhombic or monoclinic: No axial ratio is determinable.

Forms observed: Apparently two vertical pinacoids and a terminal dome or sometimes one plane of such a dome.

Angles: The crystals were not perfect enough to measure angles with exactness; the angle of the terminal dome seemed to be about 58° (normals), and the two pinacoids at right angles.

Habit ordinarily hair-like, the ends tapering to a point, without any definite plane terminations; some larger crystals were lath-shaped and showed the dome or oblique termination described above (text figure 193). The crystals grew in tufts, radiating slightly; or in groups of such tufts, sometimes radiating from a center like the spokes of a wheel; along the protein ring they shoot out normal to the surface and form a continuous mass of hairs on the inside of the ring; outside of it the crystals are larger and longer and the tufts more dense. The crystals are quite elastic, bending considerably before they break. They reach a large size, the individual tufts of hairs being easily seen with the unaided eye.

When the crystals are lath-shaped, the flat surface of the lath is usually presented; and on this surface the pleochroism is quite marked. The length of the lath is apparently $\mathfrak{c}$, the width $\mathfrak{b}$, and the thickness $\mathfrak{a}$. On this aspect above described the axes $\mathfrak{b}$ and $\mathfrak{c}$

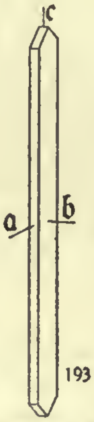
show. The crystal is very thin so that the $\mathfrak{a}$ direction is very short. The pleochroism on the flat is $\mathfrak{b}$ pale yellowish-red, $\mathfrak{c}$ pale red; or, when very thin, $\mathfrak{b}$ colorless and $\mathfrak{c}$ pale pink. On edge view the crystal shows probably from 5 to 10 times the thickness seen on the flat, and the colors are deeper; $\mathfrak{a}$ pale pink, $\mathfrak{c}$ deep red. On the flat aspect in convergent light a pair of dusky brushes of a biaxial interference figure shows; the conjugate axis is the long dimension of the lath $\mathfrak{c}$, but the brushes pass out of the field on rotation of the crystal. It seems probable from this that the acute bisectrix of the optic axes $Bx_a = \mathfrak{c}$, and the optical character is *positive.* Calling the flat side of the lath the macropinacoid, the narrow edge the brachypinacoid and the dome a brachydome, the orientation of the elasticity axes is $\mathfrak{a} = a$, $\mathfrak{b} = b$, $\mathfrak{c} = \dot{c}$; and the axial plane is the brachypinacoid.

### γ-*Oxyhemoglobin of Marmota monax.*

Monoclinic: Axial ratio $a : b : \dot{c} = 1.804 : 1 : \dot{c}$; $\beta$ near 90° (?).

Forms observed: Unit prism (110), base (001).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 58°$ (about); the angle $\beta$ was not exactly observed, but appears to be near 90°.



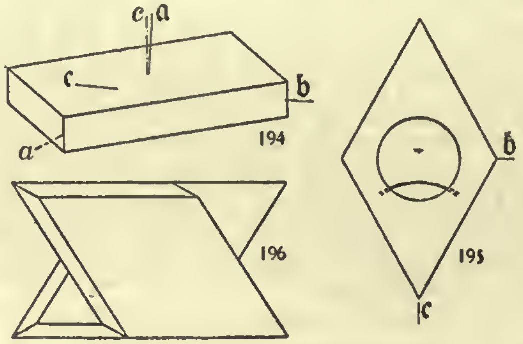


FIG. 193. *Marmota monax* β-Oxyhemoglobin. FIGS. 194, 195, 196. *Marmota monax* γ-Oxyhemoglobin.

Habit thin tabular, consisting of the basal pinacoid, bounded by the unit prism (text figures 194, 195); but the crystals rarely occur singly, they form groups radiating from a center both on edge and on the flat (see plate 50). They occurred sparingly as a second growth in preparations made from whole blood, and appeared to be more soluble than the *a* and β-crystals. The prism faces at first were curved, making measurement of the prism angle impossible, but later the crystals became more perfect. Single rhombic plates were rare, but the groups were not all irregular. Twinning is the normal type for these rhombic plates ("horse-type," text figure 196) with the twin axis in the base and normal to a prism-base edge. The composition face is the base. The crystals do not seem to elongate along the common edge so much as is usual in this type of twin, but remain symmetrical rhomboidal plates.

Pleochroism and absorption are not noticeable on the flat view; the color is bright oxyhemoglobin red. Double refraction is strong on the basal aspect as also on edge; extinction on the base is symmetrical. On edge the extinction is oblique looking along *b*, and straight looking along *a*; the extinction angle is 11° from the trace of the base. On the basal aspect, in convergent light, a biaxial interference figure is seen, with the brushes unsymmetrically placed with respect to the normal to the base. The orientation of the elasticity axes is $\mathfrak{a} \wedge \dot{c}$ about 10°, $\mathfrak{b} = b$, $\mathfrak{c} \wedge a = 11°$. The angle $\beta$ not being exactly determined, the angle $\mathfrak{a} \wedge \dot{c}$ is somewhat uncertain. The plane of the optic axes is the plane of symmetry (010) and the acute bisectrix $Bx_a = \mathfrak{a}$; the optical character is hence *negative.*

It would appear very probable that the hexagonal crystals of $\alpha$-oxyhemoglobin were simply mimetic twins of this $\gamma$-oxyhemoglobin; the twins consisting of a number of individuals twinned on the different prism-base edges, and producing a uniaxial effect as in the artificial twin of mica. Both the monoclinic and the hexagonal plates are negative; the monoclinic have their axis of greatest elasticity within 10° (about) of the normal to the plate, which becomes exactly normal, by averaging, in the multiple twins; while, in the hexagonal, the axis of greatest elasticity is normal to the plate also. The double refraction is not so strong in the hexagonal form as in the monoclinic; and this would have to be the case in such mimetic twins, as the elasticity of $\mathfrak{a}$ is diminished by its being inclined to the plate at an angle of some 80°, which would allow the influence of $\mathfrak{b}$ and $\mathfrak{c}$ to be shown in the direction normal to the plate; and, of course, these latter axes would neutralize each other to produce the apparently uniaxial character. If the $\gamma$-crystals were orthorhombic this lessening of the strength of the double refraction would not be so noticeable.

### *Reduced Hemoglobin and Metoxyhemoglobin of Marmota monax.*

As noted above, the hexagonal plates passed by paramorphous change into reduced hemoglobin and metoxyhemoglobin without any apparent change of form or of optical character. This could readily happen in such mimetic twins, even though the angles of the original rhomboidal plates should be slightly different from those of the $\gamma$-oxyhemoglobin; for the composite hexagonal crystal always changes the angles of the rhomboidal plates a few degrees, if necessary, to be exactly 60°. The change seemed to be from $\alpha$-oxyhemoglobin to reduced hemoglobin, and then from reduced hemoglobin to the metoxyhemoglobin. In the reduced hemoglobin the pleochroism on edge is quite strong; $\varepsilon$ nearly colorless, usually pale lilac; $\omega$ deep rose-pink. The elasticity was $\varepsilon > \omega$, and the optical character was *negative*. The metoxyhemoglobin showed the mixed spectrum of oxyhemoglobin and methemoglobin that is often described as methemoglobin. It seemed to be the final paramorphous change, following the change to reduced hemoglobin. The metoxyhemoglobin is also quite strongly pleochroic; $\varepsilon$ is colorless or pale yellow, $\omega$ is deep reddish-brown. The elasticity and optical character are as in the reduced hemoglobin.

## BEAVER, *Castor canadensis*. Plate 51.

The specimen was received from the Philadelphia Zoölogical Gardens in a putrid condition. The usual method of preparation was employed. Crystals formed readily after the slides were covered, and were at first long needle-like rods; but soon they became lath-shaped, and then plate-like crystals began to appear. They were not very stable, many crystals disintegrated and were dissolved within 24 hours after making the preparation. The crystals were oxyhemoglobin.

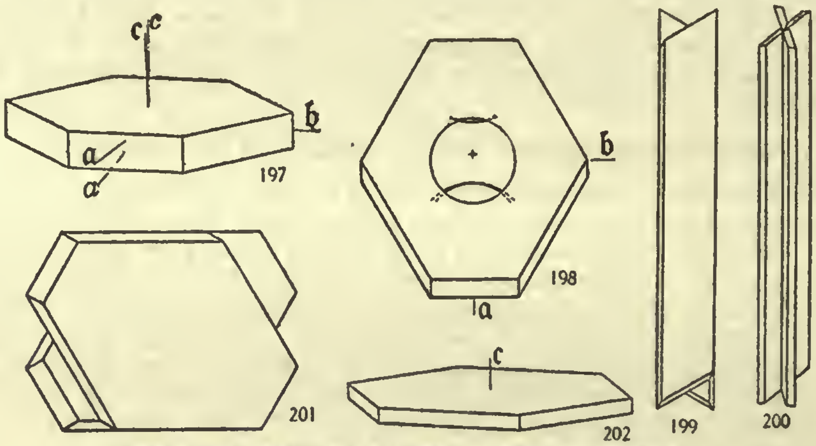
### *Oxyhemoglobin of Castor canadensis.*

Monoclinic: Axial ratio $a : b : \acute{c} = 1.732 : 1 : \acute{c}$; $\beta = 78°$ (about).

Forms observed: Unit prism (110), base (001), orthopinacoid (100).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 60°$ (or very nearly); orthopinacoid to base $010 \wedge 100 = 78°$ (about) $= \beta$; prism edge to base, edge $110\text{–}\bar{1}10 \wedge 001 = 90°$.

Habit tabular on the base, the combination being prism and base with a greater or less development of the orthopinacoid, making generally hexagonal plates or truncated lozenge-shaped plates (text figures 197, 198). The first crystals to appear are needles; when these attain dimensions to show planes they are generally seen to be twinned, and are the twin on a twin axis in the base and normal to a prism-base edge, along which the crystal appears to be elongated (text figure 199). In some cases the twin in these prismatic crystals seems to be on a unit pyramid, interpenetrant and forming an oblique cross-shape in the cross-section (text figure 200). The plates appear when these twins are visible; some are rhomboidal plates, apparently untwinned, but most of them seem to be twinned and in the twins the orthopinacoid planes seem to be more developed (text figure 201). By this twinning being several times repeated the crystals become nearly symmetrically hexagonal in outline, and perfect hexagonal plates appear sparingly (along with the obviously twinned crystals) that are apparently mimetic twins and really hexagonal in symmetry (text figure 202). The rhomboidal plates tend to grow into groups, by piling up of the plates (plate 51, fig. 303), and, as these are nearly all hexagonal in outline, due to development of (100), these groups closely resemble the similar forms seen in the hexagonal plates of other rodents, as the squirrels for example.



FIGS. 197, 198, 199, 200, 201, 202. *Castor canadensis* Oxyhemoglobin.

The color of the crystals is a bright scarlet or blood-red. Pleochroism on the basal aspect is hardly noticeable, but probably most of the crystals examined were twinned. The colors were: $\mathfrak{a}$ yellowish, $\mathfrak{b}$ yellowish-red, rather a strong color; $\mathfrak{c}$ deep blood-red. On the basal aspect the double refraction is very weak, and extinction is very hard to observe; it is, however, symmetrical. On the edge view, the double refraction is stronger, and looking along $a$ the extinction is straight; along $b$ it is 8° from the trace of the base or from the clino-axis, $a$. On the base in convergent light the interference figure is readily seen—a nearly uniaxial cross, which opens and closes as the crystal is revolved, showing the crystal to be biaxial. The angle of the optic axes, $2E$ is not above 7° or 8°.

The orientation of the elasticity axes is $\mathfrak{a} \wedge a = 8°$, the extinction angle; $\mathfrak{b} = b$, $\mathfrak{c} \wedge \acute{c} = 4°$ (about). The plane of the optic axes is the plane of symmetry, and the acute bisectrix is the axis of least elasticity, $Bx_a = \mathfrak{c}$. The optical character is hence *positive*.

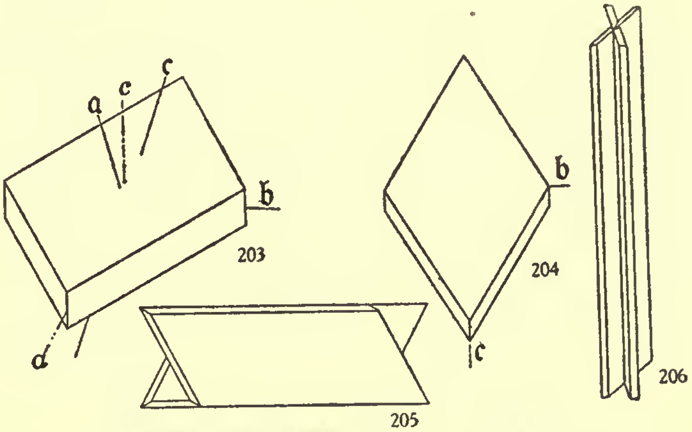
As nearly all of the crystals examined were twinned, and as these mimetic twins tend to become uniaxial, it is possible that the above-described interference figure is due to twinning; but if so, the orientation of the optic axes is not altered nor is the optical character.

### MUSKRAT, *Fiber zibethicus*. Plates 51 and 52.

The living animal was procured from a collector and bled in the laboratory. The blood was oxalated, laked with ether and centrifugalized; from the clear solution slide preparations were made in the usual manner. The crystals formed readily soon after covering the slides; at first, the crystals were fine needles, but afterwards these became lath-shaped, or flat prismatic crystals appeared amongst the needles; and, at the same time, tabular crystals began to appear. They kept well, showing no sign of dissolving. Crystallization continued after sealing the slides, until practically the entire slide was filled with crystals. The crystals are oxyhemoglobin.

### *Oxyhemoglobin of Fiber zibethicus.*

Monoclinic: Axial ratio $a : b : \acute{c} = 1.6318 : 1 : \acute{c}$; $\beta = 68°$.
Forms observed: Unit prism (110), base (001).
Angles: Prism angle $110 \wedge 1\bar{1}0 = 63°$; prism edge to base, edge $110–1\bar{1}0 \wedge 001 = 68° = \beta$.



FIGS. 203, 204, 205, 206. *Fiber zibethicus* Oxyhemoglobin.

Habit thin tabular on the base, the crystal consisting of the base (001) bounded by the prism (110). The first crystals to form are long needles or lath-shaped crystals with oblique ends; among these soon appear blade-like crystals of the same habit, often twinned; and, at about the same time, lozenge-shaped tabular crystals appear all through the slides. These latter (text figures 203 and 204), which are undistorted crystals, are very symmetrical rhombic plates, sometimes untwinned, sometimes twinned repeatedly. They do not seem to form mimetic twins and develop into hexagonal plates, as is so commonly the case with rodents. The twins are of the usual horse-type (text figure 205), on a twin axis normal to a prism-base edge and lying in the base. But in the blade-like crystals these appear as contact twins with the common prism-like edge parallel to the length of the blade-like crystal; the blades being elongated in the direction of two opposite prism faces, and consisting, therefore, of the two basal faces and two prism faces and terminated by the prism faces. This elongation produces in the untwinned crystals a triclinic appearance. In these same blade-like crystals another kind of twinning is very commonly seen, on a unit pyramid as the plane of twinning, the twin being interpenetrant and showing an X-shaped cross-section (text figure 206). These were also observed in the plates. The twins of the plates are, as stated, of the usual horse-type, but the plates being very symmetrical the group formed by the twinning has often the outline of a truncated triangle, and sometimes is nearly triangular. By parallel growth the plates become greatly elongated in the direction of the clino-axis, forming parallel growth groups, and they also grow together on the base in groups, extending in the direction of the same axis. Sometimes the rhombic plates form radiating groups by uniting on the base, the radial character showing when the plates are seen on edge.

On the base, the color of the crystals is a deep scarlet, owing to the very slight pleochroism on this aspect. Pleochroism is weak on the base, but strong when the edge aspect is presented; $\mathfrak{a}$ pale reddish-orange, $\mathfrak{b}$ blood-red; $\mathfrak{c}$ blood-red, somewhat deeper than $\mathfrak{b}$, but the two practically equal. Double refraction is so weak on the basal aspect that the quartz wedge scarcely shows the difference between $\mathfrak{b}$ and $\mathfrak{c}$. On the edge view, looking along $\mathfrak{c}$ or $\mathfrak{b}$, however, the double refraction is quite strong. On the edge view looking along $\mathfrak{b}$ the extinction is oblique, about 15° in the acute angle. The orientation of the optic axes is $\mathfrak{a} \wedge \acute{c} = 37°$, in the obtuse angle; $\mathfrak{b} = b$; $\mathfrak{c} \wedge a = 15°$, in the acute angle. The plane of the optic axes is the plane of symmetry (010), and the acute bisectrix is the axis of least elasticity, $Bx_a = \mathfrak{a}$; the optical character is hence *negative.*

### White Rat, Albino of *Mus norvegicus* (*Mus norvegicus var. albus* Hatai). Plate 53.

A number of specimens were examined at different times, the living animals being bled in the laboratory. The general method of preparation was to bleed the animal into oxalate, lake the whole blood with ether, and centrifugalize. From the clear solution slide preparations were made as usual. Modifications of the method above described, using corpuscles and adding variable amounts of plasma in excess of the normal, gave about the same results as the preparations of the whole blood. The blood crystallizes very readily, so much so that the crystals are usually small unless methods of preparation are used that retard crystallization. Being small, they show but little color; the crystals examined were, in each case, determined to be oxyhemoglobin by the microspectroscope. A superficial examination shows that the crystals are of several habits, and they look as though they were of different systems. Careful study shows, however, that they are all of the same crystallization, although one type, a hexagonal plate, seems to be sometimes a mimetic twin of the normal crystals.

#### *Oxyhemoglobin of Albino of Mus norvegicus.*

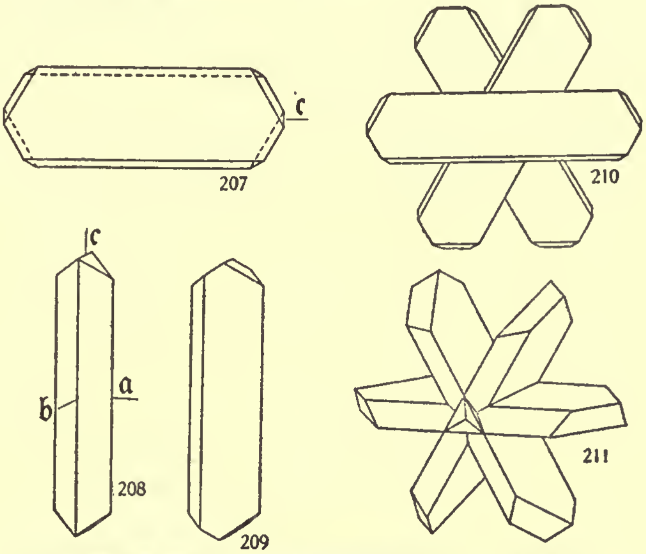
Orthorhombic: The axial ratio was calculated from the traces of the macrodome on the prism, assuming the same prism as in *Mus norvegicus*; it is $a : b : \dot{c} = 0.7829 : 1 : 0.7332$.

Forms observed: Unit prism (110), brachydome (101).

Angles: The only angles that can ordinarily be observed are the plane angles between the edges produced by a prism face intersecting the two brachydome faces, that is edges $110\text{–}011 \wedge 110\text{–}0\bar{1}1 = 120°$. The half of this angle can also often be measured, and it is 60° actual angle. In twins of the stellate shape, the edges are inclined to each other at 60°. As this dome angle on the prism is the same as in *Mus norvegicus*, the true dome angle $011 \wedge 0\bar{1}1$ is assumed to be the same also, 72° 30′, which makes the prism angle $110 \wedge 1\bar{1}0 = 76° 7'$.

Habit thin tabular, elongated along the vertical axis and the crystal tabular on two opposite faces of the unit prism (110), the end being formed by the faces of the brachydome (text figure 207). The tabular crystals are thus roughly six-sided with two sides longer than the other four. Some symmetrically developed crystals of the combination of prism and brachydome were seen (text figure 208), but they were always very small. Generally when the prism faces were equally developed, which occasionally happened, the dome faces were unsymmetrical, two opposite faces being larger and the other two smaller, but usually two opposite prism faces were larger, making the tabular crystal, and the other two prism faces were smaller (text figure 209). The prism is very nearly square, although evidently not quite so; but no cross-sections of it could be obtained for measurement. The ratio given $a : b = 0.7829 : 1$ was calculated by assuming the same prism that was determined for the Norway rat crystal, which gave the same plane angle of macrodome on prism face as in this albino variety. The usual crystals are hence like vertical sections of the prism, parallel to one pair of faces; and, as the section approaches the exterior of the symmetrical crystal, the outline of the section becomes nearly four-sided; whereas a median section is nearly regularly six-sided, with four short and two long sides. This flattening of the prism produces the tabular effect, and there is, therefore, a flat view and an edge view of each crystal possible. The above descriptions refer to the flat view, but the edge view is quite analogous. The crystals twin by growing together on a prism face either on the flat or on the edge aspect, with the prism edges of the individuals of the trilling (which it usually is), at almost exactly 60° with each other. When this is on the flat aspect the crystals seem to pile up on each other at the 60° angle (text figure

210) and there may be more than three in the combination. This kind of grouping produces roughly hexagonal plates, and even fairly regular hexagonal tabular crystals, in which the composite character can, however, usually be made out. Rarely, this composite character almost disappears when the members of the twin are many and very thin, and the crystal then becomes pseudohexagonal; this is the normal hexagonal crystal of the rodents. When, on the other hand, the crystals twin on edge, they seem to be more interpenetrant; although in these, too, there is often the appearance of piling up. The twins of crystals on edge are in the form of six-rayed star-shaped groups (text figure 211). In some cases the two aspects of the crystals are presented in the same group, as is natural, for there is no essential difference of structure on the two kinds of prism faces, the broad face and the narrow face. The twin on the flat seems to be on a brachypyramid nearly, or quite in the zone of the prism-dome edge, but the composition face is the unit prism; in the star-shaped twins, the twin plane and composition face are a pyramid of the unit series.



Figs. 207, 208, 209, 210, 211. *Mus norvegicus albus* Oxyhemoglobin.

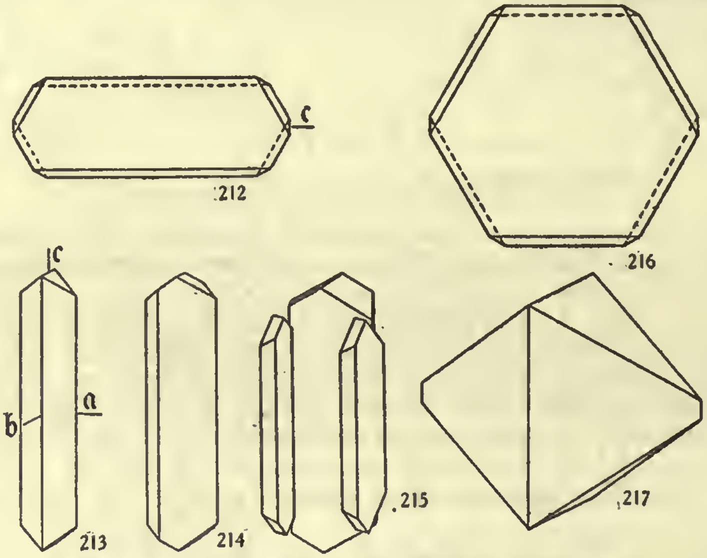
The crystals therefore occur in five habits or forms:

(1) Prismatic crystals, long or short, consisting of the symmetrically developed prism and generally unsymmetrically developed brachydome. Rather rare, but the most nearly symmetrical crystal.
(2) Elongated six-sided plates formed from (1) by flattening on two opposite prism faces. The common single crystal.
(3) Composite and rough hexagonal plates, twins of (2) on the brachypyramid, presenting the tabular aspect. The common crystal.
(4) Six-pointed star-shaped twins, produced by twinning on the unit pyramid, presenting the narrow planes or edge aspect. Almost as common as (3).
(5) More or less regular hexagonal plates, mimetic twins of the type of (3). Rather rarely observed.

The color of the crystals is rather pale, owing to their very small size, but pleochroism is quite noticeable; $\mathfrak{a}$ and $\mathfrak{b}$ nearly equal, almost colorless in these minute crystals; $\mathfrak{c}$ is reddish-orange to deeper red. The orientation of the elasticity axes is only approximately made out for $\mathfrak{a}$ and $\mathfrak{b}$, which are nearly equal, but $\mathfrak{c}=\dot{c}$. The peculiar development of the crystals renders it nearly impossible to get views along axes $\mathfrak{a}$ and $\mathfrak{b}$; there can be little doubt, however, that the acute bisectrix $Bx_a=\mathfrak{c}$, which would make the optical character *positive.*

### NORWAY OR BROWN RAT, *Mus norvegicus*. Plate 54.

The specimen of blood was received from the Wistar Institute of Anatomy, Philadelphia. The animal was bled into oxalate, and the blood used immediately. On laking, the blood at once began to crystallize, and within a few minutes a considerable amount of the crystals of oxyhemoglobin had formed in the tube. These were separated by centrifugalization, and from the clear mother-liquor the slide preparations were made. After covering the slides crystals formed rapidly, and they were quite insoluble. The color of the plasma was almost entirely discharged, showing the crystallization to be nearly complete. The crystals were small and thin, as in the case of the white rat. They kept well and showed no tendency to dissolve upon moderate increase of temperature. Even after a month the form of the crystals in the slides had not changed materially. The crystals were oxyhemoglobin. Two forms of the oxyhemoglobin appeared: one prismatic, and probably orthorhombic, like the white-rat oxyhemoglobin; the other isotropic and apparently isometric, but showing hexagonal outlines. The prismatic form was the first to appear; the isotropic form developing later may be an isomer of the first form or a mimetic twin. They are distinguished as α-oxyhemoglobin and β-oxyhemoglobin.



FIGS. 212, 213, 214, 215, 216, 217. *Mus norvegicus* α-Oxyhemoglobin.

### *α-Oxyhemoglobin of Mus norvegicus.*

Orthorhombic: Axial ratio $a : b : \dot{c} = 0.7829 : 1 : 0.7332$.

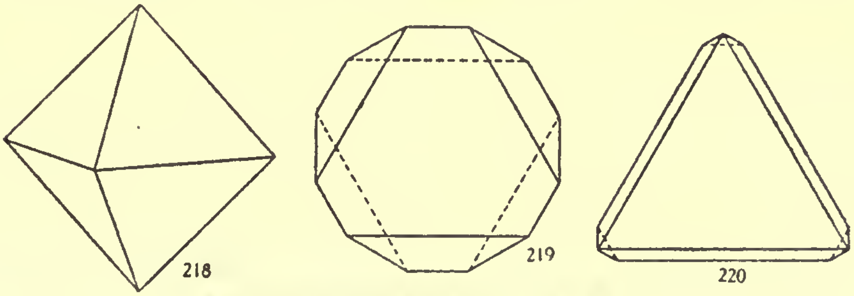
Angles: Brachydome angle $011 \wedge 0\bar{1}1 = 72° 30'$; the prism angle was not observed, but was calculated as 76° 7′; profile of dome edges over pole when the prism lies on its side, edges $110\text{–}011 \wedge \bar{1}\bar{1}0\text{–}0\bar{1}1 = 120°$; this is the plane angle of the dome on the prism.

Habit of the first crystals to form prismatic and generally flattened on two opposite prism faces, making a six-sided tabular crystal elongated on the vertical axis, as is common in the white rat (text figure 212). Some symmetrically developed prismatic crystals were observed that showed the dome termination in symmetrical development; these looked like normal orthorhombic crystals (text figure 213). But the distorted crystals, flattened on two opposite prism faces (text figure 214), have a decidedly monoclinic aspect. Nothing that was observed of the optical characters could determine that these crystals were not orthorhombic; but no end views, looking along the length of the crystal, could be obtained, and hence they may be really monoclinic. Twins on the brachy and unit pyramids formed as has been described for the white rat; on the flat aspect, twinned on the brachypyramid, they make pseudo-hexagonal groups; and twinned on the unit pyramid, with the edge of the flattened prism presented, they make six-pointed star-shaped groups (text figures 210, 211). What appears to be a twin on the prism also occurs, in some cases producing the effect of a carpenter's miterbox, where the two crystals on edge appear on either side of one presenting the flat aspect (text figure 215). As the crystals continue to develop, short prismatic crystals, flattened on two prism faces, appear, and they produce hexagonal plates, owing to the angle of 120° of the dome profile, which is of course the same as the profile of dome to prism outline (text figure 216). These crystals show much less double refraction than the elongated crystals and are sometimes practically isotropic. When the prism is symmetrically developed and in equilibrium with the dome (text figure 217) the crystals resemble octahedra, and they appear to pass into isometric octahedra, the β-oxyhemoglobin crystal.

Pleochroism is marked in the elongated prismatic crystals, but wanting in the hexagonal plates and in the equidimensional, octahedral-looking, prism-dome combinations. The colors are $\mathfrak{a}=\mathfrak{b}$ (about), pale yellowish-red to pale red; $\mathfrak{c}$ deeper red. No end views were seen, so that the pleochroism of $\mathfrak{a}$ and $\mathfrak{b}$ could not be differentiated. Double refraction is strong in the long crystals, but very weak or entirely wanting in the equidimensional crystals and in the hexagonal plates. The symmetrical crystals in convergent polarized light showed traces of the brushes of an interference figure, looking along the macro-axis, the brushes passing out of the field upon rotation of the stage, showing that the observation was being made on the obtuse bisectrix of the optic axes. The orientation of the elasticity axes is $\mathfrak{a}=b$; $\mathfrak{b}=a$; $\mathfrak{c}=\dot{c}$. No observation of the interference figure, looking along the acute bisectrix was possible, but the acute bisectrix is the axis of least elasticity, $Bx_a=\mathfrak{c}$, and the optical character is *positive.*

### *β-Oxyhemoglobin of Mus norvegicus.*

Isometric or pseudo-isometric.
Forms observed: Octahedron (111).
Angles: The angle over the pole of the octahedron 111 ∧ $\bar{1}\bar{1}1$ = 71° (about).



Figs. 218, 219, 220. *Mus norvegicus* β-Oxyhemoglobin.

Habit octahedral; symmetrical isometric octahedra, or distorted octahedra formed by the crystal lying on one face and hence developing into forms with a nearly triangular to almost hexagonal profile (text figures 218, 219, 220). These hexagonal sections of the octahedron closely resemble the hexagonal-looking tabular crystals of the α-oxyhemoglobin, but may be distinguished from them by careful examination.

The crystals show the oxyhemoglobin red of the white and Norway rats, rather a pale brownish or yellowish-red. There is no pleochroism nor double refraction; the crystals appear to be absolutely isotropic.

The passage of the orthorhombic crystal of α-oxyhemoglobin into the isometric or pseudo-isometric crystal of β-oxyhemoglobin, that was described above, depends upon the pseudo-isometric character of the α-oxyhemoglobin (its angles being near the angle of the octahedron), if no change in substance occurs in the change from the distinctly orthorhombic crystal to the pseudo-isometric crystal of the hexagonal or equidimensional type. More probably there is a change of substance and an isomer or polymer is formed (or may already exist in the solution) and then the hexagonal or the equidimensional form is a mixed crystal containing both α-oxyhemoglobin and β-oxyhemoglobin. The influence of the β-oxyhemoglobin on the α-oxyhemoglobin, or the concentration of the solution, determines the conversion of the α-oxyhemoglobin into the other isomer (or polymer) β-oxyhemoglobin.

### BLACK RAT, *Mus rattus*. Plates 54 and 55.

Specimens of the blood of the black rat were obtained from the Wistar Institute of Anatomy, of Philadelphia. The animal was bled into oxalate and the blood used immediately. The corpuscles, separated by centrifugalizing, were laked with ether, oxalated, and the solution again centrifugalized. From the clear solution thus obtained the slides were prepared. The blood crystallized very readily; in fact, it is probable that better preparations would have been obtained if the whole blood had been used. The crystals do not form so readily as those of the white rat or the Norway rat, but they are quite permanent, show no signs of dissolving on slight increase of temperature, and they keep for weeks in the slides. They are not nearly so insoluble as the crystals of the white or Norway rats, however, and upon an increase of temperature, up to a temperature of 25° C., they begin to dissolve, so that they can not be satisfactorily studied in warm weather. This character is in sharp distinction from the insolubility of the oxyhemoglobin crystals of the white and Norway rats, which are permanent at temperatures up to 35° C. The crystallization is not so complete as in the case of the other rats mentioned, so that the fluid remains of a strong red color, showing much oxyhemoglobin still in solution.

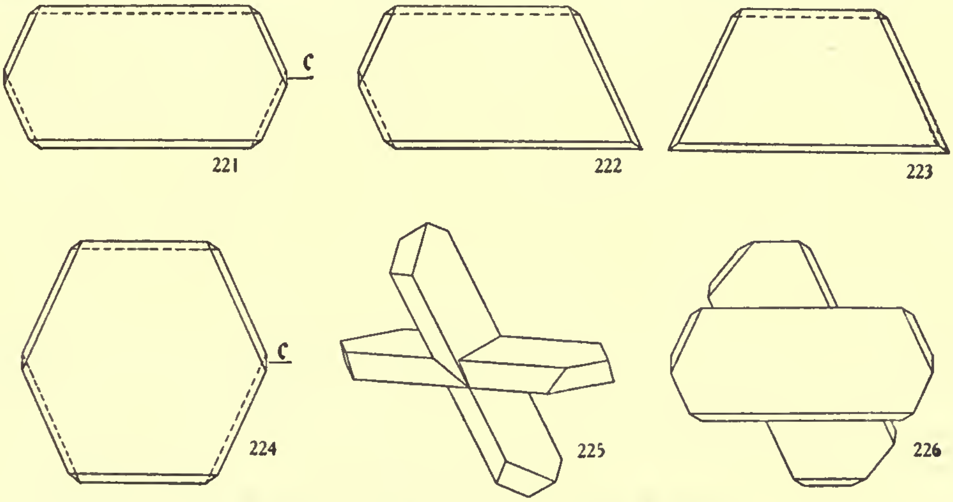
#### *Oxyhemoglobin of Mus rattus.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.7829 : 1 : 0.5864$.

Forms observed: Unit prism (110), macrodome (101).

Angles: No cross-sections of the prism could be observed. The only angle that can be determined is the plane angle of the brachydome on the prism face, edges $110\text{–}011 \wedge 110\text{–}0\bar{1}1 = 130^\circ\,26'$, average of nine measurements. Assuming the same prism for this rat that was determined for the Norway rat from the true dome angle and the plane angle of the dome on the prism, the axial ratio was calculated. This makes the macrodome of the black rat (101), the macrodome (405) on the axial ratio of the Norway rat, the average measured angle for the edges, 130° 26′, agreeing exactly with the calculated value.

Habit tabular on two faces of the prism, the crystal consisting of the prism (110) and the brachydome (011). The prism is flattened on two opposite faces, as is common in the rats (text figure 221), and the dome termination may be of four equally developed dome faces, or two large and two small dome faces, or even of two equally developed faces on one end and one large and one small face on the other end. In some crystals two dome faces appear at one end of the prism and only one at the other end, making a five-sided plate (text figure 222). When two dome faces on the same side of the crystal are developed (one at each end), the plate becomes unsymmetrically four-sided (text figure 223). By shortening of the prism the tabular crystal becomes hexagonal in outline (text figure 224); the crystals do not elongate into the long tabular crystals so common in the Norway and albino rats. Twinning on the flat and on edge, as seen in the white rat, was observed, but it did not occur so frequently as in the case of that species. The twinning is upon a pyramid in each case, as is common in the rats. The twins on edge were of two individuals only, as a rule, and did not form the six-pointed star like Norway and white rat twins (text figure 225). On the flat, the twin consists of two individuals also (text figure 226), and does not result in the formation of a hexagonal plate, as in the case of the Norway and white rat crystals. The difference in the twins is no doubt due to the fact that in the twin on the brachypyramid the prism edge of one individual is parallel to the prism-dome trace of the other; but in the corresponding twins of the white rat and the Norway rat crystals the angle of the twin of the prism edges in the three members is 120°, while in the black rat (and Alexandrine rat) the angle of the prism edges in the two members of the twin is about 130° 25′, being for the Alexandrine rat 130° 19′ and for the black rat 130° 26′. Three crystals of the Norway or white rat could twin at the angle of 120° to make a regular hexagonal plate; but three crystals of the black or Alexandrine rat so twinned could not produce a regular hexagonal plate.



FIGS. 221, 222, 223, 224, 225, 226. *Mus rattus* Oxyhemoglobin.

Pleochroism is fairly strong, but from the positions of the crystals presented 𝔞 and 𝔟 can not be directly observed. The colors of 𝔞 and 𝔟 are evidently close together, ranging from pale yellowish-red to deeper red, according to the thickness of the crystal. The color of 𝔠 is always much deeper; even in the thinner plates it is a deep red. Double refraction is strong, extinction is straight in all aspects presented. The orientation of 𝔞 and 𝔟 could not be observed; it is probably the same as in the Norway rat, 𝔞 $=b$, 𝔟 $=a$; the axis of least elasticity 𝔠 $=\dot{c}$. From the fact that the elasticities of 𝔞 and 𝔟 are nearly the same, it is probable that the axis of least elasticity 𝔠 is the acute bisectrix, $Bx_a =$ 𝔠; this makes the optical character *positive*. No interference figure could be observed.

### ALEXANDRINE RAT, *Mus alexandrinus*. Plate 55.

Specimens of the blood of the Alexandrine rat were obtained from the Wistar Institute of Anatomy, of Philadelphia. The blood was collected in oxalate and was used immediately. The corpuscles were separated by centrifugalization, laked with ether, additional oxalate added, and the solution centrifugalized. The slide preparations were made as usual. Crystallization proceeded rapidly before and after covering the slides;

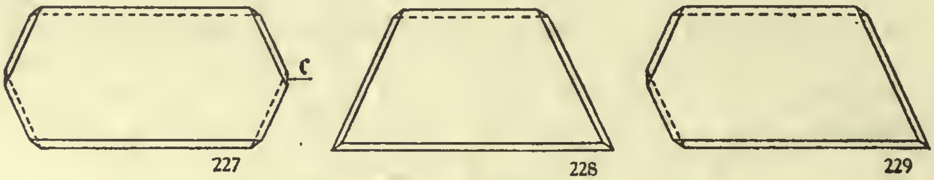
the crystals kept well at temperatures under 15° C., but did not form well at 25° C. or higher. At moderate temperatures they showed no tendency to dissolve. They were oxyhemoglobin. In solubility they resemble the crystals of the black rat, being perhaps somewhat more soluble, but they are very much more soluble than the crystals of the white rat or the Norway rat. Crystallization was not complete, the mother-liquor remaining quite strongly colored. The crystals in the slides were in good condition a month after the preparations were made. Only one kind of crystal was observed, corresponding to the α-oxyhemoglobin of the Norway rat.

### *Oxyhemoglobin of Mus alexandrinus.*

Orthorhombic: Axial ratio $a : b : \dot{c} = 0.7829 : 1 : 0.5880$.

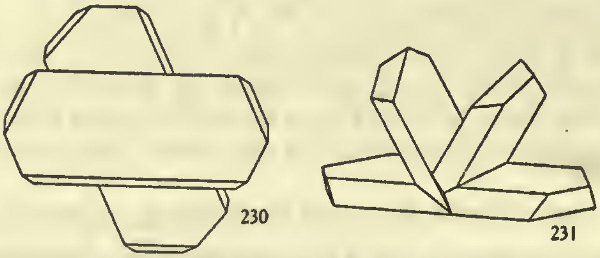
Forms observed: Unit prism (110), brachydome (011).

Angles: From the aspects presented by the crystals the prism angle could not be measured; it was assumed as the same as that determined for the Norway rat, 110 ∧ $1\bar{1}0$ = 76° 7′. The only angle that could be measured was the plane angle of the brachydome on the prism face; this was, edges 110–011 ∧ 110–$0\bar{1}1$ = 130° 19′, the average of a number of measurements. From this angle and the assumed prism angle of 76° 7′ the axial ratio was calculated. The true brachydome angle 011 ∧ $0\bar{1}1$ could not be observed.



Figs. 227, 228, 229. *Mus alexandrinus* Oxyhemoglobin.

Habit tabular on two opposite prism faces and somewhat elongated parallel to the vertical axis (text figure 227); the crystal consists of the flattened prism terminated by the flat brachydome. Distorted crystals, in which the two brachydome faces on one side of the prism are alone developed (text figure 228), or crystals with one end so developed and the other showing the two dome faces (text figure 229), are very common; these distorted crystals are rather more common in the crystals of this species than in those of the black rat. There are thus formed four, five, and six-sided plates, the four and five-sided tabular crystals having a decidedly hemimorphic aspect. By shortening of the prism the tabular crystal becomes a hexagonal plate, but owing to the two angles of 130° 19′ it is not a regular hexagon. The crystals are considerably larger than those of the black rat in preparations made under the same conditions, indicating that they are more soluble than those of the black rat. Twinning occurs on the flat aspect; two crystals united on the prism face with the orientation that of a twin in the zone of the brachypyramid, which would bevel the dome-prism edge (text figure 230); but such twins are very rare. The stellate twin on a pyramid of the unit series is more common,



Figs. 230, 231. *Mus alexandrinus* Oxyhemoglobin.

sometimes forming a six-rayed star, like text figure 211; more commonly forming X-shaped twins by the interpenetration of two prisms, like text figure 225; and also often showing interpenetration, but with one or two of the rays developed on one side of the prism only (text figure 231). The twin in the zone of the brachypyramid can not form mimetic hexagonal crystals because of the flat dome angle. The axial ratio of this species is related to that of the *Mus norvegicus* groups of rats by the same 5 : 4 ratio that was true of the black rat. Indeed, except for the difference in solubility and in habit of crystal there is no great difference between crystals of the black and the Alexandrine rats, and these rats look like varieties of the same species. The difference in angle of dome on prism, that makes the slight difference in axial ratio, is within the limit of error of the method of observation, and the axial ratios of the crystals of the two species are probably identical.

Pleochroism is rather strong, but, owing to the positions of the crystals presented, it is not possible to distinguish between $\mathfrak{a}$ and $\mathfrak{b}$, the colors of which are apparently nearly alike. The pleochroic colors are $\mathfrak{a}$ and $\mathfrak{b}$ pale to deeper yellowish-red; $\mathfrak{c}$ rather deep red. Double refraction is strong and extinction is straight in all aspects that could be observed. The orientation of $\mathfrak{a}$ and $\mathfrak{b}$ could not be observed, but may be assumed to be the same as in the Norway rat, $\mathfrak{a}=b$, $\mathfrak{b}=a$; the third axis could be observed and gave $\mathfrak{c}=c$. No interference figure could be observed, but from the fact that $\mathfrak{a}=\mathfrak{b}$ (nearly) it is probable that the axis of least elasticity $\mathfrak{c}$ is the acute bisectrix, $Bx_a=\mathfrak{c}$; and the optical character is *positive.*

Reviewing these four species of rats it will be seen that they may be arranged in two groups: (1), the *Mus norvegicus* group, comprising *Mus norvegicus* and its albino, *Mus norvegicus albus* Hatai, in which the crystals are orthorhombic and pseudo-isometric, with an axial ratio of 0.7829 : 1 : 0.7332; and (2), the *Mus rattus* group, comprising *Mus rattus* and *Mus alexandrinus,* in which the crystals are orthorhombic, but not pseudo-isometric, and the axial ratio is 0.7829 : 1 : 0.5864 or 0.7829 : 1 : 0.5880. As noted above, these ratios for the vertical axis stand in the ratio of 5 : 4; but that they are different axial ratios, and not simply different crystal habits, is shown by the twins in the brachypyramid zones following these ratios in each case, thus indicating that the difference is one of the form of structure of the crystal and not simply a difference of development. The oxyhemoglobin of the *Mus norvegicus* group is not the same substance as the oxyhemoglobin of the *Mus rattus-alexandrinus* group.

### Canadian Porcupine, *Erethizon dorsatus.* Plates 56 and 57.

The specimen was received from the Philadelphia Zoölogical Gardens and was in the form of rather hard clots. The clotted blood was ground up in sand and ether, a little oxalate and water added, and the mixture centrifugalized. From the clear liquid thus obtained the slide preparations were made as usual. Crystals began to appear within about half an hour after the slides were covered; they were rather small and thin and showed a tendency to dissolve in the solution. These are described as $\alpha$-oxyhemoglobin. Inside of 20 hours, they had mostly been dissolved from the slides, and their place was taken by the crystals of $\beta$-oxyhemoglobin; in only a few slides were there any of the $\alpha$-oxyhemoglobin crystals remaining. The second crop of crystals, $\beta$-oxyhemoglobin, appears to be less soluble and more stable than the $\alpha$-oxyhemoglobin crystals.

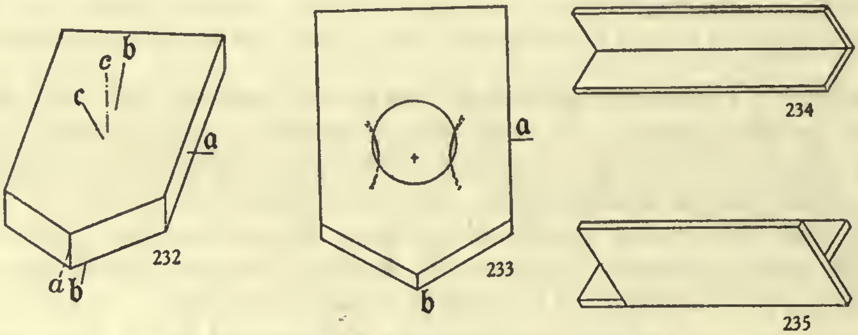
A second preparation was made the next day, a new lot of slides being mounted from the cleansed blood above described, to which an additional amount of oxalate had been added. This blood was highly charged with ether (from the method of preparation) and this prevented the development of bacteria, so that the blood kept well. In this series, the first crystals to appear were the $\alpha$-oxyhemoglobin crystals, as before, but of a different type; and soon after they appeared the $\beta$-crystals began to form, of the same habit as in the first experiment. All the crystals examined were determined to be oxyhemoglobin by the microspectroscope.

### *a-Oxyhemoglobin of Erethizon dorsatus.*

Monoclinic: Axial ratio $a : b : \acute{c} = 0.5543 : 1 : \acute{c}$; $\beta = 56°$.

Forms observed: Unit prism (110), orthopinacoid (100), clinopinacoid (010), basal pinacoid (001).

Angles: Prism angle, traces of the prism on the base, edges $110\text{–}001 \wedge 1\bar{1}0\text{–}001 =$ 58° (normals); orthopinacoid to base $100 \wedge 001 = 56° = \beta$.



FIGS. 232, 233, 234, 235. *Erethizon dorsatus a*-Oxyhemoglobin.

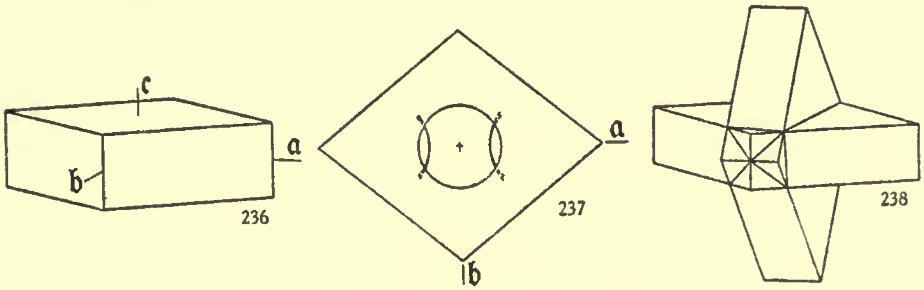
Habit tabular on the base; in the untwinned crystals of the first preparation, type (a), the combination was the base cut by one-half of the prism and one face of the orthopinacoid, with the two faces of the clinopinacoid (text figures 232, 233), much in the habit of the clinohedrite type of the monoclinic system (domatic class). In the twinned crystals of the second preparation, from blood containing more oxalate, type (b), while the corresponding angles are the same, the crystal is a twin on a normal to the prism-base edge, but so developed that it looks as though the composition plane was the normal to the base that included this common prism-base edge (text figure 234). The obtuse prism angle of 122° (or 58° normals) appears symmetrically four times on these twins, while the double of the acute angle 116° (or $58° \times 2 = 64°$ normals) appears twice in symmetrical position. In some crystals the development is as usual in this horse-type of twin; the two parts united on the base, elongated on the common prism-base edge, and with the ends overlapping (text figure 235). These twins are evidently repeated in polysynthetic order, and the optical characters for the twin are abnormal, being the summation of the optical characters of its members, which vary in their orientation. Hence the twin has a plane of symmetry for its optical characters as seen on the flat, which is normal to the base, and includes the prism-base edge, the twin plane in short.

The crystals are rather strong oxyhemoglobin red; the simple crystals, being thin, are paler than the twins. On the base, the simple crystals of type (a) are distinctly pleochroic, but not strongly so; on edge, the pleochroism is quite strong. The pleochroic colors in these thin crystals of type (a) are $\mathfrak{a}$ pale yellowish-red, $\mathfrak{b}$ rather pale scarlet, $\mathfrak{c}$ (on edge) deep red. In the second type of crystals, the pleochroism on the base is very slight, owing to the averaging of the elasticities in the composite crystal; and the apparent $\mathfrak{a}$ comes much nearer to the apparent $\mathfrak{b}$. On edge, these still show considerable pleochroism; but the contrast is not so strong as in the untwinned crystals. Extinction in type

(a) is symmetrical on the base and straight looking along the *a*-axis; but is 19° to 20° (perhaps higher) on the edge view, looking along *b*. In the twins of the second type of crystal, the extinction is symmetrical with the outline and parallel to the twin axis. In the untwinned crystals the interference may be easily seen in traces when the crystal does not lie flat; but is not readily observed when the crystal is flat, only traces of it showing. This is due to the orientation of the elasticity axes, which is $\mathfrak{a}=b$; $\mathfrak{b} \wedge a=20°$, in the acute angle; $\mathfrak{c} \wedge \acute{c}=54°$, in the obtuse angle. The plane of the optic axes is normal to the plane of symmetry and inclined to the orthopinacoid at an angle of 54°, in the obtuse angle; it is hence inclined 34° to the normal to the base. The acute bisectrix is the axis of least elasticity, $Bx_a=\mathfrak{c}$, and the optical character is *positive*.

### *β-Oxyhemoglobin of Erethizon dorsatus.*

Orthorhombic: Axial ratio $a : b : \acute{c}=0.8170 : 1 : \acute{c}$.
Forms observed: Unit prism (110), base (001).
Angles: Prism angle $110 \wedge 1\bar{1}0=78° 30'$ (normals); prism to base $110 \wedge 001=90°$.



FIGS. 236, 237, 238. *Erethizon dorsatus* β-Oxyhemoglobin.

Habit tabular on the base; consisting of the short prism (110) cut by the basal pinacoid (001) (text figures 236 and 237); the ratio of length to breadth of the prism being generally about 1 : 3, but the tabular crystals thicken and become equidimensional in some cases. The crystals are generally more or less hopper-shaped on the base and evidently grow more actively on the prism faces and edges and less so on the base; the result is often the appearance of a skeleton crystal, looking down upon (001), with the crystal axes marked out in more solid substance. On edge views and sections, the solid central part is seen to run out from the center to the four coigns of the basal face; the intervening parts, between these four directions and the outer surfaces of the prism, having a porous aspect. These crystals are relatively large, much larger than the crystals of the *α*-oxyhemoglobin that have been described. The crystals showed parallel growth; and a twin, of the interpenetrant type, on a brachydome (text figure 238), was seen, the two parts crossing at an angle of nearly 90°.

The crystals are a rather bright scarlet color, and on the flat as well as on edge they are quite pleochroic. The pleochroism is $\mathfrak{a}$ nearly colorless to yellowish-red, depending on the thickness; $\mathfrak{b}$ deep scarlet to blood-red; $\mathfrak{c}$ deep blood-red. On the base, the extinction is symmetrical; and on edge views it is straight in all aspects, when the section is normal to the base. On the base, in convergent light, a biaxial figure is seen, symmetrically placed, with the separation of the brushes wide; $2E$ is above 75°, but was not exactly measured. The orientation of the elasticity axes is: $\mathfrak{a}=b$; $\mathfrak{b}=a$; $\mathfrak{c}=\acute{c}$. The plane of the optic axes is the macropinacoid and the acute bisectrix $Bx_a=\mathfrak{c}$. The optical character is hence *positive*.

Comparing the two crystals, *α*-oxyhemoglobin and *β*-oxyhemoglobin, it is interesting to note that the true prism angle is nearly the same in each, 78° 30′ for the *β*-oxyhemoglobin against 79° 20′ in the *α*-oxyhemoglobin; it is quite possible that the orthorhombic form is some sort of a mimetic twin of the monoclinic form. The skeleton character of the crystal is an indication pointing in the same direction.

### GUINEA-PIG, A DOMESTICATED VARIETY OF *Cavia cutleri*. Plates 57 and 58.

The blood of the guinea-pig is so easy to procure and to crystallize, that it has been studied very carefully. Our specimens were obtained by bleeding the live animals in the laboratory, and were always, therefore, fresh blood. Many variations of the method of preparation were tried to see if such variations produced any change or modification of the crystals. The ordinary methods of preparation were usually followed in preparing crystals for crystallographic study. The whole blood, oxalated, was laked with ether and centrifugalized to obtain a clear solution, from which the slide preparations were made. In such a solution crystallization is very rapid, and the crystals are apt to be small. Diluting the blood with plasma from the same blood was tried, with the result that the crystals were increased in size, while remaining very perfect. In some cases, the blood (defibrinated by beating without the addition of oxalate) was thus diluted with the plasma of another portion of the sample that had been oxalated to prevent clotting; and from this, also, very large and perfect crystals were obtained. In the diluted blood, when the crystals grow to large size, there is generally more tendency to form twins than in the undiluted blood. To see whether the addition of certain inorganic salts had any effect upon the form of the crystals, preparations were made from the same specimen of blood (defibrinated by beating and laked with ether) as follows:

(1) By the usual method of adding ammonium oxalate: In this case crystals formed very rapidly, a pellicle of crystals appearing on the drop on the slide before the cover was applied. The separation of the oxyhemoglobin was so complete that the solution became colorless. Twins formed only sparingly.

(2) Substituting potassium oxalate for ammonium oxalate: The pellicle of crystals did not form on the drop, but crystallization proceeded until the solution was nearly colorless. The crystals showed a tendency to form twins and irregular aggregates, otherwise they were like the normal type.

(3) Substituting sodium oxalate for ammonium oxalate: Crystallization was slower than in cases (1) and (2), but the crystals were larger and more perfectly formed than with either of the other oxalates. Twins were very plentiful.

(4) Sodium chloride substituted for oxalate: This greatly retarded the crystallization, but the crystals that formed were very large, relatively enormous. The crystals did not show much tendency to twin.

(5) Substituting calcium chloride for an oxalate: This retarded the crystallization, as in the case of the addition of sodium chloride; but the crystals were more numerous, and not nearly so large as those obtained by method (4).

In no case were the crystals altered in form or angles, beyond the tendency, shown in some of the solutions, for twins to form; or for the relative sizes of the planes, in crystals showing two forms, to vary in the different mixtures. The retarding of crystallization may be effected by the addition of a 50 per cent solution of egg-white that has been treated with ether and centrifugalized; the clear solution being used instead of the blood plasma, and this is perhaps safer than the use of any saline solution. The addition of the plasma of the bloods of other species appears to alter the angles slightly in some cases. All of the specimens examined were oxyhemoglobin.

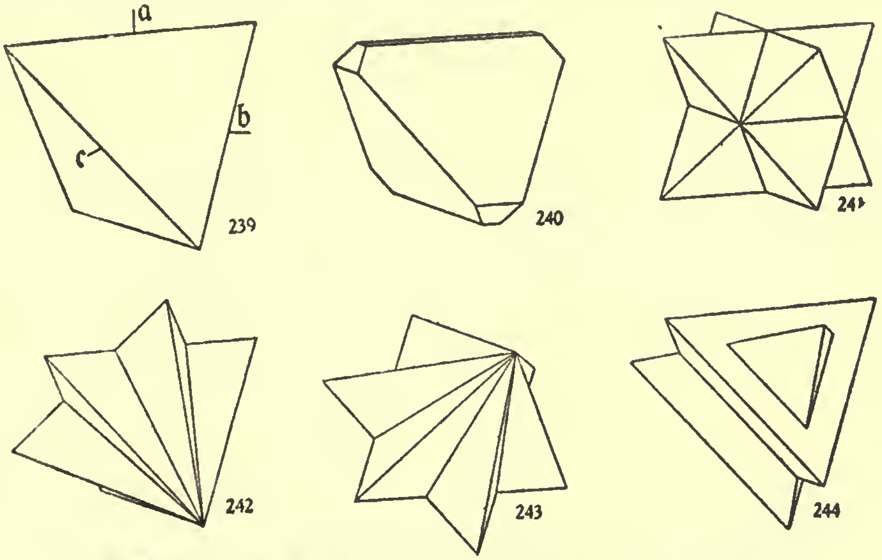
### *Oxyhemoglobin of Domesticated Variety of Cavia cutleri.*

Orthorhombic sphenoidal:* Axial ratio $a : b : \acute{c} = 0.9428 : 1 : 0.8875$.

Forms observed: Right sphenoid (111), left sphenoid $(1\bar{1}1)$, base (001).

Angles: The three plane angles on the triangular face of the sphenoid were measured as 57°, 60°, 63°, and from these angles the axial ratio was calculated.

Habit sphenoidal, the angles being such that the sphenoids can not, without measurement, be distinguished from isometric tetrahedra. Ordinarily the simple sphenoid alone (111) (text figure 239) is the only form shown except in twins, but in some preparations, and especially when considerable amounts of salts have been added, the left-handed sphenoid appears, truncating the corners of the right-handed sphenoid, but rarely forming a large plane (text figure 240); they are not seen in equilibrium. The crystals occur singly or uniting in irregular groups; or more often, when composite, uniting as twins. The two common types of twins seen in tetrahedrite occur most commonly: (a) The right-handed and left-handed sphenoids interpenetrant, with their edges crossing in the polar axis, analogous to the twins in the isometric mineral eulytite (text figure 241, also plate 57, fig. 340). (b) The two interpenetrant sphenoids having a common plane as the base of the tetrahedron, and also a common apex to the tetrahedron (text figures 242 and 243). In these the base forms a six-pointed star, and the edges from the points of the star all meet in one point (see plate 58, fig. 343). (c) The interpenetrant sphenoids uniting point to point with their bases in the same orientation; in these the apex of each sphenoid appears through the base of the other, and the bases may be quite near together (text figure 244, also plate 57, fig. 341). In the composite crystals that appear to be simply irregular aggregates, it can often be seen that two of these kinds of twinning are present and the parts are really in twin position. Only interpenetrant twins were observed. Parallel growths also occur, but much more rarely. The crystals were generally the sphenoids only, but in a few preparations the basal pinacoid occurs (text figure 240).



Figs. 239, 240, 241, 242, 243, 244. *Cavia cutleri* (domesticated variety) Oxyhemoglobin.

Pleochroism is not very strong, the shade of the red changing from paler red for light vibrating parallel to $\mathfrak{a}$ to deeper red for light vibrating parallel to $\mathfrak{c}$. The orientation

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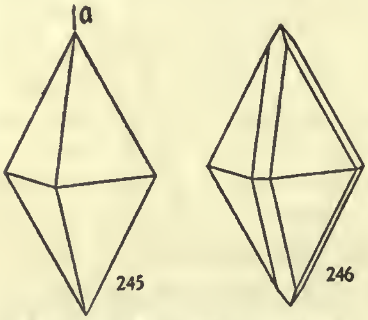
* The orthorhombic character of the tetrahedra of the guinea-pig oxyhemoglobin crystals was first determined by von Lang (Sitzungsb. d. Math.-nat. Klasse d. Kais. Akad. d. Wiss., Wien, 1862–63, xli, Heft ii, p. 85), who showed that they were orthorhombic sphenoidal. More recently, Donogány (Mathematikai és természettudomanyi ertesitö, xi, 262; Zeit. f. Kryst., 1894, xxiii, 499) confirmed von Lang's determination, and published measurements of the three plane angles of the triangular face of the sphenoid as 64° 11′, 60° 50′, and 55° 45′; but without giving any axial ratio. It will be observed that these three angles given by Donogány add up to 180° 46′, or 46′ above two right angles.

of the elasticity axes appears to be $\mathfrak{a}=\acute{c}$, $\mathfrak{b}=b$, $\mathfrak{c}=a$; and the plane of the optic axes is therefore the brachypinacoid. As the sphenoids lie on their faces, a single brush of a biaxial figure is frequently seen; and, when looking along the elasticity axis $\mathfrak{c}$, the symmetrical figure can be made out, with the axes moderately separated, the angle $2E=35°$ to 40°. The acute bisectrix is $\mathfrak{c}$, which can be determined upon the interference figure by means of the quartz wedge, etc. As the acute bisectrix $Bx_a=\mathfrak{c}$, the optical character is *positive*.

### Capybara, *Hydrochœrus capyvara*. Plates 58 and 59.

The specimen of blood was obtained from the National Zoölogical Park at Washington, District of Columbia, and was very thick, containing soft clots. The oxalated blood was diluted with an equal volume of water and laked with ether, and then centrifugalized for 3 hours. The slide preparations were made in the usual manner. Crystals formed readily at room temperature, but less readily than with normal blood of the guinea-pig. The first crystals to form were prisms; later pyramidal crystals appeared. Crystallization proceeded readily at room temperature, and the crystals showed no tendency to dissolve on slight increase of temperature. Inside of 3 hours after the slides were prepared, the crystals were large enough to give good photomicrographs. After a day, most of the prismatic crystals had lost their terminal planes through solution. The pyramidal crystals kept very well, and increased in size to many times the dimensions of the original first crystals; they were sharp and perfect after several days. All of the crystals examined were oxyhemoglobin. The normal and permanent crystal is evidently the pyramidal type; the prismatic crystal is not so insoluble and more unstable. The pyramidal crystal is distinguished as α-oxyhemoglobin; the prismatic crystal is called β-oxyhemoglobin. They evidently crystallize in different systems.



Figs. 245, 246. *Hydrochœrus capyvara* α-Oxyhemoglobin.

#### *α-Oxyhemoglobin of Hydrochœrus capyvara.*

Tetragonal: Axial ratio $a : \acute{c}=1 : 1.8184$.

Forms observed: Unit pyramid (111), second-order pyramid (201).

Angles: On the unit pyramid over the pole, $111 \wedge \bar{1}\bar{1}1=42° 30'$; angles of the pole edges of the unit pyramid, over the pole, edges $111\text{–}1\bar{1}1 \wedge \bar{1}11\text{–}\bar{1}\bar{1}1=57° 30'$ (calculated 57° 36′). The pyramid edges in the horizontal plane make an angle of 90° with each other.

Habit pyramidal; consisting of the unit pyramid alone in very symmetrical development (text figure 245), except when the crystals become large, when the cover and slide distort them. Crystals generally occur singly or more rarely in groups, interpenetrating each other, but not twinned apparently. Very rarely on some of the larger crystals the second-order pyramid was observed (text figure 246). The crystals generally lie on one pyramid face and present a lozenge-shaped profile; more rarely they are found in such a position that the angle of the pole edges can be measured.

The color is a bright oxyhemoglobin red, and pleochroism is readily observed, as the vertical axis is nearly normal to the line of sight in the usual aspect. The pleochroism is $\epsilon$ colorless, $\omega$ deep red. The vertical axis ($\epsilon$) is the direction of greatest elasticity; all directions normal to this ($\omega$) are of equal and less elasticity. Double refraction is strong; extinction is parallel to the vertical axis, or symmetrical in all of the usual aspects. Looking along the vertical axis, in convergent polarized light, the uniaxial cross is seen. The refractive indices are $\omega > \epsilon$ and hence the optical character is *negative*.

### *β-Oxyhemoglobin of Hydrochœrus capyvara.*

Orthorhombic: Axial ratio not determinable.

Forms: Unit prism (110)? terminations were wanting.

Angles: No angles could be measured.

Habit prismatic, the crystals appear to be a prism, much elongated; but perhaps the planes may be two pinacoids. When examined, a few hours after the crystals had begun to form, the terminations were imperfect and not measurable. Later they were lost by resolution.

Pleochroism was weak; 𝔠 deep red, 𝔞 (or 𝔟) somewhat paler.

Extinction is parallel to the length of the crystal in all aspects that were examined. On the side view, in some cases, a biaxial figure was seen, with the plane of the optic axes including the vertical axis, which is evidently 𝔠; this must, therefore, have been seen when looking along 𝔞 and the optical character is *negative*, as in the case of the tetragonal crystals of α-oxyhemoglobin. The crystallization is evidently orthorhombic, but the crystallographic constants can not be determined beyond those already stated.

### DOMESTIC RABBIT, *Lepus cuniculus*. Plates 59–61.

The living animal was purchased and bled into oxalate in the laboratory. The corpuscles were separated from the plasma by centrifugalizing; and the preparations were made from the corpuscles by laking with ether and centrifugalizing for 3 hours. The slide preparations were made from the clear centrifugalized blood as usual. Crystallization begins in the protein ring soon after the slides are covered, and it proceeds rapidly at room temperature. As the solution under the cover comes to an equilibrium, these first crystals dissolve and disappear from the slides. Upon putting the slides in the cold at near 0° C. a second crop of crystals appears, some of which are like the first crop and some are of a different type. These are distinguished as α-oxyhemoglobin and β-oxyhemoglobin. The α-crystals are less soluble and may be examined at near room temperature; but the β-crystals are much more soluble, and dissolve rapidly when the temperature is raised a few degrees above 0° C. They had to be examined and photographed in a room temperature near the freezing-point. Another preparation, made from the same blood, was evidently not evaporated quite to the same point as the first before applying the cover; for, while two types of crystals developed, both tended to dissolve upon increasing the temperature a few degrees above 0° C., and they therefore had to be examined at about this temperature. So long as the preparations were kept at about the freezing-point the crystals continued in excellent condition.

### *a-Oxyhemoglobin of Lepus cuniculus.*

Monoclinic: Axial ratio $a : b : \dot{c} = 0.643 : 1 : 0.797$; $\beta = 85°$.

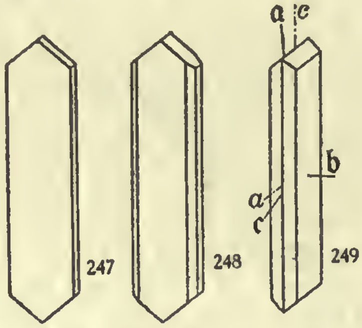
Forms observed: Unit prism (110), clinoprism (320), clinodome (011), clinopinacoid (010), orthopinacoid (100).

Angles: Unit prism $110 \wedge 1\bar{1}0 = 65° 30'$ (normals); clinoprism $320 \wedge 3\bar{2}0 = 88°$ (normals); clinodome $011 \wedge 0\bar{1}1 = 77°$; prism edge to dome edge, in the plane of symmetry $= 85°$ (normals) $= \beta$, or 95° actual angle.

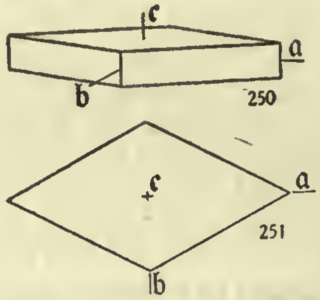
Two habits of crystals develop: (a) the first crystals to appear are usually prismatic, consisting of the two pinacoids and the clinodome, elongated vertically and flattened on (100) (text figure 247), and with or without the unit prism (110) (text figure 248); (b) the second type, which is much more symmetrically developed, consists of the clinoprism (320) in combination with the clinodome and the clinopinacoid (text figure 249). Type (a) crystals are elongated vertically and striated on the orthopinacoid;

by the disappearance of this plane and the development of the clinoprism, while, at the same time, the clinopinacoid becomes larger, they pass into the second type of crystals. Type (b) crystals are tabular on the clinopinacoid, elongated vertically, and generally smooth, not striated, as in the type (a) crystals. Both kinds of crystals of the $\alpha$-oxyhemoglobin are much smaller than the crystals of the $\beta$-oxyhemoglobin. The (b) type of crystals form parallel growths and also seem to twin on the orthopinacoid; the twinning was observed in polarized light.

The color is the usual oxyhemoglobin red, but the crystals are quite pleochroic; $\mathfrak{a}$ colorless or pale yellowish; $\mathfrak{b}$ rose-pink to pale red; $\mathfrak{c}$ deep red. Double refraction is moderately strong on most aspects; in all sections in the zone of 100–001 the extinction is straight or symmetrical; on the plane of symmetry, looking along $b$, the extinction is oblique; 15°, in the obtuse angle, from the prism edge. On this aspect in some crystals, a biaxial interference figure was seen with the above orientation, the plane of the optic axes being inclined to the vertical axis $\dot{c}$ at 15°. The orientation of the elasticity axes is $\mathfrak{a} \wedge \dot{c} = 15°$, in the obtuse angle; $\mathfrak{b} \wedge a = 10°$, in the acute angle; $\mathfrak{c} = b$. The plane of the optic axes is hence normal to the plane of symmetry. The angle between the optic axes was not accurately measured; the separation was considerable, however. The acute bisectrix emerges normal to the plane of symmetry; it is $\mathfrak{c}$ and hence the optical character is positive.



FIGS. 247, 248, 249. *Lepus cuniculus* $\alpha$-Oxyhemoglobin.



FIGS. 250, 251. *Lepus cuniculus* $\beta$-Oxyhemoglobin.

### *$\beta$-Oxyhemoglobin of Lepus cuniculus.*

Orthorhombic: Axial ratio $a : b : \dot{c} = 0.5317 : 1 : \dot{c}$.

Forms observed: Unit prism (110), base (001).

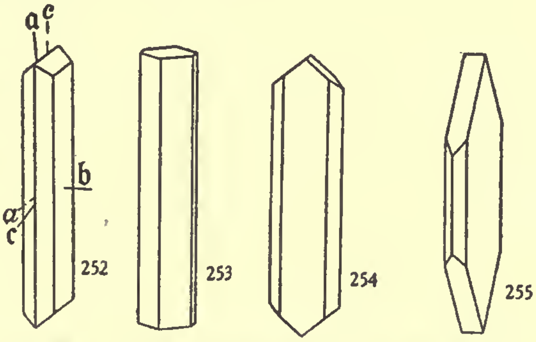
Angles: Unit prism angle $110 \wedge 1\bar{1}0 = 56°$ (normals); prism to base $110 \wedge 001 = 90°$. Many oblique sections of the prism in the position of a brachydome were produced by the slide and cover; these had the angle of the prism (210), but were oblique sections, as could be shown by their optical properties. This angle of the prism (210) is 30°.

Habit tabular on the base; or, in case of the crystals that were interfered with by the slide and cover, flattened on a brachydome. The tabular crystal consists of the very short prism cut by the base, and in some cases traces of a macrodome were seen (text figures 250 and 251). These crystals are many times the size of those of the $\alpha$-oxyhemoglobin. The oblique sections of the crystals are particularly common, but they are not always at the same angle, nor in the zone of the brachydomes. Their angle generally runs near 30°. These $\beta$-crystals are much more soluble than the $\alpha$-crystals as a rule, and they are corroded so rapidly that, in spite of the fact that they were photographed at a room temperature of about 0° C., they show the effect of solution in etching figures, which appear on their surfaces in many of the photographs.

The crystals were somewhat more of a scarlet-red color than the $\alpha$-crystals, but the absorption spectrum was the same, that of oxyhemoglobin. The difference in color is due to the difference in the pleochroic colors. The pleochroism is $\mathfrak{a}$ pale yellow-red to nearly colorless; $\mathfrak{b}$ pale scarlet-red; $\mathfrak{c}$ deep red. The orientation of the elasticity axes is $\mathfrak{a} = b$, $\mathfrak{b} = a$, $\mathfrak{c} = \dot{c}$. The macropinacoid is the plane of the optic axes and the acute bisectrix $Bx_a = \mathfrak{c}$. The optical character is hence *positive.* In the crystals presenting the basal aspect, the symmetrical interference figure is seen, in convergent light, with rather widely separated brushes; in the case of the oblique sections referred to, one brush only appears in the field. As these oblique sections greatly outnumber the normal basal sections, most of the crystals are viewed looking along an optic axis, and being nearly singly refracting in such an aspect they show no pleochroism and the color is pale scarlet, a mean between $\mathfrak{a}$ and $\mathfrak{c}$, or near $\mathfrak{b}$.

### Belgian Hare, *Lepus europæus*. Plates 61, 62.

The living animal was procured and bled in the laboratory, and the oxalated blood centrifugalized to separate the corpuscles. The corpuscles were laked with ether and centrifugalized for 2 hours; from the clear solution thus obtained the slide preparations were made in the usual way. The drops were allowed to dry until a rather thick protein ring formed, and then the preparations were covered. Crystallization proceeded at about the same rate as in the rabbit; the first-formed crystals were rather small and were gradually dissolved as the solution came to an equilibrium. They were mostly rather imperfectly formed. The crystals of the second crop were sharper and of good size; they mostly resembled the α-oxyhemoglobin crystals of the rabbit type (a). Later, soluble crystals of larger size appeared, which seemed to correspond to the β-oxyhemoglobin of the rabbit. They were very soluble, and were only obtained at a temperature near 0° C. They dissolved so quickly when the temperature rose a few degrees that they were very difficult to measure, even in a room at about the freezing-point; the heat of the body or of the breath destroyed the sharp angles. They were not photographed. These two kinds of crystals are called α-oxyhemoglobin and β-oxyhemoglobin, as in the case of the rabbit.



Figs. 252, 253, 254. *Lepus europæus* α-Oxyhemoglobin.
Fig. 255. *Lepus europæus* β-Oxyhemoglobin.

#### *α-Oxyhemoglobin of Lepus europæus.*

Monoclinic: Axial ratio $a : b : \acute{c}$ = 0.6588 : 1 : 0.8069; β = 85° approximately.

Forms observed: Unit prism (110), clinodome (011), orthopinacoid (100), clinopinacoid (010), base (001). Also questionably a pyramid and perhaps a hemiorthodome.

Angles: Prism angle $110 \wedge 1\bar{1}0 = 66°$ (normals); clinodome $011 \wedge 0\bar{1}1 = 77°$; clinopinacoid to prism $010 \wedge 110 = 57°$.

Habit of the first crystals to form appears to be prism, clinopinacoid, and clinodome (text figure 252); but these give place to crystals consisting of prism, clinopinacoid, and base (text figure 253); or prism alone with the base or a hemiorthodome; or prism and orthopinacoid with the clinodome (text figure 254). The crystals are elongated vertically and striated on the pinacoid faces (orthopinacoid and clinopinacoid) on one of which the crystal is generally flattened. They are liable to be much distorted by irregular development; and in a great many cases are terminated by one oblique face, as though one face of the clinodome.

Pleochroism is distinct, and on the clinopinacoid aspect is quite pronounced; $\mathfrak{a}$ is yellowish-red; $\mathfrak{b}$ and $\mathfrak{c}$ are nearly equal and deep red or paler red according to the thickness. On the clinopinacoid section, the extinction angle is 15°, measured from the prism edge; on sections in the zone 100–001, the extinction is straight. On the section looking along the clinodome, or along $a$ about, one brush of the biaxial interference figure is seen. From the position of this brush it looks as though the acute bisectrix was $\mathfrak{a}$ and the optical character was negative. The orientation of the elasticity axes is apparently $\mathfrak{a} \wedge \acute{c}=15°$; $\mathfrak{b}=b$; $\mathfrak{c} \wedge a=10°$ in the acute angle (approximately), the angle $\beta$ not being exactly determined, but assumed to be 85°, as in the common rabbit. The plane of the optic axes appears to be normal to what it is in case of the common domestic rabbit, being in the case of the Belgian hare the plane of symmetry, and in the rabbit normal to the plane of symmetry. In other words, $\mathfrak{c}$ and $\mathfrak{b}$ change places in the Belgian-hare crystals from what they are in the rabbit.

### *β-Oxyhemoglobin of Lepus europæus.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 1 : b : 1.376$.

Forms observed: Unit prism (110), brachydome (011), brachypinacoid (010), macrodome (101), unit pyramid (111) (?).

Angles: Macrodome angle $011 \wedge 0\bar{1}1=72°$ actual angle.

Habit tabular on the brachypinacoid; the development of the faces very irregular, producing a monoclinic habit (text figure 225); but the extinction straight in all aspects and hence probably only pseudomonoclinic. The crystals were sometimes regularly developed, and in some crystals only one or two unsymmetrical planes appeared. The tabular crystal, flattened on the brachypinacoid, is bounded by the macrodome and prism in some of the crystals examined; in others, one pair of faces of the macrodome only appear or one pair of faces of the unit prism, producing the monoclinic habit noted above. The crystals melted so rapidly that measurements were incomplete.

Pleochroic colors are shades of rose-pink or rose-red; $\mathfrak{a}$ colorless, $\mathfrak{b}$ deep rose-pink, $\mathfrak{c}$ deep red.

TABLE 42.—*Crystallographic characters of the hemoglobins of the Rodentia.*

| Name of species. | Axial ratio $a : b : \acute{c}$, etc. | Prism angle or traces of prism on base (normals). | Angle $\beta$. | Extinction angle. | Optical character. | System and class. | Substance |
|---|---|---|---|---|---|---|---|
| | | ° ′ | ° | | | | |
| Sciurus vulgaris............ | ................ | 60 0 | 90 | 0° | Weakly positive | Hexagonal or pseudohexagonal | $\alpha$-OHb. |
| Do.................... | $0.577 : 1 : \acute{c}$ | 60 0 | 90 | 0° | Negative | Orthorhombic | $\beta$-OHb. |
| Sciurus rufiventer neglectus.. | ................ | 60 0 | 90 | 0° | Weakly positive | Hexagonal | OHb. |
| Sciurus carolinensis ......... | $0.577 : 1 : \acute{c}$ | 60 0 | 90 | 0° | Negative | Orthorhombic | OHb. |
| Sciuropterus volans ......... | ................ | 60 0 | 90 | 0° | Do. | Hexagonal | OHb. |
| Tamias striatus ............ | $0.9246 : 1 : 0.589$ | 85 30 | 90 | 0° | Positive? | Orthorhombic | OHb. |
| Cynomys ludovicianus ...... | ................ | ... 0 | 90 | 0° | ......... | Do. | OHb. |
| Marmota monax ........... | ................ | 60 0 | 90 | 0° | Negative | Hexagonal or pseudohexagonal | $\alpha$-OHb. |
| Do.................... | ................ | ... 0 | .. | 0° | Positive | Orthorhombic | $\beta$-OHb. |
| Do.................... | $1.804 : 1 : \acute{c}$ | 122 0<br>(58* 0) | near 90 | $\mathfrak{c} \wedge a=11°$ | Negative | Monoclinic | $\gamma$-OHb. |
| Castor canadensis .......... | $1.732 : 1 : \acute{c}$ | 120 0<br>(60* 0) | 78 | $\mathfrak{a} \wedge a= 8°$ | Positive | Do. | OHb. |
| Fiber zibethicus............. | $1.6318 : 1 : \acute{c}$ | 117 0<br>(63* 0) | 68 | $\mathfrak{c} \wedge a=15°$ | Negative | Do. | OHb. |
| Mus norvegicus albino....... | $0.7829 : 1 : 0.7332$ | 76 7 | 90 | 0° | Positive | Orthorhombic | OHb. |
| Mus norvegicus............. | $0.7829 : 1 : 0.7332$ | 76 7 | 90 | 0° | Do. | Do. | $\alpha$-OHb. |
| Do.................... | $1 : 1 : 1$ | 90 0 | 90 | .. | Isotropic | Isometric | $\beta$-OHb. |
| Mus rattus................. | $0.7829 : 1 : 0.5864$ | 76 7 | 90 | 0° | Positive | Orthorhombic | OHb. |
| Mus alexandrinus .......... | $0.7829 : 1 : 0.5880$ | 76 7 | 90 | 0° | Do. | Do. | OHb. |
| Erethizon dorsatus.......... | $0.5543 : 1 : \acute{c}$ | 58 0 | 56 | $\mathfrak{b} \wedge a=20°$ | Do. | Monoclinic | $\alpha$-OHb. |
| Do.................... | $0.8170 : 1 : \acute{c}$ | 78 30 | 90 | 0° | Do. | Orthorhombic | $\beta$-OHb. |
| Cavia cutleri, domesticated variety.................. | ................ | ..... | 90 | 0° | Do. | Do. | OHb. |
| Hydrochœrus capyvara...... | $1 : 1.8184$ | 90 0 | 90 | 0° | Negative | Tetragonal | $\alpha$-OHb. |
| Do.................... | ................ | ..... | 90 | 0° | Do. | Orthorhombic | $\beta$-OHb. |
| Lepus cuniculus ........... | $0.643 : 1 : 0.797$ | 65 30 | 85 | $\mathfrak{a} \wedge \acute{c}=15°$ | Positive | Monoclinic | $\alpha$-OHb. |
| Do.................... | $0.5317 : 1 : \acute{c}$ | 56 0 | 90 | 0° | Do. | Orthorhombic | $\beta$-OHb. |
| Lepus europæus............ | $0.6588 : 1 : 0.8069$ | 66 0 | 85 | $\mathfrak{a} \wedge \acute{c}=15°$ | Ncgative? | Monoclinic | $\alpha$-OHb. |

* True angle of traces of prism on base.

# CHAPTER XIV.

## CRYSTALLOGRAPHY OF THE HEMOGLOBINS OF THE OTARIIDÆ, PHOCIDÆ, MUSTELIDÆ, PROCYONIDÆ, AND URSIDÆ.

Under *Carnivora* zoölogists distinguish two suborders, (1) *Carnivora vera* and (2) *Pinnipedia.* The distinction is mainly in the structure of the limbs, which in the *Carnivora vera* are normal for terrestrial animals and in the *Pinnipedia* are modified for aquatic progression. In the arrangement of species here adopted the *Pinnipedia* are considered first, and then the *Carnivora vera,* beginning with the species which, from their hemoglobin crystals, appear to be most nearly related to the *Pinnipedia,* namely the *Mustelidæ, Procyonidæ,* and *Ursidæ.*

The *Pinnipedia* are divided into the eared seals, *Otariidæ,* including the sea-lions and sea-bears, of which one species, the California sea-lion, was examined; the walruses, *Trichechidæ,* of which we had no representative; and the *Phocidæ* or earless seals, of which the harbor seal was examined. These are all evidently descendants of some terrestrial mammals, and from the resemblances between the skulls of the eared seals and the bears of the *Carnivora vera,* Mivart (Proc. Zool. Soc., 1885, p. 497) has suggested that there exists a true genetic relationship between the two groups. Mivart states that while the sea-bears may be thus related to the bears, both being derived from bear-like carnivores, the true seals may on the other hand be genetically related to the sea-otters. The true bears are a modern group, and a common bear-like ancestor for them and for the eared seals is entirely possible. It will be seen from a comparison of the hemoglobin crystals of the sea-lion and of the bears that they have a certain very remarkable character in common, namely, a habit of twinning that is very unusual, and is identical in the two groups, but which produces different-looking crystals, owing to the development of the planes of the crystals being different in the two groups. This is not the only point of resemblance between the two groups, as will be shown.

The *Mustelidæ* and *Ursidæ* are evidently closely related, as indicated by their hemoglobin crystals; the *Procyonidæ,* however, so far as our investigations have gone, do not show close relationship to the other groups above mentioned. Of the *Mustelidæ* the common ferret, *Mustela putorius* (domesticated variety); the skunk, *Mephitis mephitica putida;* the badger, *Taxidea americana,* and the otter, *Lutra canadensis,* were examined. They show strong resemblance to each other and to the bears and seals, with the exception of the skunk, which more nearly resembles the *Procyonidæ* from what data we were able to obtain in regard to its hemoglobin crystals; but in the case of this species the data were incomplete.

Of the family *Procyonidæ*, the species from which crystals were examined were the kinkajou, *Cercoleptes caudivolvulus*, and the cacomistle, *Bassariscus astuta*. The blood of the raccoon was experimented with early in our work, but no satisfactory crystals were obtained. They no doubt can be produced by the use of a suitably modified process of preparation, such as we have resorted to in our more recent work.

The family *Ursidæ* is represented by three species, the black bear, *Ursus americanus;* the polar bear, *Ursus maritimus*, and the sloth bear, *Melursus ursinus*. Their crystals all closely resemble each other, and are characterized by the peculiar cyclic trillings already alluded to as being found in this group and in the *Otariidæ*. All belong to the same class of crystals, monoclinic hemimorphic or monoclinic sphenoidal, which has thus far been seen only in this family and in the *Otariidæ* and *Phocidæ*, but may very possibly occur in the otters and ferrets also, although they have been determined as monoclinic hemihedral, or domatic (clinohedral group of Dana).

## PHOCIDÆ.

### Harbor Seal, *Phoca vitulina*. Plate 62.

Specimens of blood of the harbor seal were received from the Philadelphia Zoölogical Gardens, from the National Zoölogical Park at Washington, District of Columbia, and from the Zoölogical Garden at Detroit, Michigan. In each case the blood was not quite fresh, but all were in fairly good condition and were not putrid, except in the case of the specimen from Detroit, which was slightly putrid. The blood from Washington was frozen; all of the specimens were treated in the same manner with this exception. The bloods were oxalated, ether-laked, and centrifugalized, generally from 2 to 3 hours; and from the clear solution thus obtained the slide preparations were made. The crystals formed slowly at room temperature, and rather more rapidly at a temperature near freezing, but the color of the solution remained a deep red, showing that much of the oxyhemoglobin was still in solution. The crystals were kept at temperatures near the freezing-point, but when brought into the warm room did not appear to dissolve, and even on the stage of the photomicrographic apparatus they did not lose their form. The solution was very deeply colored and of about the color of the crystals, which were oxyhemoglobin.

#### *Oxyhemoglobin of Phoca vitulina.*

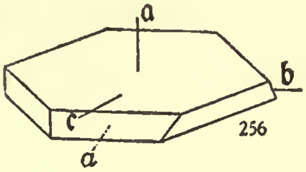
Monoclinic hemimorphic, or monoclinic sphenoidal (tartaric acid type): Axial ratio $a : b : \acute{c} = 1.2131 : 1 : 1.1970$; $\beta = 75°$.

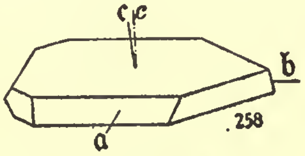
Forms observed: Unit tetartopyramid $(\bar{1}11)$, unit hemiprism $(1\bar{1}0)$, orthopinacoid (100), base (001).

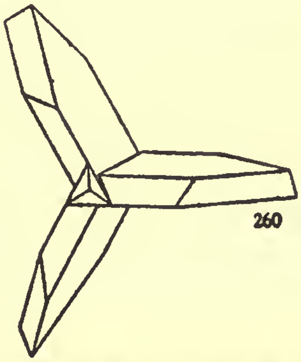
Angles: Unit prism to unit pyramid edges on base, edges $1\bar{1}0\text{–}001 \wedge 11\bar{1}\text{–}001 = 101°$, or traces of unit prism edges $1\bar{1}0\text{–}001 \wedge \bar{1}\bar{1}0\text{–}001 = 79°$, and the traces of the unit pyramid on the base give the same angle. The angle $\beta$ on sections parallel to (010) or $100 \wedge 001 = 75°$; and the angle of the trace of the unit pyramid on the same section or $\bar{1}01 \wedge 001 = 52°$.

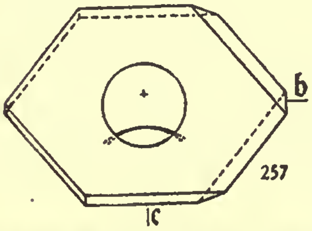
Habit tabular on the base, the crystal consisting of the basal pinacoid bounded by the positive unit pyramid at the positive extremity of the ortho-axis and by the unit prism at the negative extremity of this axis (text figures 256 and 257). In many cases the orthopinacoid developed, but it was not always present. Sometimes this orthopinacoid was so strongly developed as to produce a prismatic habit on the ortho-axis, the crystal being 5 to 10 times as long on this axis as on the clino-axis. The plates were generally rather thin; edge views showed a ratio of length on the ortho-axis to the thickness of the plate of 10 : 1 or more, but in some cases this became 5 : 1. The crystals grow singly or in radiating groups, showing a tendency to twinning on the clinodome; but this plane was not developed and there were no definite twins made out. Parallel growth by piling up on the base and the group extending along the ortho-axis was a very common habit in the larger crystals.

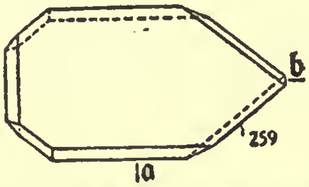
Pleochroism is hardly noticeable on the flat aspect of the plates, but quite strong on edge views. The colors are: $\mathfrak{a}$ pale red, $\mathfrak{b}$ and $\mathfrak{c}$ nearly equal and deep red. The orientation of the elasticity axes is $\mathfrak{a} = \acute{c}$, $\mathfrak{b} = b$, $\mathfrak{c} \wedge a = 15°$, in the obtuse angle. The plane of the optic axes is the plane of symmetry. On the base, in convergent polarized light, one brush of a biaxial interference figure is seen and traces of the other. The acute bisectrix of the optic axes is the axis of greatest elasticity, $Bx_a = \mathfrak{a}$ and the optical character is hence negative.











FIGS. 256, 257. *Phoca vitulina* Oxyhemoglobin. FIGS. 258, 259, 260. *Otaria gillespii* Oxyhemoglobin (first orientation).

### CALIFORNIA SEA-LION, *Otaria gillespii*. Plates 63 and 64.

Specimens of the blood of the sea-lion were received from the National Zoölogical Park at Washington and from the New York Zoölogical Park. In both cases the blood was rather stale, and in one case it was clotted. The specimens were oxalated and laked with ether, and cleared by centrifugalizing for several hours. From the clear blood slide preparations were made in the usual manner. The blood crystallized at room temperature, and the crystals did not readily dissolve on slight increase of temperature. Bacteria in the preparations destroyed the crystals after some days. A preparation of CO-hemoglobin kept well and developed very fine crystals. From these the most satisfactory measurements were obtained. The crystals grew to much larger size than was the case with the oxyhemoglobin crystals in the other preparations, and the planes of the crystals were perfectly developed. The angles, as far as they could be measured in the oxyhemoglobin crystals, corresponded to the good measurements obtained from the CO-hemoglobin crystals, and the optical characters appeared to be identical. The crystallographic and optical constants are hence derived from the CO-hemoglobin with greater exactness.

### *Oxyhemoglobin of Otaria gillespii.*

Monoclinic hemimorphic or monoclinic sphenoidal (tartaric-acid type): Axial ratio $a : b : \acute{c} = 0.7883 : 1 : 1.7314$; $\beta = 74°$.

Forms observed: Pyramid of unit series, not measured, may be called unit pyramid $(\bar{1}11)$, unit prism $(1\bar{1}0)$, orthopinacoid (100), clinodome $(0\bar{1}1)$, base (001).

Angles: Traces of unit pyramid on base, edges $11\bar{1}-001 \wedge \bar{1}11-001 = 76° 30'$, or edges $11\bar{1}-001 \wedge 1\bar{1}0-001 = 103° 30'$; traces of unit prism on base at opposite pole, edges $1\bar{1}0-001 \wedge \bar{1}\bar{1}0-001 = 76° 30'$. The angle of the clinodome was not accurately determined, but was very near 60°. The angle of the orthopinacoid to base $100 \wedge 001 = 73° 30'$ to $74° = \beta$.

Habit tabular on the base and elongated on the ortho-axis, the crystal consists of the base bounded by the orthopinacoid and at the positive end of the ortho-axis the unit pyramid, while at the negative end of this axis is found the unit prism and the clinodome (text figures 258 and 259). The crystals show a tendency to grow together in radiating groups uniting at the negative end of the ortho-axis and also in parallel growth on the base, piling upon each other in perfect orientation. These groups in parallel growth also expand along the clino-axis, frequently with the prism and clinodome planes in common, but the pyramid planes showing the parallel growth (1559). Twins on the clinodome are common, the angle being nearly 60°; they form in trillings, very frequently radiating from a common axis, the common clinodome edge (text figure 260). The hemimorphic character is found in the smallest crystals as well as in the larger ones, and there is not much change of shape of the crystals as they grow from small to large size.

Pleochroism is strong; $\mathfrak{a}$ nearly colorless, $\mathfrak{b}$ rather strong red, $\mathfrak{c}$ deep red. Double refraction is fairly strong, extinction is straight or nearly so in all ordinary aspects. The orientation of the elasticity axes is $\mathfrak{a} = a$ or very nearly, $\mathfrak{b} = b$, $\mathfrak{c} \wedge \acute{c} =$ about 16° 30′ in the obtuse angle or the angle $= 90° - \beta$; hence the axis $\mathfrak{c}$ is nearly or quite normal to the base (001). The plane of the optic axes is the clinopinacoid and the acute bisectrix is the axis of greatest elasticity; $Bx_a = \mathfrak{a}$ and the optical character is *negative*. The axial angle $2E = 35°$ to 40°.

### *CO-hemoglobin of Otaria gillespii.*

Monoclinic hemimorphic or monoclinic sphenoidal (tartaric acid type): Axial ratio $a : b : \acute{c} = 0.7883 : 1 : 1.7314$; $\beta = 74°$.

Forms observed: Unit pyramid $(\bar{1}11)$, unit prism $(1\bar{1}0)$, clinodome $(0\bar{1}1)$, orthopinacoid (100), base (001).

Angles: Traces of unit pyramid or of unit prism on base 76° 30′ actual angle, or 103° 30′ normals as measured at the ends of the ortho-axis, making the edges of these two unit forms on the base $11\bar{1}-001 \wedge 1\bar{1}0-001 = 103° 30'$ as recorded for the oxyhemoglobin; clinodome angle $0\bar{1}1 \wedge 0\bar{1}\bar{1} = 60°$ to 61° (taken as 60° in calculation of the axial ratio); orthopinacoid to base $100 \wedge 001 = 74° = \beta$. The pyramid called unit on the base, angle $111 \wedge 001$, was not obtained.

Habit tabular on the base and elongated on the ortho-axis, exactly as described for the oxyhemoglobin (text figures 258, 259). The crystals grow in groups and in regular growths as there described, but are much more perfect.

Twins and trillings are very common on the clinodome as described under the oxyhemoglobin; the angle of the trilling is generally 120°, but some were 121°, 118°, perhaps not quite in symmetrical position (text figure 260). These twins show a cyclic arrangement of the unit pyramid planes, the three planes of the unit pyramid on one side of the trilling being corresponding planes.

The crystals are strongly pleochroic; $\mathfrak{a}$ colorless, $\mathfrak{b}$ old rose, $\mathfrak{c}$ deep crimson. Double refraction is rather strong, except on edge views looking along the axis of greatest elasticity. Extinction is straight with the basal edges and symmetrical on the base, as though orthorhombic. The orientation of the elasticity axes is the same as in the oxyhemoglobin, $\mathfrak{a} = a$, $\mathfrak{b} = b$, $\mathfrak{c} \wedge \acute{c} = 16°$. The plane of the optic axes is the plane of symmetry; on the base a biaxial figure is obtained with brushes that pass out of the field indicating the obtuse bisectrix (text figure 259). Looking along $\mathfrak{a}$ the biaxial figure is seen with moderately separated brushes, and the axial angle $2E=37°$ to $40°$ as measured in white light (nearly monochromatic and red owing to the color of the crystal). The axis of greatest elasticity is, therefore, the acute bisectrix $Bx_a=\mathfrak{a}$, and the optical character is *negative.*

The trillings of the oxyhemoglobin and CO-hemoglobin of the California sea-lion noted above are apparently perfectly conformable with trillings observed in different species of the *Ursidæ* (page 259). In the case of the California sea-lion, however, the axis of the trilling was taken as the crystallographic axis $a$; in the case of the *Ursidæ* as the axis $\dot{c}$. In order better to compare the oxyhemoglobin and the CO-hemoglobin in the *Otariidæ* and the *Ursidæ* the crystals of the sea-lion were recalculated, regarding the trilling axis as $\dot{c}$, as follows:

### *Oxyhemoglobin of Otaria gillespii.*

Monoclinic sphenoidal (tartaric acid type): Axial ratio $a : b : \dot{c} = 1.8019 : 1 : 0.7883$; $\beta = 74°$.

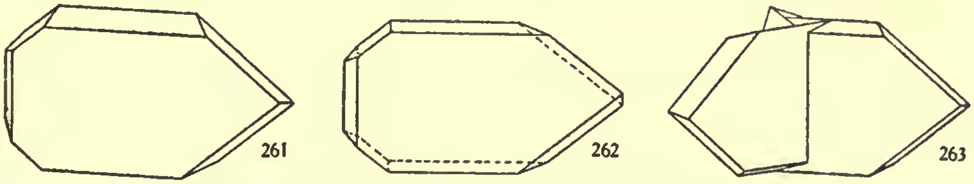
Forms observed: Unit pyramid $(\bar{1}11)$; unit prism $(1\bar{1}0)$; orthopinacoid (100); clinodome $(0\bar{1}1)$; base (001).

Angles: Traces of unit pyramid on orthopinacoid, edges $11\bar{1}–100 \wedge \bar{1}11–100 = 103° 30'$; opposite pole traces of clinodome on orthopinacoid edges $0\bar{1}1–100 \wedge 0\bar{1}\bar{1}–100 = 103° 30'$. Angle of prism $1\bar{1}0 \wedge \bar{1}\bar{1}0 = 60°$ from twins. Orthopinacoid to base $100 \wedge 001 = 74°$.

In this orientation the habit is tabular on the orthopinacoid elongated on the orthoaxis (text figure 261), instead of being tabular on the base, as in the original orientation. The orientation of the elasticity axes is $\mathfrak{a}=\dot{c}$ very nearly; $\mathfrak{b}=b$; $\mathfrak{c} \wedge a = 16° 30'$, in the obtuse angle; the axis $\mathfrak{c}$ is nearly or quite normal to the orthopinacoid. The plane of the optic axes is the clinopinacoid 010, $Bx_a=\mathfrak{a}$, and the optical character is *negative.* The axial angle $2E=35°$ to $40°$.

### *CO-hemoglobin of Otaria gillespii.*

In the changed orientation of the CO-hemoglobin crystals the description is entirely analogous to that of the oxyhemoglobin.



FIGS. 261, 262, 263. *Otaria gillespii* Oxyhemoglobin (second orientation).

Text figures 258, 259, and 260 are drawn according to the original orientation; text figures 261, 262, and 263 according to the orientation as revised.

In the summary of the crystallographic characters of the hemoglobins of the *Phocidæ, Otariidæ, Mustelidæ,* and *Ursidæ,* page 263, the axial ratio of the oxyhemoglobin and CO-hemoglobin of *Otaria gillespii* is entered according to the second orientation; the isomorphism between the crystals of oxyhemoglobin of this species and those of *Ursus americanus* and *Ursus maritimus* is there apparent.

## MUSTELIDÆ.

### FERRET, DOMESTICATED VARIETY OF *Mustela putorius*. Plates 65 and 66.

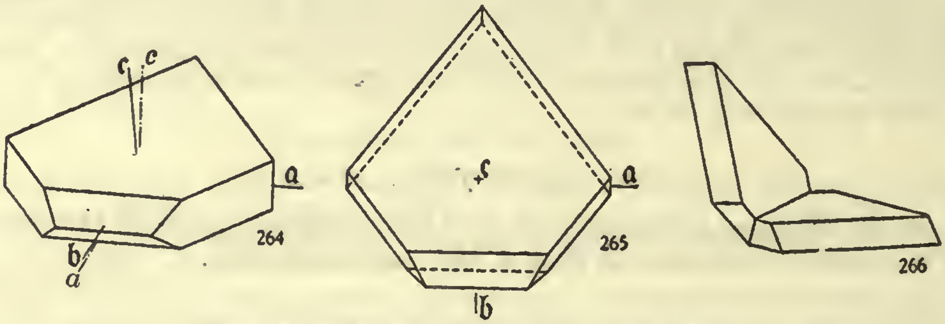
The animal was purchased from a dealer and bled in the laboratory. The blood was oxalated and repeatedly frozen and thawed, then centrifugalized, and the slide preparations made in the usual manner. Crystals formed rather slowly, but after 24 hours were sufficiently developed to photograph. Once formed, the crystals were fairly stable and could be preserved for several days at ordinary room temperature. The crystallographic examination was made on crystals 48 hours old. Only a small portion of the hemoglobin in solution crystallized, and the mother-liquor remained so strongly colored that determination of the pleochroic colors was difficult. The crystals were oxyhemoglobin, as shown by the microspectroscope and color.

*Oxyhemoglobin of Domesticated Variety of Mustela putorius.*

Monoclinic domatic (clinohedrite type): Axial ratio $a : b : \acute{c} = 1.2799 : 1 : 1.1105$; $\beta = 68°$.

Forms observed: Unit prism (110), positive hemiorthodome ($10\bar{1}$), negative hemiorthodome (101), base (001).

Angles: Prism edges on base $1\bar{1}0\text{–}001 \wedge \bar{1}\bar{1}0\text{–}001 = 104°$; negative hemiorthodome to base $101 \wedge 001 = 50°$; prism edge to base, edge $1\bar{1}0\text{–}\bar{1}\bar{1}0 \wedge 001 = 68° = \beta$.



FIGS. 264, 265, 266. *Mustela putorius* (var.) Oxyhemoglobin.

Habit tabular on the base, the plate bounded by the unit prism, and usually one face of each of the hemiorthodomes, at the positive end of the clino-axis (text figure 264), giving a clinohedral symmetry to the crystal when viewed on the base (text figure 265) or when seen on edge looking along the ortho-axis. The crystals were of various sizes, but usually developed rather regularly, so that the thickness of the plates was about one-fifth of the long diagonal on the average, and in the smaller crystals about one-fourth. The crystals twinned on the hemiorthodome (text figure 266).

Pleochroism is marked on the basal aspect and on edge views looking along the clino-axis, but not so strong on edge view looking along the ortho-axis. Good measurable views looking along the ortho-axis were not seen where the extinction could be measured. The pleochroic colors are $\mathfrak{a}$ colorless or very pale reddish, $\mathfrak{b}$ rather deep rose-red, $\mathfrak{c}$ deep red, the colors of $\mathfrak{b}$ and $\mathfrak{c}$ being near together. Extinction is symmetrical on the base and straight when looking along the clino-axis in edge view; the third critical position looking along the ortho-axis was not satisfactorily observed. As the extinction when viewed on the plane of symmetry was not determined, the exact orientation of the elasticity axes can not be given; $\mathfrak{a} = b$, $\mathfrak{b}$ is near $a$, and $\mathfrak{c}$ is near the normal to the base. The plane of the optic axes is normal to the plane of symmetry, and on the base a figure is seen with this orientation but with very widely separated brushes as though it were being observed along the obtuse bisectrix. From the character of the pleochroism it would seem that the axis of greatest elasticity should be the acute bisectrix, which would make the optical character *negative*.

### SKUNK, *Mephitis mephitica putida*. Plate 65.

The specimen was obtained by purchase from a collector in Florida, and the blood drawn from the freshly killed animal into oxalate, laked with ether, and centrifugalized. From the clear solution thus obtained the slide preparations were made as usual. The blood crystallized rather readily, and the crystals did not dissolve on slight increase of temperature. Examination with the microspectroscope showed them to be oxyhemoglobin, but the solution showed traces of reduced hemoglobin which did not form in crystals. While the blood crystallized readily, the crystals were not easily measured on account of their strong prismatic habit.

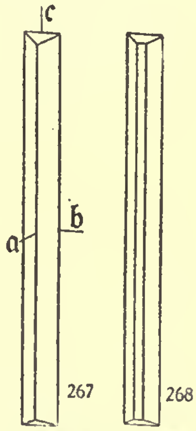
#### *Oxyhemoglobin of Mephitis mephitica putida.*

Orthorhombic: Axial ratio $a : \acute{c} = 1 : 0.4877$.

Forms observed: Unit prism (110), macrodome (101), macropinacoid (100), base (001).

Angles: The only angle obtained was that of the macrodome $101 \wedge \bar{1}01 = 52°$ approximately. The prism is squarish, but the axis *b* is distinctly longer than the axis *a*.

Habit prismatic parallel to the vertical axis, and striated in the same direction, especially on the macropinacoid and in that portion of the crystal. The prismatic character and the striated habit of the crystal as well as the habit of growth and of aggregation of the crystals recalls the genus *Canis*, although the prism appears to be more nearly square and the dome not so flat. The crystals vary greatly in length, the ratio of length to thickness in the longer crystals being often as high as 75 : 1, while in the shorter crystals it falls to 15 : 1 or less. The crystals show a tendency to aggregate into large groups in parallel orientation, parallel growths; but they also grow in spherulitic radiating clusters and in radiating sheaf-like groups. No definite twins were observed. The usual crystal is the unit prism and the macrodome (text figure 267), generally with striations marking the position of the macropinacoid; this latter plane appears as a well-developed face in some cases (text figure 268) and more rarely the base is developed on the end of the crystal.



FIGS. 267, 268. *Mephitis mephitica putida* Oxyhemoglobin.

Pleochroism is rather marked, but the crystals show a decided red color, even when the light vibrates along the elasticity axis of $\mathfrak{a}$. The colors are: $\mathfrak{a}$ pale red, $\mathfrak{b}$ stronger red, $\mathfrak{c}$ deep red. The double refraction is rather strong, especially when looking along the macro-axis. The orientation of the elasticity axes is $\mathfrak{a} = a$, $\mathfrak{b} = b$, $\mathfrak{c} = \acute{c}$. Extinction is straight in all aspects that were observed. No interference figure was seen; the optical character was not exactly determined, but it appears to be *positive*, judging from the character of the double refraction.

### BADGER, *Taxidea americana*. Plate 67.

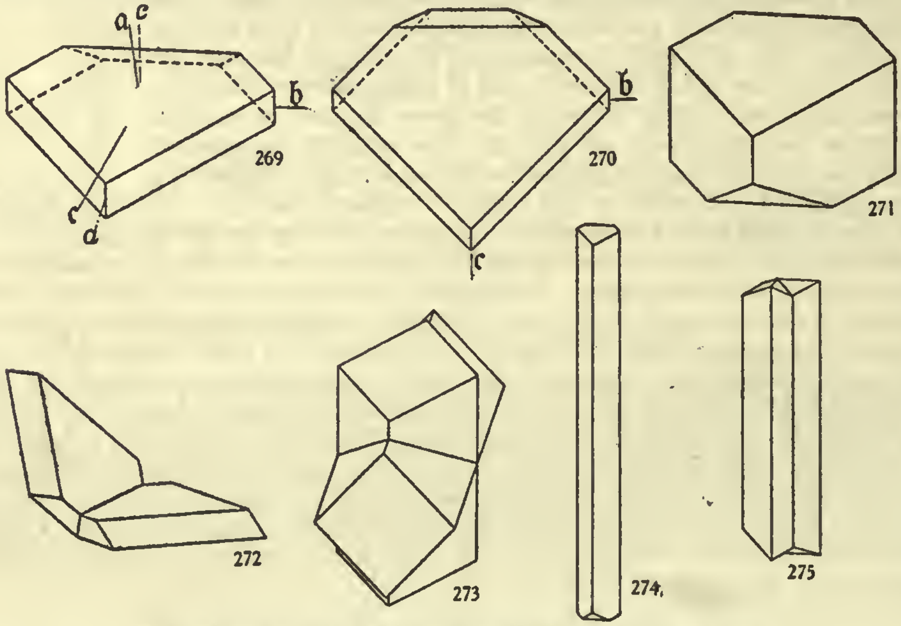
The specimen was received from the National Zoölogical Park at Washington, District of Columbia, during the summer and was kept frozen in the refrigerating plant until examined. The blood had been collected in oxalate, in our usual collecting tube; it was thawed and laked with ether, and then centrifugalized for 3 hours. From the clear solution thus obtained the slide preparations were made in the usual manner. Crystals formed readily at room temperature, about as readily as in the case of the dogs. Within 3 hours after covering, satisfactory negatives of the crystals were secured. The tabular crystals at first formed showed a slight tendency to dissolve in the solution when brought into a warm room; but when kept in the cold they were permanent for more than two weeks. After about 3 days in the cold, long rod-like crystals made their appearance; these appeared to be more soluble than the tabular crystals and dissolved when brought into the warm room. These large rods were not so permanent as the tabular crystals, and inside of 2 weeks had almost entirely disappeared from the slides; but smaller crystals of the prismatic type were still to be seen in the slides at the end of 15 days. The crystals were oxyhemoglobin, as shown by the microspectroscope; crystals of reduced hemoglobin were not observed.

### *Oxyhemoglobin of Taxidea americana.*

Monoclinic: Axial ratio $a : b : \acute{c} = 1.0355 : 1 : 1.0125$; $\beta = 54° 32'$ (calculated, or 55° measured).

Forms observed: Unit prism (110), positive hemiorthodome (101), base (001), orthoprism (430).

Angles: Unit prism, traces on the base, or edges 110–001 ∧ $1\bar{1}0$–001 = 88°; macroprism edges on base 430–001 ∧ $\bar{4}30$–001 = 75° 30′ (calculated 75° 24′); actual angle of unit prism 110 ∧ $1\bar{1}0$ = 76° 20′; positive hemiorthodome on base $10\bar{1}$ ∧ 001 = 61° 30′; prism edge on base or angle $\beta = 54° 32'$ (calculated, or 55° measured).



FIGS. 269, 270, 271, 272, 273, 274, 275. *Taxidea americana* Oxyhemoglobin.

The crystals occur in two habits which may be designated as type (a) and type (b); type (a) crystals are the first to appear.

*Crystals of type* (a): Habit at first tabular on the base and appearing as five-sided plates, consisting of the unit prism and base with one face of the unit positive hemiorthodome $(\bar{1}01)$ (text figures 269 and 270), which gives the crystal a strongly clinohedral form. These crystals grow in thickness by elongation of the prism, and the other face of the hemiorthodome $(10\bar{1})$ appears by the time they become nearly equidimensional;

the crystal has then a normal monoclinic habit (text figure 271). In this form they appear to be quite permanent, and do not disappear from the slides for more than 2 weeks. These equidimensional crystals are largely of a second growth, however, and the tabular type may persist in that form until they attain considerable size. When still in the tabular form, and within a day or two after the preparations are made, these crystals very frequently twin on the hemiorthodome as contact twins (text figure 272), showing often, too, groups of three in contact, perhaps twinned on both hemiorthodomes. As the crystals develop larger unit-prism faces they also form contact twins on the prism. Penetration twins of these short prismatic crystals, showing only the prism and base, are seen (text figure 273) twinned on the same positive hemiorthodome. Some of the plates or tabular crystals of type (a) that appeared among the first crystals, show the prism (430) instead of the unit prism; these do not seem to be common, and were not observed in the later crystals of this type that formed in the slides.

*Crystals of type* (b): These appeared, within 3 days after the preparations were made, in the slides that had been kept in the cold. The habit of these type (b) crystals is long to stout prismatic, elongated on the vertical axis (text figure 274); and they attain a length of 20 times that of the largest of the type (a) crystals. Their ratio of length to thickness varies from 25 : 1 to 4 : 1. They are evidently the unit prism terminated by the base and the hemiorthodome; but most of them are contact twins on the prism face (text figure 275), and the angle of the prism edge to base is likely to show on both sides of the termination. In some of these crystals the clinopinacoid appears to be present. Besides twinning on the prism they also appear to form penetration twins on the pyramid. These crystals are more soluble than those of type (a); they were formed in the cold and began to dissolve when brought into a warm room. They were the first crystals to disappear from the slides, and may contain more water of crystallization, or water of crystallization instead of serum of crystallization. They were oxyhemoglobin, like the type (a) crystals, but the spectrum was not measured for possible displacement of the lines, which might be expected to occur if the liquids of crystallization were not identical. The crystallographic characters appeared to be the same in both, which is to be expected with the same molecular structure, no matter what the crystal liquids may have been.

Pleochroism is marked in both types, which exhibit the same optical characters throughout, except that the prismatic type (b) usually presents one orientation with the prism face normal to the line of sight. The pleochroic colors are: $\mathfrak{a}$ nearly colorless, $\mathfrak{b}$ and $\mathfrak{c}$ nearly equal and deep red. Extinction is symmetrical on the base, or when observing the crystal in the direction of the plane of symmetry; when observing normal to the plane of symmetry, the extinction angle is 15° measured from the trace of the base or 20° 28′ from the prism edge by calculation, usually measured as about 20°. On the crystals observed normal to a prism face, the extinction is about 12° as usually observed, measured from the prism edge; in the type (b) crystals twinned on the prism this becomes 12° in each half of the twin, symmetrical with the composition plane. In convergent polarized light a brush of a biaxial interference figure was seen in some positions, especially when looking at the obtuse angle between the base and the front prism edge. The orientation of the elasticity axes is $\mathfrak{a} \wedge \acute{c} = 20^\circ 28'$, in the obtuse angle; $\mathfrak{b} = b$; $\mathfrak{c} \wedge a = 15^\circ$, in the obtuse angle. The plane of the optic axes is the plane of symmetry and the axis of greatest elasticity is the acute bisectrix, $Bx_a = \mathfrak{a}$; the optical character is hence *negative.* The axial angle is evidently large, but a single brush is seen on the cross-section of the prism and also on the basal section, but both are excentric.

### OTTER, *Lutra canadensis.* Plate 66.

The specimen of blood was received from the National Zoölogical Park of Washington, District of Columbia. The blood was oxalated and repeatedly frozen and thawed, and from this solution the slide preparations were made. The blood crystallized rather readily, and the crystals did not dissolve upon slight increase of temperature. They were oxyhemoglobin.

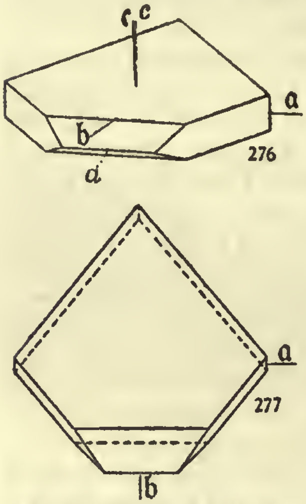
### *Oxyhemoglobin of Lutra canadensis.*

Monoclinic domatic or clinohedral: Axial ratio $a : b : \dot{c} = 1.2131 : 1 : 0.6794$; $\beta = 72°$.

Forms observed: Unit prism (110), hemiorthodomes (101) and ($\bar{1}01$), clinopinacoid (010), base (001).

Angles: Traces of the prism on the base, edges $110\text{–}001 \wedge 1\bar{1}0\text{–}001 = 101°$; orthodome on base $\bar{1}01 \wedge 001 = 49°$; prism edge on the base, edge $110\text{–}1\bar{1}0 \wedge 00\bar{1} = 72° = \beta$.

Habit tabular on the base, the crystal consisting of base (001), unit prism (110) and hemiorthodomes (101) and ($10\bar{1}$), the domes appearing at one end of the clino-axis only, indicating clinohedral symmetry (text figures 276 and 277). When the plates are very thin they appear to be orthorhombic hemimorphic, but as they increase in thickness they assume a monoclinic habit. In some cases the crystals seem to be the combination (110), (001), (111) (101) ($10\bar{1}$), but the unit pyramid was not positively established. The crystals grow singly or in aggregates of two or three together on the base in parallel growth. Twins on the orthodome, or what appears to be a contact of the orthopinacoid on the base, occur; but the orthopinacoid was not observed as a plane. A few crystals of prismatic habit were seen; they seemed to be elongated on the unit prism. The edge views of the plates also appear to be prismatic at first sight, but an inspection of the ends shows the orientation and the clinohedral habit, except when looking along the clino-axis.



**Figs. 276, 277.** *Lutra canadensis* Oxyhemoglobin.

Pleochroism is strong; $\mathfrak{a}$ nearly colorless, pale reddish, $\mathfrak{b}$ rather strong red, $\mathfrak{c}$ deep red. Double refraction is strong; extinction on the plates normal to the base is symmetrical; on edge, looking along the clino-axis, the extinction is straight. Extinctions up to 15° were observed on edge views, apparently normal to the clinopinacoid. On the base, in convergent light, the biaxial interference figure was observed with widely separated brushes, with the plane of the optic axes normal to the plane of symmetry. The orientation of the elasticity axes is $\mathfrak{a} = b$, $\mathfrak{b} \wedge a$ about 15°, $\mathfrak{c} \wedge \dot{c} = 3°$ about. The brushes of the interference figure are widely separated, passing out of the field upon rotation of the crystal; so it is very probable that the interference figure was seen when looking along the obtuse bisectrix of the optic axes and that the axis of greatest elasticity is the acute bisectrix. The character of the double refraction and of the pleochroism also points to the axis of greatest elasticity as the acute bisectrix of the optic axes; if this is correct the optical character is *negative.*

## PROCYONIDÆ.

### KINKAJOU, *Cercoleptes caudivolvulus.* Plate 68.

The specimen of blood was received from the Philadelphia Zoölogical Gardens. It was oxalated and repeatedly frozen, and from the solution thus obtained the slides were prepared as usual. Crystals formed readily at room temperature, but showed a tendency to dissolve upon an increase of temperature. The solution remained rather deeply colored. The crystals were oxyhemoglobin. They were gradually converted by paramorphous change into metoxyhemoglobin after about two weeks. Crystals of reduced hemoglobin appeared in the slides after a few days; they seemed to have the same form as the oxyhemoglobin crystals of the second type, but from the planes developed it would have been impossible to say whether the axial ratio is the same.

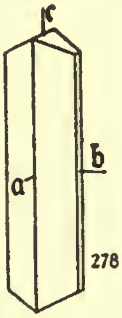
### *Oxyhemoglobin of Cercoleptes caudivolvulus.*

Orthorhombic: Axial ratio, $a : b : \dot{c} = 0.6556 : 1 : 0.4663$.

Forms observed: Unit prism (110), brachydome (011), brachypinacoid (010), base (001), macropinacoid (100).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 66° 30'$; brachydome angle $011 \wedge 0\bar{1}1 = 50°$; brachypinacoid to brachydome $010 \wedge 011 = 65°$; base to macropinacoid $001 \wedge 100 = 90°$.

The crystals occur in two habits: (a) prismatic along the vertical axis, the crystal consisting of the unit prism (110), brachydome (011), and brachypinacoid (010) (text figure 278); (b) tabular on the brachypinacoid, the crystals apparently consisting of the three pinacoids (100, 010, and 001) (text figure 279). Type (a) is the common crystal, and the crystals of the type (b) only appeared sparingly in the slides. The type (a) crystal is usually quite symmetrically developed, the planes (110) and (011) being large, but the plane (010) generally small. The dome termination is liable to develop with two faces, usually on the same side (as 011 and $01\bar{1}$), larger than the opposite pair; sometimes the larger pair are diagonally opposite each other. This unsymmetrical development occasionally goes so far as practically to suppress two of the dome faces, when the crystal has a monoclinic appearance. The ratio of length to thickness of the prism, comparing the length to the longer diagonal of the prism section, is usually about 5 : 1 and runs down to 2 : 1 or less. Definite twinning was not observed, and the crystals occur singly or simply massed together in irregular order. These type (a) crystals are very smooth and perfect and show no striation. The type (b) crystals appeared sparingly in the slides and were either nearly square plates or long lath-shaped crystals. The latter form aggregated in bundles or groups with parallel orientation, but not so regular as parallel growths; the square habit piled up on the brachypinacoid in parallel growth. This type (b) crystal seemed to be somewhat more soluble than the other type, losing its angles and especially the ends of the long lath-shaped crystals by solution, but this was probably due to the fact that they were very thin and hence showed solution corrosion sooner than the crystals of the other type. Apart from the parallel growth, these crystals showed nothing of the nature of twinning.



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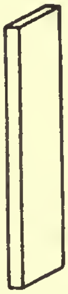
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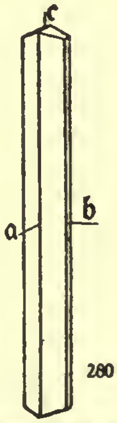

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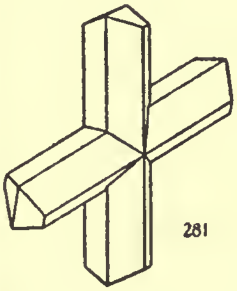
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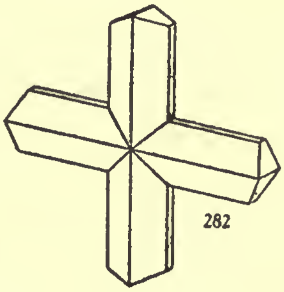
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**Figs. 278, 279.** *Cercoleptes caudivolvulus* Oxyhemoglobin. **Figs. 280, 281, 282.** *Bassariscus astuta* Oxyhemoglobin.

Pleochroism is strong when viewed on the brachypinacoid aspect in both types of crystals, but practically absent when looking along the brachy-axis. The colors are: $\mathfrak{a}$ pale yellowish-red to pinkish; $\mathfrak{b}$ and $\mathfrak{c}$ equal and deep red. Extinction is straight in all aspects. No interference figure was observed. The orientation of the elasticity axes is $\mathfrak{a} = a$, $\mathfrak{b} = b$, $\mathfrak{c} = \dot{c}$. From the character of the double refraction (very weak when $\mathfrak{b}$ and $\mathfrak{c}$ are in the section and strong when either $\mathfrak{b}$ or $\mathfrak{c}$ and $\mathfrak{a}$ are in the section) and from the pleochroism, it is evident that the axis of greatest elasticity is the acute bisectrix, $Bx_a = \mathfrak{a}$, and the optical character is hence *negative*.

### *Reduced Hemoglobin of Cercoleptes caudivolvulus.*

Orthorhombic: Axial ratio not determinable.

Forms observed: Macropinacoid (100), brachypinacoid (010), base (001).

Angles: Macropinacoid to base $100 \wedge 001 = 90°$.

Habit the same as the square tabular form of the type (b) crystals of the oxyhemoglobin (text figure 279), growing in the same parallel growth aggregates. The plates were thin and were not observed on edge.

Pleochroism on the brachypinacoid strong, as in the oxyhemoglobin; $\mathfrak{a}$ rose-pink, $\mathfrak{c}$ deep rose-red to purplish-red.

These may be only paramorphs after the oxyhemoglobin plates, type (b) crystal.

### Cacomyxl or Cacomistle, *Bassariscus astuta.* Plate 68.

The specimen of blood was received from the Philadelphia Zoölogical Gardens; and, while it had a slight odor, was in fairly fresh condition. The blood was oxalated, but the method of laking was not recorded. It was not centrifugalized. The crystals formed quite readily, and inside of 4 hours after the preparations were made satisfactory photomicrographs were obtained. The crystals do not appear to be very soluble, and retain their sharp outlines when brought from the cold into a warm room. The crystals were oxyhemoglobin.

### *Oxyhemoglobin of Bassariscus astuta.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.7399 : 1 : 0.3939$.

Forms observed: Unit prism (110), brachydome (011), brachypinacoid (010).

Angles: Unit prism $110 \wedge 1\bar{1}0 = 73°$; brachydome $011 \wedge 0\bar{1}1 = 43°$; brachypinacoid to brachydome $010 \wedge 011 = 68° 30'$.

Habit prismatic on the vertical axis, the crystal consisting of the unit prism, brachypinacoid, and brachydome (text figure 280), the brachypinacoid being in some cases very small or absent. The crystals are generally very perfect and symmetrically developed, with smooth planes and without striations. They occur as individuals, scattered through the slides, and also twinned; but do not aggregate in any regular form aside from the twins. They vary much in size and in ratio of length to thickness; generally this ratio is about 10 : 1, but short forms occur in which it runs down almost to 1 : 1. Twins occur with the unit pyramid (111) as the plane of twinning (text figure 281), also with a brachydome of about (031) as the plane of twinning (text figure 282). In the scattered crystals in the slides, a large number are seen intersecting at other angles, which may be twins; but the angles appear to vary so much that the crystals are probably in accidental orientation with each other. The crystals do not form radiating or parallel groups.

The color of the crystals in ordinary light is blood-red; in plane polarized light from one nicol they are strongly pleochroic in all aspects. The colors are: $\mathfrak{a}$ nearly colorless, $\mathfrak{b}$ rose-pink, $\mathfrak{c}$ deep rose-red. Double refraction is strong and the extinction is straight in all side views of the prism and symmetrical in cross-sections on the base. The orientation of the elasticity axes is $\mathfrak{a} = b$, $\mathfrak{b} = a$, $\mathfrak{c} = \acute{c}$. The plane of the optic axes is the macropinacoid. On basal sections, and on the brachypinacoid, the biaxial interference figure may be observed, with the brushes widely separated; but the figure seen on the basal section shows that the axis of least elasticity is the acute bisectrix $Bx_a = \mathfrak{c}$, and the optical character is hence *positive.*

## URSIDÆ.

### Black Bear, *Ursus americanus.* Plates 69 and 70.

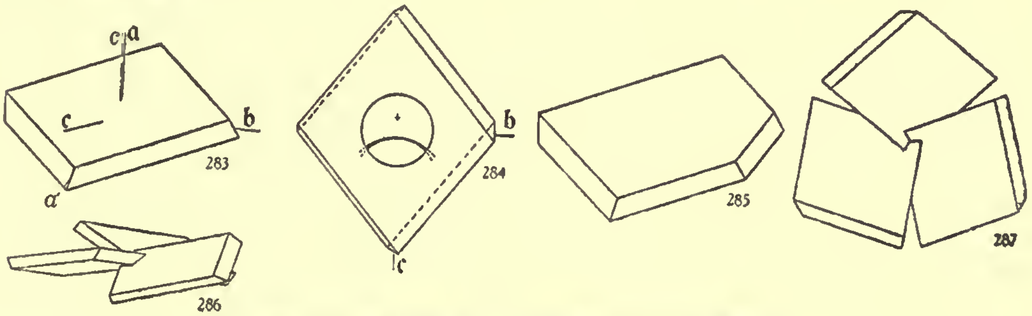
Two specimens of blood were examined, one from the Pittsburg Zoölogical Garden (specimen I) and one from the Philadelphia Zoölogical Garden (specimen II). Both specimens had been collected in oxalate in our regular tubes. Specimen I was rather thick and slightly decomposed; it was laked with ether and centrifugalized, and from the clear solution the slide preparations were made as usual. Specimen II was clotted and very slightly putrid; it was ground up in sand, with the addition of ether, and the mixture centrifugalized. This gave a clear solution from which the slide preparations were made. Both crystallized in the protein ring soon after the covers were applied, but the crystals in each case showed a tendency to dissolve; and 24 hours later crystals of much larger size appeared along the cover edge. The first crystals were of the same type, a peculiar trilling that was found in all crystals of bears that were examined; the second crop of larger crystals were single, or in parallel growth, rather than twinned. All of the crystals were at first oxyhemoglobin, but the solution showed traces of metoxyhemoglobin in specimen II, and later crystals of this material developed in both preparations. They seemed to be paramorphs of the oxyhemoglobin, however, and did not differ very much in their angles from the oxyhemoglobin crystals, although the prism angle was slightly larger, as will be seen below.

*Oxyhemoglobin of Ursus americanus.*

Monoclinic hemimorphic, monoclinic sphenoidal (tartaric-acid type): Axial ratio $a : b : \acute{c} = 1.2239 : 1 : 1.1429$; $\beta = 75° 5'$ (calculated).

Forms observed: Unit pyramid $(\bar{1}11)$, unit prism $(1\bar{1}0)$, clinopinacoid (010), base (001), and in twins, prism $(2\bar{3}0)$.

Angles: Traces of the unit prism or the unit pyramid on the base, the edges $1\bar{1}0$–$001 \wedge \bar{1}\bar{1}0$–$001 = 78° 30'$ = the angle between the edges $11\bar{1}$–$001 \wedge \bar{1}11$–$001$; from sections looking along the edges $1\bar{1}0$–$001$ or $\bar{1}11$–$001$ the angles of $1\bar{1}0 \wedge 001 = 79°$, and $\bar{1}11 \wedge 001 = 46° 47°$.



FIGS. 283, 284, 285, 286, 287. *Ursus americanus* Oxyhemoglobin.

Habit of the first crystals to form (in and about the protein ring) squarish tabular on the base and consisting of the forms, base (001), unit prism $(1\bar{1}0)$ on one end of the binary axis, and unit pyramid $(\bar{1}11)$ on the other end (text figures 283 and 284), the one being the analogous pole and the other the antilogous pole. These crystals are found occurring singly or in trillings (text figures 286 and 287), the three individuals in the trilling growing with their unit prism edges pointing in towards the center of the group and with the edges formed by the unit pyramid pointing outward, but each member of the group overlapping or tilted in the same sense against the adjacent member, in cyclic order, the relative position of the three individuals being comparable to that of the three blades of a screw-propeller (text figure 286). The vertical axes of the three individuals are parallel, and the composition face is evidently the prism $(2\bar{3}0)$, whose angle, calculated from the axial ratios, is 60° 35′. This form was not observed as a crystal plane.

The first-formed crystals do not attain large size, and are gradually dissolved into the solution, as larger crystals of a second crop (text figure 285) begin to appear along the cover edge. These latter do not appear to twin as do those of the first crop; they grow singly, or united into irregular aggregates, or forming larger groups in parallel growth. As they attain a large size, they develop many false planes due to the interference of the cover and slide with the growth of the crystals; and in many cases such false planes are symmetrical with the principal axis. In these crystals the form (010), the clinopinacoid, is sometimes observed, appearing on the end of the binary axis that is cut by the unit pyramid $(\bar{1}11)$ (text figure 285). The hemimorphic character of these crystals is very noticeable; not only in the perfect crystals, but also in sections, which show at the one end the unit pyramid and at the other the unit prism, and these sections appear to be most commonly in the zone of the normal to the prism-base edge.

The color of the crystals is the usual oxyhemoglobin scarlet, and pleochroism is quite marked; $\mathfrak{a}$ is colorless or nearly so; $\mathfrak{b}$ is almost equal to $\mathfrak{c}$, and both are deep scarlet-red. A few sections were seen, evidently in the zone of $100 \wedge 001$, but showing the hemimorphic character very markedly, that gave straight extinction, because such a section is parallel to the binary axis. All other sections of the plate gave oblique extinction, the maximum extinction angle being reached on the clinopinacoid section, and giving about 19°, measured from *a*. The usual cross-sections of the tabular crystals are normal to the edge $\bar{1}11$–001 or nearly so and give an extinction angle of 12° to 13°, about. On the base, the extinction is symmetrical with the outline of the crystal. The biaxial interference figure is indicated by one brush of the figure appearing on the base in convergent light, with traces of the second brush on revolution of the crystal. The orientation of the elasticity axes is $\mathfrak{c} \wedge a = 19°$ (about), in the obtuse angle; $\mathfrak{a} \wedge \acute{c} = 6°$ (about), in the obtuse angle; $\mathfrak{b} = b$. The plane of the optic axes is the clinopinacoid, and the acute bisectrix of the axes is the axis of greatest elasticity, $Bx_a = \mathfrak{a}$. The optical character is hence *negative*. The angle $2E$ could not be measured, but was apparently not very large, perhaps about 60°.

### *Metoxyhemoglobin of Ursus americanus.*

Monoclinic hemimorphic (monoclinic sphenoidal), like the oxyhemoglobin, and the angles about the same. One or two gave an angle of the plate of 80°, but most of them ran about 78° 30′, as in the oxyhemoglobin. The habit of twinning and the forms observed were the same; the optical characters are identical in the metoxyhemoglobin and in the oxyhemoglobin; the extinction angles, the negative character of the double refraction, and the relative elasticities could not be distinguished in the two. The crystals which developed in slides that had been kept for a considerable time were probably paramorphous. The thin crystals showed the oxyhemoglobin bands only, but the thicker crystals showed the characteristic band in the red and the shortening of the blue end of the spectrum characteristic of metoxyhemoglobin.

### POLAR BEAR, *Ursus maritimus*. Plate 70.

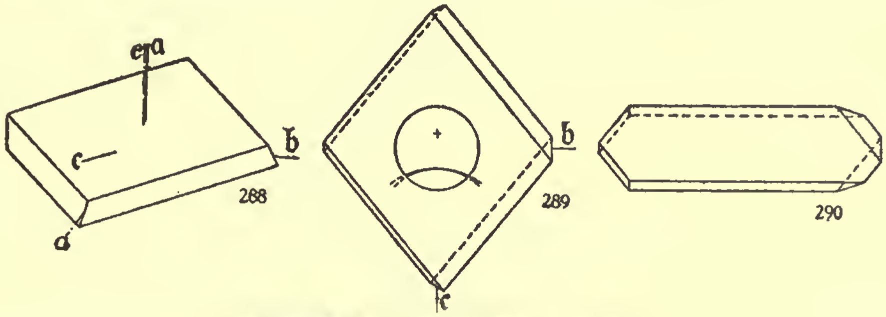
The specimen was received from the National Zoölogical Park at Washington, District of Columbia, and was the blood from a young bear that had died in the Park. The blood was quite thick and slightly putrid. It was oxalated, laked with ether, and centrifugalized, and from the clear solution thus obtained the slide preparations were made. Crystals began to form in the protein ring at room temperature, and the blood crystallized readily; but as the solution came to equilibrium in about 1 hour these first-formed crystals were partly dissolved, and a second crop began to form along the cover edge. The first crop was almost entirely dissolved in the solution, but the second crop of crystals was more permanent. As in the case of the black bear, the crystals of the first crop were mostly trillings, and those of the second crop were mainly single, untwinned crystals. The color of the blood and of the crystals was decidedly brownish, but the spectrum was that of oxyhemoglobin. The solution probably contained metoxyhemoglobin, which seems to be common in bear blood, at least during the winter.

*Oxyhemoglobin of Ursus maritimus.*

Monoclinic hemimorphic (monoclinic sphenoidal): Axial ratio $a : b : \acute{c} = 1.2088 : 1 : \acute{c}$; $\beta = 73° 2'$ (calculated).

Forms observed: Unit pyramid $(\bar{1}11)$, imperfectly developed; unit prism $(1\bar{1}0)$, prism $(2\bar{3}0)$, in twins only; basal pinacoid (001); also rarely (100) ? or (101).

Angles : Traces of the unit prism on the base, edges $110\text{–}001 \wedge \bar{1}10\text{–}001 = 79° 12\tfrac{1}{2}'$, average of a number of measurements; $110 \wedge 001 = 77°$ (about); $\bar{1}11 \wedge 001 = 48° 30'$. The angle of the dome (101), or orthopinacoid (100), on the base was not determined.



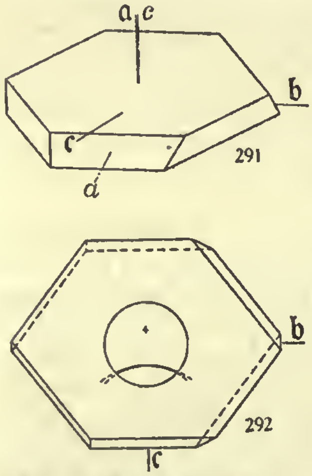
FIGS. 288, 289, 290. *Ursus maritimus* Oxyhemoglobin.

Habit tabular on the base, the crystal consisting of the basal pinacoid (001) cut by the unit prism at one end of the ortho-axis and by the unit pyramid at the other end (text figures 288 and 289). The first crystals to form are of this habit, porous-looking tabular crystals that are proportionately rather thick, but they do not attain very large size. They are usually in trillings as already described for the black bear oxyhemoglobin (text figures 286 and 287) and this kind of twinning is normal in the bears. As these crystals of the first crop disappear, they are succeeded by crystals of the second crop, in which the base (001) and the unit prism (110) are well formed, and usually have sharp angles and smooth faces; but the unit-pyramid planes are generally imperfect in these second-crop crystals. Nevertheless, the angle of the unit pyramid on the base was obtained from crystals of the second crop. The tabular crystals of the first crop are proportionately much thicker than those of the second crop, the ratio of the long diagonal of the plate to its thickness being about 5 : 1; while in the second-crop crystals this ratio will average nearer to 25 : 1. The crystals of the second crop produce parallel growths, but do not twin, as do those of the first crop. A few crystals were observed in the second crop that were prismatic on the ortho-axis, by development of planes in the zone 100–001, which seemed to be (100) or (101), and (001) (text figure 290); but the angle on the base was not measurable, owing to the position in which the crystals were lying.

Pleochroism was rather marked in positions where the axis of greatest elasticity appeared in the section; the colors were: $\mathfrak{a}$ nearly colorless, $\mathfrak{b}$ and $\mathfrak{c}$ about equal and deep red, somewhat brownish-red. The crystals of the first crop, which look soft and porous, do not show as strong double refraction as those of the second crop; the extinction on the flat is symmetrical with the outline of the plate; on edge views of the plate the extinction runs up to 20° with the axis $a$, or the long dimension of the plate. The orientation of the elasticity axes is $\mathfrak{c} \wedge a = 20°$, in the obtuse angle; $\mathfrak{b} = b$, $\mathfrak{a} \wedge \acute{c} = 0° 6'$ (calculated); or $\mathfrak{a}$ is parallel to $\acute{c}$ as nearly as can be measured. On the flat aspect, in convergent light, a rather dusky interference figure was observed, showing one brush of the biaxial figure, with traces of the other brush in certain positions. The bisectrix of the optic axes is evidently the axis of greatest elasticity $Bx_a = \mathfrak{a}$, and the optical character is hence *negative.*

### SLOTH BEAR, *Melursus ursinus.* Plates 71 and 72.

The specimen was received from the Philadelphia Zoölogical Gardens and was in a very putrid condition. The blood was oxalated, laked with ether, and centrifugalized to obtain the clear solution from which the slide preparations were made. The blood crystallized readily at room temperature, and the crystals were fairly permanent. These first crystals were frequently, but not always, twinned, as was the case with the crystals from the bloods of other bears examined. As in other cases, too, a second crop of crystals formed that were more perfect than those of the first crop; and these were not so frequently twinned. The crystals, especially those of the second crop, kept well and did not dissolve in the solution. They were of the monoclinic sphenoidal class (tartaric-acid type) as in the other bears, but occasionally a slide would contain a few crystals which appeared to be of more symmetrical character. They all appeared to be oxyhemoglobin.



FIGS. 291, 292. *Melursus ursinus* Oxyhemoglobin.

#### *Oxyhemoglobin of Melursus ursinus.*

Monoclinic hemimorphic, monoclinic sphenoidal (tartaric-acid type): Axial ratio $a : b : \acute{c} = 1.2857 : 1 : 1.498$; $\beta = 68° 40'$ (calculated).

Forms observed: Unit pyramid ($\bar{1}11$), unit prism ($1\bar{1}0$), orthopinacoid (100), orthodome (101), base (001); in twins, prism ($2\bar{3}0$).

Angles: Traces of unit prism or unit pyramid on the base, angle of the edges $1\bar{1}0\text{–}001 \wedge 1\bar{1}\bar{1}\text{–}001 = 75° 35'$; prism to base $\bar{1}10 \wedge 001 = 77°$, unit pyramid to base $\bar{1}11 \wedge 001 = 48° 15'$.

Habit thin tabular on the base, the bounding planes being on one end of the ortho-axis the unit prism ($1\bar{1}0$), and on the other end the unit pyramid ($\bar{1}11$), with very often the orthopinacoid or an orthodome, (100) or (101), making a six-sided plate (text figures 291 and 292). The first crystals to form were frequently the trilling, which appears to be common in the bears, and which is described under the black bear (text figures 286 and 287). But this form of twinning was not so common and characteristic as is the case with the two species of *Ursus* examined. Single crystals were much more common in this species than these trillings; and the twins were almost absent from the crystals of the second crop. These latter were larger and more perfect than those of the first crystals to form, and they were mainly seen along the cover edge. They grow into groups, sometimes quite arborescent in appearance, and all in parallel growth position; the group elongates along the ortho-axis and the faces of the orthopinacoid are likely to develop prominently in these groups. They usually have the appearance of overlapping, roughly hexagonal plates, the overlapping being where the unit-prism edge grows over the unit-pyramid edge. All sorts of irregular groups are thus produced, the crystals appearing to unite on the base with the orientation the same throughout the entire group. Edge views of these second-crop crystals often give the angles of the unit-prism and unit pyramid on the base in measurable condition. The individual tabular crystals, not in parallel growth, frequently grow together in the zone of the pyramid base in radiating groups as seen on edge; some of these may be in twinned position.

Pleochroism is strong, especially when the axis $\mathfrak{a}$ is visible; the colors are: $\mathfrak{a}$ nearly colorless, pale reddish; $\mathfrak{b}$ and $\mathfrak{c}$ nearly equal and deep red, but the color of $\mathfrak{b}$ distinctly lower than that of $\mathfrak{c}$. Double refraction is strong, and on the base the extinction is symmetrical; on edge views the extinction varies from 0° up to 20° as a maximum, and most of the edge views of the plates give an extinction angle of 7° or 8°, measured from the trace of the base (001), or the long dimension of the plate. In convergent light, the basal aspect shows a dusky biaxial cross; or one brush of such a cross, with traces of the other. The orientation of the elasticity axes is $\mathfrak{c} \wedge a = 20°$, $\mathfrak{b} = b$, $\mathfrak{a} \wedge \acute{c} = 1° 20'$. The crystals with the orthodome developed appear to be orthorhombic, owing to straight extinction along the apparent prism; but this is only straight on the aspects in the zone 001–101, and the orientation of the elasticity axes appears to be the same in these as in the others. The plane of the optic axes is the clinopinacoid; the binary axis is the axis of mean elasticity. The acute bisectrix of the optic axes is evidently the axis of greatest elasticity, $Bx_a = \mathfrak{a}$, and the optical character is hence *negative*.

TABLE 43.—*Crystallographic characters of the hemoglobins of the Phocidæ, Otariidæ, Mustelidæ, Procyonidæ, and Ursidæ.*

| Name of species. | Axial ratio. | Prism angle or traces of prism on base (real angle). | Angle β. | Extinction angle. | Optical character. | System and class. | Substance. |
|---|---|---|---|---|---|---|---|
| Phocidæ: | | ° ′ | ° ′ | | | | |
| Phoca vitulina .......... | 1.2131 : 1 : 1.1970 | 79 0 | 75 0 | $\mathfrak{a} = \acute{c}$<br>$\mathfrak{c} \wedge a = 15°$ | Negative | Monoclinic hemimorphic | OHb. |
| Otariidæ: | | | | | | | |
| Otaria gillespii........... | $(\frac{3}{2})$1.2012 : 1 : $(\frac{2}{3})$1.1825<br>1.8019 : 1 : 0.7883 | 76 30 | 74 0 | $\mathfrak{c} \wedge \acute{c} = 16° 30'$ | Do. | Do. | OHb. |
| Do................. | 1.8019 : 1 : 0.7883 | 76 30 | 74 0 | $\mathfrak{c} \wedge \acute{c} = 16°$ | Do. | Do. | COHb. |
| Mustelidæ: | | | | | | | |
| Mustela putorius, var .... | 1.2799 : 1 : 1.1105 | 76 0 | 68 0 | ............<br>0° | Do. | Monoclinic domatic | OHb. |
| Mephitis mephitica putida | $a : \acute{c} = 1 : 0.4877$ | ...... | 90 0 | $\mathfrak{c} \wedge a = 15°$ | Positive | Orthorhombic | OHb. |
| Taxidea americana ...... | 1.0355 : 1 : 1.0125 | 88 0 | 54 32 | $\mathfrak{a} \wedge \acute{c} = 20° 28'$ | Negative | Monoclinic | OHb. |
| Lutra canadensis ........ | 1.2131 : 1 : 0.6794 | 79 0 | 72 0 | $\mathfrak{b} \wedge a = 15°$<br>$\mathfrak{c} \wedge \acute{c} = 3°$ | Do. | Monoclinic domatic | OHb. |
| Procyonidæ: | | | | | | | |
| Cercoleptes caudivolvulus | 0.6556 : 1 : 0.4663 | 66 30 | 90 0 | 0° | Do. | Orthorhombic | OHb. |
| Bassariscus astuta....... | 0.7399 : 1 : 0.3939 | 73 0 | 90 0 | 0° | Positive | Do. | OHb. |
| Ursidæ: | | | | | | | |
| Ursus americanus ....... | 1.2239 : 1 : 1.1429 | 78 30 | 75 5 | $\mathfrak{c} \wedge a = 19°$ | Negative | Monoclinic hemimorphic | OHb. |
| Ursus maritimus ........ | 1.2088 : 1 : $\acute{c}$ | 79 12½ | 73 2 | $\mathfrak{c} \wedge a = 20°$ | Do. | Do. | OHb. |
| Melursus ursinus ........ | 1.2857 : 1 : 1.498 | 75 35 | 68 40 | $\mathfrak{c} \wedge a = 20°$ | Do. | Do. | OHb. |

# CHAPTER XV.

## CRYSTALLOGRAPHY OF THE HEMOGLOBINS OF THE CANIDÆ—DOGS, WOLVES, AND FOXES.

Twelve members of the family *Canidæ* were studied, representing 10 distinct species, and two varieties or crosses. Some of the species were represented by several specimens, so that their crystals could be studied under different methods of preparation. As a group, the dogs possess hemoglobins of a rather insoluble character, the crystals form readily and they do not readily dissolve. All members of the family furnished oxyhemoglobin crystals which closely resembled each other, so that the differences between species were not readily made out. All the crystals, without exception were orthorhombic, and the optical character was negative in each case. The axial ratios were so nearly alike, especially the ratio of $a : \dot{c}$, which was determinable in each case, that, in spite of the fact that the zoölogists place the 10 species examined in three different genera, they show much less difference among themselves than is common with the species of a single genus.

Six species and two varieties of the genus *Canis* were studied, also three species of the genus *Vulpes*, and the gray fox *Urocyon*. Of the genus *Canis*, the species examined included the common dog, *Canis familiaris;* the chow dog, a variety of the same species; a cross between the coyote and a collie dog; the gray wolf, *Canis lupus mexicanus;* the coyote, *Canis latrans;* the jackal, *Canis aureus;* the dingo, *Canis dingo;* and Azara's wild dog, *Canis azaræ*. This list of dogs includes animals from Europe, Asia, and Australia, besides those from North and South America. In spite of this wide range, however, the species examined show a remarkable resemblance in their crystals. The four foxes studied were the Swiss fox, *Vulpes vulpes,* the American red fox, *Vulpes fulvus;* the Arctic or blue fox, *Vulpes lagopus,* and the gray fox, *Urocyon cinereoargenteus.*

The general type of crystal, common to all species of this family, is a more or less elongated prism, with a diamond-shaped cross-section, and usually strongly striated longitudinally, terminated by a rather flat dome. The striation of the prism is due to the tendency of the crystals to form in needles and aggregate into bundles of crystals; and this makes the dome terminations rather small, so that the angles, on which the crystallographic constants are based, are only determined with some difficulty. The prism angle is still more difficult to observe, as cross-sections are hard to find that are sufficiently symmetrical for trustworthy measurements; and all examinations of angles must be made with a moderately high-power objective. On account of these difficulties of measurement, the complete axial ratio was not always obtained; but, as noted above, the dome angle, giving the ratio of $a : \acute{c}$, was made out in each case. It is very constant in the entire group, as will be seen from table 44, page 279; and the common dog and the chow dog, domesticated varieties, include the extremes of variation noted in this ratio. Different strains of the common dog vary among themselves in such a way as to lead to the conclusion that they are a polyphyletic group; some individuals seem to approach the wolf, others the jackal, etc. But this part of the subject has not been worked out with sufficient detail to warrant any final conclusions. In all cases but one the material examined was oxyhemoglobin; and as the material in this one case was metoxyhemoglobin, into which the oxyhemoglobin was in several other cases seen to pass by paramorphous change, it may safely be said that all of the crystals examined were strictly comparable. It is well known that crosses between the dogs are readily obtained; and, from the close resemblance of the crystals in the species examined, it would seem probable that any one of these species could cross with any other.

## CANIDÆ.

### DOG, *Canis familiaris*. Plate 72.

Specimens of dog blood were obtained from living animals in the laboratory. Preparations were made from the whole blood defibrinated by beating; from blood kept liquid by oxalating; and also from mixtures of whole blood and the blood plasma. The blood, either defibrinated or oxalated, was laked with ether and centrifugalized; and from the clear solution thus obtained, with or without the addition of plasma, the slide preparations were made. Two forms of crystallization were observed in the oxyhemoglobin; the first, distinguished as α-oxyhemoglobin, is the normal form and crystallizes in the orthorhombic system; the second, which is designated as β-oxyhemoglobin, is monoclinic. The α-oxyhemoglobin is readily obtained by any of the methods of preparation above mentioned and is very insoluble, the crystals continuing to form until the color is practically discharged from the solution, and the slide is filled with a mass of needles in most cases. When the preparation is thick, the crystals become so massed together in the slides that the preparations are useless for crystallographic investigation (see plate 72, fig. 429). The β-oxyhemoglobin crystals were only occasionally observed (in perhaps one out of a dozen slides), and appeared to develop more readily in the blood to which no oxalate had been added. Aside from this, no difference was noted in preparations made with and without oxalate. All crystals became brownish on standing under the cover for some days, and passed by paramorphism into the metoxyhemoglobin, but without any change in their angles.

#### *a-Oxyhemoglobin of Canis familiaris.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.6745 : 1 : 0.2863$; $a : c = 1 : 0.4245$.

Forms observed: Unit prism (110), macrodome (101), base (001).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 68°$ (normals); macrodome angle $101 \wedge \bar{1}01 = 46°$ (normals) (also measured on some specimens as 44°).

Habit prismatic, elongated parallel to the vertical axis, and the prism terminated by the flat dome (101) (text figure 293) or sometimes by the base (text figure 294). The first crystals to form are usually hair-like, but stouter crystals later develop along the protein ring and the cover edge. These stouter crystals may also appear in the body of the slide, and may even be doubly terminated. They are often seen to be composite, by parallel growth in the zone of the vertical axis; and are strongly striated in this direction, because of the parallel growth. Cross-sections are rare, and almost never appear in measurable form until the slides are several days old. The ratio of length to thickness varies from 500 : 1 in the hair-like crystals, down to 20 : 1 in some of the stouter crystals. Twinning was not observed, but a parallel growth, in which the crystals grow together upon the brachypinacoid, is very commonly seen. In such groups two prisms are seen in perfectly parallel orientation, and united side by side on the brachypinacoid. This is a character quite common in all of these rod-like orthorhombic crystals in other species also.

Pleochroism is marked; $\mathfrak{a}$ nearly colorless, $\mathfrak{b}$ rather pale red, $\mathfrak{c}$ deep red. Double refraction is strong; and extinction is straight in all side views and symmetrical on cross-sections of the prism. The orientation of the optic axis $\mathfrak{a} = a$, $\mathfrak{b} = b$, $\mathfrak{c} = \acute{c}$. No interference figure was observed; but, from the relative elasticities in different directions, it would appear probable that the optical character was *negative* and the acute bisectrix $Bx_a = \mathfrak{a}$.

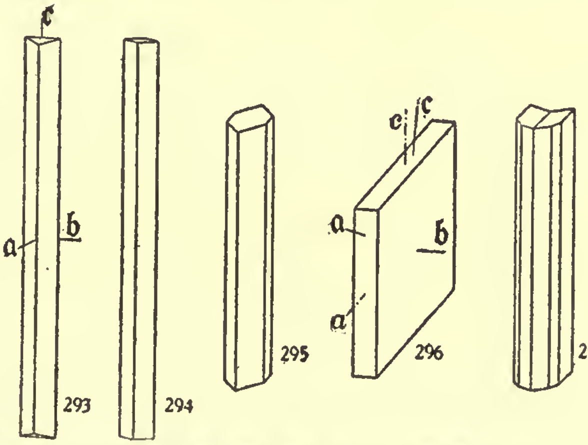
### *β-Oxyhemoglobin of Canis familiaris.*

Monoclinic: Axial ratio not determined, $\beta = 78°$.

Forms observed: Unit prism (110), clinopinacoid (010), orthopinacoid (100), basal pinacoid (001).

Angles: Orthopinacoid to base $100 \wedge 001 = 78° = \beta$; the prism angle appears to be acute on $a$ or $a > b$, but no cross-sections were found from which this angle of the prism could be obtained.

Habit (a) prismatic on the vertical axis, the crystal consisting of the unit prism, clinopinacoid, and base (text figure 295); the crystals rather large and well-formed; also (b) tabular on the clinopinacoid, the crystal consisting of the three pinacoids only, with the base and orthopinacoid in equilibrium; thus making a rhomboidal plate (text figure 296). Both kinds of crystals, types (a) and (b), were found very sparingly, in a preparation of defibrinated blood without oxalate; the plate-like crystals of type (b) were seen still more sparingly in a preparation in which oxalate was used. Often preparations of defibrinated blood failed to develop these crystals.



Figs. 293, 294. *Canis familiaris* α-Oxyhemoglobin.
Figs. 295, 296, 297. *Canis familiaris* β-Oxyhemoglobin.

Pleochroism is very strong; $\mathfrak{a}$ pale yellowish-red, $\mathfrak{b}$ rose-pink, $\mathfrak{c}$ deep blood-red. Double refraction is strong, and the extinction, measured from the prism edge, is 15°. The orientation of the optic axes is as follows: $\mathfrak{a} \wedge a = 27°$ in the obtuse angle; $\mathfrak{b} = b$; $\mathfrak{c} \wedge \acute{c} = 15°$, in the acute angle. Looking at the crystal, normal to the orthopinacoid, in convergent light, one brush of the interference figure is seen, showing that the axis of greatest elasticity is the acute bisectrix $Bx_a = \mathfrak{a}$, and the optical character is hence *negative*. The plane of the optic axes is the plane of symmetry, and the axis of mean elasticity is the ortho-axis. The crystals twin on a prism, and are frequently seen so twinned, in such a position that the extinction is symmetrical to the twin plane (text figure 297). In such twins, the extinction angle is, of course, less than that recorded above, about 7° or 8°.

### CHOW DOG, *Canis familiaris var.* Plate 73.

The specimen of blood was received from the Philadelphia Zoölogical Gardens, and was in a clotted and rather putrid condition. The specimen was ground in sand and etherized and then centrifugalized for several hours; and from the clear solution thus obtained the slide preparations were made as usual. Crystals form rapidly and readily at room temperature, and show no sign of dissolving. Within 3 hours after the slide preparations were made, satisfactory photographs were procured. The crystals were oxyhemoglobin, and resemble those of the common domestic dogs very closely; appearing, however, to differ slightly in angles.

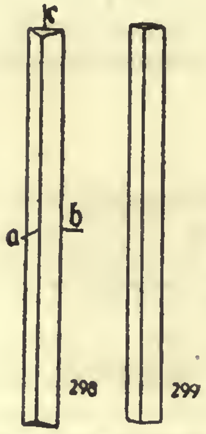
*Oxyhemoglobin of Canis familiaris var.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.6696 : 1 : 0.2878$; $a : \acute{c} = 1 : 0.4348$.

Forms observed: Unit prism (110), macrodome (101), base (001).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 67°$; macrodome angle $101 \wedge \bar{1}01 = 47°$.

Habit long prismatic on the vertical axis, the crystals consisting of the unit prism terminated by the macrodome and sometimes also by the base (text figures 298 and 299). The first crystals to form are long and hair-like or needle-like; but, as they grow, they develop greater thickness; so that the normal and fully developed crystal has a ratio of length to thickness of about 25 : 1 to 15 : 1, and sometimes somewhat less, down to 10 : 1. Doubly terminated crystals of measurable quality are comparatively common and the crystals are well developed and sharp in outline. Cross-sections of the crystals are, however, difficult to find, owing to the great length of the crystals in proportion to their thickness. Parallel growth is normal on the prism faces and on the brachypinacoid, and the crystals flatten in this way in the macropinacoid direction. A very common feature is the development of double crystals in this way, two growing side by side in parallel orientation and united on the brachypinacoid. The hair-like crystals which have a ratio of length to thickness of 100 : 1, or even 200 : 1, do not show this tendency to parallel growth until they have considerably increased in thickness; the most perfect crystals are generally found in the ratio of about 20 : 1. The crystals grow singly through the slide, or in irregular groups, and often in radiating clusters; small thin rods, attached in such radiating groups to larger composite crystals, being particularly common. In the protein ring, and along the cover edge, they grow usually more or less normal to the surface from which they spring; but radiating tufts are common here also. Twinning was not definitely observed; but indications of twinning upon the brachydome and upon the pyramid were seen; and twinning on the prism appeared to be present in many of the composite groups. These last could hardly be made out with certainty without observations of cross-sections, and these were impossible to find.



FIGS. 298, 299. Oxyhemoglobin of "Chow Dog."

Pleochroism is marked and is as follows: $\mathfrak{a}$ yellowish-red, $\mathfrak{b}$ pale red (medium oxyhemoglobin red), $\mathfrak{c}$ deep red. Double refraction is strong, and extinction is parallel to the vertical axis in all side views; on cross-sections extinction is symmetrical, and parallel to the crystal axes. Looking along the brachy-axis in convergent light, the interference figure is seen, with widely separated brushes, the plane of the axes being the brachypinacoid. The orientation of the elasticity axes is $\mathfrak{a} = a$; $\mathfrak{b} = b$; $\mathfrak{c} = \acute{c}$. The acute bisectrix of the optic axes is the axis of greatest elasticity, $Bx_a = \mathfrak{a}$, and the optical character is hence *negative.*

### CROSS BETWEEN COLLIE DOG AND COYOTE, *Canis familiaris* AND *Canis latrans.* Plate 73.

This specimen of the blood of a hybrid coyote was from an animal 8 years of age that was killed fighting with the other coyotes with which it was confined; and was received from the Zoölogical Garden at Lincoln Park, Chicago. The blood was not clotted; it was dark purplish in color and quite putrid. It was ether-laked, mixed with an equal volume of 50 per cent solution of egg-white, and centrifugalized. From the clear solution thus obtained the slide preparations were made in the usual manner. The blood crystallized very readily, and inside of 2 hours after the slides were covered satisfactory photomicrographs were obtained. The crystallization was very complete, and the crystals showed no signs of dissolving. The crystals were oxyhemoglobin.

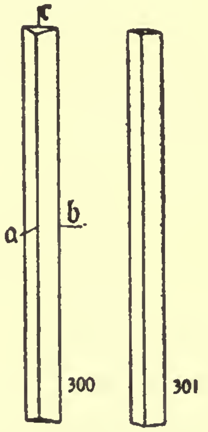
*Oxyhemoglobin of cross between C. familiaris and C. latrans.*

Orthorhombic: Axial ratio $a : b : \dot{c} = 0.6619 : 1 : 0.2912$; $a : \dot{c} = 1 : 0.4400$.

Forms observed: Unit prism (110), macrodome (101), (706), base (001).

Angles: Prism angle about 67°, not measured exactly; macrodome angle $101 \wedge \bar{1}01 = 47° 30'$; macrodome $706 \wedge 706 = 54° 30'$; prism to base $110 \wedge 001 = 90°$.

Habit medium and long prismatic to capillary; the first-formed crystals are trichites without much dimension, aside from length; these soon increase in diameter, and become measurable crystals, with a ratio of length to thickness of 20 : 1 or less (down to 10 : 1) (text figure 300); some of the first-formed crystals, even, show such relative dimensions. The crystals of large size show, usually, parallel growth on the prismatic axis, producing the usual groups of two, side by side, and united on the brachypinacoid; but much more often they form irregular groups in parallel growth, looking like bundles of crystals; and these, especially, grow in such a manner that the dome faces are suppressed and a pseudo-basal pinacoid develops. These groups appear cut off square on the ends by this apparent base (text figure 301). Whether the real basal pinacoid actually occurs, or whether the apparent base is always this pseudo-plane, could not be determined. Both the capillary and the stouter crystals are found growing in irregularly radiating tufts, as is common in the dog crystals. Twins were only doubtfully observed; the apparent twins seemed to be on the pyramid.

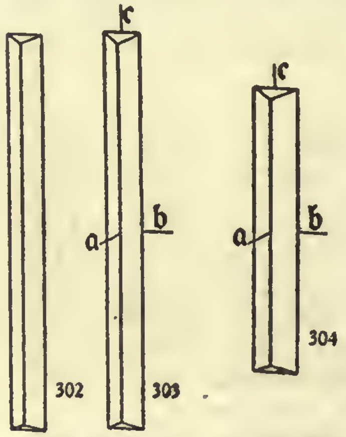


FIGS. 300, 301. Oxyhemoglobin of Cross of Collie and Coyote.

Pleochroism is rather pronounced; $\mathfrak{a}$ nearly colorless, $\mathfrak{b}$ rather strong red, $\mathfrak{c}$ rather deep red; but the colors of $\mathfrak{b}$ and $\mathfrak{c}$ are not so very different. Double refraction is strong and extinction straight in all side views. In convergent light, looking along the brachyaxis, the biaxial interference figure is seen, with widely separated brushes, and the orientation such that the plane of the optic axes is the brachypinacoid. The orientation of the elasticity axes is $\mathfrak{a} = a$, $\mathfrak{b} = b$, $\mathfrak{c} = \dot{c}$. The acute bisectrix of the optic axes is the axis of greatest elasticity, $Bx_a = \mathfrak{a}$, and the optical character is *negative.*

### GRAY WOLF, *Canis lupus mexicanus.* Plate 74.

This specimen was received from the Philadelphia Zoölogical Gardens. The blood was of a brownish color and was rather thin and watery. It was oxalated and ether-laked and then centrifugalized for several hours. From the clear solution thus obtained the slide preparations were made as usual. The blood crystallized very readily, and the crystals showed no tendency to dissolve in the solution. They were brownish in color, and the spectroscope showed them to be metoxyhemoglobin. Later, a sort of second crop of crystals appeared, of a somewhat different habit; but evidently the same material, and with the same axial ratio. The morphological characters of these crystals, including the angles, compare very closely with those of other species of *Canis*, and evidently this metoxyhemoglobin crystallizes much as the oxyhemoglobin does in this species, as is found to be the case in other genera where both substances were observed in one species and could be directly compared.



Figs. 302, 303. *Canis lupus mexicanus* Metoxyhemoglobin.
Fig. 304. *Canis latrans* Oxyhemoglobin.

### *Metoxyhemoglobin of Canis lupus mexicanus.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.6576 : 1 : 0.2863$; $a : \acute{c} = 1 : 0.4272$.

Forms observed: Prism (670), macrodomes (101), (403).

Angles: Brachy-prism angle $670 \wedge 670 = 75°$; unit prism (computed) $110 \wedge \bar{1}10 = 66° 40'$; macrodome $403 \wedge \bar{4}03 = 53°$; macrodome $101 \wedge 101$ (computed) $46° 16'$; measured roughly as 46°.

Habit long prismatic on the vertical axis (text figures 302 and 303), the crystals as they increase in size becoming strongly striated, due to composition of many individuals in parallel growth. Capillary crystals not so common as is usual in the *Canidæ*, but the thicker crystals rather long in proportion to the thickness, with a ratio of length to thickness ranging from 50 : 1 to 15 : 1. The second-crop crystals are proportionately longer, and many of them have a ratio exceeding 100 : 1. Among these rod-like crystals one or two oblique sections of the prism (670) were seen developed as plates. The crystals of the first crop showed a decided tendency to arrange themselves in groups, radiating in all directions from a center; or frequently the rods would develop brush-like ends, due to the same tendency to form divergent groups. In the second-crop crystals, this tendency to form brush-like ends was especially pronounced; and they also formed various tufted arborescent groupings, but without producing the circular radiating clusters found so commonly in those of the first crop. The long, slightly divergent tufts of the second-crop crystals, and the spherulitic radiating groups of the first crop, are characteristic of this species; as are also the particular forms that are present, the prism (670) and the dome (403). The unit macrodome was seen in crystals from a second preparation from the same blood, and probably also the unit prism, but this last was not measured.

Pleochroism is not very marked, as the colors are not bright; it is quite noticeable, however, and in some positions rather strong; the colors are shades of brownish, as follows: $\mathfrak{a}$ pale brownish, $\mathfrak{b}$ deeper brownish, $\mathfrak{c}$ deep brown. Double refraction is fairly strong and extinction is straight in all aspects normal to the prism, and symmetrical on cross-sections. The orientation of the elasticity axes is $\mathfrak{a} = a$, $\mathfrak{b} = b$, $\mathfrak{c} = \acute{c}$. The interference figure was not observed, but the relative elasticities appear to indicate that the axis of greatest elasticity is the acute bisectrix, $Bx_a = \mathfrak{a}$, and hence the optical character is *negative*.

### Coyote or Prairie Wolf, *Canis latrans*. Plate 74.

The specimen of blood was received from the National Zoölogical Park at Washington, District of Columbia. The blood was stale, but not putrid. It was laked with ether and centrifugalized for several hours;

and from the clear solution thus obtained slide preparations were made as usual. Crystallization proceeded rapidly after covering the slides, the blood crystallizing more readily than that of the dog. The crystals were small, but gradually increased in size and showed no tendency to dissolve for several days. Finally, however, they did dissolve in the plasma. They were oxyhemoglobin.

### *Oxyhemoglobin of Canis latrans.*

Orthorhombic: Axial ratio $a : b : \dot{c} = 1 : 0.4254$, from (504).

Forms observed: Prism, probably (110), macrodome (504).

Angles: The only angle obtained satisfactorily was that of the macrodome (504) = 56°. The prism angle was not observed in measurable position. From (504) the angle $101 \wedge \bar{1}01 = 46° 5'$ was calculated.

Habit of the crystals at first comparatively short capillary, elongated on the prism and terminated by a dome which appears to be (504). Later, the crystals along the protein ring and the cover edge, as well as scattered crystals throughout the body of the slide, became thicker, without increase of length, until the ratio of length to thickness was about 5 : 1 (text figure 304); whereas in the capillary crystals at first developed this ratio was 200 : 1 or more. The larger crystals show very distinctly the striated character, parallel to the length, that is common in the genus *Canis*, and this is evidently due, as usual, to parallel growth on the vertical axis. The capillary crystals form dense felted masses throughout the slide, or grow in spherulitic tufts, radiating from a common center. The larger crystals are much shorter than is common in the dog tribe, but they were never so thick as is common with most of the dogs. It is evident that the oxyhemoglobin is more insoluble than is usual in this genus, and this fact would account for the differences noted.

Pleochroism is marked and double refraction strong. The colors are: $\mathfrak{a}$ pale yellowish-red, $\mathfrak{b}$ deeper red, $\mathfrak{c}$ deep red. Extinction is straight in all aspects seen; no cross-sections of the prism were observed. The interference figure was not observed. The orientation of the elasticity axes appears to be $\mathfrak{a} = a$, $\mathfrak{b} = b$, $\mathfrak{c} = \dot{c}$ as usual. The axis of greatest elasticity is probably the acute bisectrix, $Bx_a = \mathfrak{a}$, which would make the optical character *negative*.

## JACKAL, *Canis aureus*. Plate 75.

The specimen was received from the National Zoölogical Park at Washington, District of Columbia, and was not putrid, but had the color of stale blood. The blood was oxalated, frozen and thawed, ether-laked, and centrifugalized for several hours, and from the clear solution thus obtained the usual slide preparations were made. The blood crystallized very readily and the crystals appeared to be rather insoluble, showing no tendency to dissolve in the solution. Inside of a few hours the crystals had reached measurable dimensions, and after 24 hours many of them were quite large. They formed at first along the protein ring and the cover edge, but soon the entire body of the slide became filled with crystals, and deposition continued until the solution was nearly colorless. The crystals were oxyhemoglobin.

### *Oxyhemoglobin of Canis aureus.*

Orthorhombic: Axial ratio $a : \dot{c} = 1 : 0.4245$.

Forms observed: Prism, probably the unit prism (110), macrodome (101).

Angles: The only angle that was satisfactorily determined was that of the macrodome, $101 \wedge 101 = 46°$. The prism angle was not made out.

Habit at first (when the crystallization is proceeding rapidly) capillary, long trichites which are quite flexible and have a ratio of length to thickness of 1000 : 1 or more; as the crystals begin to form more slowly the thickness increases and in crystals in the protein ring and along the cover edge, as well as in scattered crystals throughout the body of the slide, this ratio falls to 10 : 1 and even to 5 : 1. The hair-like crystals grow felted together irregularly and in spherulitic groupings radiating from a common center (see plate 75, fig. 445). In some cases these trichites are resolved, but in most of the slides they persisted for days. The thicker normal crystals are strongly striated parallel with the length (parallel to the vertical axis, $\dot{c}$), as is usual in this genus; and they were evidently bundles of crystals aggregated together in parallel growth. But some of the crystals appearing throughout the body of the slide were apparently single crystals (text figure 305), although of measurable size. They were not vertically striated. All of the larger crystals showed only the long prism (110) and the macrodome (101); but frequently in the groups the dome became so reduced that the crystal was seen to be terminated by a pseudo-base due to the parallel grouping. When the parallel growth resulted in the members of the group uniting on the brachypinacoid (and hence the group flattening parallel to the macropinacoid) the domes were not so noticeable, and again the crystal appeared to be terminated by the base. No definite twins were observed.

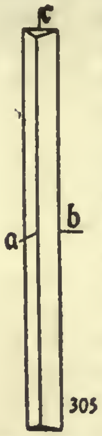


Fig. 305. *Canis aureus* Oxyhemoglobin.

Pleochroism was not very marked when looking normal to the macropinacoid, but when looking along the macro-axis it was very strong. The colors were: $\mathfrak{a}$ pale yellowish-red; $\mathfrak{b}$ rose-pink; $\mathfrak{c}$ pale to deep red, according to the thickness. When the crystal was observed on the brachypinacoid aspect, looking along the macro-axis and with $\mathfrak{a}$ and $\mathfrak{c}$ in the field, the colors were: $\mathfrak{a}$ pale yellowish-red, $\mathfrak{c}$ deep (scarlet) red. In the aspect at 90° to this, with $\mathfrak{b}$ and $\mathfrak{c}$ in the field, the colors were: $\mathfrak{b}$ rose-pink, $\mathfrak{c}$ pale scarlet, and the colors of $\mathfrak{b}$ and $\mathfrak{c}$ were nearly equally strong. Extinction is straight in all aspects normal to the vertical axis and no cross-sections were seen. The double refraction is rather strong. No interference figure was observed. The orientation of the elasticity axes is $\mathfrak{a} = a$; $\mathfrak{b} = b$; $\mathfrak{c} = \dot{c}$. The acute bisectrix of the optic axes is evidently the axis of greatest elasticity, $Bx_a = \mathfrak{a}$, and the optical character is *negative.*

### DINGO OR AUSTRALIAN WILD DOG, *Canis dingo.* Plates 75 and 76.

The specimen of blood was received from the National Zoölogical Park at Washington, District of Columbia, and was from a pup. The blood was clotted and putrid, and was evidently stale when placed in our collecting tube with oxalate. The specimen was ground in sand with ether to destroy the clot, a little normal saline solution added, and the ground mixture centrifugalized for several hours. From the clear solution thus obtained the slide preparations were made as usual. Crystallization proceeds very rapidly after the slides are covered, the first crystals to form being rather short rods, but later longer crystals developed. The crystals formed at room temperature show no signs of dissolving, but the slides kept overnight at a temperature below freezing developed crystals that dissolved until equilibrium in the solution was reëstablished. When kept at room temperature fairly large crystals formed in the protein ring and along the cover edge, and short trichites appeared through the body of the slide. The crystals are evidently quite as insoluble as is commonly the case in this genus. They are oxyhemoglobin as determined by the microspectroscope.

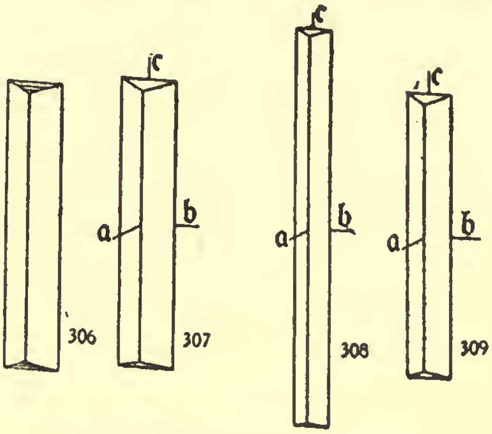
### *Oxyhemoglobin of Canis dingo.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.6009 : 1 : 0.2582$; $a : \acute{c} = 1 : 0.4296$.

Forms observed: Unit prism (110), macrodomes (101), (504), (302); basal pinacoid (001).

Angles: Unit prism angle $110 \wedge 1\bar{1}0 = 61° 30'$; macrodome $101 \wedge \bar{1}01 = 46°$; macrodome $504 \wedge \bar{5}04 = 56° 30'$; macrodome $302 \wedge \bar{3}02 = 66°$, about (65° 36′ calculated).

Habit prismatic on the vertical axis, the crystal consisting of the unit prism (110) terminated by a zone of macrodomes (101), (504), (302) (text figure 306), of which either one may predominate, but the common termination is the unit dome (101) (text figure 307). The crystals are normally much shorter than is common in this genus and vary in ratio of length to thickness from 20 : 1 to 4 : 1 or even less. The normal crystals usually range between 20 : 1 and 15 : 1. Along the protein ring and the cover edge the crystals are much larger and stouter, and are vertically striated, due to parallel growth. Groups of two crystals united on the brachypinacoid in parallel growth are particularly common among these larger crystals, but the majority of them are more complicated groups. The greater part of the crystals occurring in the body of the slide are simple and unstriated, and doubly terminated. They appear to twin upon a pyramid face as contact twins, but do not unite in spherulitic or radiating groups, as is common in the dogs.



Figs. 306, 307. *Canis dingo* Oxyhemoglobin.
Figs. 308, 309. *Canis azaræ* Oxyhemoglobin.

Pleochroism is marked when $\mathfrak{a}$ and $\mathfrak{c}$ lie in the section, but weak when $\mathfrak{b}$ and $\mathfrak{c}$ are in the section, indicating that $\mathfrak{b}$ and $\mathfrak{c}$ are near together in index, but $\mathfrak{a}$ is rather far from either. The pleochroic colors are: $\mathfrak{a}$ pale rose; $\mathfrak{b}$ deep rose, $\mathfrak{c}$ deep blood-red. In ordinary (unpolarized) light the color of the crystals is the usual oxyhemoglobin red. Looking along $\mathfrak{a}$ the double refraction is quite weak, but in other aspects it is moderately strong. Extinction is straight in all aspects. On the flat of the prism, looking along $\mathfrak{a}$ the biaxial interference figure is seen, with not very widely separated brushes. The orientation of the elasticity axes is $\mathfrak{a} = a$; $\mathfrak{b} = b$; $\mathfrak{c} = \acute{c}$. The plane of the optic axes is the brachypinacoid. The acute bisectrix of the optic axes is the axis of greatest elasticity, $Bx_a = \mathfrak{a}$, and the optical character is hence *negative*.

### AZARA'S WILD DOG, *Canis azaræ*. Plate 76.

The specimen of blood was received during the summer from the New York Zoölogical Park, and was kept frozen in the refrigerating plant until examined. The blood was very putrid and was practically a mass of crystals when the collecting tube was taken from the cold storage. The blood was mixed with an equal volume of a 50 per cent solution of egg-white and subjected to action of oxygen until thoroughly saturated and the color had changed to bright red. It was then centrifugalized for 2 hours, and the slide preparations made as usual. Crystals of oxyhemoglobin formed very readily and showed no signs of dissolving. About 20 hours after the slides were prepared the photomicrographs were made. Spectroscopic examination showed the presence of reduced hemoglobin in the solution, but the crystals showed only the spectrum of oxyhemoglobin.

### *Oxyhemoglobin of Canis azaræ.*

Orthorhombic: Axial ratio $a : \acute{c} = 1 : 0.4328$ from (706) or $1 : 0.4348$ from (101).

Forms observed: Unit prism (110), macrodomes (101), (706), (304).

Angles: The prism angle was not obtained. The most satisfactory angle was the macrodome $706 \wedge \bar{7}06 = 53° 35'$; the other macrodomes were $101 \wedge \bar{1}01 = 47°$ measured, 46° 50′ computed from (706); also the dome $304 \wedge \bar{3}04 = 36°$, which agrees almost exactly with calculation from dome (706), as $a : \acute{c}$ for this dome from (706) is $1 : 0.3246$ and from 36° measured is $1 : 0.3249$.

Habit at first long capillary, later stout prismatic crystals form (text figure 309). The normal crystals are long prismatic (text figure 308), elongated on the vertical axis and longitudinally striated; as is usual in the genus *Canis*, the striations are produced by parallel growth. Double crystals, two prisms growing side by side in parallel growth and united on the brachypinacoid, are common in the normal crystals. Owing to this tendency to parallel growth, the crystals become flattened in the direction of the macropinacoid, and the square-ended aspect is the common one; the crystal on edge is less frequently seen. Cross-sections were not observed, so that the angle of the unit prism was not obtained. The capillary crystals grow in somewhat radiating tufts, but the tendency of all crystals, whether capillary or thicker, is to aggregate into masses in nearly parallel growth, so that quite large groups are formed, the crystals growing as though united on the brachypinacoid. The circular radiating tufts and spherulitic groups, usually seen in this genus, were not observed in this species. The crystals that formed after the solution came to an equilibrium, which were hence formed more slowly, were shorter and stouter than the larger crystals formed during the first crystallization. The ratio of length to thickness in the normal crystals may be taken at about 20 : 1 on the average; but in these later crystals it was often as low as 5 : 1 or even less. No definite twins were observed.

The color of the crystals in ordinary light is the usual oxyhemoglobin red. Pleochroism is not very strong; the colors are: $\mathfrak{a}$ pale pink; $\mathfrak{b}$ and $\mathfrak{c}$ nearly equal and ranging from pale to deep red. The double refraction is fairly strong, and the extinction is straight in all positions. The orientation of the elasticity axes is $\mathfrak{a} = a$, $\mathfrak{b} = b$, $\mathfrak{c} = \acute{c}$. The interference figure was not observed, but the pleochroism and the double refraction indicate that the acute bisectrix is the axis of greatest elasticity, $Bx_{\mathfrak{a}} = \mathfrak{a}$, and the optical character is hence probably *negative*.

## SWISS FOX,* *Vulpes vulpes* (?). Plate 78.

The specimen of blood was received from the National Zoölogical Park at Washington, District of Columbia. The blood was very thick and clotted and quite putrid. The clots were destroyed by grinding in sand and the ground mass was diluted with twice its volume of a 50 per cent aqueous solution of egg-white, 1 : 1; the mixture was then centrifugalized, and from the clear solution thus obtained the slides were prepared as usual. Decomposition continued, however, and considerable granular matter separated, due to breaking down of the materials in the solution. Crystallization proceeded rapidly and well-formed crystals of oxyhemoglobin formed along the protein ring and the cover edge, as well as throughout the body of the slide. After 24 hours many of these crystals had passed into reduced hemoglobin by paramorphous change, and many were partly dissolved along the protein ring. Along the cover edge, the crystals were in rather good condition when the photomicrographs were made, about 28 hours

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[*] This may be the swift fox, *Vulpes velox*, but was marked "Swiss fox," which presumably would be the European fox, *Vulpes vulpes*.

after the slides were prepared. No difference in form or optical characters was observed between the crystals of oxyhemoglobin and the paramorphs of reduced hemoglobin.

### *Oxyhemoglobin of Vulpes vulpes.*

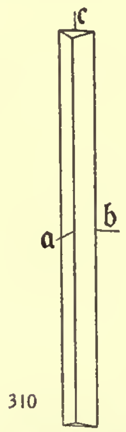
Orthorhombic: Axial ratio $a : \dot{c} = 1 : 0.4245$.

Forms observed: Unit prism (110), macrodome (101).

Angles: The prism angle could not be observed as no cross-sections were seen. A fair measurement of the macrodome angle gave $101 \wedge \bar{1}01 = 46°$.

Habit long prismatic on the vertical axis, the crystals ranging from long hairs, 1,000 times as long as thick, to more nearly normal crystals with the ratio of length to thickness varying from 40 : 1 to 15 : 1 (text figure 310). The larger crystals are vertically striated along the length, due to parallel growth; but they appear to grow together on the prism planes as well as on the brachypinacoid, so that the composite crystals, produced by parallel growth, are not so flattened on the macropinacoid as they often are in this genus. The hair-like crystals grow throughout the body of the slide and form felted masses of hairs, but do not grow in the spherulitic and radiating tufts, as is usual in this genus. The larger crystals grow in parallel groups, rather than radiating, and the tufts of crystals thus formed are only slightly divergent. Twins were not observed.

Pleochroism was noticeable, the double refraction was distinct and extinction straight in all aspects. The orientation of the elasticity axes appears to be as usual in the dogs, $\mathfrak{a}=a$, $\mathfrak{b}=b$, $\mathfrak{c}=\dot{c}$. The optical character is probably *negative.*



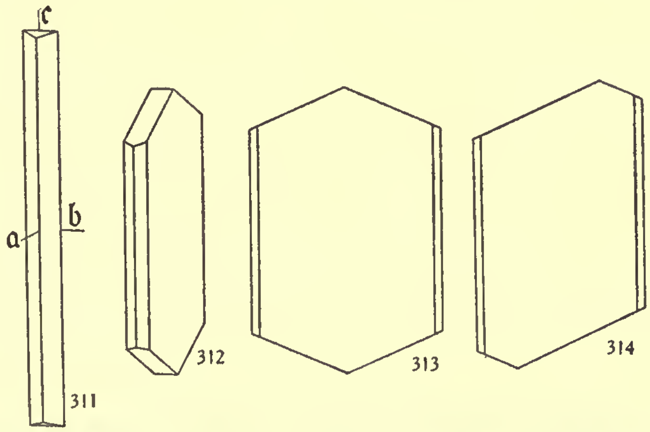


FIG. 310. *Vulpes vulpes* Oxyhemoglobin. FIGS. 311, 312, 313, 314. *Vulpes fulvus* Oxyhemoglobin.

### RED FOX, *Vulpes fulvus.* Plate 77.

Examination was made of two specimens, both of fresh blood, the first specimen being obtained from a zoölogical garden and the second by purchase of the living animal from a collector. The first specimen was oxalated and repeatedly frozen and thawed and then ether-laked and centrifugalized; and from the clear solution the slides were prepared. The crystals obtained in this series of preparations were rather more perfect than those obtained by either of the other methods of preparation used. The blood from the second specimen was treated in several ways: (*a*) The whole blood was oxalated and ether-laked as in the regular method of preparation, (*b*) the corpuscles were centrifugalized and a mixture of three-fourths plasma and one-fourth corpuscles was oxalated and ether-laked;

both were then centrifugalized, and from the clear solution the slides were prepared. The blood crystallized very rapidly in all cases, and the slides soon became filled with crystals. These were seen to vary in habit somewhat according to the method of preparation, but not more than the crystals varied in the same slide, according as to how long after covering they appeared. In other words, the habit of the crystals was conditioned by the strength of solution, rather than by its composition. The first crystals to form are capillary, as a rule; later these grow thicker, or stouter crystals appear; but in the first preparation some well-formed tabular crystals finally made their appearance, which at first sight seemed to be different from the prisms. After a study of their angles and optical characters they were finally determined to be identical with the prisms. All of the crystals observed were oxyhemoglobin.

### *Oxyhemoglobin of Vulpes fulvus.*

Orthorhombic: Axial ratio $a : b : \grave{c} = 0.6494 : 1 : 0.2824$; $a : \grave{c} = 1 : 0.4348$.

Forms observed: Unit prism (110), macrodome (101), brachypinacoid (010).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 66°$; macrodome $101 \wedge \bar{1}01 = 47°$.

Habit prismatic, elongated on the vertical axis (text figure 311), and the prism striated in the same direction, due to parallel growth; or tabular on the brachypinacoid, the crystal in the prismatic habit consisting of the unit prism with the macrodome, and, in the tabular habit, the same faces with the brachypinacoid (text figures 312, 313); but in some cases one pair of opposite dome faces much developed while the alternate pair are much reduced in size or even wanting, giving the crystal a monoclinic aspect (text figure 314).

The first crystals to form are generally capillary; and in some of the preparations, especially in those that were diluted with plasma, nearly all of the crystals retained the relative dimensions of the capillary crystals, until they attained a length of more than 3 mm. As the crystals continue to deposit from the solution, stouter crystals appear, and these resemble more closely the usual crystals seen in the blood of the species of this genus. The thin prisms are usually single crystals until they attain large size; they grow in slightly divergent tufts or more rarely form groups radiating in all directions from a center; in very many cases the groups are so slightly divergent that the individual crystals appear parallel, and the group looks like a parallel growth. Single crystals become covered by smaller ones that are actually arranged in parallel growth; but they do not seem to grow together on the brachypinacoid and hence flatten on the macropinacoid; in some cases the groups may contain individuals in twin position on the prism. It is this tendency to form composite groups, with the vertical axes parallel, that produces the characteristic striation in this direction.

Pleochroism is moderately strong when observed along $\mathfrak{b}$ with $\mathfrak{a}$ and $\mathfrak{c}$ in the field; but when looking along $\mathfrak{a}$ it is rather weak. The colors are $\mathfrak{a}$ pale reddish, somewhat yellowish-red; $\mathfrak{b}$ and $\mathfrak{c}$ nearly equal and deeper red. The crystals are not highly colored, as they are slender. Double refraction is not very strong except when $\mathfrak{a}$ and $\mathfrak{c}$ are in the field; the extinction is straight in all aspects. Traces of a biaxial interference figure were observed. The orientation of the elasticity axes is $\mathfrak{a} = a$, $\mathfrak{b} = b$, $\mathfrak{c} = \grave{c}$; and the axis of greatest elasticity appears to be the acute bisectrix, $Bx_a = \mathfrak{a}$; the optical character is hence *negative.*

### Blue or Arctic Fox, *Vulpes lagopus.* Plate 78.

Two specimens of the blood of this species were received from the National Zoölogical Park at Washington, District of Columbia, one during warm weather and the other during the winter. The former was kept frozen in the refrigerating plant until examined; it was in good condition, and evidently was collected when the animal was but recently dead. The second specimen was somewhat putrid and thick; and had probably been taken some time after death. Both had been collected in oxalate, in our regular collecting tubes. The two specimens were treated in the same manner; laked with ether and centrifugalized, and from the clear solution thus obtained the slide preparations were made in the usual way. The blood crystallizes very readily, and within a few hours after the preparations were made the slides were in condition for examination. The stale blood crystallized rather faster than the fresh specimen, due no doubt to its being in a more concentrated condition. The portion of clear solution remaining in the tube after the slides were prepared from this stale specimen became a mass of crystals within 2 hours after the preparations were made. The crystals keep well and show no tendency to dissolve in the solution. They were oxyhemoglobin in the case of the second sample of blood, which was the one from which the measurements were obtained on which the crystallographic constants were determined. The other sample appeared to have been converted into the acid form of metoxyhemoglobin. Its crystallographic constants were the same as those of the second specimen, so far as they were recorded, but the habit of growth of the crystals was somewhat different.

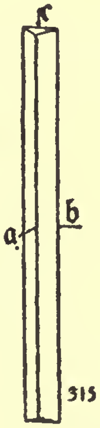
*Oxyhemoglobin of Vulpes lagopus.*

Orthorhombic: Axial ratio $a : \acute{c} = 1 : 0.4265$.

Forms observed: Unit prism (110), macrodomes (101), (405).

Angles: The prism angle was not obtained; macrodomes $101 \wedge \bar{1}01 = 46° 12'$ (average); $405 \wedge \bar{4}05 = 56°$.

Habit long prismatic on the vertical axis, the crystals at first almost capillary, but later becoming stouter, and even short prismatic in the case of the second specimen. The ratio of length to thickness of the larger normal crystals was about 20 : 1 in the first specimen and 10 : 1 in the second (text figure 315). The crystals do not show very much tendency to form large parallel growths, but are single crystals, in which the tendency to parallel growth so common in this genus is only indicated by vertical striation on the prism and flattening on the macropinacoid. This produces rather flattened lath-shaped and striated crystals, which nearly always present the flat aspect, and hence are square on the end which is cut by one of the macrodomes. Usually only one of these domes develops on a single crystal, but sometimes both may be seen in the same crystal. The unit dome seems to predominate.



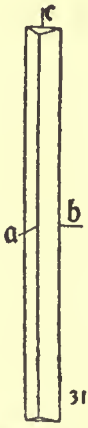


Fig. 315. *Vulpes lagopus* Oxyhemoglobin.
Fig. 316. *Urocyon cinereoargenteus* Oxyhemoglobin.

The crystals from the first specimen, which appeared to be metoxyhemoglobin, showed a decided tendency to grow into circular or spherulitic groups of crystals, radiating from a common center; and this was particularly true of the crystals that formed in the protein ring; those from the second specimen did not show this tendency, but were usually single crystals matted together into a felt, and occasionally grouped as though twinned on a pyramid.

Pleochroism is rather marked; for the oxyhemoglobin the colors were: $\mathfrak{a}$ pale reddish or pinkish; $\mathfrak{b}$ and $\mathfrak{c}$ nearly equal, varying from paler to deeper red according to the thickness. Looking along $\mathfrak{a}$, the double refraction was rather weak; along $\mathfrak{b}$ it was very strong. Extinction was straight in all aspects. No interference figure was made out, but the orientation of the elasticity axes is $\mathfrak{a}=a$, $\mathfrak{b}=b$, $\mathfrak{c}=\dot{c}$. The plane of the optic axes is, as usual in this genus, the brachypinacoid; the bisectrix of the optic axes is evidently the axis of greatest elasticity $Bx_{\mathfrak{a}}=\mathfrak{a}$, and the optical character is hence *negative.*

### Gray Fox, *Urocyon cinereoargenteus.* Plate 79.

Two specimens of the gray-fox blood were examined, both from the National Zoölogical Park at Washington, District of Columbia. In the blood from specimen I, the usual method of laking with ether, oxalating and centrifugalizing was followed; and in the blood from specimen II one series of preparations was made in the same way. As these crystallized rather more rapidly than seemed to be desirable, a second preparation was made from specimen II by adding one volume of a 50 per cent solution of egg-white to the centrifugalized blood before making the slide preparations. This resulted in rather sharper crystals than those at first obtained from this specimen, and also much better than those obtained from specimen I. In all of these preparations the crystals formed very rapidly and showed no signs of dissolving. They were oxyhemoglobin in all of the preparations made, as determined by the microspectroscope. When received both specimens were somewhat putrid, but still contained mainly oxyhemoglobin as indicated by the color.

#### *Oxyhemoglobin of Urocyon cinereoargenteus.*

Orthorhombic: Axial ratio $a : b : \dot{c} = 0.6619 : 1 : 0.2809$; $a : \dot{c} = 1 : 0.4245$.

Forms observed: Prisms, unit prism (110), macroprism (650), macrodomes (101), (201), macropinacoid (100), base (001) (?).

Angles: Unit prism $110 \wedge 1\bar{1}0 = 67°$; macroprism $650 \wedge 6\bar{5}0 = 57° 45'$; unit macrodome $101 \wedge \bar{1}01 = 46°$; macrodome $201 \wedge \bar{2}01 = 80°$ to 81° (about) (calculated 80° 40′).

Habit of the crystals long prismatic on the vertical axis, but varying somewhat according to the method of preparation. In normal whole blood prepared in the usual manner, as in specimen I, and in the first undiluted preparation of specimen II, the habit is capillary or very long prismatic, as is usual in the crystals formed from concentrated solutions in the genus *Canis.* A small number of shorter and thicker crystals appeared in these slides made from the whole blood, which were measurable, and they gave the measurements of the crystals obtained in the blood diluted by addition of egg-white; but these larger, thicker crystals are not so fine as those obtained in the preparations of diluted blood. The prism obtained in the whole blood from specimen I was the unit prism (text figure 316), but from specimen II in the diluted blood the prism (650) seemed to be common in the larger crystals. The capillary crystals formed first in all cases, and gradually became thicker until the ratio of length to thickness might be about 50 : 1; but the thicker crystals which appeared in preparations of the whole blood had a ratio of perhaps 20 : 1, and did not develop from the original capillary crystals. These capillary crystals grow singly, forming a loose felted mass throughout the slide; or in tufts growing from the protein ring and the cover edge, the fibers in the tufts being slightly divergent or nearly parallel. The thicker crystals showed a more nearly normal development, in the preparations of blood diluted with egg-white, and in this state they closely resembled the crystals from the blood of other species of the genus *Canis.* They are elongated on the vertical axis and striated longitudinally, evidently on account of the tendency which the crystals have to aggregate in parallel growth. Instead of growing in slightly divergent groups they form masses of many individuals, fifty or more in a bundle in absolutely parallel growth, and frequently showing a strong tendency to flatten upon the macropinacoid. The cross-sections of these prisms often show the composite character very distinctly, and in what looks like single crystals it can often be seen that these are a number of individuals in parallel growth. The observed macropinacoid appeared to be a definite plane; but it could readily be a pseudo-plane, produced by this tendency of the crystals to grow together on the brachypinacoid and to flatten on the macropinacoid. In the retarded crystallizations (the diluted preparations of specimen II) both the capillary crystals and also the normal crystals show a tendency to form into more divergent groups, in some cases, as well as to form the parallel groups already described; but the spherulitic masses of fibers, seen in many species of *Canis*, are not common. These larger crystals are rarely short; and are very frequently single individuals, with a ratio of length to thickness of 30 : 1 or 20 : 1; the tendency to produce parallel growths results rather in the formation of bundles or masses of such single crystals of rather irregular outlines, than in the formation of striated single crystals. While the number of individuals in these bundles is usually only about 50 to 60, many of them contain hundreds of crystals in parallel position, all extinguishing simultaneously.

Pleochroism is marked when the crystals are seen with the smaller angle of the prism pointing forward, or on edge; but not so strong when seen on the flat, looking along $\mathfrak{a}$. The pleochroic colors are $\mathfrak{a}$ pale reddish, $\mathfrak{b}$ and $\mathfrak{c}$ nearly equal and deep red to pale red according to the thickness. Extinction is straight in all side views of the prisms and symmetrical on cross-sections. The orientation of the elasticity axes is $\mathfrak{a} = a$; $\mathfrak{b} = b$; $\mathfrak{c} = \acute{c}$. The axis of greatest elasticity is evidently the acute bisectrix of the optic axes, $Bx_a = \mathfrak{a}$, and the optical character is hence *negative*.

TABLE 44.—*Crystallographic characters of the hemoglobins of the Canidæ.*

| Name of species. | Axial ratio. | Prism angle. | Macrodome angle. | Ratio $a : \acute{c}$. | Optical character. | System. | Substance. |
|---|---|---|---|---|---|---|---|
| | | ° ′ | ° ′ | | | | |
| Canis familiaris ............ | 0.6745 : 1 : 0.2863 | 68 0 | 46 0 | 1 : 0.4245 | Negative | Orthorhombic | $\alpha$-OHb. |
| Do.................... | ................ | ..... | ($\beta$=78°) | ........ | Do. | Monoclinic | $\beta$-OHb. |
| Chow dog, C. familiaris, var.. | 0.6696 : 1 : 0.2878 | 67 0 | 47 0 | 1 : 0.4348 | Do. | Orthorhombic | OHb. |
| Cross between C. familiaris and C. latrans ............ | 0.6619 : 1 : 0.2912 | 67 0 | 47 30 | 1 : 0.4400 | Do. | Do. | OHb. |
| Canis lupus mexicanus ...... | 0.6576 : 1 : 0.2863 | 66 40 | 46 16 | 1 : 0.4272 | Do. | Do. | MOHb. |
| Canis latrans .............. | ................ | ..... | 46 5 | 1 : 0.4254 | Do. | Do. | OHb. |
| Canis aureus................ | ................ | ..... | 46 0 | 1 : 0.4245 | Do. | Do. | OHb. |
| Canis dingo................. | 0.6009 : 1 : 0.2582 | 61 30 | 46 30 | 1 : 0.4296 | Do. | Do. | OHb. |
| Canis azaræ ................ | ................ | ..... | 46 50 | 1 : 0.4328 | Do. | Do. | OHb. |
| Vulpes vulpes............... | ................ | ..... | 46 | 1 : 0.4245 | Do. | Do. | OHb. |
| Vulpes fulvus............... | 0.6494 : 1 : 0.2824 | 66 0 | 47 | 1 : 0.4348 | Do. | Do. | OHb. |
| Vulpes lagopus ............. | ................ | ..... | 46 12 | 1 : 0.4265 | Do. | Do. | OHb. |
| Urocyon cinereoargenteus ... | 0.6619 : 1 : 0.2809 | 67 0 | 46 | 1 : 0.4245 | Do. | Do. | OHb. |

# CHAPTER XVI.

## CRYSTALLOGRAPHY OF THE HEMOGLOBINS OF THE FELIDÆ AND VIVERRIDÆ—CATS AND CIVETS.

Nine species of the cats and one species of civet were studied, including, of the genus *Felis,* the lion, Bengal tiger, jaguar, puma or mountain-lion, leopard-cat, ocelot, and common domestic cat; and of the genus *Lynx* the wild cat or bob-cat and the lynx. The civet examined was the binturong, *Arctictis binturong.*

As in the case of the dogs, the hemoglobin crystals of the cat tribe form a strictly isomorphous group; but in the case of the *Felidæ* it is common to obtain the crystals of reduced hemoglobin, which is not the case of the dogs. The fresh blood of the cats, especially of the Old World species that were examined, gave crystals of reduced hemoglobin, along with those of oxyhemoglobin, in the first crop of crystals to form; or frequently the reduced hemoglobin crystallized alone, with no oxyhemoglobin. The oxidation of the blood by exposure to air or to pure oxygen would generally produce crystals of oxyhemoglobin in such cases. The blood of the common cat, freshly obtained, crystallizes first as reduced hemoglobin, and crystals of oxyhemoglobin are apparently not produced. But the solution contains much oxyhemoglobin, and the probability is that the oxyhemoglobin crystals are much more soluble than those of the reduced hemoglobin, as appears to be the case in all species of cats. The lion, tiger, leopard-cat, and ocelot also gave the crystals of reduced hemoglobin very readily, but, in the blood of the lion and leopard-cat, crystals of oxyhemoglobin were also observed. In general, the Old World cats seemed to develop reduced hemoglobin very readily in their blood, and there seemed to be a large amount of it present. The New World cats, the jaguar and puma, as well as the bob-cat and the lynx, all gave crystals of oxyhemoglobin readily, and while these were more soluble than the crystals of the reduced hemoglobin of the same species, they formed readily in fresh or oxygenated blood and grew to large size. In the case of the mountain-lion, two forms of oxyhemoglobin were observed, and the same was true of the jaguar; while two forms of reduced hemoglobin were observed in the case of the lynx.

The oxyhemoglobin in the cats is thus dimorphous or trimorphous, and the reduced hemoglobin also appears to be dimorphous. The reduced hemoglobin crystals, being formed more constantly than the oxyhemoglobin crystals, give a better basis for comparison of the species; but, even with these reduced-hemoglobin crystals forming in large quantity, they were often so imperfectly formed that the axial ratios could not be made out.

While frequently of quite a different shape from the crystals of oxyhemoglobin, a comparison of the axial ratios will show that the oxyhemoglobin and the reduced hemoglobin must have nearly the same form of structure. The prism angle that develops in the reduced hemoglobin crystals is usually about 88° to 89°; this angle also appears in the tabular crystals of oxyhemoglobin in some cases. The macrodome is usually present in the reduced hemoglobin crystals, and can always be calculated when the complete axial ratio is determined; it runs from 41° 30′ to 45° in the different species. The following table shows the crystallographic characters of the reduced hemoglobins of the cats:

TABLE 45.—*Crystallographic characters of the reduced hemoglobins of the Felidæ.*

| Name of species. | Axial ratio. | Angle of unit prism (normals). | Angle of macrodome (normals). | Optical character. | Crystal system. |
|---|---|---|---|---|---|
| | | ° ′ | ° ′ | | |
| Felis leo | 0.9742 : 1 : 0.3707 | 88 30 | 41 50 | Positive | Orthorhombic |
| Felis tigris | 0.9742 : 1 : 0.3838 | 88 30 | 43 0 | Do. | Do. |
| Felis onca | ................ | ..... | ..... | ........ | Orthorhombic(?) |
| Felis concolor | ................ | ..... | ..... | Positive | Orthorhombic |
| Felis bengalensis | 0.9657 : 1 : 0.3667 | 88 0 | 41 35 | Do. | Do. |
| Felis pardalis | 0.9489 : 1 : 0.3931 | 87 0 | 45 0 | Do. | Do. |
| Felis domestica | 0.9656 : 1 : 0.3839 | 88 0 | 43 20 | Do. | Do. |
| Lynx rufus | 0.9869 : 1 : 0.3914 | 89 15 | 42 46 | Do. | Do. |
| Lynx canadensis | 0.9605 : 1 : 0.3944 | 87 42* | 41 30 | Do. | Do. |

* Computed.

A glance at the above table will show that the data for the reduced hemoglobin for the Old World cats are complete, while those for the New World cats are not. If the oxyhemoglobins were compared, the reverse would be found to be the case; the data for the oxyhemoglobin crystals of the Old World cats would be incomplete while that for the New World cats would be complete. The two species of *Lynx* gave reduced hemoglobin crystals that varied more from each other than they did from the species of the genus *Felis* proper. This would indicate that, so far as the reduced hemoglobin is concerned, they belong strictly in the genus *Felis*.

As the prism angle approaches 90° in the normal unit crystals, twinning, especially mimetic twinning, produces forms that appear to be tetragonal; examples of this probable mimetic twinning are to be found in the oxyhemoglobin of *Felis bengalensis* and the reduced hemoglobin of *Lynx canadensis*. The more obtuse prism (430) that was frequently observed, has an angle that ranges from 75° to near 72° and thus it approaches the angle of the octahedron; from this are probably developed the isotropic forms with isometric, or pseudo-isometric, development that were seen in the $\beta$-oxyhemoglobin of the jaguar, *Felis onca*, and the $\beta$-oxyhemoglobin of the puma, *Felis concolor*.

The crystals from the blood of the civet examined (*Arctictis binturong*) did not show the prism, and the terminations were wanting. No axial ratio could be obtained, therefore, for comparison with the cats; and while, like the cats, it is orthorhombic and positive, it can not be stated that the crystals resemble those of any of the cats examined.

## FELIDÆ.

### LION, *Felis leo*. Plates 80, 81, and 82.

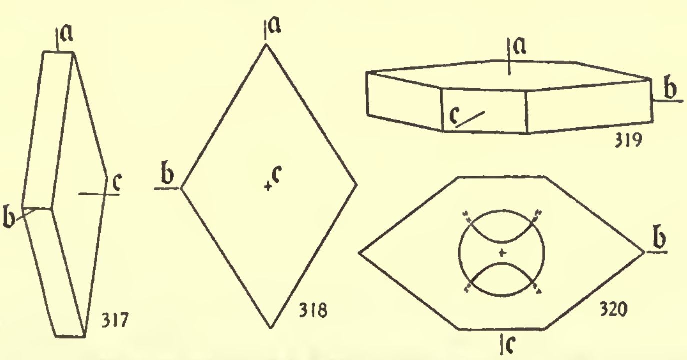
The first of the specimens examined was from a young lion, about 18 months old, that had been shot and afterwards killed with chloroform. The specimen was received from the New York Zoölogical Park. The blood was 24 hours old and had been drawn into oxalate. It was ether-laked and centrifugalized, and from the clear solution the preparations were made. After covering, the blood soon begins to crystallize, but in this preparation only crystals of reduced hemoglobin were obtained. The laked blood crystallized in the test-tube. The second specimen was from the Philadelphia Zoölogical Gardens, and was from an adult animal. In this blood, after the usual preparation as outlined above, there appeared at first crystals of reduced hemoglobin in the shape of rods or prisms; and later, as a sort of second crop, there appeared two types of crystals of oxyhemoglobin, both evidently with the same axial ratio, but with different development and a different prism. From these oxyhemoglobin crystals, and especially from this latter form, there developed, by paramorphous change, crystals of the metoxyhemoglobin that retained the form of the original oxyhemoglobin. The crystallographic characters did not appear to be influenced by the change from the one to the other material.

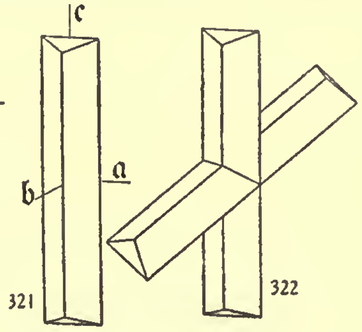
#### *Oxyhemoglobin of Felis leo.*

Orthorhombic: Axial ratio $a : b : \dot{c} = 0.765 : 1 : 1.235$.

Forms observed: Unit prism (110), macrodome (101), base (001), macropinacoid (100), brachypinacoid (010).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 74° 50'$ (normals); macrodome angle $101 \wedge \bar{1}01 = 63° 32'$; prism to base $110 \wedge 001 = 90°$, macropinacoid to brachypinacoid $100 \wedge 010 = 90°$.





FIGS. 317, 318, 319, 320. *Felis leo* Oxyhemoglobin. FIGS. 321, 322. *Felis leo* Reduced Hemoglobin.

The crystals occur in two habits: (a) very thin tabular on the brachypinacoid, in rhombic plates consisting of the brachypinacoid bounded by the macrodome (text figures 317 and 318); (b) somewhat thicker, tabular on the basal pinacoid (001), and consisting of this pinacoid in combination with the unit prism (110) and the macropinacoid (100) (text figures 319 and 320). The crystals of type (a) occur scattered through the body of the slide, singly or in groups formed by the piling of the plates one upon another on the brachypinacoid in regular growth; they also occur in irregular, radiating heterogeneous aggregates. Type (b) crystals occur mainly along the cover edge; they are in groups of regular growth along the brachy and macro-axes, and as single crystals are elongated on the macro-axes. They also aggregate into groups by piling up on the base, but these crystals are much thicker than those of type (a) and do not pile up to the same extent. They are not very plentiful, not nearly so much so as those of the first type. Actual twins of either of these types were not observed. As no forms are found in the one type that occur in the other, the correlation of the two rests upon the correspondence of the optical characters, and the orientation of the elasticity axes is assumed to be the same in each type. On this basis, the complete axial ratio is made out; $\acute{c}$ being determined from type (a) and $a$ from type (b).

Pleochroism is marked in type (a) crystals, but not very noticeable in type (b) when each is examined on the large face. This of course depends upon which of the elasticity axes lie in the plane of this large face, in each case. When all edge views are considered, as well as the flat views, the pleochroism is the same in both types. Crystals of type (a) are rather pale in ordinary light, and with one nicol prism they appear either deeply colored or nearly colorless; the crystals of type (b), on the other hand, when viewed on the flat, are deep scarlet in ordinary light; and with one nicol are much the same color, owing to the two elasticity axes in this plane (001) being nearly of the same elasticity. Pleochroism is: $\mathfrak{a}$ nearly colorless to pale pinkish, $\mathfrak{b}$ rather deep scarlet-red, $\mathfrak{c}$ deep red; the colors of $\mathfrak{b}$ and $\mathfrak{c}$ are nearly equal. Extinction is straight in all aspects, and in both types of crystals. In type (a) crystals the interference figure is difficult to observe, on account of the position of the acute bisectrix; and it was only made out in exceptional cases, when the crystals were in a proper position for observation. But in type (b) the figure is easily seen, as the acute bisectrix is normal to the large plane of the plate. The orientation of the elasticity axes is $\mathfrak{a}=\acute{c}$, $\mathfrak{b}=a$, $\mathfrak{c}=b$. The plane of the optic axes is the macropinacoid, and the acute bisectrix is the vertical axis, $Bx_a=\mathfrak{a}$. The optical character is therefore *negative*. This is, of course, true of both types of crystals, and it is one of the reasons for identifying them as the same substance. The biaxial figure is easily observed in the type (b) crystals and is seen on the base; the brushes do not open very widely and the axial angle $2E=25°$. In some slides in which the type (b) crystals appeared, there were seen long needle-like crystals of oxyhemoglobin, which showed the characters of these type (b) crystals on edge. They are probably the same crystals, with the development prismatic on the two pinacoids (100) and (010), and elongated along the vertical axis.

### *Metoxyhemoglobin of Felis leo.*

The crystals of oxyhemoglobin passed by paramorphous change into brownish crystals, giving the spectrum of metoxyhemoglobin, but without any alteration in the angles or change in the optical characters.

### *Reduced Hemoglobin of Felis leo.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.9742 : 1 : 0.3707$.

Forms observed: Unit prism (110), macrodome (101).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 88° 30'$ (normals); macrodome angle $101 \wedge \bar{1}01 = 41° 50'$ (normals).

Habit prismatic; the normal crystals consisting of the unit prism and macrodome, elongated on the prism and with the ratio of length to thickness very variable; about 5 : 1 is an average (text figure 321). Some crystals are enormously elongated and even needle-like, others are abnormally short until the above ratio becomes 2 : 3. The crystals become, in some cases, relatively enormous, and, under the cover, are often more than 2 mm. long by more than 0.5 mm. thick. The needles also attain a length of 2 mm. and more, but remain very thin. As in the cats generally, preparations of the whole blood of the lion, fresh and not exposed to the air, first develop crystals of reduced hemoglobin and then later crystals of oxyhemoglobin, even when the spectrum of the blood does not show reduced hemoglobin at all. Therefore, in both of the specimens of blood examined, crystals of reduced hemoglobin were the first to appear and were very plentiful. The shorter crystals with a ratio of length to thickness of 5 : 1 to 3 : 1 appeared first; later, along with the crystals of oxyhemoglobin, and especially when these began to change to metoxyhemoglobin, the very long needles of the reduced hemoglobin appeared in brush-like tufts; and still later, the relatively enormous crystals noted above. The larger the crystals grow, the more they tend to become flattened by the slide and cover, and, as they usually lie on a prism face, this flattening is generally on a pair of prism faces. The dome angle thus developed, which is a section of the dome faces along the diagonal plane parallel to a prism face, is of course more obtuse than the true angle and in these sections it appears as an angle near 30° (normals). The crystals appear to twin on the unit pyramid, in contact and interpenetrant twins (fig. 322).

Pleochroism is very marked; $\mathfrak{a}$ pale rose-pink, $\mathfrak{b}$ deeper rose-pink, $\mathfrak{c}$ deep carmine-red; the colors of $\mathfrak{a}$ and $\mathfrak{b}$ are not far apart. The orientation of the elasticity axes is $\mathfrak{a}=b$, $\mathfrak{b}=a$, $\mathfrak{c}=\acute{c}$. On all aspects extinction is straight or symmetrical. In convergent light on cross-sections of the prism the biaxial interference figure can be seen with the brushes widely separated; the angle $2E$ is about 100° to 105°. The plane of the optic axes is the macropinacoid, and the vertical axis is the acute bisectrix; $Bx_a=\mathfrak{c}$; hence the optical character is *positive.*

### Bengal Tiger, *Felis tigris.* Plates 82 and 83.

The specimen of blood was received from the Philadelphia Zoölogical Gardens. After oxalating, the blood was repeatedly frozen and thawed to dissolve the hemoglobin from the corpuscles; finally, upon thawing, a mass of broken crystals was obtained. Water was then added in small amount, the blood warmed to room temperature and centrifugalized. From the clear solution thus obtained the slide preparations were made as usual. Crystals of reduced hemoglobin began to appear very soon after the slides were covered; the crystals formed rapidly, and were at first, when small, very even in size and shape; later, some grew to relatively enormous dimensions, while the others remained small. The crystals are very insoluble, evidently, and remain very sharp and well formed for days, showing no tendency to dissolve in the plasma. Only crystals of reduced hemoglobin appeared in these slides, the blood being somewhat stale and putrescent, a condition which it probably acquired inside of the animal before the specimen was collected.

#### *Reduced Hemoglobin of Felis tigris.*

Orthorhombic: Axial ratio $a:b:\acute{c}=0.9741:1:0.3838$.

Forms observed: Unit prism (110), macrodome (101).

Angles: Prism angle, $110 \wedge 1\bar{1}0 = 88° 30'$ (normals) (also measured 89°, giving axial ratio $0.9827:1:0.3871$); macrodome angle, $101 \wedge \bar{1}01 = 43°$ (normals).

Habit short prismatic, elongated along the vertical axis; a nearly square prism (110) cut by a rather flat macrodome (101) (text figure 323); the ratio of length to thickness of the prism, whether large or small (except the most minute crystals), ranging from 3 : 1 to 2 : 1. In very minute crystals this ratio may rise to 5 : 1, and in very large crystals it falls to 3 : 2. In some exceptional cases the ratio was 1 : 2, the crystal becoming almost tabular on one of the macrodome faces, and hence distorted. But in general the crystals were very symmetrically developed. The usual aspect presented is that of the crystal lying on one prism face and showing the dome in an oblique position. The cross-section of the crystal was also occasionally seen, but generally in a somewhat oblique position. The exact measurement of the prism angle was therefore not easy, and it may vary towards 90° by some half-degree (89° normals). The profile view of the macrodome was seen frequently enough to allow of rather exact measurement. Twins were rare, but an interpenetrant twin on a unit pyramid was apparently seen (compare text figure 322).

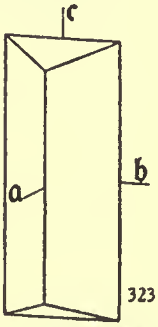


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FIG. 323. *Felis tigris* Reduced Hemoglobin.

Pleochroism is usually rather marked in all aspects, except the cross-sections of the prism; $\mathfrak{a}$ pale rose-pink, $\mathfrak{b}$ deep rose-pink, $\mathfrak{c}$ deep blood-red, inclining to carmine. Extinction is straight or symmetrical in all aspects. The orientation of the elasticity axes is $\mathfrak{a}=a$, $\mathfrak{b}=b$, $\mathfrak{c}=\acute{c}$. The plane of the optic axes is the brachypinacoid; on the basal sections, the biaxial figure is seen with the axes widely separated; the angle $2E$ was not exactly measured, but was above 75°. The vertical axis is the acute bisectrix $Bx_a=\mathfrak{c}$, and the optical character is *positive.*

### JAGUAR, *Felis onca.* Plates 84 and 85.

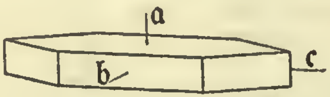
The specimen of blood was obtained from the National Zoölogical Park at Washington, District of Columbia, and was dark in color and in a slightly putrid condition. It was oxalated and repeatedly frozen and thawed to lake it; finally, a little ether was added to complete the laking, and the blood centrifugalized for several hours. From the clear solution thus obtained drop preparations were made on slides as usual. Only crystals of oxyhemoglobin formed at first; these were of two forms, plates or tabular crystals, α-oxyhemoglobin, and octahedral crystals, β-oxyhemoglobin. The slides were kept at a temperature near the freezing-point, and when brought into a warm room the crystals rapidly dissolved; this was especially the case with the octahedral crystals. All examinations, therefore, had to be made at about the freezing-point, and the photographs were taken in a room temperature of 0° C. Rod-like crystals, evidently sections of the plates on edge, were seen along with the plates. From a second preparation, made later from the same prepared blood, crystals of reduced hemoglobin were obtained in the form of rods with imperfect ends. These reduced hemoglobin crystals were not measurable.

#### *α-Oxyhemoglobin of Felis onca.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.7813 : 1 : 1.2146$.

Forms observed: Unit prism (110), macropinacoid (100), brachypinacoid (010), base (001); rarely the macrodome (101) and brachydome (025).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 76°$ (normals); macropinacoid to base $100 \wedge 001 = 90°$; macrodome angle $101 \wedge \bar{1}01 = 65° 30'$; brachydome $025 \wedge 0\bar{2}5 = 51° 45'$.



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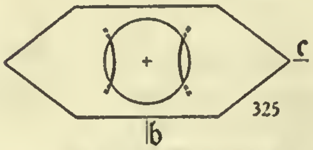

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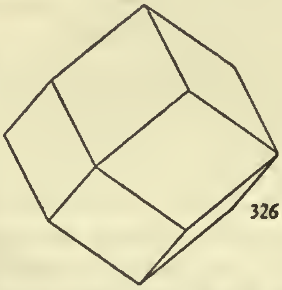

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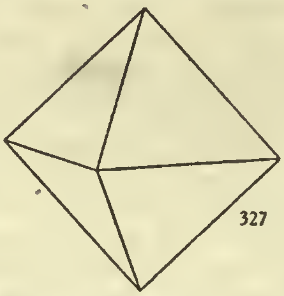

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FIGS. 324, 325. *Felis onca* α-Oxyhemoglobin. FIGS. 326, 327. *Felis onca* β-Oxyhemoglobin.

Habit tabular on the base, the usual crystal consisting of this plane, bounded by the macropinacoid and the unit prism (text figures 324 and 325). The tabular crystals are elongated in the direction of the macro-axis and grow together in parallel growth in the direction of the two axes in the basal pinacoid. They also pile up on the base. By this method of parallel growth large groups occur with the same orientation throughout the groups. The macrodome was not observed, except in the case of a very few plates similar to those taken as macrodome and brachypinacoid in the case of the lion. From these the macrodome angle was obtained and the axial ratio computed. The only form of crystal aggregate noted was the parallel growth described; no form of twinning was observed. The tendency towards elongation along the macro-axis produces long parallel growths and drawn-out crystals; when these are on edge they appear to be prismatic crystals.

The color on the basal pinacoid face is a rather deep scarlet-red, owing to the slight pleochroism in this aspect; but when seen on the edge views the pleochroism is rather strong. The colors are: $\mathfrak{a}$ pale yellowish-red, $\mathfrak{b}$ rather strong red, $\mathfrak{c}$ deeper red. Extinction in all aspects is straight or symmetrical. In convergent light, on the base, the biaxial interference figure is seen, with the brushes widely separated and the plane of the optic axes the macropinacoid. The orientation of the elasticity axes is $\mathfrak{a}=\grave{c}$; $\mathfrak{b}=a$; $\mathfrak{c}=b$. The acute bisectrix of the optic axes is the axis of greatest elasticity; $Bx_a=\mathfrak{a}$, the optical character is hence *negative.*

### *β-Oxyhemoglobin of Felis onca.*

Isometric, normal (perhaps pseudo-isometric only).

Forms observed: Octahedron (111); cube (100); dodecahedron (110).

Angles: Octahedron angle $111 \wedge \bar{1}\bar{1}1=70° 30'$ (actual angle); dodecahedron angles $110 \wedge 1\bar{1}0=90°$.

Habit octahedral or dodecahedral; in minute octahedra or dodecahedra (text figures 326 and 327), rarely combined with the cube. These crystals appeared after the crystals of *a*-oxyhemoglobin, but were found with them in the same slide; they developed along the cover edge.

Color rather bright scarlet-red; not pleochroic. They show no double refraction and do not polarize in any position. Convergent light had no effect upon them. They are evidently isotropic.

### *Reduced Hemoglobin of Felis onca.*

Orthorhombic (?): No axial ratio could be determined.

Forms: A prism or two vertical pinacoids, the prismatic crystals have a nearly square section.

Angles: No good measurements of angles were obtained.

Habit prismatic, long square prisms, often ending in a brush of fibers at the end and sometimes looking like a bundle of crystals.

Rather pleochroic, extinction parallel to the length in all aspects. These crystals resemble the reduced-hemoglobin needles that appeared in lion blood; and, like them, they appeared in blood that had been kept for some days.

The *β*-oxyhemoglobin crystals were very likely mimetic isometric, but no evidence of this was seen. Similar, apparently isometric, and perfectly isotropic crystals were seen to develop in the blood of the mountain-lion, and all stages of the development of these crystals were observed in that species, so that their mimetic character was determined. In the bob-cat, or wild cat, similar mimetic crystals, also apparently isometric, were noted. In all of these American cats this character of forming mimetic twins seems to be common and normal.

## MOUNTAIN-LION OR PUMA, *Felis concolor.* Plates 85 and 86.

The specimen of blood was received from the National Zoölogical Park at Washington, and was in a very fresh condition. It was evidently fresh blood and showed only oxyhemoglobin. The blood was laked with ether, and centrifugalized for several hours; from the clear solution thus obtained the slide preparations were made in the usual manner. The blood crystallized quite readily, about as fast as in the case of the domestic cat. The first crystals to develop are short prismatic crystals; these are followed by small pyramidal crystals and very thin tabular crystals, almost simultaneously. All of these forms appear to be the same substance, and to have the same axial ratio. They were all oxyhemoglobin as determined by the microspectroscope. They will be designated types (a), (b), and (c). From the pyramidal type, by twinning in sixlings, are produced mimetic crystals, that finally become isometric in angles and isotropic in structure. These are distinguished from the first three kinds enumerated as $\beta$-oxyhemoglobin, while they—types (a), (b), and (c)—are designated as $\alpha$-oxyhemoglobin.

The first crystals to appear, type (a), have a somewhat porous aspect; they tend to be dissolved, and the same is true of the tabular crystals; but the pyramidal-looking crystals of the $\alpha$-oxyhemoglobin and their mimetic twins, the $\beta$-oxyhemoglobin, are more permanent. The thin plates designated as type (c) of the $\alpha$-oxyhemoglobin are somewhat more soluble than the type (a) prisms; and the latter recrystallize readily.

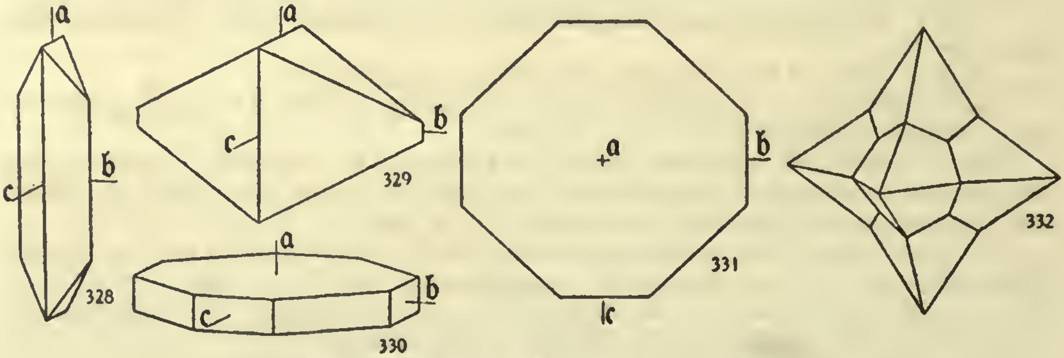
Later, crystals of reduced hemoglobin developed in the slides; they were well formed and showed no tendency to dissolve, but they only appeared very sparingly and in a few slides.

### *α-Oxyhemoglobin of Felis concolor.*

Orthorhombic: Axial ratio $\breve{a} : b : \acute{c}$ =0.9489 : 1 : 1.5546.

Forms observed: Unit prism (110), macroprism (210), brachydomes (011), (013); macropinacoid (100), brachypinacoid (010), base (001).

Angles: Prism angle 110 ∧ $1\bar{1}0$ =87° (normals); also measured as 85° on some crystals; brachydomes 011 ∧ $0\bar{1}1$ =65° 30′, 013 ∧ $0\bar{1}3$ =54° (about).



Figs. 328, 329, 330, 331, 332. *Felis concolor* α-Oxyhemoglobin.

Habit at first prismatic on the vertical axis, the crystal consisting of the unit prism and brachydome, type (a) (plate 85, figs. 507 and 508; also text figure 328). In these crystals the ratio of length to thickness is about 4 : 1. These are succeeded by type (b) crystals (see text figure 329 and plate 85, figs. 509 and 510), consisting of the macroprism prism (210) with the brachydome (013) in about equal development, the combination making a rather pyramidal-looking crystal resembling a regular tetragonal pyramid. With these also appear very thin plates, type (c) (plate 86, fig. 511); four, six, and eight-sided; consisting of the base bounded by the unit prism and the two vertical pinacoids (001) (110) (100) (010) (text figures 330 and 331). These type (c) crystals are evidently the same as the large plates found in the blood of the jaguar and lion and several other cats; but in this case they are not the normal type of crystal. Of these three types of crystal the type (b) appears to be the most permanent, the other two seem to show a tendency to dissolve and recrystallize as type (b).

The type (a) crystals did not appear to twin, and in the type (c) crystals twinning was only doubtfully observed; but in the type (b) crystals they formed sixlings, apparently by twinning on a pyramid (233), the six being arranged with the pseudo-tetragonal pyramid points all pointing outward or in the direction of the axes (text figure 332). The group of six thus formed continued to grow, and the somewhat sunken faces filled up; at the same time, the polar edges of the group became straight lines, so that, finally, the crystal became apparently isometric in form. The group also lost all trace of double refraction and became isotropic, passing into the mimetic form of $\beta$-oxyhemoglobin, which may be an isomer of the $\alpha$-oxyhemoglobin.

Pleochroism is not very strong in any of these three types of crystals; but they are all small, so that the colors are necessarily pale. The color varies inversely as the elasticity, as usual; $\mathfrak{a}$ nearly colorless, $\mathfrak{b}$ deeper red, but rather pale, $\mathfrak{c}$ rather deep red. In the type (c) crystals the change of color is very slight. The mimetic crystals show no pleochroism at all. Extinction is straight in type (a) and type (b), but the tabular crystals of the third type are too thin to polarize. On the type (a) crystals, looking along the brachy-axis, the biaxial interference figure is seen, with the separation of the brushes very slight. The orientation of the elasticity axes is $\mathfrak{a} = \acute{c}$, $\mathfrak{b} = b$, $\mathfrak{c} = a$. The plane of the optic axes is the brachypinacoid; the axis of least elasticity is the acute bisectrix, $Bx_a = \mathfrak{c}$, and the optical character is *positive*. The crystals are nearly uniaxial, the separation of the axes is quite small, $2E = 15°$ about.

### *β-Oxyhemoglobin of Felis concolor.*

Pseudo-isometric: A mimetic twin of the orthorhombic $\alpha$-oxyhemoglobin.

Forms observed: The only form is the apparent octahedron.

Angles: As well as the angle could be measured it was about 71°.

Habit octahedral; the apparent octahedra being, in some cases, very symmetrically developed and the faces very smooth (text figure 333). Their development from the pyramidal-looking crystals of the $\alpha$-oxyhemoglobin has been described under that substance.

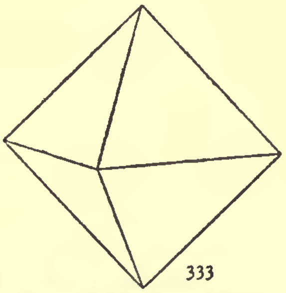


Fig. 333. *Felis concolor* β-Oxyhemoglobin.

The crystals are a very bright scarlet red, as is usually the case in oxyhemoglobin crystals that show little or no pleochroism. In this case, there is no trace of any pleochroism; nor is there any double refraction. The crystals have absolutely no effect upon polarized light; evidently the twinning has produced a structure in which the elements of the twin are submicroscopic, or it is a true mimetic twin.

### *Reduced Hemoglobin of Felis concolor.*

Orthorhombic: Axial ratio not determined.

Forms observed: Unit prism (110), macrodome (101), brachypinacoid (010).

Angles: The prism is nearly square; but neither the prism nor dome angles could be measured in the few crystals seen, because of the positions in which they were lying.

Habit prismatic on the vertical axis, the combination consisting of the unit prism and the macrodome; very frequently one of the dome faces is much more developed than the other, and this produces a monoclinic aspect. Some of the crystals are tabular on the brachypinacoid, but most of them are flattened on two planes of the macrodome. No twins were observed.

Pleochroism is very marked; $\mathfrak{a}$ nearly colorless, $\mathfrak{b}$ deep rose, $\mathfrak{c}$ deep red. The extinction is straight with the direction of the crystal axes, in all aspects. On the oblique end section of one crystal, a single brush of the interference figure was seen, and it gave the orientation of the axes as follows: $\mathfrak{a} = a$, $\mathfrak{b} = b$, $\mathfrak{c} = \acute{c}$. The plane of the optic axes is the brachypinacoid, the acute bisectrix $Bx_a = \mathfrak{c}$. The optical character is hence *positive*.

### Leopard-cat, *Felis bengalensis.* Plates 86 and 78.

The specimen of blood was received from the New York Zoölogical Park, and was clotted and very putrid. The clots were ground in sand and subjected to an atmosphere of oxygen before centrifugalizing. No ether was used for fear of breaking down the hemoglobins, as the blood was so putrid. From the clear centrifugalized solution the slide preparations were made as usual. Crystals of reduced hemoglobin formed slowly, and with them a few imperfect crystals of oxyhemoglobin. The crystals of reduced hemoglobin were at first small but very numerous; as more crystals developed, some of these grew to rather large size and a few reached relatively enormous proportions. The crystals of oxyhemoglobin were small, pyramidal in shape, and were rare. The reduced-hemoglobin crystals did not show any tendency to dissolve and remained sharp, but the crystals of oxyhemoglobin gradually disappeared from the slides by solution.

#### *Reduced Hemoglobin of Felis bengalensis.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.9657 : 1 : 0.3667$.

Forms observed: Unit prism (110), macrodomes (101), (403).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 88°$: macrodomes $101 \wedge \bar{1}01 = 41° 35'$; $403 \wedge \bar{4}03 = 53° 30'$.

Habit prismatic on the vertical axis, consisting of the square prism of 88° and the flat macrodome (text figure 334); the ratio of length to thickness of the prism is about 5 : 1 on the average, but many are much shorter, and 2 : 1 is common. Cross-sections of the prism are frequently seen, and also other irregular sections, that are sometimes quite symmetrical, and produce false planes, by the cover and slide interfering with the development of the crystal. Thus, many oblique sections of the square prism are seen, which look quite monoclinic, and some even rhombohedral. This sometimes occurs by the growth of a crystal that rests upon one of the dome faces; as it grows larger, it loses the other pair of dome faces, and an apparent rhombohedron results. Parallel growth is normal in some crystals and skeleton groups are formed, by extension of the crystal group in the plane of the macropinacoid, and in such a way that the diagonals of the single crystal in this plane become the growth axes of the group in parallel growth. Thus, the X-shaped groups are formed shown on plate 87, fig. 519. Twins do not appear to form commonly, but a few interpenetrant twins on the pyramid as twin plane were observed (text figure 335).

Pleochroism is marked, particularly on the side views of the prism; when seen in end view the pleochroism is not so strong. The colors are: $\mathfrak{a}$ nearly colorless or with a tinge of pinkish-lilac, $\mathfrak{b}$ deep rose pink, $\mathfrak{c}$ deep purplish-red, or, when thinner, deep purplish-rose color. Double refraction is strong, and the extinction is straight in all aspects. On the cross-sections of the prism the biaxial interference figure is seen with well-separated brushes, with the angle $2E$ about 50°; but the cross is rather dusky. The orientation of the elasticity axes is $\mathfrak{a} = a$, $\mathfrak{b} = b$, $\mathfrak{c} = \acute{c}$. The plane of the optic axes is the brachypinacoid; the acute bisectrix of the optic axes is the axis of least elasticity, $Bx_a = \mathfrak{c}$, hence, the optical character is *positive.*

#### *Oxyhemoglobin of Felis bengalensis.*

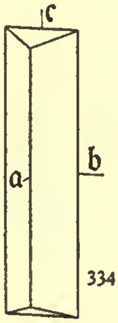
Tetragonal: Axial ratio $a : \acute{c} = 1 : 1.9253$.

Forms observed: Unit pyramid (111).

Angles: Angle over apex $111 \wedge \bar{1}\bar{1}1 = 40° 20'$ (actual angle); angle of pole edges over apex $= 54° 50'$, observed (computed 54° 54'); angle of horizontal edges $= 90°$.

Habit pyramidal, apparently a tetragonal unit pyramid (text figure 336), but the crystals are not very sharp. They have a granular appearance as though undergoing solution, and all look somewhat soft and porous. They occurred sparingly, scattered singly or in small groups through the slides.

Color scarlet, no pleochroism and apparently no double refraction. As the crystals have a definitively tetragonal aspect and angles, this absence of double refraction is very likely due to mimetic twinning. The angle of 54° 50′ and near 55° is a common dome angle in the cats. An interpenetrant twin showing this dome angle only and twinned on the macrodome would produce the effect. The macro-axis is the axis of mean elasticity 𝔟 and the others 𝔞 and 𝔠 would neutralize each other and the resultant would about equal 𝔟, so that double refraction would disappear.



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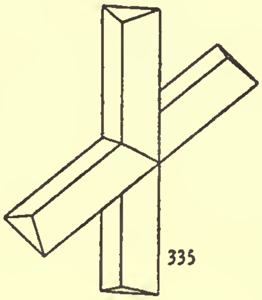

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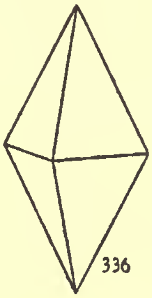

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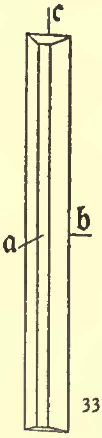

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Figs. 334, 335. *Felis bengalensis* Reduced Hemoglobin. Fig. 336. *Felis bengalensis* Oxyhemoglobin. Fig. 337. *Felis pardalis* Reduced Hemoglobin.

### OCELOT, *Felis pardalis.* Plate 88.

The specimen was received from the National Zoölogical Park at Washington, District of Columbia, during the summer, and was kept frozen in the refrigerating plant until examined in the following winter. When thawed, the blood was found to be full of clots and amorphous matter and quite putrid. The freezing having laked the blood, the mixture was diluted with water and centrifugalized to separate the impurities, and the aqueous solution thus obtained was evaporated until sufficiently concentrated for good preparations, when the slides were prepared in the usual manner. The blood crystallized very readily at room temperature, and, inside of 2 hours after the slides were covered, satisfactory photographs were obtained of the crystals. The crystals showed no tendency to dissolve on keeping, and they remained in good condition for a considerable time. Only crystals of reduced hemoglobin were made from this blood.

#### *Reduced Hemoglobin of Felis pardalis.*

Orthorhombic: $a : b : \dot{c} = 0.9489 : 1 : 0.3931$.

Forms observed: Unit prism (110), macrodome (101), macropinacoid (100).

Angles: Prism angle 110 $\wedge$ $1\bar{1}0 = 87°$; macrodome 101 $\wedge$ $\bar{1}01 = 45°$.

Habit long prismatic on the vertical axis, the crystal consisting of the unit prism, and generally the macropinacoid in the prismatic zone, and terminated by the macrodome (text figure 337). The crystals are much longer in proportion to the thickness than is common with the cats; in this species, the ratio of length to thickness is from 15 : 1 to 20 : 1. These ratios apply to the crystals after 24 hours; those that form first are shorter with a ratio of length to thickness of about 8 : 1. The first crystals to form do not show the macropinacoid; this appears on the larger crystals later. The slides soon become filled with a network of crystals, in which, besides the usual side views, numerous cross-sections, mostly oblique, can be observed, and on these the macropinacoid may be readily distinguished. The larger crystals frequently become covered over with tufts and growths of smaller crystals, usually radiating more or less from certain points along the larger crystals. Twinning seems to occur on the pyramid, both contact and penetration twins.

Pleochroism is marked; $\mathfrak{a}$ pale rose-pink, $\mathfrak{b}$ rose-pink, somewhat darker than $\mathfrak{a}$, but rather pale; $\mathfrak{c}$ deep rose-red. Double refraction is strong; extinction is straight in all aspects. The orientation of the elasticity axes is $\mathfrak{a}=a$, $\mathfrak{b}=b$, $\mathfrak{c}=\dot{c}$; the plane of the optic axes is the brachypinacoid and the acute bisectrix appears to be the axis of least elasticity, $Bx_a=\mathfrak{c}$. The optical character is hence *positive.*

### Domestic Cat, *Felis domestica.* Plates 88, 89, and 90.

The animal was killed in the laboratory and bled into oxalate. Several preparations were made from different specimens. Whole blood, ether-laked and centrifugalized, was mounted as slide preparations and was found to crystallize very slowly; but quite good crystals of reduced hemoglobin formed 30 hours after the preparations were made. The habit of these crystals is different from those prepared by the other methods described, but the forms, and the angles of the corresponding forms are the same. Several preparations were made from corpuscles settled from the plasma, both of the simply ether-laked corpuscles, and of the ether-laked corpuscles diluted with an equal volume of water. These all gave satisfactory preparations that crystallized very readily. Laking by repeated freezing and thawing, instead of laking with ether, gave perhaps the best crystals; but all of the preparations made from the corpuscles alone gave good results. The solution was of the color of oxyhemoglobin, and the first crystals to appear were minute needles that seemed to be oxyhemoglobin. Almost at the same time, irregular groups of crystals, which were evidently reduced hemoglobin, began to form; and these were the first crystals to attain any considerable size and definite shape. Prismatic crystals of a purplish color, but apparently showing the absorption spectrum of oxyhemoglobin, appeared soon after the crystals of undoubtedly reduced hemoglobin. All seemed to have the same axial ratio. The spectroscopic examination was interfered with by the oxyhemoglobin solution, so that the oxyhemoglobin lines might have come from the solution. The crystals that appeared to be oxyhemoglobin were not at all of the same color as the undoubtedly oxyhemoglobin crystals of other cats, nor did they show the forms usual in such crystals in the cats. They will all be described as reduced hemoglobin, provisionally.

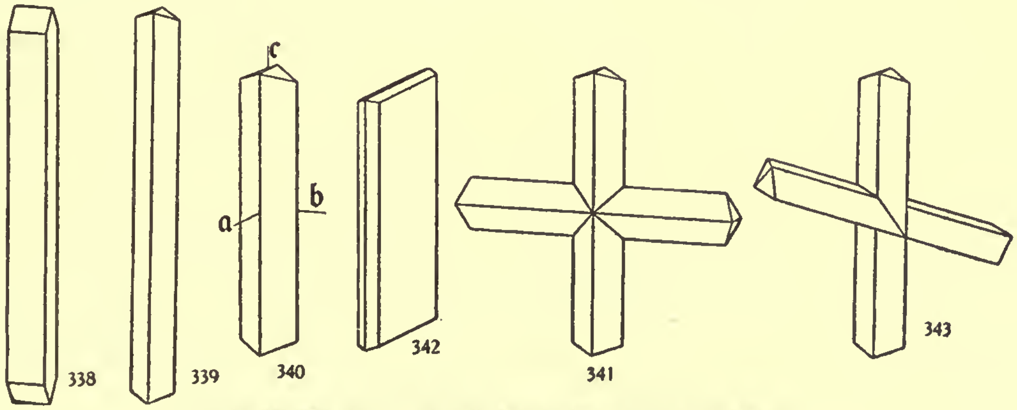
#### *Reduced Hemoglobin of Felis domestica.*

Orthorhombic: Axial ratio $a : b : \dot{c} = 0.9656 : 1 : 0.3839$.

Forms observed: Unit prism (110), brachydome (011), brachypinacoid (010), macroprism (320), macrodome (301), macropinacoid (100).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 88°$; macroprism angle $320 \wedge 3\bar{2}0 = 65° 30'$; brachydome angle $011 \wedge 0\bar{1}1 = 42°$; macrodome angle $301 \wedge \bar{3}01 = 100°$, all normals.

Habit different in the different types of crystals, but in general prismatic. The first crystals to appear are long needles, so thin that their forms can not be determined with any certainty. With them appeared groups of hemoglobin crystals growing in arborescent forms and in parallel growths (see plate 88, fig. 527), evidently the two vertical pinacoids (100) and (010), terminated by the macrodome (301) (text figure 338), sometimes with only one plane of the brachydome (011) developed and looking quite monoclinic in habit. Later, prismatic crystals of the usual cat-type appeared, showing the macroprism (320) and the brachydome (011) (text figure 339); and, from preparations of the corpuscles, generally the unit prism (110) with the same brachydome (011) (text figure 340). The dimensions of the prismatic crystals varied considerably and the ratio of length to thickness ran from 8 : 1 to 30 : 1. From corpuscles alone, without any dilution of the blood this ratio was 5 : 1 to 3 : 1 or even less (see plate 90, fig. 535), and when the square prism was cut by the cover and slide the crystals looked like cubes or rhombohedra (see plate 90, fig. 536). Frequently these crystals become covered with small second-growth crystals, sprouting out in every direction; they are generally proportionately longer, as is usual in the small prismatic crystals. When the longer crystals meet each other, confined in the thin layer of solution between slide and cover, they frequently are opposed or interpenetrate, thus simulating contact and interpenetrant twins; but most of these are probably only adventitious orientations and not true twins. Two kinds of twinning appeared to occur, however; one on a brachydome, probably about (052), which, when interpenetrant (text figure 341), produces a twin like the square staurolite cross; and the other kind of twin apparently on a pyramid, the two parts making an angle with each other of about 72° (text figure 342) like the oblique staurolite cross. Both were ordinarily seen as interpenetrant twins.



FIGS. 338, 339, 340, 341, 342, 343. *Felis domestica* Reduced Hemoglobin.

Pleochroism was very strong in all of the crystals, except when seen in cross-section of the prism, when it was weak. The colors varied with the thickness, but were usually: $\mathfrak{a}$ pale rose-pink to purplish-lilac or nearly colorless, $\mathfrak{b}$ various shades of rose-red, paling to rose-pink, $\mathfrak{c}$ deep rose-red. Extinction was straight, or symmetrical, in all sections. Double refraction is strong. The interference figure is easily seen in convergent light on cross-sections; and the axial angle is evidently large. The orientation of the elasticity axes is $\mathfrak{a}=b$, $\mathfrak{b}=a$, $\mathfrak{c}=\dot{c}$. The plane of the optic axes is the macropinacoid (100); the acute bisectrix is the axis of least elasticity $Bx_a=\mathfrak{c}$. The optical character is hence *positive.*

As the first-formed crystals of reduced hemoglobin increased in size, they became quite tabular on the brachypinacoid (011), and the prism developed on the vertical edge that at first appeared to be occupied by the macropinacoid (100) (text figure 343). These tabular crystals showed a tendency to develop into somewhat radiating groups, growing together in the zone of the brachydome and nearly on the brachypinacoid. The crystals of this type appeared to show more of a purplish color than those of the prismatic type; but this was probably due to the fact that there was less of the oxyhemoglobin solution covering the crystal, than in the case of the prisms, and hence less absorption of the reduced-hemoglobin color by the scarlet oxyhemoglobin solution.

### WILD CAT OR BAY LYNX, *Lynx rufus*. Plates 90, 91, and 92.

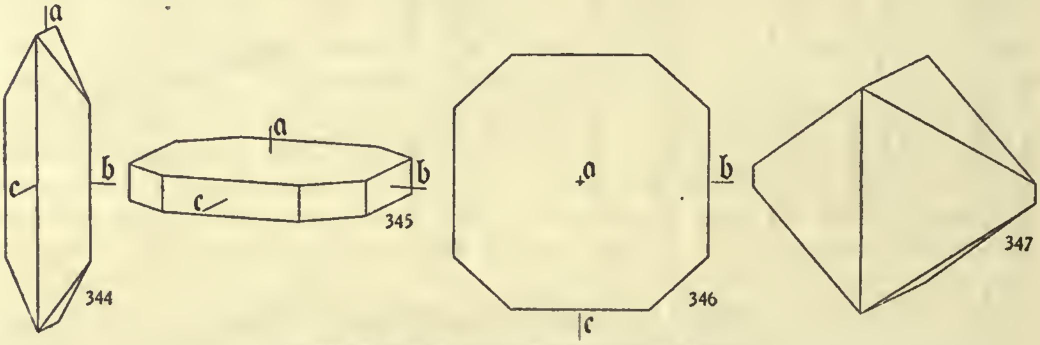
The specimen of blood was received from the National Zoölogical Park at Washington, District of Columbia. The blood, which had been collected in oxalate, was dark and thick. It was laked with ether and centrifugalized for several hours; and from the clear solution the slide preparations were made as usual. The crystals of reduced hemoglobin formed very readily, but negatives were not made until the following day, by which time some crystals of oxyhemoglobin had appeared in the form of very thin plates and also in the form of prismatic crystals. The crystals kept well, the reduced-hemoglobin crystals especially being quite insoluble; and after several days they were rather sharper and more perfect than at the end of the first 24 hours. The crystals of oxyhemoglobin were found rather sparingly in the slides, but developed in nearly all cases. They showed three habits, of which the tabular type was perhaps the most common.

#### *Oxyhemoglobin of Lynx rufus.*

Orthorhombic: Axial ratio 0.9866 : 1 : 0.3849.

Forms observed: Unit prism (110), macroprism (430), brachydome (041), pyramid (991), macropinacoid (100), brachypinacoid (010), basal pinacoid (001).

Angles: Unit prism angle, $110 \wedge 1\bar{1}0 = 89^\circ 15'$; macroprism angle, $430 \wedge 4\bar{3}0 = 73^\circ$; brachydome angle, $041 \wedge 0\bar{4}1 = 114^\circ$; pinacoids $100 \wedge 010 = 90^\circ$; pyramid edges of (991), actual angle over pole $= 32^\circ 15'$.



FIGS. 344, 345, 346, 347. *Lynx rufus* Oxyhemoglobin.

Habit of the prismatic crystals a rather short prism (430) with the brachydome (041) (text figure 344), the ratio of length to thickness (on *b*) about 3 : 1 or less. These prismatic crystals are generally rather small, but are very numerous in the slides in which they develop. The tabular crystals are flattened on the base and very thin; their bounding planes are the unit prism (110) and the two vertical pinacoids (100) and (010), with the pinacoids usually predominating (text figures 345 and 346); but sometimes only the two pinacoids or the unit prism form the bounding planes, which makes a square tabular crystal. The third type of crystal (text figure 347), which was observed in a few slides only, resembled an isometric octahedron and appeared to be a mimetic twin showing the prism faces only, the two interpenetrant prisms (430) being twinned on a macrodome, but possibly it may be only the prism (430) and the dome (041) in equilibrium. The only angles that could be measured corresponded to the angle of the prism of the ratio (430). Aside from the possible twin in these isometric-looking crystals, no definite twins were observed in the oxyhemoglobin crystals.

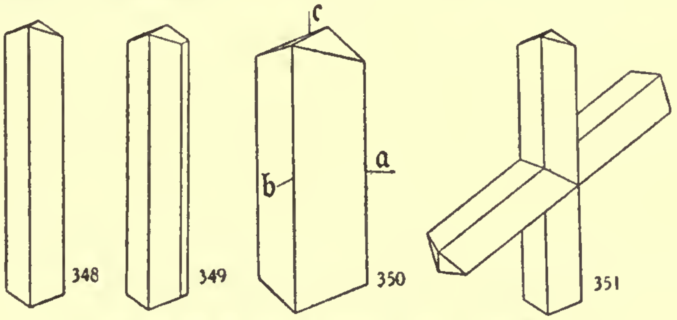
The color of these crystals was a bright scarlet, the usual color of oxyhemoglobin when the crystals are not strongly pleochroic. Pleochroism was weak and hardly noticeable, except in the prismatic type of crystals. In the isometric type it was absent, which would indicate mimetic twinning. The thin plates were not pleochroic. The change in color was very slight in the prismatic crystals, mainly a variation in shade of color. Extinction was straight in the prismatic crystals; the other types did not have any action upon the polarized light. The interference figure was not observed. The orientation of the elasticity axes was $\mathfrak{a} = \dot{c}$, $\mathfrak{b} = b$, $\mathfrak{c} = a$; the elasticity of $\mathfrak{a}$ and $\mathfrak{b}$ was nearly equal. The tabular crystals were too thin to show any polarization characters, but did not give an interference figure. It is therefore probable that the acute bisectrix of the optic axes is the axis of least elasticity $Bx_a = \mathfrak{c}$; this makes the optical character probably *positive.*

### *Reduced Hemoglobin of Lynx rufus.*

Orthorhombic: Axial ratio $a : b : \dot{c} = 0.9863 : 1 : 0.3914$.

Forms observed: Unit prism (110), macroprisms (320), (210), brachydome (011), brachypinacoid (010).

Angles: Unit prism 110 ∧ $1\bar{1}0$ = 89° 15′; macroprism 320 ∧ $3\bar{2}0$ = 66° 45′; macroprism 210 ∧ $2\bar{1}0$ = 52° 30′; brachydome 011 ∧ $0\bar{1}1$ = 42° 45′. The angle of prism to brachypinacoid was not measured.



FIGS. 348, 349, 350, 351. *Lynx rufus* Reduced Hemoglobin.

Habit at first (a) the macroprism (210) and the dome (011) (text figure 348) elongated on the prism, which is very variable in length, the ratio of length or thickness varying from 4 : 1 to 20 : 1 or more. Later crystals (b) showing the prism (320) with (010) and (011) appear (text figure 349); and also, sparingly, crystals (c) consisting of the unit prism (110) and the dome (011) only (text figure 350). These last (c) are usually short with ratio of length to thickness of 3 : 1 or less; but the other two types (a) and (b) are more frequently long. Twinning is very common in these crystals; apparently the twins are on the unit pyramid as a rule (text figure 351), but some seem to be on a brachydome. Irregular radiating groups of crystals, and second growths of small crystals in tufts on the larger crystals, are very frequently observed. A very common habit is the unequal development of the two brachydome faces; one being sometimes almost suppressed, while the other is greatly developed on the two ends of the crystals; these are usually the faces on one side as 011, $01\bar{1}$; but when only one end of the crystal appears, this oblique termination of the single dome face produces a very monoclinic aspect. In twins it is common to see the oblique ends arranged symmetrically with respect to the twin plane. In some cases a long pointed pyramid, perhaps (991) as in the oxyhemoglobin, was observed. The angle of its edges over the pole was about 30°, but the measurement was very inexact.

The color of the crystals is the usual purplish-red of reduced hemoglobin. Pleochroism is very pronounced; $\mathfrak{a}$ pale lilac, $\mathfrak{b}$ purplish, rather pale, $\mathfrak{c}$ rose-purple, much deeper than $\mathfrak{b}$. Double refraction is strong and extinction is straight or symmetrical in all axial aspects. On cross-sections of the prism the biaxial interference figure is seen in convergent light, with widely separated brushes. The orientation of the elasticity axes is $\mathfrak{a} = b$, $\mathfrak{b} = a$, $\mathfrak{c} = \dot{c}$. The plane of the optic axes is the macropinacoid and the acute bisectrix of the axes is the axis of least elasticity, $Bx_a = \mathfrak{c}$. The optical character is hence *positive.*

### Florida Lynx, *Lynx canadensis var.* Plates 92 and 93.

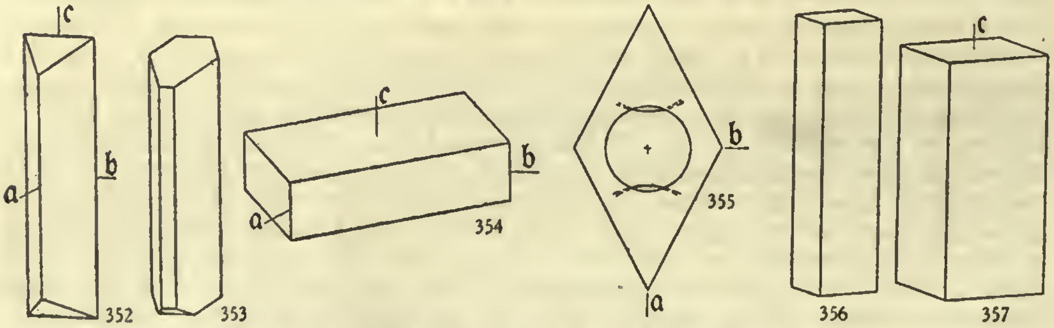
The specimen was received from the National Zoölogical Park at Washington, District of Columbia. The blood was somewhat clotted and was prepared by grinding the clot in sand with ether and centrifugalizing for several hours. From the clear solution thus obtained slide preparations were made as usual. The color of the blood was red, and it showed the oxyhemoglobin spectrum; but, as is usual in the cats, the crystals that formed most plentifully were reduced hemoglobin. The blood crystallized readily; small trichites and baculites of apparently oxyhemoglobin formed first, but soon good crystals of reduced hemoglobin appeared. Even in the tube in which the stock of centrifugalized prepared blood was kept, crystals gradually formed. After about 24 hours the slides were filled with well-formed crystals of reduced hemoglobin, and in a number of slides there were small crystals of oxyhemoglobin in the form of thin plates. These last were dissolved by the next day, and only the crystals of reduced hemoglobin remained. These continued to increase in size; and inside of 4 days they had reached dimensions that were relatively enormous, their form being readily seen with the naked eye. A very small number of crystals of a second form of reduced hemoglobin were observed, which had different optical properties from the normal crystal; but these may be some form of twin of the normal reduced hemoglobin. These two forms of reduced hemoglobin are designated as α-reduced hemoglobin (the normal crystal) and β-reduced hemoglobin (the second form).

#### *α-Reduced Hemoglobin of Lynx canadensis var.*

Orthorhombic: Axial ratio $a : b : \acute{c}$ = 0.9605 : 1 : 0.3944.

Forms observed: Unit prism (110), rare; brachyprism (120), macrodome (101), base (001), macropinacoid.

Angles: Brachyprism 120 ∧ $\bar{1}20$ = 55°, unit prism 110 ∧ 110 = about 88° (computed, 87° 42′), macrodome 101 ∧ $\bar{1}01$ = 41° 30′.



Figs. 352, 353, 354, 355, 356. *Lynx canadensis* α-Reduced Hemoglobin.
Fig. 357. *Lynx canadensis* β-Reduced Hemoglobin.

Habit type (a) short prismatic, consisting of the brachyprism (120) and the macrodome (text figure 352); with occasionally the macropinacoid also; these are the common crystals, and range in ratio of length to thickness from 3 : 2 to 5 : 1, with some that are much longer, and a few shorter, down to 2 : 3. They grow to very large size as single crystals and in parallel growth along the prism faces. The dome faces are often very unequally developed, giving an oblique aspect to the crystal (text figure 353); and in some cases only one parallel pair show at all, when the crystal looks monoclinic. Type (b) tabular on the base, with the brachyprism (120) and the base only developed (text figures 354 and 355); in these the length of the prism may be one-fourth of its short diagonal. These type (b) crystals are rather rare. Type (c), the unit prism (110) and the base (text figure 356); these are rare; when the dome appears instead of the base, the crystal looks like a rhombohedron. Aside from the tendency of the crystals of type (a) to unite on the prism faces in parallel growth or to grow along the macro-axis in the same kind of arrangement, aggregate crystals with regular arrangement do not appear to occur; no undoubted twins were observed.

The crystals are of the typical reduced-hemoglobin color and show marked pleochroism; $\mathfrak{a}$ pale rose-pink or pale lilac, $\mathfrak{b}$ rose-red, $\mathfrak{c}$ deep purplish-red. Extinction is straight or symmetrical; and in the unsymmetrical, monoclinic-looking crystals it is parallel to the prismatic zone. Double refraction is strong. On accidental cross-sections of the prism, or on the tabular crystals of type (b) when the basal aspect is presented, the biaxial interference figure is seen in convergent light. The orientation of the elasticity axes is $\mathfrak{a}=a$; $\mathfrak{b}=b$; $\mathfrak{c}=\dot{c}$. The plane of the optic axes is the brachypinacoid; and the acute bisectrix of the optic axes is the axis of least elasticity, $Bx_a=\mathfrak{c}$. The angle of the optic axes is wide, $2E=82°$. The optical character is *positive.*

### *β-Reduced Hemoglobin of Lynx canadensis var.*

Tetragonal or pseudo-tetragonal. No axial ratio was determinable.

Forms observed: The only forms seen were the unit prism (110) and the base (001).

Angles: The prismatic angle was 90°, as nearly as it could be observed; and a like angle was measured from the prism to the base.

Habit short prismatic, cubical, or sometimes rhombohedral-looking crystals. The apparent prism was in equilibrium with the base or somewhat longer, with a ratio of length to thickness of 1 : 1 or 3 : 2 (text figure 357).

The crystals were not very strongly pleochroic. Extinction took place in the direction of the prism axis. On cross-sections showing the basal aspect, the crystals were singly refracting. When this aspect was presented, a uniaxial cross was seen. The vertical axis is the axis of least elasticity, and the crystal is hence optically *positive.*

Except for the uniaxial character of these crystals they closely resemble crystals of type (c) of the *α*-reduced hemoglobin. They occurred very sparingly in the slides.

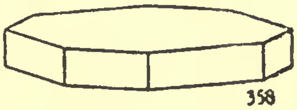
### *Oxyhemoglobin of Lynx canadensis var.*

Orthorhombic: Axial ratio $a : b : \dot{c} = 0.9657 : 1 : \dot{c}$.

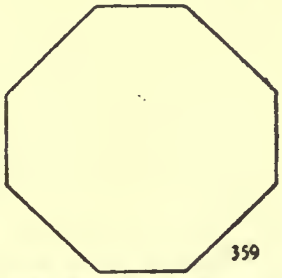
Forms observed: Unit prism (110), base (001), brachypinacoid (010), macropinacoid (100).

Angles: Unit prism 110 ∧ 1$\bar{1}$0 = 88°; pinacoids 110 ∧ 010 = 90°.

Habit very thin tabular on the base, the crystal consisting of the base (001) cut by the unit prism (110) and sometimes also by the two vertical pinacoids (100) and (010) (text figures 358 and 359). The crystals grow in groups on the base; also in radiating tufts and spheroidal masses, by growing together on the base, or in the zone of the base and one of the vertical pinacoids. They are frequently curved, at least in the groups, and when seen on edge form various arborescent shapes. Sometimes, when on edge, they present the appearance of radiating rods. They were only found in the slides for about 24 hours after the preparations were made.



358



359

FIGS. 358, 359. *Lynx canadensis* Oxyhemoglobin.

The color was the usual scarlet-red of oxyhemoglobin. The crystals were not noticeably pleochroic and did not have sufficient thickness to polarize, so that definite optical characters could be determined. (See plate 92, figs. 551 and 552.)

## VIVERRIDÆ.

### BINTURONG, *Arctictis binturong.*

The specimen was received from the New York Zoölogical Park during the summer, and the blood kept frozen in the refrigerating plant until the preparations were made. The blood was diluted with a little water before centrifugalizing, and the clear liquid obtained was rather dilute. The slide preparations were made in the usual manner. Crystals formed readily, but, probably owing to the diluted solution, were not very perfect. They were oxyhemoglobin. They kept fairly well and did not appear to be very soluble, but no photographic records were obtained.

#### *Oxyhemoglobin of Arctictis binturong.*

Orthorhombic: No axial ratio was obtained.

Forms observed: Macropinacoid (100), brachypinacoid (010); the terminations were simply tapering to a point or irregular, apparently corrosion forms only. They represented very acute pyramidal planes.

Angles: The two pinacoids were at 90°, as nearly as they could be measured.

Habit acicular, elongated on the vertical axis, often hair-like, but some rather lath-shaped. As the crystals increased in thickness they grew together in bundles and parallel growths along the vertical axis, often with brush-like tufted ends. Some bundles aggregated into rough prismatic-looking crystals, but no measurable terminal planes were developed.

The color was oxyhemoglobin red; pleochroism was rather marked. Evidently 𝔞 and 𝔟 were the two directions normal to the vertical axis; their colors were both pale yellowish-red; 𝔠 was parallel to the vertical axis and its color was deep red. Extinction was parallel to the prismatic direction, or straight in all aspects normal to the vertical axis; no end views were obtained as the prisms were too thin. No interference figures were obtained for the same reasons, but from the fact that 𝔞 and 𝔟 are near together, it follows that the axis of least elasticity, 𝔠, must probably be the acute bisectrix, which would make the optical character *positive.*

There is a possibility that these prisms are tetragonal; but it is very unlikely, as the flattened crystals are usually orthorhombic. There appeared to be a difference also between 𝔟 and 𝔞, but in absence of cross-sections this is uncertain. The optical character would be positive in any case, whether the crystals were orthorhombic or tetragonal. The characters observed agree best with orthorhombic crystallization.

TABLE 46.—*Crystallographic characters of the hemoglobins of the Felidæ and Viverridæ.*

| Name of species. | Axial ratio. | Prism angle. | Dome angle. | Optical character. | System. | Substance. |
|---|---|---|---|---|---|---|
| | | ° ′ | ° ′ | | | |
| Felis leo | 0.765 : 1 : 1.235 | 74 50 | 63 32 | Negative | Orthorhombic | OHb. |
| Do | 0.9742 : 1 : 0.3707 | 88 30 | 41 50 | Positive | Do. | Hb. |
| Felis tigris | 0.9742 : 1 : 0.3838 | 88 30 | 43 0 | Do. | Do. | Hb. |
| Felis onca | 0.7813 : 1 : 1.2146 | 76 0 | 65 30 | Negative | Do. | α-OHb. |
| Do | 1 : 1 : 1 | 90 0 | 90 0 | Isotropic | Isometric (?) | β-OHb. |
| Do | ..... | ..... | ..... | ..... | Orthorhombic (?) | Hb. |
| Felis concolor | 0.9489 : 1 : 1.5546 | 87 0 | 65 30 | Positive | Orthorhombic | α-Hb. |
| Do | 1 : 1 : 1 | 90 0 | 90 0 | Isotropic | Pseudo-isometric | β-OHb. |
| Do | ..... | ..... | ..... | Positive | Orthorhombic | Hb. |
| Felis bengalensis | a : ċ = 1 : 1.9253 | 90 0 | 54 50 | Nearly isotropic | Tetragonal ± | OHb. |
| Do | 0.9657 : 1 : 0.3667 | 88 0 | 41 35 | Positive | Orthorhombic | Hb. |
| Felis pardalis | 0.9489 : 1 : 0.3931 | 87 0 | 45 0 | Do. | Do. | Hb. |
| Felis domestica | 0.9656 : 1 : 0.3839 | 88 0 | 43 20 | Do. | Do. | Hb. |
| Lynx rufus | 0.9869 : 1 : 0.3849 | 89 15 | 42 6 | Do. | Do. | OHb. |
| Do | 0.9869 : 1 : 0.3914 | 89 15 | 42 46 | Do. | Do. | Hb. |
| Lynx canadensis, var. | 0.9657 : 1 : ċ | 88 0 | ..... | Nearly isotropic | Do. | OHb. |
| Do | 0.9605 : 1 : 0.3944 | 87 42 | 41 30 | Positive | Do. | α-Hb. |
| Do | ..... | 90 0 | ..... | Do. | Tetragonal ± | β-Hb. |
| Arctictis binturong | ..... | ..... | ..... | Do. | Orthorhombic | OHb. |

# CHAPTER XVII.

## CRYSTALLOGRAPHY OF THE HEMOGLOBINS OF THE INSECTIVORA AND CHIROPTERA.

One species of the *Insectivora,* the mole, *Scalops aquaticus,* and two species of the *Chiroptera,* or bats, were studied. One of the bats belonged to the *Megachiroptera,* the flying-fox or fruit-bat of India, *Pteropus medius;* and the other to the *Microchiroptera,* the common brown bat, *Vespertilio fuscus.* In each case but a very small amount of blood was available, so that the clearing of the blood from extraneous matter was difficult. But the hemoglobins of the three species examined were all rather insoluble, so that fairly good crystals were obtained. As the three species mentioned are rather widely separated zoölogically, their hemoglobin crystals would not necessarily bear much resemblance to each other; nevertheless, the crystals of the two bats show some resemblance to each other, both being apparently monoclinic.

All of the species are widely separated zoölogically from the other animals examined, and both groups are highly specialized animals; so that the crystals do not show much resemblance to others that were studied, and the crystals obtained from the mole are practically unique. Like the hemoglobins of other animals living largely underground, the crystals of the mole are very insoluble, and they keep, in the presence of ether, for a long period. But unlike the hemoglobins of other burrowing animals that crystallize in the monoclinic or orthorhombic systems, these mole crystals were fairly well formed and sharp, not simply hairs or trichites, as is so common in these insoluble hemoglobins. This comes from the fact that the habit of the crystals is pyramidal and not prismatic.

The blood of the flying-fox was in a putrid condition when received, and while it crystallized readily, the crystals were not stable. So far as form is concerned they are quite different from those of the little brown bat; but if the blood had been fresh, it is possible that a different habit of crystal might have developed that would have shown more resemblance to those obtained from the blood of the brown bat. This fruit-bat blood was examined early in our work, and before we had perfected methods for regenerating putrid blood.

The very few examples of these two orders examined preclude any attempt at generalization as to their affinities, but it may be remarked that there is a strong superficial resemblance between the lath-shaped monoclinic crystals of the oxyhemoglobin of the brown bat, *Vespertilio fuscus,* and the similarly shaped α-oxyhemoglobin crystals of the genus *Papio* of the *Primates.* These latter, however, are orthorhombic.

## INSECTIVORA.

### MOLE, *Scalops aquaticus*. Plate 94.

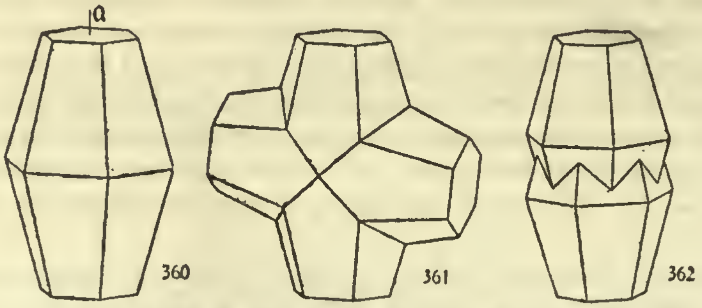
Two specimens of mole blood were obtained from animals procured in the suburbs of Philadelphia. One was bled in the laboratory. The fresh blood from this latter specimen was oxalated, ether-laked and centrifugalized as usual. The other was from a dead animal, and the clotted blood from the heart and larger vessels was removed, the clots ground in sand with ether and a little oxalate, and the resulting mixture centrifugalized. The slide preparations were made as usual. The preparations from the fresh blood crystallized so quickly that only very small crystals were obtained; that from the clotted blood crystallized somewhat less rapidly and furnished larger crystals. The crystals in any given preparation varied only slightly in size, when obtained from the fresh blood; and they kept for a very long time, showing no tendency to dissolve. The crystallization of the oxyhemoglobin was almost complete, and no color remained in the solution in the slides. In a test-tube, with a little ether, the crystals of the fresh blood in the plasma kept for several months. From the stale blood, on the other hand, the crystals in the slides were attacked by bacteria inside of a few days, and completely honeycombed by their action, the interior of the crystal being converted into reduced hemoglobin inside of a shell of oxyhemoglobin. In some cases the crystals were completely converted into reduced hemoglobin, no shell of oxyhemoglobin remaining. The change was, however, a paramorphous one, and the crystals were not at all altered in form, only the optical characters being changed by the alteration to reduced hemoglobin. The preparation of fresh blood preserved in a tube at ordinary temperature for as much as 3 months did not produce any larger crystals, but the habit changed slightly after a long period.

*Oxyhemoglobin of Scalops aquaticus.*

Hexagonal (pyramidal ?): Axial ratio $a : \acute{c} = 1 : 3.2931$.

Forms observed: Unit pyramid (1011), base (001).

Angles: Angle of the pole edges of the unit pyramid, measured over the apex, average, gave 33° 47′.



FIGS. 360, 361, 362. *Scalops aquaticus* Oxyhemoglobin.

Habit, rather short pyramidal crystals; consisting of the tapering unit pyramid, cut off by the base and making a "barrel-shaped" crystal (text figure 360). The basal planes are rather large so that the pyramid is truncated to about one-third of what its length would be if complete. In crystals formed in the fresh blood, however, the truncation is much less and amounts to not over one-third of the length, perhaps one-fourth; the resulting crystal being then about two-thirds to three-fourths of the normal pyramid, if complete and not truncated. In some cases the truncation is even more, so that the crystal is about equidimensional. The basal surfaces were sometimes perfect, but more often depressed as though the crystals grew more rapidly on the prism-base edges, and the depression was frequently quite funnel-shaped. This was the common point of attack for the bacteria, which converted the oxyhemoglobin to reduced hemoglobin as above described. Twins of two types were noted: (a) interpenetrant twins on the second-order pyramid (1123) (text figure 361) with the hexagonal axes inclined at 84° and 96° approximately, producing twins similar to those formed in quartz; (b) interpenetrant twins on the third-order prism (text figure 362), which would seem to indicate that the pyramid taken as unit may be a third-order pyramid and the twin a combination of the plus and minus third-order pyramids with the principal axis coincident in the two members of the twin. The exact orientation was not definitely determined in this case, however.

Pleochroism is strong; $\mathfrak{a} = \epsilon$, very pale red, nearly colorless; $\mathfrak{c} = \omega$, deep red. Extinction is straight in all side views and the crystals are singly refracting on the base. In convergent light a faint uniaxial figure is seen on the base. The axis of greatest elasticity is the vertical axis $\dot{c}$. Hence $\omega > \epsilon$ and the optical character is *negative.*

### *Hemoglobin of Scalops aquaticus.*

Hexagonal, only observed in paramorphs after the oxyhemoglobin. The forms and angles are hence identical in the two substances. The reduced hemoglobin paramorph is produced by bacterial action. The bacteria enter at the basal depressions and frequently penetrate the crystal from end to end, which then becomes like a short hexagonal bead with a central perforation. They work through the substance of the crystal and completely honeycomb it, but usually leave a shell of unaltered oxyhemoglobin on the exterior, including the pyramidal planes, but not the base, which is completely eaten away. In some cases the crystals were thus completely converted to reduced hemoglobin and the channels made by the bacteria were even repaired and filled up by recrystallized hemoglobin, making quite perfect crystals.

Pleochroism was very strong, $\mathfrak{a} = \epsilon$, nearly colorless, pale lilac; $\mathfrak{c} = \omega$, deep purplish-red. The double refraction is strong and extinction straight. The axis of greatest elasticity is the vertical axis, $\omega > \epsilon$; hence the optical character is *negative,* the same as in the oxyhemoglobin.

## CHIROPTERA.

### FOX-BAT OR FLYING-FOX, *Pteropus medius.* Plate 94.

The specimen was received from the Philadelphia Zoölogical Gardens, and was in a putrid condition. It was oxalated, a little ether added, and preparations made as usual. The blood crystallized readily, and the crystals did not appear to dissolve at first, but after a few hours they began to break down and by the next day had disappeared from the slides. The photographs were taken inside of 4 hours after the preparations were made. The crystals were oxyhemoglobin. The examination of the crystals was incomplete, owing to their disappearing from the slides so rapidly; and hence the crystallographic constants were imperfectly determined.

### *Oxyhemoglobin of Pteropus medius.*

Monoclinic (or perhaps triclinic): Axial ratio $a : b : \dot{c} = 1 : b : 1.2808$; $\beta = 56° 30'$.

Forms observed: Unit prism (110), orthodome ($\bar{1}01$), clinopinacoid (010), base (001).

Angles: The prism angle was not obtained. Hemiorthodome to prism edge or orthopinacoid $\bar{1}01 \wedge 100 = 50°$; hemiorthodome to base $101 \wedge 001 = 73° 30'$; orthopinacoid to base (or prism-edge to base) $100 \wedge 001 = 56° 30' = \beta$.

Habit generally tabular on the clinopinacoid (text figures 363 and 364); also short prismatic to tabular on the base. The planes seem to be irregularly developed, and may represent triclinic symmetry. The cover-edge crystals are elongated, apparently on the vertical axis, and generally flattened on the plane of symmetry; they grow crowded together, but as irregular aggregates, not apparently twinned.

Pleochroism was only observed on the clinopinacoid aspect, and is $\mathfrak{a}$ pale yellowish, nearly colorless; $\mathfrak{c}$ deep red. Double refraction is strong; extinction is nearly or quite parallel to the prism edges. The plane of the optic axes appears to be the plane of symmetry, or parallel to the plane taken as the clinopinacoid. The optical character can not be determined because of insufficient data.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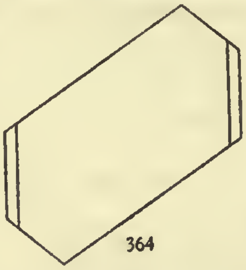
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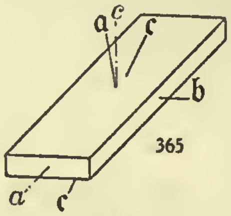

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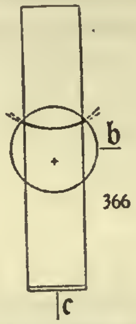


366

Figs. 363, 364. *Pteropus medius* Oxyhemoglobin.  Figs. 365, 366. *Vespertilio fuscus* Oxyhemoglobin.

### Brown Bat, *Vespertilio fuscus*. Plate 95.

The specimen was bled in the laboratory. The few drops of blood obtained were caught in oxalate, and ether-laked. The quantity was not enough to centrifugalize. The slide preparations were made with the laked blood in the usual manner. Crystallization proceeded rapidly, and the crystals appeared to be rather insoluble, keeping well and showing no tendency to dissolve. Crystallization was quite complete, but little color remaining in the solution. The crystals were shown to be oxyhemoglobin by the microspectroscope.

#### *Oxyhemoglobin of Vespertilio fuscus.*

Monoclinic: Axial ratio not determinable; $\beta = 81°$.

Forms observed: The three pinacoids only, orthopinacoid (100), clinopinacoid (010), base (001).

Angles: Base to orthopinacoid, $001 \wedge 100 = 81° = \beta$; clinopinacoid, $010 \wedge 100 = 90°$.

Habit tabular on the base, and elongated on the clino-axis, producing broad lath-shaped crystals (text figures 365 and 366), with a ratio of length to width on the base of about 8 : 1 to 5 : 1. The tabular crystals are thin, the thickness is one-twentieth of the length or less. They usually grow singly or sometimes in parallel growth on the base. In some cases the plates pile up on the base into parallel groups or bundles of crystals. No evidence of twinning was observed.

Pleochroism moderate; $\mathfrak{a}$ pale yellowish-red, $\mathfrak{b}$ rather deep red, $\mathfrak{c}$ very deep red. Double refraction fairly strong, extinction straight on the basal aspect; on the clinopinacoid aspect the extinction is 16°, measured from the basal edge. The orientation of the elasticity axes is $\mathfrak{a} \wedge \dot{c} = 25°$, in the obtuse angle; $\mathfrak{b} = b$; $\mathfrak{c} \wedge a = 16°$, in the acute angle. On the base, in convergent light, a single brush of a biaxial interference figure is seen, which passes out of the field upon rotation. As the normal to the base is 16° from $\mathfrak{a}$ it would appear that the acute bisectrix $Bx_a = \mathfrak{c}$, and the optical character is hence *positive*.

TABLE 47.—*Crystallographic characters of the hemoglobins of the Insectivora and of the Chiroptera examined.*

| Name of species. | Axial ratio. | Prism angle. | Angle β. | | Extinction angle. | Optical character. | System. | Substance. |
|---|---|---|---|---|---|---|---|---|
| Insectivora: | | ° | ° | ' | | | | |
| Scalops aquaticus . | 1:3.2931 | 60 | 90 | 0 | 0° | Negative | Hexagonal | OHb. |
| Do........... | 1:3.2931 | 60 | 90 | 0 | 0° | Do. | Paramorphous, after OHb | Hb. |
| Megachiroptera: | | | | | | | | |
| Pteropus medius.. | 1:*b*:1.2808 | .. | 56 | 30 | 0° (nearly) | ........ | Monoclinic (or perhaps triclinic) | OHb. |
| Microchiroptera: | | | | | | | | |
| Vespertilio fuscus.. | .......... | .. | 81 | 0 | $c \wedge a = 16^{\circ}$ | Positive | Monoclinic | OHb. |

# CHAPTER XVIII.

## CRYSTALLOGRAPHY OF THE HEMOGLOBINS OF THE PRIMATES—LEMURS, BABOONS, AND MAN.

The specimens of blood of the *Primates* received were not very representative of the order. They comprised one lemuroid, *Lemur catta*, the ring-tailed lemur; 6 species of the *Cercopithecidæ*, all members of the genus *Papio* (baboons); and the blood of man, *Homo sapiens*. The 6 species of baboons were the yellow baboon, *Papio babuin;* the drill, *Papio leucophæus;* the Guinea baboon, *Papio sphinx;* the long-armed baboon, *Papio langheldi;* the chacma, *Papio porcarius*, and the Anubis baboon, *Papio anubis*.

In the case of several of the baboons, the supply of blood was sufficient to allow several methods of preparation to be used, and the crystals obtained were satisfactory for study. Three kinds of oxyhemoglobin crystals were observed in baboon blood, which are distinguished as $\alpha$-, $\beta$-, and $\gamma$-oxyhemoglobin, respectively. Two of these types were observed in human blood. All species of the baboons did not develop these three kinds of oxyhemoglobin crystals, but when all three kinds were not observed it was due to the condition of the blood, or to lack of sufficient material. In comparing the hemoglobins of the species of *Primates*, the corresponding kind of oxyhemoglobin should be used.

TABLE 48.—*The three kinds of oxyhemoglobin observed in baboons and in man, with their optical characters.*

| Name of species. | $\alpha$-oxyhemoglobin, orthorhombic. | | $\beta$-oxyhemoglobin, monoclinic. | | $\gamma$-oxyhemoglobin, orthorhombic. | |
|---|---|---|---|---|---|---|
| Papio babuin | .......... | Negative | 1.6801 : 1 : ć | Positive | 0.5317 : 1 : ć | Negative. |
| Papio leucophæus | .......... | Do. | .......... | ......... | .......... | ......... |
| Papio sphinx | .......... | Do. | 1.8418 : 1 : ć | Negative | 0.3346 : 1 : ć | Negative. |
| Papio langheldi | $a : 1 : 0.543$ | Do. | 1.655 : 1 : ć | Positive | .......... | ......... |
| Papio porcarius | .......... | Do. | 1.732 : 1 : ć | Do. | .......... | ......... |
| Papio anubis | .......... | ......... | 1.737 : 1 : ć | Do. | 0.3268 : 1 : ć | Negative. |
| Homo sapiens | .......... | Negative | .......... | ......... | Optical character not determined. | |

The $\alpha$-oxyhemoglobin of the baboons and of man showed only the pinacoidal planes, so that the axial ratio could not be determined; but, in the monoclinic $\beta$-oxyhemoglobin crystals, the prism angle gave the ratio of $a : b$. The optical characters were determined in practically every case. The crystals of $\gamma$-oxyhemoglobin were not observed so frequently as those of the $\alpha$- and $\beta$-oxyhemoglobin; in these $\gamma$-oxyhemoglobin crystals, also, the prism was developed and gave the axial ratio of $a : b$. Table 48 shows the distribution of these three kinds of oxyhemoglobin in the baboons and in man, as they were observed in our experiments.

From table 48 it will be seen that the α-oxyhemoglobin was observed in all species except in *Papio anubis*, and in this species the blood was not fresh. The β-oxyhemoglobin was observed in all species except the drill and man; when it did not develop, it was probably a question of its solubility. The γ-oxyhemoglobin was observed in three species of baboons and in man, this last a rather doubtful observation, as the angles of the crystals could not be definitely measured. As the orthorhombic forms, α- and γ-oxyhemoglobin, are both optically negative, it is possible that they are the same substance; this does not seem likely from the way that they developed, but there are no data to prove that they are the same or different substances. The γ-oxyhemoglobin of the yellow baboon, the guinea baboon, and the anubis, the three species in which this form yielded a partial axial ratio, show ratios of *Papio babuin*=0.5317 : 1 : ċ, *Papio sphinx*= 0.3346 : 1 : ċ, and *Papio anubis*=0.3268 : 1 : ċ. This apparent discrepancy disappears if the prism in *Papio babuin* is regarded as (230); then the ratio becomes for *Papio babuin*=0.3545 : 1 : ċ. It will be noticed that while the two orthorhombic forms of the oxyhemoglobin are, as stated above, both negative, the monoclinic form is always (with the exception of this form in *Papio sphinx*) optically positive. The orthorhombic crystals of the *Lemur catta* are optically negative.

## PRIMATES.

### Ring-tailed Lemur, *Lemur catta*. Plate 95.

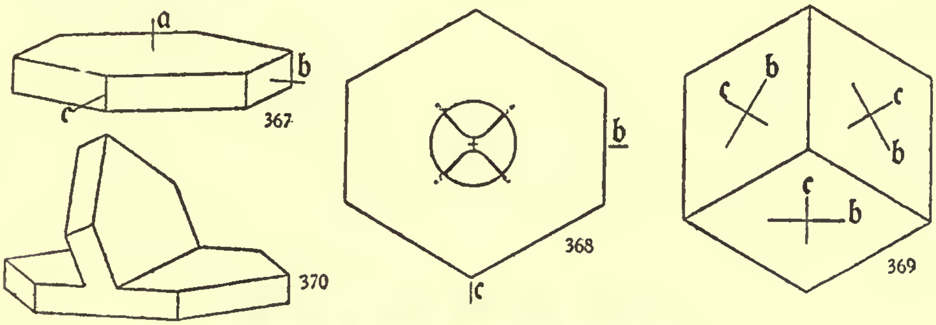
The specimen was obtained from the Philadelphia Zoölogical Garden, and was in the form of clots, slightly putrid. The clots were ground in sand, with a little ether, diluted with a little normal saline solution, and centrifugalized for 2 hours. The specimen had been collected in oxalate, hence no addition of this salt was necessary. From the clear solution obtained after the centrifugalizing, the slide preparations were made in the usual manner. The blood crystallized very slowly; after 2 hours only very small crystals began to appear, and these were very poorly formed and only irregular aggregates of rods. After standing in the cold for some time, tabular crystals appeared; but these dissolved rapidly when the slides were brought into a warm room. The tabular crystals improved in character, and became quite large and perfect in 4 days. They dissolved rapidly when the temperature was raised only slightly, and all examinations and photographs had to be made in a room temperature near the freezing-point. The rods at first formed were so poorly developed that their crystallographic characters could not be made out. The plates developed later are probably the same substance as the rods, and were well-formed crystals. They, as well as the rods, were found to be oxyhemoglobin by the spectroscope. Fine hair-like rods were seen with the tabular crystals; these were evidently of the same crystallization, but were not of sufficiently definite form to determine the crystallographic constants with certainty.

#### *Oxyhemoglobin of Lemur catta.*

Orthorhombic, pseudo-hexagonal by twinning: Axial ratio $a:b:\dot{c}$ = 0.5832 : 1 : 0.3860.

Forms observed: Unit prism (110), macrodome (101) (in twins), brachypinacoid (010), base (001); also, in the prismatic crystals in twins, the macrodome (403).

Angles: Unit prism angle, 101 ∧ 1$\bar{1}$0 = 60° 30′; prism to brachypinacoid, 110 ∧ 010 = 59° 45′, macrodome to base (from twin) 101 ∧ 001 = 32° 30′; angle of twin 67°; brachypinacoid to base 010 ∧ 001 = 90°; prism to base 110 ∧ 001 = 90°.



Figs. 367, 368, 369, 370. *Lemur catta* Oxyhemoglobin.

Habit tabular on the base (text figures 367 and 368), the crystal consisting of the unit prism and the brachypinacoid, cut by the base. The crystals twin three together on the prism faces, as is common in aragonite, and form almost perfect hexagonal plates (text figure 369). The simple crystals, when the prism and brachypinacoid are in equilibrium, are also almost perfect hexagonal plates. The plates pile up on the base, and evidently produce twins in that way also, in the same orientation as the twins on the prism, simply producing an overgrowth of one crystal on another in the composite group. This produces such an averaging of the elasticities, when the light passes through a number of such lamellæ, that the crystal appears to be uniaxial; and, the angles averaging also, it becomes, around the edges, where the reaction of one layer upon the next is most pronounced, practically hexagonal (mimetic hexagonal). The crystals grow in rosette-shaped groups (really sphèrulitic aggregates) and also in single crystals; this formation of rosette-shaped groups is perhaps partly due to the tendency to twin on the macrodome, the second type of twinning. These twins of the second type are the contact hemitrope twins on the macrodome (text figure 370) that have furnished the data for calculating the vertical axis $\dot{c}$. The angle of the bases in the two members of this twin is about 67°, but was not obtained within less than a degree. The prismatic crystals look like bundles of needles; they were seen crossing each other at definite angles of about 66° to 67° corresponding to the angle of the macrodome and probably represented the base and brachypinacoid, forming a pseudo-prism. Other prismatic groups were seen crossing at a definite angle of 83°; this would correspond to twins on the macrodome (403), and the angle of this macrodome on the base would be 41° 30′.

Pleochroism on the base of the tabular crystals is not noticeable. On edge it is more definite, but the pleochroism is weak. Colors are: $\mathfrak{a}$ rather strong red; $\mathfrak{b}=\mathfrak{c}$, deep red; but they are difficult to observe on account of the deeply colored plasma. The double refraction is weak on the flat, and often, in this position, the crystals are singly refracting, owing to the tendency to mimetic twinning which makes them appear uniaxial. This tendency to mimetic twinning is stronger near the edges of the plate, and in the center the double refraction is generally noticeable. Probably the edge is a true mimetic twin by rearrangement of the crystal molecules, while the center has not been so much affected. In plane polarized light, the extinction is symmetrical on the base and straight in edge views. In convergent light, a simple uniaxial cross is seen in many cases near the edge of the plate; but the biaxial figure usually shows in the central position, with the brushes close together, and $2E=15°$ about. The orientation of the elasticity axes is $\mathfrak{a}=\dot{c}$, $\mathfrak{b}=b$, $\mathfrak{c}=a$. The plane of the optic axes is the brachypinacoid (010), the acute bisectrix is the axis of greatest elasticity, $Bx_{\mathfrak{a}}=\mathfrak{a}$, and the optical character is hence *negative.*

### Yellow Baboon, *Papio babuin*. Plate 96.

Blood of the yellow baboon was sent to us at different times from the Philadelphia Zoölogical Gardens and from the National Zoölogical Park at Washington. From the latter source two specimens of blood were received, one of which was quite fresh; the others were rather putrid when examined. The stale bloods were laked by repeated freezing and thawing, the laked blood centrifugalized, and from the clear solution obtained the slides were prepared. The sample of fresh blood was ether-laked, centrifugalized for 3 hours, and preparations made as usual. The stale and putrid blood crystallized rather slowly, and the crystals first formed showed a tendency to dissolve in the solution. The preparations from the fresh blood crystallized rather readily at room temperature, but showed the same tendency to dissolve and appeared to be rather porous.

Two distinct kinds of oxyhemoglobin were observed, besides rods of reduced hemoglobin that were only partly examined. The two kinds of oxyhemoglobin recorded are the $\alpha$-oxyhemoglobin and the $\beta$-oxyhemoglobin of the genus *Papio*. The third kind of oxyhemoglobin, seen in *Papio sphinx* and *Papio anubis*, is probably represented by the $\gamma$-oxyhemoglobin recorded for this species. In the stale blood both $\alpha$-oxyhemoglobin and $\beta$-oxyhemoglobin were well developed, and it was in this stale blood that the reduced hemoglobin crystals were seen. The $\gamma$-oxyhemoglobin developed in the fresh blood.

#### *a-Oxyhemoglobin of Papio babuin.*

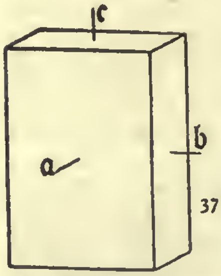


Fig. 371. *Papio babuin* $\alpha$-Oxyhemoglobin.

Orthorhombic: Axial ratio not determinable from the planes developed on the crystals.

Forms observed: The three pinacoids are the only forms present; macropinacoid (100), brachypinacoid (010), base (001).

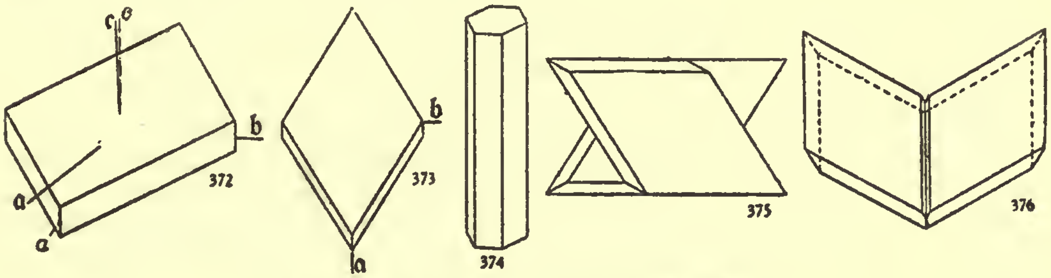
Angles: All of the angles between the pinacoids appear to be 90°, exactly, indicating the orthorhombic character.

Habit of the crystals, medium to thick tabular, or square prismatic, with square ends. The crystal seems to be the three pinacoids (100), (010), and (001) (text figure 371); sometimes one predominates, sometimes another. In the prismatic crystals that are the first to appear, the apparent prism is nearly square; in the later crystals it becomes flattened on the brachy-axis and the crystals become tabular on (100). In some cases, the crystals developed are practically cubes in form, the development being equidimensional. They occur singly and do not aggregate into regular groups of any kind, nor do they form twins.

Pleochroism is strong in some aspects and weak in others, so that the crystals, in ordinary light, appear light or dark red, according to the position in which they are viewed. The colors are: $\mathfrak{a}$ nearly colorless, pale yellowish-red; $\mathfrak{b}$ and $\mathfrak{c}$ nearly equal and deep scarlet-red. Double refraction is strong when the axis of greatest elasticity occurs in the section, but is weak when $\mathfrak{b}$ and $\mathfrak{c}$ are in the plane of the section. Extinction is straight with the pinacoid edges in all aspects. No interference figure was seen, but from the double refraction and the pleochroism the acute bisectrix should be the axis of greatest elasticity, $Bx_a = \mathfrak{a}$, and the optical character is *negative*. The orientation of the axes of elasticity may be taken as $\mathfrak{a} = a$, $\mathfrak{b} = b$, $\mathfrak{c} = \dot{c}$.

### *β-Oxyhemoglobin of Papio babuin.*

Monoclinic: Axial ratio $a : b : \dot{c} = 1.6808 : 1 : \dot{c}$; $\beta = 72°$ to 72° 30′.
Forms observed: Unit prism (110), orthopinacoid (100), base (001).
Angles: Traces of prism on base edges 110–001 ∧ 1$\bar{1}$0–001 = 60° 30′ actual angle; base to prism edge, or to orthopinacoid, 001 ∧ edge 110–1$\bar{1}$0 = 72° to 72° 30′ = $\beta$.



Figs. 372, 373, 374, 375, 376. *Papio babuin* β-Oxyhemoglobin.

Habit tabular on the base, usually rather thin, but also thick tabular (text figures 372 and 373); and, in some cases, becoming prismatic by development of (110). The tabular crystal consists of the unit prism (110) and base (001) only, unless the pseudo-plane (010) is formed by contact with slide and cover; but the prismatic crystal frequently develops also the orthopinacoid, (100), and then the combination is (110), (100), (001) (text figure 374); the crystal in this type of prismatic development becomes almost a hexagonal prism but with oblique ends. When the prism and base occur alone, in equilibrium, the form assumed is that of a regular rhombohedron.

Twinning is normal in this β-oxyhemoglobin; it follows the horse-type of twinning, in which the axis of the twin is normal to a prism-base edge, and the composition face is generally the base (text figure 375), but may be a plane normal to the base, and including the prism-base edge (text figure 376). The tabular crystals showing this twin, and with the base as composition face, become quite similar in appearance to the horse twin; they tend to form trillings or more complicated groups in the same way; but no very regular groups result, such as the six-rayed stars seen in twins of the oxyhemoglobin of *Papio sphinx*, the guinea baboon.

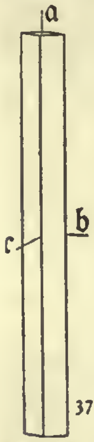
Pleochroism on plates is strong; $\mathfrak{a}$ colorless or nearly so, $\mathfrak{b}$ deep red, $\mathfrak{c}$ very deep red. Double refraction is strong; extinction is symmetrical on the plate, but oblique on the side view, looking along the symmetry axis. The extinction angle was not exactly obtained, but ran near 12° to 14°. Calling it the mean 13°, the orientation of the elasticity axes becomes $\mathfrak{a} \wedge a = 13°$, in the obtuse angle ; $\mathfrak{b} = b$; $\mathfrak{c} \wedge \dot{c} = 4° 30'$ in the obtuse angle. In the twins, positions on edge are often seen, in which the two extinctions are nearly symmetrical with the composition plane, and they then run near 8° to 10°. The interference figure was not observed. Apparently the acute bisectrix is the axis of least elasticity, $Bx_a = \mathfrak{c}$. This would make the optical character *positive*.

### *γ-Oxyhemoglobin of Papio babuin.*

Orthorhombic: Axial ratio $a : b : \dot{c} = 0.5317 : 1 : \dot{c}$, or $\frac{3}{2}$ (0.3544) : 1 : $\dot{c}$.
Forms observed: Unit prism (110), base (001).
Angles: Prism angle 110 ∧ 1$\bar{1}$0 = 56° (about); prism to base 110 ∧ 001 = 90° (about).

Habit long prismatic along the vertical axis, usually terminated by the base (text figure 377); but the crystals very soluble and the ends usually lost by solution. The crystals had to be observed in the cold; but, even with this precaution, satisfactory measurements of the terminal plane with respect to the prism were not obtained. The crystals grew singly or in loose aggregates. Twins were not observed. Some of the crystals seemed to show an oblique terminal plane, but, as stated, the ends dissolved so rapidly on slightly warming the slide that this was not confirmed.

Pleochroism was marked; $\mathfrak{a}$ nearly colorless, $\mathfrak{b}$ rose pink, $\mathfrak{c}$ deep red. Extinction was straight in all aspects. The orientation of the elasticity axes is $\mathfrak{a} = \dot{c}$, $\mathfrak{b} = b$, $\mathfrak{c} = a$. On the cross-sections, a biaxial figure with slightly separated brushes was seen, looking along the axis of greatest elasticity. The acute bisectrix of the optic axes is hence the axis of greatest elasticity, $Bx_a = \mathfrak{a}$, and the optical character is *negative*. This may be the prism of *a*-oxyhemoglobin, but the optical characters do not agree very well. It corresponds quite well with the *γ*-oxyhemoglobin of *P. sphinx* and *P. anubis* if the prism here developed is taken as (230).



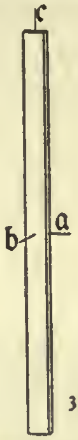


Fig. 377. *Papio babuin* γ-Oxyhemoglobin.
Fig. 378. *Papio leucophæus* Oxyhemoglobin.

### *Reduced Hemoglobin of Papio babuin.*

The crystals of reduced hemoglobin were prismatic with a nearly square cross-section, and they were sometimes seen as tabular crystals. They were not well developed, and their characters were not definitely made out. They recall the reduced-hemoglobin crystals in man.

### Drill, *Papio leucophæus*. Plate 96.

The specimen of blood was received from the Philadelphia Zoölogical Gardens, and was somewhat putrid and contained extraneous matters. It had been collected in oxalate in one of our collecting tubes. The blood was laked with ether and centrifugalized, and from the clear solution thus obtained the slide preparations were made. Fine needle-like crystals began to form soon after the slides were covered. The slides were kept overnight at a temperature near freezing, and on examination in the morning only the needle-like crystals were found. They began to dissolve when the slides were taken into a warm room, and dissolved or melted rapidly on the stage of the photomicroscope. The crystals were oxyhemoglobin. Owing to the fact that they dissolve so readily on slight increase of temperature, the crystallographic data are very incomplete.

### *a-Oxyhemoglobin of Papio leucophæus.*

Orthorhombic: Axial ratio not determinable with the crystals examined.

Forms observed: Macropinacoid (100), brachypinacoid (010), brachydome (011) (?), base (001) (?).

Angles: No satisfactory angles were obtained.

Habit prismatic on the vertical axis (text figure 378), the apparent prism consisting of the two vertical pinacoids and the termination, in most of the crystals examined, being formed of corrosion planes only. The crystals grow in divergent tufts and in irregular tufted aggregates; the ratio of length to width is about 30 : 1 on the broader side, and about 75 : 1 on the narrow edge view. When the ends become dissolved away they present a rounded or, in some cases, a wedge-shaped appearance, but no measurable planes are formed.

Pleochroism is moderate; $\mathfrak{a}$ pale reddish to colorless, $\mathfrak{b}$ and $\mathfrak{c}$ near together and deeper rose-red. Double refraction is easily observed, and the extinction is straight in all aspects that could be examined, namely, on the flat and edge views of the apparent prism. The orientation of the elasticity axes is $\mathfrak{a} = b$; $\mathfrak{b} = a$; $\mathfrak{c} = \dot{c}$; as well as it could be made out. Apparently the axis of greatest elasticity is the acute bisectrix, judging from the pleochroism. If this is correct the optical character is *negative*, $Bx_a = \mathfrak{a}$.

While this substance is only partially investigated, it seems probable that it is a first stage in the crystallization of the *a*-oxyhemoglobin of the baboons.

### GUINEA BABOON, *Papio sphinx*. Plate 97.

The specimen was received from the National Zoölogical Park at Washington, District of Columbia, and was largely clotted. The blood was rubbed in sand with addition of ether. The ground mass was centrifugalized, obtaining a clear solution from which the slide preparations were made. Crystals of α-oxyhemoglobin formed at first; but were soon dissolved and replaced by crystals of β-oxyhemoglobin; and these in turn finally gave place (in part) to γ-oxyhemoglobin; these three corresponding to the three kinds of oxyhemoglobin observed in other species of baboons. Unfortunately, only the γ-oxyhemoglobin was photographed, and the α-oxyhemoglobin, which disappeared soon after it was formed, was not fully examined crystallographically. The other modifications were fully examined, and quite complete data obtained in regard to them.

#### *α-Oxyhemoglobin of Papio sphinx.*

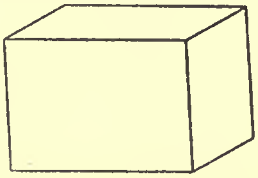


FIG. 379. *Papio sphinx* α-Oxyhemoglobin.

Orthorhombic: no axial ratio determinable from the crystals.

Forms observed: Macropinacoid (100), brachypinacoid (010), base (001).

Angles: The angles of the pinacoids, taken over the pinacoid edges, measured 90° in each case.

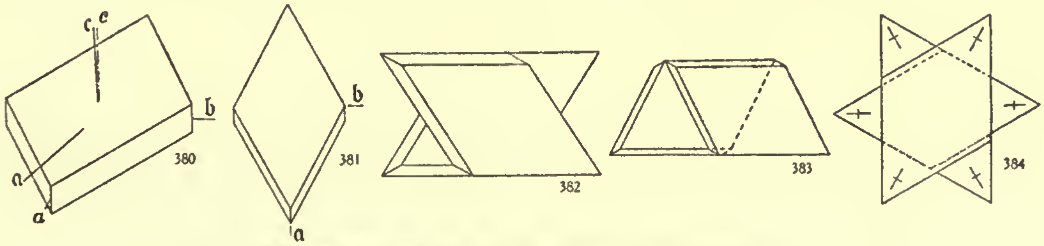
Habit square tabular to almost cubic (text figure 379), generally relatively thick tabular crystals. As the optical characters were not obtained, the principal plane in the tabular crystal can not be determined for comparison with other species of baboons. The crystals occurred singly and appeared along the protein ring, as well as through the body of the slides. They were dissolved as the β-oxyhemoglobin crystals formed, and did not persist until the optical characters could be determined. They were, however, pleochroic; and probably corresponded closely to this type of crystal as seen in the long-armed baboon and the chacma.

#### *β-Oxyhemoglobin of Papio sphinx.*

Monoclinic: Axial ratio $a : b : \dot{c} = 1.8418 : 1 : \dot{c}$; $\beta$ = about 70°, but not measured exactly.

Forms observed: Unit prism (110), base (001).

Angles: Prism angle, traces of the prism on base, edges 110–001 ∧ 1$\bar{1}$0–001 = 57° actual angle. The angle β was only approximately determined as about 70°.



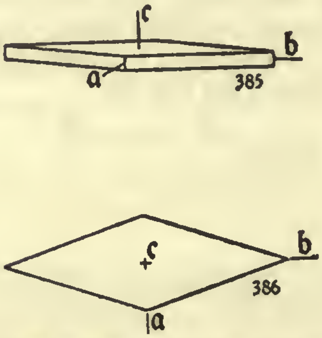
FIGS. 380, 381, 382, 383, 384. *Papio sphinx* β-Oxyhemoglobin.

Habit tabular on the base; the crystal consisting of the unit prism cut by the base (text figures 380 and 381). Some more prismatic crystals were observed, and these showed the habit of developing on one pair of prismatic planes until they became tabular in that respect. In the normal crystals tabular on the base, the length of the prism, as compared with its long diagonal, showed a ratio of about 1 : 5. Twinning is common, following the horse-type of twin, as described in the case of the yellow baboon. In this twin, where the twin-axis is a normal to a common prism-base edge, the two parts generally match exactly on that edge, so that the group is trapezoidal in outline, without reëntrant angles (text figure 382). But in many cases the two members of the twin overlap each other symmetrically and produce symmetrical reëntrant angles (text figure 383). Trillings produced by three individuals in this position were observed. In these, the composition plane instead of being the base, as is usual in this type of twin, becomes the plane normal to the base, which includes the common prism-base edge; there is thus produced a symmetrical six-pointed star composed of six sections in which the orientation in opposite sectors is the same (text figure 384). In polarized light this became very evident, the opposite sectors extinguishing simultaneously.

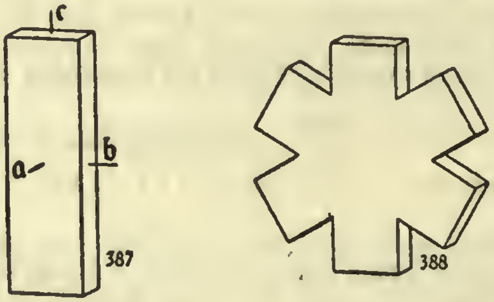
Pleochroism is quite marked; $\mathfrak{a}$ pale yellowish-red, nearly colorless, $\mathfrak{b}$ rather strong scarlet-red, $\mathfrak{c}$ deep red. The extinction is symmetrical on the base and oblique on edge views of the tabular crystal, unless they are in the zone of the orthopinacoid-base. The extinction, when looking along the symmetry axis, is about 14°, measured from the profile of the base. The orientation of the elasticity axes is $\mathfrak{a} \wedge a = 14°$ in the obtuse angle; $\mathfrak{b} = b$; $\mathfrak{c} \wedge \acute{c} = 6°$, in the obtuse angle (taking $\beta$ at 70°; it is probable this angle is somewhat less and $\beta$ a little more than 70°). The interference figure was not observed. The double refraction and the character of the pleochroism appear to indicate the axis of greatest elasticity as the acute bisectrix of the optic axes, $Bx_a = \mathfrak{a}$, and this would make the optical character *negative.*

### *γ-Oxyhemoglobin of Papio sphinx.*

Orthorhombic: Axial ratio $a : b : \acute{c} = 0.3346 : 1 : \acute{c}$.
Forms observed: Unit prism (110), base (001).
Angles: Unit prism angle $110 \wedge 1\bar{1}0 = 37°$; prism to base $110 \wedge 001 = 90°$.



Figs. 385, 386. *Papio sphinx* γ-Oxyhemoglobin.



Figs. 387, 388. *Papio langheldi* α-Oxyhemoglobin.

Habit thin tabular, the crystal consisting of the very long diamond-shaped section of the prism, cut by the base (text figures 385 and 386). The crystals grow from the protein ring and from the cover edge in tufts, attached by one end of the macro-axis; the groups of crystals are divergent or radiating, and the individual crystals are very thin. They did not appear to form definite twins. These crystals, when they begin to appear, develop in great numbers, so that the protein ring and cover edge become fringed with them. They appear to be more soluble than the $\beta$-crystals.

Pleochroism was not so strong as in the $\beta$-oxyhemoglobin. The colors were : $\mathfrak{a}$ pale yellowish-red, $\mathfrak{b}$ deeper red, $\mathfrak{c}$ rather deep red. Extinction is symmetrical on the basal section and straight on all edge views observed. The orientation of the elasticity axes appears to be $\mathfrak{a} = a$, $\mathfrak{b} = b$, $\mathfrak{c} = \acute{c}$. No interference figure was observed; but, from the indications of the pleochroism and double refraction, it would seem probable that the axis of greatest elasticity is the acute bisectrix, $Bx_a = \mathfrak{a}$, and the optical character would then be *negative.*

### LONG-ARMED BABOON, *Papio langheldi*. Plates 97 and 98.

The specimen was received from the New York Zoölogical Park. The blood was clotted and rather putrid. The clots were ground in sand and etherized, the resulting mixture was centrifugalized, and from the clear solution the slide preparations were made. Crystals formed readily and were at first the α-oxyhemoglobin of the genus *Papio*; later, crystals of the β-oxyhemoglobin appeared. The first crystals to appear (α-oxyhemoglobin) showed a tendency to dissolve, and they gradually disappeared as the β-crystals developed. Both were in the slides at the same time when the crystals first formed, but inside of 4 hours after the preparations were made the β-crystals greatly predominated. These β-crystals were more permanent than the α-crystals and kept for several days.

#### *α-Oxyhemoglobin of Papio langheldi.*

Orthorhombic: No axial ratio determinable from the single crystals, $a : b : \acute{c} = a : 1 : 0.543$ from twins.

Forms observed: The three pinacoids (100) (010) (001) in the single crystals, and (011) in twins.

Angles: The angles between the pinacoids were measured as 90°; from twins, $010 \wedge 011 = 61^\circ\, 30'$.

Habit tabular on the macropinacoid and elongated on the vertical axis (text figure 387), taking the large plane as the macropinacoid. They appear to be laminated, parallel to the large face (100); and, on this macropinacoid face, the crystal is marked with lines parallel to the two edges of the plates, due to lamination by parallel growth; on edge views, looking along the axis $b$ the lamination is also visible, the crystal being striated vertically. All cross-sections are rectangular. The lamination of the crystal produced a micaceous structure, and the crystal parted, or cleaved, parallel to the macropinacoid. The crystals grew singly, or massed together into irregular aggregates; but were also found in parallel growths of the plates, piled upon each other on the macropinacoid. Some of the groups showed interpenetrant and contact twins on the brachydome, making an angle of about 60°; or a six-pointed star, when the twin included both dome faces (text figure 388). The best measurements (which were not very good) gave the angle between the brachypinacoid and the brachydome as 61° 30′, making the value of $\acute{c} = 0.543$ or the ratio of $b : \acute{c} = 1 : 0.543$.

Pleochroism strong; $\mathfrak{a}$ colorless, $\mathfrak{b}$ old-rose red, $\mathfrak{c}$ deep red; the colors of $\mathfrak{b}$ and $\mathfrak{c}$ being nearly of the same depth, in sections of equal thickness. Double refraction is strong when $\mathfrak{a}$ is in the section. Extinction is straight in all aspects. Looking along $\mathfrak{a}$, a biaxial interference figure is seen, with the brushes not very widely separated. The orientation of the elasticity axes is $\mathfrak{a} = b$, $\mathfrak{b} = \acute{c}$, $\mathfrak{c} = a$. The acute bisectrix of the optic axes is the axis of greatest elasticity, $Bx_a = \mathfrak{a}$, and the optical character is *negative*.

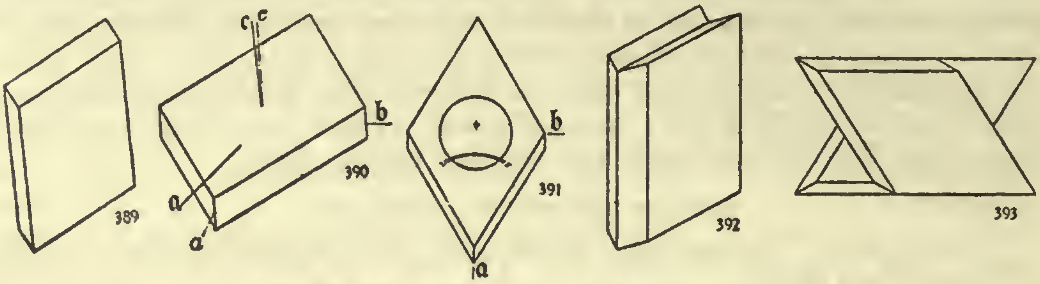
#### *β-Oxyhemoglobin of Papio langheldi.*

Monoclinic: Axial ratio $a : b : \acute{c} = 1.655 : 1 : \acute{c}$; $\beta = 70^\circ\, 30'$.

Forms observed: Unit prism (110), base (001).

Angles: Prism angle, traces of the edges $110\text{–}001 \wedge 1\bar{1}0\text{–}001 = 62^\circ\, 16'$; prism edge to base, edge $110\text{–}1\bar{1}0 \wedge 001 = 70^\circ\, 30'$; in twins, the dihedral angle of the twin edges, corresponding to the above prism edges, is 39°.

Habit tabular, the crystals sometimes flattened on a prism face and elongated on the vertical axis (text figure 389), and sometimes flattened on the base and symmetrically developed on the prism (text figures 390 and 391). The flattening on the prism face gives the unsymmetrical crystals a triclinic appearance, but the symmetrical crystals show distinctly the monoclinic character. The crystals grow singly and in irregular groupings; very frequently twinning on the large plane developed, whether the prism or the base. Contact twins on the prism are common (text figure 392); also twins of the horse-type on the base (text figure 393). The crystals are of all forms of the combination of prism and base; equidimensional, elongated on the prism or vertical axis, and flattened in this direction or tabular on the base. Cleavage is prismatic. These crystals appear later than those of the α-oxyhemoglobin and seem to develop at the expense of the latter, but both were seen together. The β-crystals begin as plates, flattened on the base, but grow into equidimensional blocks or short prismatic crystals resembling rhombohedra; or, by suppression of one prism face which is attached to the slide or cover, they become unsymmetrically developed on the prism.



FIGS. 389, 390, 391, 392, 393. *Papio langheldi* β-Oxyhemoglobin.

Pleochroism is rather strong; $\mathfrak{a}$ pale yellowish-red, $\mathfrak{b}$ rather strong scarlet-red, $\mathfrak{c}$ deep red. Double refraction strong; on basal sections the extinction is symmetrical; on edge views, looking along the ortho-axis, the extinction is 13° from the basal trace in the obtuse angle. The orientation of the elasticity axes is $\mathfrak{a} \wedge a = 13°$ in the obtuse angle; $\mathfrak{b} = b$; $\mathfrak{c} \wedge \dot{c} = 6° 30'$, in the obtuse angle. On the base, one brush of an interference figure is seen with traces of the other, the brushes widely separated. The acute bisectrix of the optic axes is the axis of least elasticity, $Bx_a = \mathfrak{c}$, and the optical character is *positive*.

The γ-oxyhemoglobin, observed in *P. babuin*, *P. sphinx*, and *P. anubis*, did not appear in the preparations from the blood of this species.

### CHACMA, *Papio porcarius*. Plates 98 and 99.

The specimen was received from the National Zoölogical Park at Washington, District of Columbia. Slide preparations of the blood were made in the usual way. The crystals formed readily and were not dissolved, but kept in good condition for more than a month, gradually passing by paramorphous change into methemoglobin, but without loss of form or of sharpness of angles. The first crystals to form were the α-oxyhemoglobin plates; these were soon followed by the β-oxyhemoglobin, which developed into very large crystals and groups, and became the predominant crystal; but the two forms appeared together in the same slide. The third form, γ-oxyhemoglobin, seen in some other species of *Papio*, did not develop in this species.

#### *α-Oxyhemoglobin of Papio porcarius.*

Orthorhombic: Axial ratio not determinable.

Forms observed: Macropinacoid (100), brachypinacoid (010), base (001).

Angles: The angles between the pinacoids were measured and gave 90° in each case.

Habit tabular, or long or short prismatic; the crystal consisting of the three pinacoids only. Small crystals are square tables, or, by becoming equidimensional, they look like cubes; others are elongated square prisms. The larger crystals are usually tabular. Taking the orientation of the axes, not from the laminated habit, but on the basis of the arrangement of the elasticity axes adopted with *Papio langheldi*, the crystals become tabular on the basal pinacoid (text figure 394). The tabular face (001) shows growth lines meeting at right angles and, in many cases, distinct piling of the crystals into groups, united on this face, the groups being parallel growths. Where the parallel growth becomes marked in this way the outline becomes irregular and the composite character of the crystal is apparent (plate 98, fig. 588). The crystals growing together on this flat face (001) often develop into radiating or fan-shaped aggregates, perhaps due to a tendency to twin on the brachydome, but actual twins of this kind were not observed.

Pleochroism in some positions is strong, in others is scarcely noticeable; $\mathfrak{a}$ pale yellowish-red, $\mathfrak{b} = \mathfrak{c}$ deep red. Double refraction is strong when $\mathfrak{a}$ is in the section; extinction is straight in all aspects. In a thick crystal looking along $\mathfrak{a}$ the biaxial figure was observed. The orientation of the elasticity axes is $\mathfrak{a} = b$, $\mathfrak{b} = \acute{c}$, $\mathfrak{c} = a$. The acute bisectrix of the optic axes is the axis of greatest elasticity, $Bx_a = \mathfrak{a}$, and the optical character is *negative.*

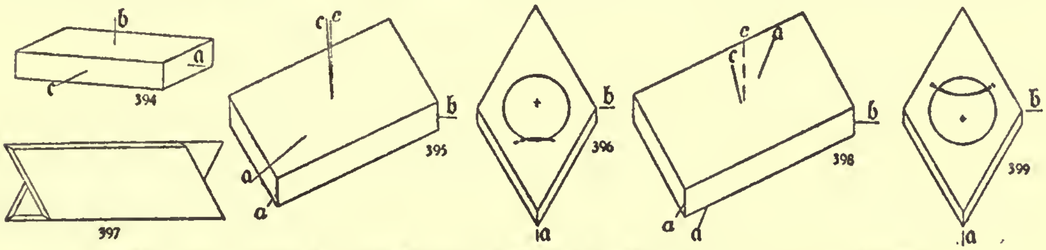


FIG. 394. *Papio porcarius* α-Oxyhemoglobin. FIGS. 395, 396, 397. *Papio porcarius* β-Oxyhemoglobin. FIGS. 398, 399. *Papio porcarius* β-Methemoglobin.

### *β-Oxyhemoglobin of Papio porcarius.*

Monoclinic: Axial ratio $a : b : \acute{c} = 1.732 : 1 : \acute{c}$; $\beta = 75°$.

Forms observed: Prism (110), base (001).

Angles: Prism angle, traces of the prism on the base, edges $110\text{–}001 \wedge 1\bar{1}0\text{–}001 = 60°$ (measurements ran from 59° 30′ to 60° 30′ and the average of the series was 60° 2′); angle $\beta$, measured from the prism edge to the base, edge $110\text{–}1\bar{1}0 \wedge 001 = 75°$. (But compare the $\beta$-methemoglobin angle $\beta$ which was 72° 30′.)

Habit tabular on the base in the simple crystals, the tabular crystals rather thin, and generally rather symmetrically developed (text figures 395 and 396), or with two larger and two smaller prism faces. The thickness was from one-fifth to one-eighth of the long diagonal of the plate. The plates aggregate into parallel growths, by extending along the ortho-axis (plate 99, fig. 592); and also form twins of the horse-type, the composite crystal elongating along the direction of the common prism-base edge (text figure 397). More irregular aggregates are formed, showing radiating and arborescent groupings.

Pleochroism is strong; $\mathfrak{a}$ pale yellowish, $\mathfrak{b}$ scarlet, $\mathfrak{c}$ deep red. Double refraction strong; extinction on the base is symmetrical; on the edge view, looking along the ortho-axis, the extinction angle is 10° from the trace of the base. The orientation of the optic axes is $\mathfrak{a} \wedge a = 10°$, in the obtuse angle; $\mathfrak{b} = b$; $\mathfrak{c} \wedge \acute{c} = 5°$, in the obtuse angle. On the basal section one brush of the interference figure is seen, unsymmetrical. On sections that are nearly normal to $\acute{c}$ the two brushes are seen, the angle $2E$ being about 110°. The acute bisectrix of the optic axes is the axis of least elasticity, $Bx_a = \mathfrak{c}$, and the optical character is hence *positive.* No crystals of $\gamma$-oxyhemoglobin were observed in this species.

### *a-Methemoglobin of Papio porcarius.*

Orthorhombic: No axial ratio determinable.

Forms observed: Macropinacoid 100, brachypinacoid (010), base (001).

Angles: The angles between the pinacoids were measured as 90° in each case.

Habit the same as described under *a*-oxyhemoglobin, which may alter into this methemoglobin by paramorphism.

The optical characters are different from those of the *a*-oxyhemoglobin. Pleochroism is very strong: $\mathfrak{a}$ pale yellowish, with a slightly greenish tinge; $\mathfrak{b} = \mathfrak{c}$ deep brown. In all sections that show $\mathfrak{a}$ the double refraction is very strong, in those showing only $\mathfrak{b}$ and $\mathfrak{c}$ it is weak; the same is true of the pleochroism. The interference figure is seen looking along $\mathfrak{c}$ and the traces of it may be seen looking along $\mathfrak{a}$ also. Looking along $\mathfrak{c}$

the two brushes are observed, widely separated; the angle $2E$ was measured as 105°. The acute bisectrix is evidently the axis of least elasticity, in spite of the indications of the pleochroism, $Bx_a = \mathfrak{c}$, and the optical character is hence *positive.*

### *β-Methemoglobin of Papio porcarius.*

Monoclinic: Axial ratio $a : b : \acute{c} = 1.720 : 1 : \acute{c}$; $\beta = 72° 30'$.

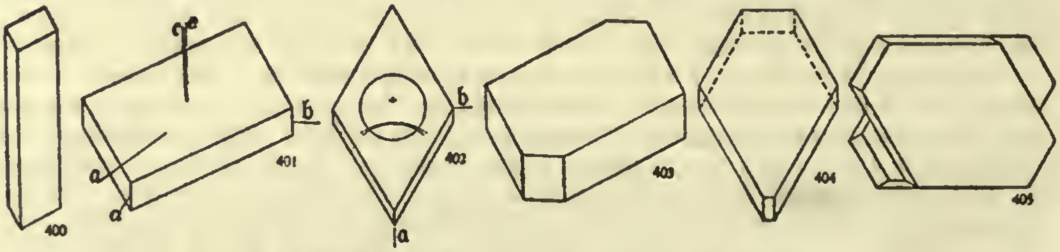
Forms observed: Unit prism (110), base (001).

Angles: Prism angle, traces of prism on base, edges $110\text{–}001 \wedge 1\bar{1}0\text{–}001 = 60° 20'$; prism edge to base, edge $110\text{–}1\bar{1}0 \wedge 001 = 72° 30' = \beta$.

Habit the same as that for the β-oxyhemoglobin, of which it may be a paramorph, but the angles appear to be slightly different. The optical characters differ from those of the β-oxyhemoglobin mainly in the colors and in the orientation of the elasticity axes. Pleochroism is strong; $\mathfrak{a}$ is nearly colorless, pale yellowish; $\mathfrak{b}$ and $\mathfrak{c}$ are nearly alike and deep brown, the absorption of $\mathfrak{c}$ being greater than that of $\mathfrak{b}$. The double refraction is strong. The orientation of the elasticity axes (text figures 398 and 399) is $\mathfrak{a} \wedge a = 15°$, in the acute angle; $\mathfrak{b} = b$; $\mathfrak{c} \wedge \acute{c} = 32° 30'$ in the obtuse angle. The interference figure is seen on the basal section, with one brush showing, and unsymmetrical. The angle between the optic axes was measured as about $80° = 2E$. The acute bisectrix of the optic axes is evidently the axis of least elasticity, $Bx_a = \mathfrak{c}$, and the optical character is hence *positive.*

### ANUBIS BABOON, *Papio anubis.* Plate 100.

The specimen of blood was received from the National Zoölogical Park at Washington, District of Columbia, and was putrid and clotted. The clot was ground in sand, etherized and centrifugalized, yielding a clear solution from which the slide preparations were made. Inside of 24 hours the crystals were sufficiently well developed to photograph. The first crystals to form were the usual monoclinic type of the baboons, β-oxyhemoglobin; and later, as a second crop, the γ-oxyhemoglobin appeared. The α-oxyhemoglobin, which was observed in *Papio babuin, P. langheldi, P. porcarius,* etc., did not make its appearance in this specimen. Only crystals of oxyhemoglobin were observed, no reduced hemoglobin or methemoglobin crystals developed.



FIGS. 400, 401, 402, 403, 404, 405. *Papio anubis* β-Oxyhemoglobin.

### *β-Oxyhemoglobin of Papio anubis.*

Monoclinic: Axial ratio $1.737 : 1 : \acute{c}$; $\beta = 71° 30'$.

Forms observed: Prism (110), orthopinacoid (100), base (001).

Angles: Prism angle, traces of prism on base, edges $110\text{–}001 \wedge 1\bar{1}0\text{–}001 = 59° 49'$, average; prism edge to base or angle $\beta = 71° 30'$.

Habit of crystals thin to thick tabular or prismatic; the smaller crystals are usually prismatic with a length on $\acute{c}$ equal to $2a$ (text figure 400) or even longer; the larger crystals are usually rather thick tabular with a thickness, in the direction of $\acute{c}$, of one-fourth of $a$ (text figures 401 and 402). But many of the larger crystals are well developed in the prismatic zone. The usual crystal is the prism-base combination; and, in the prismatic habit of crystal, these are the only forms present; but, in the tabular type, the orthopinacoid (100) is frequently present also (text figure 403), usually with one of its faces larger than the other and giving a rather unsymmetrical aspect to the crystal (text figure 404). Twins of the horse-type are rather common (text figure 405); but, as the plates are thick, the twin does not have the usual appearance. It is often seen in edge view only; when seen on the flat, it is usually more obviously an overlap than in the case of the horse.

Pleochroism is strong; $\mathfrak{a}$ colorless, $\mathfrak{b}$ deep red, about the color of the plasma in a moderately thick slide; $\mathfrak{c}$ deep red, of a deeper shade than the plasma. Double refraction is strong in all ordinary aspects. Orientation of the elasticity axes is $\mathfrak{a} \wedge a = 13°$, in the obtuse angle; $\mathfrak{b} = b$; $\mathfrak{c} \wedge \dot{c} = 5° 30'$, in the obtuse angle. On the base, in convergent light, an interference figure is seen, with the optic axes lying in the plane of symmetry. The acute bisectrix is the axis of least elasticity, $Bx_a = \mathfrak{c}$, and the optical character is hence *positive*. The angle of the optic axes is wide, $2E = 75°$ to $80°$. Dispersion of the optic axes is red > violet.

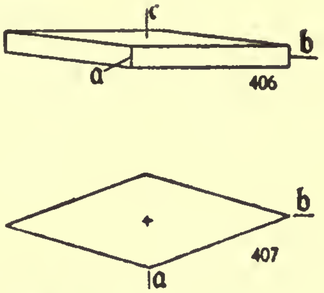
### *γ-Oxyhemoglobin of Papio anubis.*

Orthorhombic: Axial ratio $a : b : \dot{c} = 0.3268 : 1 : \dot{c}$.

Forms observed: Unit prism (110), base (001).

Angles: Prism angle $110 \wedge 1\bar{1}0 = 36° 12'$ average; prism to base $110 \wedge 001 = 90°$.

These crystals appeared as a "second crop" about three days after the first preparations were made. Habit thin tabular, consisting of the very oblique prism cut by the base (text figures 406 and 407), and with a thickness of plate of one-fifteenth to one-twentieth of the length of the $b$-axis. The crystals grow in tufts, often radiating, and occasionally are seen isolated; they are very thin and show scarcely any difference in the color from the solution when viewing a single crystal on the basal aspect. They do not appear to twin. They developed usually around the edge of the protein ring.



FIGS. 406, 407. *Papio anubis* γ-Oxyhemoglobin.

On account of the thinness of the plates, pleochroism is not very noticeable on the basal aspect, on edge view it is stronger; the colors are shades of the OHb red, with $\mathfrak{a}$ the palest color and $\mathfrak{c}$ the deepest. Double refraction is easily noted and extinction is straight on the edge view and symmetrical on the base. The orientation of the optic axes is $\mathfrak{a} = b$; $\mathfrak{b} = a$; $\mathfrak{c} = \dot{c}$. The plane of the optic axes is the macropinacoid. In convergent light no definite interference figure could be observed in the basal aspect, which was the only observable direction. It would appear probable, therefore, that the acute bisectrix is the axis of greatest elasticity, $Bx_a = \mathfrak{a}$, and the optical character is *negative*.

TABLE 49.—*β-Oxyhemoglobins of the baboons, genus Papio; monoclinic.*

| Name of species. | Axial ratio. | Angle β. | Prism angle. | Optical character. | Forms observed, etc. |
|---|---|---|---|---|---|
| | | | ° ′ | | |
| Papio babuin | 1.6808 : 1 : ċ | 72°–72° 30′ | 118 30 | Positive | (110), (100), (001). |
| Papio leucophæus | ........... | .......... | ....... | ........ | (No satisfactory data). |
| Papio sphinx | 1.8418 : 1 : ċ | 70° | 123 0 | Negative | (110), (001). |
| Papio langheldi | 1.655 : 1 : ċ | 70° 30′ | 117 44 | Positive | (110), (001). |
| Papio porcarius | 1.732 : 1 : ċ | 75° | 120 0 | Do. | (110), (001). |
| Papio porcarius (MHb) | 1.720 : 1 : ċ | 72° 30′ | 119 40 | Do. | (110), (001). |
| Papio anubis | 1.737 : 1 : ċ | 71° 30′ | 120 11 | Do. | (110), (100), (001). |

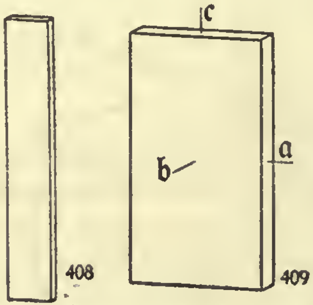
## MAN, *Homo sapiens africanus.*

The specimen in which crystals of oxyhemoglobin were successfully developed was from a negro woman. The blood was clotted, being obtained from an afterbirth. The clot was ground in sand and etherized, then centrifugalized and treated as usual. The drops were evaporated until granular oxyhemoglobin formed in the protein ring; and then, after covering, set aside in the cold to crystallize. Crystallization began after 24 hours in the cold, apparently at the expense of the granular oxyhemoglobin that had separated; the first crystals to appear were rather imperfectly formed, but later well-shaped plates were observed. The general type of crystals is that of the $\alpha$-oxyhemoglobin, as seen in the genus *Papio.*

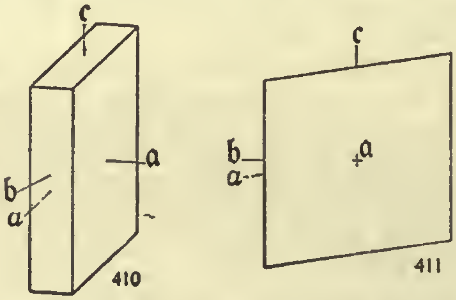
In another experiment on human blood a few imperfect crystals of oxyhemoglobin were obtained that agreed well in type with the $\gamma$-oxyhemoglobin crystals of the genus *Papio.* Crystals of reduced hemoglobin are readily obtained in human blood, and have been described, more or less exactly, by a number of observers.

### *a-Oxyhemoglobin of Homo sapiens africanus.*

Orthorhombic: Axial ratio not determinable from the faces that are developed.
Forms: Macropinacoid (100), brachypinacoid (010), base (001).
Angles: All angles between edges or between planes are 90°.



FIGS. 408, 409. *Homo sapiens africanus* a-Oxyhemoglobin.



FIGS. 410 411. *Homo sapiens africanus* Reduced hemoglobin

Habit, at first long lath-shaped (text figure 408); growing singly or in more or less closely aggregated bundles, with the large faces in contact; the smaller crystals are shreddy, with imperfect ends; and the ends are sometimes rounded as though due to erosion. Later larger and broader plates develop, which are quite well formed (text figure 409). These also showed the tendency to pile up on the large face (100); and on edge views, looking along *b* for instance, the lamination is quite marked. No planes except the pinacoids were developed, which is the rule in *Papio* also. No twins were observed.

The crystals are so thin that pleochroism is not noticeable, but the double refraction is fairly strong, and the relative elasticities can readily be observed. Extinction is straight in all aspects. The orientation of the elasticity axes is $\mathfrak{a} = b$; $\mathfrak{b} = a$; $\mathfrak{c} = c$. The elasticity of $\mathfrak{b}$ and $\mathfrak{c}$ is nearly the same; hence the acute bisectrix is probably the axis of greatest elasticity, $Bx_a = \mathfrak{a}$, and the optical character is *positive.* In convergent light, on the macropinacoid, or looking along $\mathfrak{b}$, no trace of the interference figure was seen; this was the only aspect in which the convergent light could be used.

In another experiment, in which the preparation was made from corpuscles washed with neutral saline and separated by centrifugalizing, then laked with ether, etherized and centrifugalized again, and the covers not sealed with balsam, crystals of oxyhemoglobin developed after two days in the cold, at a temperature of 0° C. These crystals were long, acicular, tapering to a sharp point at the ends, and they occurred in parallel and radiating groups. They polarize light and extinguish straight, showing greater elasticity normal to the length. In some cases the groups of crystals or the imperfect crystals showed a form that closely resembled elongated rhombic plates, very much like the $\gamma$-oxyhemoglobin crystals of the genus *Papio.* The angle of the plates was about 28°, but they were very imperfect, and no trustworthy measurements could be made. These crystals dissolved at room temperature, but rather slowly; the examination could be carried on with a low power for 20 minutes before the melting became very noticeable. Under a high power the acicular rods showed rounded ends, evidently indicating incipient solution. No satisfactory optical characters, beyond the straight extinction, were observed; the solution was so deeply colored, and so near the color of the crystals, that the pleochroism was not observable.

### *Reduced hemoglobin of Homo sapiens africanus.*

Monoclinic: Axial ratio not determined.

Forms observed: Clinopinacoid (010), orthopinacoid (100), base (001).

Angles: Orthopinacoid to base, $100 \wedge 001 = 81° 30' =$ angle $\beta$; clinopinacoid to base $010 \wedge 001 = 90°$.

Habit tabular on the clinopinacoid or plane of symmetry (text figures 410 and 411); the tabular crystals almost rectangular plates. The crystals show a ratio of thickness to length of the rhomboidal edge of about 1 : 5. The crystals formed rather sparingly in the slides in which the oxyhemoglobin crystals had been observed, also in other slides prepared from stale human blood, and in slides of fresh blood that had been kept sealed for a number of days. In each case the crystals were of the same type, the squarish tabular crystals above described. The short prisms and rhombic plates described as orthorhombic by von Lang with 54° 6′ prism angle, and the orthorhombic crystals with prism angle of 73° to 73° 35′ as described by Funke were not observed. The crystals were often so large as to be interfered with in their growth by the slide, but showed a tendency to aggregate into groups, so that the entire outlines of the individual crystals could not be made out in many cases.

Pleochroism is rather strong; $\mathfrak{a}$ nearly colorless to pale purplish, $\mathfrak{b}$ dark purplish red, $\mathfrak{c}$ dark red. Double refraction is strong; the extinction of the plate on edge views is straight in all aspects; and on the flat view, or the plane of symmetry, the extinction is nearly or quite parallel to one edge of the plate, but it makes an acute angle with the other edge. Orientation of the elasticity axes is $\mathfrak{a} = b$; $\mathfrak{b} \wedge a = 8° 30'$, in the acute angle; $\mathfrak{c} \wedge \acute{c} = 0°$. The interference figure was not observed.

TABLE 50.—*Crystallographic characters of the hemoglobins of the Primates.*

| Name of species. | Axial ratio. | Prism angle. | Angle β. | Extinction angle. | Optical character. | System. | Substance. |
|---|---|---|---|---|---|---|---|
| Lemuridæ: | | ° ′ | ° ′ | | | | |
| Lemur catta | 0.5832 : 1 : 0.3860 | 60 30 | 90 0 | 0° | Negative | Orthorhombic | OHb. |
| Cercopithecidæ: | | | | | | | |
| Papio babuin | ................ | ..... | 90 0 | 0° | Do. | Do. | α-OHb. |
| Do. | 1.6801 : 1 : ć | 61 30* | 72 0 | $\mathfrak{a} \wedge a = 13°$ | Positive | Monoclinic | β-OHb. |
| Do. | 0.5317 : 1 : ć | 56 0 | 90 0 | 0° | Negative | Orthorhombic | γ-OHb. |
| Papio leucophæus | ................ | ..... | 90 0 | 0° | Do. | Do. | α-OHb. |
| Papio sphinx | ................ | ..... | 90 0 | 0° | ........ | Do. | α-OHb. |
| Do. | 1.8418 : 1 : ć | 57 0* | 70 0 | $\mathfrak{a} \wedge a = 14°$ | Negative | Monoclinic | β-OHb. |
| Do. | 0.3346 : 1 : ć | 37 0 | 90 0 | 0° | Do. | Orthorhombic | γ-OHb. |
| Papio langheldi | a : 1 : 0.543 | ..... | 90 0 | 0° | Do. | Do. | α-OHb. |
| Do. | 1.655 : 1 : ć | 62 16* | 70 30 | $\mathfrak{a} \wedge a = 13°$ | Positive | Monoclinic | β-OHb. |
| Papio porcarius | ................ | ..... | 90 0 | 0° | Negative | Orthorhombic | α-OHb. |
| Do. | 1.732 : 1 : ć | 60* | 75 0 | $\mathfrak{a} \wedge a = 10°$ | Positive | Monoclinic | β-OHb. |
| Do. | ................ | ..... | 90 0 | 0° | Do. | Orthorhombic | α-MHb. |
| Do. | 1.720 : 1 : ć | 60 20* | 72 30 | $\mathfrak{a} \wedge a = 15°$ | Do. | Monoclinic | β-MHb. |
| Papio anubis | 1.737 : 1 : ć | 59 49* | 71 30 | $\mathfrak{a} \wedge a = 13°$ | Do. | Do. | β-OHb. |
| Do. | 0.3268 : 1 : ć | 36 12 | 90 0 | 0° | Negative | Orthorhombic | γ-OHb. |
| Homo sapiens africanus | ................ | ..... | 90 0 | 0° | Positive | Do. | α-OHb. |
| Do. | ................ | ..... | 90 0 | 0° | ........ | Do. | γ-OHb. |
| Do. | ................ | ..... | 81 30 | $\mathfrak{b} \wedge a = 8° 30'$ | ± Negative | Monoclinic | Hb. |

* Traces of the prism on base.

# CHAPTER XIX.

## SUMMARY AND CONCLUSIONS.

### MODE OF PREPARATION OF HEMOGLOBINS.

*The hemoglobins from different species examined were so prepared as to be strictly comparable:*

In order that the crystals examined should be as nearly comparable with each other as possible, a uniform method of procedure was adopted in their preparation, which, as described under the methods of preparation used in this research (page 141), consisted in *adding oxalate* to the blood when collected to prevent coagulation, *laking* the blood with ether to free the hemoglobins, *centrifugalizing* to separate extraneous matters and get a clear solution, and *crystallizing* on slides under covers sealed with Canada balsam. This method of procedure was followed whenever it was possible to do so, but it had to be modified in many cases to meet the individual requirements of the specimen. The hemoglobins vary so much in solubility, often even in the same genus, that when but one preparation could be made, owing to the small amount of blood, it might happen that it was not always crystallized at the best dilution for the blood of that particular species. Still, by endeavoring to obtain the best condition of dilution, and following a constant method of procedure, it was possible in most cases to obtain a series of products that were strictly comparable.

The specimens were received from various sources, mostly from the Zoölogical Gardens, and were very variable as to quantity and condition of the blood. In the majority of cases the blood was obtained when the *post mortem* of the animal was made, and as this was usually hours, and in some cases days, after death, many of the specimens were of clotted or partially clotted blood that was removed from the heart and larger vessels when the animal was examined; in some cases they were diluted with serum or lymph; and frequently they were in a more or less putrid condition. Generally, the blood was partly changed to reduced hemoglobin, but shaking with oxygen or air would usually convert this to oxyhemoglobin; or simply the exposure to the air, during the evaporation of the drops of prepared blood on the slide, in most cases permitted the introduction of enough oxygen to make the blood yield crystals of oxyhemoglobin. In cases when the blood had become putrid by exposure to air, the hemoglobin was frequently partly changed to the acid form (metoxyhemoglobin), and it crystallized as such in the slide preparations. The blood was usually liquid, sometimes partly or entirely clotted. The clots had to be broken up, and concentrated solutions were usually obtained in such cases. When the specimen was liquid, it sometimes was mainly serum or lymph, and in such cases the preparations were dilute solutions.

In the great majority of cases only one preparation could be made, owing to the smallness of the specimen, and in unknown bloods in such cases the proper dilution to obtain the best results was not always arrived at. Hence, it frequently happened that the crystals were not formed under the best conditions of dilution. When a sufficient quantity was available for making several preparations, the blood was crystallized at different dilutions, in order that the one most favorable for normal crystallization should be used in the preparation that was employed in the crystallographic examination. In general the whole blood was used when but a single preparation could be made. When the quantity of blood was sufficient, or its condition was such as to allow of a separation of the corpuscles, we often made preparations of the corpuscles as well as of the whole blood. The most concentrated solutions were obtained by separating the corpuscles by centrifugalization, and using these without any added plasma or other fluid. The whole blood gave a solution of medium concentration. The addition of plasma or serum to the whole blood, gave dilute solutions. For the very soluble forms of the hemoglobins, the corpuscles alone were used; for those of moderate solubility, the whole blood was taken; while for the more insoluble hemoglobins the blood was preferably diluted with the plasma or egg-white. In one or another of these various ways the conditions most favorable to the formation of crystals were arrived at, and, as far as possible, all of the bloods examined were studied when crystallized under the most favorable conditions. They were in general, therefore, strictly comparable. Crystallization under different degrees of dilution, and under different temperatures, alters crystal habit rather than form, and even when the preparations were not made under the most favorable conditions for the formation of typical crystals, the crystallographic characters were not altered, so far as the system, the axial ratio, and the optical characters were concerned. The crystals were kept under examination as long as possible, in order that any different forms of the hemoglobins that might develop could be studied. The specificity of the hemoglobins of a genus was often seen in such different forms of hemoglobin being found to run through a series of species.

### THE DIFFERENT KINDS OF HEMOGLOBINS FOUND IN THE BLOODS.

In most cases it was possible to produce crystals of oxyhemoglobin in the bloods examined, but sometimes oxyhemoglobin did not crystallize and some of the other hemoglobins were observed. These were metoxyhemoglobin, reduced hemoglobin, and methemoglobin. In many cases when a sufficient supply of blood was available, all these substances could be prepared and examined in one blood. In some cases, especially when the oxyhemoglobin was rather soluble, it was found advantageous to make carbonmonoxide hemoglobin in order to obtain better crystals for examination. In several cases in the account of our experiments this substance has been described, but it was also prepared and examined in a number of instances which have not been mentioned in the above descriptions. The substance called metoxyhemoglobin is, as pointed out by Menzies (Journal of Physiology, 1894, XVII, 402), the substance usually described as "methemoglobin," but pure methemoglobin, as Menzies shows, has quite a different spectrum. The material that we have described as methemoglobin gave the spectrum of the substance described by Menzies as "pure methemoglobin." He is inclined to regard the metoxyhemoglobin as a mixture of methemoglobin and oxyhemoglobin, but as it crystallizes in a different form from either, in the same blood, it probably should be regarded as a separate substance. It may be identical with the substance that has been described as "acid hemoglobin." It is converted into oxyhemoglobin by the addition of a little alkali, such as ammonia, but exposure to air does not change it to oxyhemoglobin; on the contrary, exposure of oxyhemoglobin to air converts it into this metoxyhemoglobin. It seems to be a condition of the hemoglobin in which the oxygen is held more closely than in oxyhemoglobin, a sort of "resting stage" of the hemoglobin, in which it is inactive as either an oxidizing or a reducing agent.

It was found in the case of many species that the fresh blood would first crystallize in one form of oxyhemoglobin; that later a second crop of crystals would appear having a totally different habit and even crystal system, or, in other words, different constitution; and that sometimes this would be succeeded by a third crop having a still different form. Many examples of this dimorphism or trimorphism are recorded in the foregoing descriptions of species. They are distinguished as $\alpha$-oxyhemoglobin, $\beta$-oxyhemoglobin, $\gamma$-oxyhemoglobin, etc. For instance, in the baboons three distinct crops of oxyhemoglobin crystals developed: (1) tabular or lath-shaped orthorhombic crystals; (2) short prismatic to tabular monoclinic crystals; and (3) tabular orthorhombic crystals. It is possible that (1) and (3) may be the same substance, but very unlikely; (2) is evidently a different substance. In the same genus two forms of methemoglobin were observed, one orthorhombic and one monoclinic, corresponding to the $\alpha$- and $\beta$-oxyhemoglobins, but varying from them in angles and axial ratio. Among the rodents, two forms of oxyhemoglobin were observed in a number of species; in some cases these may be really the same substance, as will be explained later on; in others, they are evidently different substances. Thus, in the blood of the domestic rabbit, an orthorhombic and a monoclinic form of oxyhemoglobin are found that are evidently different; and the same is true of the blood of the capybara, porcupine, and ground-hog. Indeed, in the blood of this last species, *Marmota monax*, three forms of oxyhemoglobin develop: (1) $\alpha$-oxyhemoglobin, hexagonal; (2) $\beta$-oxyhemoglobin, orthorhombic; and (3) $\gamma$-oxyhemoglobin, monoclinic; but two of these may possibly be the same substance. Two kinds of oxyhemoglobin were observed in the blood of the jaguar, *Felis onca*, and in the puma, *Felis concolor*; and two forms of reduced hemoglobin were found in the blood of the lynx, *Lynx canadensis*. In the jaguar and puma the two kinds are, one orthorhombic, and the other, isometric or pseudo-isometric; in the lynx, the two kinds are orthorhombic and tetragonal.

In several of the ungulates two forms of oxyhemoglobin or CO-hemoglobin were observed. In the horse and mule the oxyhemoglobin crystallizes (1) in orthorhombic prisms and (2) in monoclinic tabular crystals; the CO-hemoglobin is also dimorphous and crystallizes in the same respective systems. Among the antelopes, the blood of the duickerbok, *Cephalophus grimmi,* gives two forms of oxyhemoglobin, α-oxyhemoglobin which crystallizes in the tetragonal system, and β-oxyhemoglobin which crystallizes in the hexagonal system.

The marsupials show the same tendency to form several kinds of hemoglobins. Thus the opossum blood furnished an α-oxyhemoglobin, monoclinic, and a β-oxyhemoglobin, hexagonal or pseudo-hexagonal. The CO-hemoglobin of the opossum is also dimorphous, crystallizing in the monoclinic and hexagonal systems in forms analogous to the oxyhemoglobin. The oxyhemoglobin of the Tasmanian wolf, *Thylacynus cynocephalus,* is dimorphous, the α-oxyhemoglobin is monoclinic, and the β-oxyhemoglobin is isometric. Other examples of dimorphism or trimorphism of the hemoglobins will be found in the detailed descriptions of species. The occurrence of these different kinds of crystals of these substances in the same species demonstrates the possibility of the presence normally of two or more kinds of oxyhemoglobin, reduced hemoglobin, etc., in the same blood.

It has been generally stated that the oxyhemoglobin, reduced hemoglobin, methemoglobin, etc., of any given species crystallize in the same form, but a glance at the tables of the crystallographic characters of the different species examined will show that this is not always the case. The mistake has arisen in several ways. In the first place, crystals of oxyhemoglobin may be converted by paramorphous change into metoxyhemoglobin (which has generally been described as methemoglobin) and into reduced hemoglobin, without alteration of angles. Such altered crystals are analogous to the paramorphs and pseudomorphs observed in minerals. Then the above-mentioned confounding of metoxyhemoglobin with methemoglobin (both of which substances we have observed in the same species) has led to a further confusion. The metoxyhemoglobin of a given species generally crystallizes in forms that are near those observed in the oxyhemoglobin, the angular differences being such that exact measurements of the angles are often necessary to show the differences in form of the two substances; but often, while the angles may approach each other in the two substances, the optical characters may be quite different.

That the oxyhemoglobin, reduced hemoglobin, metoxyhemoglobin, and methemoglobin in one species may differ in crystallization can be illustrated by many examples from the bloods examined. But in order that the crystals shall differ in form, they must not be paramorphs, but must be crystallized *de novo* from solution. These four substances were observed in the blood of the shad, *Alosa sapidissima,* with the following characters: *Oxyhemoglobin,* monoclinic, axial ratio $1.804 : 1 : \dot{c}$, $\beta = 68°$, and an extinction angle $\mathrm{a} \wedge a = 6°$; *metoxyhemoglobin,* monoclinic, axial ratio $1.786 : 1 : \dot{c}$, $\beta = 70°$, with an extinction angle of $\mathrm{a} \wedge a = 10°$; *reduced hemoglobin,* monoclinic, axial ratio $1.786 : 1 : \dot{c}$, $\beta = 70°$, as in the metoxyhemoglobin, but with an extinction angle $\mathrm{a} \wedge a = 14°$; *methemoglobin,* hexagonal, axial ratio not determinable, but with straight extinction.

When the CO-hemoglobin was examined it generally was found to approach the oxyhemoglobin in its crystallographic characters, but differences were usually to be noted. For example, in the horse the oxyhemoglobin and the CO-hemoglobin are both dimorphous, and the homologous forms are very nearly alike, as may be seen by comparing some of their characters:

$\alpha$-oxyhemoglobin, orthorhombic, axial ratio 0.7467 : 1 : 0.4097
$\alpha$-CO-hemoglobin, orthorhombic, axial ratio 0.7332 : 1 : 0.4106
$\beta$-oxyhemoglobin, monoclinic, axial ratio 1.600 : 1 : $\dot{c}$, $\beta = 72°$, $\alpha \wedge a = 13°$
$\beta$-CO-hemoglobin, monoclinic, axial ratio 1.664 : 1 : $\dot{c}$, $\beta = 68°$, $\alpha \wedge a = 15°$

Other examples of crystallographic differences in these five substances examined may be found by reference to the tabulations of crystallographic characters of the hemoglobins, given at the end of each chapter in which the hemoglobins of the species are described.

It will be seen therefore that not only is it possible for several different kinds of oxyhemoglobin, metoxyhemoglobin, reduced hemoglobin, CO-hemoglobin, and methemoglobin to occur in the same species, but that these five different substances may be distinguished from each other by crystallographic characters as well as by spectroscopic examination.

## SPECIFICITY IN GENERIC AND SPECIFIC CHARACTERS.

*Constancy of generic characters:*

The crystals of the species of any genus belong to the same crystallographic system and generally to the same crystallographic group; and they have approximately the same axial ratios, or their ratios are in simple relation with each other. In other words, *the hemoglobin crystals of any genus are isomorphous.* In some cases this isomorphism may be extended to include several genera, but this is not usually the case, unless, as in the case of the dogs and foxes for example, the genera are very closely related. Isomorphism implies, however, more than mere correspondence of crystal system and axial ratio. The members of an isomorphous group have approximately the same forms of structure; they should, as a consequence, have approximately the same constitution. Any of the genera that are represented by a number of species may be taken as examples of this isomorphism of the hemoglobin crystals from the blood of animals of the same genus. Such are, for example, the genera *Felis, Canis, Papio,* each of which is represented by a number of species, but to which may be added closely related genera, which by some zoölogists are united with these genera, as *Felis* and *Lynx, Canis* and *Vulpes* and *Urocyon.* Other genera in which a smaller number of species were examined show the same isomorphism, as for instance the genera *Mus, Sciurus, Cervus, Ovis,* etc. Where several kinds of oxyhemoglobin occur in one species of a genus they are to be looked for in other species of the same genus, and if the conditions are favorable they can presumably all be developed in each species. These genera have crystals that are isodimorphous or isotrimorphous, a more exact test of the generic specificity. For example, the genus *Papio* has three forms of oxyhemoglobin, and, as regards this substance, the hemoglobin crystals of this genus are isotrimorphous.

The oxyhemoglobins of the genus *Canis,* with those of the related genera *Vulpes* and *Urocyon,* form a very remarkable isomorphous group in which the variation in the axial ratio between the oxyhemoglobin crystals of the most widely separated species is hardly more than the variations that occur in the different varieties of the domestic dogs. In the crystals from the cats, also, we find an isomorphous group of an equally remarkable character. In this case either the oxyhemoglobin crystals or the reduced hemoglobin crystals may be compared, and even the oxyhemoglobin crystals with the reduced-hemoglobin crystals. But while they show axial ratios that closely resemble each other, the forms of the crystals that develop do not always look alike. In the cats, too, we see an instance, common among minerals, where different species have crystals that show different prisms or pyramids, which while having the same axial ratio do not have the same form, but are multiples the one of the other. Both the dogs and the cats have hemoglobins that crystallize in the orthorhombic system, but with very different axial ratios.

Perhaps a more remarkable instance of isomorphism is exhibited in the three species of bears examined, *Ursus americanus*, *Ursus maritimus*, and *Melursus ursinus*. These vary considerably in axial ratio, but the two species of *Ursus* are close in ratio of $a:b$ and in prism angle; all three, however, belong to the *monoclinic sphenoidal* or monoclinic hemimorphic group, and all three have a very remarkable habit of twinning on a prism of about 60°. The isomorphism, due to the differences of development of the crystals, sometimes appears to be only partial, but in the case of these three bears it will be seen that the extinction angles are all very nearly alike. Thus the angle $c \wedge a$, the extinction angle in the crystals of these three species, is 19° in the black bear and 20° in each of the others.

From this isomorphism of the crystals of any genus we may infer a correspondence of structure of the molecules of the homologous hemoglobins derived from that genus.

*Constancy and specificity of the crystallographic characters of individual species*:

The oxyhemoglobin obtained from the same blood crystallizes in the same form, with the same axial ratio, though often with different habit, when obtained by different methods of preparation. When several forms exist, each form, $\alpha$-oxyhemoglobin, $\beta$-oxyhemoglobin, etc., appears always in its own proper form and axial ratio when the bloods of different individuals of the species are examined. The same is true of the other hemoglobins—metoxyhemoglobin, reduced hemoglobin, methemoglobin; so that the hemoglobins of any species are definite substances for that species. But upon comparing the corresponding substances in different species of a genus it is generally found that they differ the one from the other to a greater or less degree; the differences being such that when complete crystallographic data are available the different species can be distinguished by these differences in their hemoglobins. As these hemoglobins crystallize in isomorphous series, the differences between the angles of the crystals of the species of a genus are not, as a rule, great; but they are as great as is usually found to be the case with minerals or chemical salts that belong to an isomorphous group. How much the crystals of the corresponding hemoglobins vary from each other in different species of a genus may be seen by reference to the tabulated crystallographic characters of the hemoglobins of different species given at the end of each chapter in the descriptions of species.

Good examples of these variations of the crystals from the different species of a genus may be seen by comparing the axial ratios of the crystals of $\alpha$-oxyhemoglobin of the *Felidæ* or the crystals of reduced hemoglobin of the same family, or the ratios of the crystals of $\beta$-oxyhemoglobin of the genus *Papio*. As already stated, the crystallographic characters of the hemoglobin crystals seem to indicate that in case of the *Canidæ* the separation of the old genus *Canis* into *Canis, Vulpes,* and *Urocyon* is perhaps not justified; but in the separation of the wild cat, *Lynx rufus*, from the genus *Felis* some support may be found in the characters of the crystals, although they return to the normal form of the cats in the lynx, *Lynx canadensis*.

The differences between the species are also shown in the habit of the crystals, and this is dependent in part at least upon the differences in solubility. The development of large crystals requires that the blood should be moderately soluble, but when it is rather insoluble only small crystals are liable to appear. Thus, in the four species of rats examined, two, the Norway rat and the white rat, their oxyhemoglobins being insoluble, normally produce only small crystals, while the black and Alexandrine rats, having a more soluble oxyhemoglobin, show much larger crystals. But the habit of certain planes developing in the crystals of some species and not in those of others, and the different forms of growth of the crystal, prismatic, tabular, etc., may produce very great differences in appearance of the crystals, even when there may not be much difference in their angles.

It is recognized that differences of crystal habit may depend upon differences of composition as well as concentration of the solution from which the crystals form, and, therefore, differences in substances that may exist in the blood plasma of different species could influence the habit of crystals in different species. In some cases the differences are mainly in the way the crystals aggregate, and in the way they are related to each other in the aggregates, and this would depend upon the rate of deposition and upon the composition of the solution. In many cases two or more habits of crystals of the same substance are recorded in one species, which differences seem to depend upon concentration of the solution and the pressure under which the crystals form.

The specific character of the crystals from any species may perhaps be best seen by referring to the photographic plates, where the above-mentioned differences and similarities of the rats, for instance, are well shown. The squirrels are another group in which the distinctions between the crystals depending upon habit are quite marked, but the form and other crystallographic characters are, of necessity, the same throughout the genus.

The crystals obtained from different species of a genus are characteristic of that species, but differ from those of other species of the genus in angles or axial ratio, in optical characters, and especially in those characters comprised under the general term of *crystal habit*, so that one species can usually be distinguished from another by its hemoglobin crystals. But these differences are not such as to preclude the crystals from all species of a genus being placed in an isomorphous series.

### GENERAL CRYSTALLOGRAPHIC CHARACTERS OF THE HEMOGLOBIN CRYSTALS.

*The constant recurrence of certain angles in the hemoglobin crystals of different species, even when the species are widely separated zoölogically and when their crystals belong to various systems:*

On examining the tabulations of the crystallographic characters given at the end of Chapters IX to XVIII, and comparing the prism angles there recorded, it will be noticed that the majority of these angles are found to approximate to a few angles or to lie in one of a few groups. The most common angles are those lying near 88°, 76°, 66°, and 60°. On examining these angles more carefully they can be grouped a little more exactly. A considerable number are exactly 60° and 90° (mainly of crystals of the hexagonal and tetragonal systems), but leaving these out of account for the moment, the rest (monoclinic and orthorhombic) may be arranged into groups that approximate 88°, 82°, 73°, 66°, 61°, 58°, and 36° to 37°. On averaging these groups, and comparing the average axial ratios that may be computed from them, they may all be reduced to a series of ratios; when it may be seen that the ratios computed from these angles stand in a simple relation to each other and form a series. From averaging of these angles in the groups near 88°, 76°, 66°, and 58°, in each of which groups there are many examples, a normal value for the ratio underlying the series which may be taken as of unity for the angles near 88°, the following average values for these angles may be computed: 88° 36′, 75° 56′, 66° 5′, and 58° 17′. These stand in simple relation with each other as follows: Calling the ratio from the angle 88° 36′ a ratio of 1:1, then the angle 75° 56′ gives the ratio 5:4; the angle 66° 5′ gives the ratio 3:2, and the angle 58° 17′ gives the ratio 7:4.

The series can readily be extended to include the other simple ratios, and table 51 gives the ratios that have been computed on the basis of the above mean angles, beginning with the angle 88° 36′ as having the ratio of 1:1. The figures from which the ratios are established are the cotangents of the semi-angles of the prisms, which are also added to the table. All of these ratios here tabulated, with the exception of those given in parentheses, are ratios of the fifth complexity series of Victor Goldschmidt, as derived by his Law of Complication:

TABLE 51.—*Simple ratios and mean angles computed from them for ratio of* 1 : 1=88° 63′.

| Simple ratios. | Prism angles. | | Cotangents of half angle. | Simple ratios. | Prism angles. | | Cotangents of half angle. |
|---|---|---|---|---|---|---|---|
| | ° | ′ | | | ° | ′ | |
| 1 : 1 | 88 | 36 | 1.0249 | (9 : 4) | (46 | 54) | (2.3055) |
| (9 : 8) | (81 | 52) | (1.1530) | 7 : 3 | 45 | 23 | 2.3914 |
| 5 : 4 | 75 | 56 | 1.2811 | 5 : 2 | 42 | 39 | 2.5617 |
| 4 : 3 | 72 | 24 | 1.3667 | 8 : 3 | 40 | 12 | 2.7331 |
| 7 : 5 | 69 | 45 | 1.4349 | 3 : 1 | 36 | 2 | 3.0742 |
| 3 : 2 | 66 | 5 | 1.5374 | 7 : 2 | 31 | 9 | 3.5867 |
| 8 : 5 | 62 | 46 | 1.6398 | 4 : 1 | 26 | 25 | 4.0991 |
| 5 : 3 | 60 | 41 | 1.7083 | 5 : 1 | 22 | 5 | 5.1240 |
| 7 : 4 | 58 | 17 | 1.8031 | (6 : 1) | (18 | 28) | 6.1489 |
| 2 : 1 | 52 | 1 | 2.0493 | | | | |

Angles that run close to these angles given under the column of prism angles, on the basis of 88° 36′ as the ratio 1:1, are found in the prism or dome angles that are recorded; and practically all of the angles that have been recorded lie near one of these points represented by the simple ratios given in the table.

A more exact method of comparison would be to compare first the angles of the species of one genus with those of the species of a related genus, and from these derive a similar table of mean angles, which would vary from the above recorded angles somewhat. As far as this has been done, as for the dogs and the cats for instance, it shows a closer correspondence of the simple ratios than would be seen by simply taking angles from the recorded angles in the tables of crystallographic characters at random and comparing them with those given in this table, which latter are, of course, only very approximate.

The fact that practically all angles of the crystals (except those which can not belong in this series, as 60°, 90°, 70° 30′) run so close to the computed angles for the series, indicates that all of the hemoglobins are members of an isomorphous series, an isopolymorphous series in fact, and the isomorphism is at least partial if not complete. As will be shown later, the isometric, tetragonal, and hexagonal crystals may be, and probably are in all cases, the mimetic twins of the orthorhombic and monoclinic crystals. These, in turn, are related in the same way by simple ratios with the triclinic hemin,* the angles of which, as we have found them in various species, are also members of the above series of angles as given in the column of prism angles for 88° 36′ = ratio 1 : 1. As far as this work of comparing the angles of the crystals from different species, and of different substances from the same species, has gone, it clearly indicates the partial or complete isomorphism of all the hemoglobins, and also their isomorphism with the triclinic hemin.

Such series, isodimorphous or isotrimorphous at least, are known among minerals. The Pyroxene-Amphibole group is such a one, in which orthorhombic, monoclinic, and triclinic minerals are found in two series which are related to each other by the simple ratio of 1:2; that is, calling the pyroxene ratio 1:1, then the amphibole ratio becomes 1:2. These minerals vary widely in chemical composition, and some are very complex while others are simple, but all may be related by this simple ratio, and all have substantially the same axial ratio. It is in this sense that the hemoglobins may be said to be isomorphous.

*Mimesie, and the angles of* 60° *and* 90° *in the crystals:*

It was stated above that crystals of the hemoglobins showing the angles 90°, 60°, etc., that did not fall into this table of angles, could still be seen to be related to the main series by considering that the angles might

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* During the past two years we have devised methods for preparing relatively large crystals of hemin, and we have examined the hemins of a number of species of animals and measured the crystals, of which we have about 350 negatives. From these records, the above conclusions as to the crystallographic relations of the hemoglobins and hemin have been arrived at. We are now engaged in an active study of hemins and expect to announce the results in the near future.

become 90°, 60°, etc., by mimetic twinning. Such mimesie is very commonly found in such polymorphous substances, whether the polymorphism be due to physical isomerism or to chemical isomerism, and is generally designated as polysymmetry. The polysymmetric crystals of hemoglobins appear in some cases to be the result of pseudosymmetry in the monoclinic and orthorhombic forms that produces a tendency to a form of twinning that favors such mimesie, and makes pseudo-tetragonal and pseudo-hexagonal crystals; or it may be due to the pseudosymmetry being of such a nature that externally applied force will cause a rearrangement of the structure, with the development of a higher grade of symmetry.

Many examples of this mimetic twinning or pseudosymmetry will be found in the descriptions of the hemoglobins. It is especially common in those groups which show normally the crystals of the hexagonal system, as, for example, in the rodents.

In the species of squirrel examined, the European red squirrel's crystals of oxyhemoglobin are seen to be orthorhombic with a prism angle of 60° and hexagonal (or pseudohexagonal) with, of course, the same angle of the prism. By twinning on the prism (really on the base as composition face, but with the prism-base edge as the common direction in the crystals), three of the orthorhombic crystals produce by their overlapping an essentially uniaxial structure. As the orthorhombic crystals have the hexagonal angle already, they naturally form hexagonal plates, which is the characteristic tendency among the rodents in general. Probably all of the squirrel crystals are thus twinned, when they show the characters of a uniaxial substance, or when they are "hexagonal."

A more striking example of such mimesie is seen in the case of the ground-hog, *Marmota monax*. The $\gamma$-oxyhemoglobin of the ground-hog is monoclinic with a prism angle of 58° and an angle $\beta$ near 90°. The twinning is of the ordinary "horse-type," fully described under horse and mule. Three such crystals twinned on each other produce again a uniaxial substance where the three overlap. The optical character is negative. The axis of least elasticity $\mathfrak{a}$ ($=Bx_a$) is within 10° of normal to the base. In the averaging of the three axes to one in the pseudo-hexagonal twin, the other axes $\mathfrak{b}$ and $\mathfrak{c}$ also average to a mean value, that of the ordinary ray of the hexagonal crystal, and the optical character of the composite crystal, considered as uniaxial, is also negative. Crystallizing *de novo* from the solution appear large, perfectly formed, hexagonal plates of $\alpha$-oxyhemoglobin, which can not be distinguished by any power of the microscope as not of homogeneous hexagonal texture. Their character is that of the mimetic crystal that should develop from such a monoclinic crystal with an angle of nearly 60° for its prism and an angle $\beta$ nearly 90°. Like the obviously composite crystals, the optical character is negative, but these show a perfect uniaxial figure throughout, which the other only does in the center of the overlapping group.

Similar examples are seen in the case of the rats, when an obviously composite hexagonal plate built up of orthorhombic individuals assumes a hexagonal form by a similar sort of twinning; these are the hexagonal plates that are to be seen in the photomicrographs of the rat crystals. In this case the habit of the crystal is tabular on two opposite prism faces of the orthorhombic crystal, and three of these overlapping each other form a hexagonal plate, as may be seen by reference to text figure 210. It only requires the substance of the composite to fill up the reentrant angles to make a perfect hexagonal plate.

In the crystals of many other groups of animals, similar development of angles of 60° on account of pseudo-hexagonal structure of the substances has been noted, and many examples of this kind of mimetic twinning will be found in the descriptions of the crystallography of the hemoglobin of the species.

The angle of 90° occurs in many groups as a characteristic angle of the crystals. Thus, in the birds, the *Anseres*, *Gallinæ* (with the exception of the guinea-fowl, which probably does not belong to this family), and *Columbæ* show probable examples of the formation of tetragonal crystals by the mimetic twinning of orthorhombic crystals with angles that approximate 90°, and in a few cases the mechanism of this twinning is apparent. The orthorhombic members are the whistling swan with an angle of prism of 88° (92°), the chicken with an angle of 87° (93°), the quail with an angle of 88° (92°), and the pigeon with an angle of 89° 10′ (90° 50′). In the goose and trumpeter swan the only crystals observed were tetragonal crystals with angles of 90°, but these were probably mimetic twins of an orthorhombic form. The same kind of twinning that has been described in the rodents as the "horse-type," in which the crystals grow together on the base with a common prism-base edge, seems to be the common form here. Averaging of the angles 88° and 92° or 87° and 93° produces a composite crystal of 90° prism angle, and at the same time the elasticities for light are averaged so that a mean uniaxial structure is produced. The crystals twin on the base, and when the twin lamellæ are thin, as they finally become, the twin structure finally becomes ultra-microscopic and the symmetry is tetragonal. Examples of this were seen especially in the crystals from the whistling swan and the pigeon.

Another way in which a higher grade of symmetry is simulated was noticed in several cases, but here in general the measurements of the angles were imperfect and the indications were therefore less certain. This kind of mimesie occurred where the angles of the crystal, orthorhombic for example, approximated those of a higher grade of symmetry, isometric for example. A prism and dome combination, with angles near 71°, growing under pressure would have the prism so shortened that the prism and dome come into equilibrium; then the crystal would appear to be an isometric octahedron. The pressure that shortened the prism would be acting upon the axis of greatest elasticity, and when equilibrium of form was reached an equilibrium in the optical elasticities might also be effected and the substance would appear isotropic. In this case no obvious twinning took place, but twinning under pressure may still be the explanation of the phenomenon. The crystals of the rats, especially of the Norway rat, showed this characteristic, but unfortunately the angles of the prism and dome were not obtained very accurately for this species.

There are evidently two ways in which a higher grade of symmetry may be attained in these crystals, first by *twinning,* producing hexagonal and tetragonal crystals from orthorhombic or monoclinic crystals, the hexagonal structure requiring at least three individuals forming a triplet, and generally having a very much larger number in the same orientation as the three of the triplet; the tetragonal structure requiring a doublet, and generally having a very much larger number of individuals, as in the case of the hexagonal structure. The second way in which a higher grade of symmetry is attained is by application of external pressures which neutralize the internal tensions and produce isometric or sometimes tetragonal structure. But when the mimetic crystal finally acquires a structure in which the twin lamellæ are ultra-microscopic, or the structure is so involved by the plates not being continuous planes through the crystal that complete averaging of the asymmetries takes place, it would seem possible that the structure *really changes* to that of the higher grade of symmetry. That this may take place in the molecule itself by change from one isomer to another is rendered probable by the observation that the apparently pseudosymmetric modification may develop *de novo* from the solution from which the more unsymmetrical modification has been crystallizing, and without the formation of any intermediate forms that are obviously twinned. This is more likely to occur in chemical isomers than in physical isomers, and would indicate that the apparently pseudosymmetric modifications were in many cases chemical isomers. In large molecules like those of the hemoglobins, *plasticity of the molecule* is very likely; moreover, there is no doubt from the recorded observations of the practical *plasticity of the crystal structure.*

The crystals of the hemoglobins form a general isomorphous series in which the members are related to each other by simple ratios. They may crystallize in any crystal system. Their elementary compositions may be various or they may be stereoisomers of the same centesimal composition, but all are connected by the common nucleus hemin, whose crystals show angles that belong in the same isomorphous series. By mimesie a higher grade of symmetry is attained, and perhaps this apparently mimetic substance may be an isomer of a parent substance.

## THE ZOÖLOGICAL APPLICATIONS OF THIS METHOD OF RESEARCH.

This method of studying the hemoglobins of various species of animals furnishes the systematic zoölogist a means of testing his findings in regard to the relationships of different species and genera, and should prove a valuable adjunct to the morphological data upon which he relies for establishing such relationships. The crystallographic characters that have been recorded in the descriptions of the hemoglobins of species, and especially the tabulations of them for the different groups of animals studied in this research, show, as has been pointed out, that the characters of the crystals of a genus are specific for each genus. The comparison of the crystals of related species or genera show how closely they may approach each other as regards this character.

If we take for example the genera *Canis, Vulpes,* and *Urocyon* of the family *Canidæ,* they will be found to be as closely related to each other in regard to the crystallography of their hemoglobins as are the species of a single genus in other cases; for example, as the crystals from the baboons of the genus *Papio* are to one another. Indeed, the dingo (*Canis dingo*) varies more from the normal dog type in regard to these characters than the species of the genus *Canis* in general vary from the species of *Vulpes* and *Urocyon.* Compare, for example, the axial ratios of the following species of *Canidæ* (table 52):

TABLE 52.—*Comparison of axial ratios of species of Canidæ.*

| Specific name. | Common name. | Axial ratio. |
|---|---|---|
| | | *a* *b* *ċ* |
| Canis familiaris | Common dog | 0.6745 : 1 : 0.2863 |
| Canis familiaris, var. | Chow dog | 0.6696 : 1 : 0.2878 |
| Canis lupus mexicanus | Wolf | 0.6576 : 1 : 0.2863 |
| Canis dingo | Dingo | 0.6009 : 1 : 0.2582 |
| Vulpes fulvus | Red fox | 0.6494 : 1 : 0.2824 |
| Urocyon cinereoargenteus | Gray fox | 0.6619 : 1 : 0.2809 |

An examination of these figures would seem to indicate that, in so far as the crystallographic characters of the hemoglobins are concerned, the *Canidæ* examined should all belong to one genus. The fact that they readily cross with each other is another argument in the same direction.

Similarly, in the genus *Felis* and the related genus *Lynx,* the very close relationship will be seen by a comparison of the crystallographic characters of the hemoglobin. Take, for instance, the reduced hemoglobin crystals of the cats and compare the axial ratios of this substance in the principal species of *Felis* with the two species of *Lynx* studied:

TABLE 53.

| Specific name. | Common name. | Axial ratio. |
|---|---|---|
| | | *a* *b* *ċ* |
| Felis leo | Lion. | 0.9742 : 1 : 0.3838 |
| Felis tigris. | Tiger | 0.9742 : 1 : 0.2838 |
| Felis bengalensis | Leopard-cat | 0.9657 : 1 : 0.3667 |
| Felis pardalis | Ocelot | 0.9489 : 1 : 0.3931 |
| Felis domestica | Cat | 0.9656 : 1 : 0.3839 |
| Lynx canadensis | Lynx | 0.9605 : 1 : 0.3944 |
| Lynx rufus | Wild cat | 0.9869 : 1 : 0.3914 |

Here again the correspondence is very close between the characters of the reduced hemoglobins of the two related genera, and they have moreover the same crystal habit as regards their oxyhemoglobin, which indicates that this substance in the two genera is nearly the same material.

If it is possible by an examination of such characters as the crystallography of common vital substances in related genera or species to establish systematic zoölogical relationships between them, it should be possible to use such characters to test phylogenetic relationships. Some examples of this sort of comparison of the crystallographic characters have already been cited. The relation of the seals with the otters and of the sea-lions with the bears, and probably the interrelation of the whole group, has already been pointed out. In this case the comparison of the axial ratios alone shows similarities, but the form of structure of the crystal and the crystal habit are even more important. Thus, the bear crystals all have a habit of twinning on a prism of 60° and the crystals of the sea-lion show the same habit, each forming trillings on such a prism, which do not resemble any other crystals observed. This "bear-type twin," in so far as our investigations have gone, is found only in the genera *Ursus*, *Melursus*, and *Otaria*. Comparing the axial ratios of the bears with that of the one species of sea-lion examined, we find that the correspondence is not complete, but it involves a $\frac{2}{3}$ and $\frac{3}{2}$ ratio, as follows:

TABLE 54.

| Specific name. | Common name. | Axial ratio. |
|---|---|---|
| | | *a* *b* *ć* |
| Ursus americanus | Black bear | 1.2239 : 1 : 1.1429 |
| Ursus maritimus | Polar bear | 1.2088 : 1 : *ć* |
| Melursus ursinus | Sloth bear | 1.2857 : 1 : 1.498 |
| Otaria gillespii | California sea-lion | $(\frac{3}{2})$1.2012 : 1 : $(\frac{2}{3})$1.1825 or |
| Do | Do | 1.8019 : 1 : 0.7883 |

The axial ratios with this 2 : 3 relation are not closer than those of some quite unrelated species, and perhaps are only an expression of the general isomorphism of all hemoglobins that has already been stated, but the fact that the crystals of the genera *Ursus*, *Melursus*, and *Otaria* are all *monoclinic sphenoidal*, a very uncommon crystal class in hemoglobins, indicates a close similarity in the forms of structure in the crystals of these genera; and this is perhaps a better test of relationship than even the habit of twinning. This crystal class, the monoclinic sphenoidal, was observed also in the hemoglobins of the earless seals, in the harbor seal, *Phoca vitulina*, and it perhaps may be the class to which the otter crystals belong, although they were recorded as *monoclinic domatic*. On comparing the crystals of the harbor seal with those of the otter by axial ratios, a fairly close correspondence is seen. The prism angles are identical, 79°, and the angle $\beta$ is 72° for the otter and 75° for the harbor seal. The vertical axes, however, stand approximately in a 7 : 4 ratio with each other, as may be seen by a comparison of the axial ratios:

TABLE 55.

| Specific name. | Common name. | Axial ratio. |
|---|---|---|
| | | *a* *b* *ć* |
| Phoca vitulina | Harbor seal | 1.2131 : 1 : 1.1970 |
| Lutra canadensis | Otter | 1.2131 : 1 : $(\frac{4}{7})$1.1889 |
| | | 1.2131 : 1 : 0.6794 |

That is to say, in the prismatic zone the isomorphism is exact, but in the zone including the base and orthodome the relation of the vertical axes is as 7 : 4.

Zoölogists commonly regard the dogs as being closely related to the bears, but in so far as a comparison of their hemoglobin crystals is concerned no close relation is indicated. The crystals of the dogs all belong to the orthorhombic system, normal group, while those of the bears, as above stated, are monoclinic sphenoidal, thus indicating very different forms of structure for the two. On the other hand, the bears appear to be related to the *Mustelidæ*, as well as to the *Otariidæ* and *Phocidæ*. So small a number of species of these groups have been examined that any positive statements of relationships are probably premature, but the above indicated relationships are certainly suggested by the crystals.

Among the birds certain anomalies were noted. The guinea-fowl is classed as belonging to the *Gallinæ*, but its blood crystals seem to be more nearly like those of the ostrich, which is placed in a separate subclass. The other two members of the *Gallinæ*, the chicken, *Gallus domestica*, and the quail, *Colinus virginianus*, have crystals that closely resemble each other. A comparison of the crystallographic characters of the hemoglobins of the four species above mentioned will make these relations clear:

Table 56.

| Specific name. | Common name. | Orthorhombic. | Prism angle. |
|---|---|---|---|
| | | $a:b:\grave{c}$ | ° |
| Gallus domestica | Chicken | 0.949 : 1 : $\grave{c}$ | 87 |
| Colinus virginianus | Quail | 0.9657 : 1 : $\grave{c}$ | 88 |
| Numida meleagris | Guinea-fowl | 0.554 : 1 : $\grave{c}$ | 58 |
| Struthio camelus | Ostrich | 0.5658 : 1 : $\grave{c}$ | 59 |

These figures perhaps rather indicate the *non-relationship* between the guinea-fowl and the chicken or the quail than the relationship between the guinea-fowl and the ostrich; although, as the ostrich is a bird in which the wingless character, which places it in a separate subclass, is evidently the result of degeneration, there is no reason to believe that it and the guinea-fowl may not have had a common ancestor.

On comparing the crystals of the few species of primates that were examined, a striking similarity may be seen between the α-oxyhemoglobin crystals of the baboons and those of man. The β-oxyhemoglobin crystals found in the baboons were not observed in the crystals from the human species, but the γ-oxyhemoglobin crystals of the baboons closely resemble the corresponding crystals observed in human blood. In this case the parallelism of the crystals from the two genera is shown in two kinds of oxyhemoglobin crystals.

By some zoölogists the bats have been placed among the primates, and the relationship of the two groups has been claimed by a number of zoölogists. The crystals of oxyhemoglobin from the brown bat do show a considerable resemblance to the oxyhemoglobin crystals of the genus *Papio*, but on the other hand the fruit-bat examined showed quite a different type of crystal.

A striking example of the application of this method of comparing the crystals of the hemoglobins of different species to demonstrate relationships is shown in the rats. The first species of rat examined was the domesticated white rat. Later, the Norway rat and the black and Alexandrine rats were also studied. It has been generally stated in the zoölogies that the white rat is an albino of the black rat. From our examination of its crystals it is evident that the white rat is closely related to the brown or Norway rat, but it can not be closely related to the black or Alexandrine rats. On the other hand, the black and Alexandrine rats are very closely related to each other, but are probably distinct varieties of the same species.

Unfortunately (for phylogenetic studies) the species examined were not as a rule those which might be regarded as forms of which the origin was very uncertain, and but few examples of the bearing of this method of research upon the tracing of the phylogeny of species are to be found in the list of species examined. Enough examples have, however, been enumerated to show the application of this method to the study of phylogenetic and zoölogical relationships and to demonstrate its value.

### THE INFLUENCE OF CERTAIN PHYSIOLOGICAL CONDITIONS UPON THE COMPOSITION AND COLORING MATTER OF THE BLOOD.

The normal coloring matter of vertebrate blood may be regarded as being a mixture of variable amounts of oxyhemoglobin, reduced hemoglobin, metoxyhemoglobin, methemoglobin, etc. The percentages of these substances vary with the activity or inactivity of the animal, with the amount of oxygen and carbon dioxide in the blood, and with the intensity of oxidation and other conditions. These in turn are conditioned, in part at least, by food and environment.

Hibernating animals seem to have a relatively large proportion of the hemoglobin in the form of metoxyhemoglobin, which seems to be an inert, resting stage of this substance, neither taking up nor giving off oxygen very readily. The bears, for example, show a large proportion of this substance in their blood, and the blood has a brownish color. In most of the rodents the oxyhemoglobin changes readily to metoxyhemoglobin upon standing, at least in the covered preparations. A similar condition is probably to be found in the shad during the breeding season, when the fish does not feed. The shad caught in our rivers during the spawning season show a large proportion of metoxyhemoglobin in their blood, if the blood is obtained from the living fish. It may, however, change to oxyhemoglobin in case of fish that have been exposed to the air in the market. As less oxygen is required for the physiological processes of the fish during this time when it is not feeding, it is reasonable to suppose that a part of the hemoglobin is converted into this more or less inert, modified form, during this period.

Among the cats, the American species show a much larger percentage of oxyhemoglobin in their blood, or at least crystals of this substance develop much more readily in their blood, than in the blood of the species of the Old World cats. In the lion, tiger, leopard-cat, and common cat of the Old World cats, the first crystals to appear are reduced hemoglobin, and the oxyhemoglobin is produced with some difficulty or by further oxidation of the blood. But in the wild cat, mountain-lion, jaguar, and the lynx the oxyhemoglobin crystals form readily as a first crop in the fresh blood.

This may be due to the fact that the cats of the New World in general lead a more active life than those of the Old World, or that they live in general in a cooler climate, and therefore use more oxygen than the Old World species.

The influence of food may be seen in the bloods of animals that are herbivorous as compared with those which are graminivorous. Among the rodents, the hares and rabbits are herbivorous, and their hemoglobins are rather soluble, being comparable in solubility to the hemoglobins of the herbivorous ungulates. The hemoglobins of the squirrels, on the other hand, which are rodents that live largely on seeds and nuts, are relatively much less soluble, while the hemoglobins of species of rodents that live on the leaves and roots of plants and also on seeds, such as the porcupine and the ground-hog, have hemoglobins that are intermediate between these two extremes. When we compare other animals, such as the gallinaceous birds, which live on seeds very largely, with the seed-eating rodents, we see, however, that the hemoglobins are as soluble or even more soluble than those of the hares and rabbits.

*The condition of the hemoglobins in the corpuscle:*

It would seem strange that with substances such as the hemoglobins, which in many cases are relatively rather insoluble, crystallization is not a common occurrence in the erythrocytes in the living animal or in freshly drawn blood. In fact, crystallization under these conditions rarely occurs, although it is common, judging from the records of various investigators, in blood that is not fresh. The question then arises, what keeps this substance, even in cases where it is readily crystallizable, from solution, in this *uncrystallized* form in the corpuscles? The typical condition of matter of definite composition is crystalline, but the typical condition of living matter is the amorphous condition, or, as it is generally called, the "colloidal" condition. In inorganic compounds and in non-living organic compounds amorphous and colloidal conditions can be maintained by preventing the composition from becoming definite, as by adding to it or withdrawing from it substances that would make it definite. To prevent glass from crystallizing, care must be taken to keep the fusion of a composition that does not approach too nearly to a definite silicate. In alloys, crystallization is prevented in much the same way by avoiding mixtures in which the ratio of the metals is a regular molecular ratio. In substances which crystallize with water of crystallization, an amorphous or colloidal condition can often be produced by not having enough water present to form the normal crystal.

The hemoglobin in the corpuscle is almost universally regarded as being in some way combined with the stroma of the corpuscle, and it seems to us probable that this union is in the nature of a compound comparable to an alloy or to glass in that it is of indefinite composition; or, that the hemoglobin may be held in the corpuscle in such a way that, owing to the osmotic properties of the stroma, there is a deficiency of the fluid of crystallization to form the definite compound that can crystallize. In either case the material does not crystallize because it is not of the proper composition to form crystals. By altering the osmotic conditions, as by changing the composition of the blood plasma by the introduction of a soluble salt, by dilution with water, etc., the amount of fluid in the corpuscles may be so changed that crystals may develop inside of the corpuscle. It is recognized that the amount of hemoglobin in the corpuscle is much too large in many cases for all to go in solution in the amount of liquid that may be obtained by breaking down the hypothetical union of the hemoglobin and stroma. In such a case the hemoglobin remains amorphous in the corpuscles, or is "colloidal," because it has not enough plasma to make up the amount of plasma of crystallization necessary for the formation of crystals. It can not form a hydrate of definite composition.

In the ordinary preparation of hemoglobin crystals on slides, according to the method already described, this colloidal form of the hemoglobin is produced in the dense protein ring which forms at the margin of the drops. Upon giving this ring the necessary amount of fluid to form crystals, they are at once produced in the readily crystallizable bloods, the entire ring being rapidly converted into a crystalline mass.

It seems probable therefore that hemoglobin does not crystallize in the corpuscle because of the osmotic properties of the stroma, which keep the solvent at too low a percentage in the corpuscle to allow of crystallization, while it does not crystallize from the plasma in the living animal because the solution is too dilute or the temperature too high for that dilution. The stroma may have a selective absorption for hemoglobin, which is equivalent to saying that hemoglobin forms a combination with the stroma.

1

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3

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1. Oxyhemoglobin of the Barndoor Skate (*Raia laevis*), showing sheaf-shaped crystal aggregates seen on edge.
2, 3. Same, seen in the flat aspect.
4. Same, showing twinning.
5, 6. Oxyhemoglobin of the Sturgeon (*Acipenser sturio*), showing brachypinacoid aspect.

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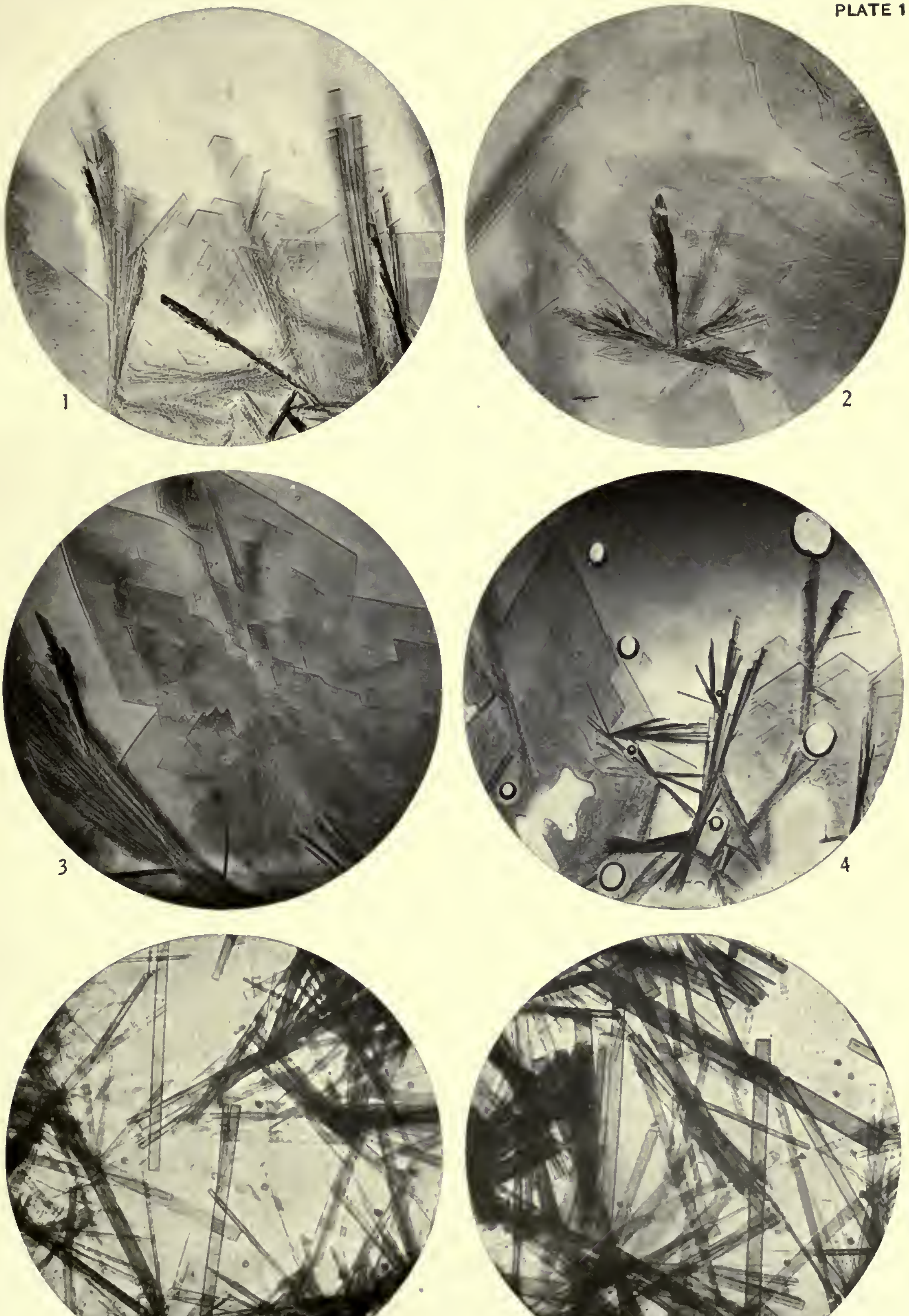
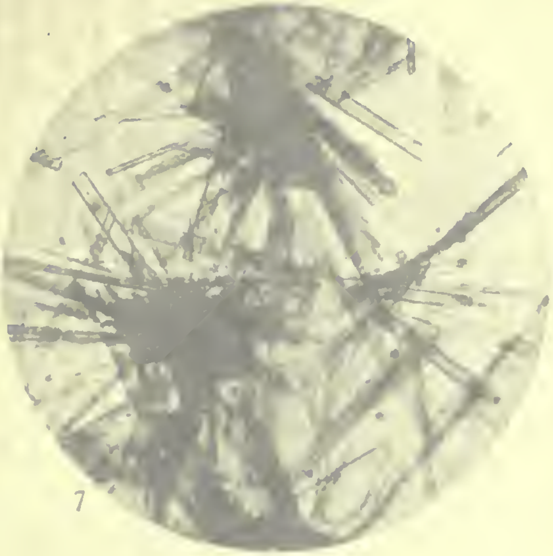
PLATE 1



1. Oxyhemoglobin of the barndoor Skate (*Raia lævis*), showing sheaf-shaped crystal aggregates seen on edge.
2, 3. Same, seen in the flat aspect.
4. Same, showing twinning.
5, 6. Oxyhemoglobin of the Sturgeon (*Acipenser sturio*), showing brachypinacoid aspect.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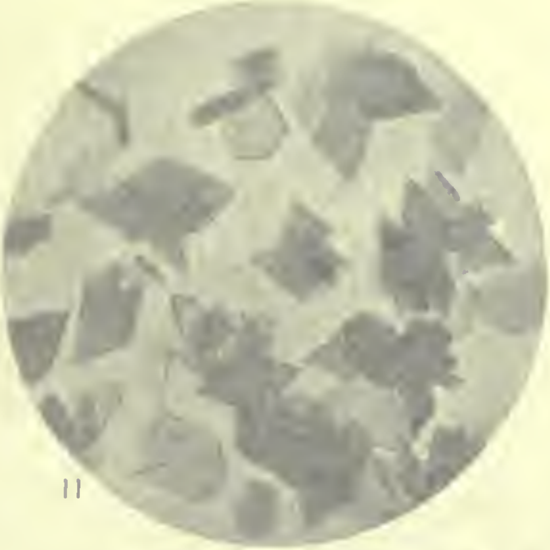
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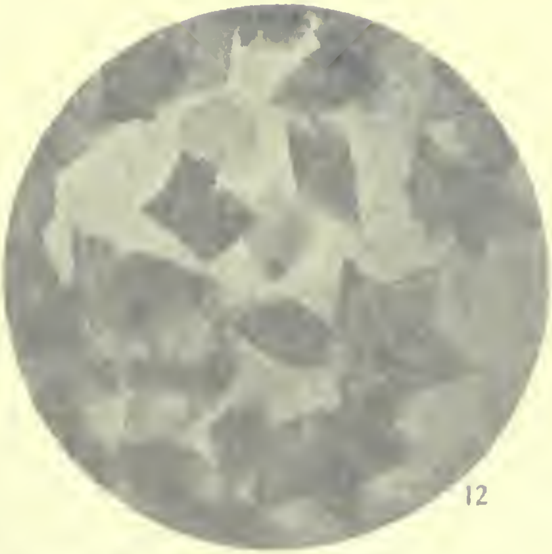
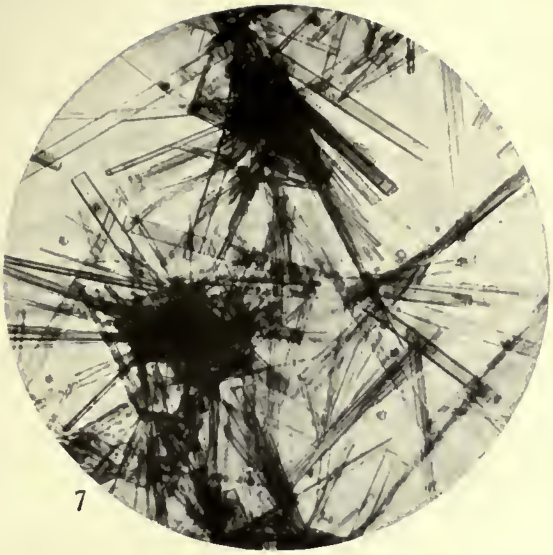

12

7. Oxyhemoglobin of the Sturgeon (*Acipenser sturio*), showing general view of smaller crystals.
8. Same, showing brachypinacoid aspect in large crystals.
9. Oxyhemoglobin of the Shad (*Alosa sapidissima*), showing aggregates produced by twinning, horse-type.
10. Same, showing large twin aggregate, horse-type.
11. Oxyhemoglobin and Methemoglobin of the Shad, showing twins of oxyhemoglobin.
12. Same, showing star-shaped twins of horse-type.

PLATE 2



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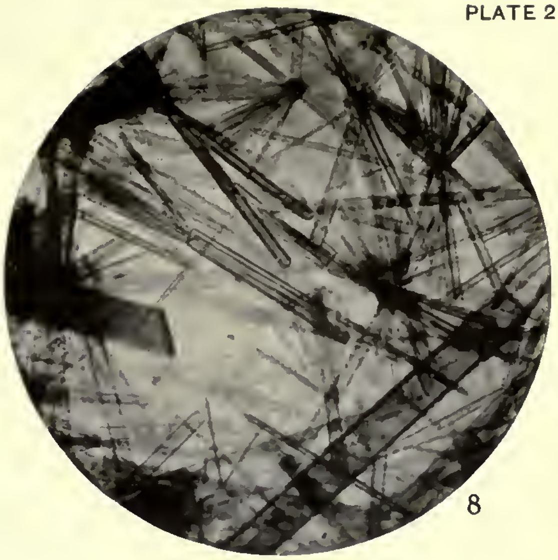

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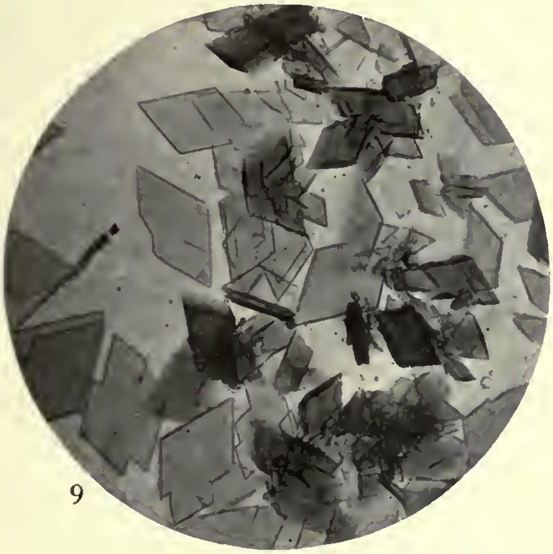

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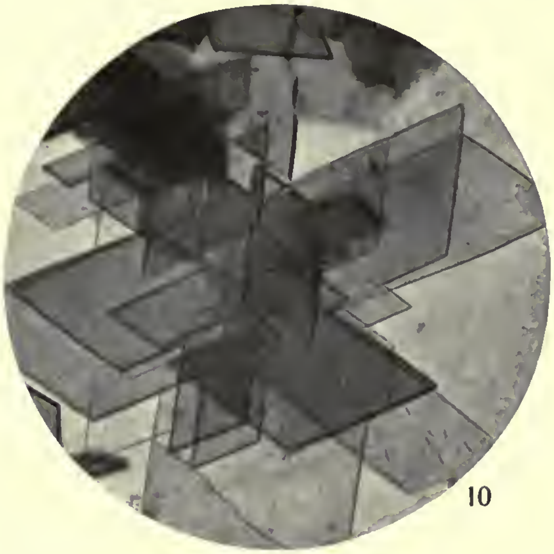

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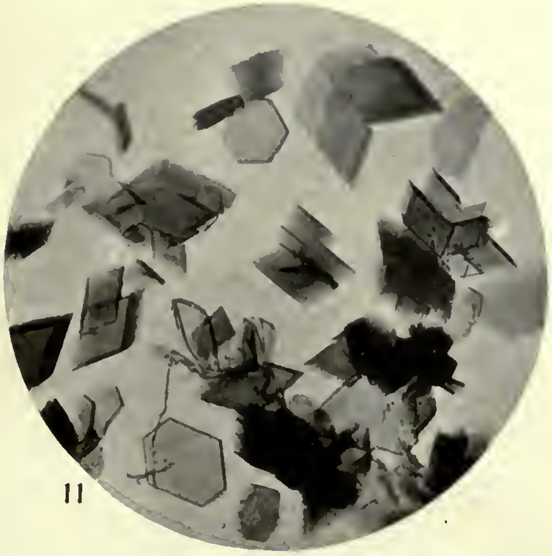

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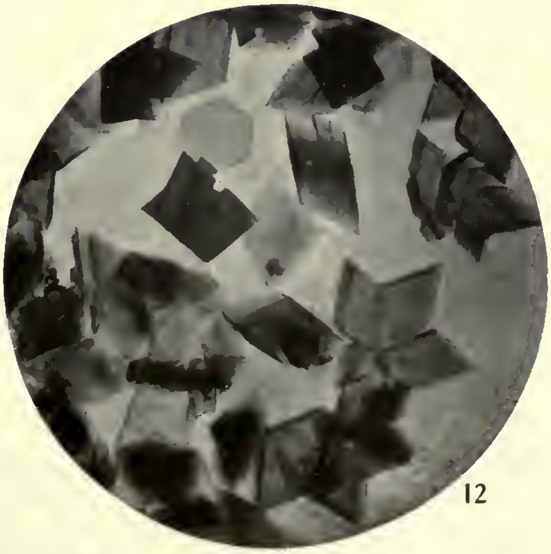

12

7. Oxyhemoglobin of the Sturgeon (*Acipenser sturio*), showing general view of smaller crystals.
8. Same, showing brachypinacoid aspect in large crystals.
9. Oxyhemoglobin of the Shad (*Alosa sapidissima*), showing aggregates produced by twinning, horse-type.
10. Same, showing large twin aggregate, horse-type.
11. Oxyhemoglobin and Methemoglobin of the Shad, showing twins of oxyhemoglobin.
12. Same, showing star-shaped twins of horse-type.


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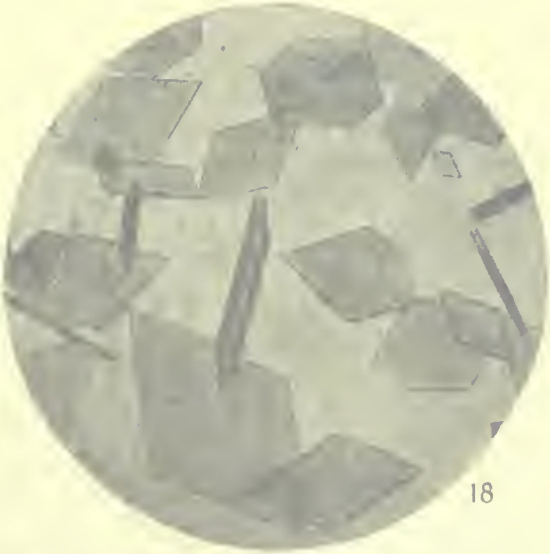
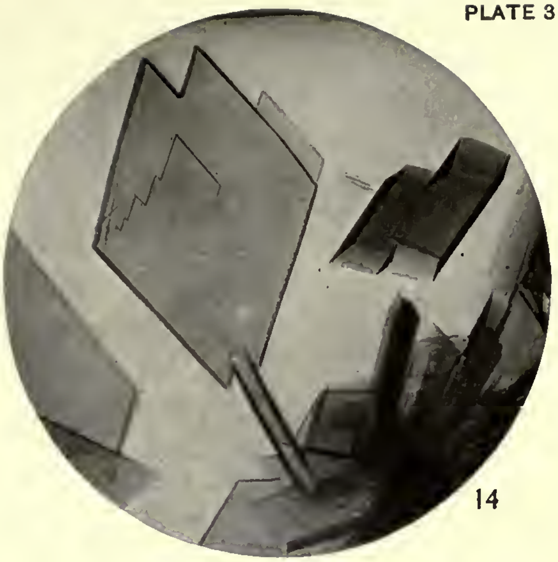

18

13. Metoxyhemoglobin of the Shad (*Alosa sapidissima*), showing aggregate produced by parallel growth and twinning.
14. Same, showing single crystals and twins.
15. Same, showing aggregate group of twinned crystals in various aspects.
16. Same, showing parallel growth and twinning.
17, 18. Reduced Hemoglobin of the Shad, showing various aspects of simple crystals.


13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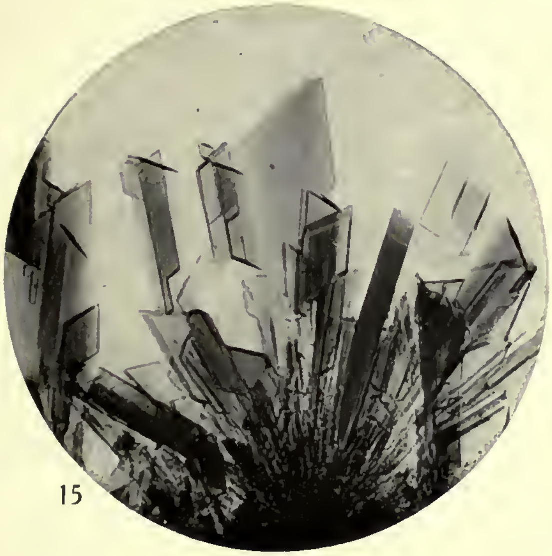
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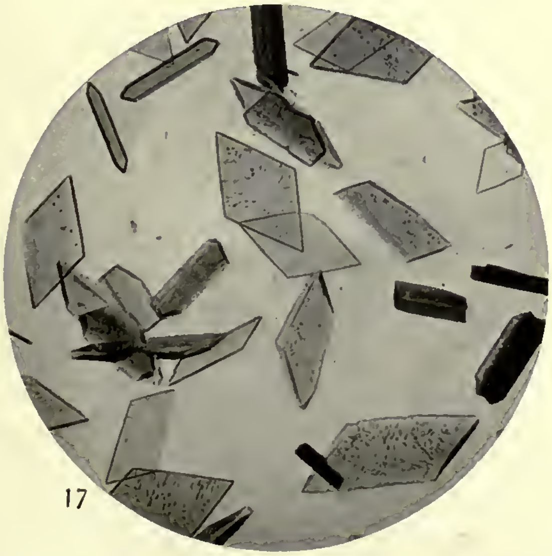
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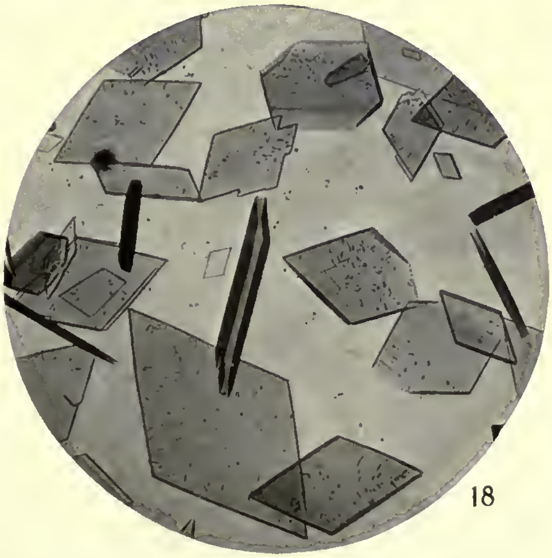
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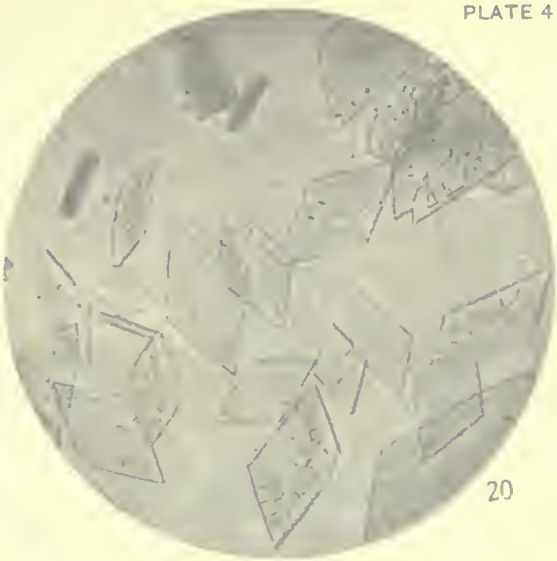
13. Metoxyhemoglobin of the Shad (*Alosa sapidissima*), showing aggregate produced by parallel growth and twinning.
14. Same, showing single crystals and twins.
15. Same, showing aggregate group of twinned crystals in various aspects.
16. Same, showing parallel growth and twinning.

17, 18. Reduced Hemoglobin of the Shad, showing various aspects of simple crystals.

19



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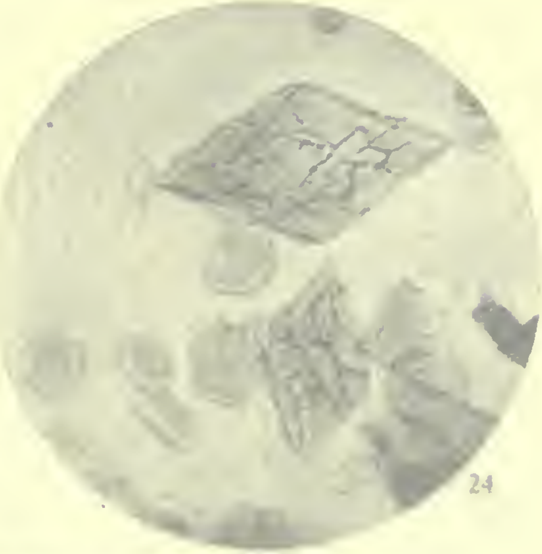


24

19, 20. Reduced Hemoglobin of the Shad (*Alosa sapidissima*), showing [illegible] crystals.

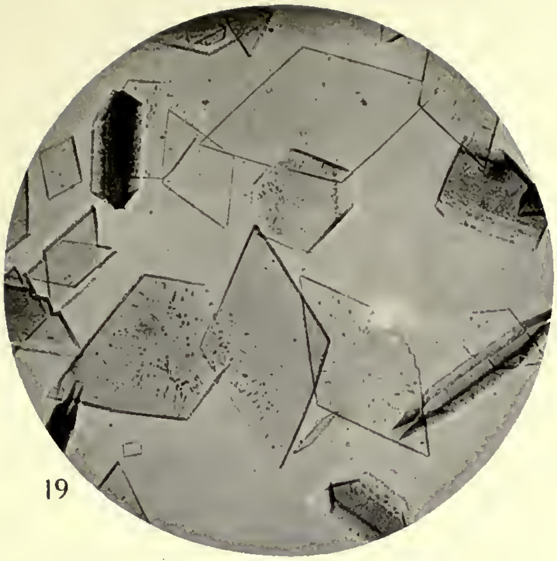
21. Oxyhemoglobin and Methemoglobin of the Shad, showing basal aspect of regular growth.

22. Oxyhemoglobin and Methemoglobin of the Shad, showing edge aspect of a crystal of oxyhemoglobin inclosed by methemoglobin in regular growth [illegible].

23. Oxyhemoglobin and Methemoglobin of the Shad, showing the two substances crystallized separately, and also in regular growth.

24. Reduced Hemoglobin and Methemoglobin of the Shad, showing [illegible] crystals, [illegible] in regular growth; also prismatic cleavage of reduced hemoglobin.

PLATE 4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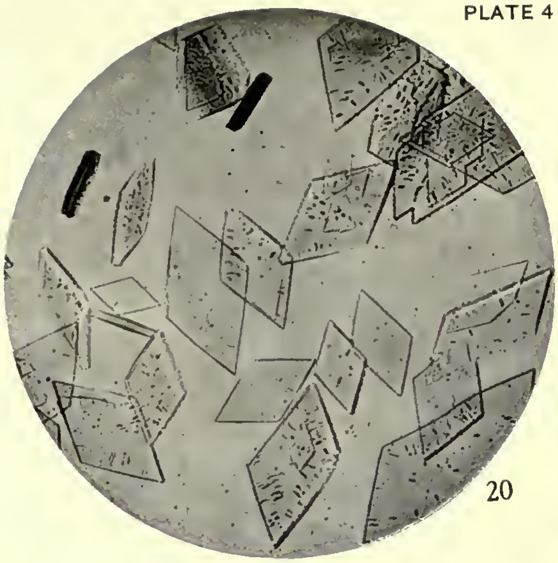
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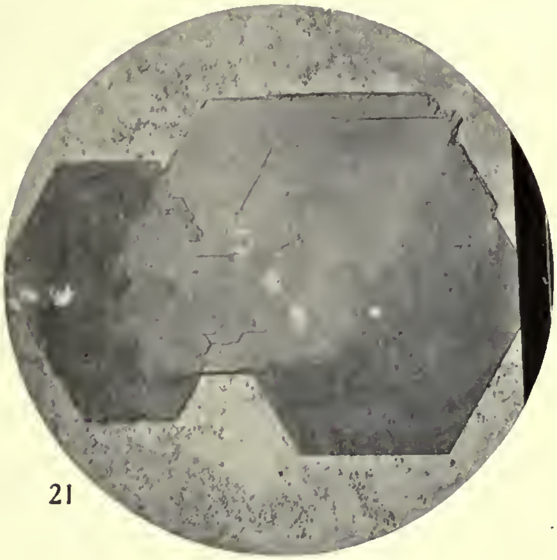
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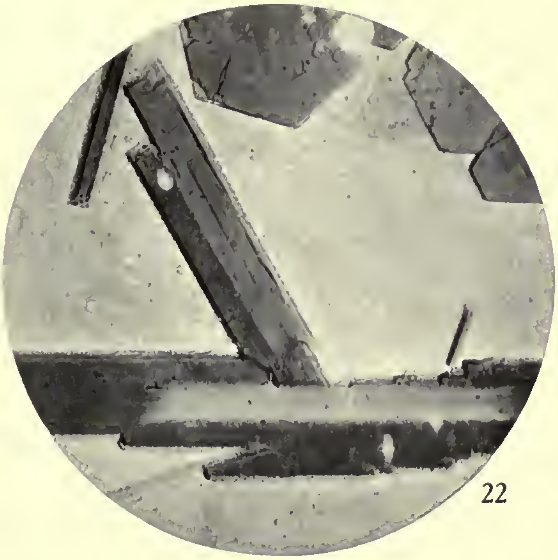
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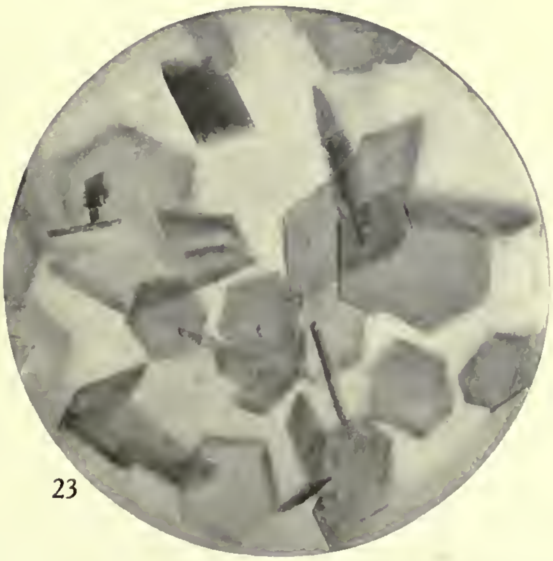
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24

19, 20. Reduced Hemoglobin of the Shad (*Alosa sapidissima*), showing simple crystals.
21. Oxyhemoglobin and Methemoglobin of the Shad, showing basal aspect of regular growth.
22. Oxyhemoglobin and Methemoglobin of the Shad, showing edge aspect of a crystal of oxyhemoglobin inclosed by methemoglobin in regular growth and twinned.
23. Oxyhemoglobin and Methemoglobin of the Shad, showing the two substances crystallized separately and also in regular growth.
24. Reduced Hemoglobin and Methemoglobin of the Shad, showing separate crystals, not in regular growth; also prismatic cleavage of reduced hemoglobin.


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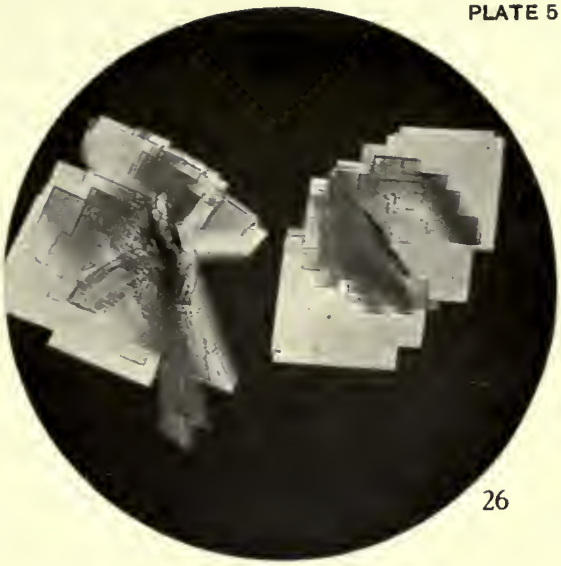
25. Metox[illegible], showing sheaf-like aggregates
26. Same, showing [illegible] polarized light.
27. Same, showing [illegible] by piling up of plates.
28. Same, showing [illegible] crystals.
29. Reduced Hem[illegible] prismatic crystals with acute macrodome (401), and also tabular [illegible]
30. Same, showing p[illegible] sheaf-shaped tufts.

25



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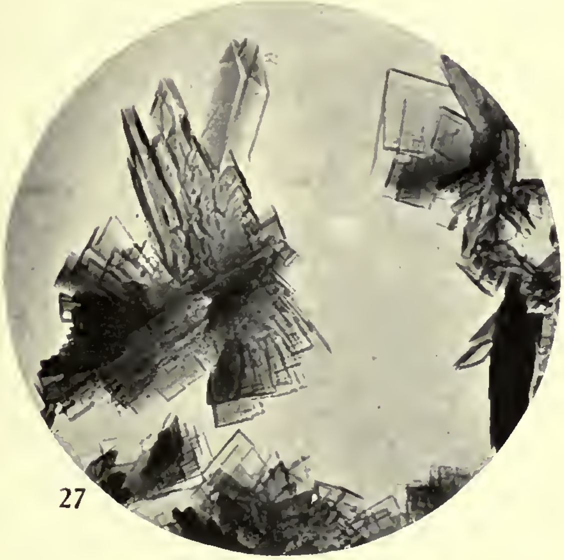

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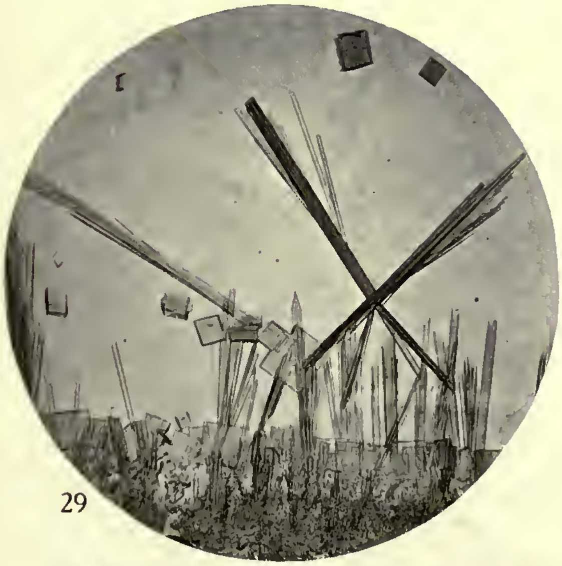

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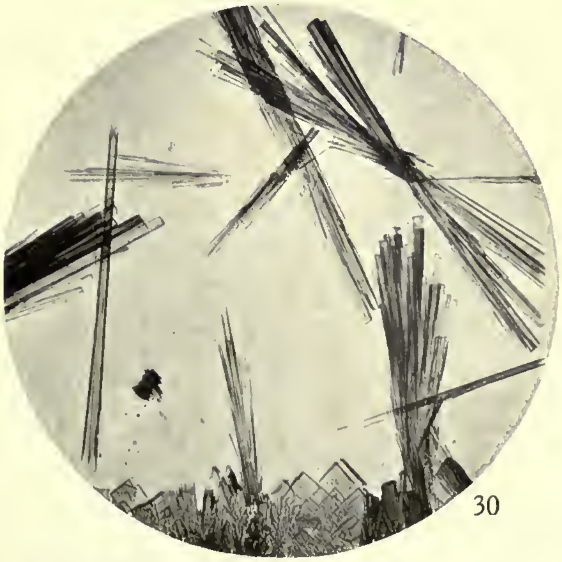
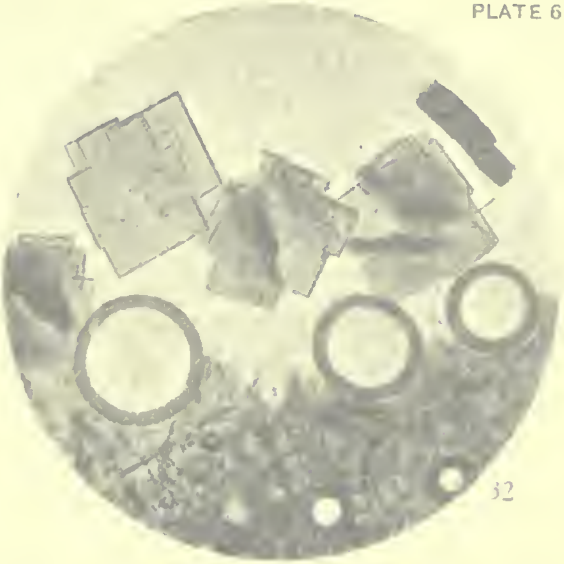


30

25. Metoxyhemoglobin of the Carp (*Cyprinus carpio*), showing sheaf-like aggregates.
26. Same, showing parallel growth of plates, in polarized light.
27. Same, showing irregular aggregates, produced by piling up of plates.
28. Same, showing parallel growth of tabular crystals.
29. Reduced Hemoglobin of the Carp, showing long prismatic crystals with acute macrodome (401), and also tabular crystals.
30. Same, showing prismatic crystals growing in sheaf-shaped tufts.

31



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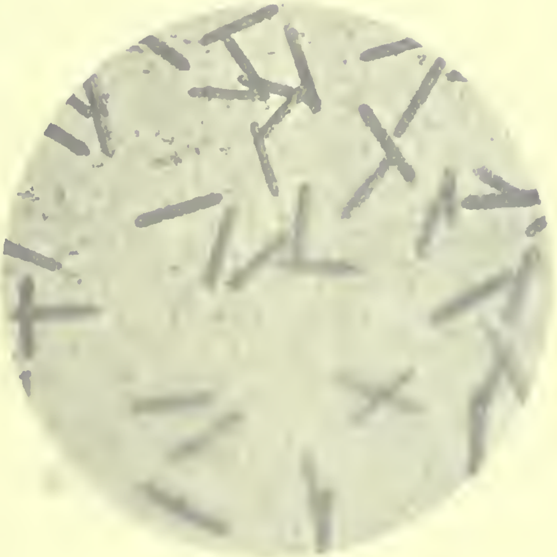

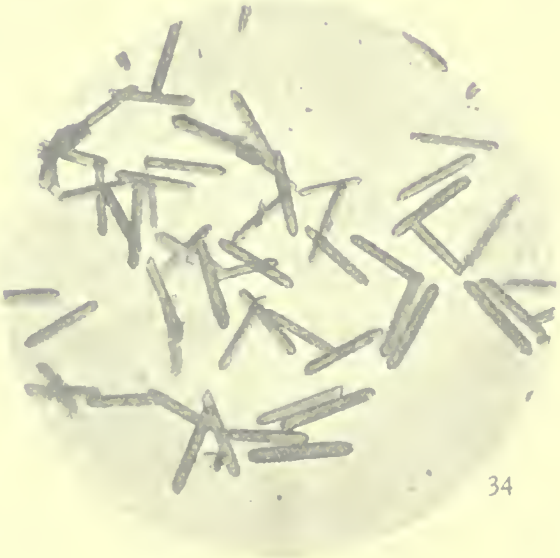

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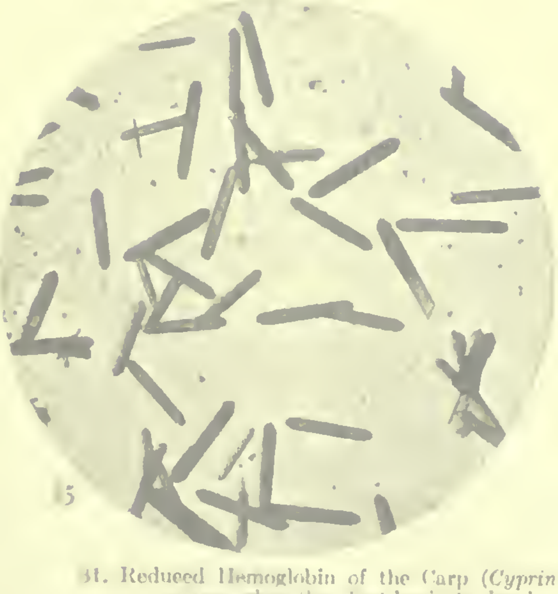

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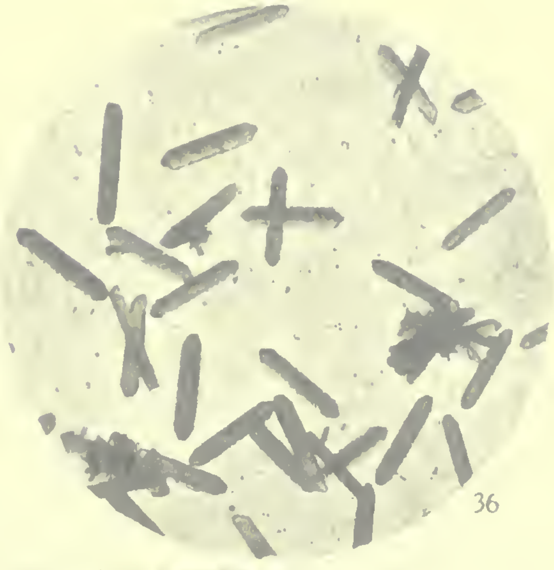
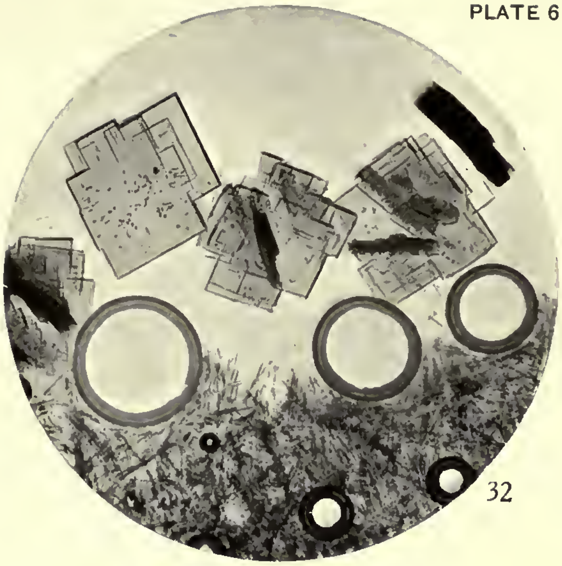


36

31. Reduced Hemoglobin of the Carp (*Cyprinus carpio*), showing smaller tabular crystals as they appear when they first begin to develop.
32. Same, showing larger tabular crystals aggregated in parallel growth.
33-36. Oxyhemoglobin of the Necturus (*Necturus maculatus*), showing normally developed crystals, some twinned.

PLATE 6


31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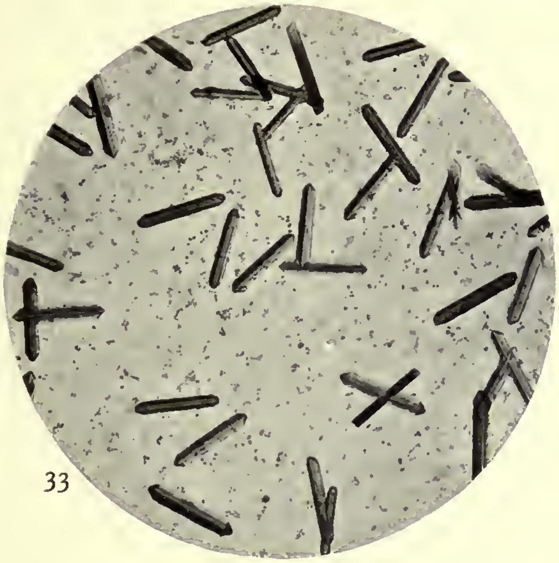
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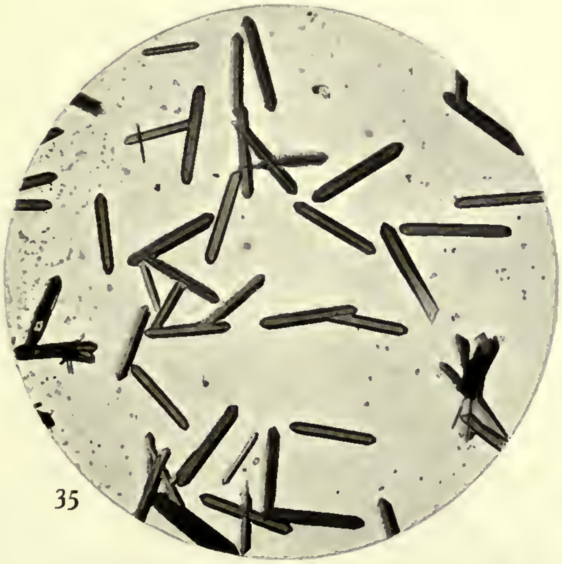
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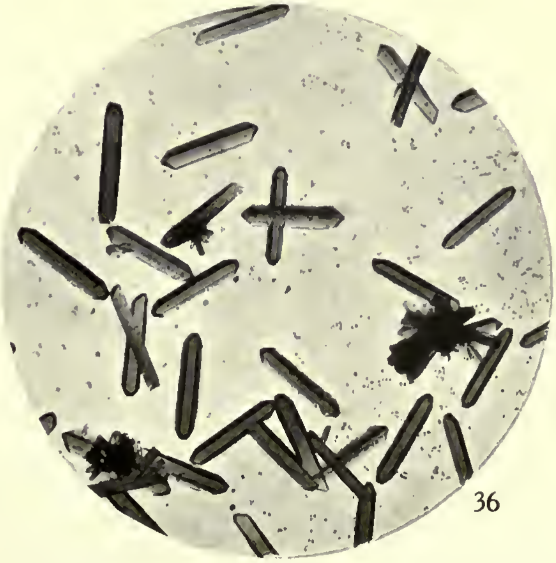
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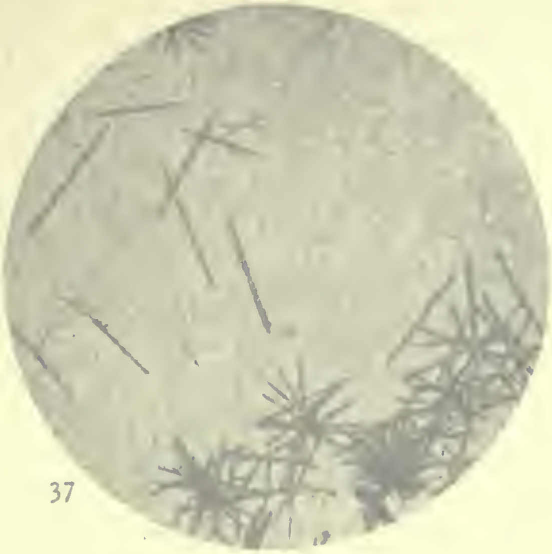
31. Reduced Hemoglobin of the Carp (*Cyprinus carpio*), showing smaller tabular crystals as they appear when they first begin to develop.
32. Same, showing larger tabular crystals aggregated in parallel growth.
33–36. Oxyhemoglobin of the Necturus (*Necturus maculatus*), showing normally developed crystals, some twinned.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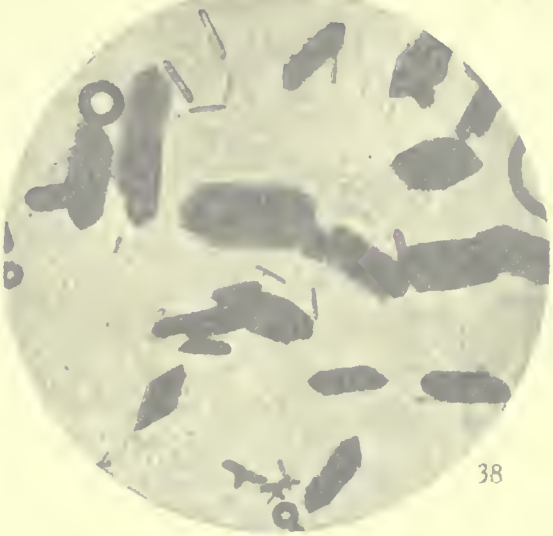
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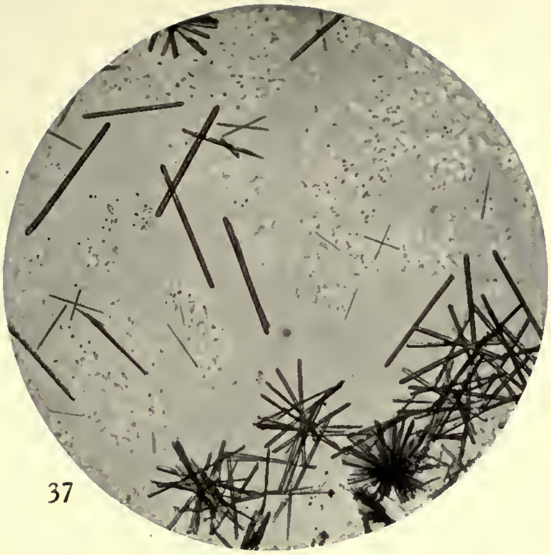
37. Oxyhemoglobin of the Necturus (*Necturus maculatus*) showing [illegible]
38. Same, showing short prismatic development.
39. α-Oxyhemoglobin of the Python (*Python molurus*), showing habit [illegible] view, and oblique section of crystal.
40. α-Oxyhemoglobin of the Python, habit (b), showing parallel growth [illegible]
41, 42. α-Oxyhemoglobin of the Python, habit (b), showing flat and edge views.

PLATE 7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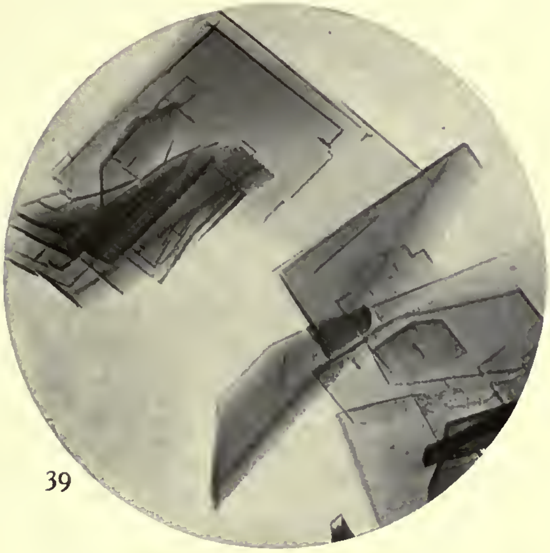
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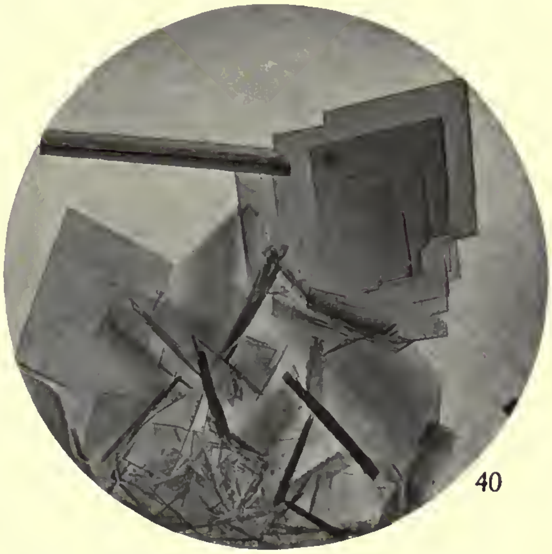
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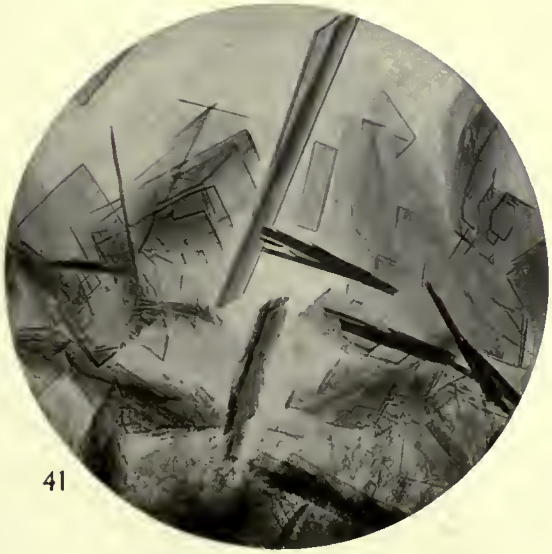
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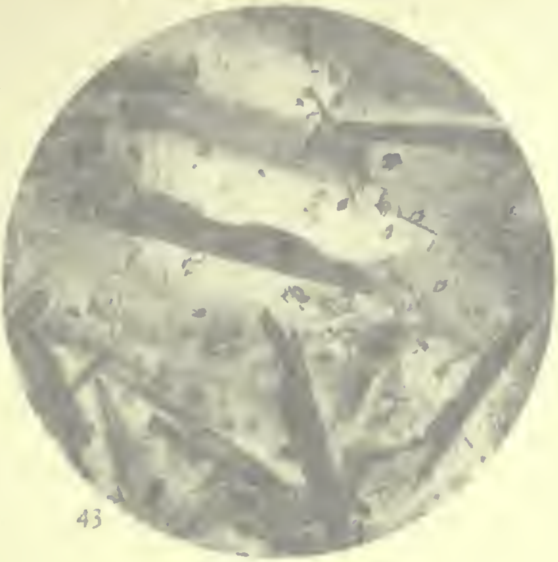
42

37. Oxyhemoglobin of the Necturus (*Necturus maculatus*), showing long prismatic type of crystal.
38. Same, showing short prismatic development.
39. $a$-Oxyhemoglobin of the Python (*Python molurus*), showing habit (b), the tabular crystal in flat view, and oblique section of crystal.
40. $a$-Oxyhemoglobin of the Python, habit (b), showing parallel growth on base.
41, 42. $a$-Oxyhemoglobin of the Python, habit (b), showing flat and edge views.



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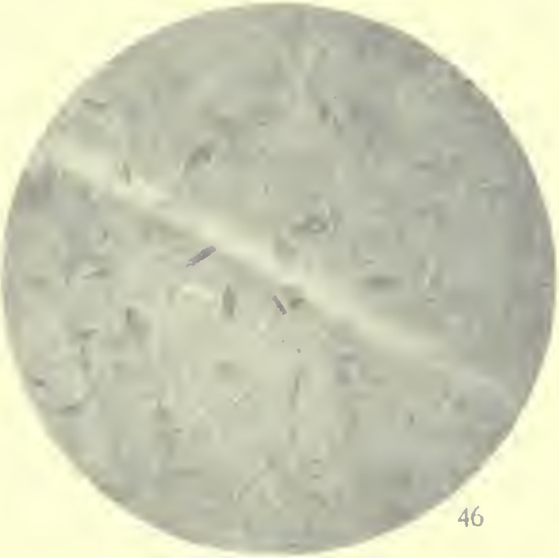

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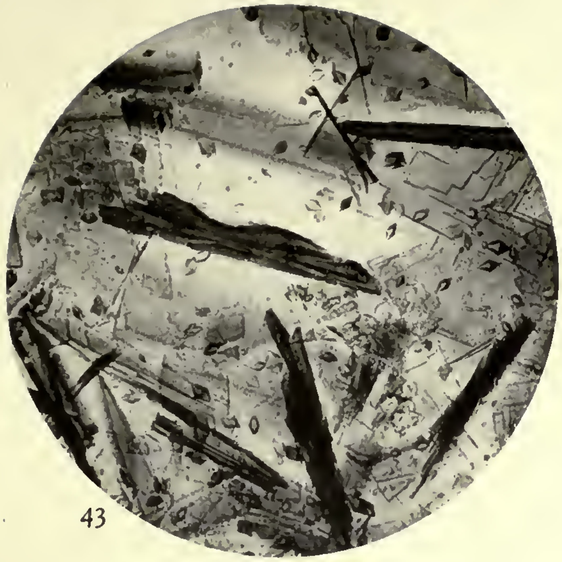
43, 44. β-Oxyhemoglobin of the Python (*Python molurus*) showing small pyramidal crystals as second crop on α-oxyhemoglobin crystals.
45, 46. Oxyhemoglobin of the Ostrich (*Struthio camelus*), showing small rhombic tabular crystals.
47, 48. Oxyhemoglobin of the Cassowary (*Casuarius galeatus*), showing prismatic crystals (dark) with crystals of ammonium oxalate (white).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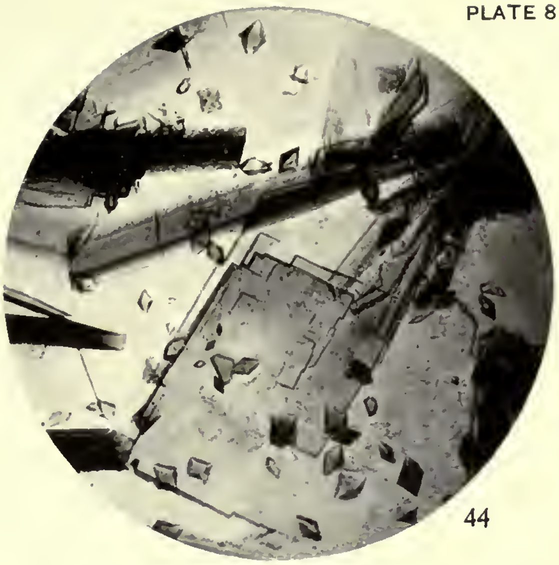
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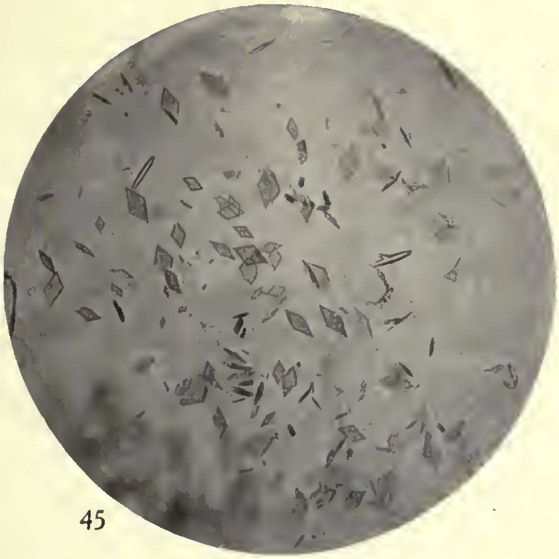
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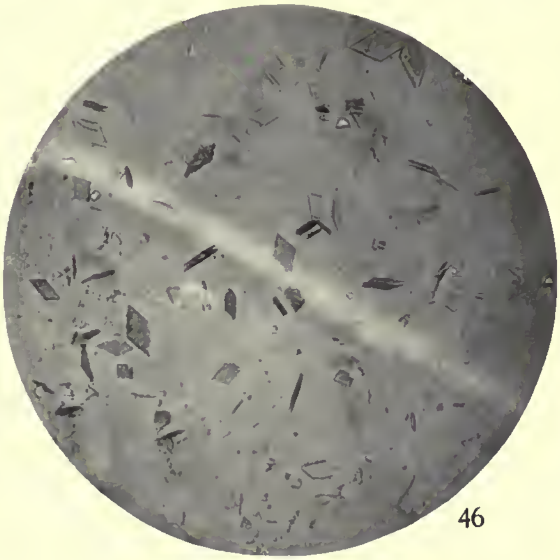
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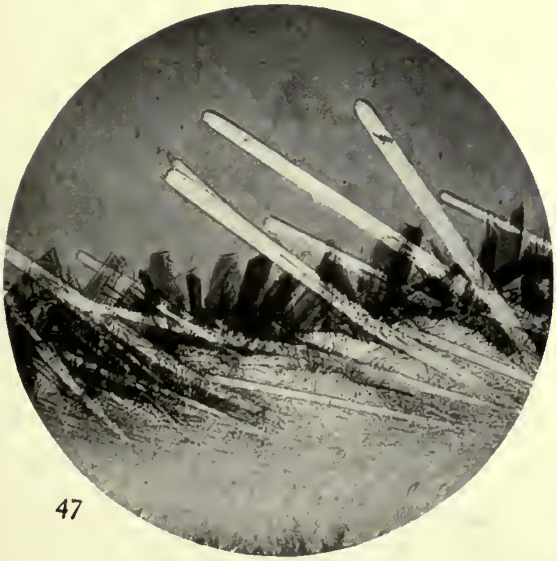
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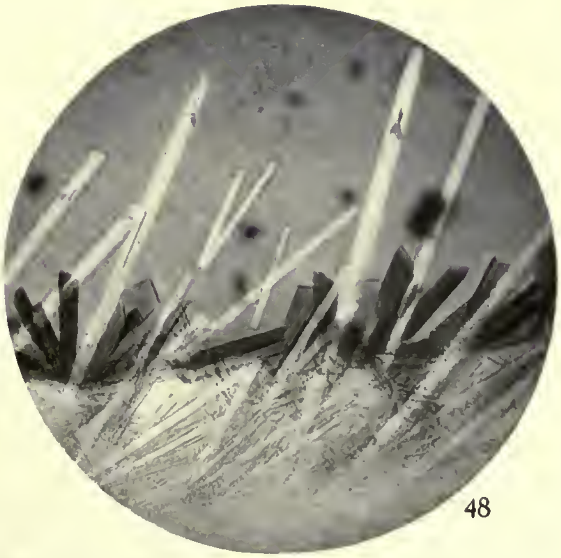
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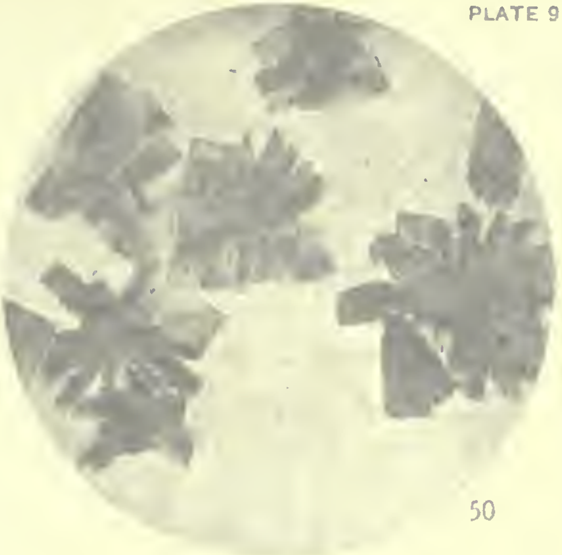
43, 44. β-Oxyhemoglobin of the Python (*Python molurus*), showing small pyramidal crystals as second crop on α-oxyhemoglobin crystals.
45, 46. Oxyhemoglobin of the Ostrich (*Struthio camelus*), showing small rhombic tabular crystals.
47, 48. Oxyhemoglobin of the Cassowary (*Casuarius galeatus*), showing prismatic crystals (dark), with crystals of ammonium oxalate (white).


49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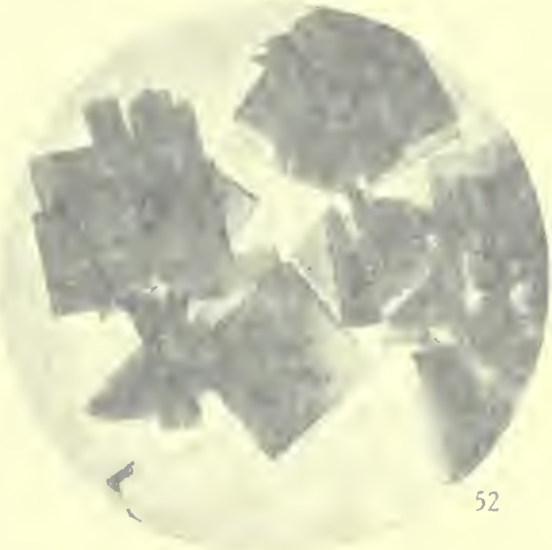
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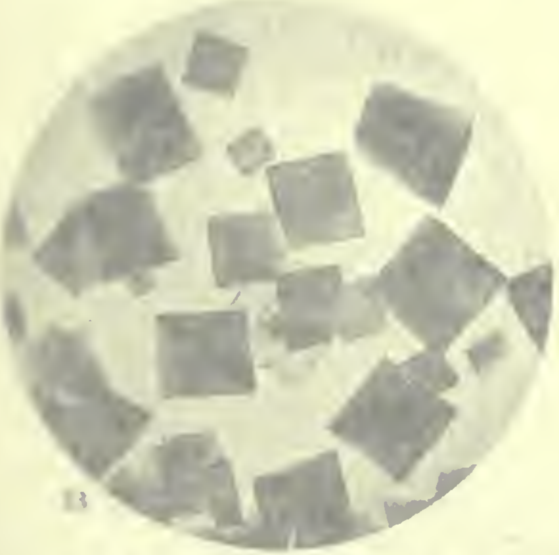
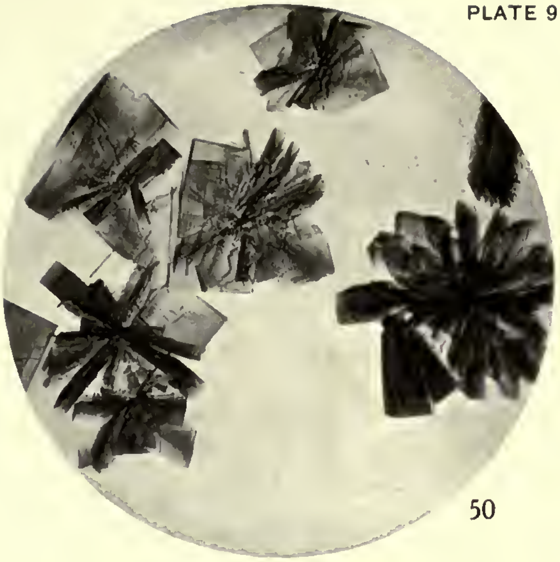

53


54

49. Oxyhemoglobin of the Goose (*Anser anser*), showing clusters of tabular crystals, [illegible] of pyramid.
50. Same, showing aggregates like figure 49, but with more individuals, producing [illegible] group
51, 52. Same, tabular crystals seen on the basal aspect.
53. Oxyhemoglobin of the Trumpeter Swan (*Olor buccinator*), showing first-formed tabular crystals.
54. Same, showing large irregular aggregate of later growth

49



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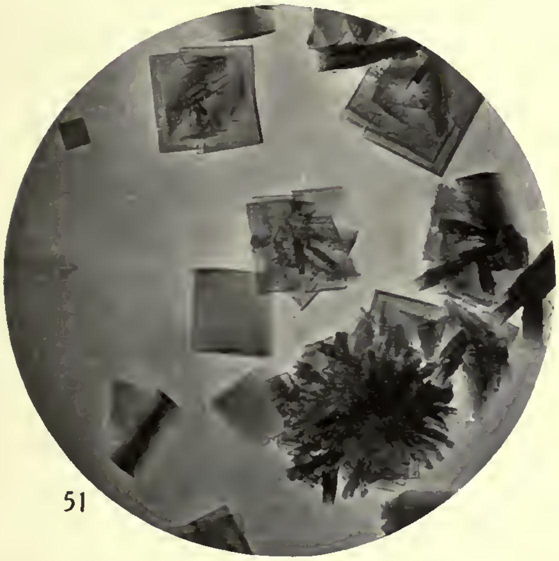

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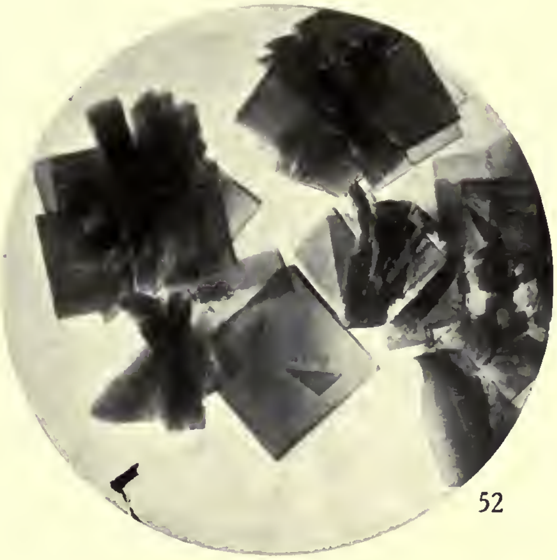

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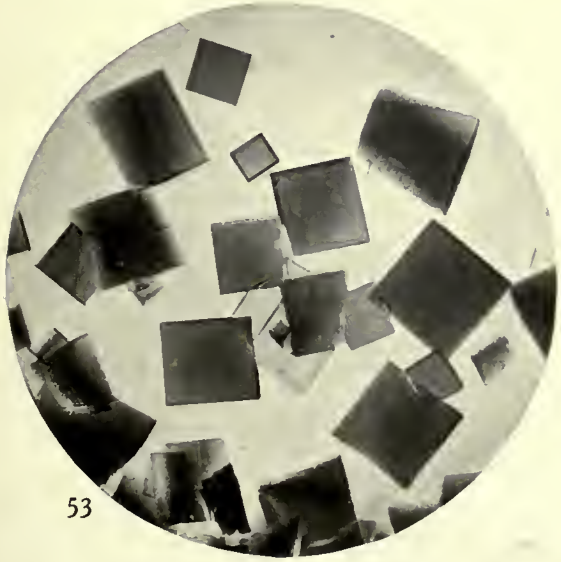

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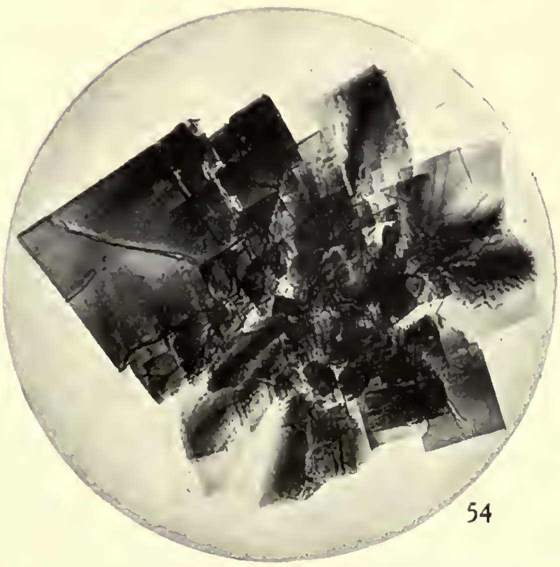

54

49. Oxyhemoglobin of the Goose (*Anser anser*), showing clusters of tabular crystals, twinned in zone of pyramid.
50. Same, showing aggregates like figure 49, but with more individuals, producing rosette-shaped group.
51, 52. Same, tabular crystals seen on the basal aspect.
53. Oxyhemoglobin of the Trumpeter Swan (*Olor buccinator*), showing first-formed simple crystals.
54. Same, showing large irregular aggregate of later growth.

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55. Oxyhemoglobin of the Trumpeter Swan (*Olor buccinator*), showing large simple crystals.
56. Same, showing arborescent aggregates.
57, 58. Oxyhemoglobin of the Whistling Swan (*Olor columbianus*), showing large [illegible] and edge aspects.
59, 60. Same, showing large composite crystals as seen on basal aspect.

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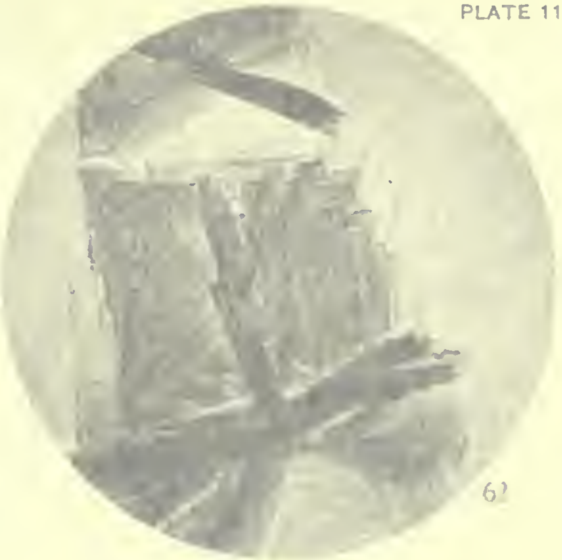
55. Oxyhemoglobin of the Trumpeter Swan (*Olor buccinator*), showing large simple crystal.
56. Same, showing arborescent aggregates.
57, 58. Oxyhemoglobin of the Whistling Swan (*Olor columbianus*), showing large twinned crystals in flat and edge aspects.
59, 60. Same, showing large composite crystals as seen on basal aspect.


61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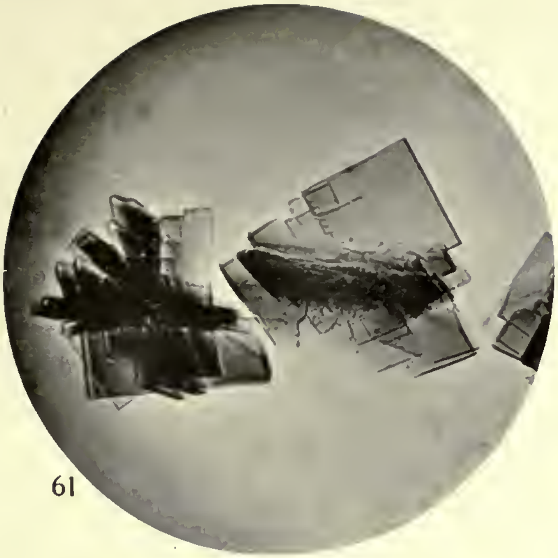
61-63. Oxyhemoglobin of the Chicken (*Gallus domestica*), [illegible]
64. Same, showing single twinned crystal.
65, 66. Same, showing composite crystals in groups show[illegible]



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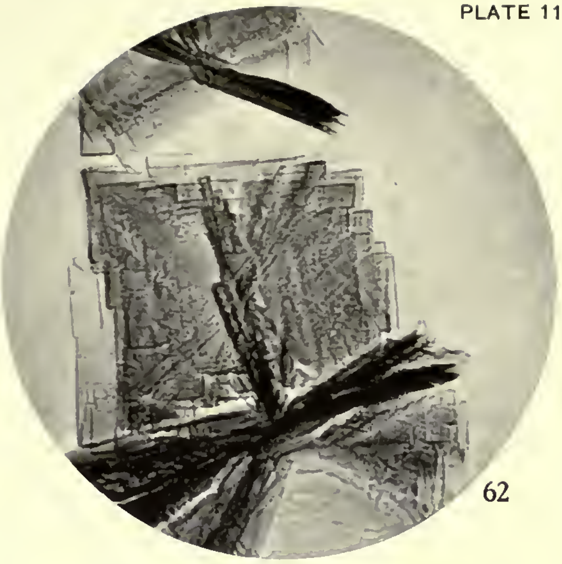

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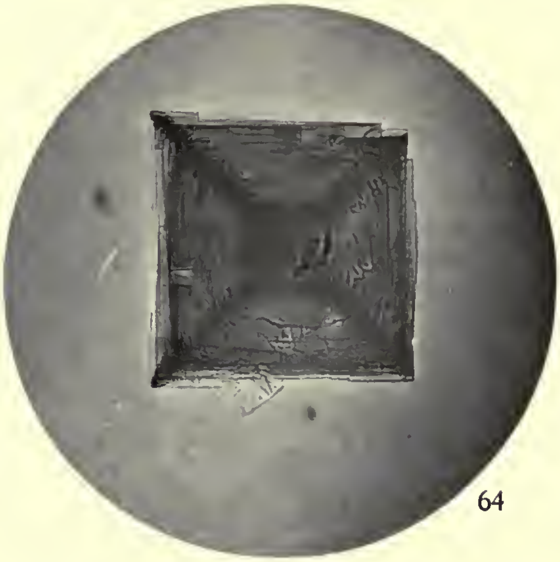


64

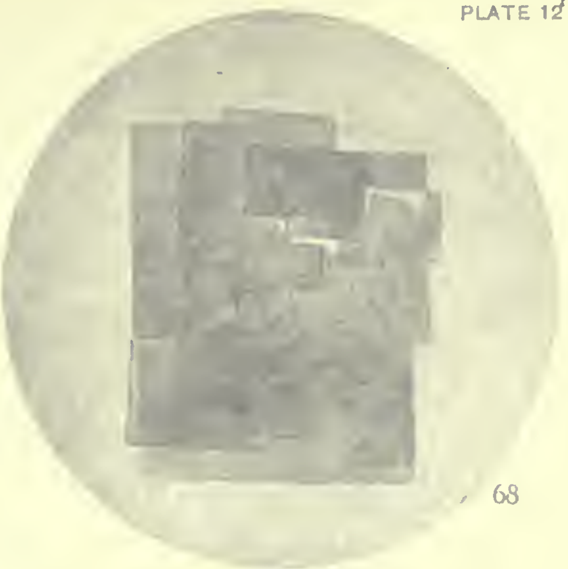
65

66

61–63. Oxyhemoglobin of the Chicken (*Gallus domestica*), showing tabular composite crystals.
64. Same, showing single twinned crystal.
65, 66. Same, showing composite crystals in groups showing basal and edge aspects.

PLATE 12


67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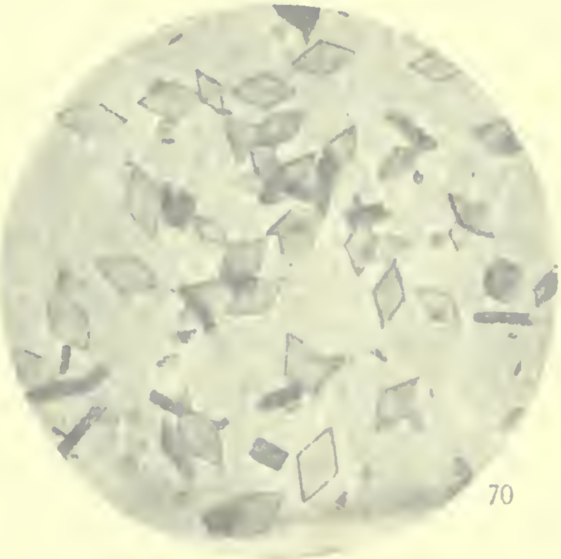
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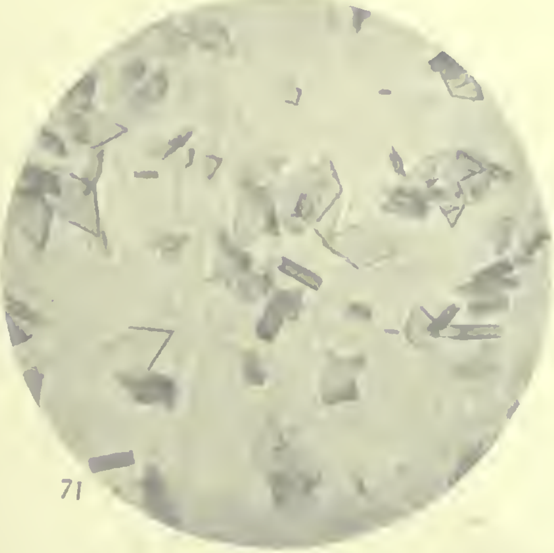
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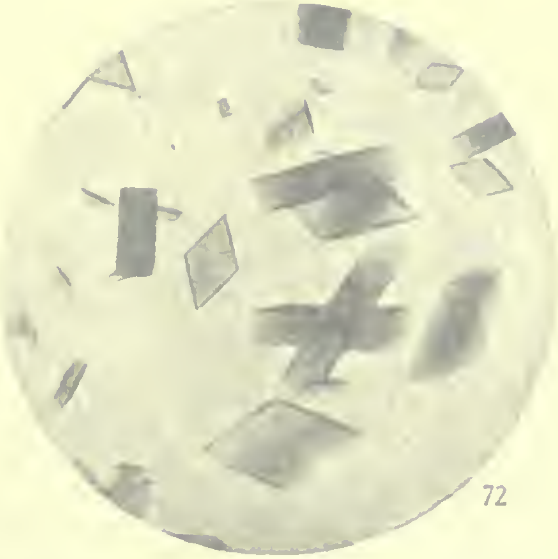
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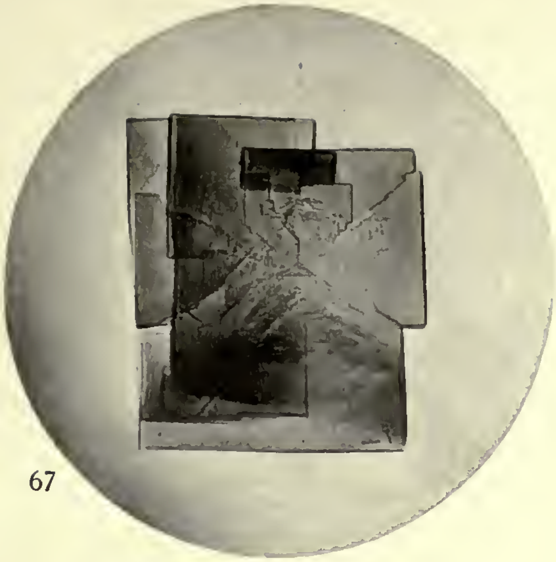
67, 68. Oxyhemoglobin of the Quail (*Colinus virginianus*), showing same composite tabular crystal in two different stages of development.
69. Same, showing single and composite tabular crystals.
70, 71. Oxyhemoglobin of the Guinea-fowl (*Numida meleagris*), showing small simple crystals.
72. Same, larger crystals, showing twin on pyramid.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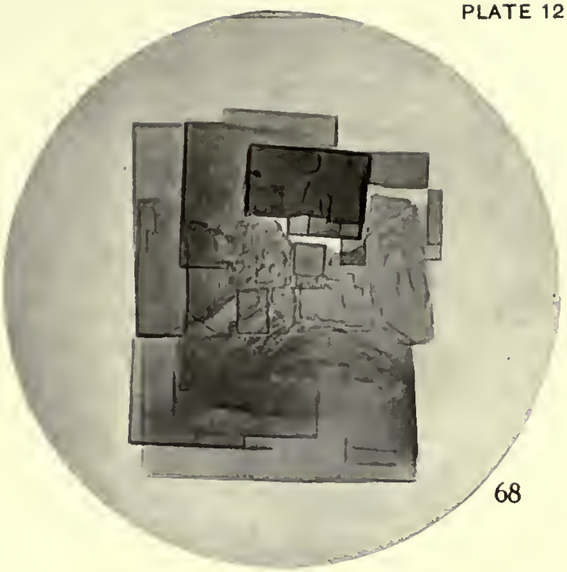
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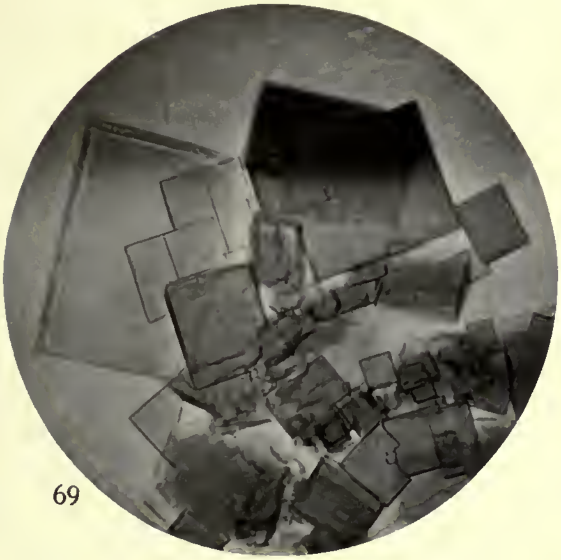
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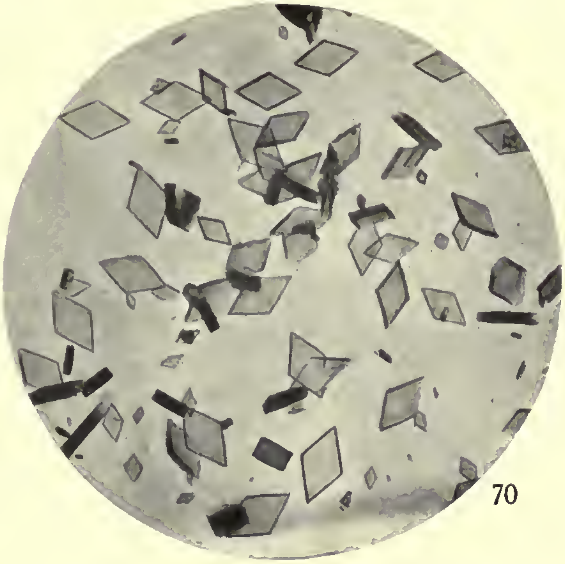
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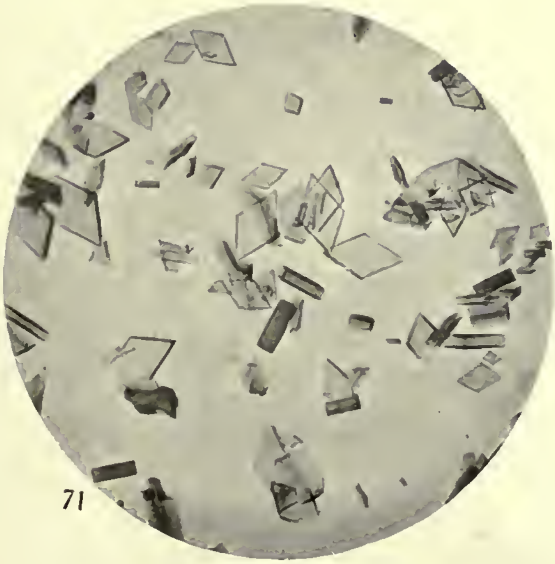
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72

67, 68. Oxyhemoglobin of the Quail (*Colinus virginianus*), showing same composite tabular crystal in two different stages of development.
69. Same, showing single and composite tabular crystals.
70, 71. Oxyhemoglobin of the Guinea-fowl (*Numida meleagris*), showing small simple crystals.
72. Same, larger crystals, showing twin on pyramid.


73


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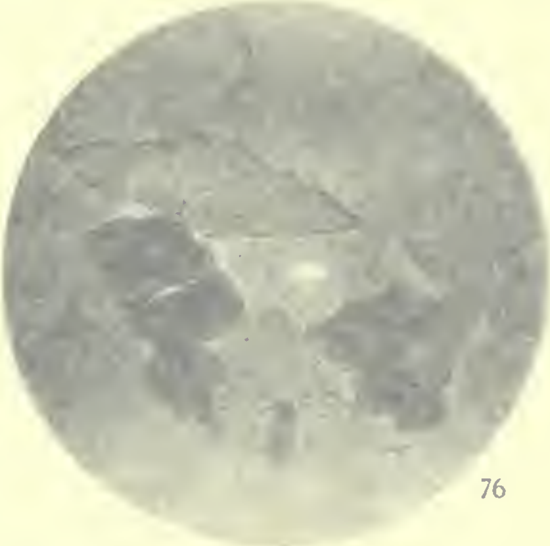
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78

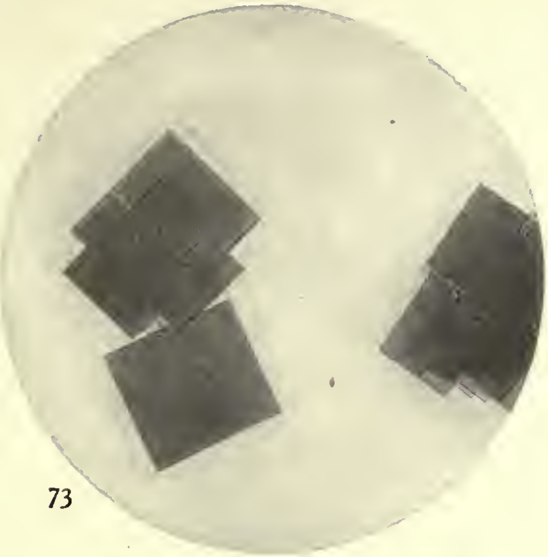
73. Oxyhemoglobin of the Pigeon (*Columba livia*), showing comparatively simple twinned aggregate, in the basal aspect.

74, 75. Same, showing more complex aggregates, some on edge or inclined.

76. Metoxyhemoglobin and Oxyhemoglobin of the Pigeon, showing disintegration of oxyhemoglobin crystals as metoxyhemoglobin crystals develop.

77, 78. Metoxyhemoglobin and Reduced Hemoglobin of the Pigeon, showing the needle-like crystals of reduced hemoglobin growing in tufts from crystals of metoxyhemoglobin.

PLATE 13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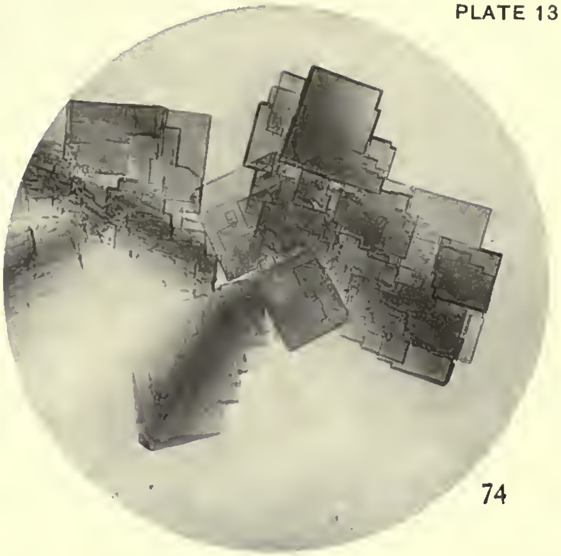
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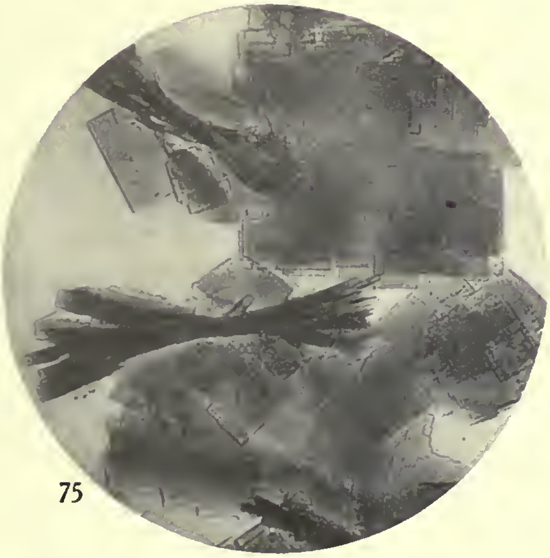
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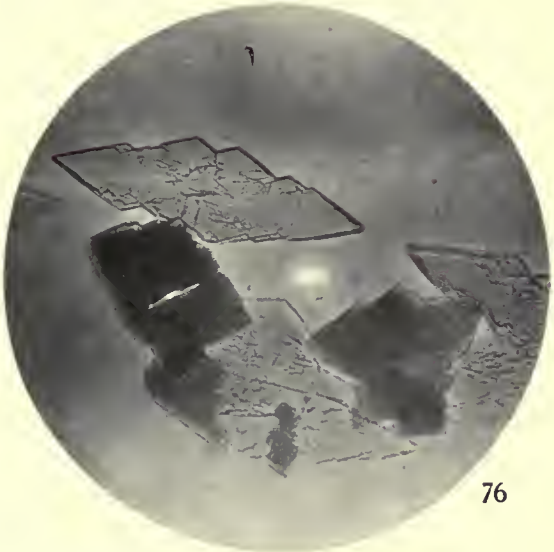
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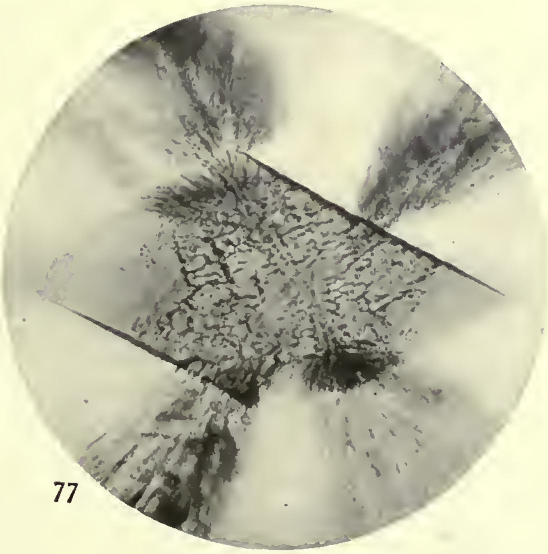
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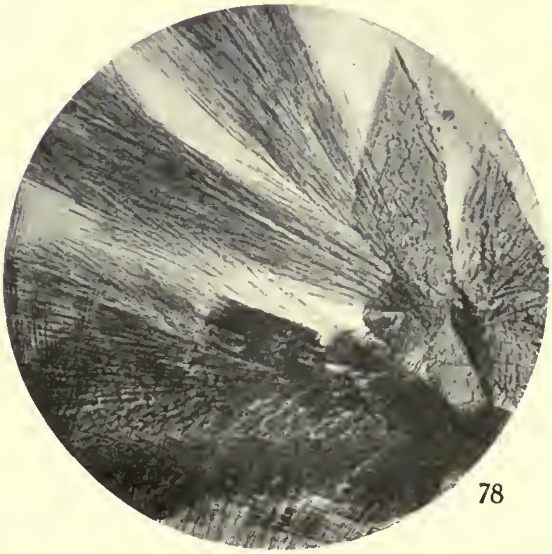
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73. Oxyhemoglobin of the Pigeon (*Columba livia*), showing comparatively simple twinned aggregate, in the basal aspect.

74, 75. Same, showing more complex aggregates, some on edge or inclined.

76. Metoxyhemoglobin and Oxyhemoglobin of the Pigeon, showing disintegration of oxyhemoglobin crystals as metoxyhemoglobin crystals develop.

77, 78. Metoxyhemoglobin and Reduced Hemoglobin of the Pigeon, showing the needle-like crystals of reduced hemoglobin growing in tufts from crystals of metoxyhemoglobin.


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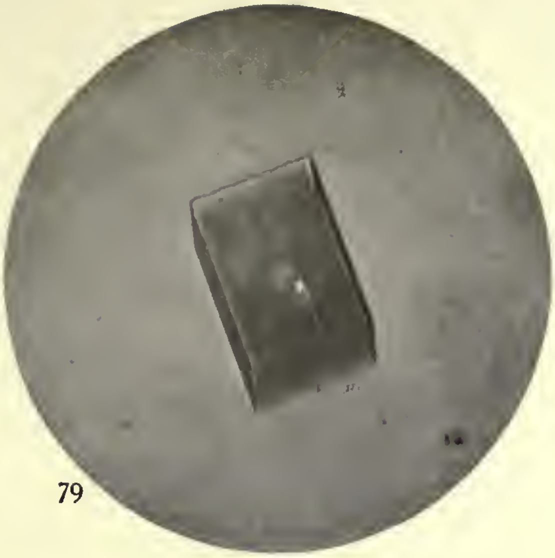
79. Oxyhemoglobin of the Crow (*Corvus americanus*), showing single [illegible] [illegible] [illegible] by false plane near (100).

80-82. Same, showing groups of crystals presenting various [illegible].

83. Reduced Hemoglobin of the Crow, showing group of elongated crystals.

84. Same, showing usual tabular crystal.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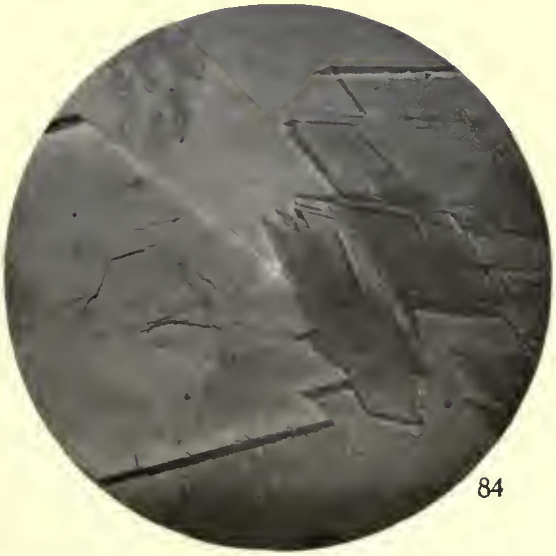
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79. Oxyhemoglobin of the Crow (*Corvus americanus*), showing single, nearly equidimensional crystal attached to cover by false plane near (100).
80–82. Same, showing groups of crystals presenting various orientations.
83. Reduced Hemoglobin of the Crow, showing group of elongated crystals.
84. Same, showing usual tabular crystal.


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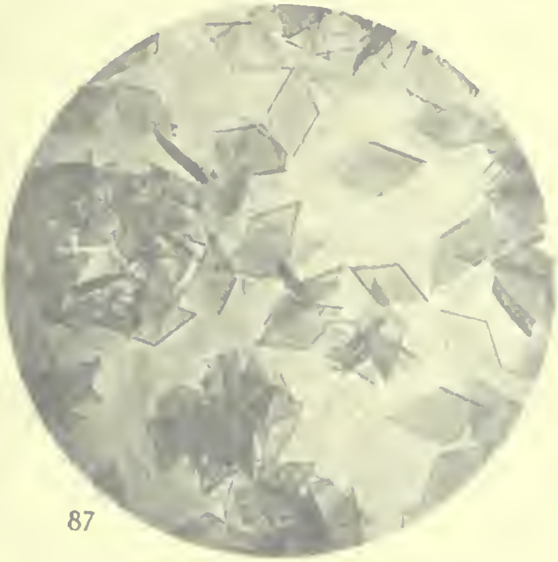
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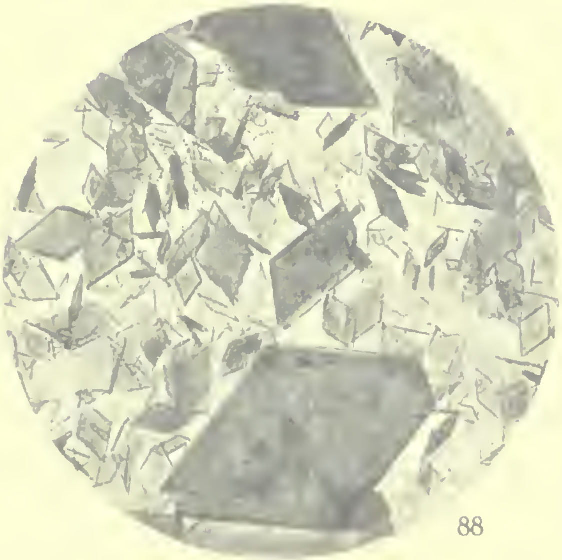
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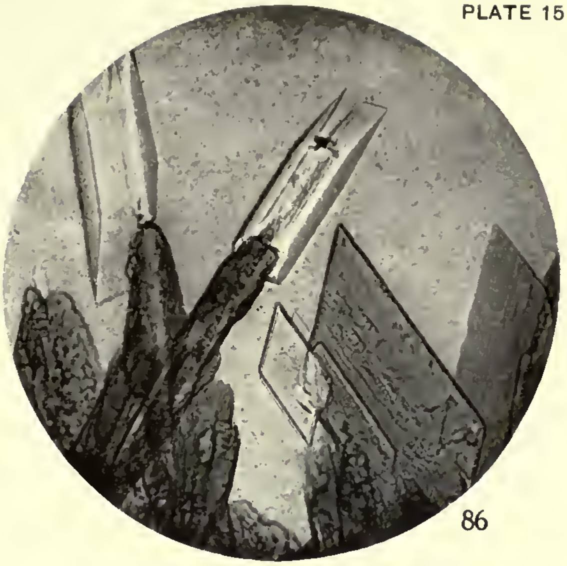
85. Oxyhemoglobin and Reduced Hemoglobin of the Crow (*Corvus americanus*), showing regular growth of reduced hemoglobin on oxyhemoglobin, in basal aspect.
86. Oxyhemoglobin and Reduced Hemoglobin of the Crow, showing regular growth of reduced hemoglobin on oxyhemoglobin in edge and basal aspects. Reduced hemoglobin crystals seen on ends of oxyhemoglobin crystals in parallel position.
87. $a$-Oxyhemoglobin of the Opossum (*Didelphis virginiana*), showing small untwinned crystals.
88. Same, showing large and small crystals. One large crystal shows unsymmetrical development of the hemiorthodome.
89. Same, showing simple crystal (100) (001).
90. Same, tabular crystals in parallel growth, showing hemiorthodome.

85



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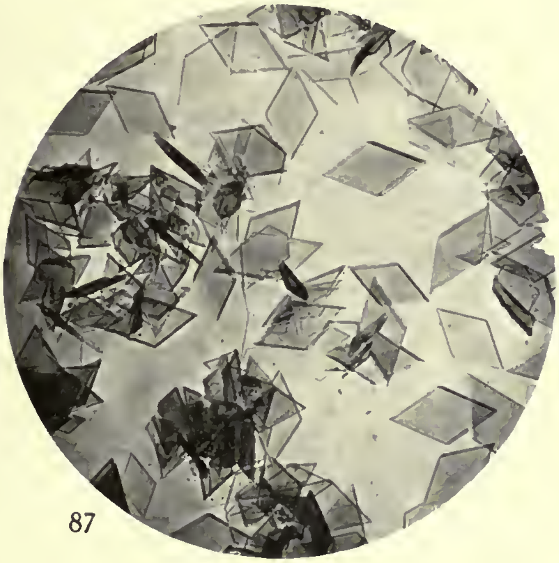

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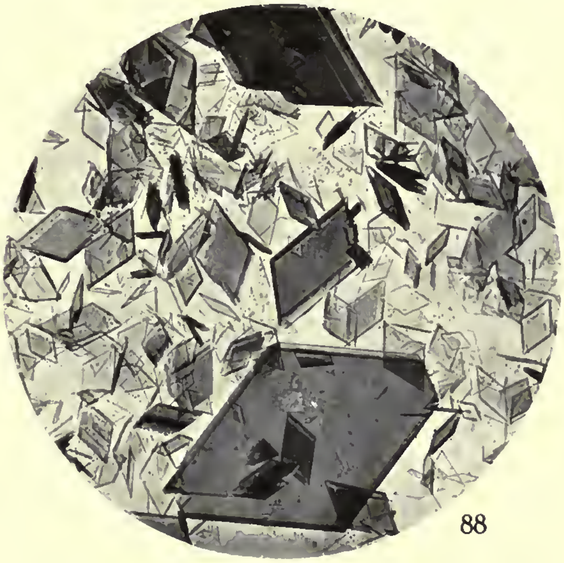

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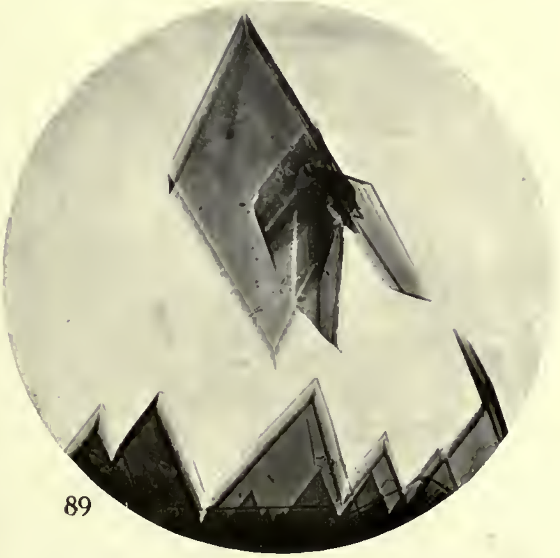

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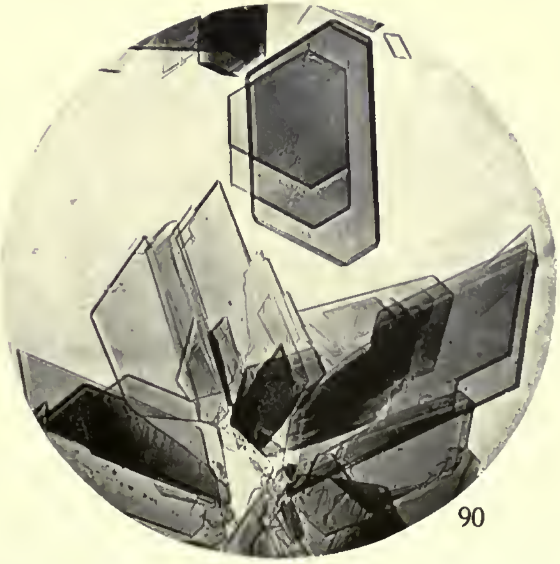

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85. Oxyhemoglobin and Reduced Hemoglobin of the Crow (*Corvus americanus*), showing regular growth of reduced hemoglobin on oxyhemoglobin, in basal aspect.
86. Oxyhemoglobin and Reduced Hemoglobin of the Crow, showing regular growth of reduced hemoglobin on oxyhemoglobin in edge and basal aspects. Reduced hemoglobin crystals seen on ends of oxyhemoglobin crystals in parallel position.
87. *a*-Oxyhemoglobin of the Opossum (*Didelphis virginiana*), showing small untwinned crystals.
88. Same, showing large and small crystals. One large crystal shows unsymmetrical development of the hemiorthodome.
89. Same, showing simple crystal (100) (001).
90. Same, tabular crystals in parallel growth, showing hemiorthodome.

PLATE 16


91


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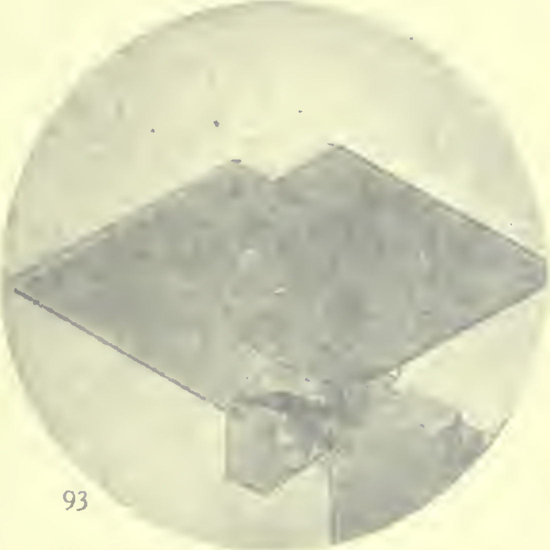
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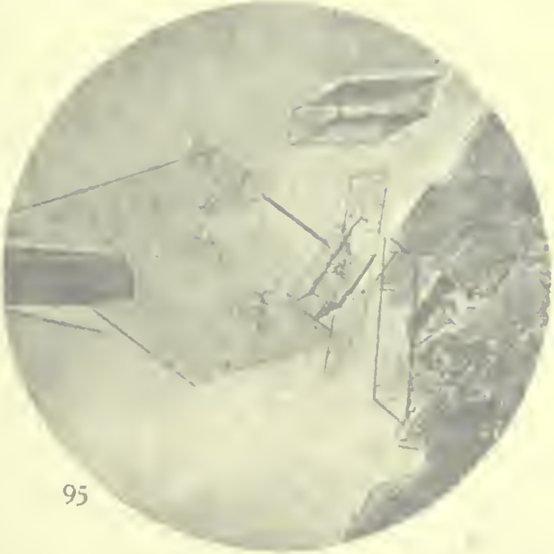
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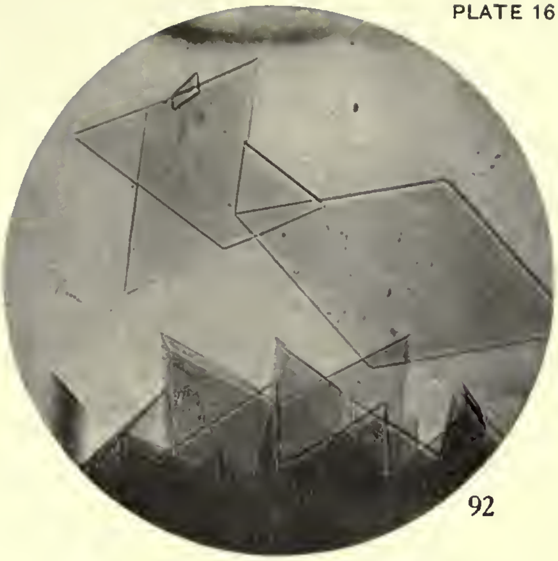
96

91. *a*-Oxyhemoglobin of the Opossum (*Didelphis virginiana*), large crystal showing parallel growth and unsymmetrical development of hemiorthodome.
92. Same, showing large simple crystal and horse-type twin.
93. Same, showing twin on hemiorthodome and unsymmetrical development of this dome.
94. Same, showing unsymmetrical crystals and a twin on edge.
95. Reduced Hemoglobin of the Opossum, showing thick and thin tabular crystals and parallel growth.
96. Same, showing Reduced Hemoglobin with crystals of oxyhemoglobin.


91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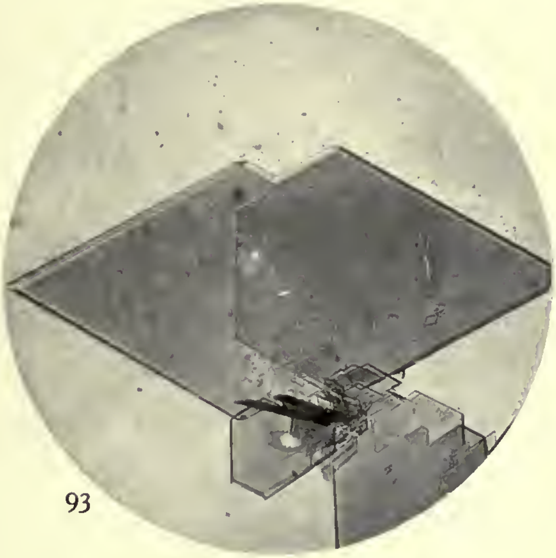
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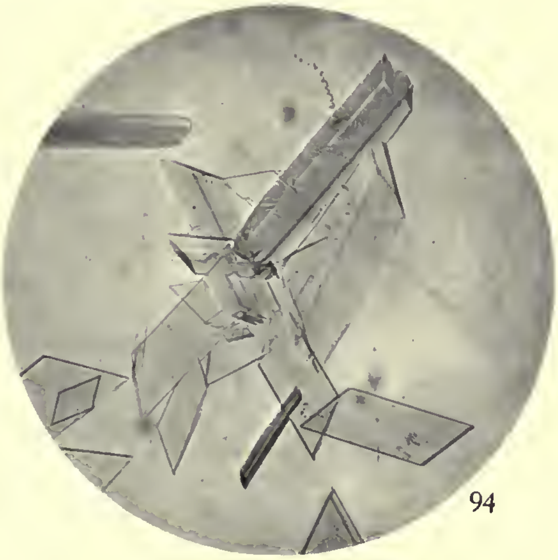
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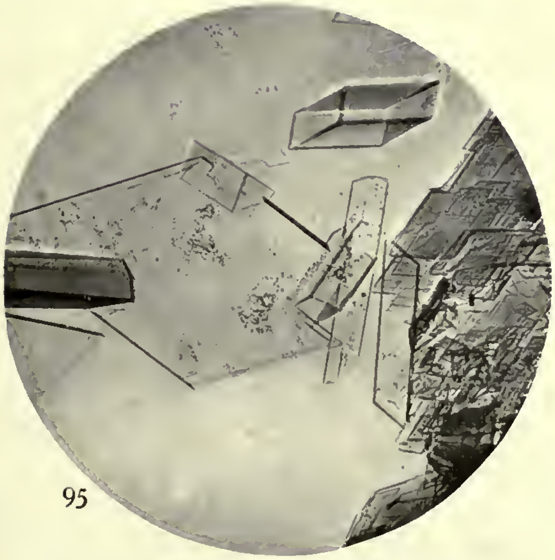
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91. *a*-Oxyhemoglobin of the Opossum (*Didelphis virginiana*), large crystal showing parallel growth and unsymmetrical development of hemiorthodome.
92. Same, showing large simple crystal and horse-type twin.
93. Same, showing twin on hemiorthodome and unsymmetrical development of this dome.
94. Same, showing unsymmetrical crystals and a twin on edge.
95. Reduced Hemoglobin of the Opossum, showing thick and thin tabular crystals and parallel growth.
96. Same, showing Reduced Hemoglobin with crystals of oxyhemoglobin.

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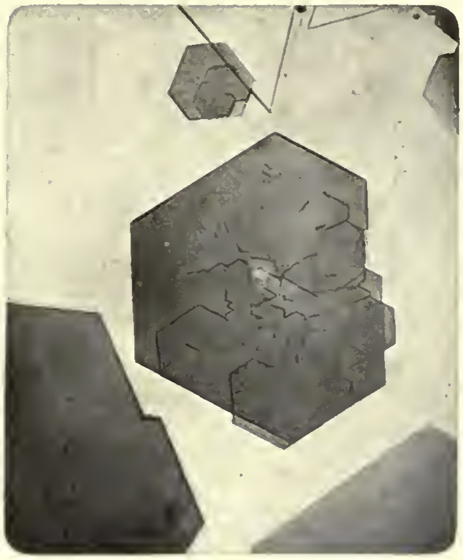
102

97. β-Oxyhemoglobin of the Opossum, showing tabular crystals in various orientations
98. Same, showing large composite crystal.
99. Same, showing basal aspect of tabular crystals with a crystal of α-oxyhemoglobin in partial orientation with it, also edge view of a larger crystal of the β-oxyhemoglobin
100. Same, showing single large tabular crystal.
101, 102. α-CO-Hemoglobin of the Opossum, showing thin tabular crystals in different aspects

97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99



100

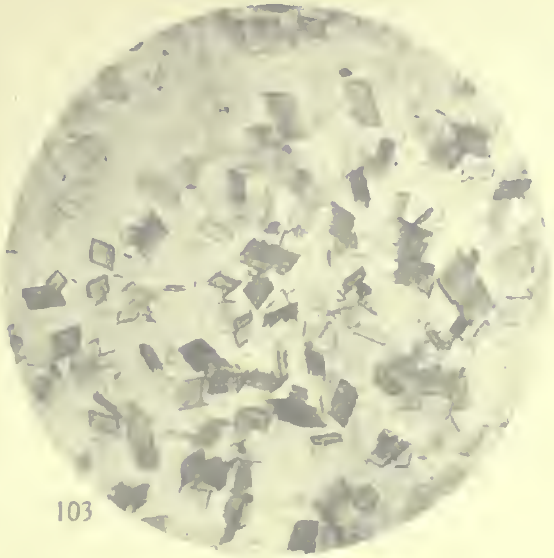
101



102

97. β-Oxyhemoglobin of the Opossum, showing tabular crystals in various orientations.
98. Same, showing large composite crystal.
99. Same, showing basal aspect of tabular crystals with a crystal of α-oxyhemoglobin in partial orientation with it, also edge view of a larger crystal of the β-oxyhemoglobin.
100. Same, showing single large tabular crystal.
101, 102. α-CO-Hemoglobin of the Opossum, showing thin tabular crystals in different aspects.

PLATE 18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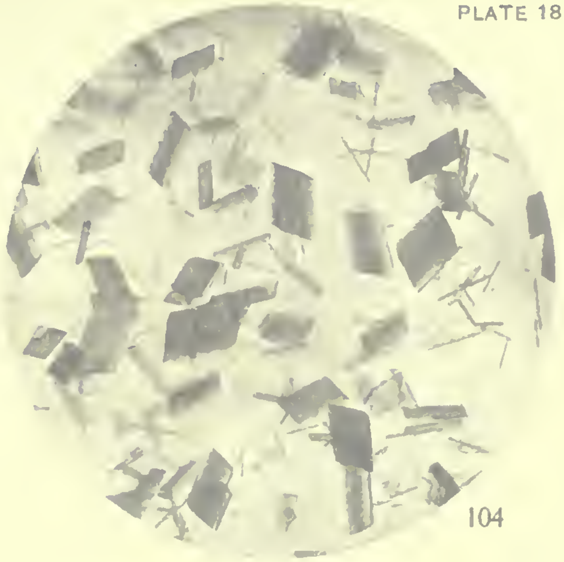
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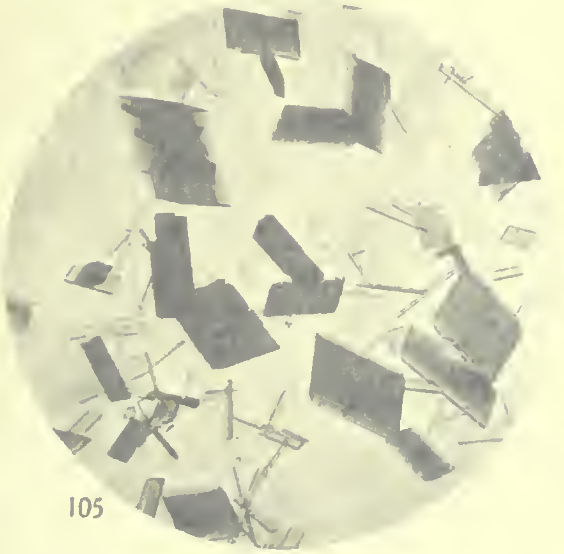
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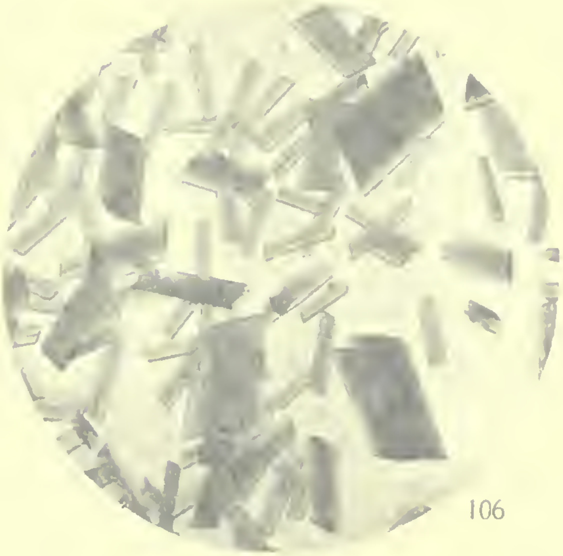
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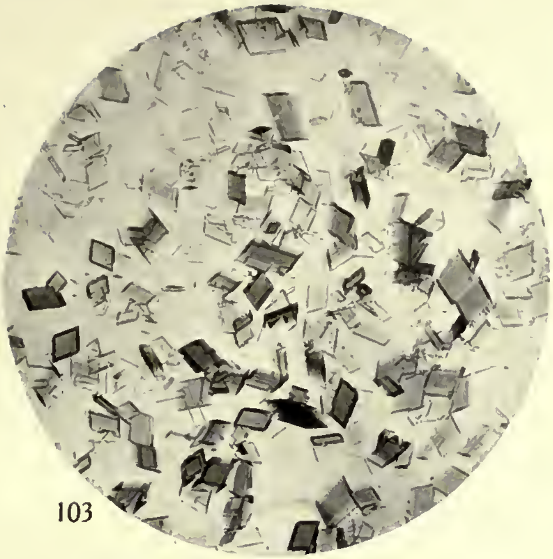
107


108

103. Oxyhemoglobin of the Tasmanian Devil (*Sarcophilus ursinus*), showing tabular and short prismatic types of crystals.
104, 105. Same, showing short prismatic crystals with slender, long prismatic type.
106. Same, showing different types of prismatic crystals, some twinned.
107. Reduced Hemoglobin of the Spotted Dasyure (*Dasyurus maculatus*), showing prismatic and tabular types of crystals.
108. Same, showing prismatic type of crystals.



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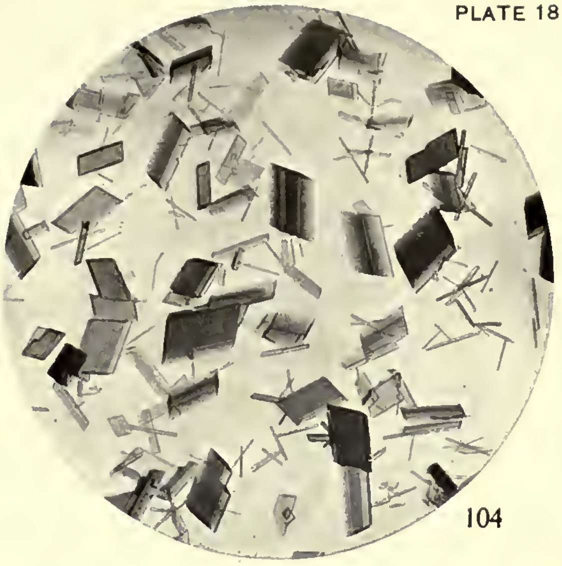

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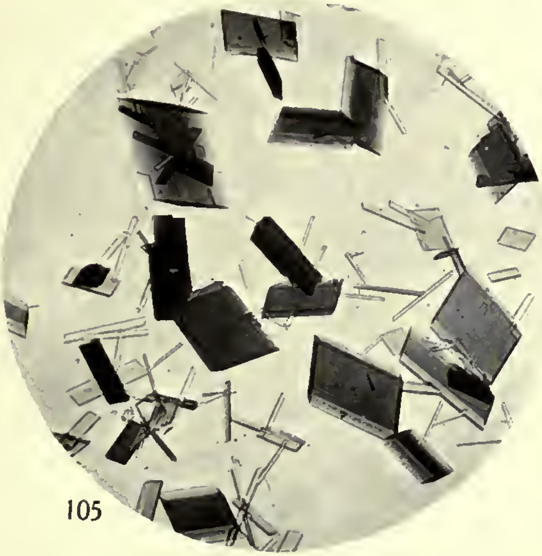

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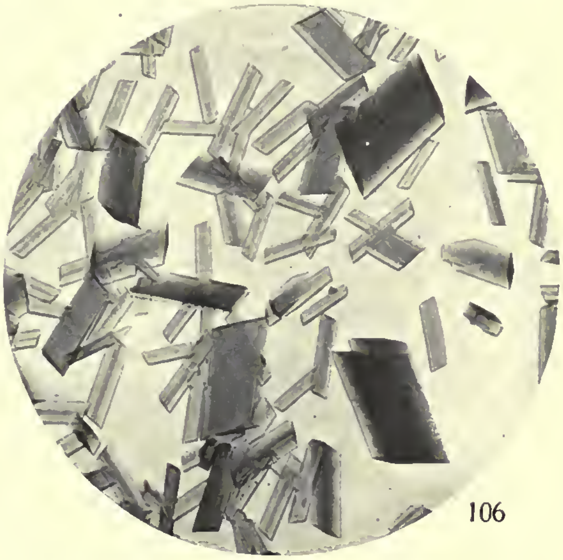

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107

108

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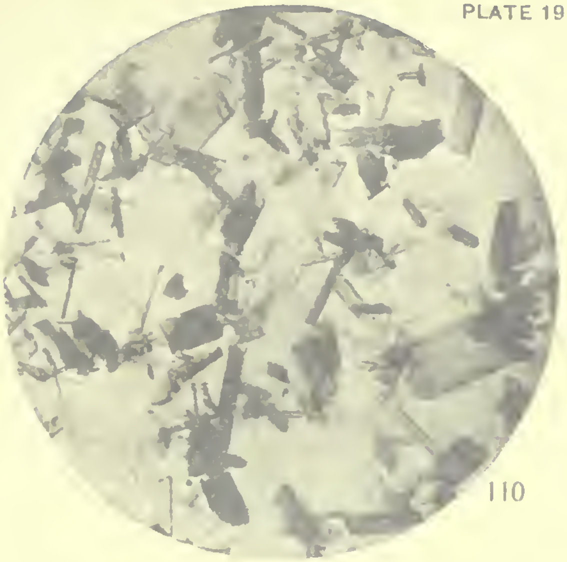
104, 105. Same, showing short prismatic crystals with slender, long prismatic type.
106. Same, showing different types of prismatic crystals, some twinned.
107. Reduced Hemoglobin of the Spotted Dasyure (*Dasyurus maculatus*), showing prismatic and tabular types of crystals.
108. Same, showing prismatic type of crystals.

PLATE 19


109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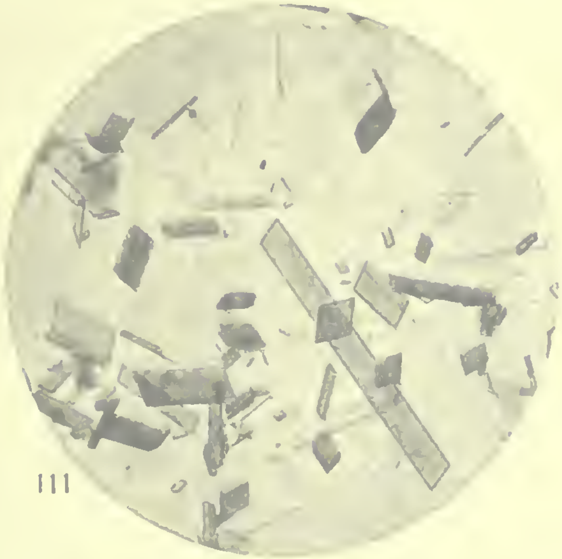
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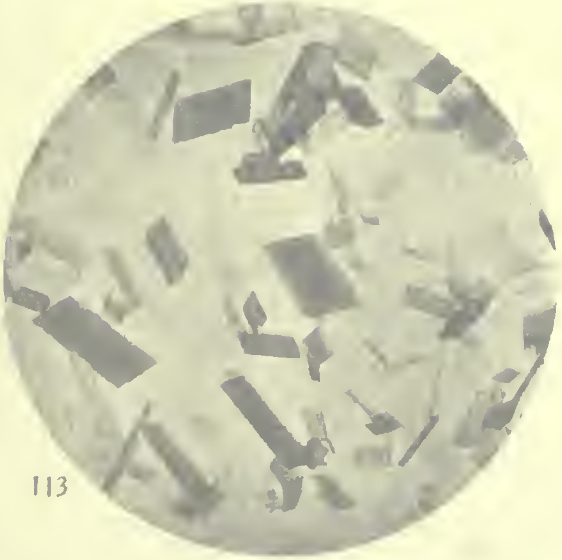
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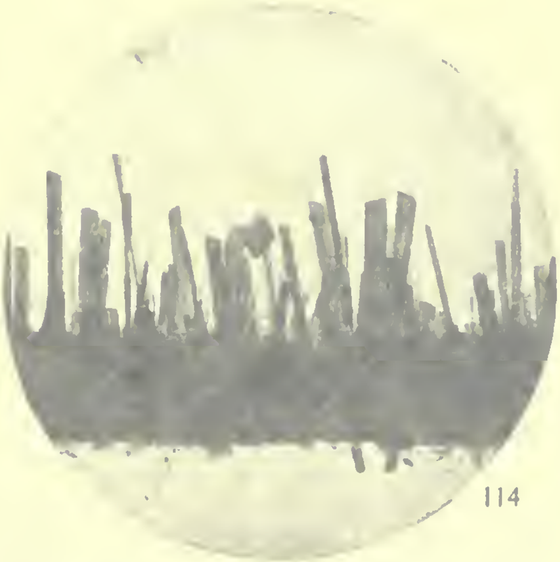
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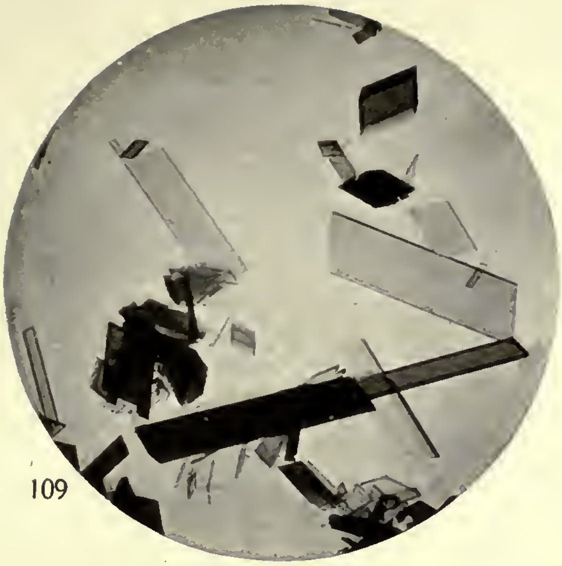
109. Oxyhemoglobin of the Australian Cat (*Dasyurus viverrinus*), showing larger crystals, tabular on two opposite prism faces. V-shaped twin seen in one crystal.
110. Same, showing crystals in different aspects.
111. 112. Same, showing very much flattened crystals and V-shaped twin on prism.
113. Same, showing symmetrical and distorted prisms.
114. Same, showing group of long prismatic crystals from protein ring.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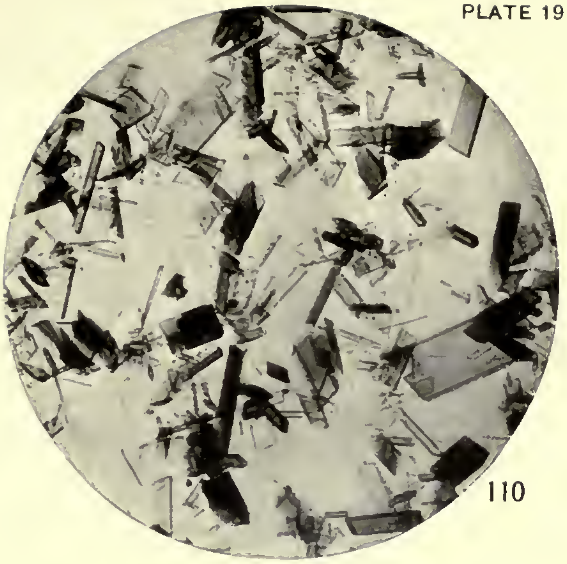
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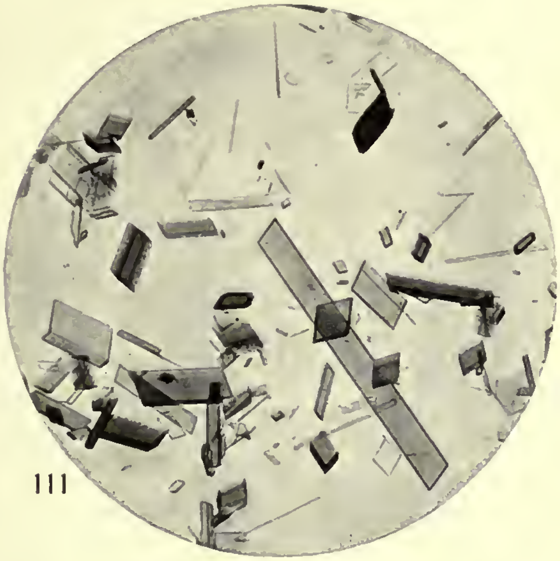
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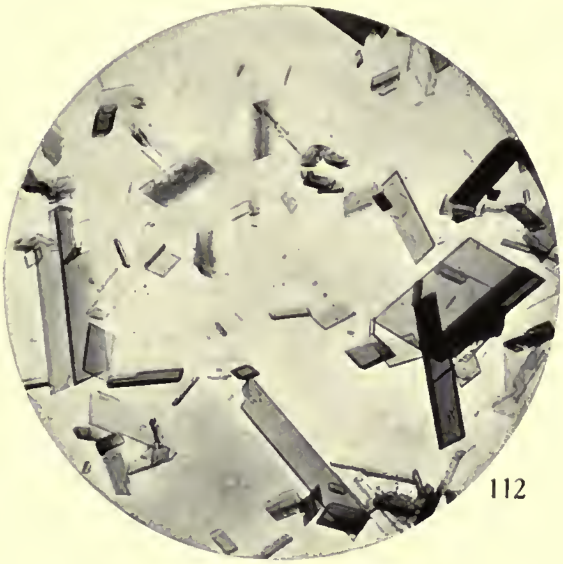
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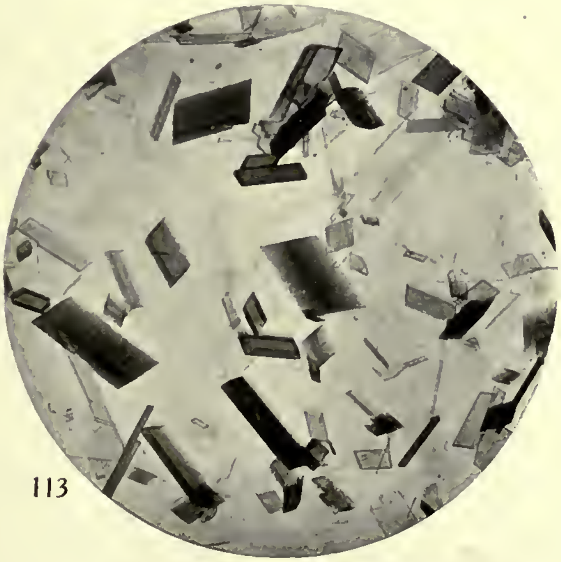
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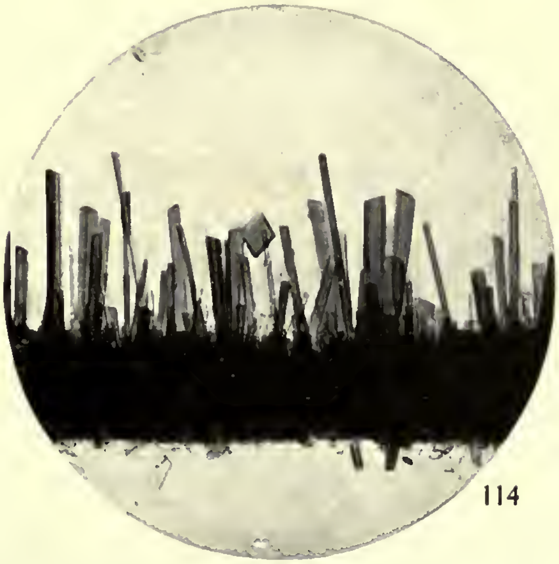
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109. Oxyhemoglobin of the Australian Cat (*Dasyurus viverrinus*), showing larger crystals, tabular on two opposite prism faces. V-shaped twin seen in one crystal.
110. Same, showing crystals in different aspects.
111, 112. Same, showing very much flattened crystals and V-shaped twin on prism.
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114. Same, showing group of long prismatic crystals from protein ring.

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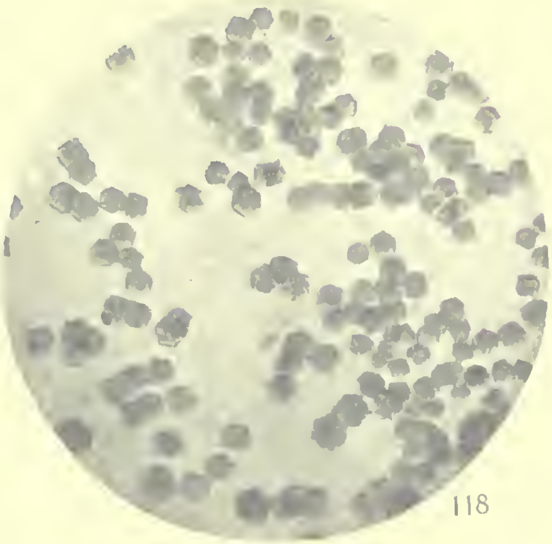
116

117



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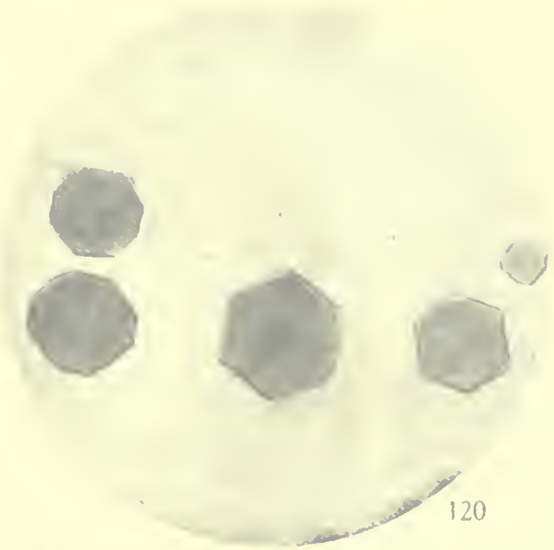

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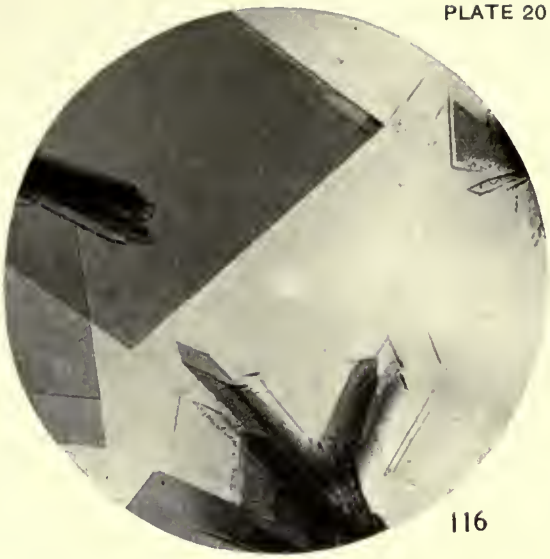
115. α-Oxyhemoglobin of the Tasmanian Wolf (*Thylacynus cynocephalus*), showing groups of plates in parallel growth.
116. Same, showing large tabular crystals, presenting basal and oblique aspects.
117. Same. showing group of crystals along protein ring, some showing edge aspect.
118. β-Oxyhemoglobin of the Tasmanian Wolf, showing small dodecahedral crystals.
119. Same, showing dodecahedron in combination with the cube.
120. Same, showing large dodecahedra flattened by the cover and some in combination with the cube.


115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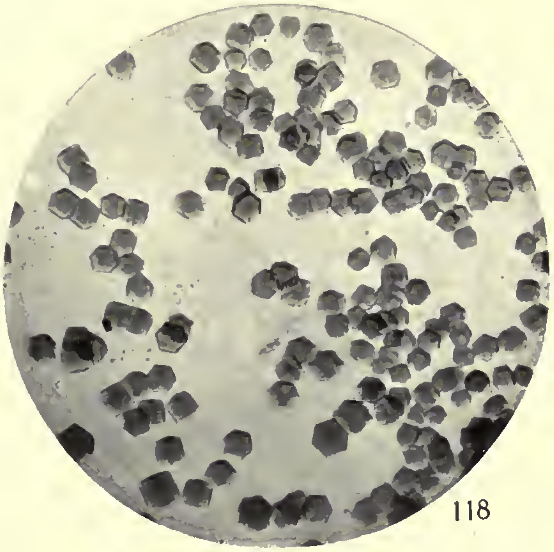
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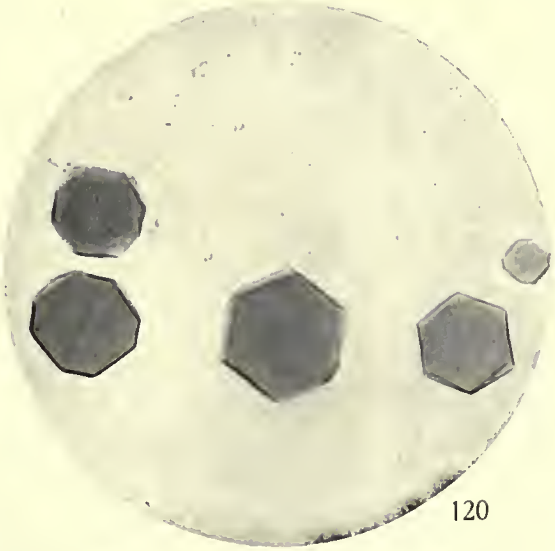
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115. α-Oxyhemoglobin of the Tasmanian Wolf (*Thylacynus cynocephalus*), showing groups of plates in parallel growth.
116. Same, showing large tabular crystals, presenting basal and oblique aspects.
117. Same, showing group of crystals along protein ring, some showing edge aspect.
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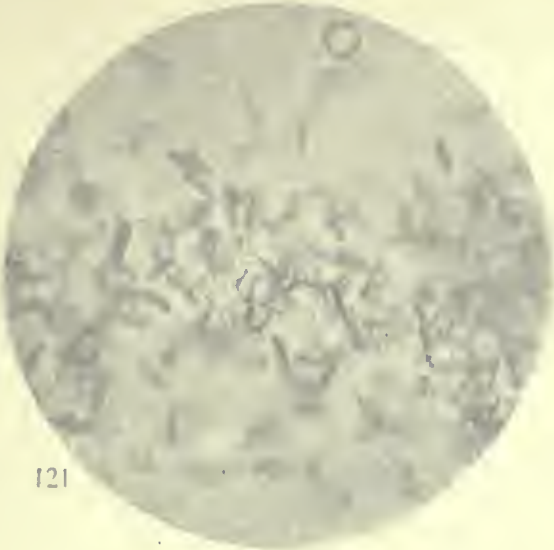
119. Same, showing dodecahedron in combination with the cube.
120. Same, showing large dodecahedra flattened by the cover and some in combination with the cube.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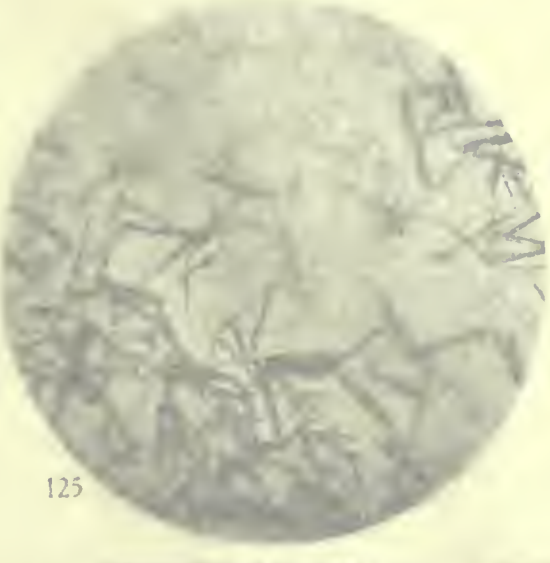
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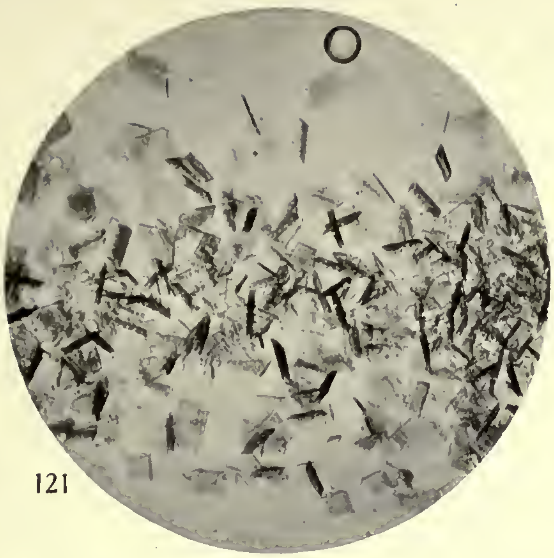
121. Ox[illegible] e V[illegible]pine Phalanger [illegible]
122. Same, [illegible] tic habit.
123. Same, [illegible] tic crystals and [illegible]
124. Same, [illegible] ls in region of [illegible]
125. Oxyhemog[illegible] -kangaroo (E[illegible] [illegible]
twinned [illegible]
126. Same, showing [illegible] d crystals, some [illegible]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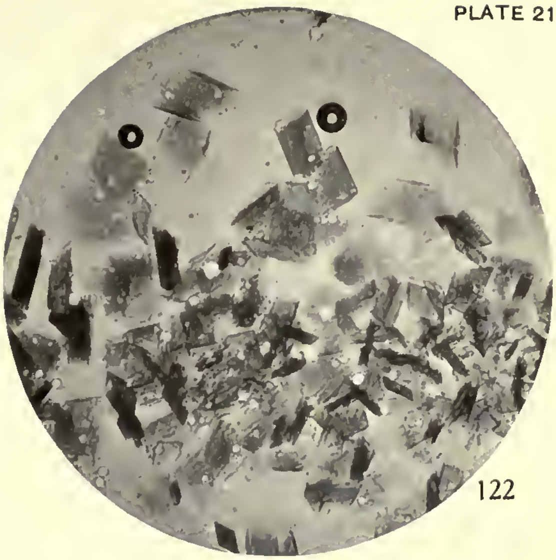
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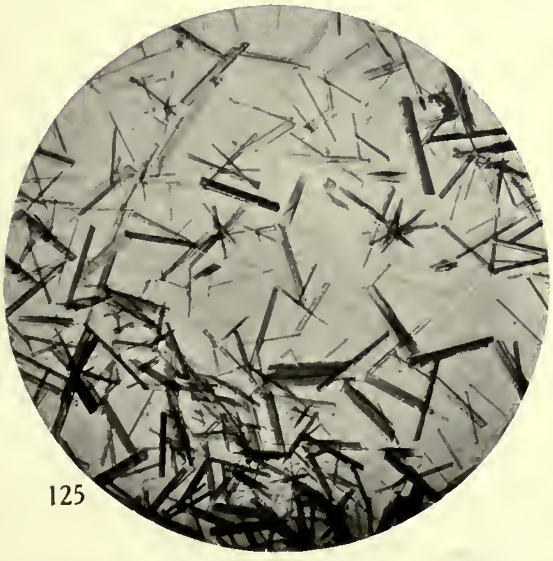
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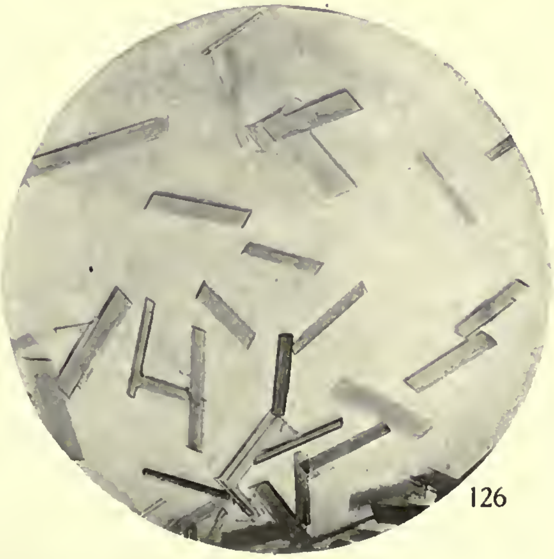
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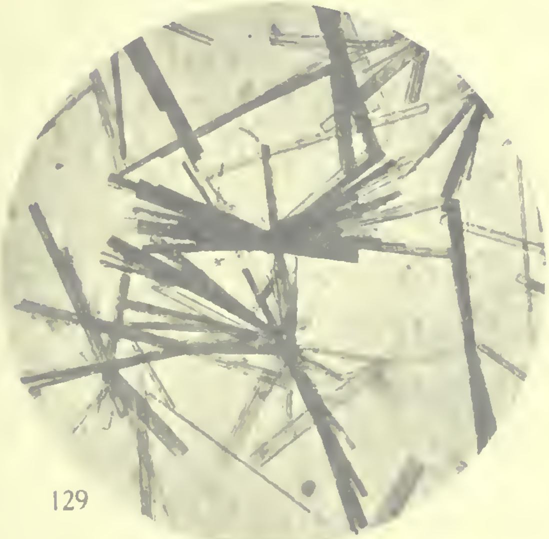
121. Oxyhemoglobin of the Vulpine Phalanger (*Trichosurus vulpecula*), showing tabular habit.
122. Same, showing short prismatic habit.
123. Same, showing long prismatic crystals and edge views of plates growing from cover edge.
124. Same, showing stout crystals in region of cover edge.
125. Oxyhemoglobin of the Rat-kangaroo (*Æpyprymnus rufescens*), showing smaller crystals, many in twinned position.
126. Same, showing medium-sized crystals, some twinned on the orthodome.


127


128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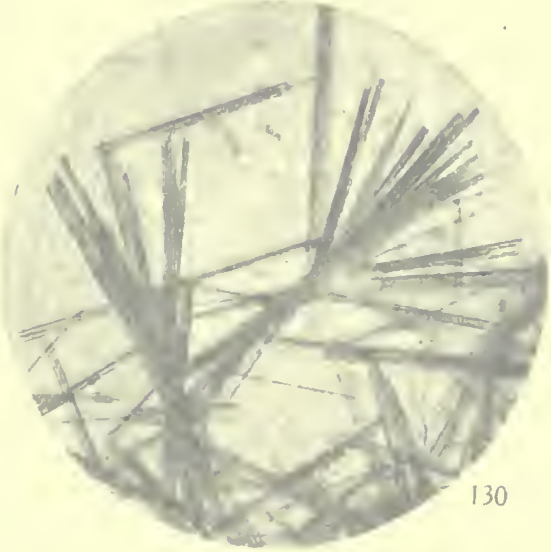
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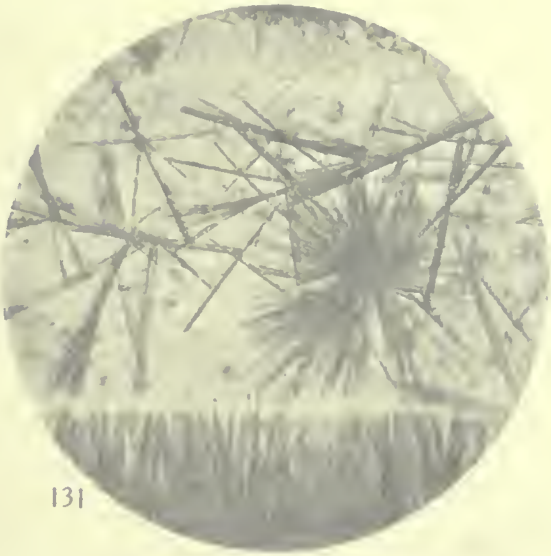
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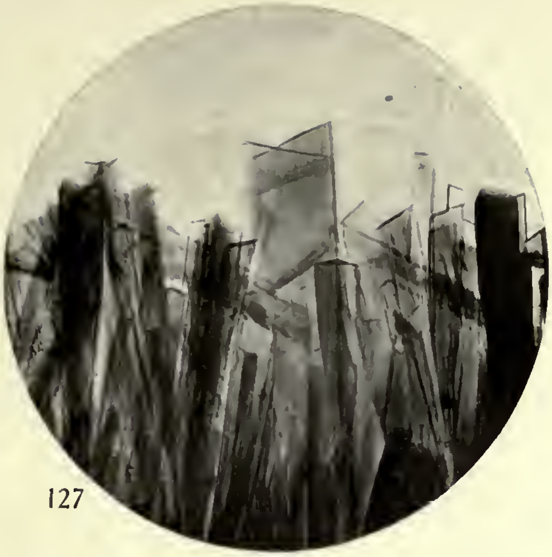
132

127. Oxyhemoglobin of the Rat-kangaroo (*Æpyprymnus rufescens*), large crystals growing from cover edge and some showing interpenetrant twin on prism in the flattened crystals.
128. Same, showing gypsum type of twin. These large crystals are flattened between cover and slide.
129, 130. Oxyhemoglobin of the Kangaroo (*Macropus giganteus*), showing lath-shaped crystals growing singly and aggregated into sheaf-like bundles.
131. Same, showing stellate group of crystals along cover edge.
132. Same, showing larger lath-shaped crystals of parallel growth.



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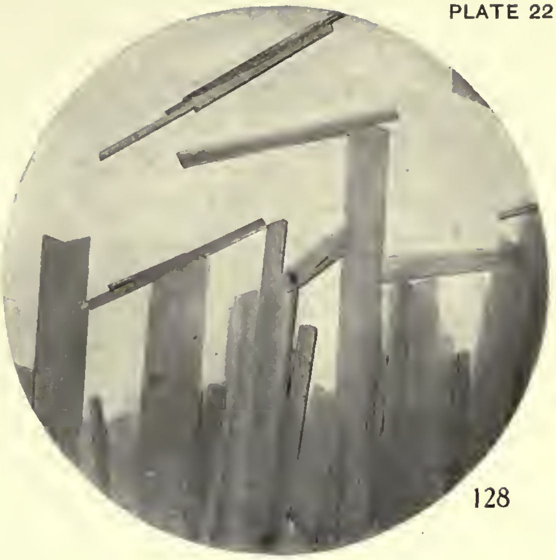

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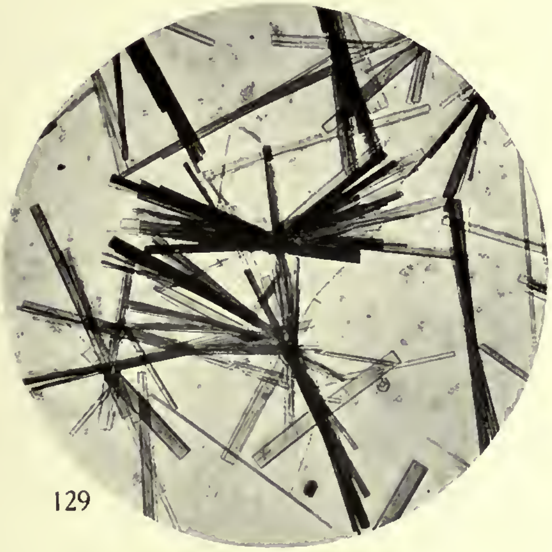

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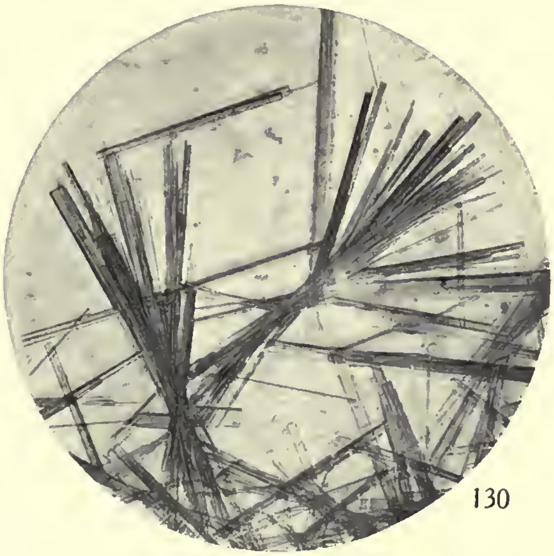

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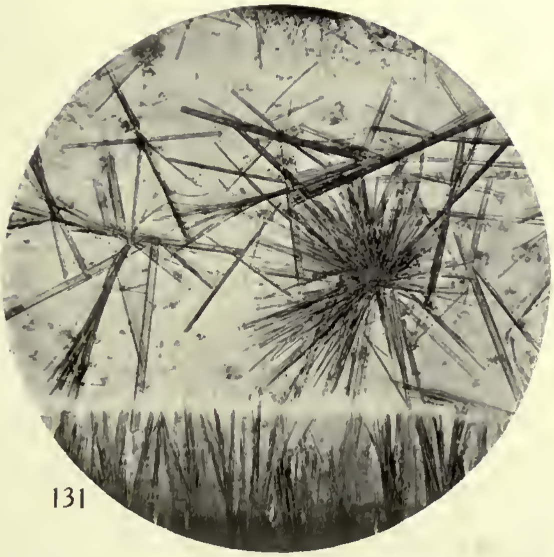

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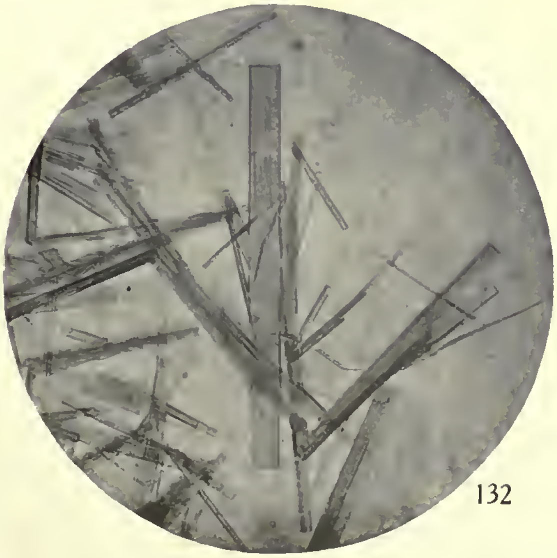

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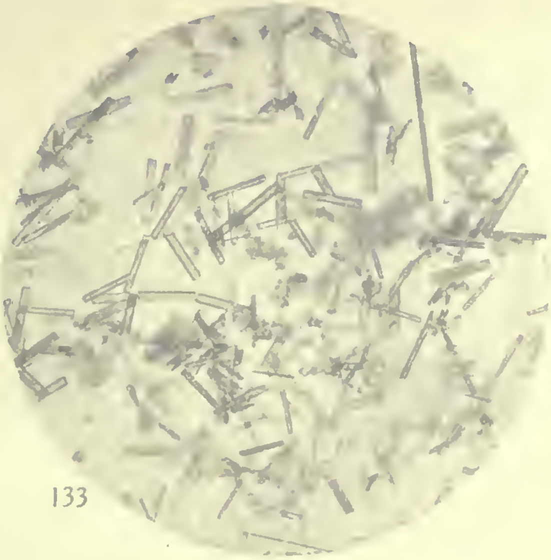
127. Oxyhemoglobin of the Rat-kangaroo (*Æpyprymnus rufescens*), large crystals growing from cover edge and some showing interpenetrant twin on prism in the flattened crystals.
128. Same, showing gypsum type of twin. These large crystals are flattened between cover and slide.
129, 130. Oxyhemoglobin of the Kangaroo (*Macropus giganteus*), showing lath-shaped crystals growing singly and aggregated into sheaf-like bundles.
131. Same, showing stellate group of crystals along cover edge.
132. Same, showing larger lath-shaped crystals of parallel growth.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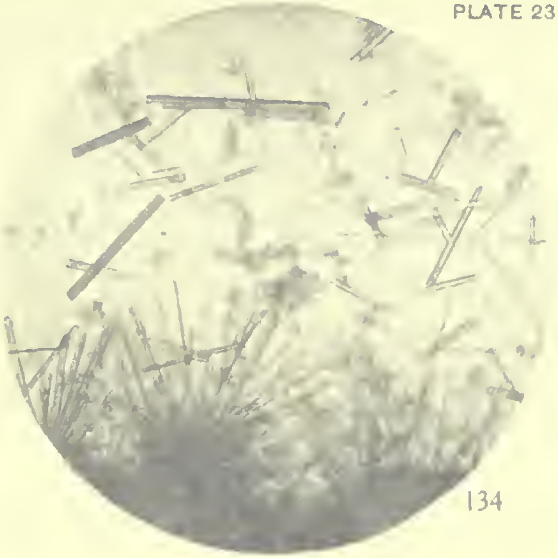
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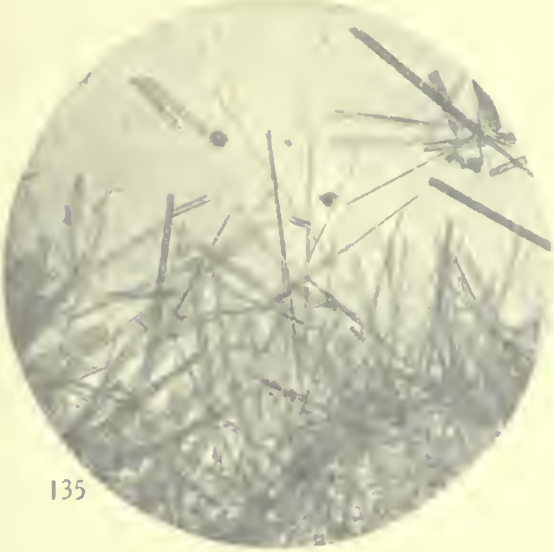
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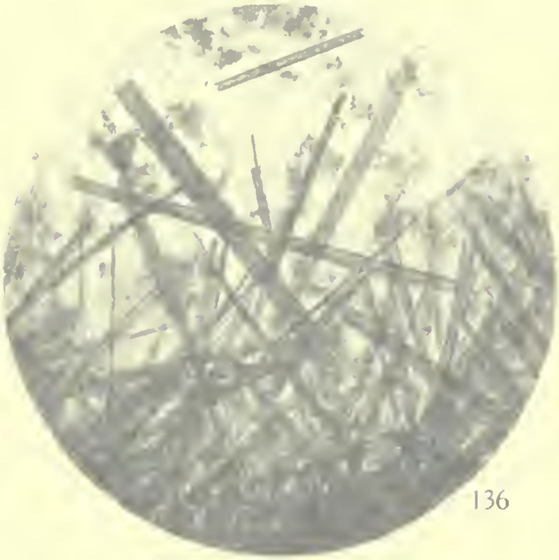
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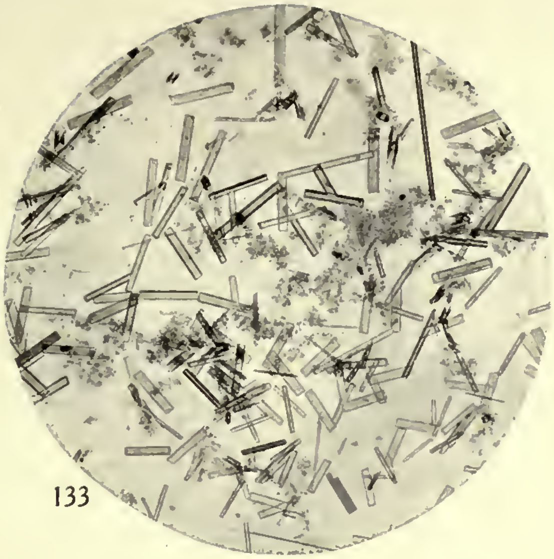
137


138

133. Oxyhemoglobin of the Rock-kangaroo (*Petrogale* sp.), showing short lath-shaped crystals.
134, 135. Same, showing long and short lath-shaped crystals and capillary crystals.
136. Same, large crystals of long, lath-shaped type, showing parallel growth.
137. Oxyhemoglobin of the Ant-eater (*Myrmecophaga?*), showing capillary crystals growing from protein ring and large lath-shaped crystals.
138. Same, showing large lath-shaped crystals growing from cover edge.

PLATE 23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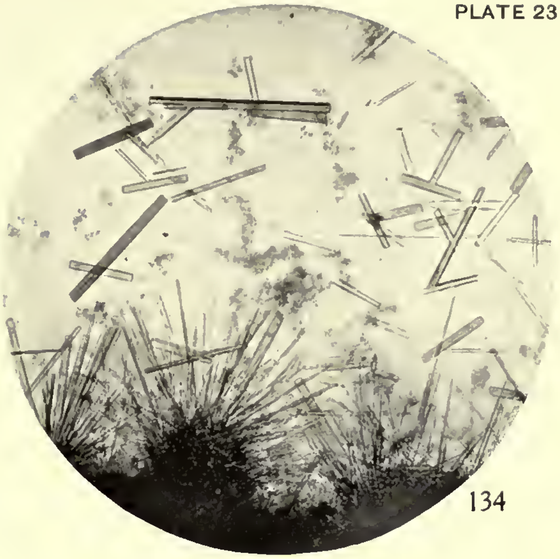
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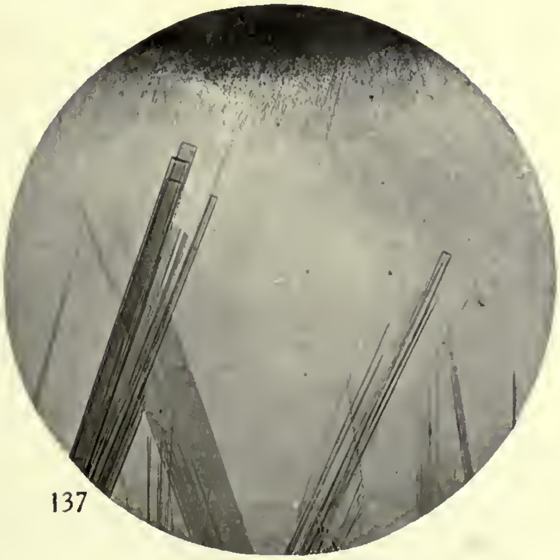
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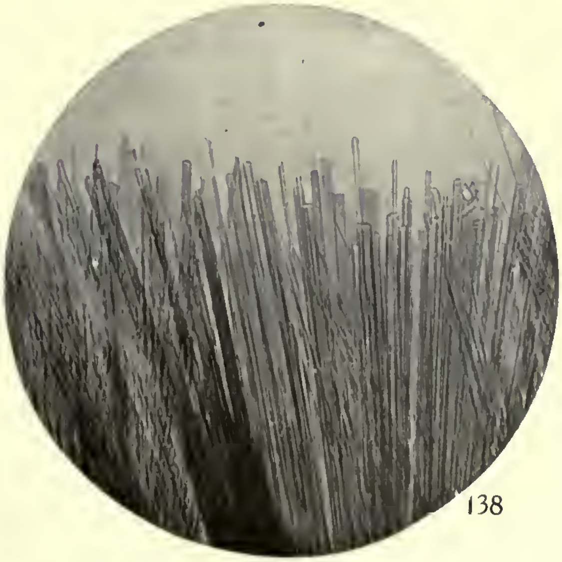
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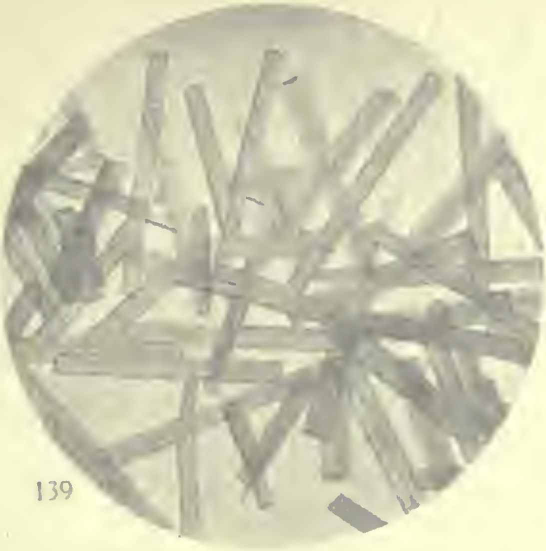
133. Oxyhemoglobin of the Rock-kangaroo (*Petrogale* sp.), showing short lath-shaped crystals.
134, 135. Same, showing long and short lath-shaped crystals and capillary crystals.
136. Same, large crystals of long, lath-shaped type, showing parallel growth.
137. Oxyhemoglobin of the Ant-eater (*Myrmecophaga ?*), showing capillary crystals growing from protein ring and large lath-shaped crystals.
138. Same, showing large lath-shaped crystals growing from cover edge.



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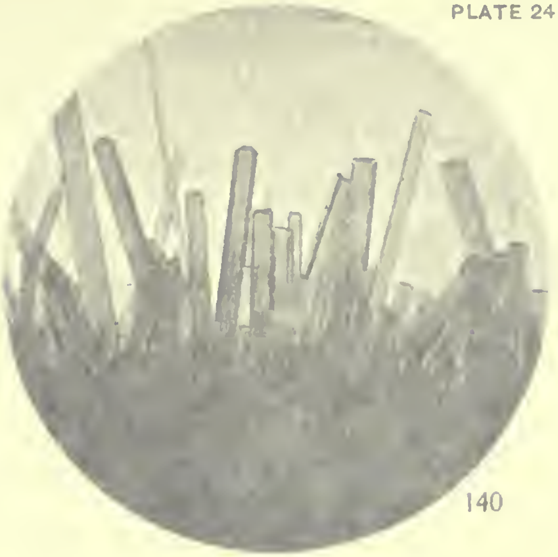

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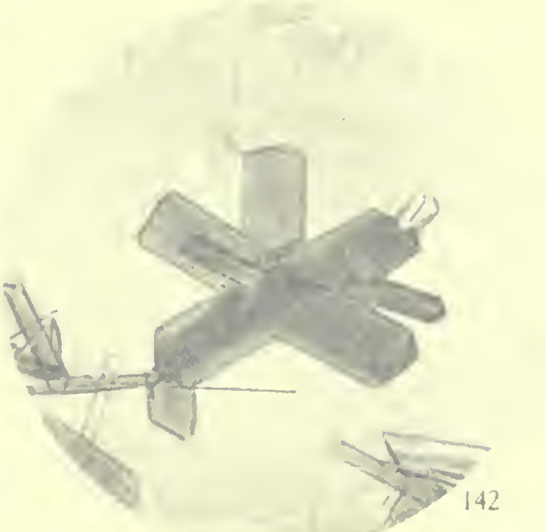

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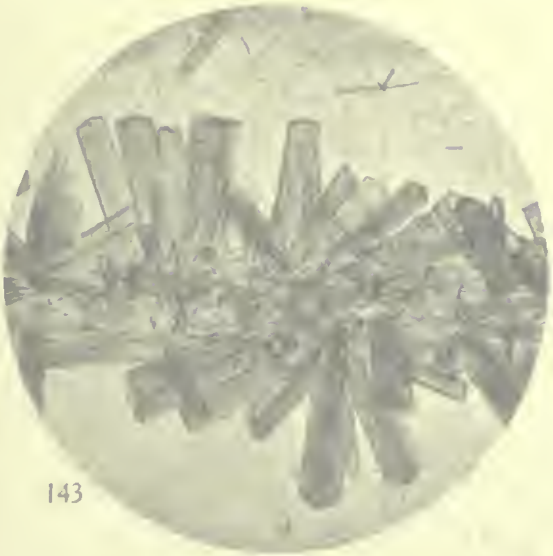
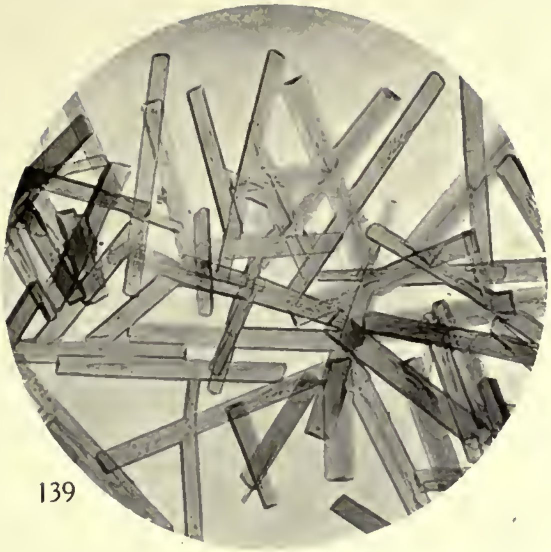


143

144

139. $\alpha$-Oxyhemoglobin of the Horse (*Equus caballus*), showing group of longer prismatic crystals (preparation without oxalate).
140. Same, showing long prismatic crystals growing from protein ring (preparation without oxalate).
141. Same, showing shorter prismatic crystals near protein ring (preparation with oxalate).
142. Same, showing twin on pyramid (preparation with oxalate). Some crystals of the $\beta$-oxyhemoglobin are seen near edge of field.
143, 144 Same, showing stout prismatic crystals along protein ring and tufts of first-formed capillary crystals (preparation with oxalate).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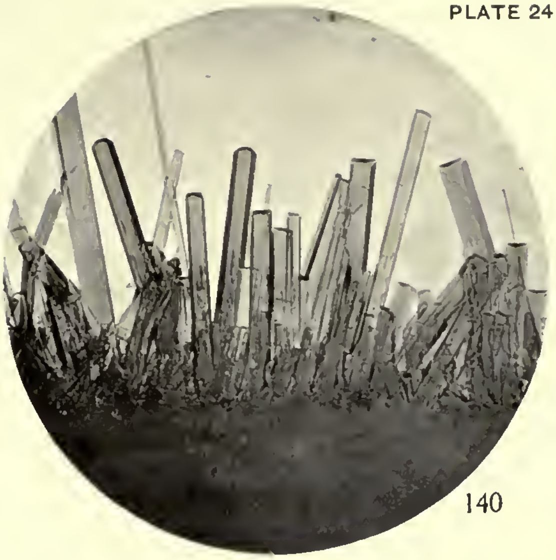
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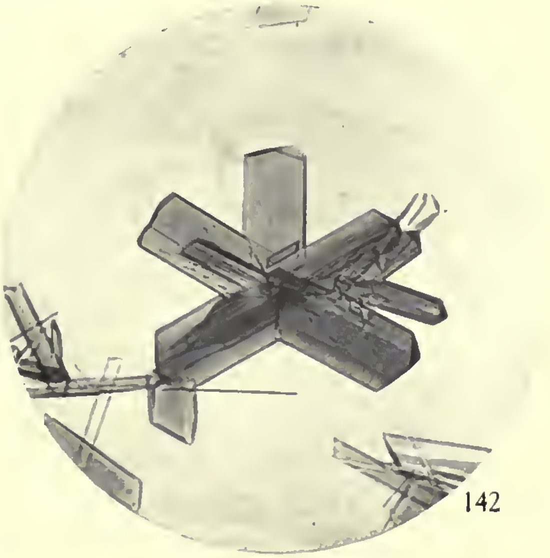
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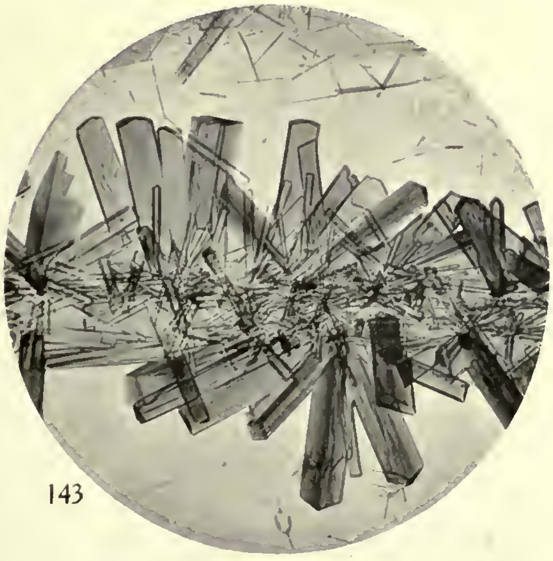
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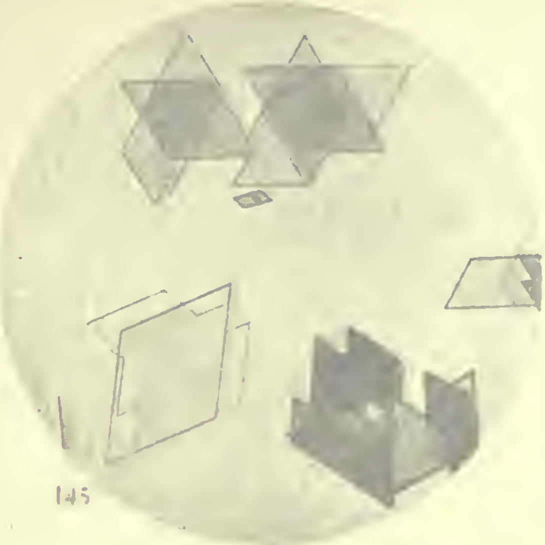
139. $\alpha$-Oxyhemoglobin of the Horse (*Equus caballus*), showing group of longer prismatic crystals (preparation without oxalate).
140. Same, showing long prismatic crystals growing from protein ring (preparation without oxalate).
141. Same, showing shorter prismatic crystals near protein ring (preparation with oxalate).
142. Same, showing twin on pyramid (preparation with oxalate). Some crystals of the $\beta$-oxyhemoglobin are seen near edge of field.
143, 144. Same, showing stout prismatic crystals along protein ring and tufts of first-formed capillary crystals (preparation with oxalate).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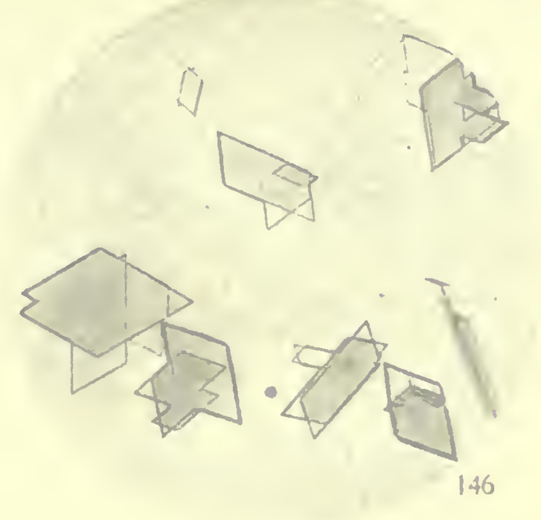
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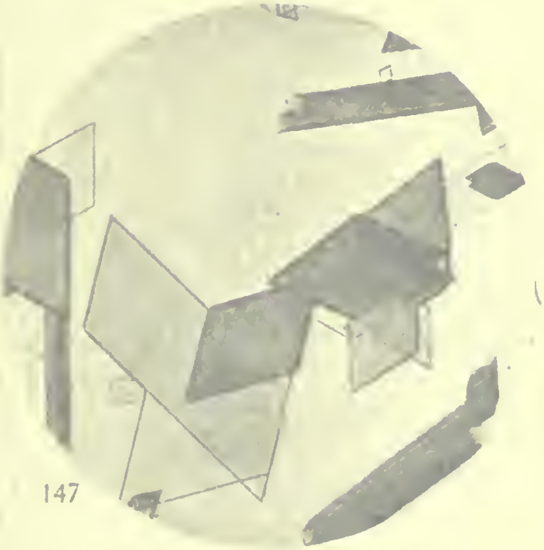
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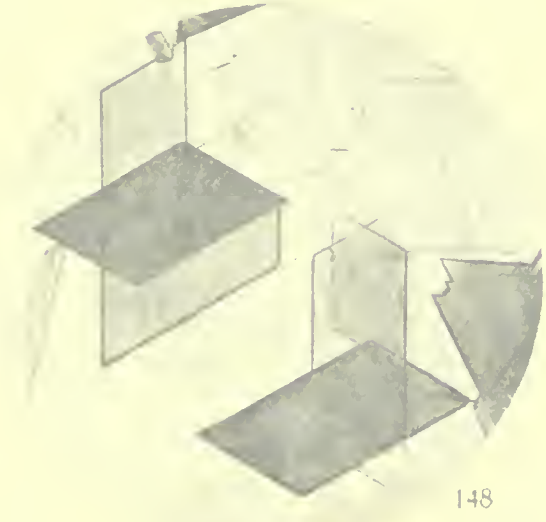
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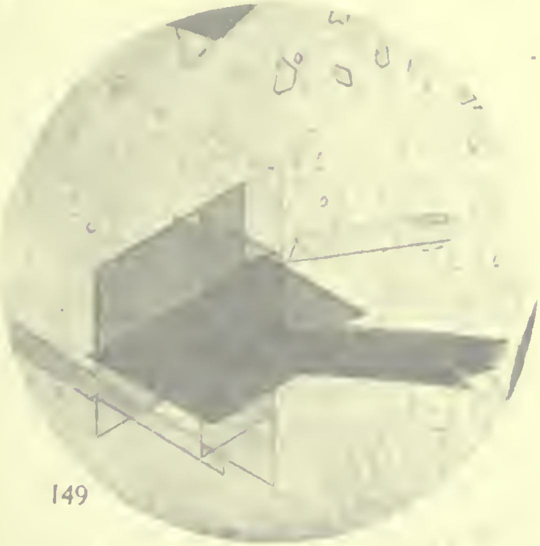
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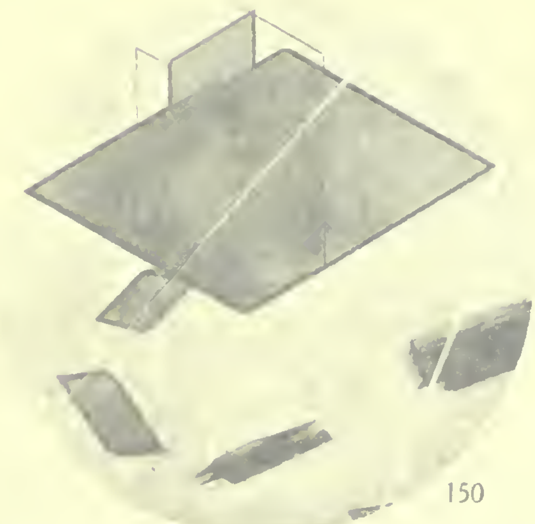
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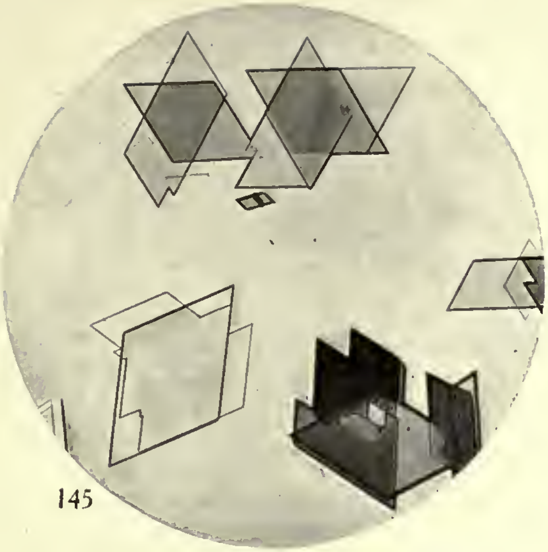
145. β-Oxyhemoglobin of the Horse (*Equus caballus*), showing horse-type twins. Twin on the right above consists of four individuals, that on the left above, of two individuals.
146. Same, showing small composite horse-type twins.
147. Same, showing large composite horse-type twins, some seen in edge view.
148. Same, showing irregular composite horse-type twins.
149. Same, showing large composite twinned group and elongated horse-type twin on right.
150. Same, showing single crystals and large twinned group, with smaller twins on edge.

PLATE 25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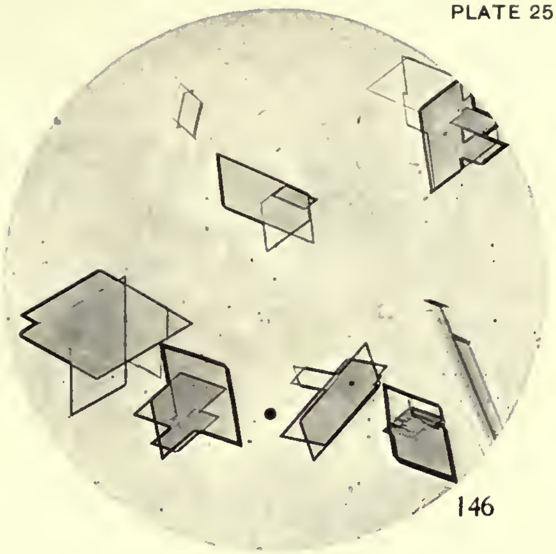
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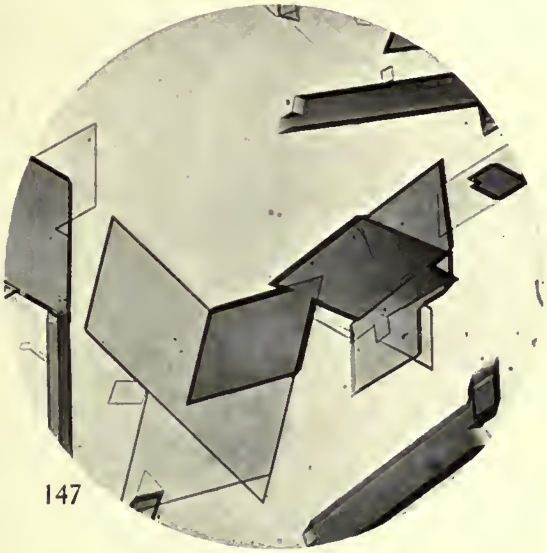
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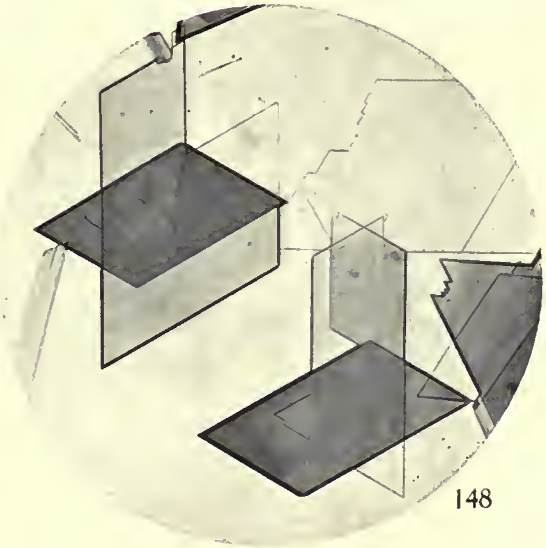
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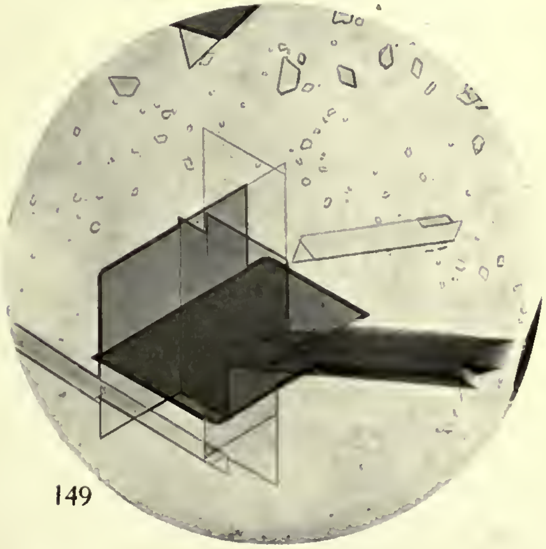
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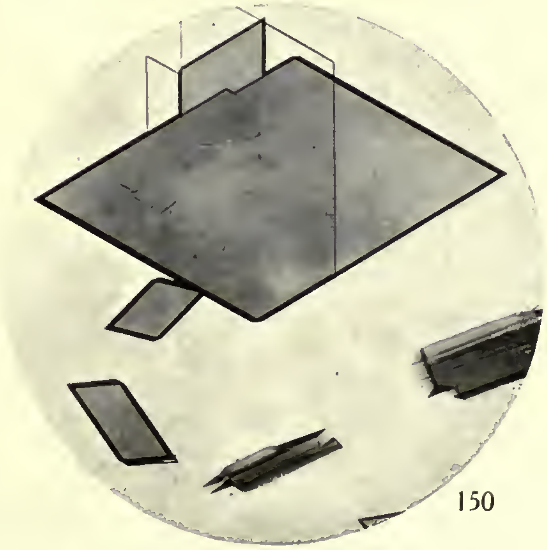
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145. β-Oxyhemoglobin of the Horse (*Equus caballus*), showing horse-type twins. Twin on the right above consists of four individuals, that on the left above, of two individuals.
146. Same, showing small composite horse-type twins.
147. Same, showing large composite horse-type twins, some seen in edge view.
148. Same, showing irregular composite horse-type twins.
149. Same, showing large composite twinned group and elongated horse-type twin on right.
150. Same, showing single crystals and large twinned group, with smaller twins on edge.


151


152


153

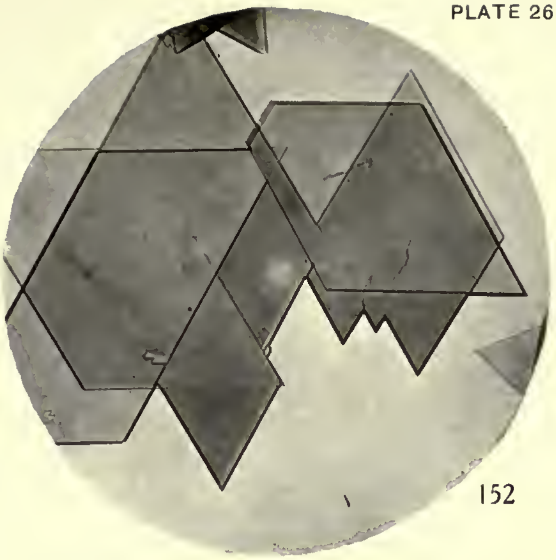

154


155


156

151. α-Oxy[illegible] of the [illegible] showing the [illegible] crystals. The [illegible] type twin
152. β-Oxyhem[illegible] of the [illegible] ortho[illegible]
153. α-CO-Hem[illegible] of the Horse, showing long prismatic crystals [illegible]
154. Same, showing short prismatic crystals growing from [illegible]
155. β-CO-Hem[illegible] of the Horse, showing composite [illegible]
156. Same, showing elongated [illegible]-type twin.


151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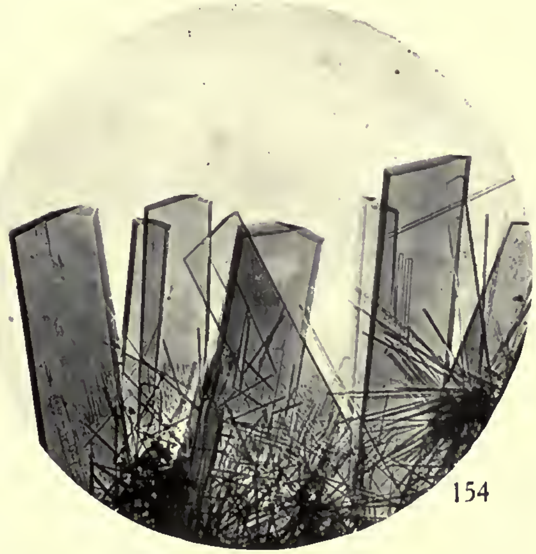
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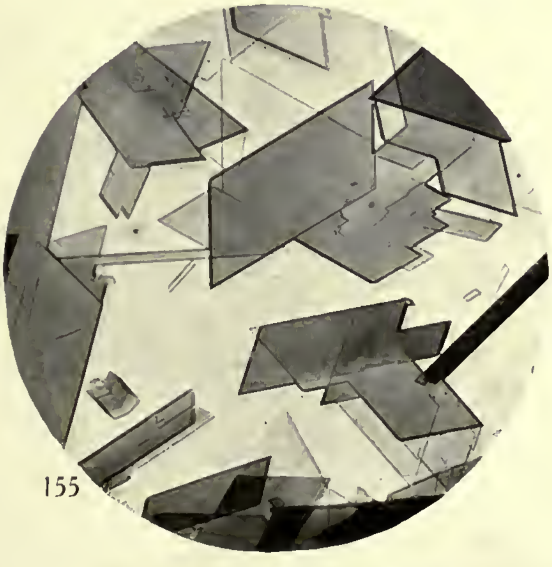
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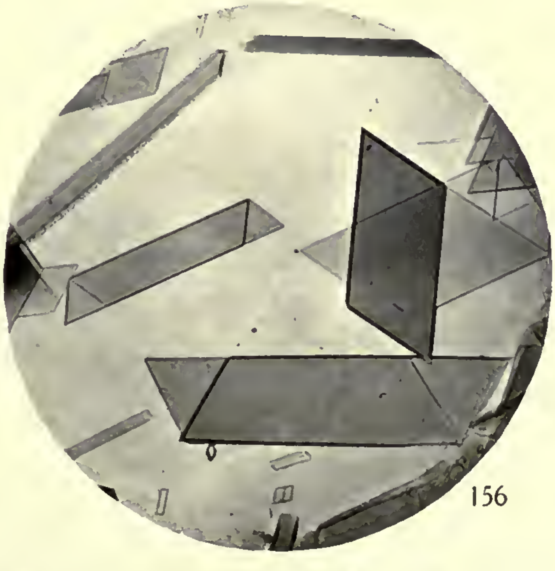
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151. α-Oxyhemoglobin and β-Oxyhemoglobin of the Horse (*Equus caballus*), showing the two in large crystals. The α-oxyhemoglobin shows twin on pyramid, the β-oxyhemoglobin shows usual horse-type twin.
152. β-Oxyhemoglobin of the Horse, showing large twinned group. Large crystal above to right shows orthopinacoid on one end.
153. α-CO-Hemoglobin of the Horse, showing long prismatic crystals growing from protein ring.
154. Same, showing stout prismatic crystals growing from protein ring and also capillary crystals.
155. β-CO-Hemoglobin of the Horse, showing composite twins of horse-type in various aspects.
156. Same, showing elongated horse-type twin.


157


158


159


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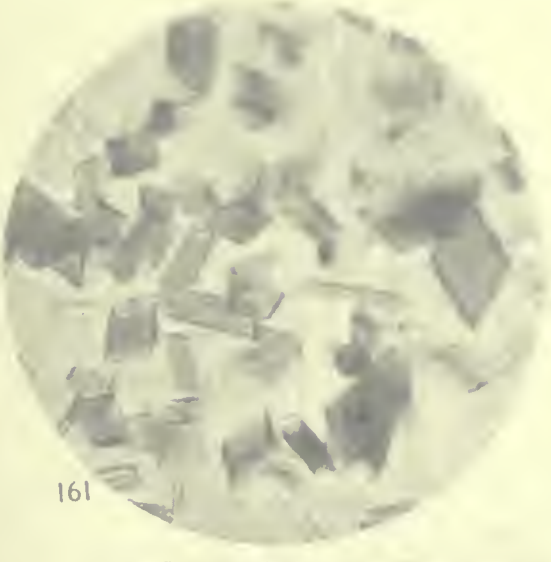
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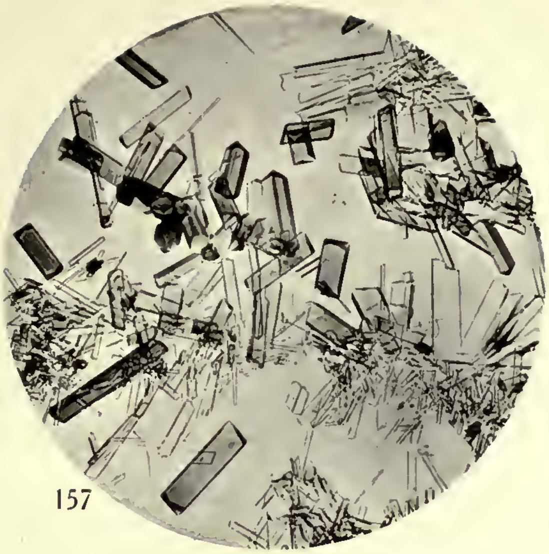
162

157. α-Oxyhemoglobin of the Mule (*Equus asinus* ♂ × *Equus caballus* ♀), showing short prismatic crystals.
158. Same, showing long prismatic crystals growing from protein ring.
159, 160. Same, showing large stout prismatic crystals growing along cover edge.
161. β-Oxyhemoglobin of the Mule, showing equidimensional development of crystals, mostly untwinned.
162. Same, showing elongated horse-type twins.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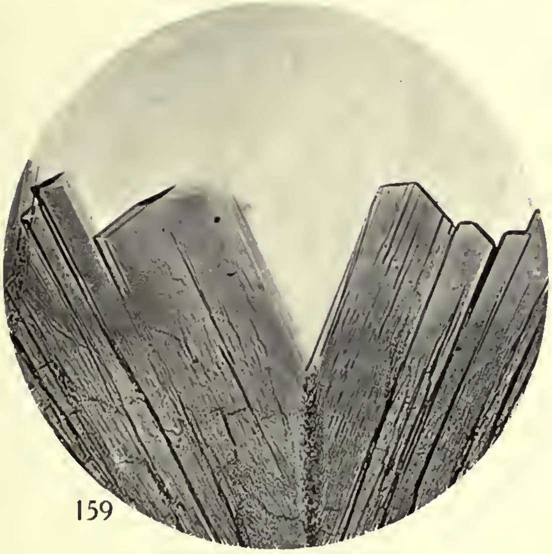
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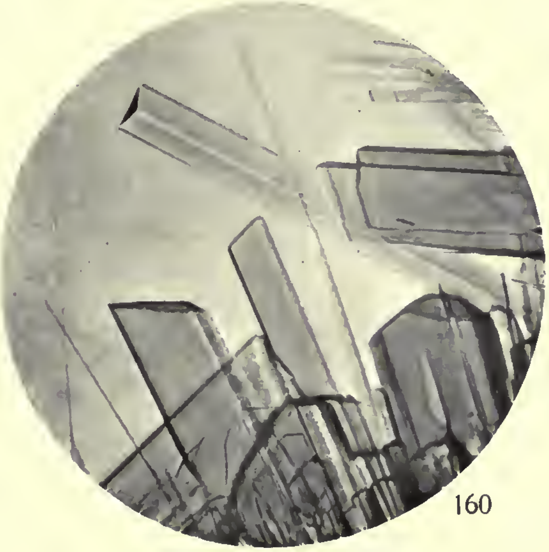
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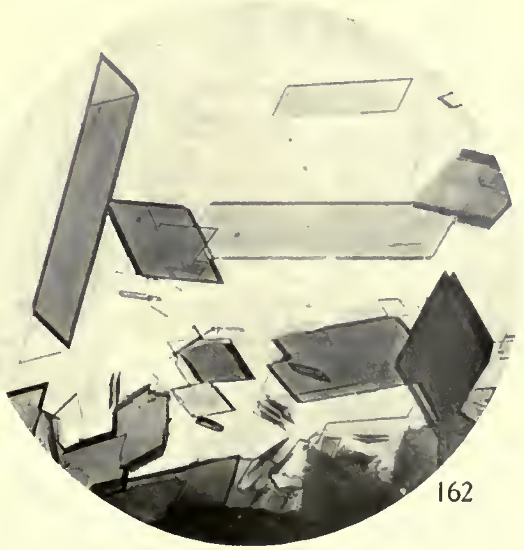
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157. *a*-Oxyhemoglobin of the Mule (*Equus asinus* ♂ × *Equus caballus* ♀), showing short prismatic crystals.
158. Same, showing long prismatic crystals growing from protein ring.
159, 160. Same, showing large stout prismatic crystals growing along cover edge.
161. *β*-Oxyhemoglobin of the Mule, showing equidimensional development of crystals, mostly untwinned.
162. Same, showing elongated horse-type twins.

PLATE 28

163

164

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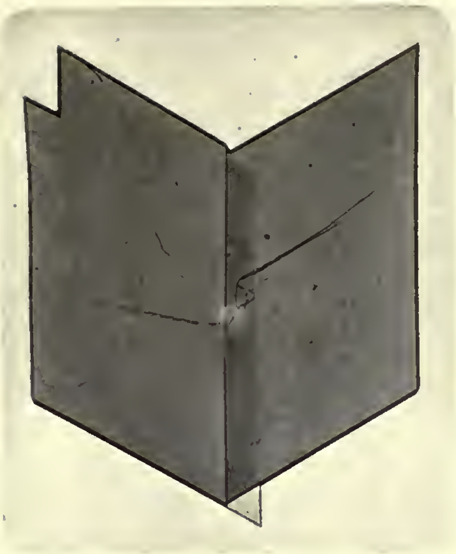
163. β-Oxyhemoglobin of the Mule (*Equus* [illegible]
164. Same, showing large horse-type twin with [illegible]
     prism-base edge.
165. Same, showing large horse-type twin in various aspects.
166. Same, showing smaller horse-type twins.
167. Same, showing large elongated horse-type twins.
168. Same, showing large horse-type twins.

PLATE 28


163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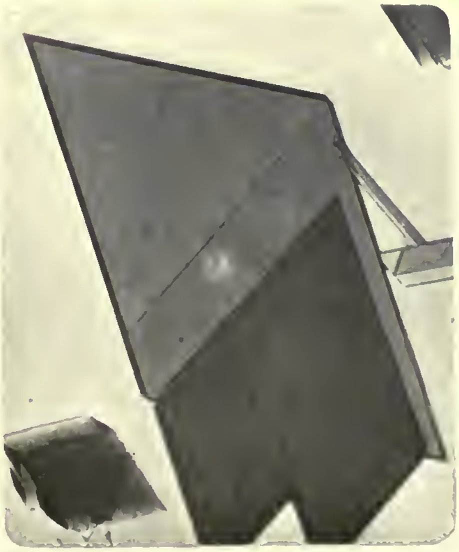
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163. β-Oxyhemoglobin of the Mule (*Equus asinus* ♂ × *Equus caballus* ♀), showing a large single crystal.
164. Same, showing large horse-type twin with composition face normal to base and including common prism-base edge.
165. Same, showing large horse-type twin in various aspects.
166. Same, showing smaller horse-type twins.
167. Same, showing large elongated horse-type twins.
168. Same, showing large horse-type twins.


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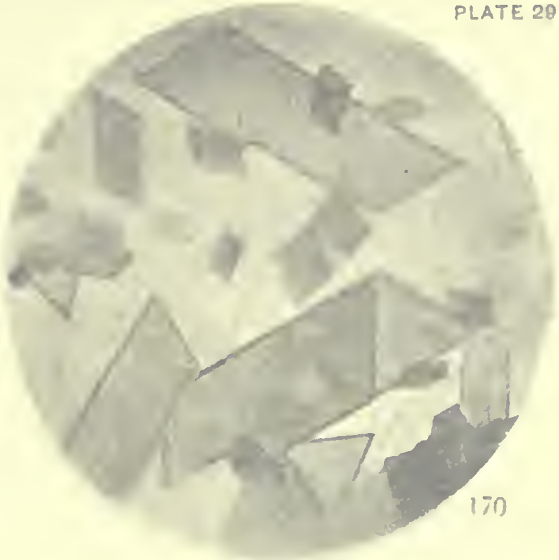
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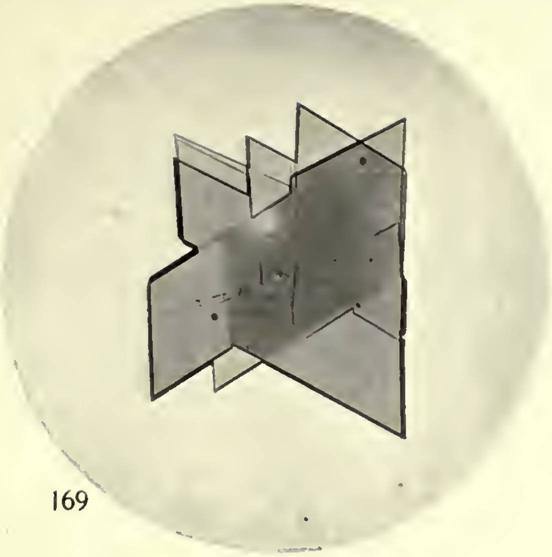
169. β-Oxyhemoglobin of the Mule (*Equus asinus* ♂ × *Equus caballus* ♀), showing composite twin of horse-type.
170. Same, showing prismatic and tabular types of crystals.
171, 172. β-CO-Hemoglobin of the Mule, showing composite horse-type twins and [illegible] [illegible] type.
173. Same, showing elongated horse-type twin.
174. α-CO-Hemoglobin and β-CO-Hemoglobin of the Mule, showing [illegible] of α-CO-hemoglobin crystals growing from protein ring, and elongated horse-type twin of β-CO-hemoglobin.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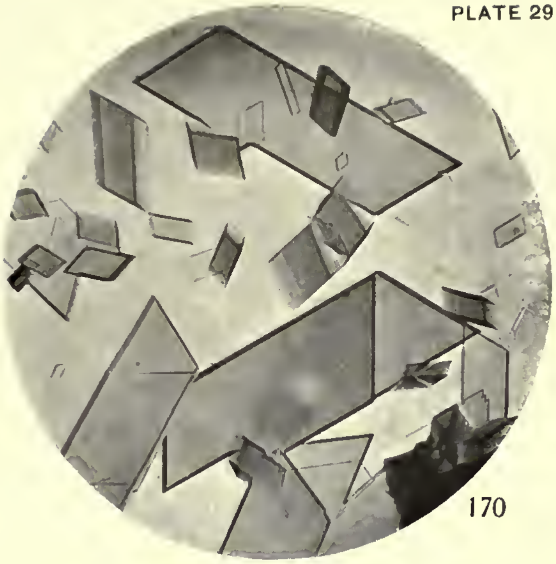
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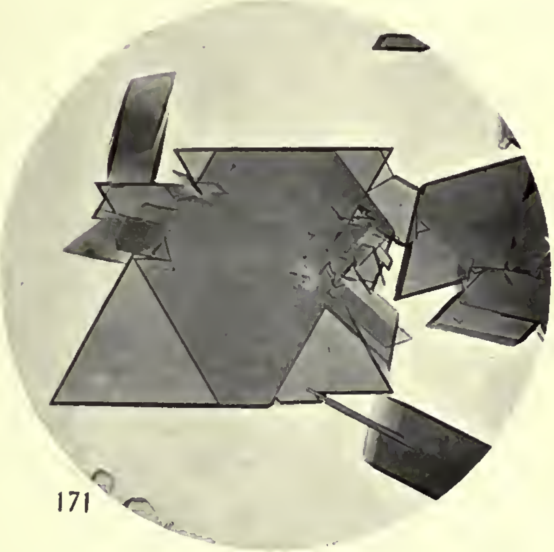
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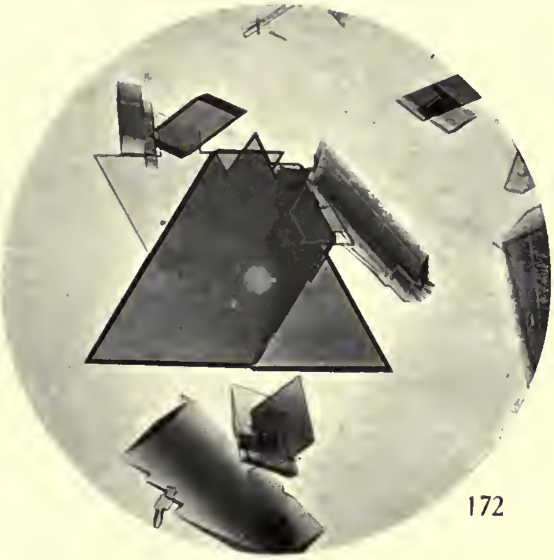
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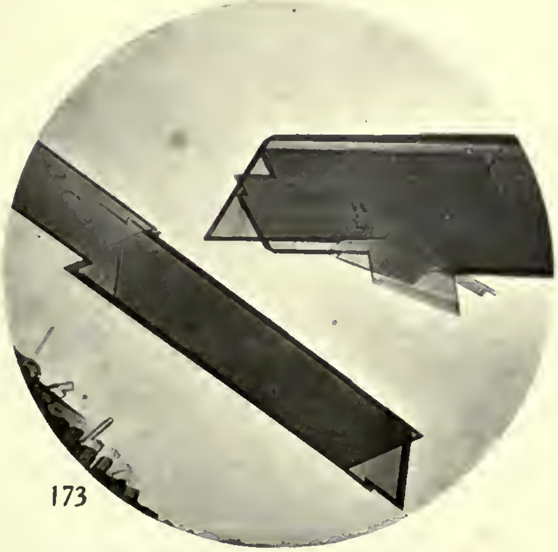
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174

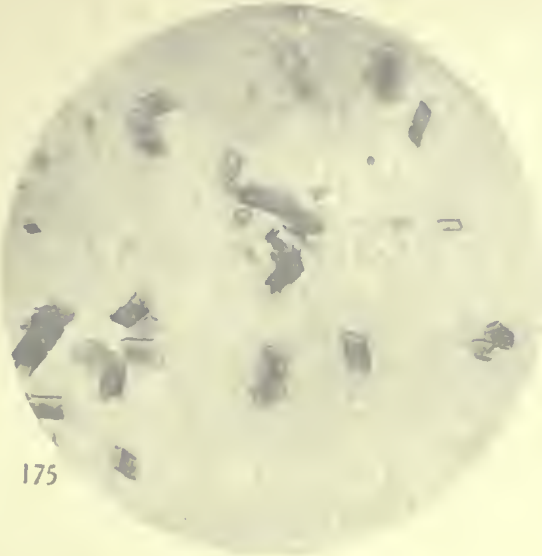
169. β-Oxyhemoglobin of the Mule (*Equus asinus* ♂ × *Equus caballus* ♀), showing composite twin of horse-type.

170. Same, showing prismatic and tabular types of crystals.

171, 172. β-CO-Hemoglobin of the Mule, showing composite horse-type twins and simple crystals, normal type.

173. Same, showing elongated horse-type twin.

174. α-CO-Hemoglobin and β-CO-Hemoglobin of the Mule, showing ends of α-CO-hemoglobin crystals growing from protein ring, and elongated horse-type twin of β-CO-hemoglobin.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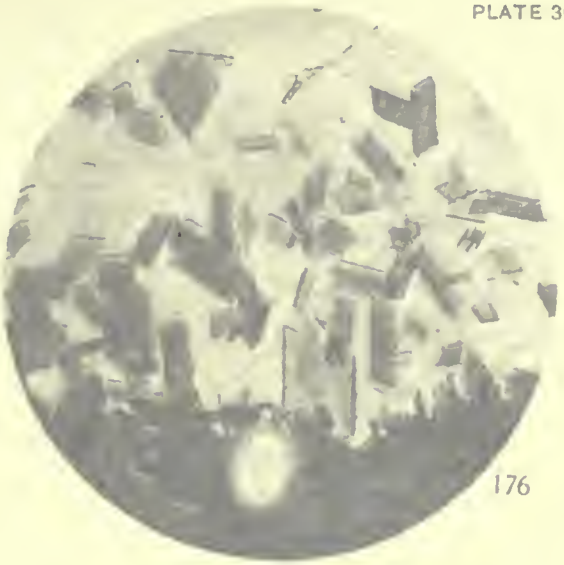
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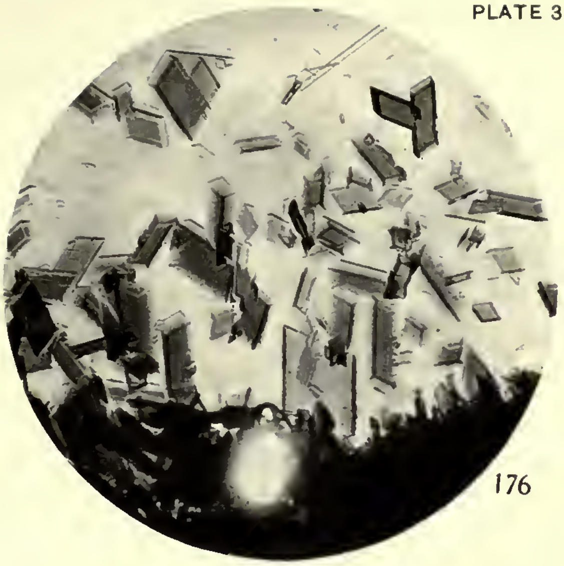
175. Oxyhemoglobin of the Hippopotamus (*Hippopotamus amphibius*), showing smaller prismatic [illegible] crystals, some tabular, some resembling rhombohedra and some longer prismatic.
176. Same, showing smaller prismatic type of crystals, some twinned.
177. Same, showing trapezoidal form of horse-type twin.
178. Same, showing large twinned aggregates.
179, 180. Same, showing apparently hemimorphic crystals produced by elongated union of trapezoidal horse-type.

175



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175. Oxyhemoglobin of the Hippopotamus (*Hippopotamus amphibius*), showing smaller prismatic untwinned crystals, some tabular, some resembling rhombohedra and some longer prismatic.
176. Same, showing smaller prismatic type of crystals, some twinned.
177. Same, showing trapezoidal form of horse-type twin.
178. Same, showing large twinned aggregates.
179, 180. Same, showing apparently hemimorphic crystals produced by elongated twins of trapezoidal horse-type.

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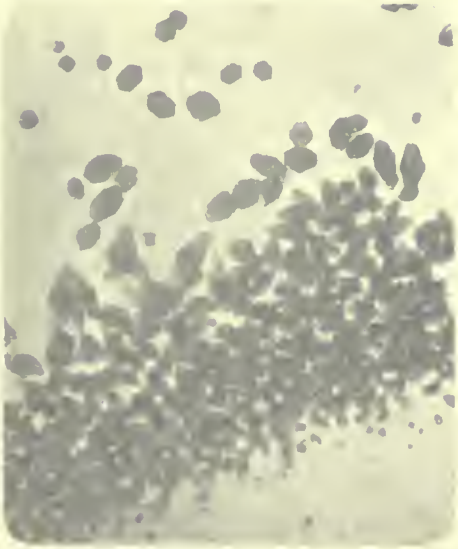

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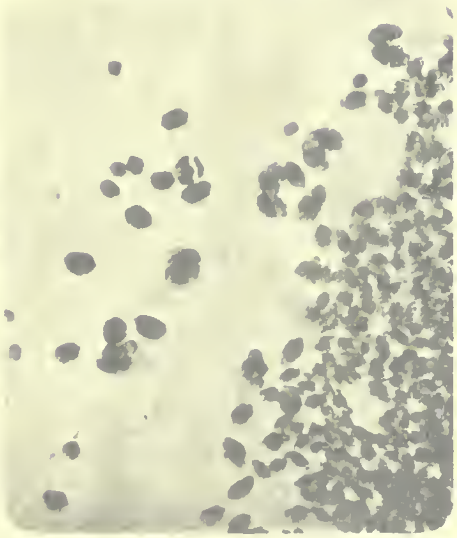

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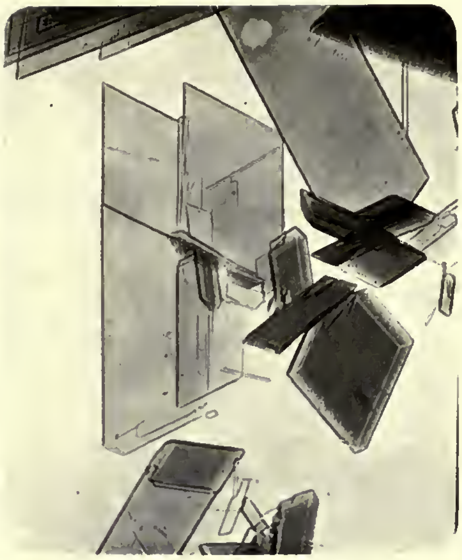
181, 182. Oxyhemoglobin of the Hippopotamus (*Hippopotamus amphibius*), showing groups of large twinned crystals.

183. Same, showing single large twinned group of horse-type twin.

184. Same, showing twin aggregates and ladder-like form produced by twinning.

185, 186. Oxyhemoglobin of the Peccary (*Dicotyles labiatus*), showing dodecahedron-like combination of short diametral prism with unit pyramid.


181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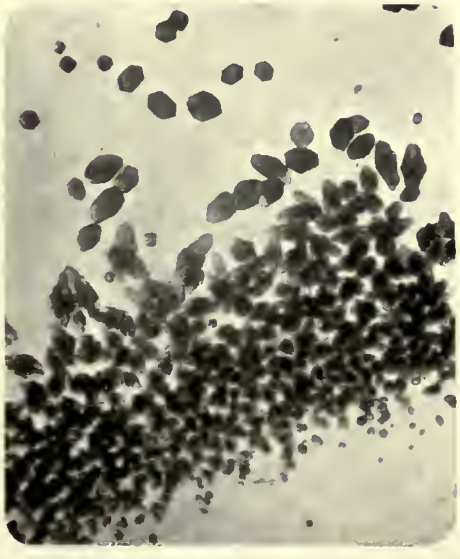
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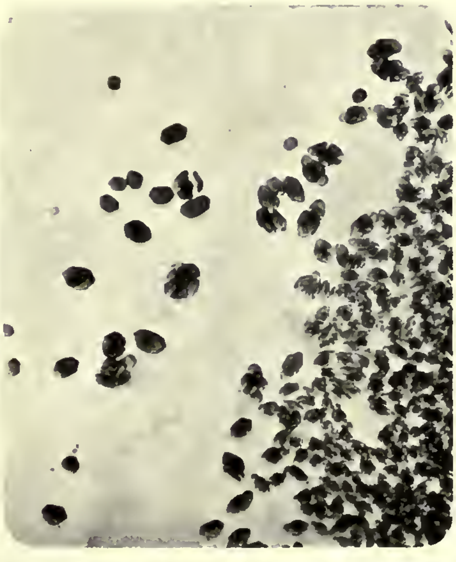
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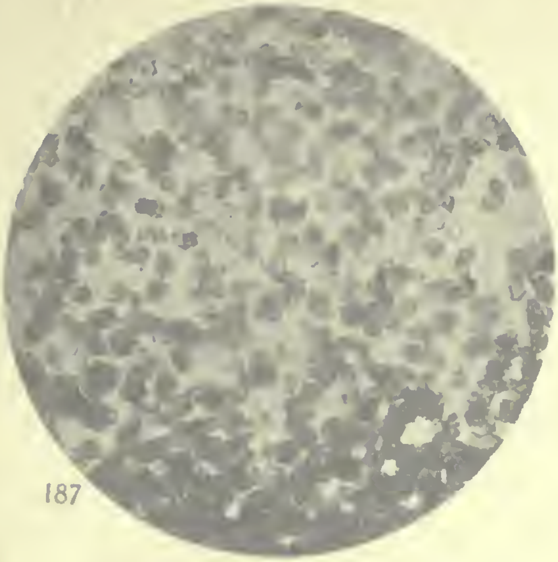
181, 182. Oxyhemoglobin of the Hippopotamus (*Hippopotamus amphibius*), showing groups of large twinned crystals.

183. Same, showing single large twinned group of horse-type twin.

184. Same, showing twin aggregates and ladder-like form produced by twinning.

185, 186. Oxyhemoglobin of the Peccary (*Dicotyles labiatus*), showing dodecahedron-like combination of short diametral prism with unit pyramid.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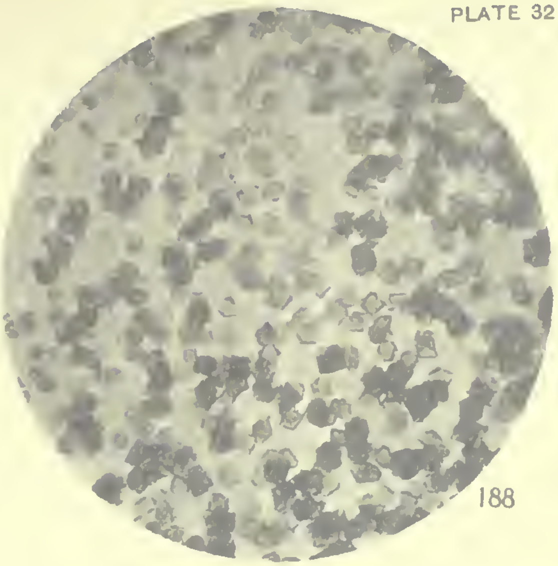
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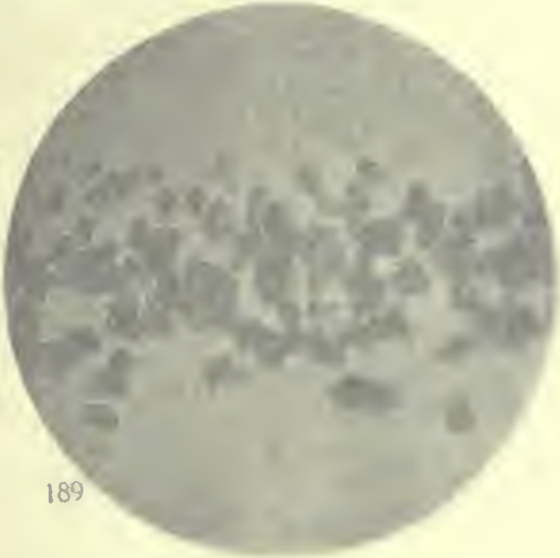
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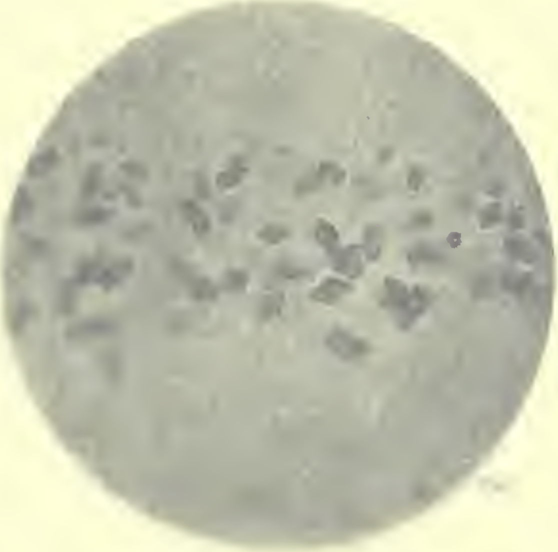
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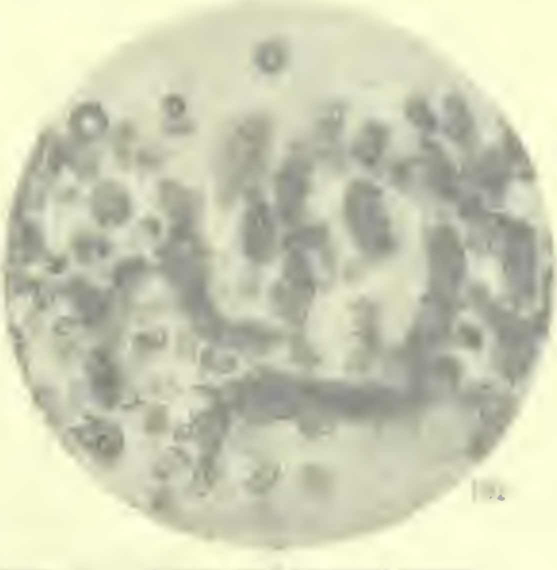
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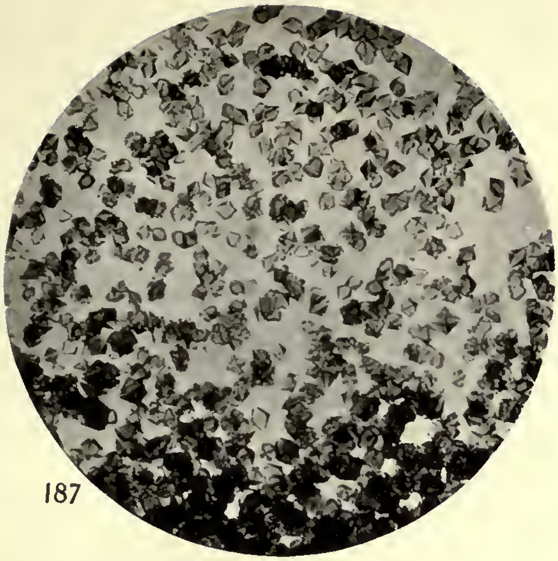
187, 188. Oxy[illegible] of the Collared Peccary (*D[illegible] ta[illegible]acu*), sh[illegible] [illegible].
189, 190. Oxy[illegible] of the Pig (*Sus scrofa*), showing single c[illegible] [illegible] br[illegible] growing in protein ring
191. Same, showing large crystals in various orientations.
192 Same, showing group of crystals and aggregates from protein ring.



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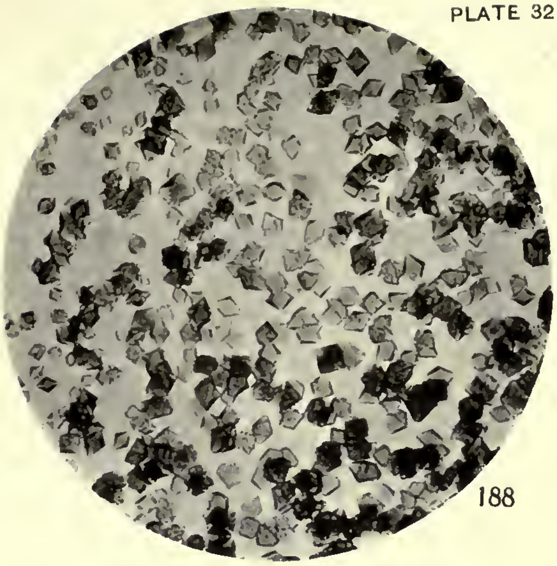

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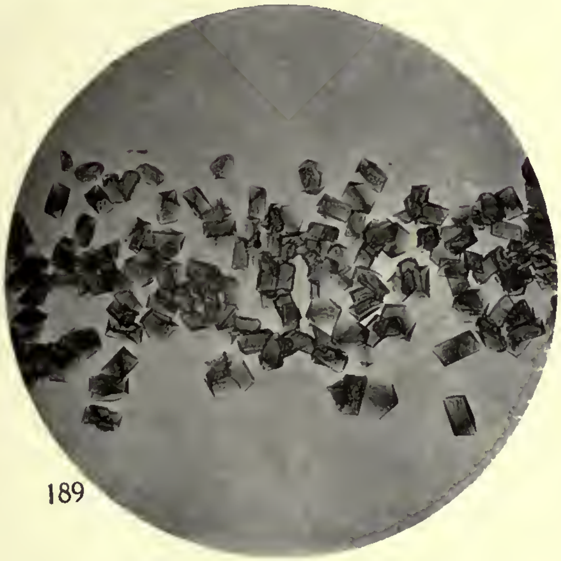

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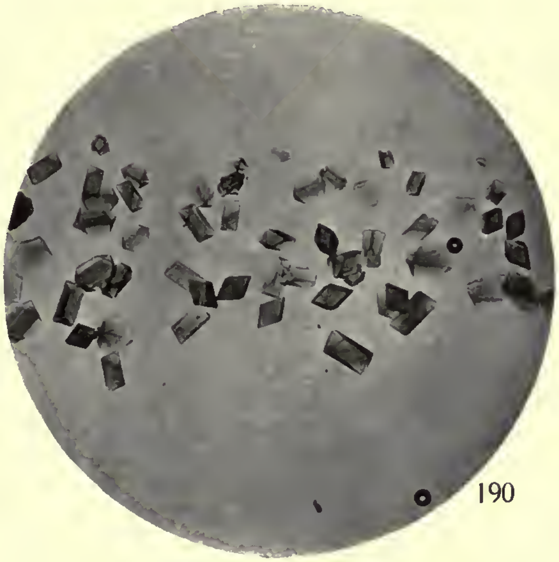

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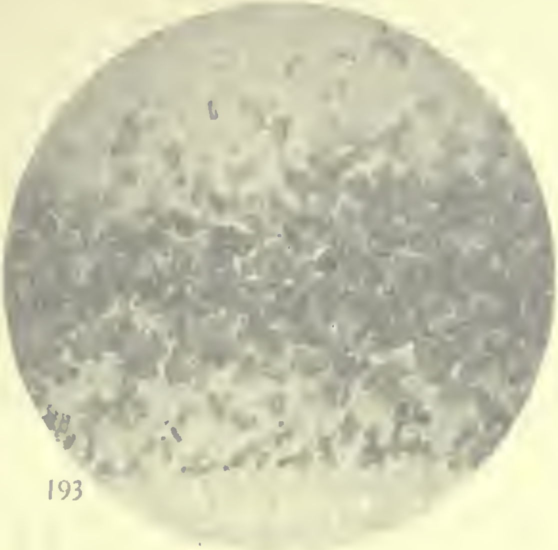
187, 188. Oxyhemoglobin of the Collared Peccary (*Dicotyles tajacu*), showing small pyramidal crystals.
189, 190. Oxyhemoglobin of the Pig (*Sus scrofa*), showing single crystals consisting of unit prism and brachydome, growing in protein ring.
191. Same, showing large crystals in various orientations.
192. Same, showing group of crystals and aggregates from protein ring.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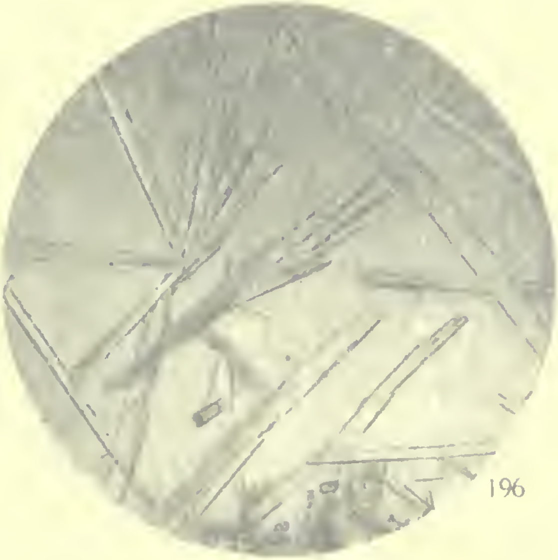
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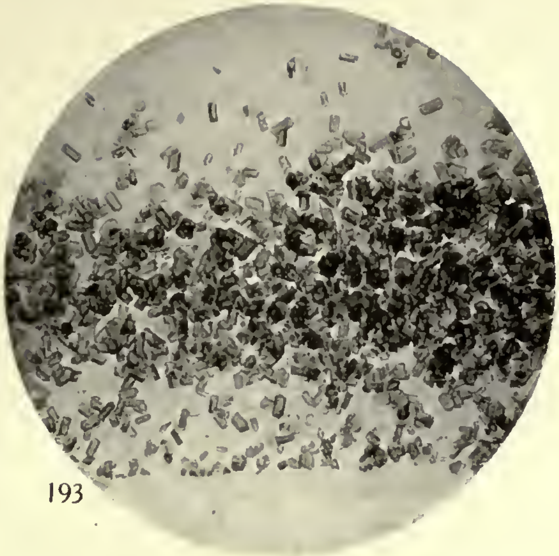
193. Oxyhemoglobin of the Pig (*Sus scrofa*), showing small crystals from protein ring
194-196. Reduced Hemoglobin of the Pig, showing long prismatic crystals some terminated by base, growing in sheaf-like groups and sometimes twinned.
197. Same, showing thick and thin prismatic crystals at basal termination.
198. Same, showing feathery groups of crystals.

PLATE 33



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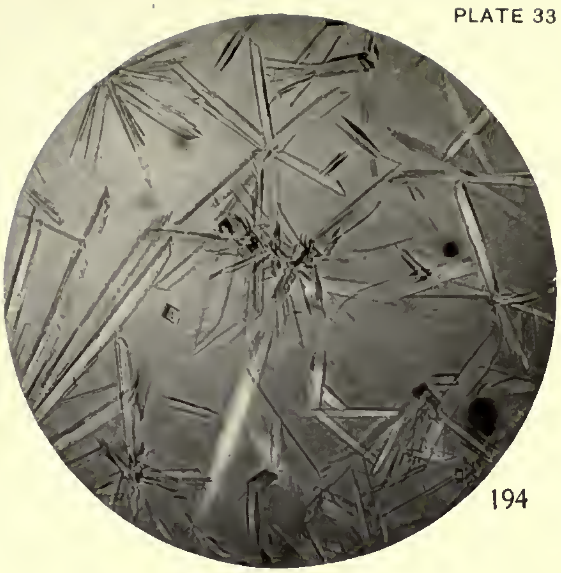

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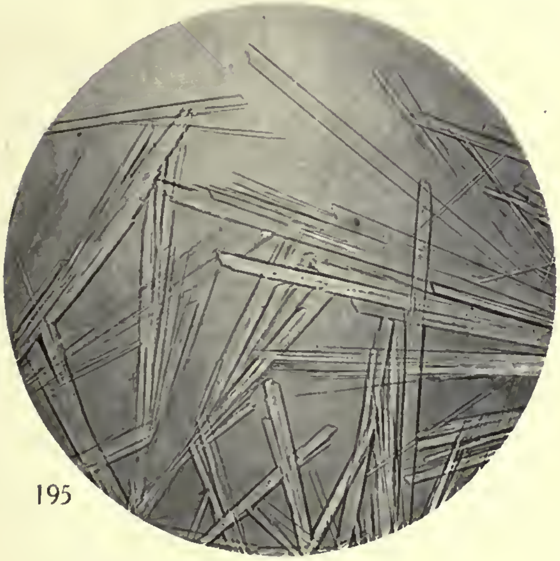

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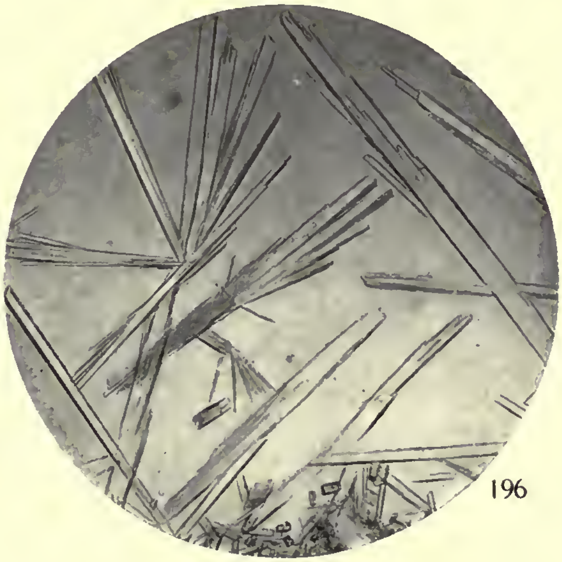

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193. Oxyhemoglobin of the Pig (*Sus scrofa*), showing small crystals from protein ring.
194-196. Reduced Hemoglobin of the Pig, showing long prismatic crystals, some terminated by base, growing in sheaf-like groups and sometimes twinned.
197. Same, showing thick and thin prismatic crystals at basal termination.
198. Same, showing feathery groups of crystals.

199

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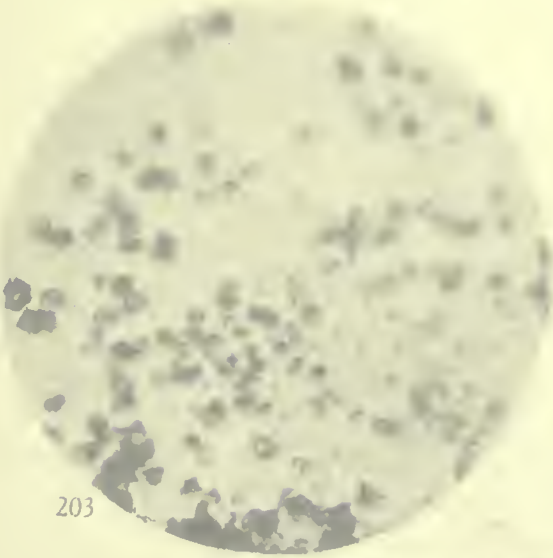

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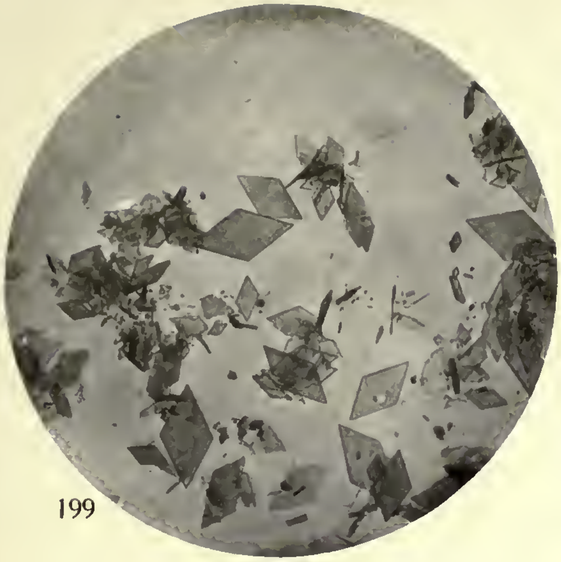
204

199. Oxyhæmoglobin of the Muis Deer (*Tragulus* [illegible]), showing [illegible].
200, 201. Same, showing [illegible]-type twin.
202. Reduced Hæmoglobin of the Muis Deer, showing part of very large [illegible].
203, 204. Oxyhæmoglobin of the Elk (*Cervus canadensis*), showing [illegible].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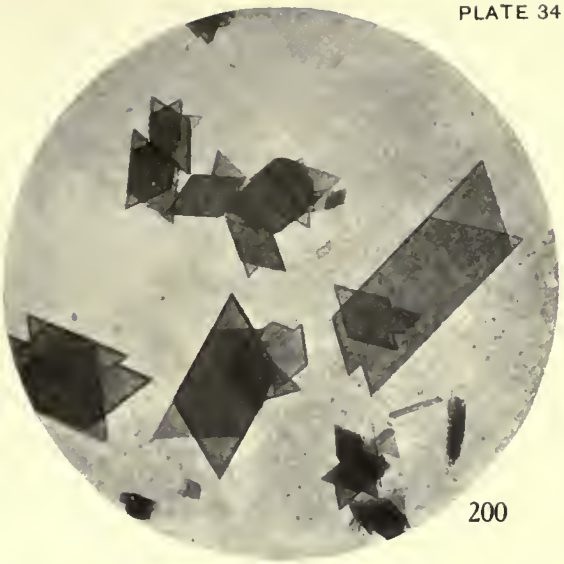
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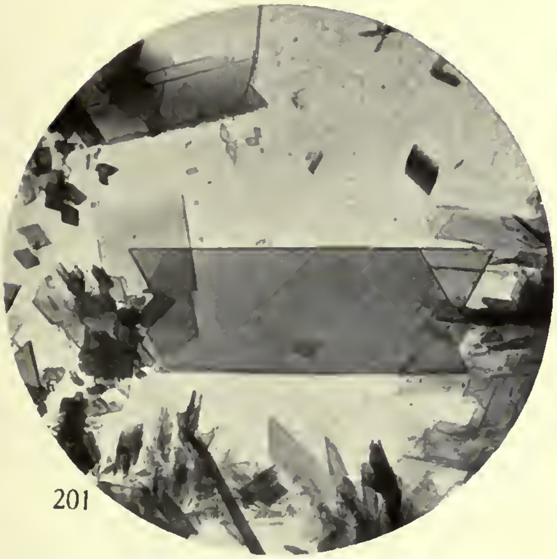
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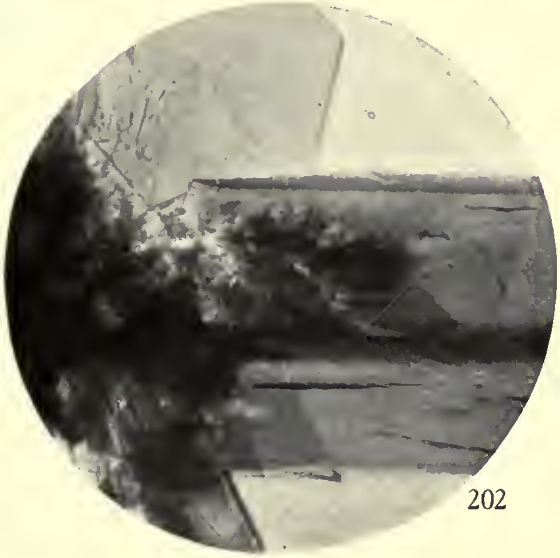
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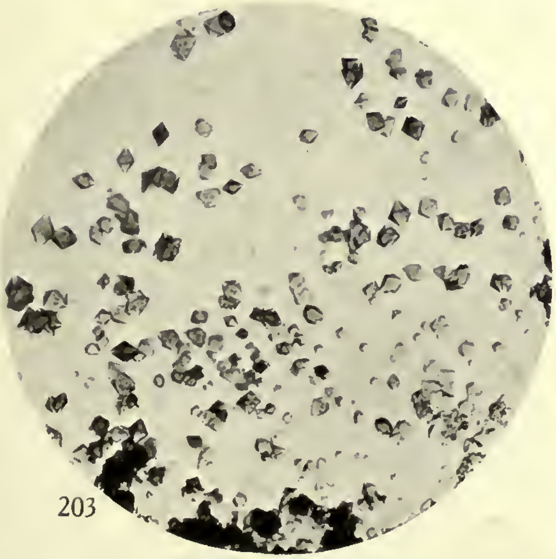
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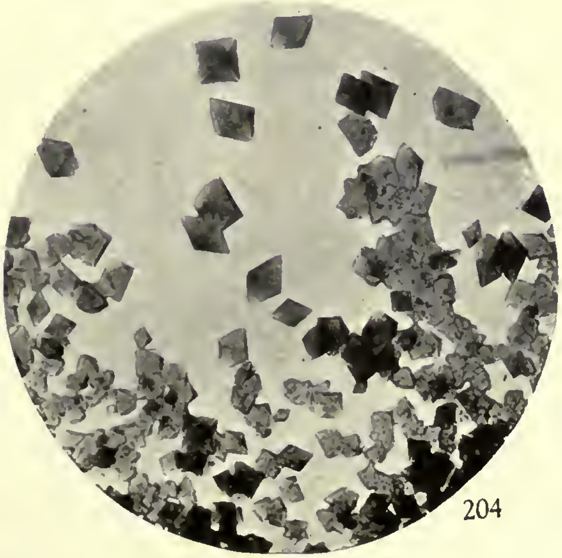
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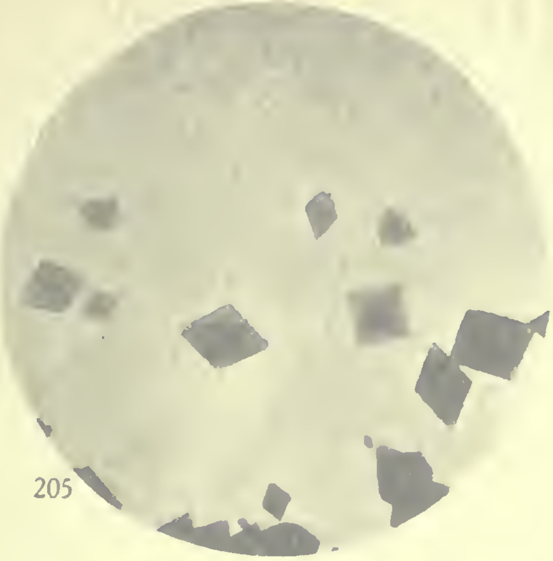
199. Oxyhemoglobin of the Muis Deer (*Tragulus meminna*), showing single tabular crystals.
200, 201. Same, showing horse-type twin.
202. Reduced Hemoglobin of the Muis Deer, showing part of very large crystal.
203, 204. Oxyhemoglobin of the Elk (*Cervus canadensis*), showing simple pyramidal form of crystals.

PLATE 35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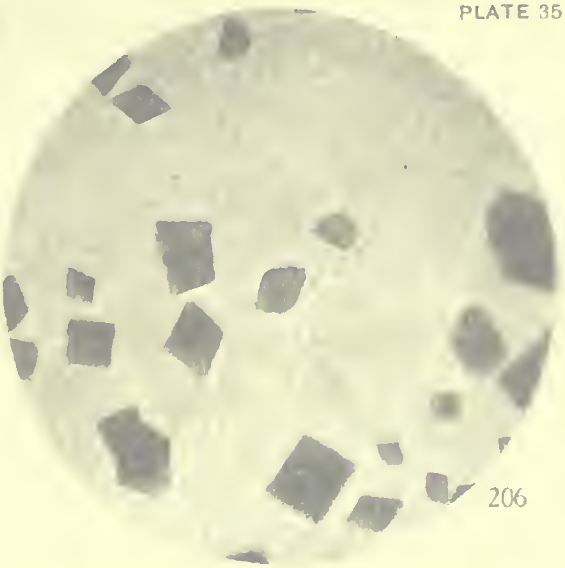
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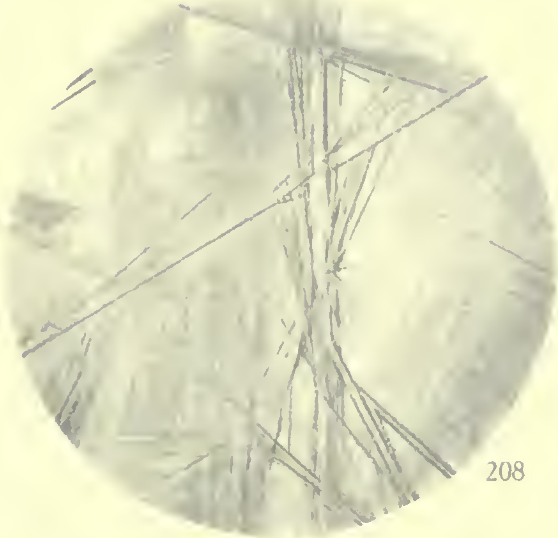
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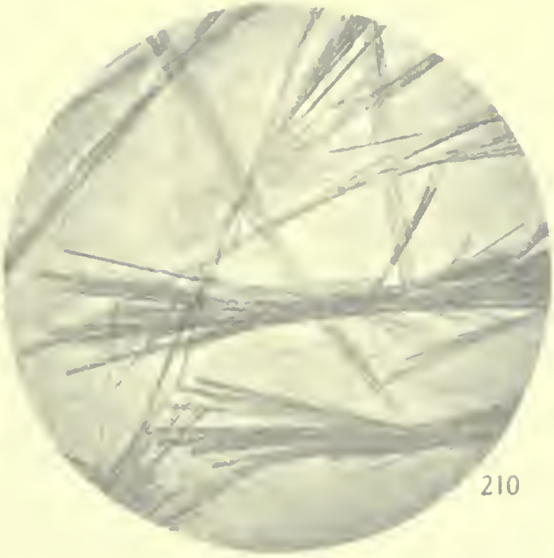
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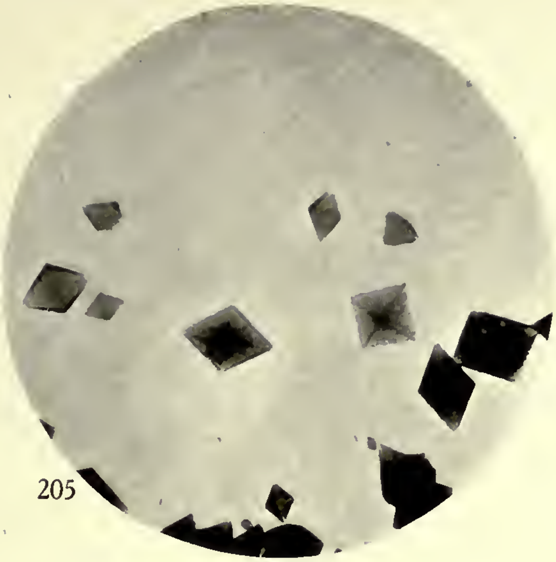
205, 206. Oxyhemoglobin of the Elk (*Cervus canadensis*), showing flat pyramid in three aspects, plan and side elevations.

207, 208. Reduced Hemoglobin of the Red Brocket (*Cariacus rufus*), showing composite tabular crystals of parallel growth; also narrow lath-shaped crystals.

209. Same, showing simple tabular crystals and lath-shaped crystals.

210. Same, showing rods growing in sheaf-like tufts.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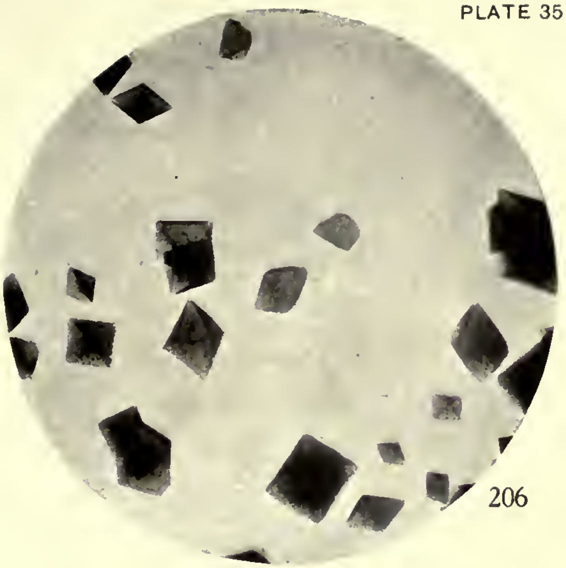
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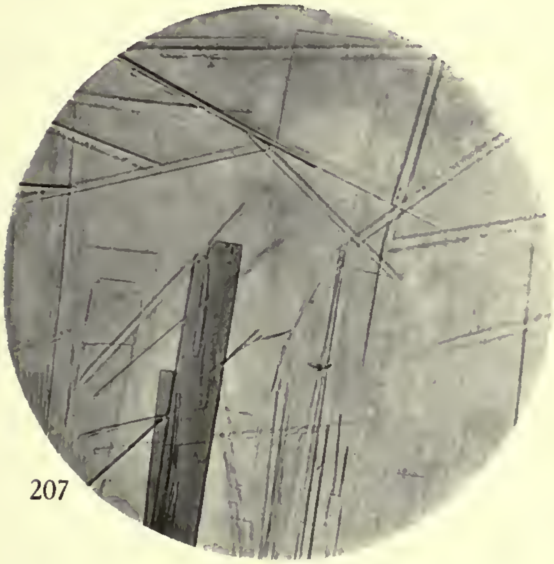
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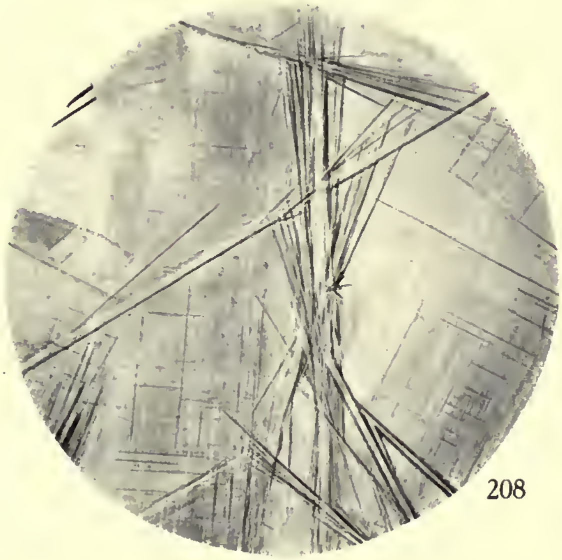
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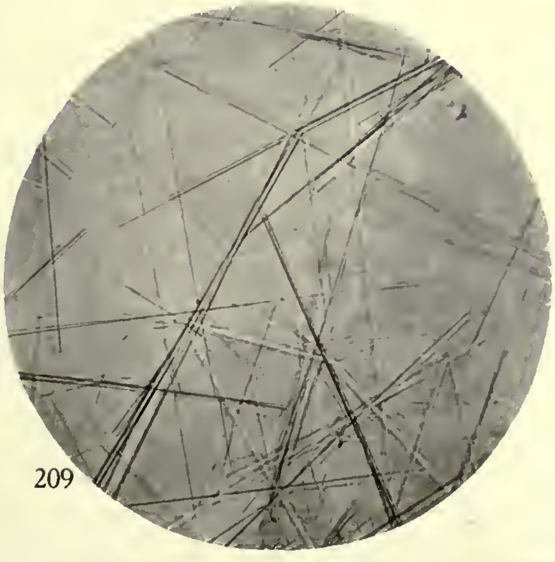
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205, 206. Oxyhemoglobin of the Elk (*Cervus canadensis*), showing flat pyramid in three aspects, plan and side elevations.
207, 208. Reduced Hemoglobin of the Red Brocket (*Cariacus rufus*), showing composite tabular crystals of parallel growth; also narrow lath-shaped crystals.
209. Same, showing simple tabular crystals and lath-shaped crystals.
210. Same, showing rods growing in sheaf-like tufts.


211


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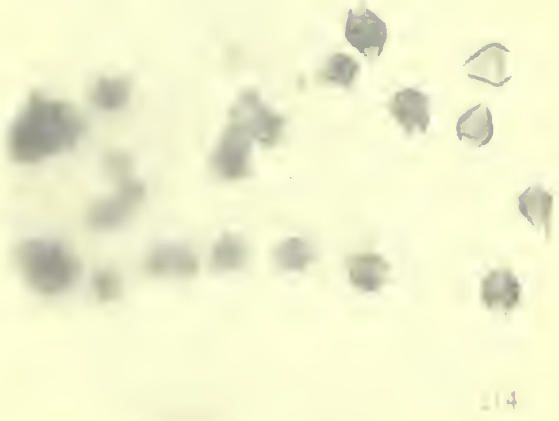
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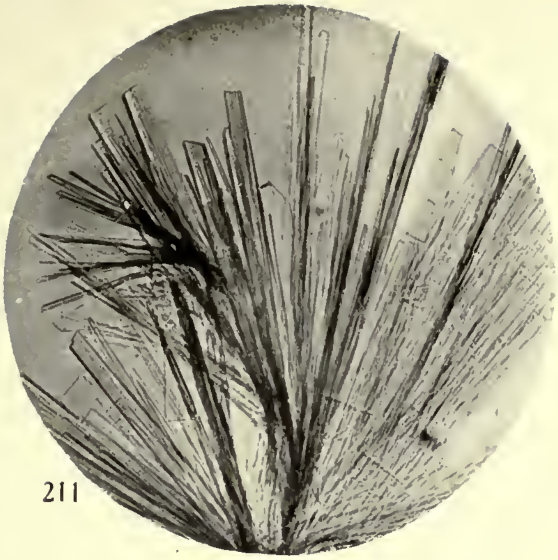
211. Reduced Hemoglobin of the [illegible] Deer ([illegible]), showing [illegible] group of narrow [illegible].
212. Same, showing group of broader [illegible].
213. Same, showing edge and flat views of broader crystals.
214. Oxyhemoglobin of the Fallow Deer ([illegible]), showing simple pyramidal crystals.
215, 216. Oxyhemoglobin of the Muntjak ([illegible]), showing prism terminated obliquely to base.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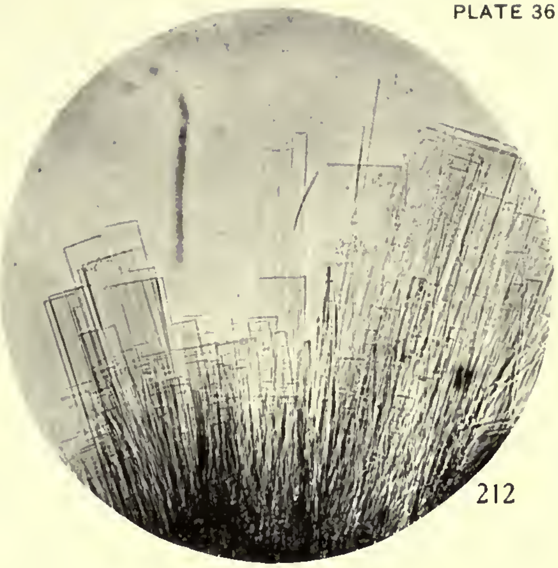
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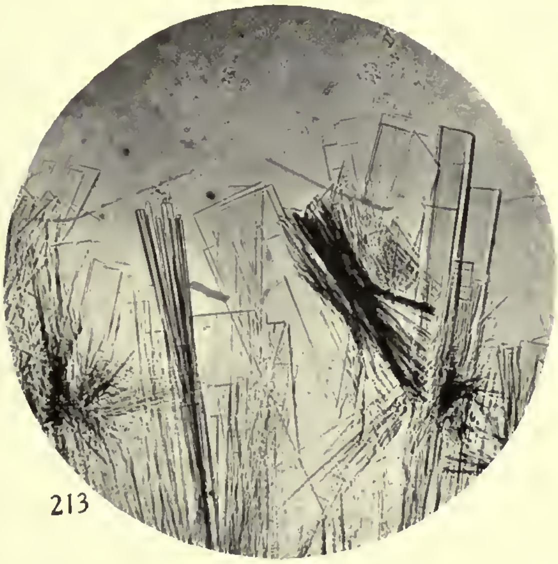
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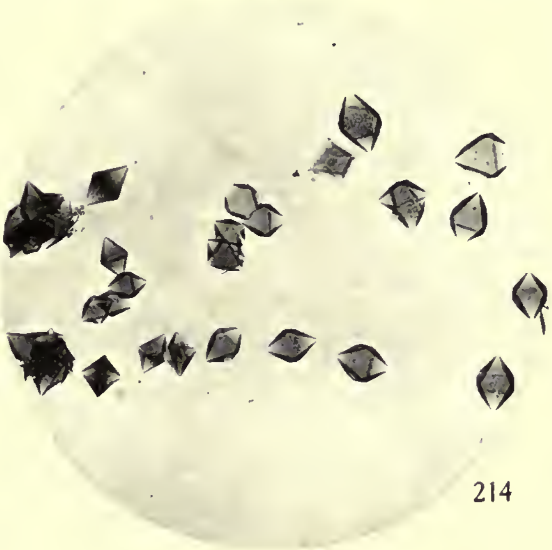
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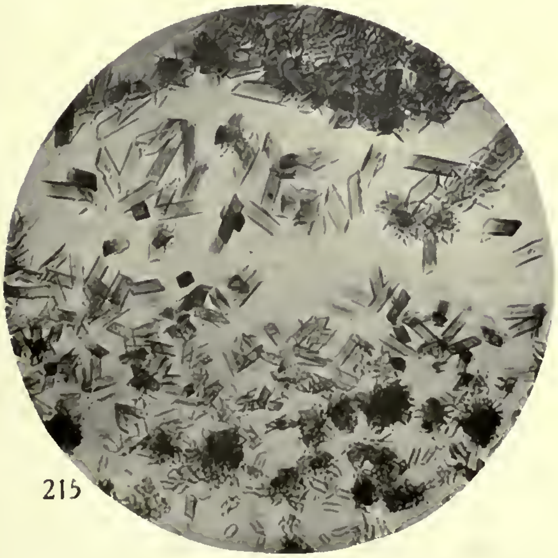
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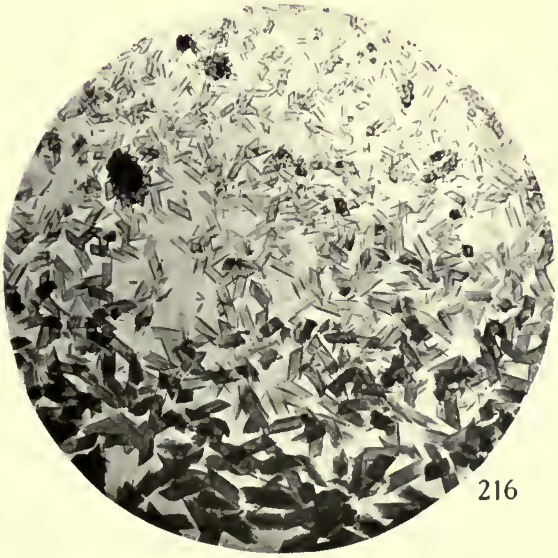
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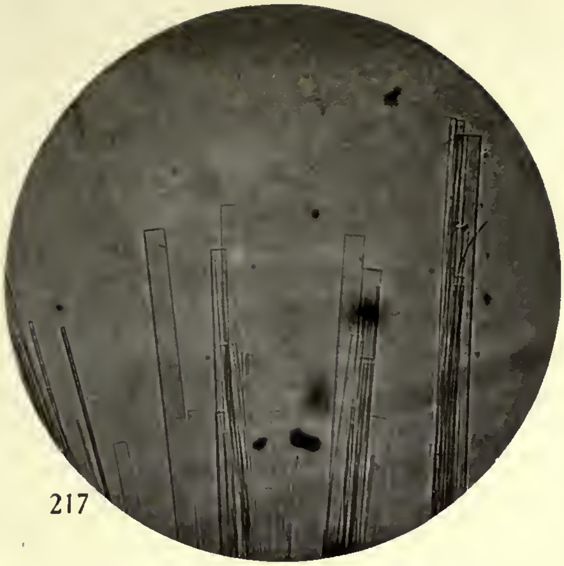
211. Reduced Hemoglobin of the Venezuela Deer (*Mazama americana savannarum*), showing divergent group of narrow lath-shaped crystals.
212. Same, showing group of broader tabular crystals.
213. Same, showing edge and flat views of broader crystals.
214. Oxyhemoglobin of the Fallow Deer (*Cervus dama*), showing simple, pyramidal crystals.
215, 216. Oxyhemoglobin of the Muntjak (*Cervulus muntjak*), showing prism terminated obliquely by base.

217

221

222

217. [illegible] Hemoglobin of the Muntjak (*Cervulus* [illegible]
218. Same, showing hair-like crystals in [illegible] groups.
219–222. Oxyhemoglobin of the Indian Antelope (*Antilope* [illegible]
terminated by base.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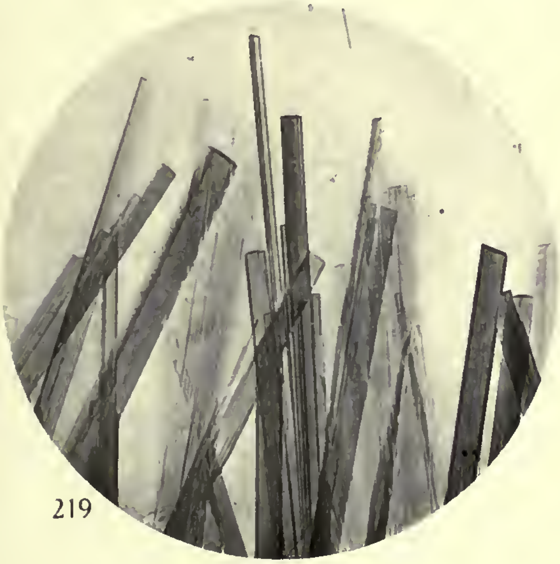
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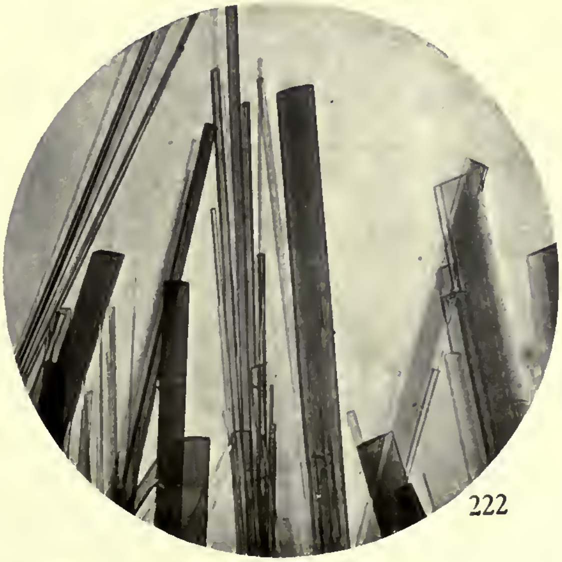
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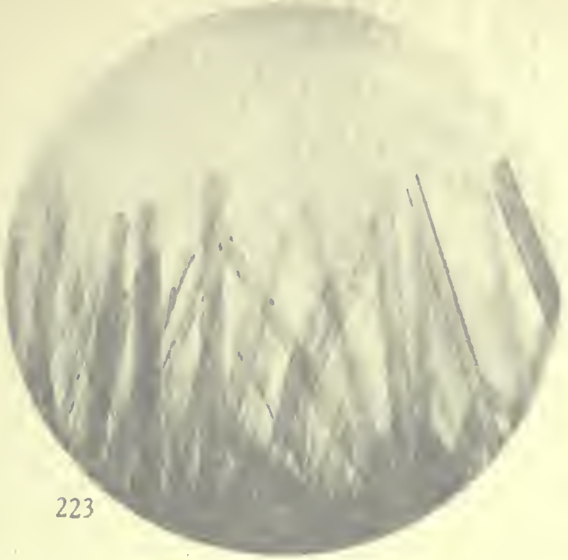
217. Reduced Hemoglobin of the Muntjak (*Cervulus muntjak*), showing lath-shaped crystals.
218. Same, showing hair-like crystals in feathery groups.
219–222. Oxyhemoglobin of the Indian Antelope (*Antilope cervicapra*), showing long prismatic crystals terminated by base.



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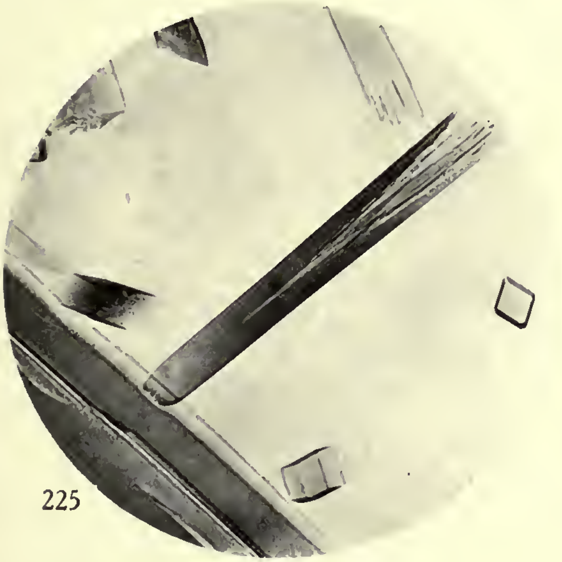
228

223. Oxyhemoglobin of the Redunca or Nagor (*Cervicapra redunca*), showing long prismatic crystals of the first crop, growing from protein ring.
224. Same, showing short crystals of second crop developed in protein ring.
225. Same, showing cross-sections of the prismatic crystals.
226. Same, shorter prismatic crystals of second crop, showing macrodome termination.
227. Same, showing large tabular crystals of second crop, flattened on brachypinacoid.
228. Same, showing large prismatic crystals of second crop, flattened on brachypinacoid.


223


224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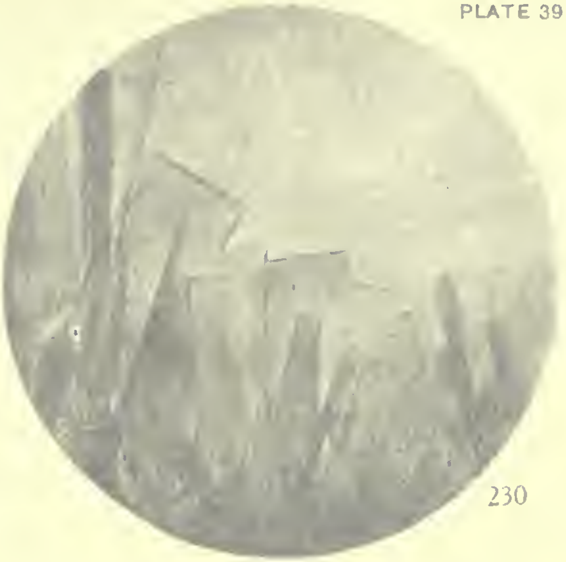
227


228

223. Oxyhemoglobin of the Redunca or Nagor (*Cervicapra redunca*), showing long prismatic crystals of the first crop, growing from protein ring.
224. Same, showing short crystals of second crop developed in protein ring.
225. Same, showing cross-sections of the prismatic crystals.
226. Same, shorter prismatic crystals of second crop, showing macrodome termination.
227. Same, showing large tabular crystals of second crop, flattened on brachypinacoid.
228. Same, showing large prismatic crystals of second crop, flattened on brachypinacoid.

229



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232

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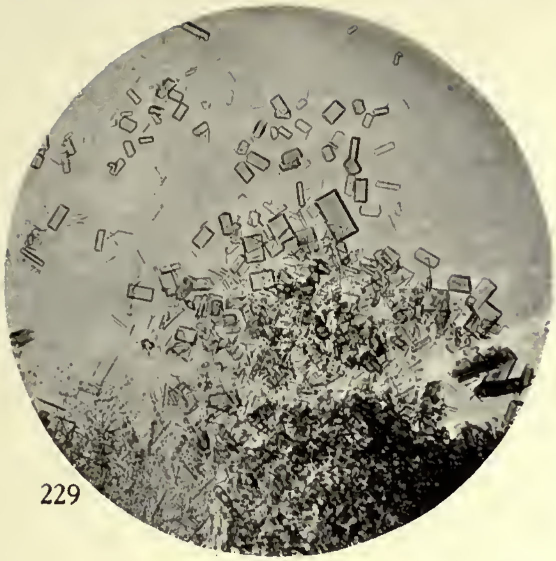
234

229. Oxyhemoglobin of the Dorcas Gazelle (*Gazella dorcas*), showing small first-formed crystals in different orientations. Prism and dome can be seen in many crystals.

230–232. Same, showing larger crystals growing from the protein ring, presenting brachypinacoid and edge views.

233, 234. Same, showing large tabular crystals in parallel growth.



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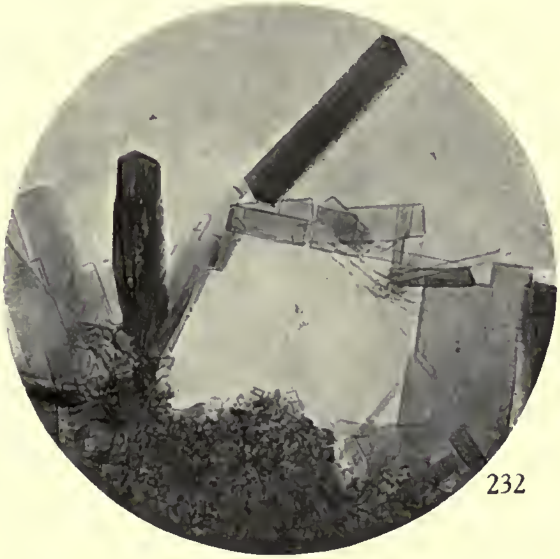

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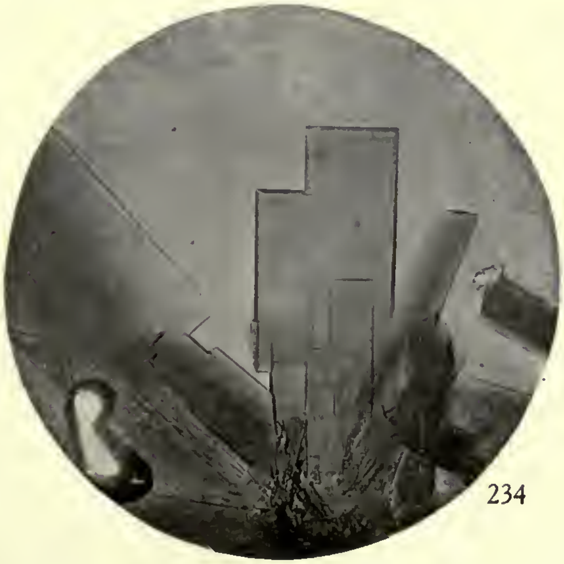

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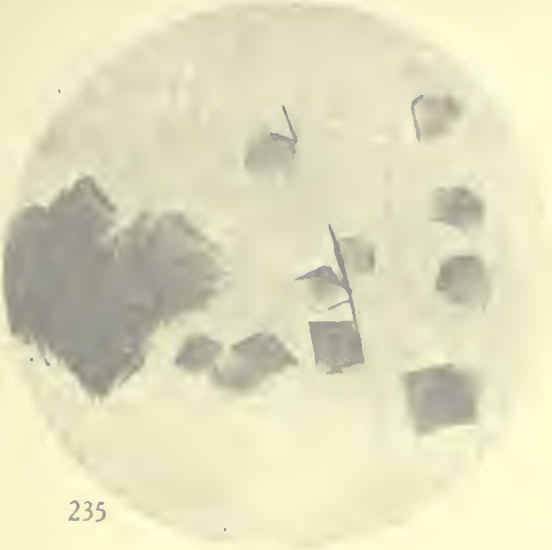
229. Oxyhemoglobin of the Dorcas Gazelle (*Gazella dorcas*), showing small first-formed crystals in different orientations. Prism and dome can be seen in many crystals.

230–232. Same, showing larger crystals growing from the protein ring, presenting brachypinacoid and edge views.

233, 234. Same, showing large tabular crystals in parallel growth.



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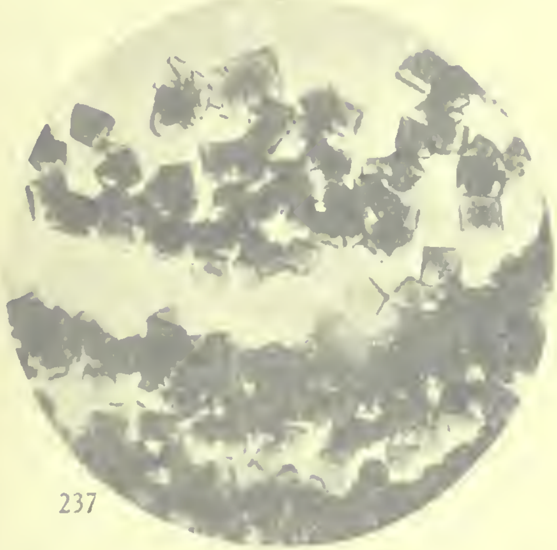

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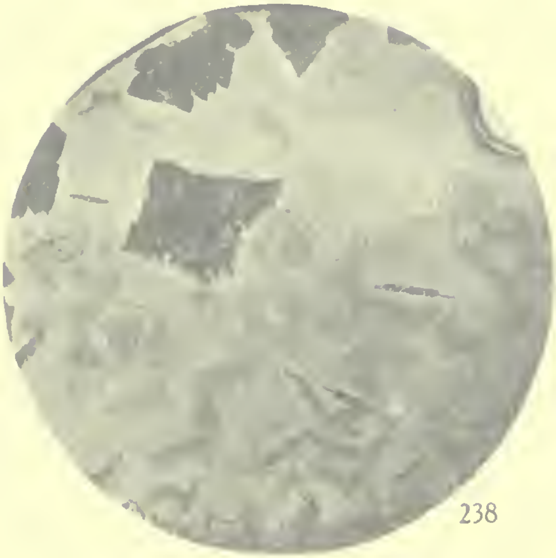

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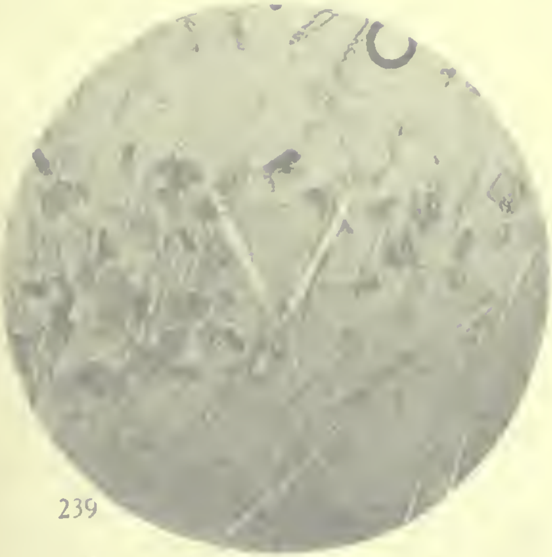

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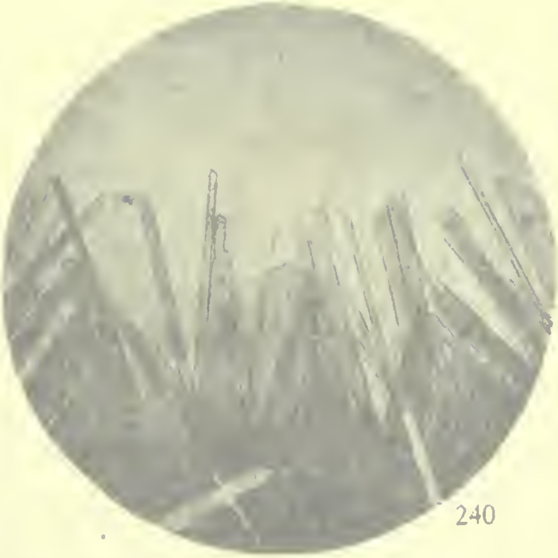


240

235–237. *a*-Oxyhemoglobin of the Duickerbok (*Cephalophus grimmi*), showing simple, pyramidal crystals.

238. *β*-Oxyhemoglobin of the Duickerbok, showing hexagonal plates on flat and on edge, also several groups of *a*-oxyhemoglobin crystals in parallel growth.

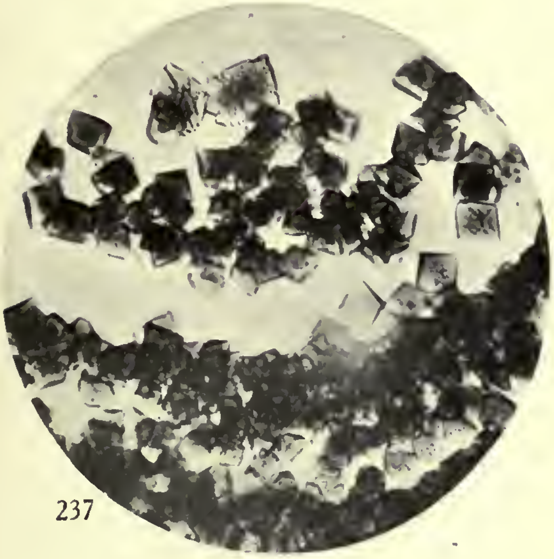
239. Oxyhemoglobin of the Sheep (*Ovis aries*), showing first-formed needle-like crystals and small tabular crystals.

240. Same, showing long prismatic crystals that develop after 24 hours.

PLATE 40


235


236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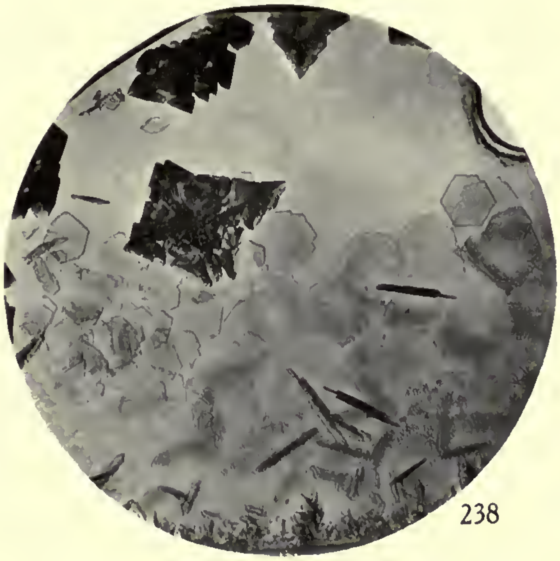
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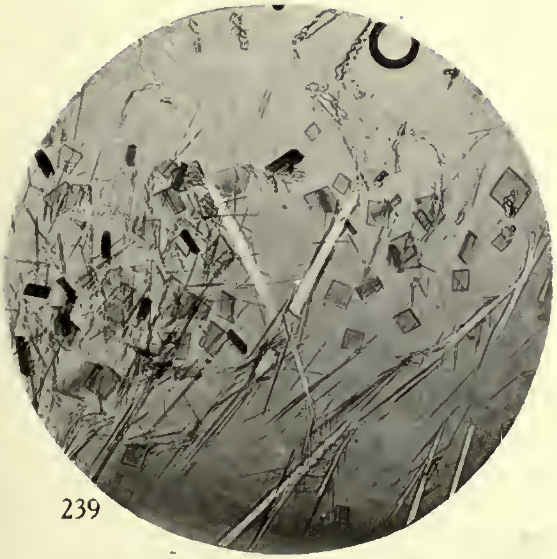
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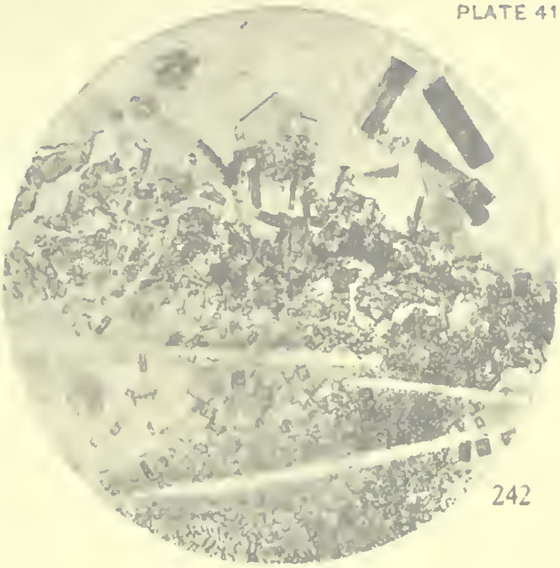
240

235–237. α-Oxyhemoglobin of the Duickerbok (*Cephalophus grimmi*), showing simple, pyramidal crystals.
238. β-Oxyhemoglobin of the Duickerbok, showing hexagonal plates on flat and on edge, also several groups of α-oxyhemoglobin crystals in parallel growth.
239. Oxyhemoglobin of the Sheep (*Ovis aries*), showing first-formed needle-like crystals and small tabular crystals.
240. Same, showing long prismatic crystals that develop after 24 hours.


241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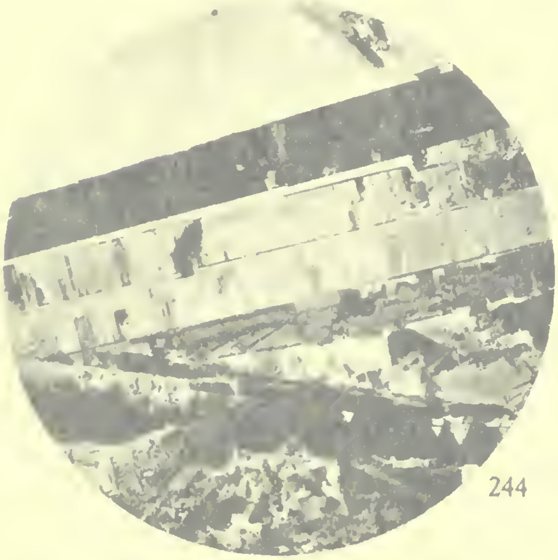
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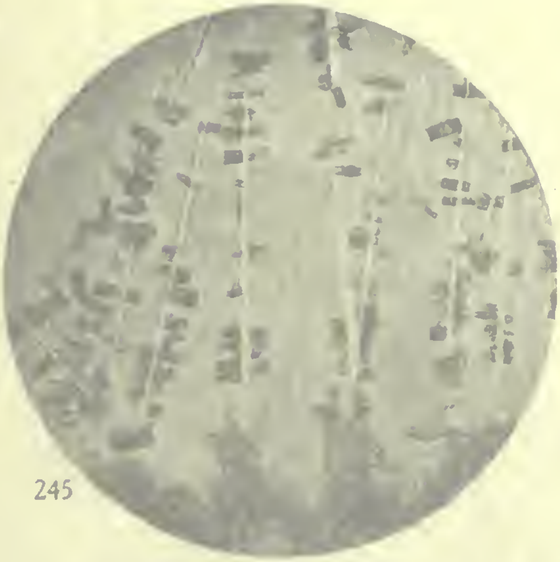
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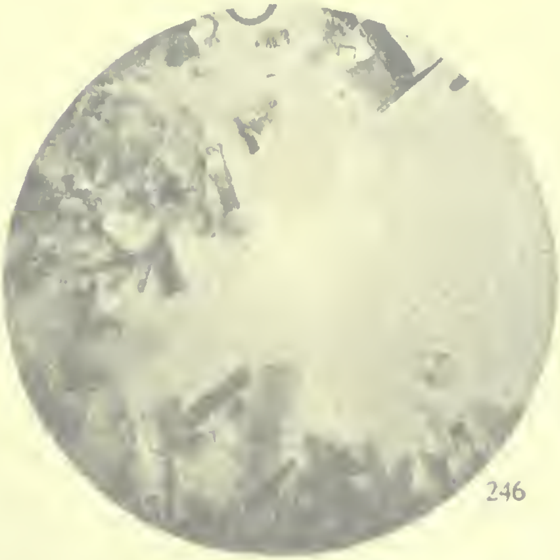
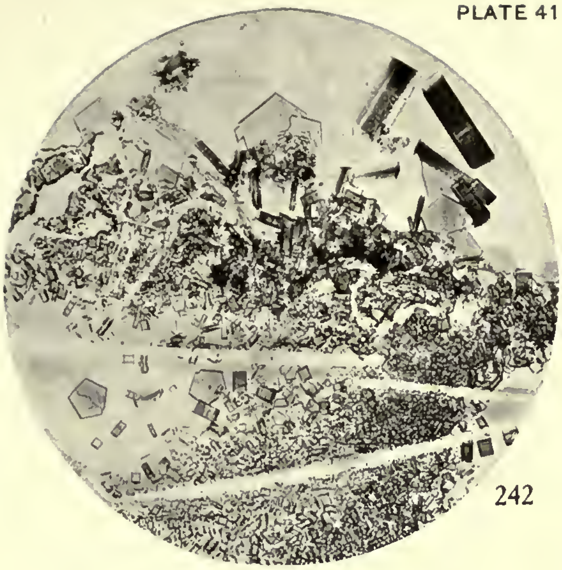

246

241. Oxyhemoglobin of the Sheep (*Ovis aries*), showing network produced by twinning and beginning of pentagon twins.
242. Same, showing isolated pentagon twins in side and edge views.
243. Same, showing composite groups produced by twinning.
244. Same, showing cross-banded effect on pinacoid produced by twinning.
245. Same, showing isolated pentagon twins strung like beads on needles of oxalate.
246. Same, showing pentagon twins seen in polarized light.

PLATE 41


241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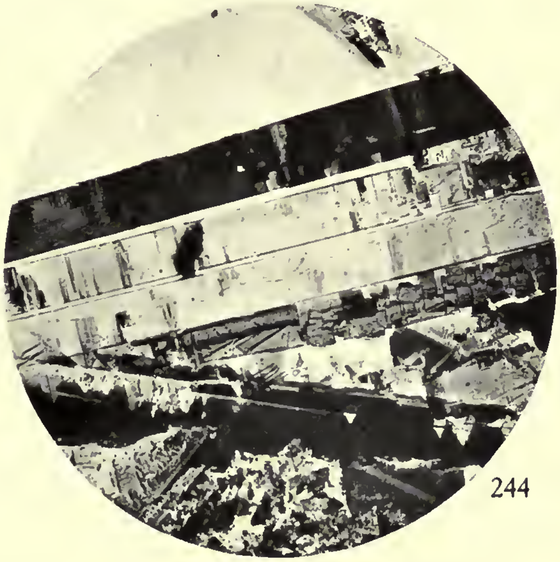
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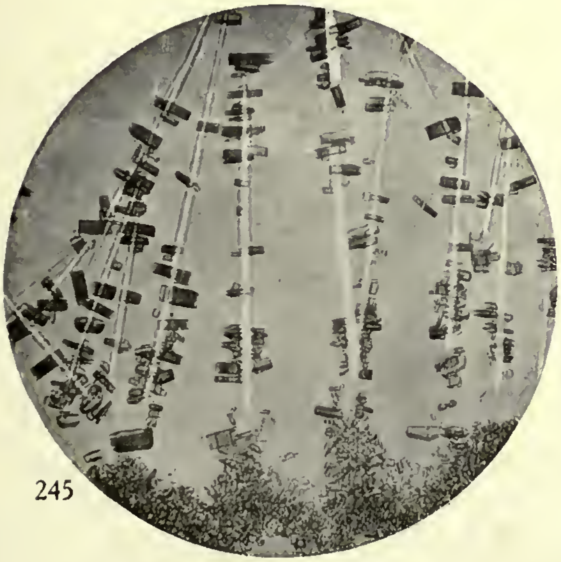
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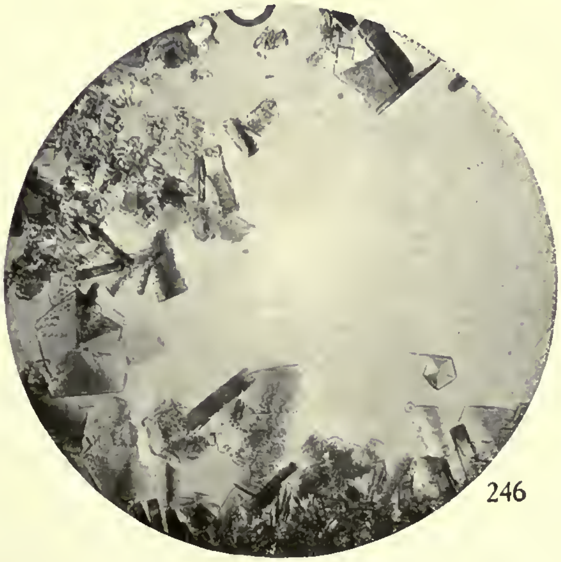
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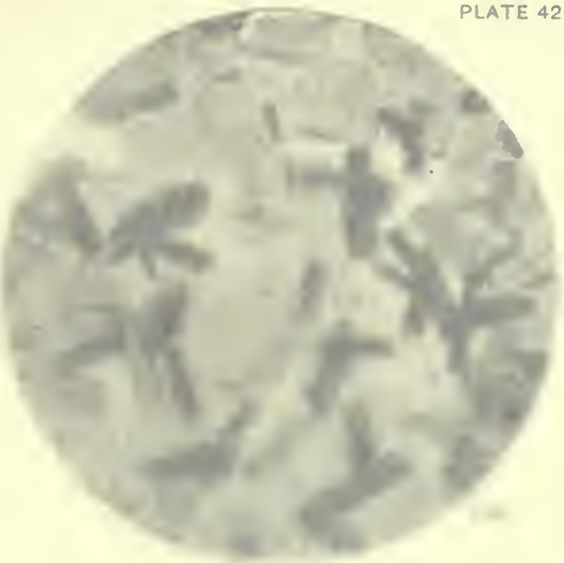
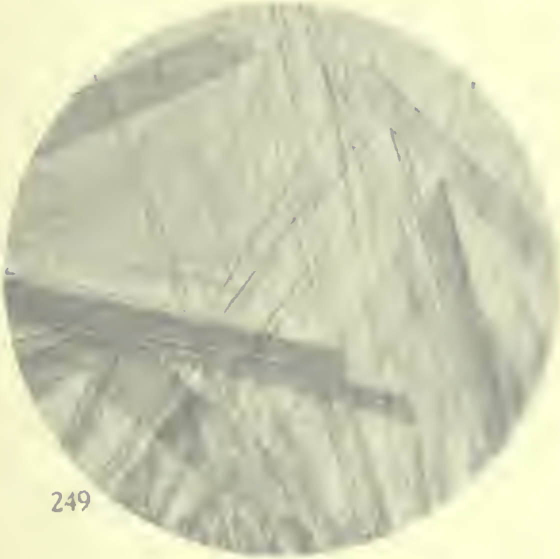
241. Oxyhemoglobin of the Sheep (*Ovis aries*), showing network produced by twinning and beginning of pentagon twins.
242. Same, showing isolated pentagon twins in side and edge views.
243. Same, showing composite groups produced by twinning.
244. Same, showing cross-banded effect on pinacoid produced by twinning.
245. Same, showing isolated pentagon twins strung like beads on needles of oxalate.
246. Same, showing pentagon twins seen in polarized light.

247





249

251

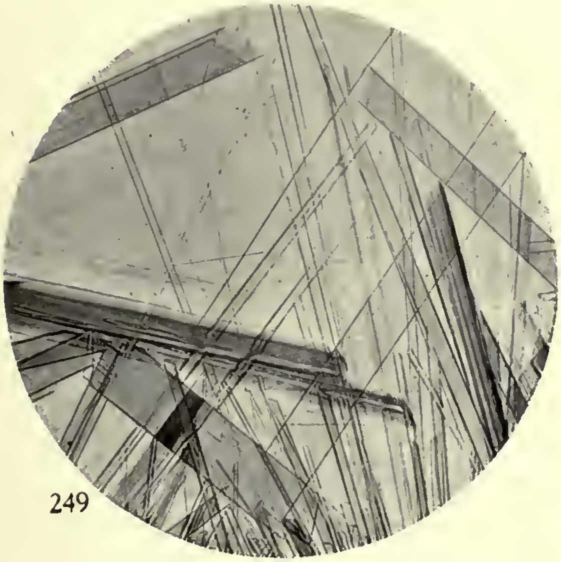
252

247, 248. R[illegible] in various orientations.
249-251. Ox[illegible] crystals, many flattened on [illegible]

252. [illegible] plates.


247


248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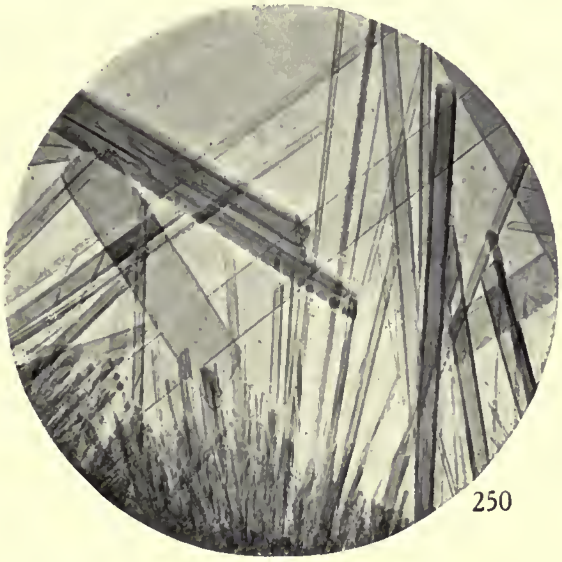
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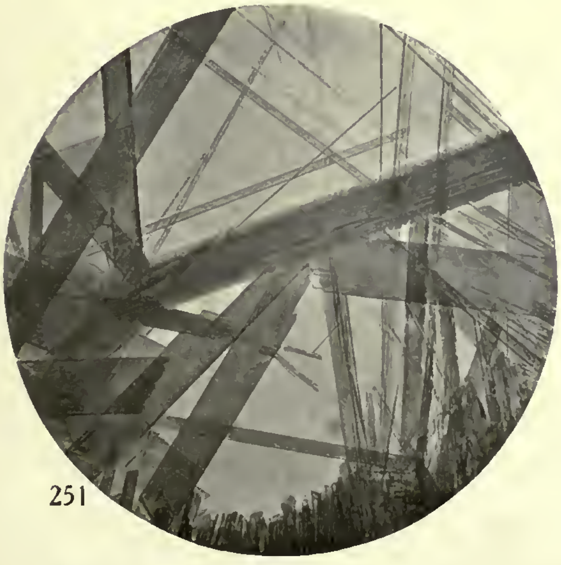
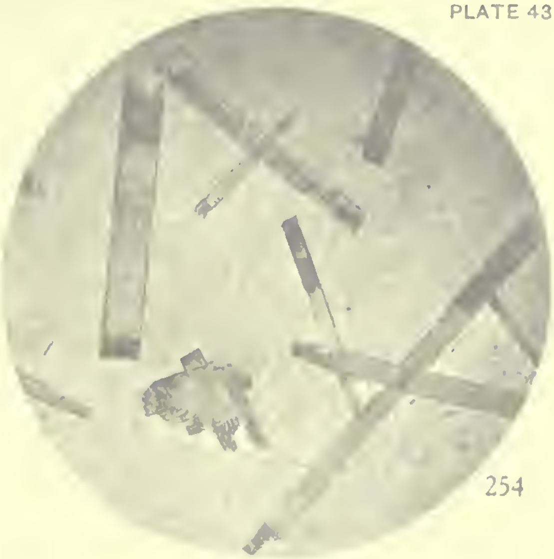

251


252

247, 248. Reduced Hemoglobin of the Sheep (*Ovis aries*), showing tabular crystals in various orientations.
249–251. Oxyhemoglobin of the Bharal (*Ovis nahura*), showing long prismatic crystals, many flattened on two opposite prism faces, thus producing a triclinic aspect.
252. Same, showing flattened prisms shortened to almost rhombic plates.


253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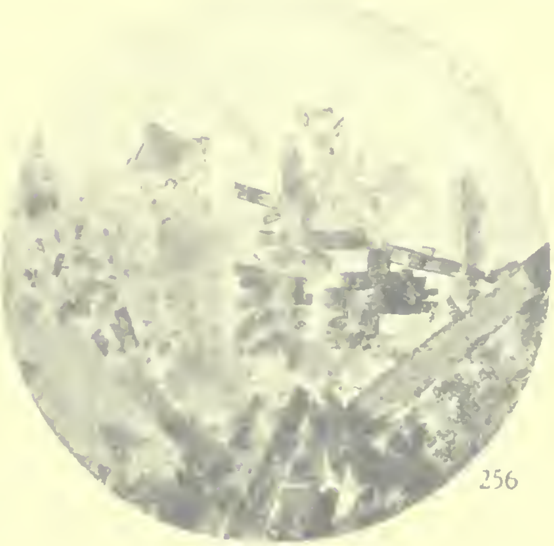
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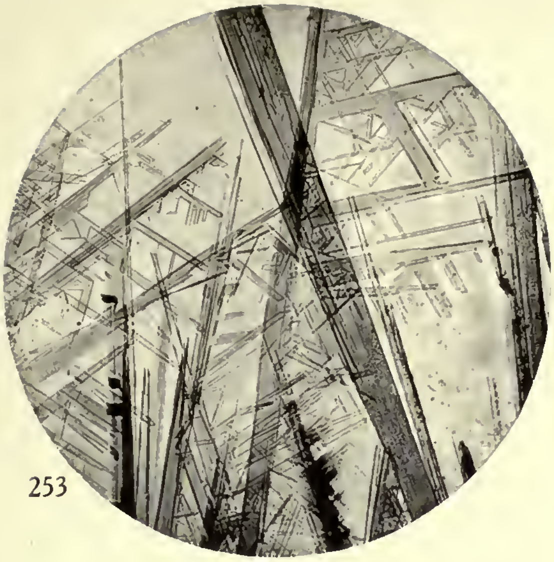
257


258

253. Oxyhemoglobin of the [illegible]
254. Same, showing [illegible]
255, 256. Same, showing twisted [illegible]
257, 258. Same, showing [illegible]



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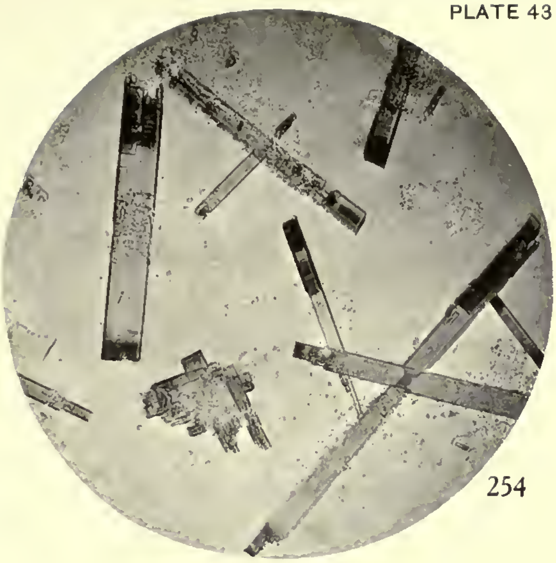

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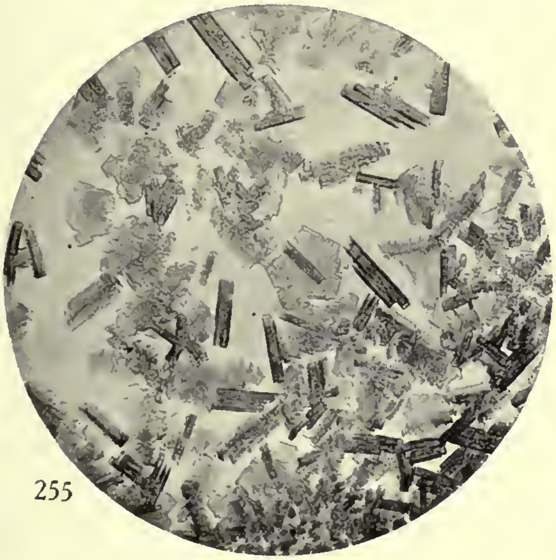

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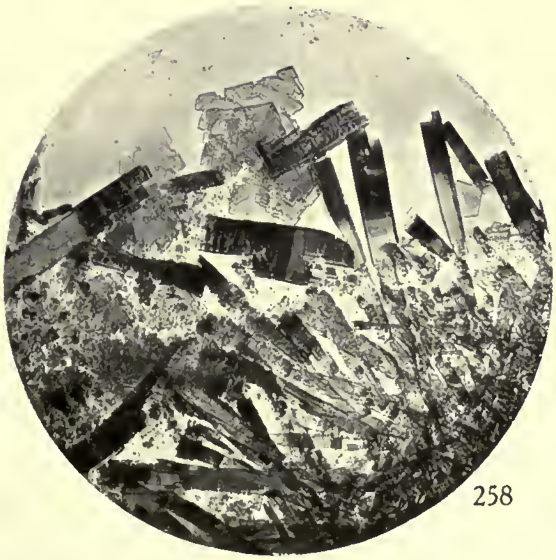
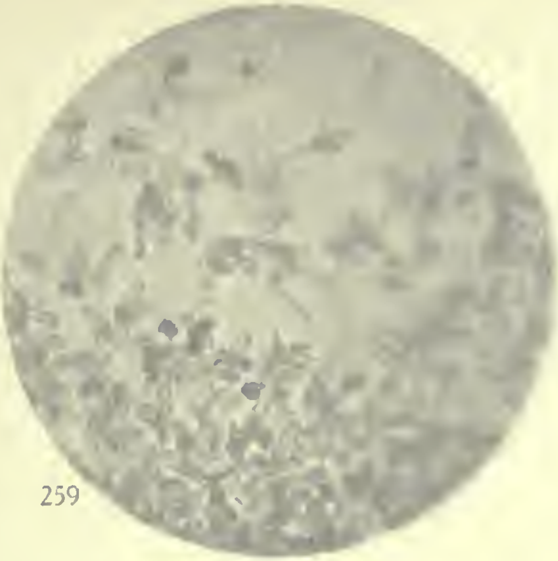


258

253. Oxyhemoglobin of the Bharal (*Ovis nahura*), showing network of prisms produced by twinning.
254. Same, showing barred effect produced on prisms by twinning.
255, 256. Same, showing isolated pentagon twins in various aspects.
257, 258. Same, showing pentagon twins growing on and capping prisms.



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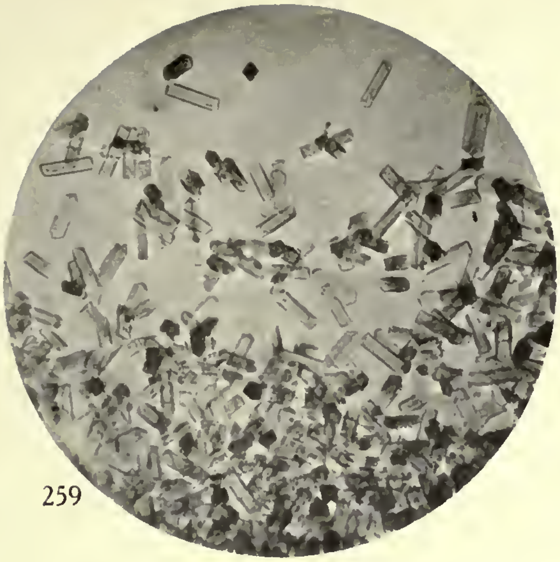
264

259. Oxyhemoglobin of the Bullock (*Bos taurus*), showing small, first-formed crystals consisting of unit prism and brachydome.
260. Same, showing larger prismatic crystals with unequally developed dome faces.
261. Same, short stout crystals, some showing brachyprism in combination with [illegible]
262. Same, showing group of crystals growing attached to an oxalate crystal.
263. Oxyhemoglobin of the Bison (*Bos bison*), showing irregular aggregate of thin, [illegible] protein ring.
264. Same, showing long crystals growing in tufts from protein ring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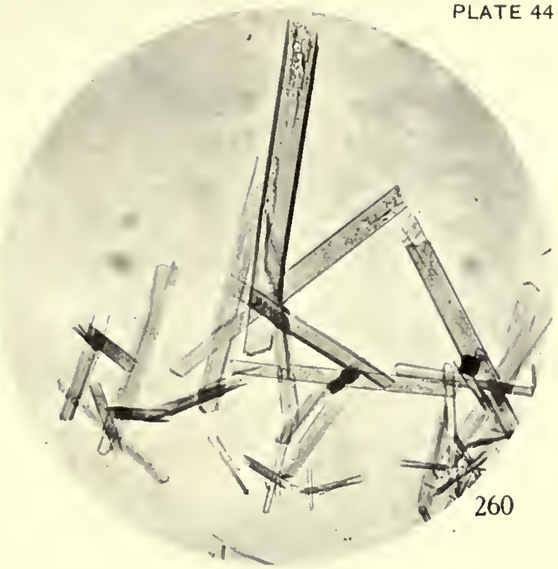
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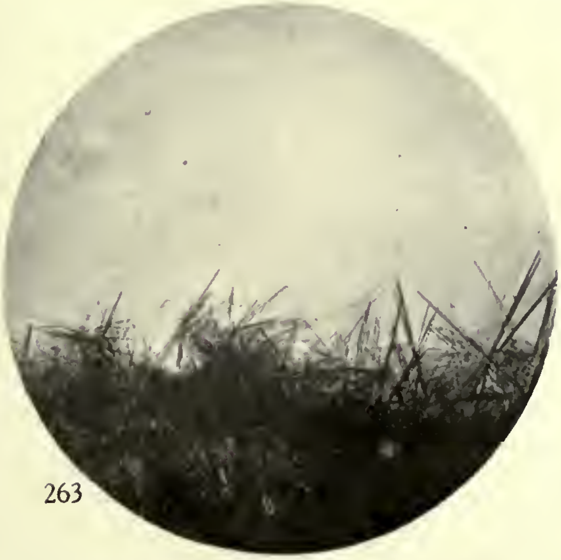
262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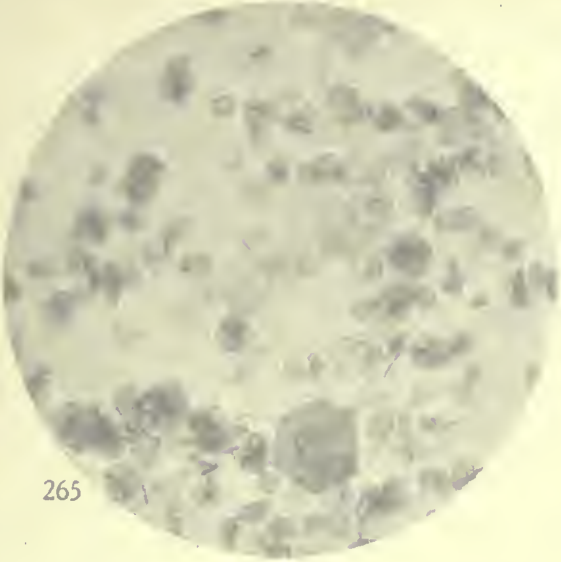
264

259. Oxyhemoglobin of the Bullock (*Bos taurus*), showing small, first-formed crystals consisting of unit prism and brachydome.
260. Same, showing larger prismatic crystals with unequally developed dome faces.
261. Same, short stout crystals, some showing brachyprism in combination with unit prism.
262. Same, showing group of crystals growing attached to an oxalate crystal.
263. Oxyhemoglobin of the Bison (*Bos bison*), showing irregular aggregate of thin lath-shaped crystals in protein ring.
264. Same, showing long crystals growing in tufts from protein ring.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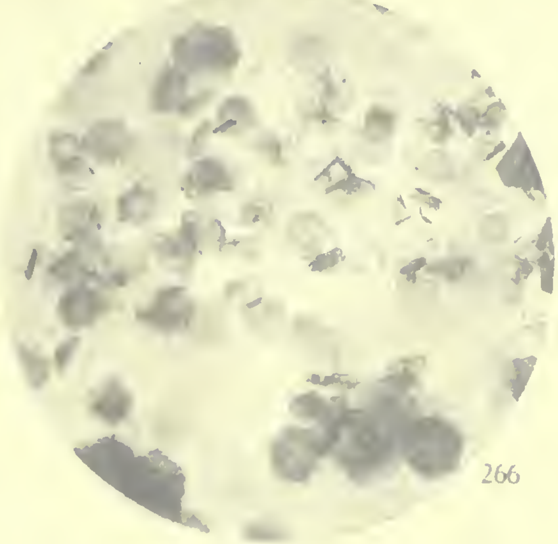
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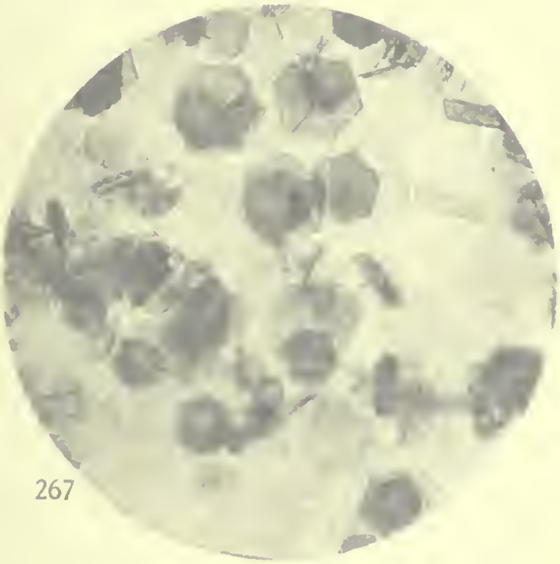
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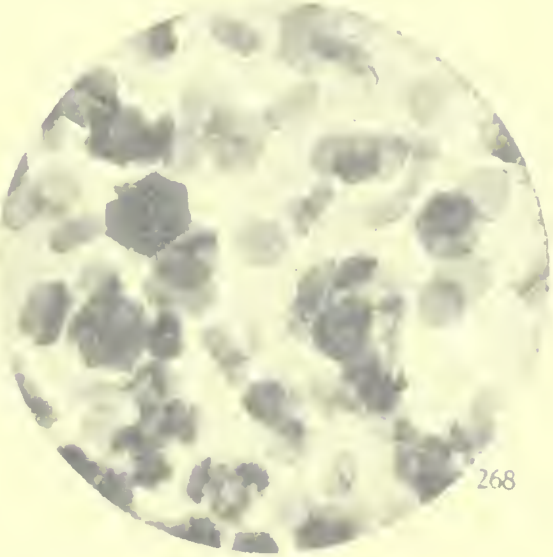
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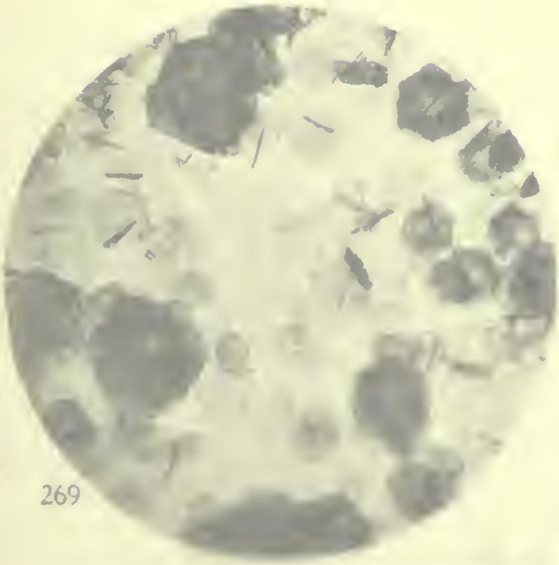
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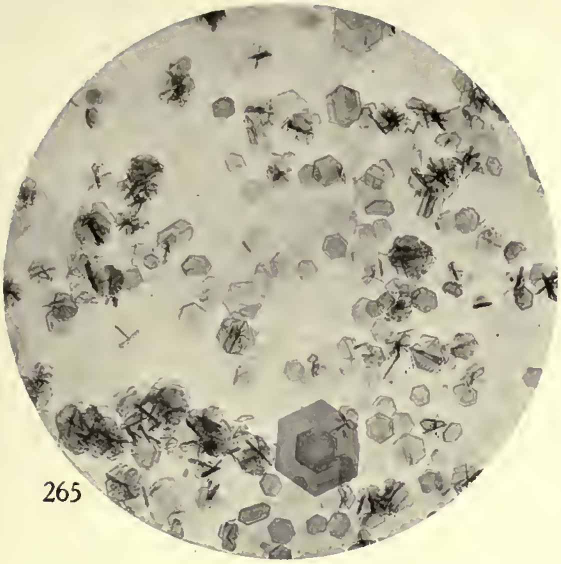
270

265–269. [illegible] of the [illegible]
[illegible]
270. [illegible] of the [illegible]



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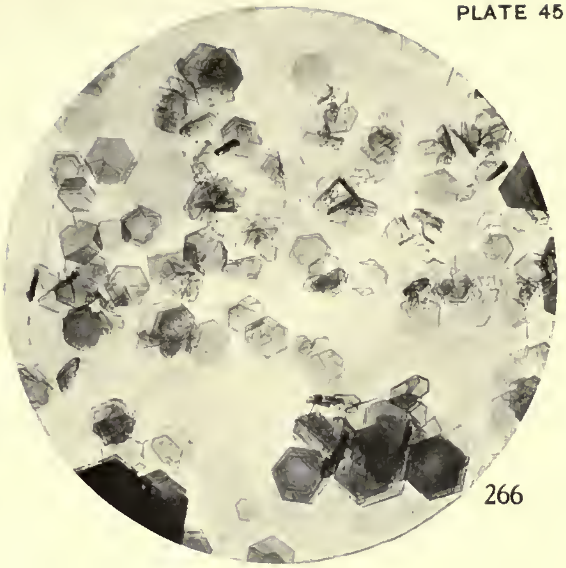

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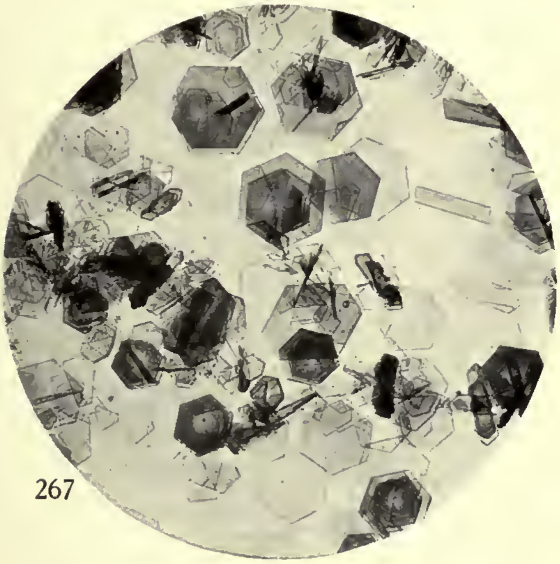

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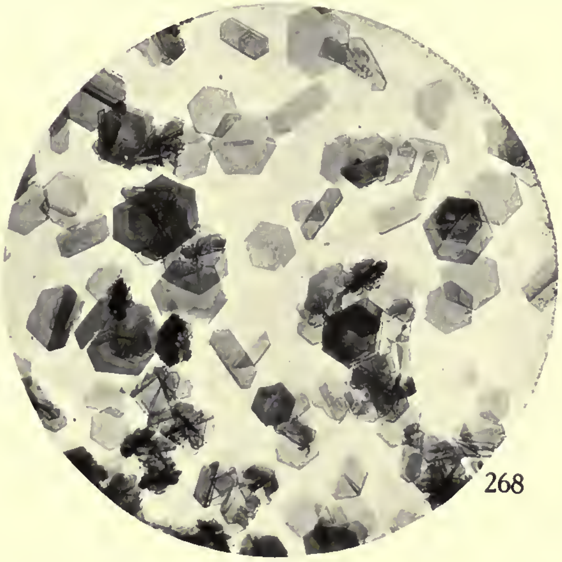

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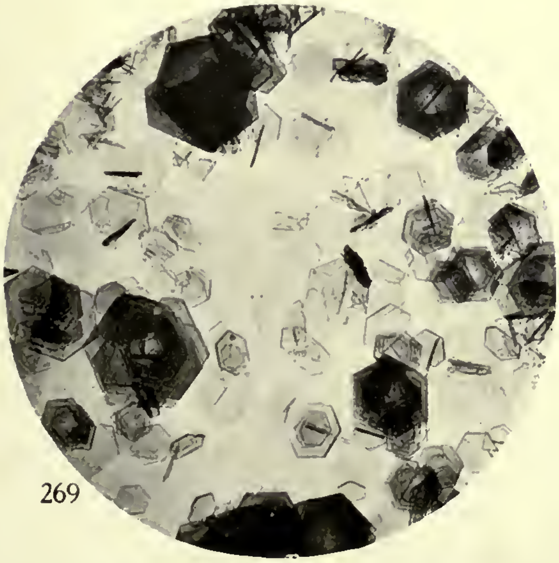

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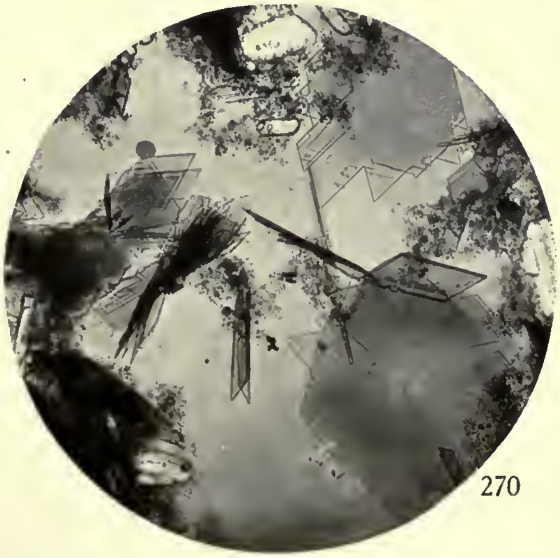
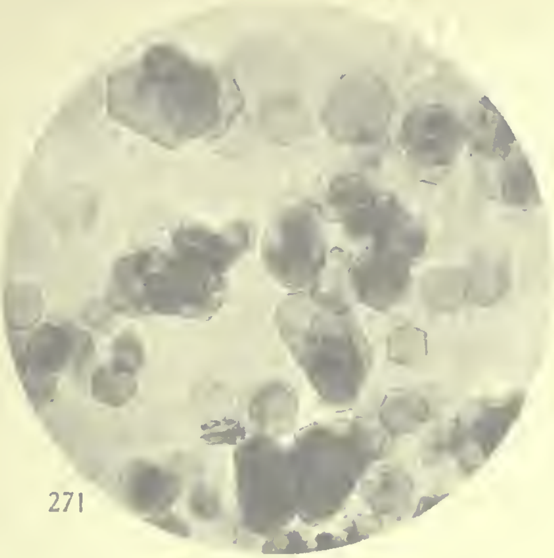


270

265–269. $\alpha$-Oxyhemoglobin of the European Red Squirrel (*Sciurus vulgaris*), showing hexagonal tabular crystals, many in parallel growth.

270. $\beta$-Oxyhemoglobin of the European Red Squirrel, showing composite crystals.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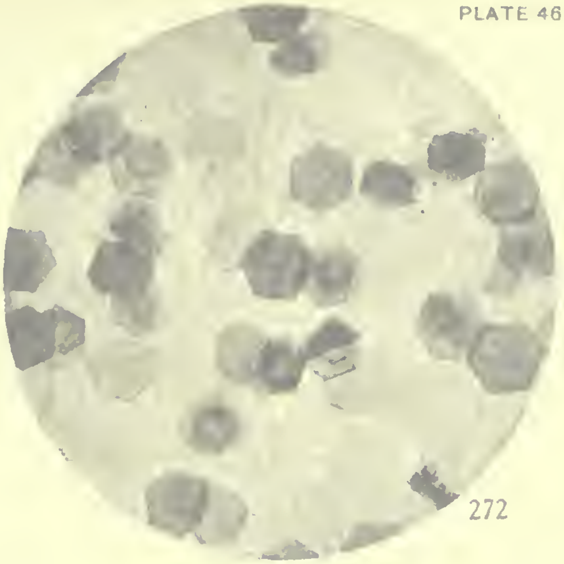
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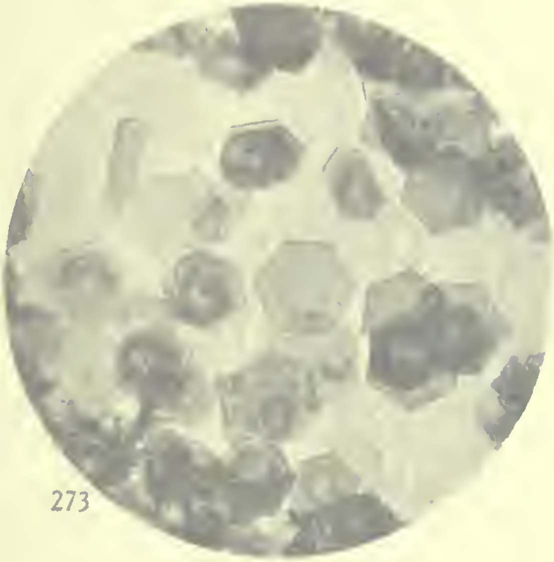
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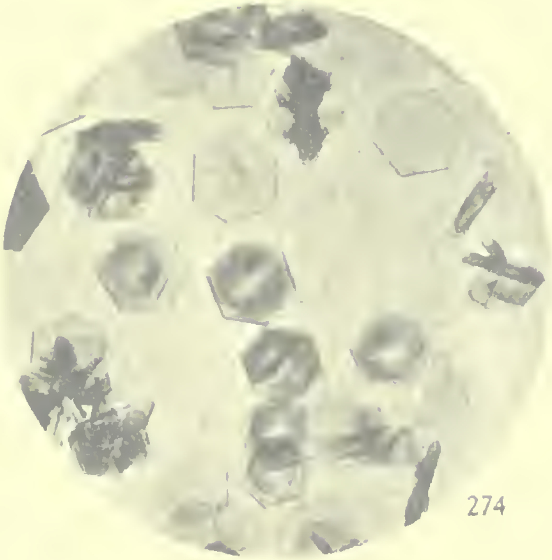
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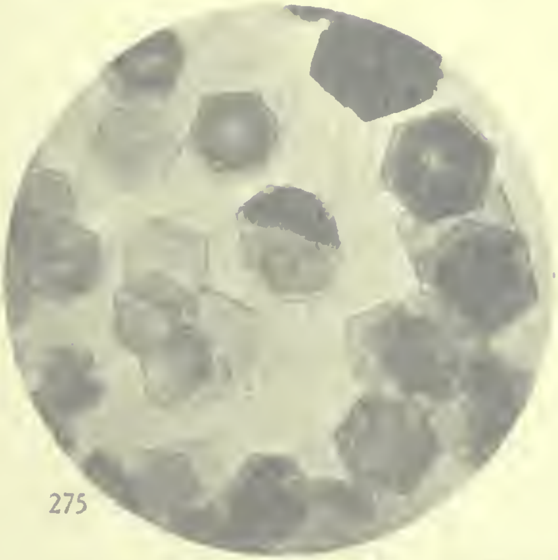
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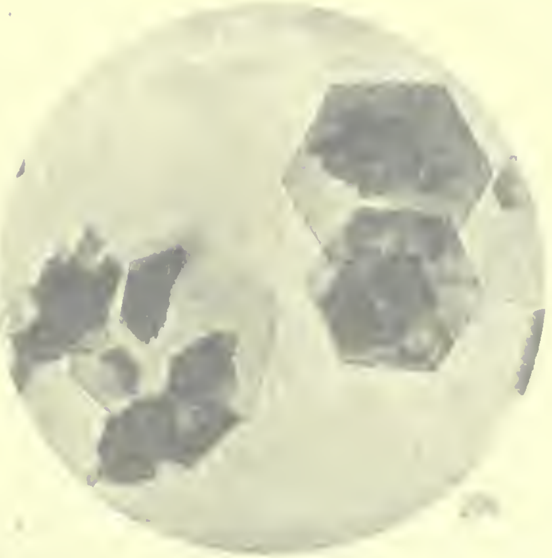
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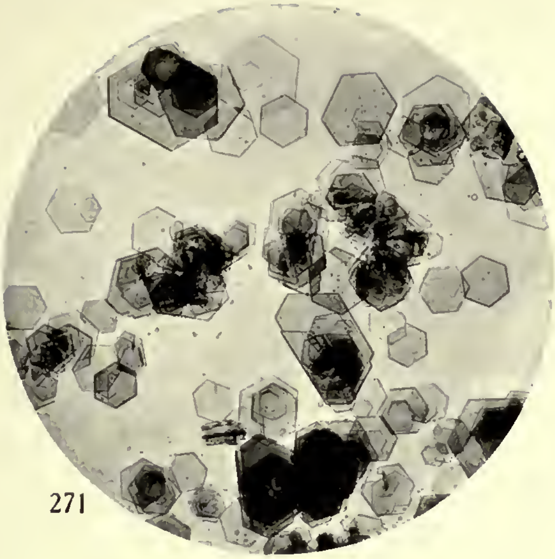
271–274. Oxyhemoglobin of the Fox-squirrel (*Sciurus rufiventer* [illegible] groups in parallel growth.

275. Oxyhemoglobin of the Gray Squirrel (*Sciurus caroline*[illegible] crystals and groups in parallel growth.

276. Same, showing large single crystals with smaller groups [illegible] producing a sheaf-like appearance.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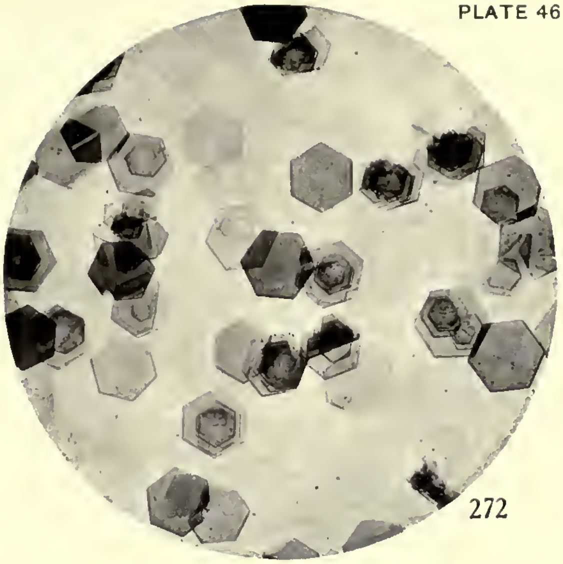
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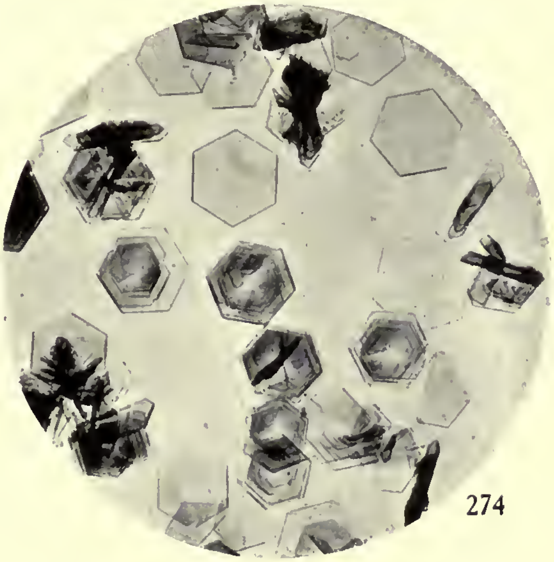
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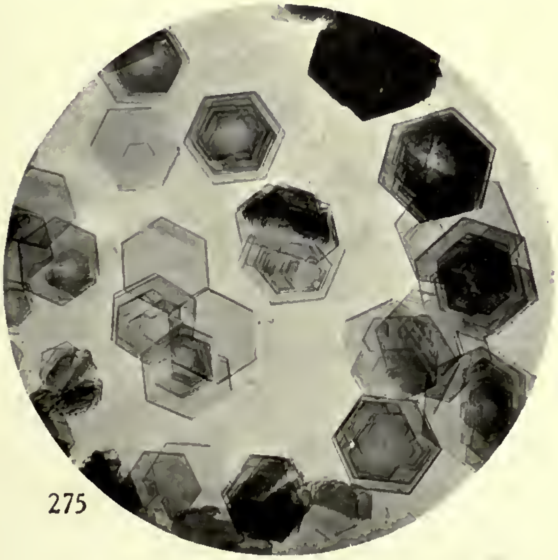
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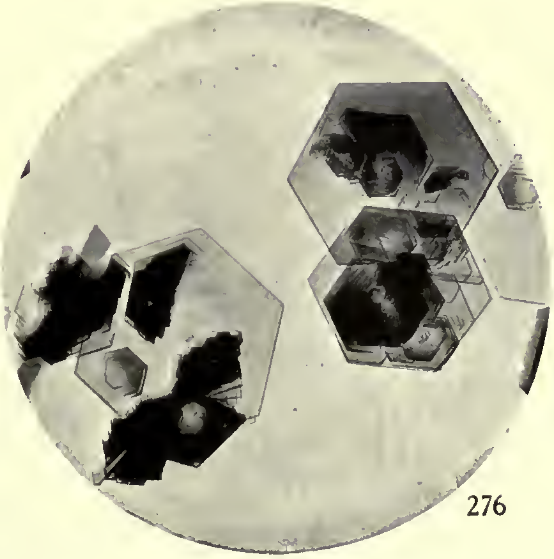
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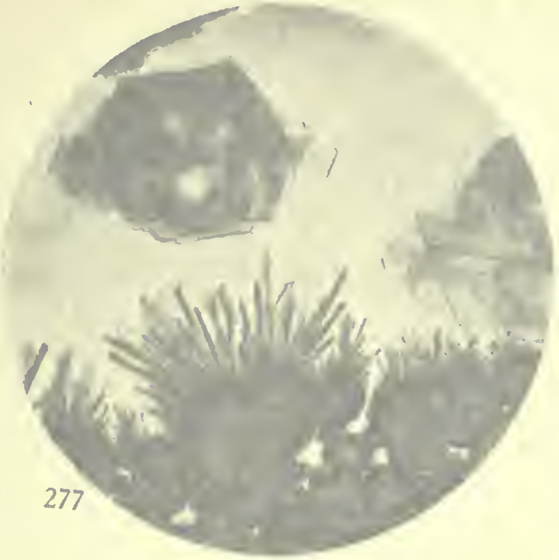
271–274. Oxyhemoglobin of the Fox-squirrel (*Sciurus rufiventer neglectus*), showing single crystals and groups in parallel growth.

275. Oxyhemoglobin of the Gray Squirrel (*Sciurus carolinensis*), showing pseudohexagonal single crystals and groups in parallel growth.

276. Same, showing large single crystals with smaller groups attached in parallel growth and radiating, producing a sheaf-like appearance.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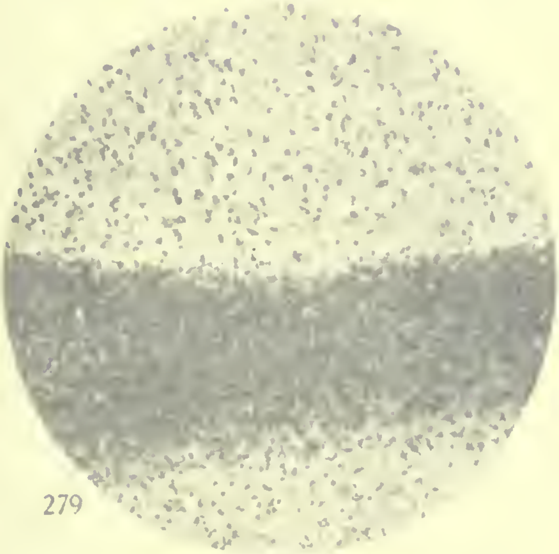
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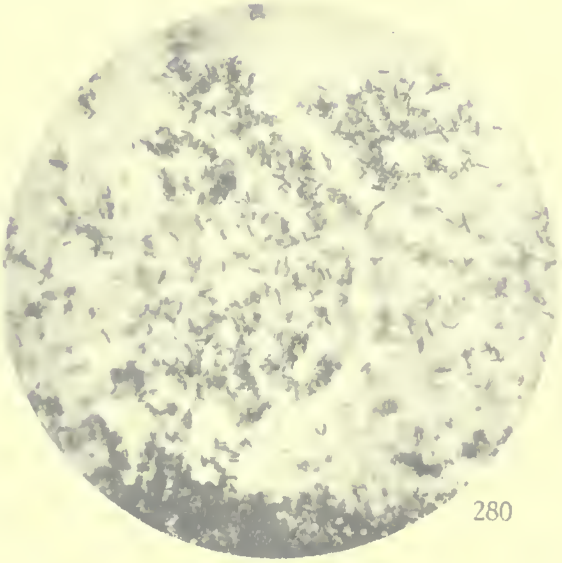
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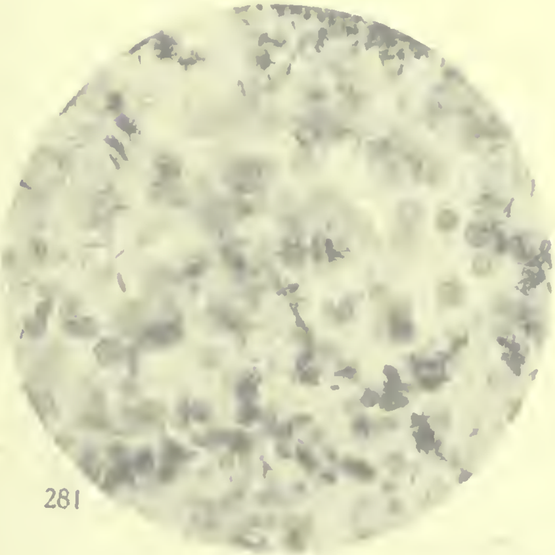
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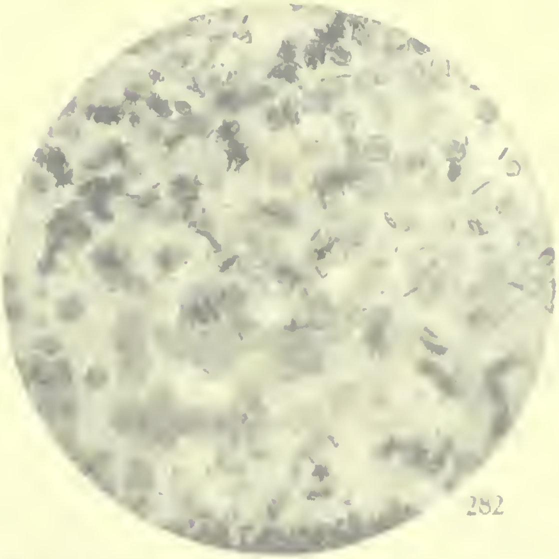
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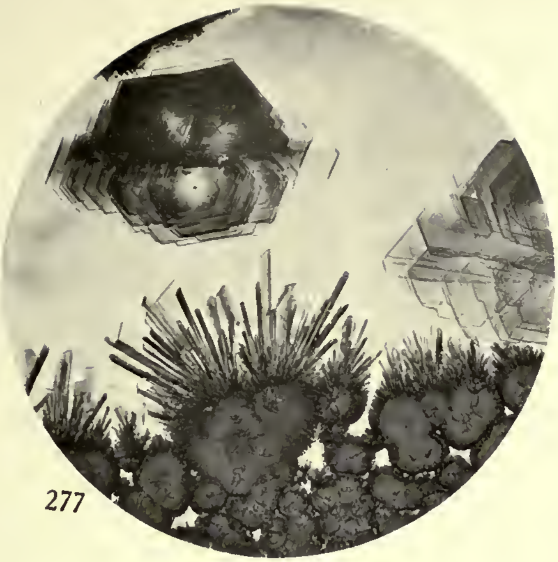
277. Oxyhemoglobin of the Gray Squirrel (*Sciurus carolinensis*), showing [illegible] and sphenelitic masses of crystals in protein ring.
278. Same, large crystals showing parallel growth.
279-282. Oxyhemoglobin of the Flying Squirrel (*Sciuropterus volans*), [illegible] crystals characteristic of the species.



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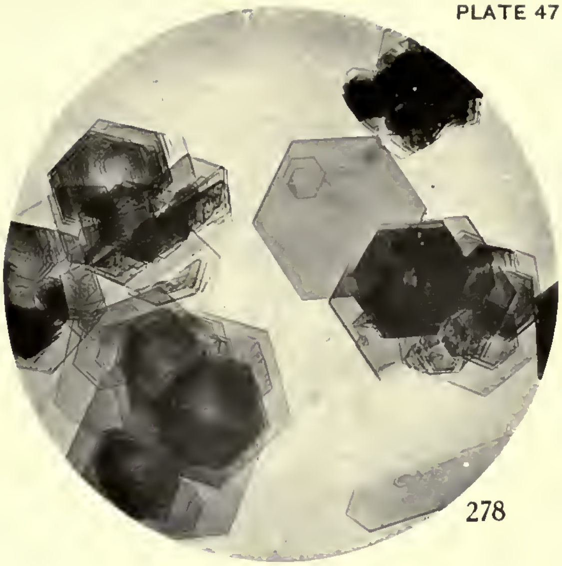

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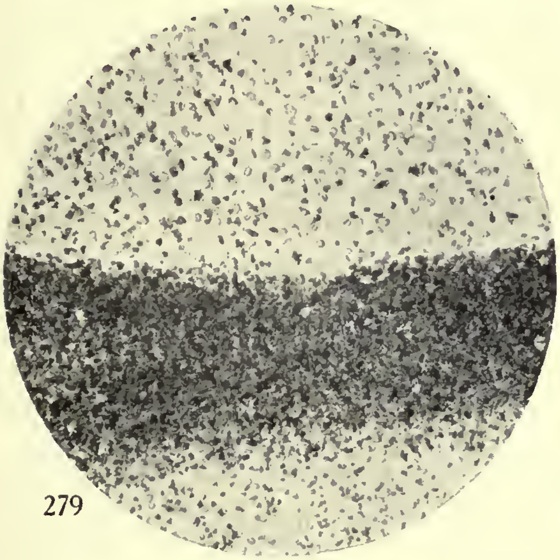

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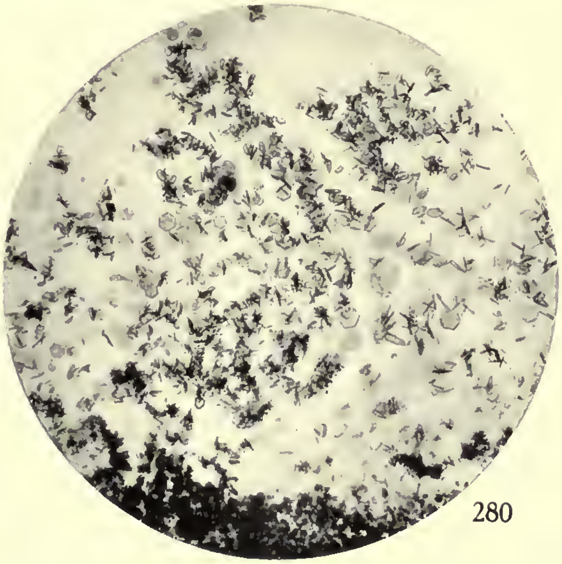

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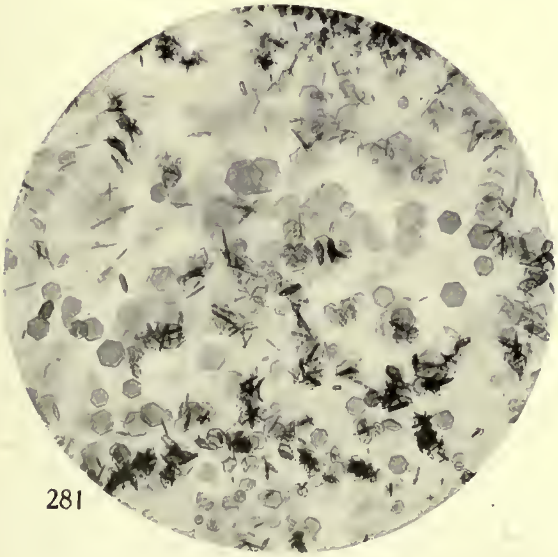

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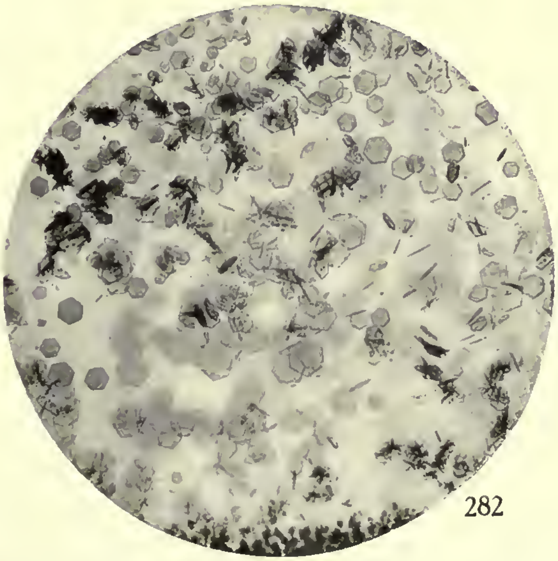

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277. Oxyhemoglobin of the Gray Squirrel (*Sciurus carolinensis*), showing "eisen rose" groups and sphenclitic masses of crystals in protein ring.

278. Same, large crystals showing parallel growth.

279–282. Oxyhemoglobin of the Flying Squirrel (*Sciuropterus volans*), showing small, simple hexagonal crystals characteristic of the species.

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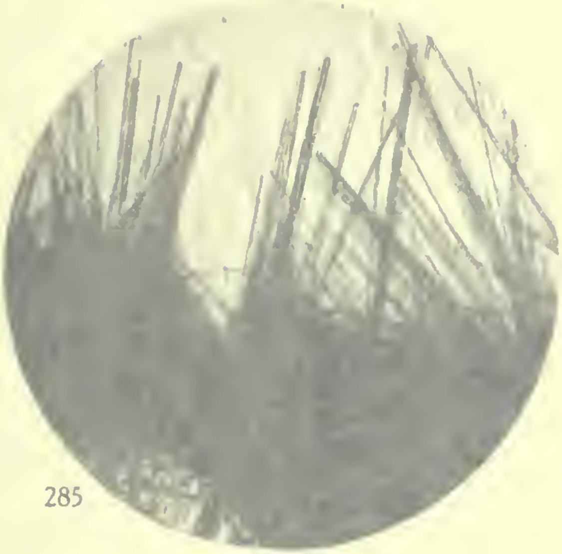

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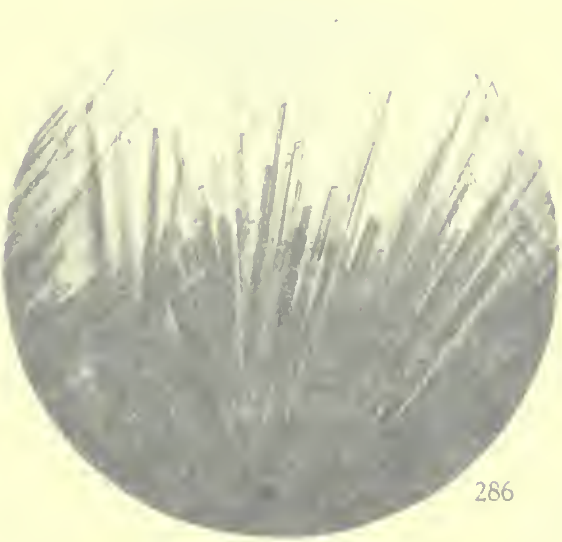

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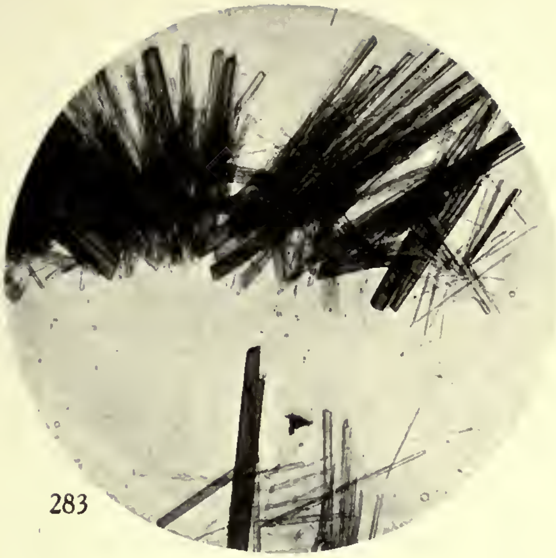
283, 284. Oxyhemoglobin of the Ground-squirrel (*Tamias striatus*), showing [illegible] growing in protein ring.

285, 286. Same showing long crystals growing in radiating groups [illegible]

287. Oxyhemoglobin of the Prairie Dog (*Cynomys* [illegible] like crystals, growing from cover edge.

288. Same, showing divergent groups of larger acicular cry[illegible]

PLATE 48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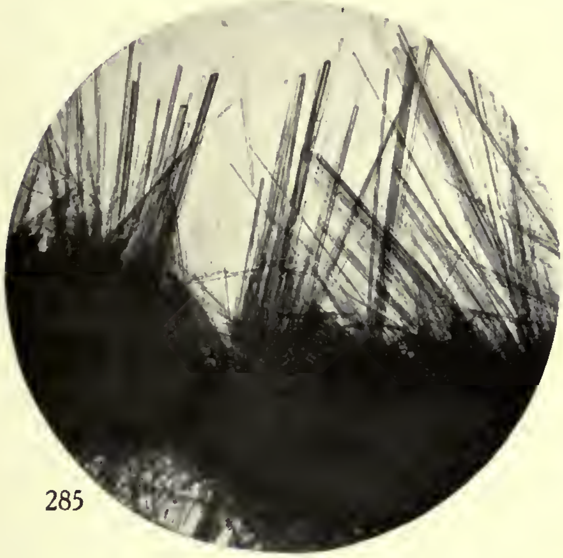
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283, 284. Oxyhemoglobin of the Ground-squirrel (*Tamias striatus*), showing irregular groups of crystals growing in protein ring.

285, 286. Same showing long crystals growing in radiating groups from protein ring.

287. Oxyhemoglobin of the Prairie Dog (*Cynomys ludovicianus*), showing divergent tufts of hair-like crystals, growing from cover edge.

288. Same, showing divergent groups of larger acicular crystals, seen in polarized light.

PLATE 49


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289. β-Oxyhemoglobin of the Ground-hog (*Marmota monax*), showing radiating tufts of hair-like crystals

290. α-Oxyhemoglobin and β-oxyhemoglobin of same, showing tufts of β-oxyhemoglobin and small hexagonal plates of first-formed α-oxyhemoglobin crystals.

291. Same, showing single large crystal with groups of smaller crystals on it in parallel growth.

292 293. Same, showing large simple and composite crystals, the parallel growth preserving general hexagonal outline.

294. Same, showing irregular aggregate of hexagonal plates, all in parallel growth.

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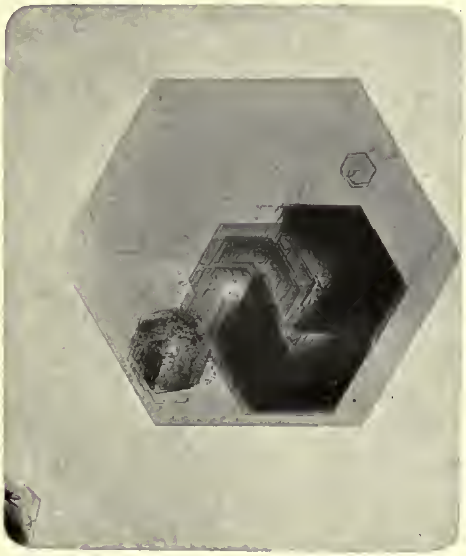

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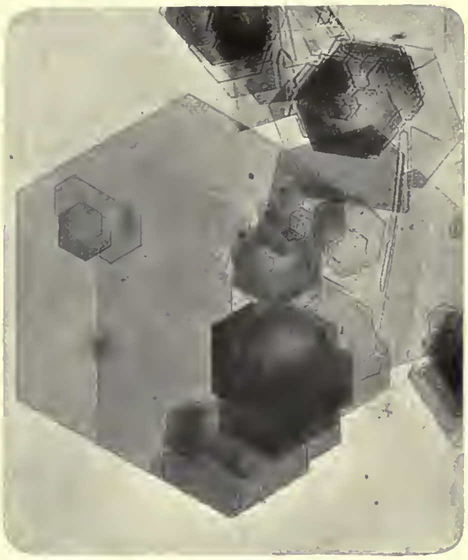

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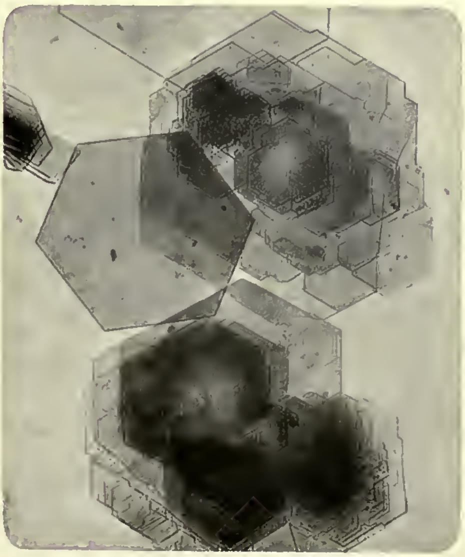

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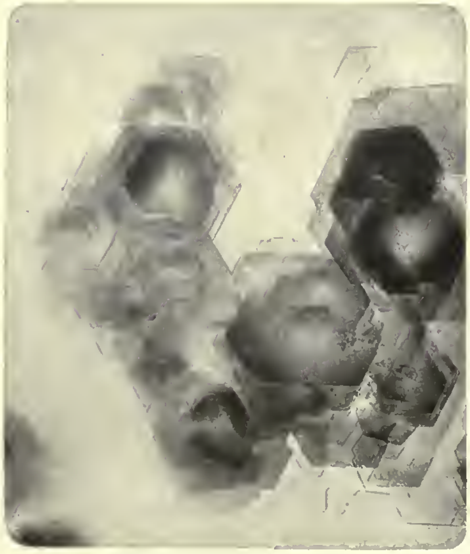

294

289. $\beta$-Oxyhemoglobin of the Ground-hog (*Marmota monax*), showing radiating tufts of hair-like crystals.
290. $\alpha$-Oxyhemoglobin and $\beta$-oxyhemoglobin of same, showing tufts of $\beta$-oxyhemoglobin and small hexagonal plates of first-formed $\alpha$-oxyhemoglobin crystals.
291. Same, showing single large crystal with groups of smaller crystals on it in parallel growth.
292, 293. Same, showing large simple and composite crystals, the parallel growth preserving general hexagonal outline.
294. Same, showing irregular aggregate of hexagonal plates, all in parallel growth.

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295. α-Oxy[illegible]

296. Same, [illegible]

297. Same, [illegible] of group.

298. γ-O[illegible] of groups of crystals.

299. Same, [illegible]

300. Same, [illegible] In 299 and 300 the imperfect curved outlines of crystals [illegible]

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295. *a*-Oxyhemoglobin of the Ground-hog (*Marmota monax*), showing two groups of hexagonal plates in partial orientation, looking complete as seen on base, and one group seen in side view, showing radiating character due to partial orientation.
296. Same, showing larger group in this partial orientation, as seen on base and on edge. In these two figures the lath-shaped section of plates is illustrated.
297. Same, a very complex group in edge view, showing arborescent form of group.
298. $\gamma$-Oxyhemoglobin, showing arborescent and feathery forms of groups of crystals.
299. Same, showing simpler group of crystals on base and on edge.
300. Same, showing twinned group of crystals in polarized light. In 299 and 300 the imperfect curved outlines of crystals are shown.

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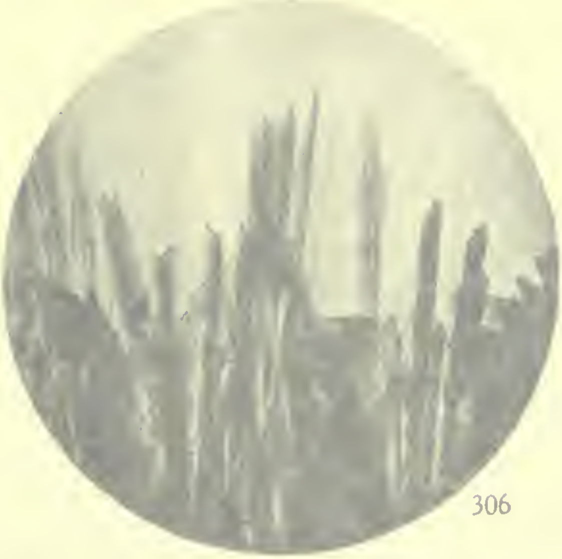
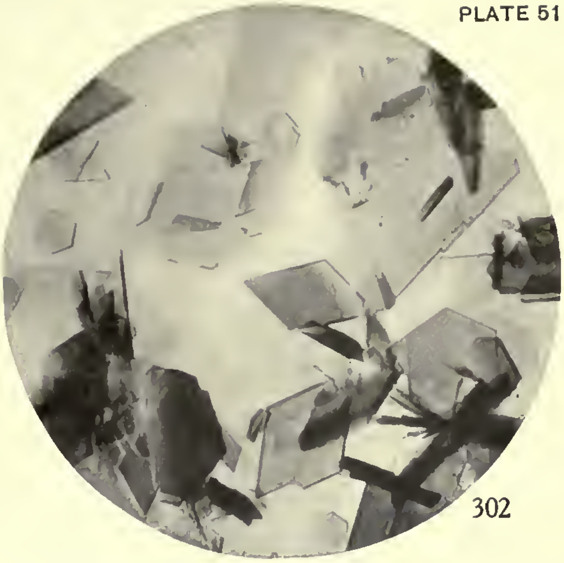


306

301. Oxyhemoglobin of the Beaver (*Castor canadensis*), showing rhomboidal and hexagonal tabular crystals, on base and edge.
302. Same, showing four and six-sided tabular crystals in different aspects and small hexagonal plates of mimetic twin.
303. Same, large hexagonal plates, showing twinning and parallel growth.
304. Same, elongated crystals showing twinning.
305, 306. Oxyhemoglobin of the Muskrat (*Fiber zibethicus*), showing needle-like first-formed crystals with elongated horse-type twins of lath-shaped crystals.


301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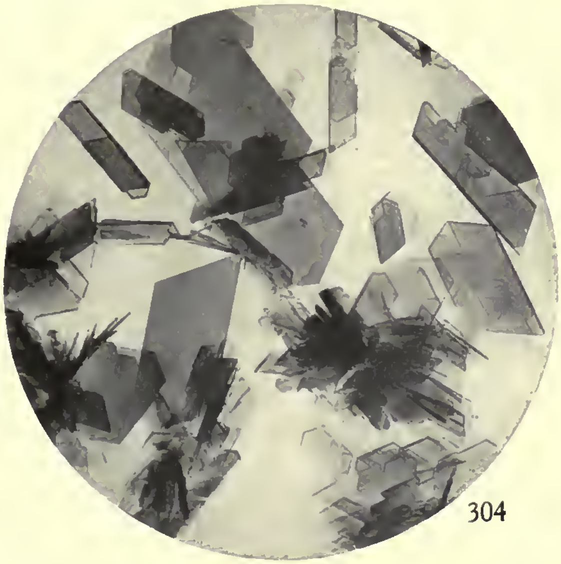
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306

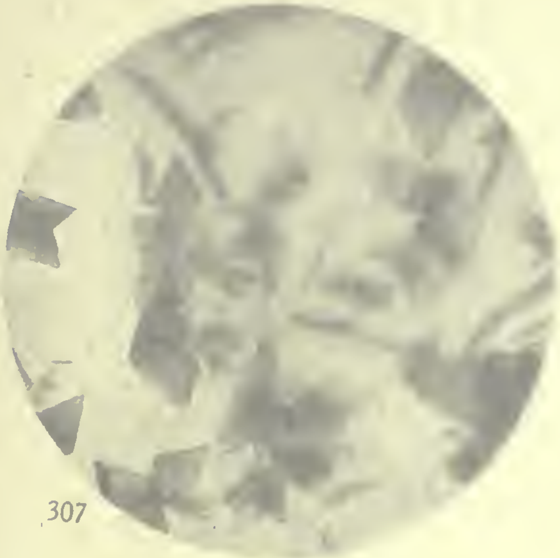
301. Oxyhemoglobin of the Beaver (*Castor canadensis*), showing rhomboidal and hexagonal tabular crystals, on base and edge.

302. Same, showing four and six-sided tabular crystals in different aspects and small hexagonal plates of mimetic twin.

303. Same, large hexagonal plates, showing twinning and parallel growth.

304. Same, elongated crystals showing twinning.

305, 306. Oxyhemoglobin of the Muskrat (*Fiber zibethicus*), showing needle-like first-formed crystals with elongated horse-type twins of lath-shaped crystals.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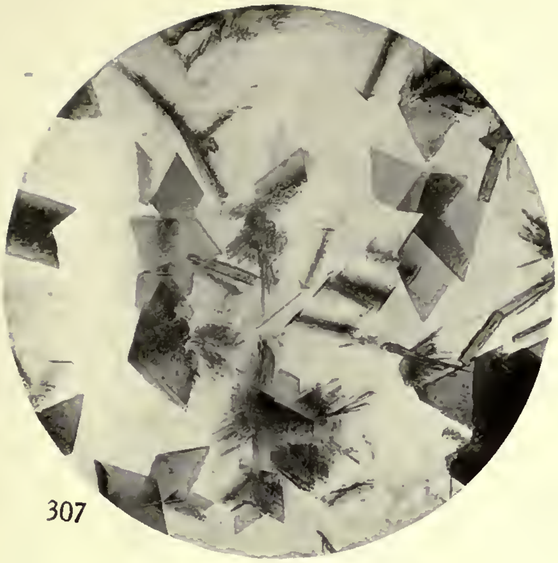
307–309. [illegible] Muskrat (*Fiber zibethicus*), showing [illegible] some horse-type twins.

310–312. [illegible] kinds of crystal aggregates [illegible] being produced.



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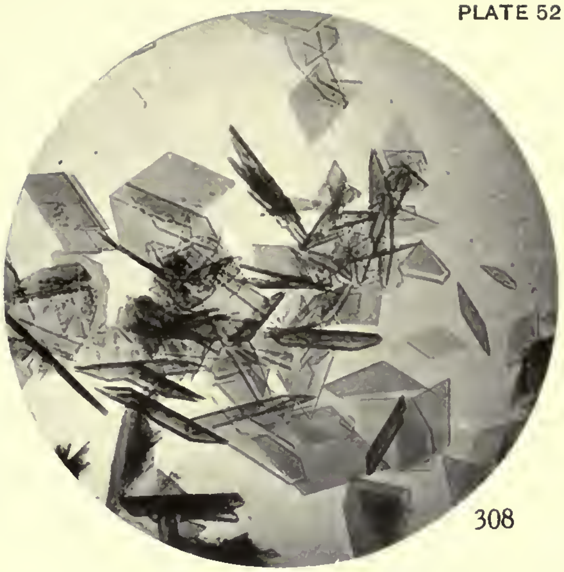

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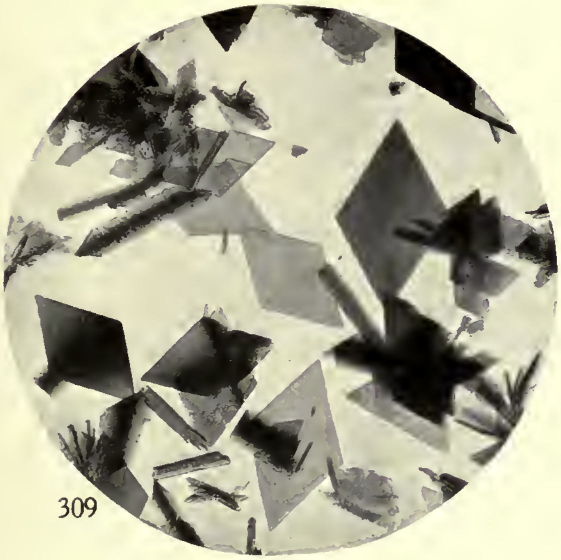

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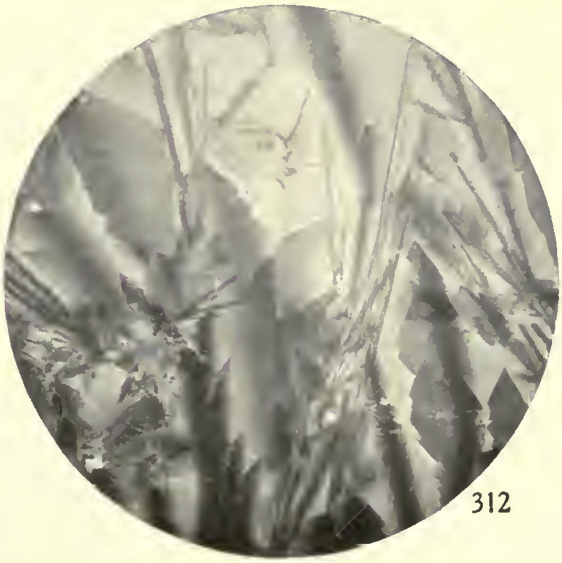

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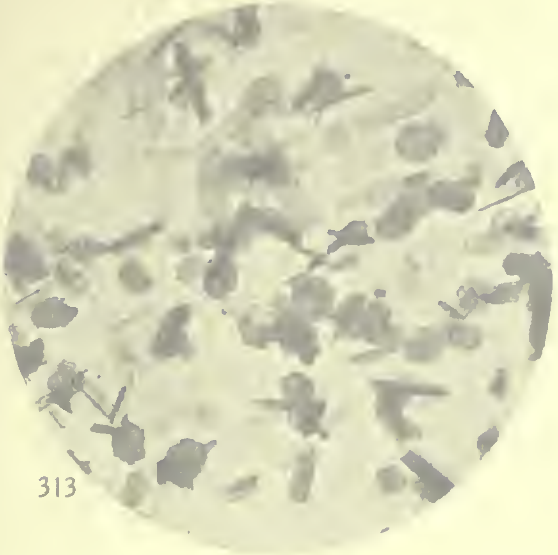
307–309. Oxyhemoglobin of the Muskrat (*Fiber zibethicus*), showing lozenge-shaped tabular crystals in different orientations and some horse-type twins.
310–312. Same, showing different kinds of crystal aggregates in parallel growth, arborescent grouping and sheaf-like forms being produced.



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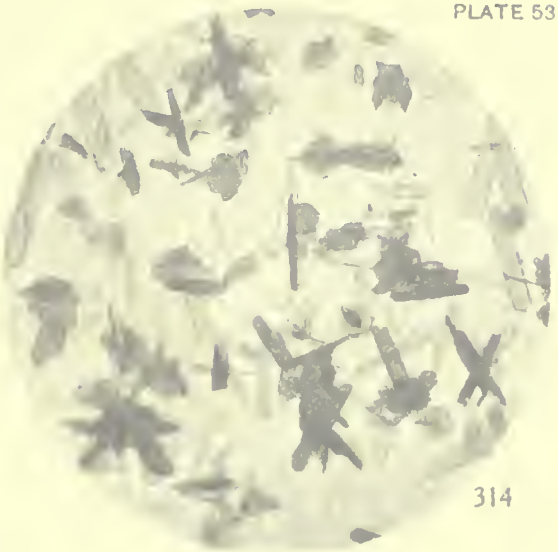

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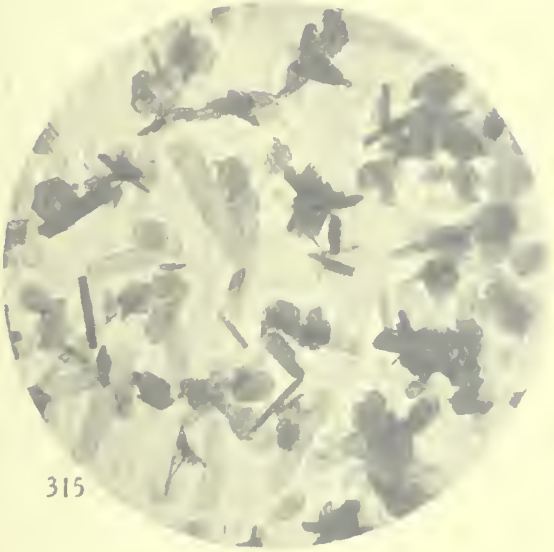

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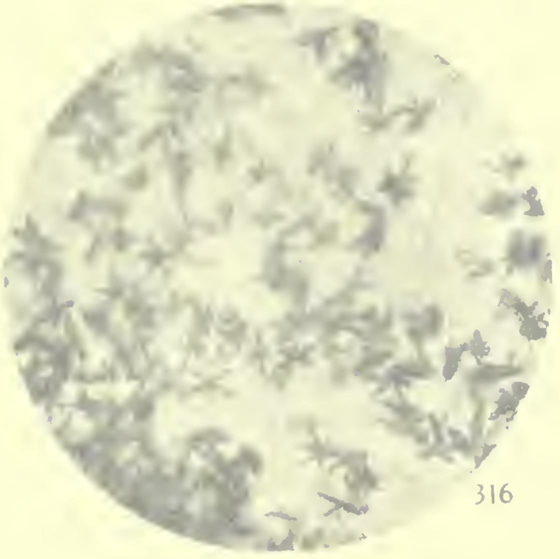

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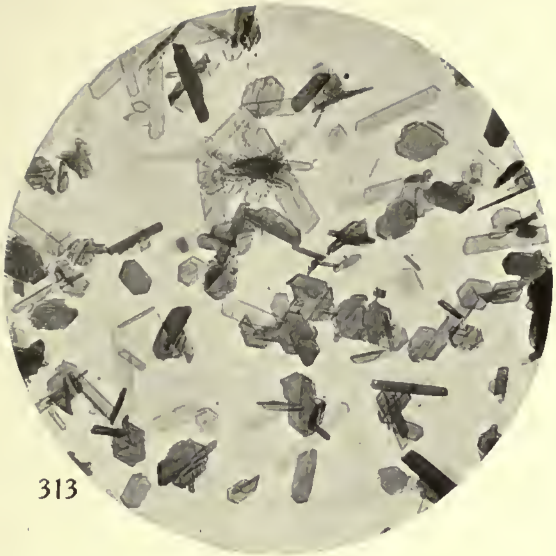
318

313-316. Oxyhemoglobin of the White Rat (Albino of *Mus norvegicus*), showing elongated six-sided plate produced by flattening of prism, roughly hexagonal groups due to twinning on brachy-pinacoid and interpenetrant twins on unit pyramids.
316. Same, showing star-shaped twins on unit pyramid.
317. Same, showing hexagonal composites with higher magnification.
318. Same, showing star-shaped twin with higher magnification.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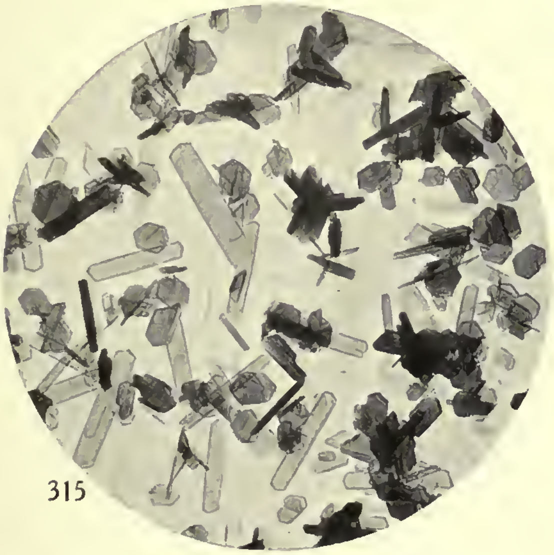
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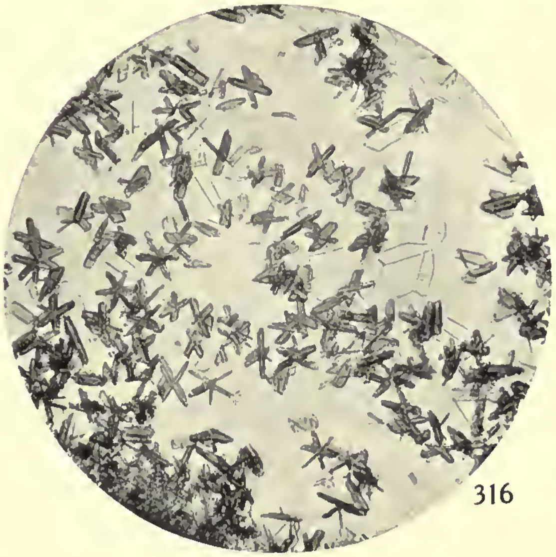
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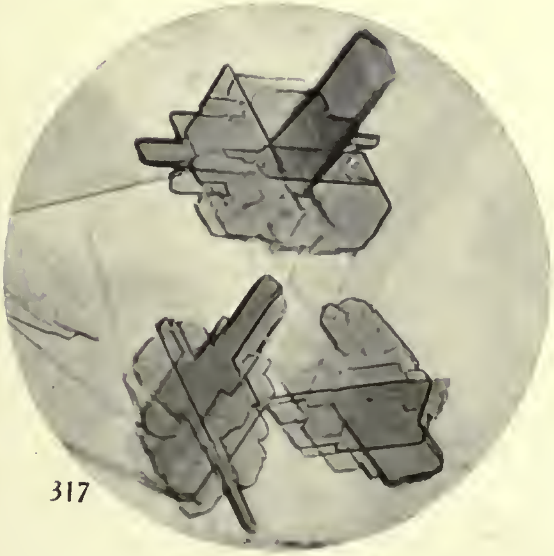
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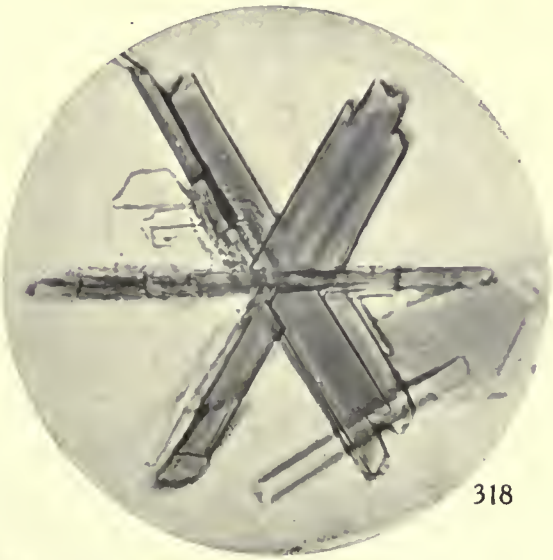
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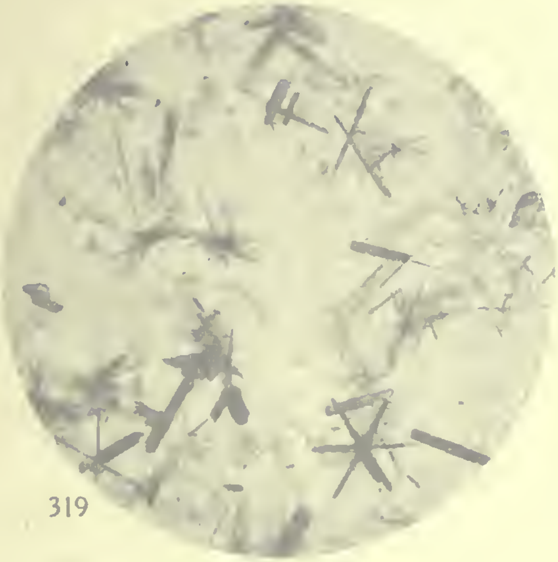
313-315. Oxyhemoglobin of the White Rat (Albino of *Mus norvegicus*), showing elongated six-sided plate produced by flattening of prism; roughly hexagonal groups due to twinning on brachy-pyramid and interpenetrant twins on unit pyramids.

316. Same, showing star-shaped twins on unit pyramid.

317. Same, showing hexagonal composites with higher magnification.

318. Same, showing star-shaped twin with higher magnification.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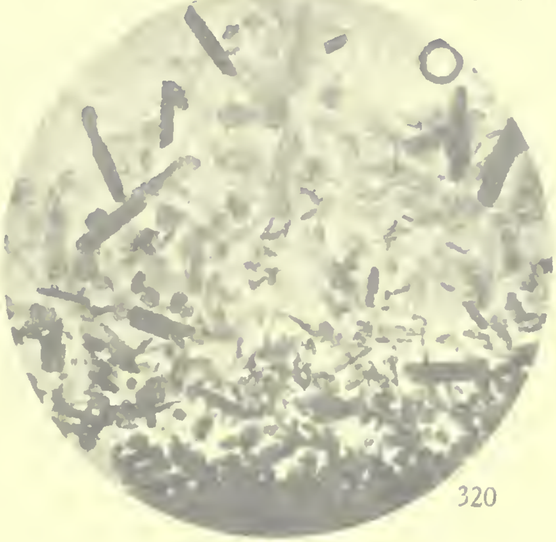
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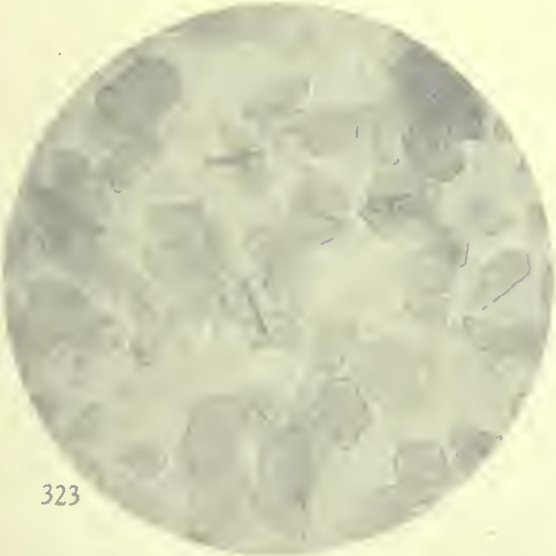
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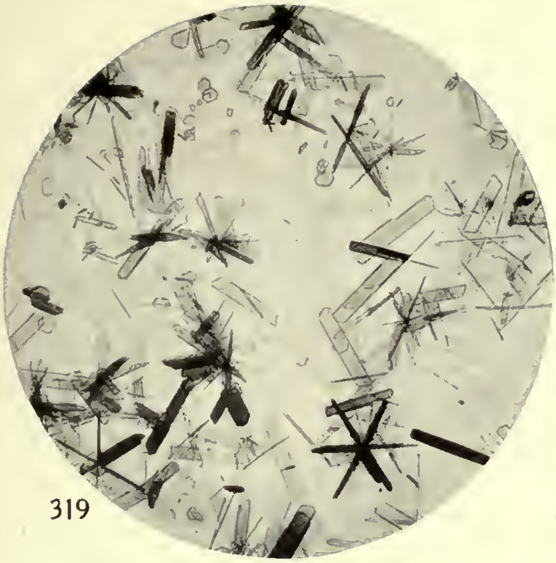
324

319. *α*-Oxyhemoglobin of the Norway Rat (*Mus norvegicus*), showing symmetrical and flattened prisms and hexagonal plates produced by shortening of prism. Star-shaped twin on unit pyramid seen at two places in lower part of field.
320. Same, showing especially nearly hexagonal plates produced by shortening of flattened prism.
321. *β*-Oxyhemoglobin of the Norway Rat, showing symmetrical and flattened octahedra in different aspects, with higher magnification.
322. *α*-Oxyhemoglobin of the Norway Rat, showing symmetrical and unsymmetrical prismatic crystals and pseudo-hexagonal plates, with some hexagonal plates of *β*-oxyhemoglobin due to flattening of octahedron.
323, 324. Oxyhemoglobin of Black Rat (*Mus rattus*), showing flattened prism terminated by dome, with dome faces sometimes unequally developed and producing four-, five-, and six-sided plates.

PLATE 54



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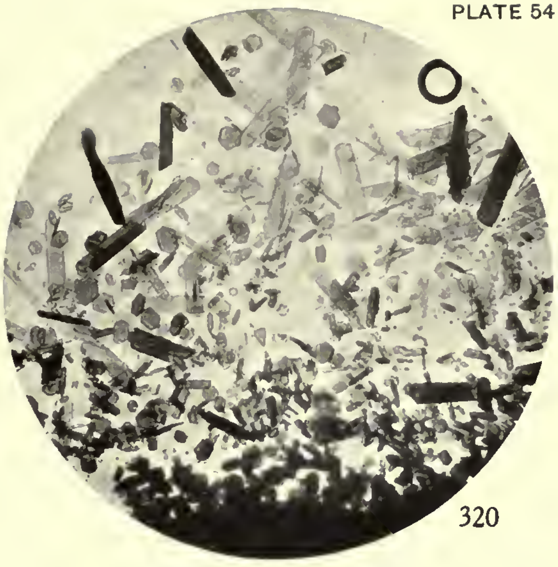

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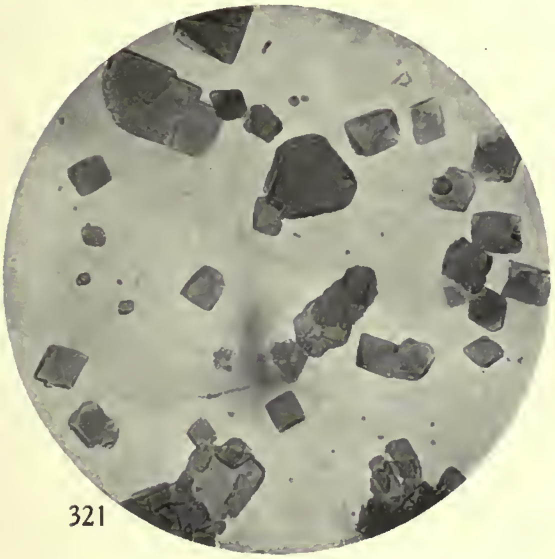

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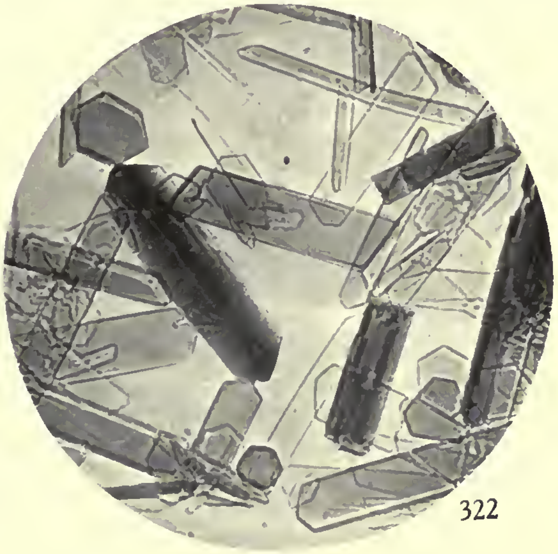

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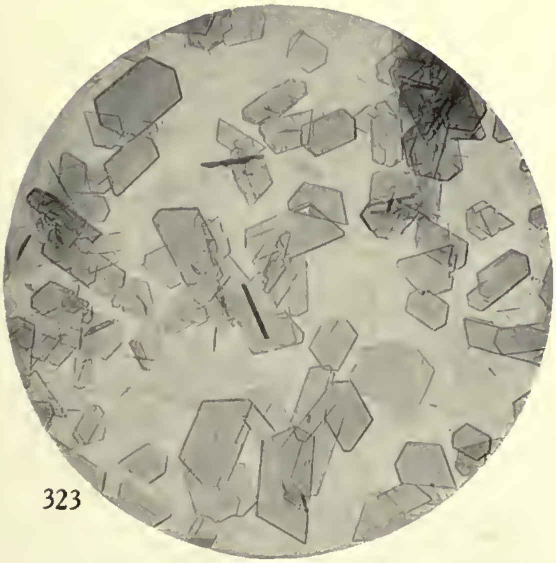

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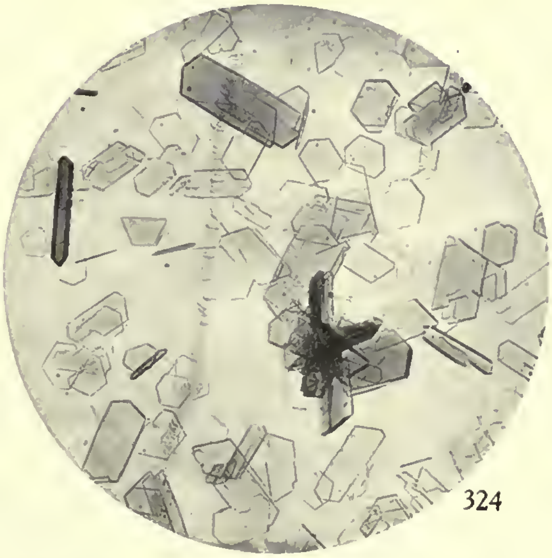

324

319. $\alpha$-Oxyhemoglobin of the Norway Rat (*Mus norvegicus*), showing symmetrical and flattened prisms and hexagonal plates produced by shortening of prism. Star-shaped twin on unit pyramid seen at two places in lower part of field.

320. Same, showing especially nearly hexagonal plates produced by shortening of flattened prism.

321. $\beta$-Oxyhemoglobin of the Norway Rat, showing symmetrical and flattened octahedra in different aspects, with higher magnification.

322. $\alpha$-Oxyhemoglobin of the Norway Rat, showing symmetrical and unsymmetrical prismatic crystals and pseudo-hexagonal plates, with some hexagonal plates of $\beta$-oxyhemoglobin due to flattening of octahedron.

323, 324. Oxyhemoglobin of Black Rat (*Mus rattus*), showing flattened prism terminated by dome, with dome faces sometimes unequally developed and producing four-, five-, and six-sided plates.


325


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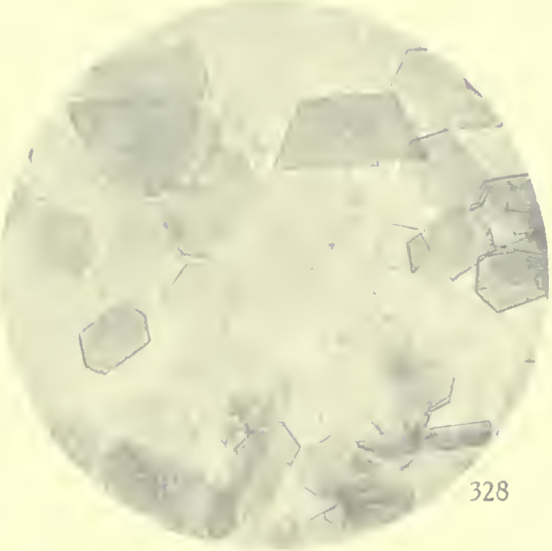
327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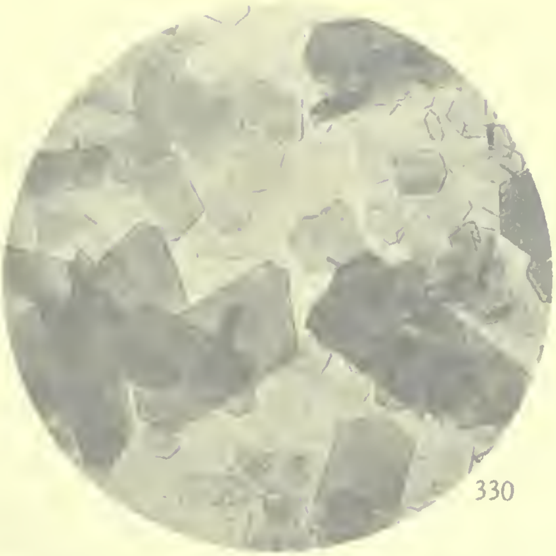
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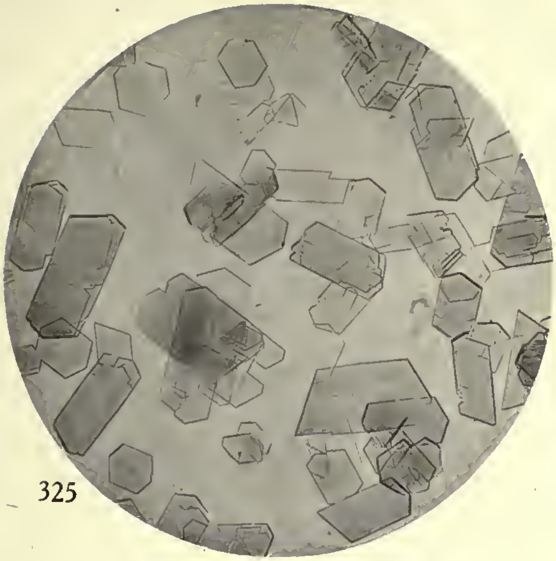
325. Oxyhemoglobin of the Black Rat (*Mus rattus*), showing twin on the flat, consisting of two individuals and not producing a hexagonal plate as in White Rat twin.
326. Same, showing thicker crystals and oblique termination of dome faces.
327. Oxyhemoglobin of the Alexandrine Rat (*Mus alexandrinus*), showing unsymmetrical flattened prisms and a twin on flat aspect to lower left of field.
328. Same, showing four-, five-, and six-sided tabular crystals, due to unsymmetrical development of dome faces.
329. Same, showing star-shaped twins.
330. Same, showing larger crystals.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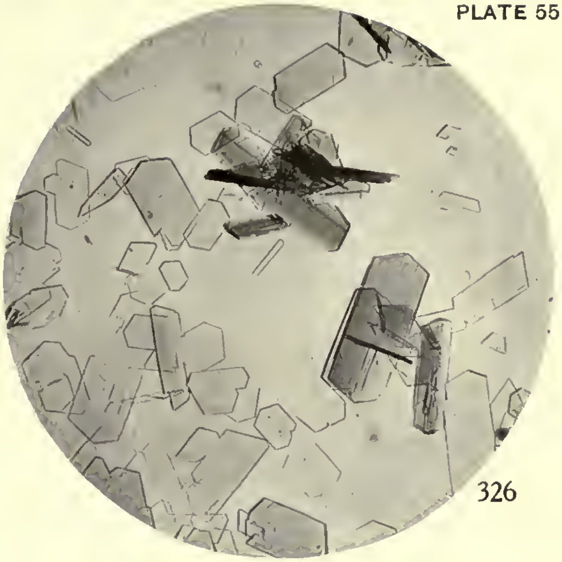
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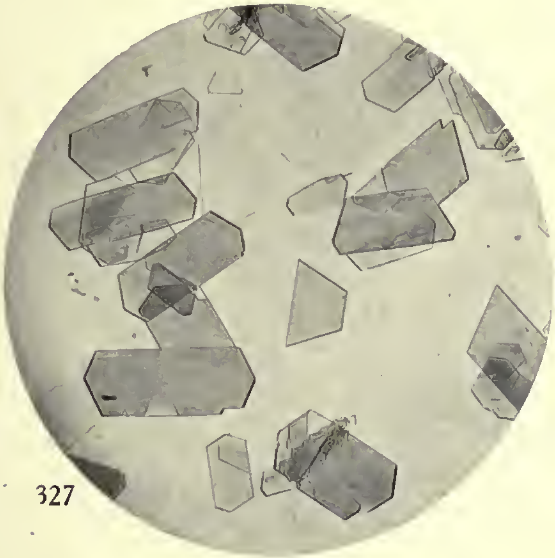
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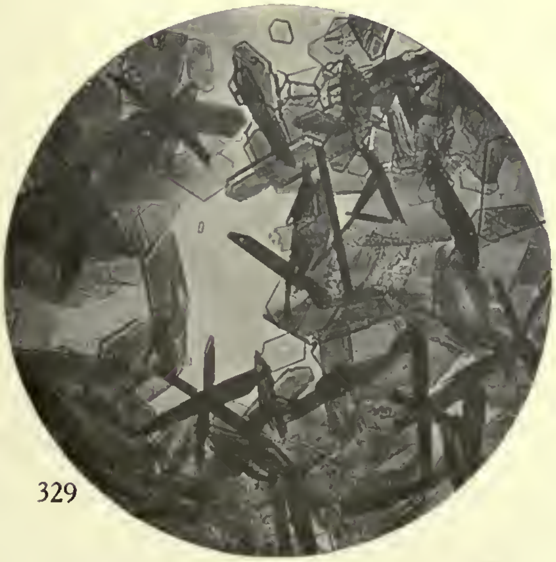
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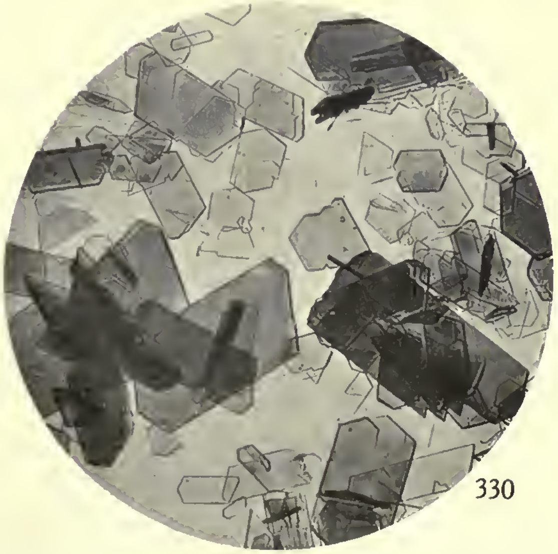
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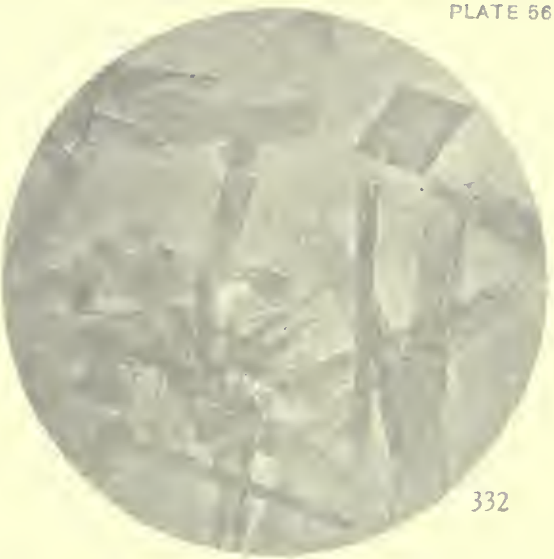
325. Oxyhemoglobin of the Black Rat (*Mus rattus*), showing twin on the flat, consisting of two individuals and not producing a hexagonal plate as in White Rat twin.
326. Same, showing thicker crystals and oblique termination of dome faces.
327. Oxyhemoglobin of the Alexandrine Rat (*Mus alexandrinus*), showing unsymmetrical flattened prisms and a twin on flat aspect to lower left of field.
328. Same, showing four-, five-, and six-sided tabular crystals, due to unsymmetrical development of dome faces.
329. Same, showing star-shaped twins.
330. Same, showing larger crystals.

331



332

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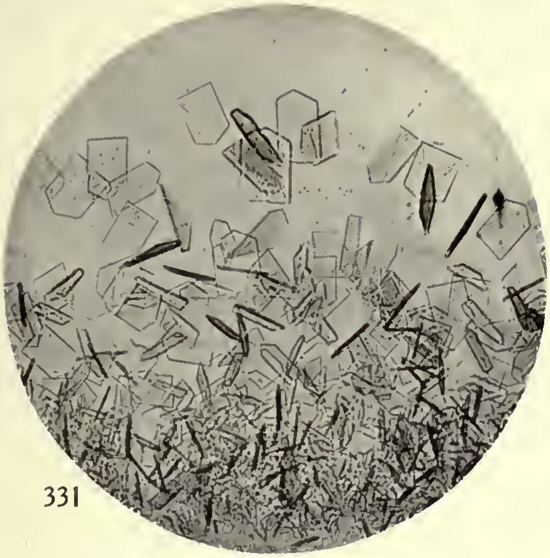
336

331. α-Oxyhemoglobin of the Porcupine (*Erethizon dorsatus*), showing [illegible] crystals.
332, 333. α-Oxyhemoglobin and β-Oxyhemoglobin of the Porcupine, showing [illegible] type twins of α-oxyhemoglobin and their [illegible] crystals of β-oxyhemoglobin.
334. β-Oxyhemoglobin of the Porcupine, showing [illegible] crystal [illegible].
335, 336. Same, showing large crystals in various orientations.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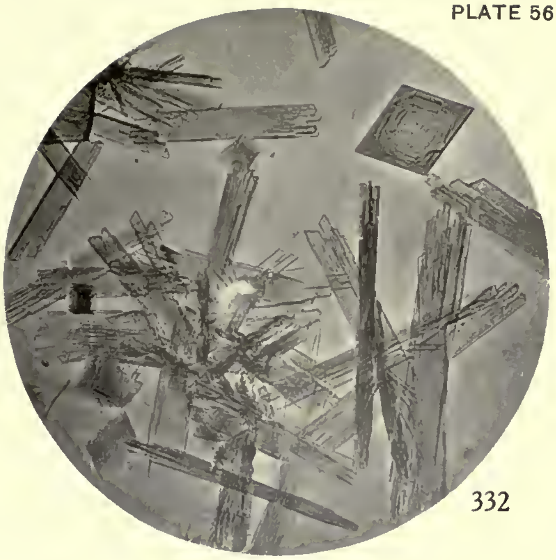
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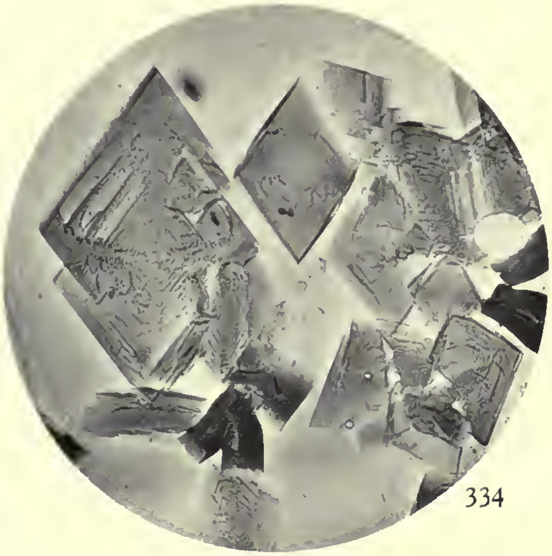
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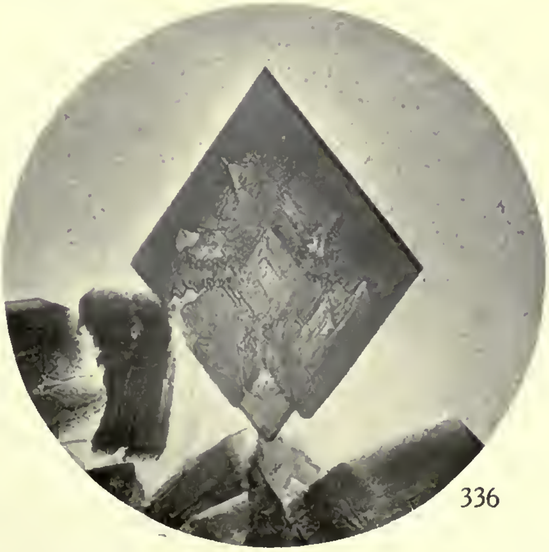
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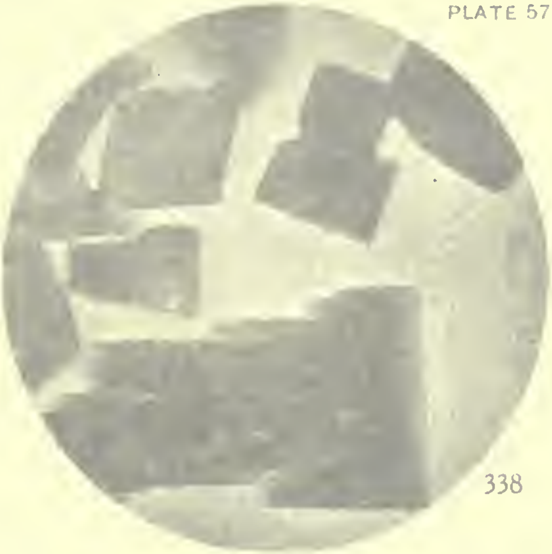
331. $\alpha$-Oxyhemoglobin of the Porcupine (*Erethizon dorsatus*), showing first-formed tabular crystals.
332, 333. $\alpha$-Oxyhemoglobin and $\beta$-Oxyhemoglobin of the Porcupine, showing bundles of elongated horse-type twins of $\alpha$-oxyhemoglobin and thick tabular crystals of $\beta$-oxyhemoglobin.
334. $\beta$-Oxyhemoglobin of the Porcupine, showing skeleton crystal appearance.
335, 336. Same, showing large crystals in various orientations.


337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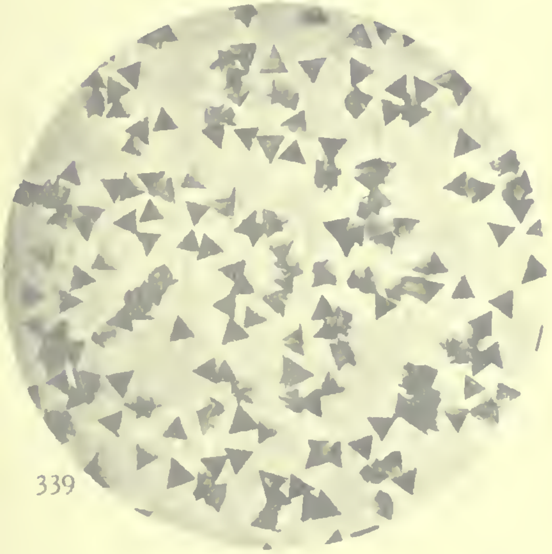
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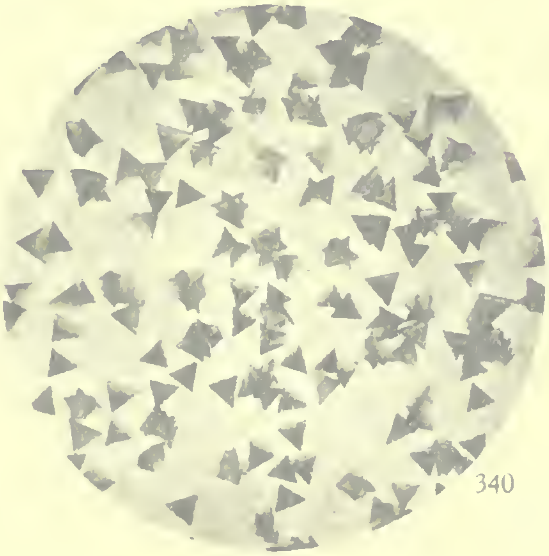
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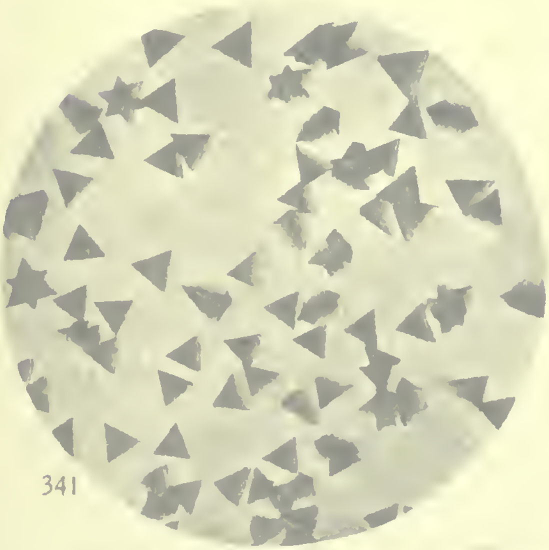
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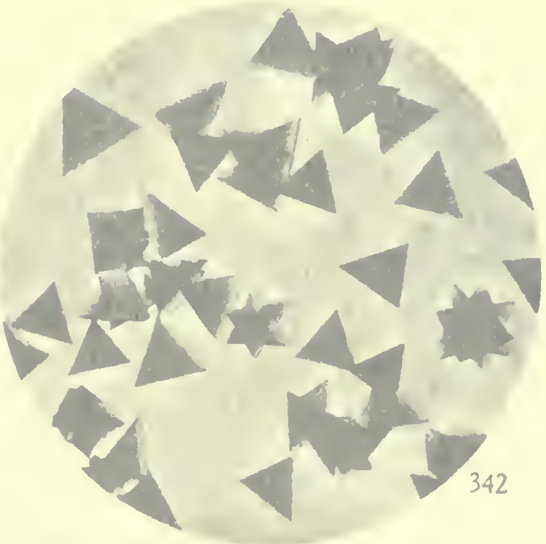
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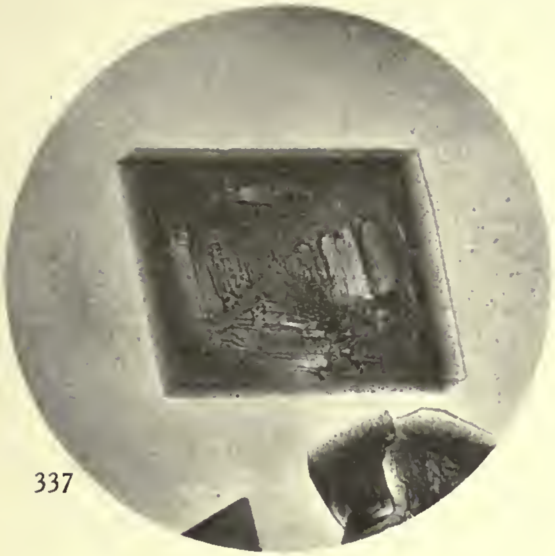
337. β-Oxyhemoglobin of the Porcupine (*Erethizon dorsatus*), showing large crystals in basal aspect
338. Same, showing basal and edge views.
339, 340. Oxyhemoglobin of the Guinea-pig (*Cavia cutleri*), showing small single crystals and twins of types *a* and *c*.
341. Same, medium-size crystals, some twinned, showing types *a*, *b*, and *c*
342. Same, showing large crystals with twins of types *a* and *b*.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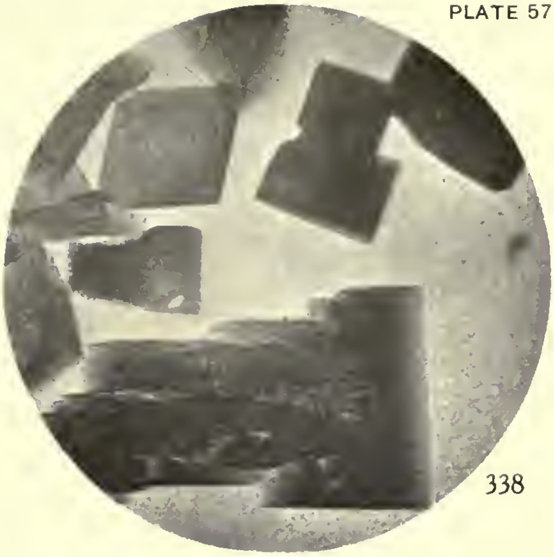
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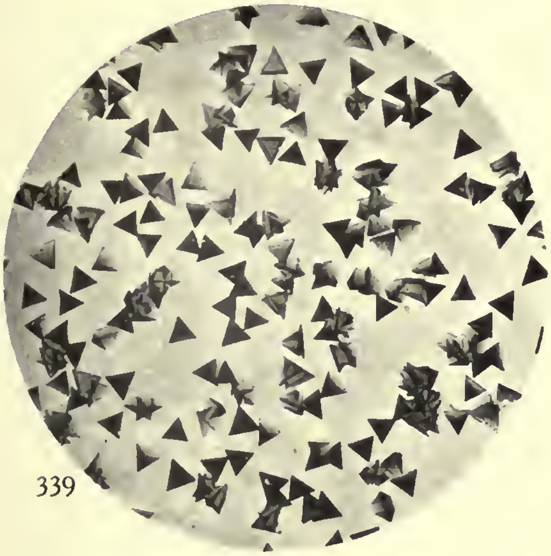
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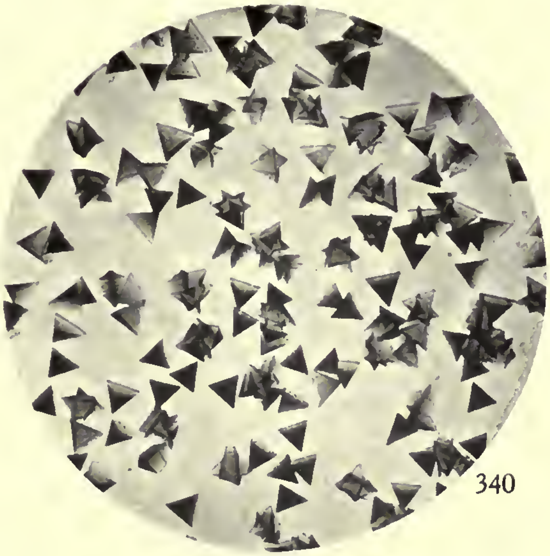
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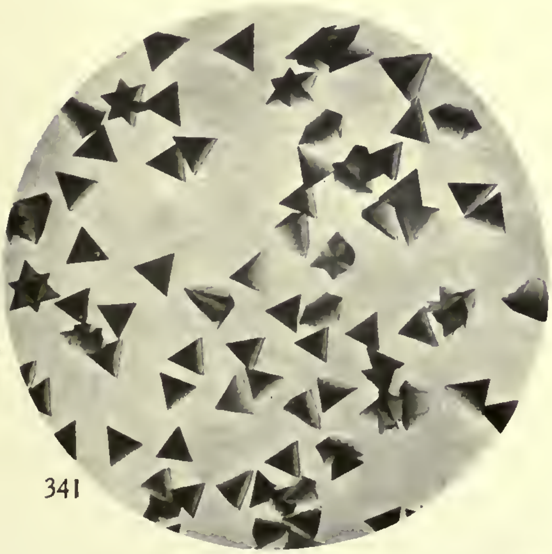
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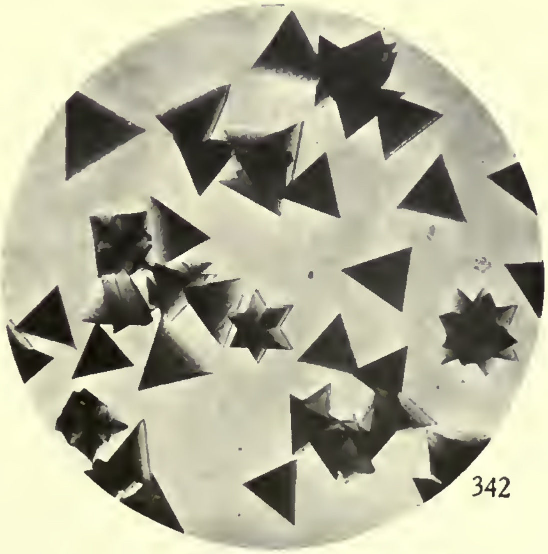
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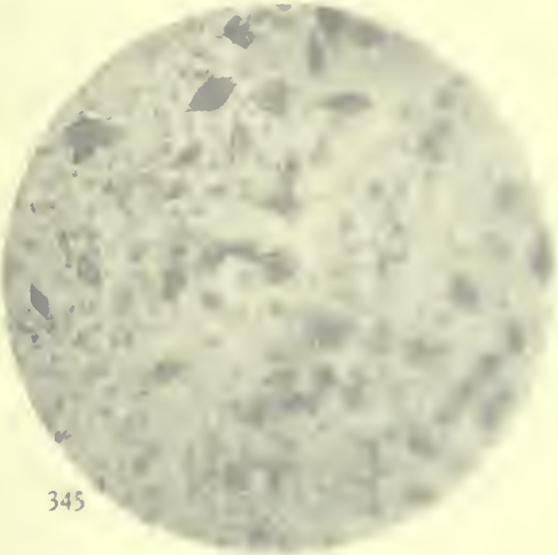
337. β-Oxyhemoglobin of the Porcupine (*Erethizon dorsatus*), showing large crystals in basal aspect.
338. Same, showing basal and edge views.
339, 340. Oxyhemoglobin of the Guinea-pig (*Cavia cutleri*), showing small single crystals and twins of types *a* and *c*.
341. Same, medium-size crystals, some twinned, showing types *a*, *b*, and *c*.
342. Same, showing large crystals with twins of types *a* and *b*.


343


344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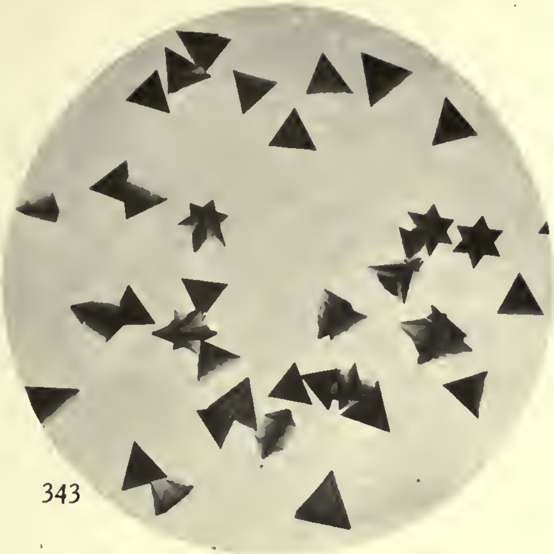
347


348

343. Oxyhemoglobin of the [illegible]
344. Same, showing twi[illegible]
345-347. α-Oxyhemoglob[illegible]
orientat[illegible]
348. β-Oxyhemogl[illegible]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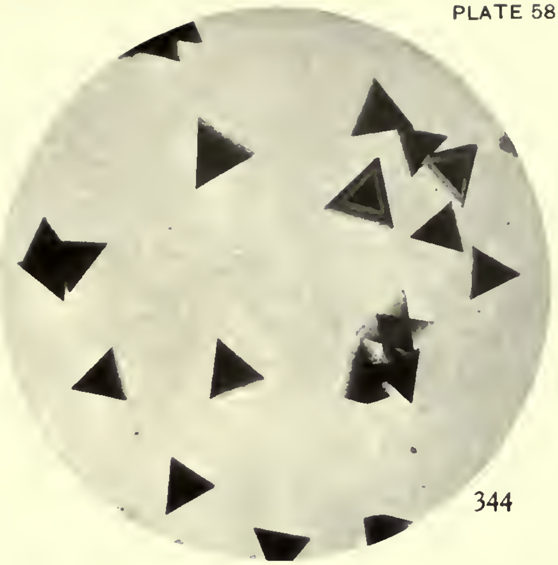
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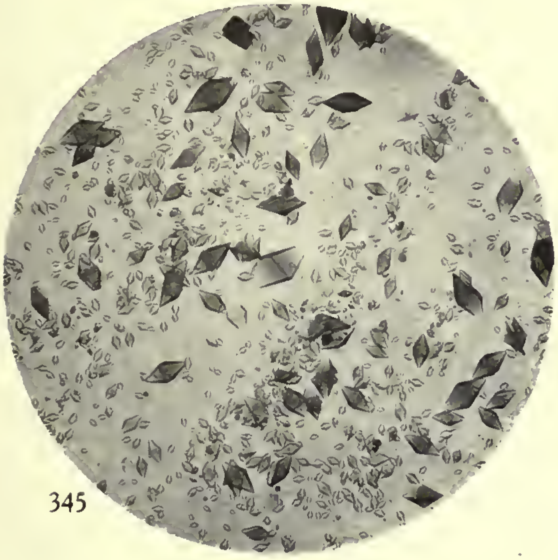
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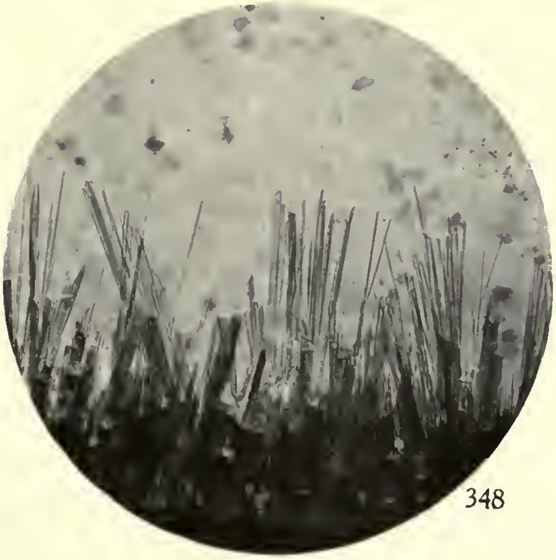
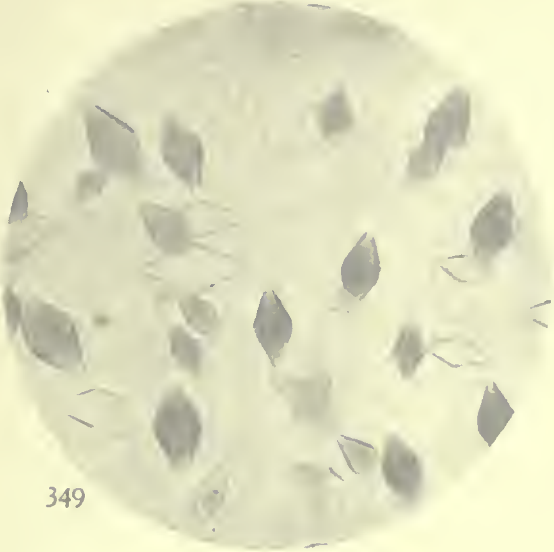

348

343. Oxyhemoglobin of the Guinea-pig (*Cavia cutleri*), showing single crystals and twins of types *b* and *c*.
344. Same, showing twin of type *a* in side view to right center of field.
345–347. *a*-Oxyhemoglobin of the Capybara (*Hydrochærus capyvara*), showing pyramidal crystals in various orientations.
348. β-Oxyhemoglobin of the Capybara, showing long prismatic crystals.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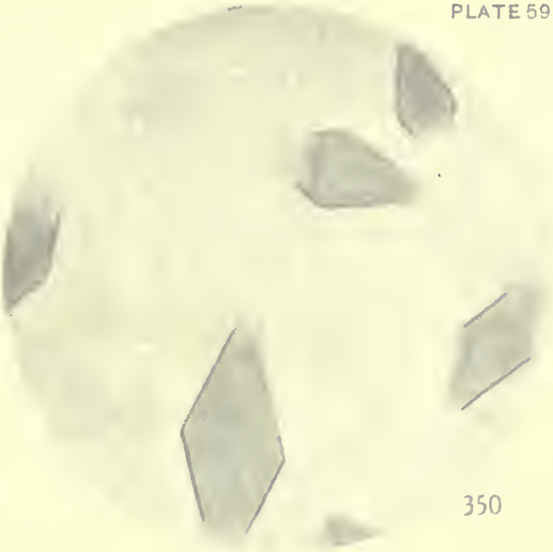
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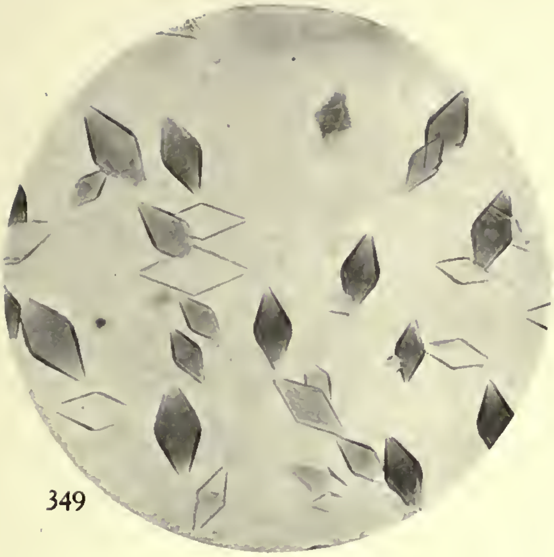
353


354

349. α-Oxyhemoglobin of the Capybara (*Hydrochœrus capybara*), photographed in polarized [illegible] used to show pleochroism.
350. Same, showing larger crystals.
351. Same, showing large crystals in ordinary light.
352. Same crystals seen in 351, in polarized light with one nicol showing [illegible]
353. α-Oxyhemoglobin of the Rabbit (*Lepus cuniculus*), showing prismatic type of crystals.
354. Same, showing prismatic and tabular types of crystal.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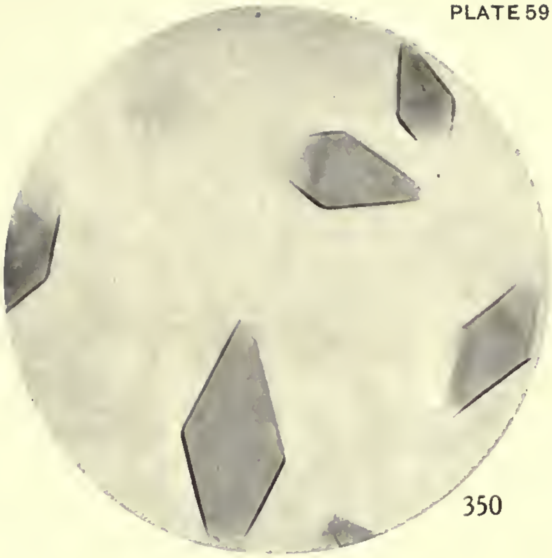
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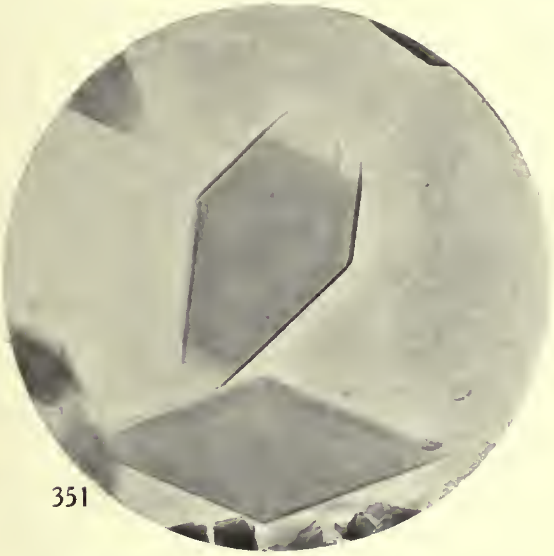
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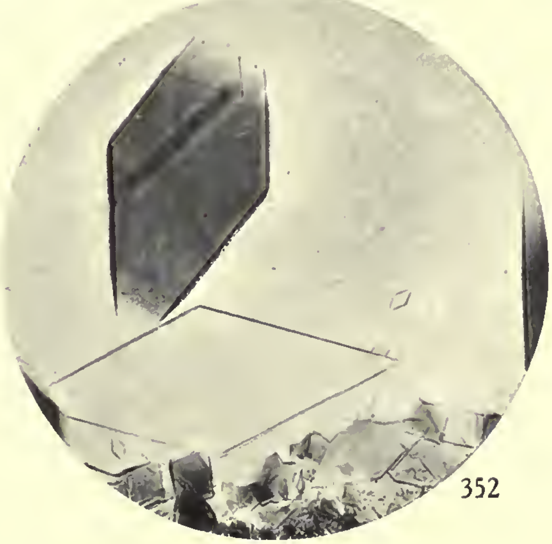
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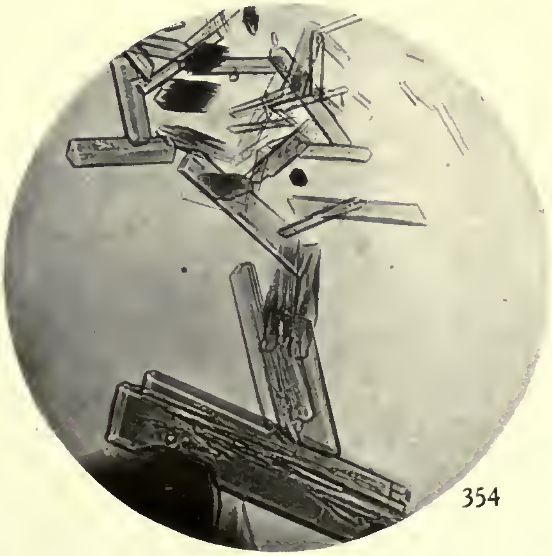
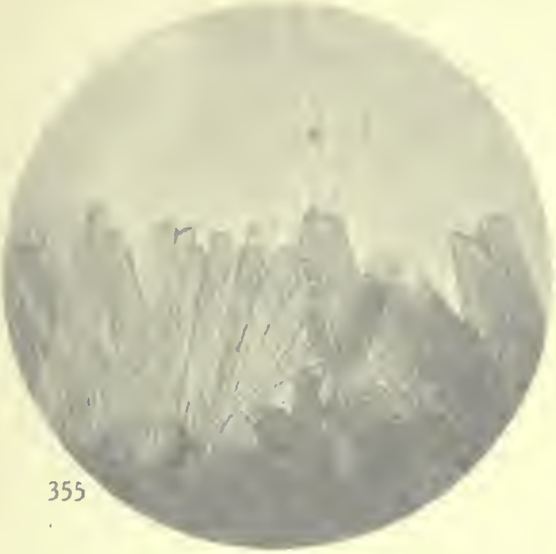
353


354

349. *a*-Oxyhemoglobin of the Capybara (*Hydrochærus capyvara*), photographed in polarized light with one nicol to show pleochroism.
350. Same, showing larger crystals.
351. Same, showing large crystals in ordinary light.
352. Same crystals seen in 351, in polarized light with one nicol showing pleochroism.
353. *a*-Oxyhemoglobin of the Rabbit (*Lepus cuniculus*), showing prismatic type of crystal.
354. Same, showing prismatic and tabular types of crystal.



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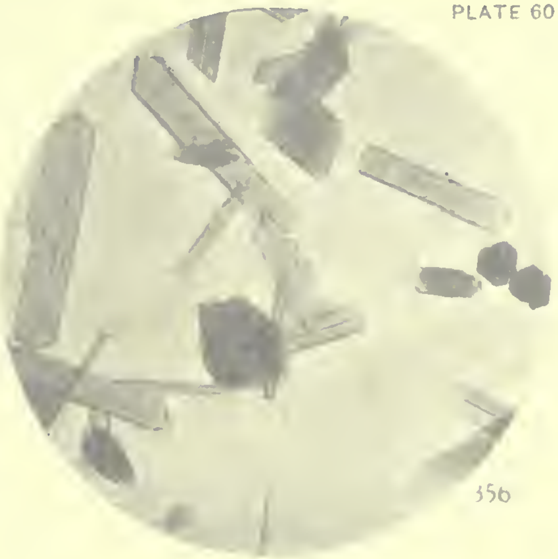

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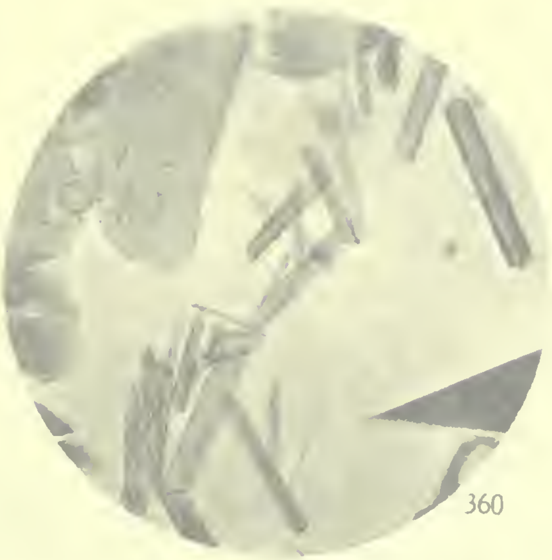
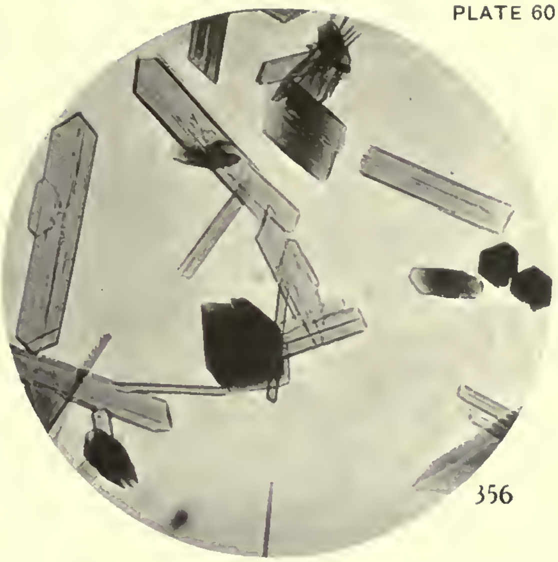


360

355. α-Oxyhemoglobin of the Rabbit (*Lepus cuniculus*), showing prismatic (type *a*) crystals growing from protein ring.
356. Same, showing prismatic (type *a*) crystals in various orientations.
357–359. Same, showing tabular (type *b*) crystals.
360. α-Oxyhemoglobin type *a* and β-Oxyhemoglobin of the Rabbit, showing pleochroism of β-Oxyhemoglobin crystals in ordinary light.


355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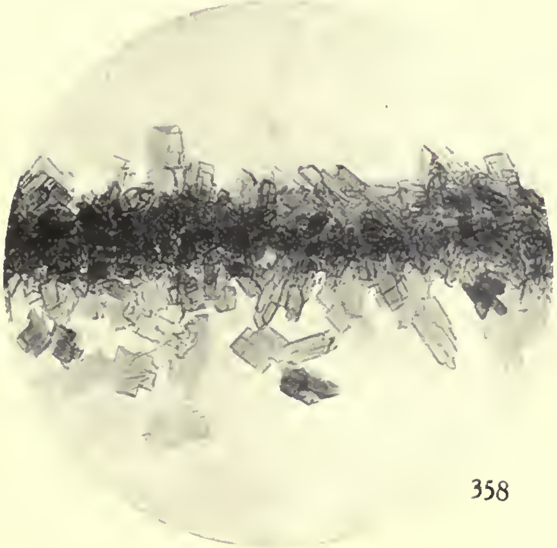
357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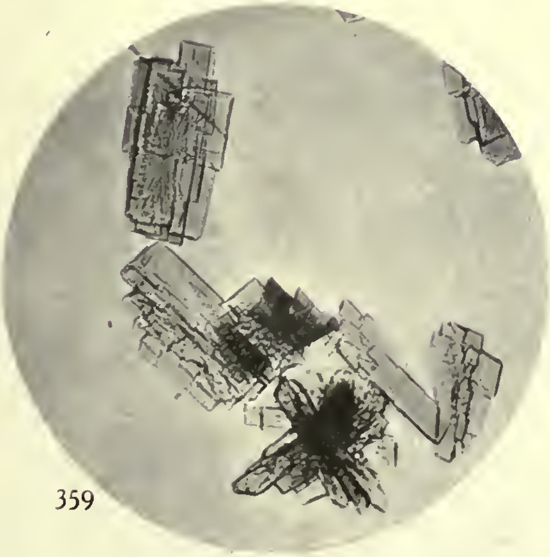
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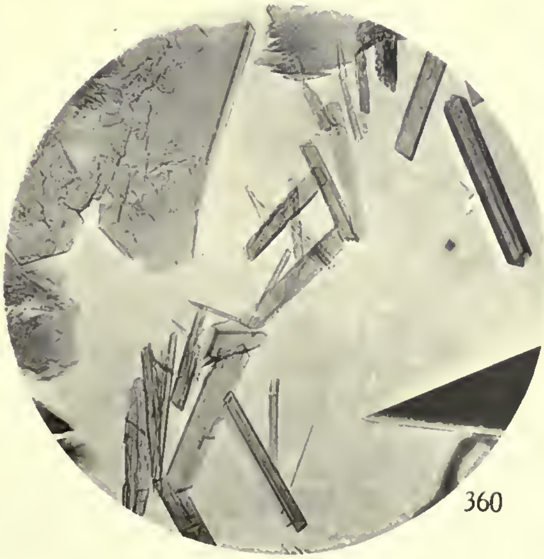
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355. *a*-Oxyhemoglobin of the Rabbit (*Lepus cuniculus*), showing prismatic (type *a*) crystals growing from protein ring.
356. Same, showing prismatic (type *a*) crystals in various orientations.
357–359. Same, showing tabular (type *b*) crystals.
360. *a*-Oxyhemoglobin type *a* and *β*-Oxyhemoglobin of the Rabbit, showing pleochroism of *β*-Oxyhemoglobin crystals in ordinary light.


361


362


363


364


365


366

361. [illegible]
362. [illegible]
363 364. [illegible]
365. [illegible]
[illegible] terminations.
366. [illegible]


361


362


363


364

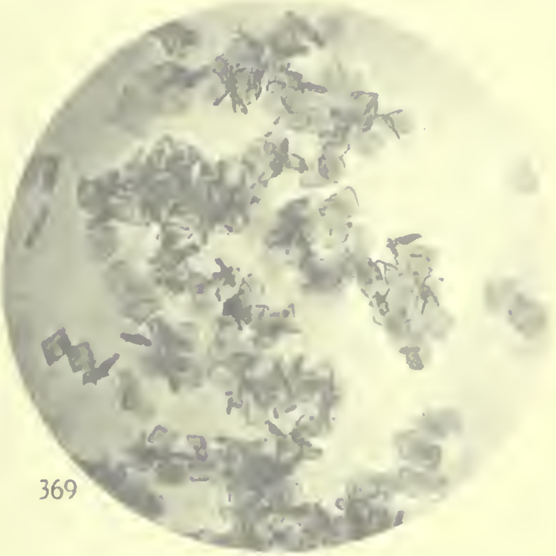

365


366

361. *β*-Oxyhemoglobin of the Rabbit (*Lepus cuniculus*), showing oblique sections of crystals.
362. Same, showing oblique sections; crystals are pleochroic in ordinary light.
363, 364. Same, showing groups of large crystals in oblique section. 364 shows decided pleochroism.
365. *a*-Oxyhemoglobin of the Belgian Hare (*Lepus europæus*), showing prismatic crystals of second crop, growing in radiating groups from protein ring. A few show single oblique termination.
366. Same, showing doubly terminated prismatic crystals from protein ring.


367


368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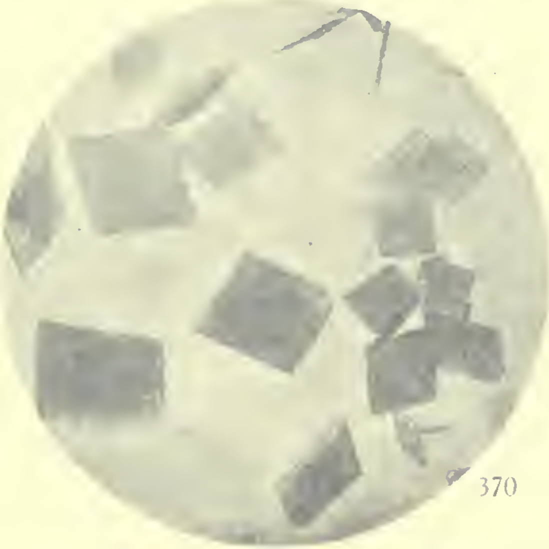
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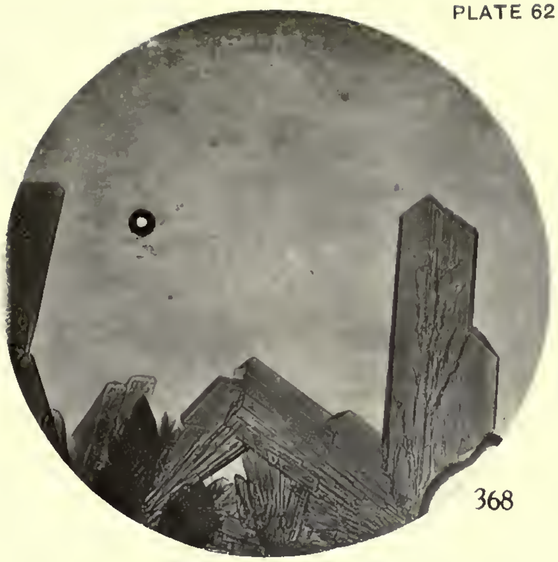
371


372

367, 368. *a*-Oxyhemoglobin of the Belgian Hare (*Lepus europæus*), showing large prismatic crystals of second crop.
369. Oxyhemoglobin of the Harbor Seal (*Phoca vitulina*), showing first-formed, small tabular crystals. Their hemimorphic character is easily observed.
370. Same, showing larger tabular crystals, some exhibiting parallel growth. Hemimorphism is evident in large central crystal
371. Same, showing crystals elongated into prisms by development of orthopinacoid.
372. Same, showing simple symmetrical crystals consisting of base (001), unit prism (110), and unit pyramid (111).

367



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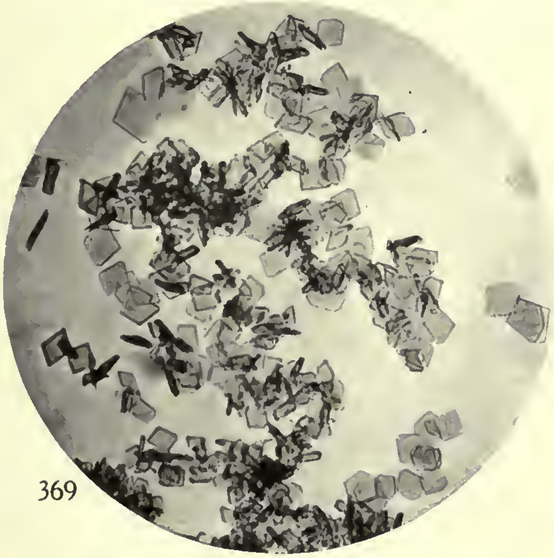

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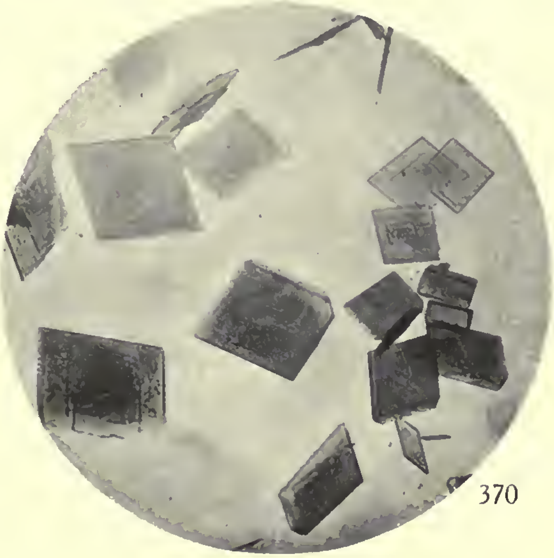

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371

372

367, 368. *a*-Oxyhemoglobin of the Belgian Hare (*Lepus europæus*), showing large prismatic crystals of second crop.

369. Oxyhemoglobin of the Harbor Seal (*Phoca vitulina*), showing first-formed, small tabular crystals. Their hemimorphic character is easily observed.

370. Same, showing larger tabular crystals, some exhibiting parallel growth. Hemimorphism is evident in large central crystal.

371. Same, showing crystals elongated into prisms by development of orthopinacoid.

372. Same, showing simple symmetrical crystals consisting of base (001), unit prism (110), and unit pyramid (111).


373


374


375

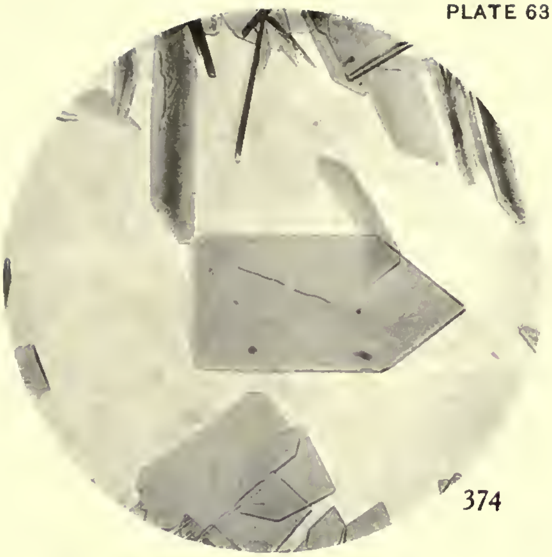

376


377


378

373. [illegible] of California Sea-lion ([illegible]), [illegible] untwinned.
374. Same, showing larger single crystal. [illegible]
375. Same, showing Sea-lion twin in [illegible]
376. Same, showing parallel growth [illegible]
377. Same, showing flat view of crystals [illegible] of individuals in [illegible]
378. Same, showing [illegible] barring when [illegible]


373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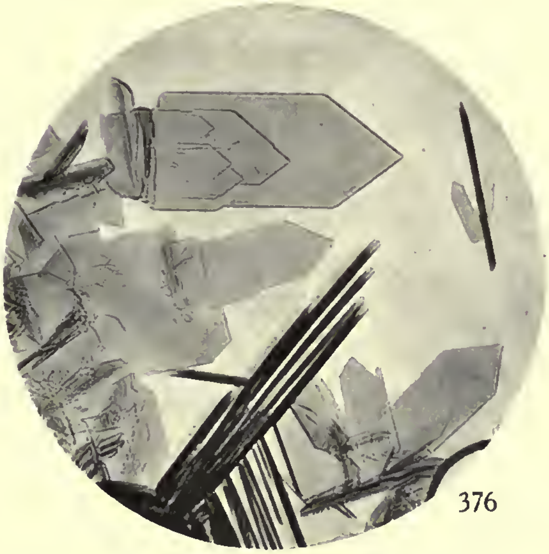
375

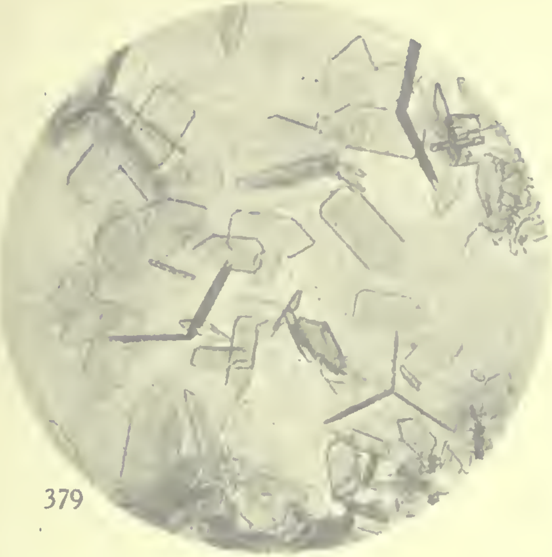

376


377


378

373. Oxyhemoglobin of California Sea-lion (*Otaria gillespii*), showing small, first-formed crystals, mostly simple and untwinned.
374. Same, showing larger single crystal. Hemimorphic character well shown in this crystal.
375. Same, showing Sea-lion twin in edge view, consisting in this case of two individuals.
376. Same, showing parallel growth and an edge view of Sea-lion twin in parallel growth.
377. Same, showing flat view of crystals in parallel growth. Cross-barring is due to twinning of a number of individuals in parallel position.
378. Same, showing parallel growth in twins seen on edge, producing comb-like appearance and cross-barring when seen on the flat.

PLATE 64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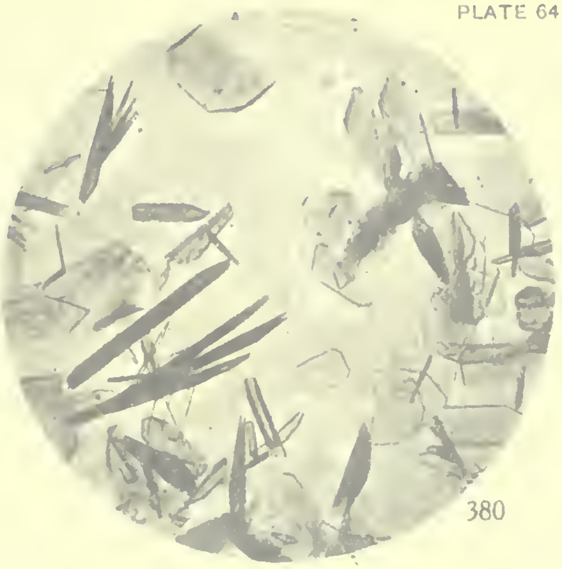
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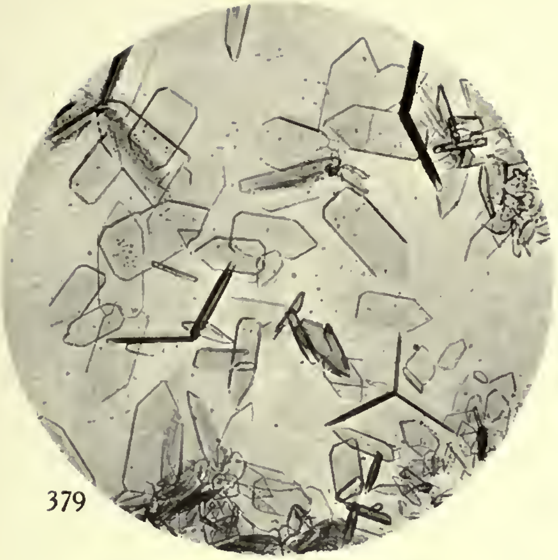
384

379. CO-Hemoglobin of the California Sea-lion (*Otaria gillespii*), showing small, first-formed crystals, some twinned.
380. Same, showing the crystals seen on edge and in section.
381. Same, large crystals showing parallel growth.
382. Same, showing Sea-lion twin in edge view, consisting of three nearly symmetrically developed individuals.
383. Same, showing parallel growth.
384 Same, showing single large crystal in symmetrical position. Small crystal below to the left shows profile view looking along ortho-axis.



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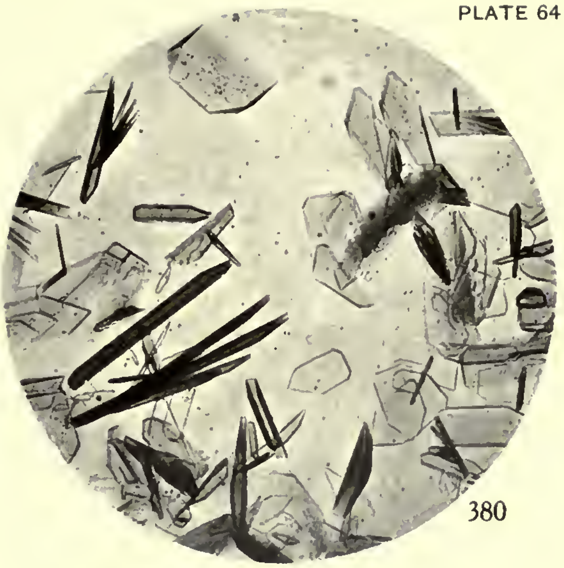

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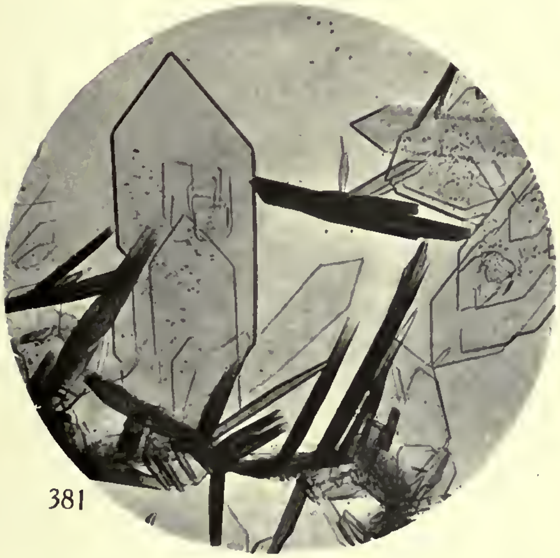

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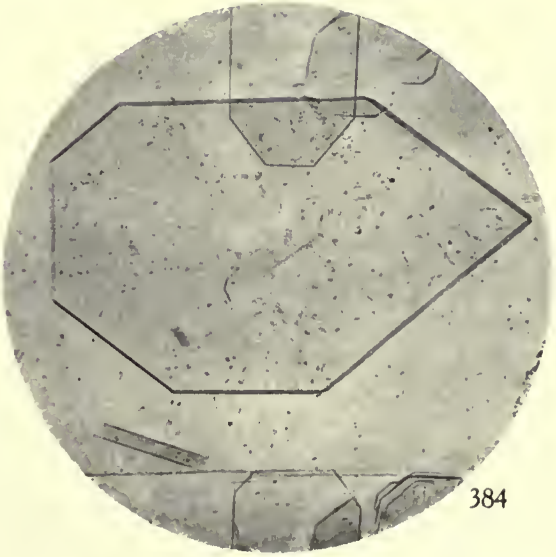

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379. CO-Hemoglobin of the California Sea-lion (*Otaria gillespii*), showing small, first-formed crystals, some twinned.
380. Same, showing the crystals seen on edge and in section.
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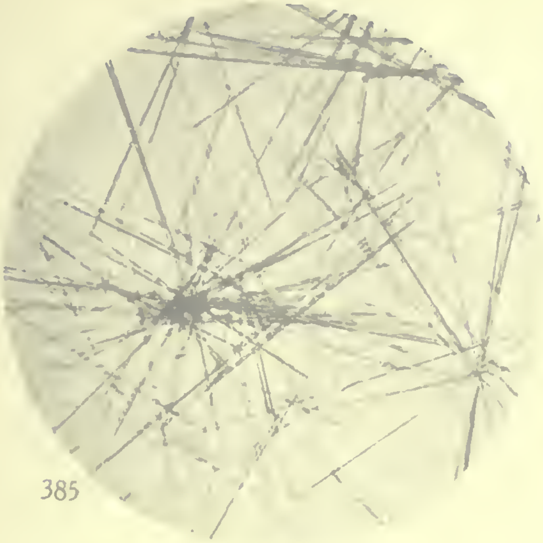
384 Same, showing single large crystal in symmetrical position. Small crystal below to the left shows profile view looking along ortho-axis.



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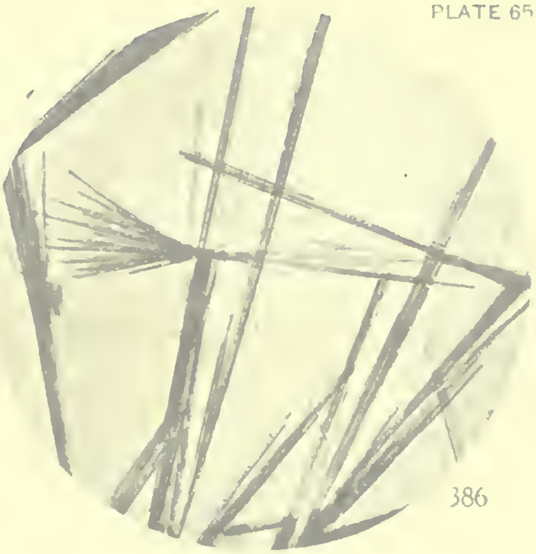

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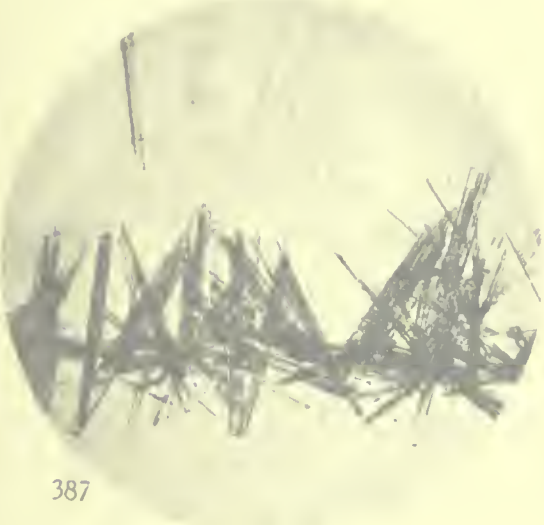

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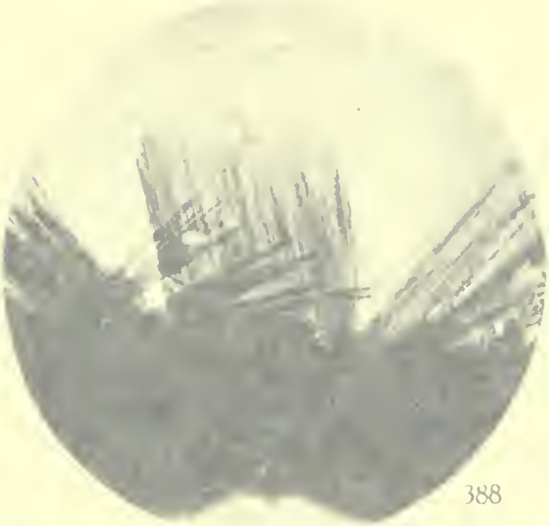

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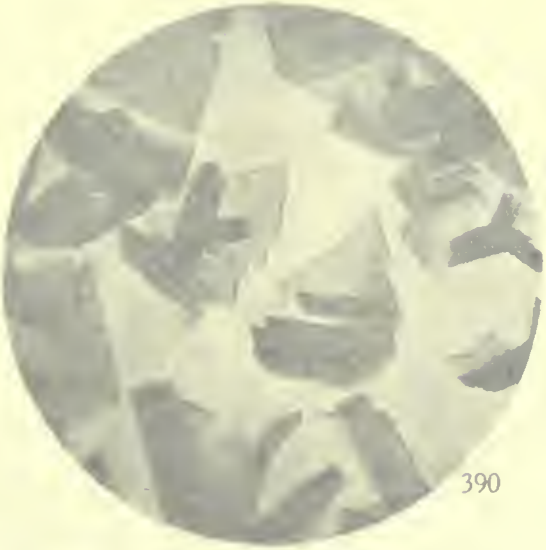
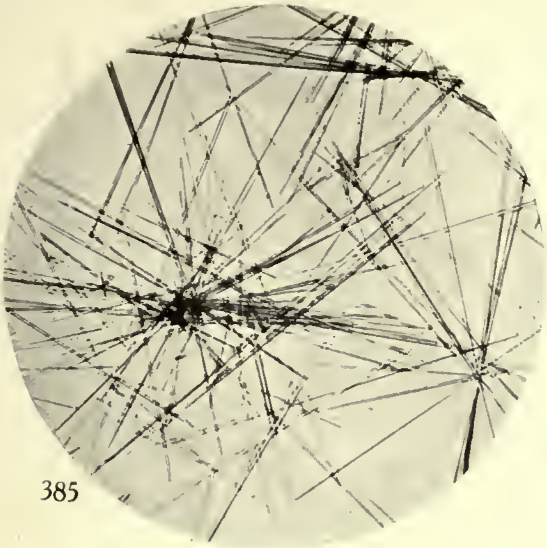


390

[illegible]. Oxyhemoglobin of the Skunk (*Mephitis mephitica putida*), showing long prismatic crystals growing in radiating groups.
[illegible]. Same, showing long prismatic crystals and brush-like tuft.
387. Same, showing irregular aggregate of shorter prismatic crystals growing in protein ring.
388. Same, showing large groups growing in parallel orientation
389, 390. Oxyhemoglobin of the Ferret (*Mustela put* [illegible] tabular crystals and twins.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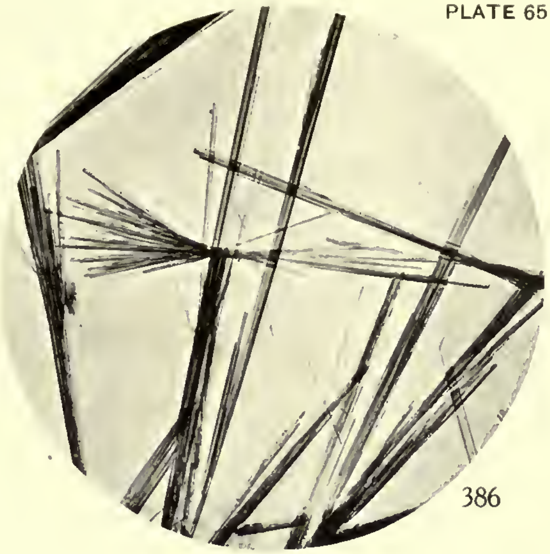
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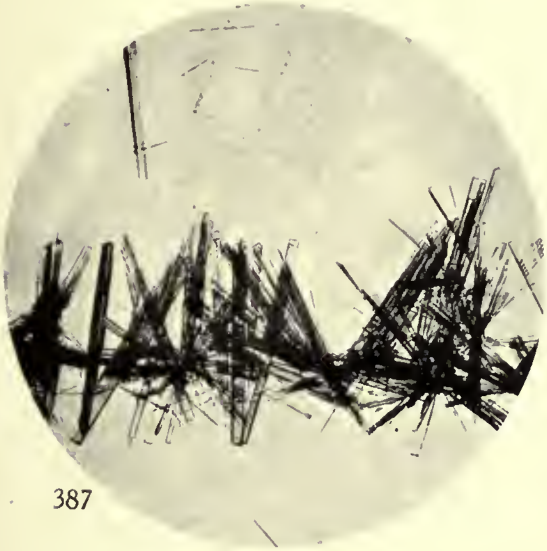
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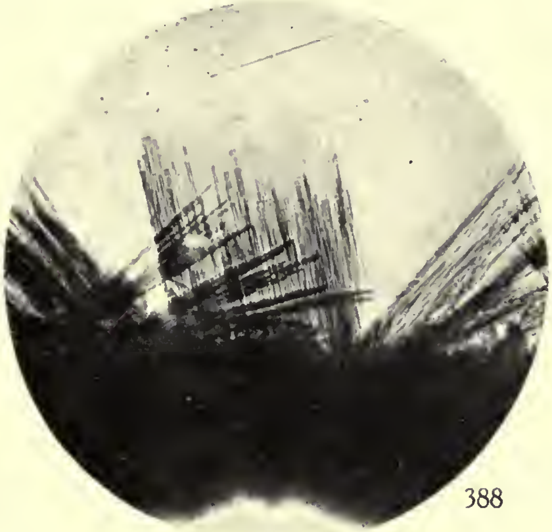
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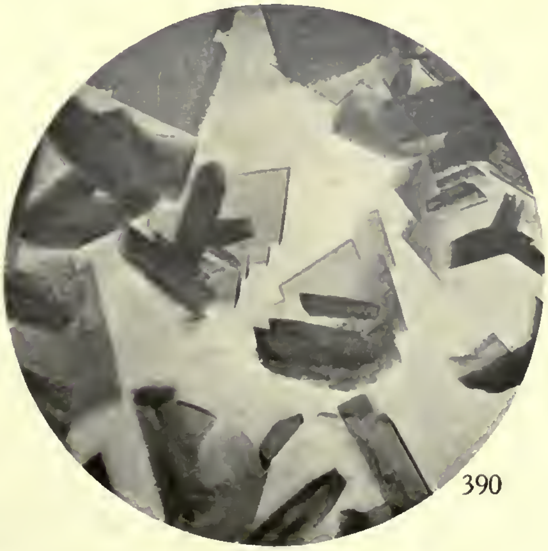
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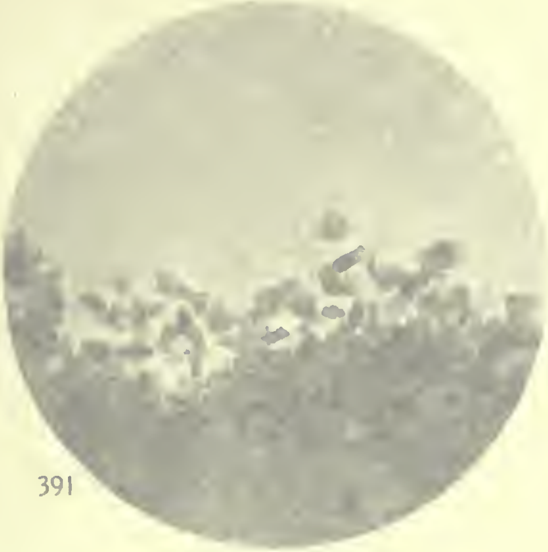
385. Oxyhemoglobin of the Skunk (*Mephitis mephitica putida*), showing long prismatic crystals growing in radiating groups.
386. Same, showing long prismatic crystals and brush-like tuft.
387. Same, showing irregular aggregate of shorter prismatic crystals growing in protein ring.
388. Same, showing large groups growing in parallel orientation.
389, 390. Oxyhemoglobin of the Ferret (*Mustela putorius*), showing tabular crystals and twins.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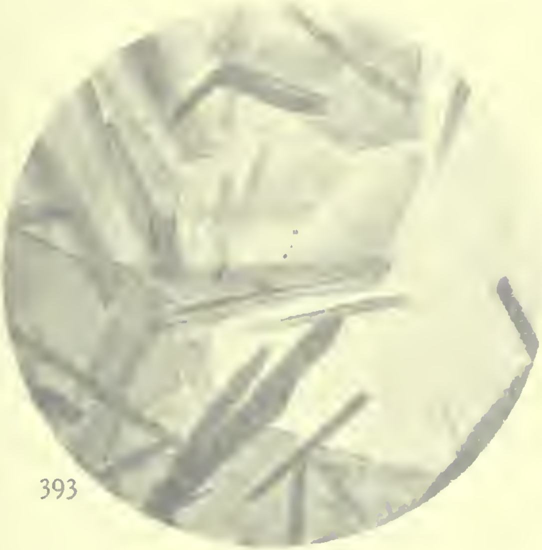
393


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396

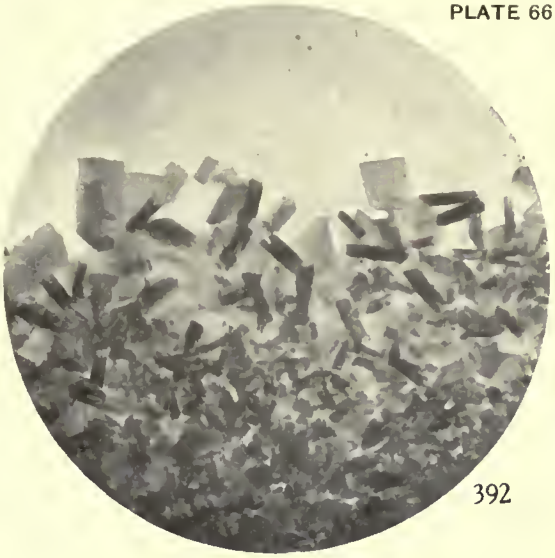
391. Oxyhemoglobin of the Ferret (*Mustela p[illegible]*), showing small, [illegible] tabular crystals in protein ring.

392. Same, showing larger tabular crystals in various combinations, growing in protein ring.

393, 394. Oxyhemoglobin of the Otter (*L[illegible]*), showing tabular crystals in different positions, many in twin position. Sp[illegible] character may be seen in crystal on edge near middle of field in 393.

395. Same, showing thicker tabular crystals [illegible] shows parallel growth.


391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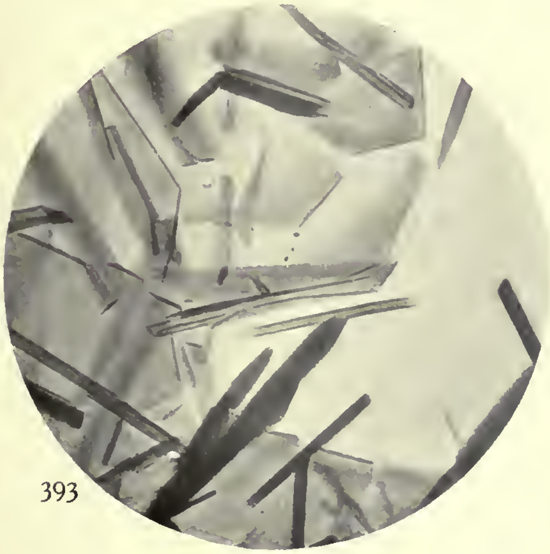
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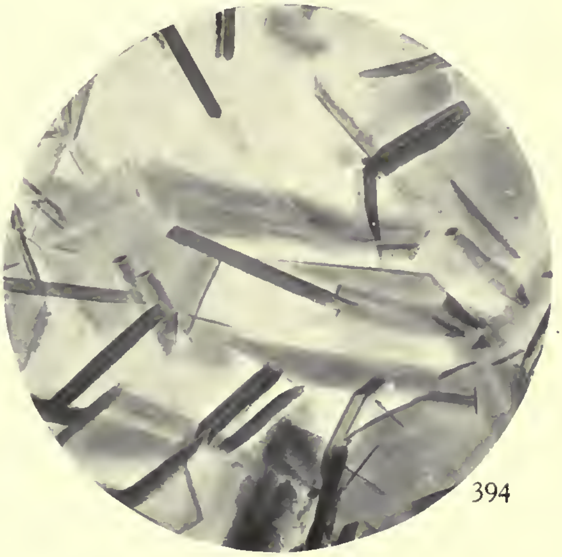
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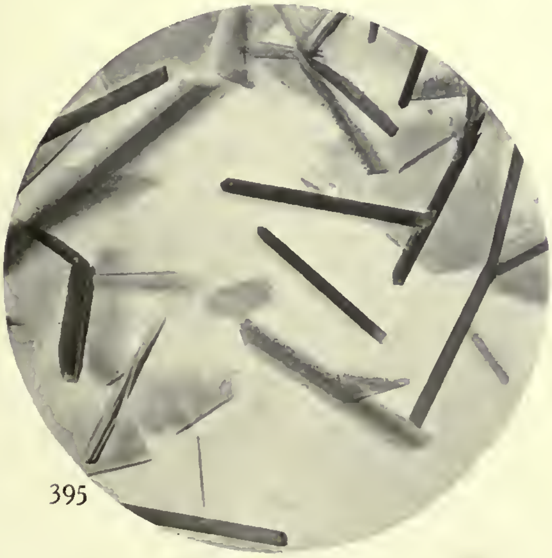
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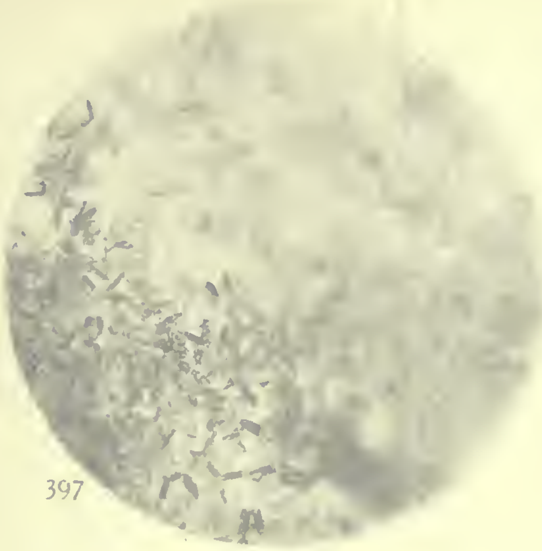
396

391. Oxyhemoglobin of the Ferret (*Mustela putorius*), showing small, first-formed somatic crystals in protein ring.

392. Same, showing larger tabular crystals in various orientations, growing in protein ring.

393–395. Oxyhemoglobin of the Otter (*Lutra canadensis*), showing tabular crystals in different positions, many in twin position. Sphenoidal or hemimorphic character may be seen in crystal on edge near middle of field in 395.

396. Same, showing thicker tabular crystals in basal and edge views; basal aspect shows parallel growth.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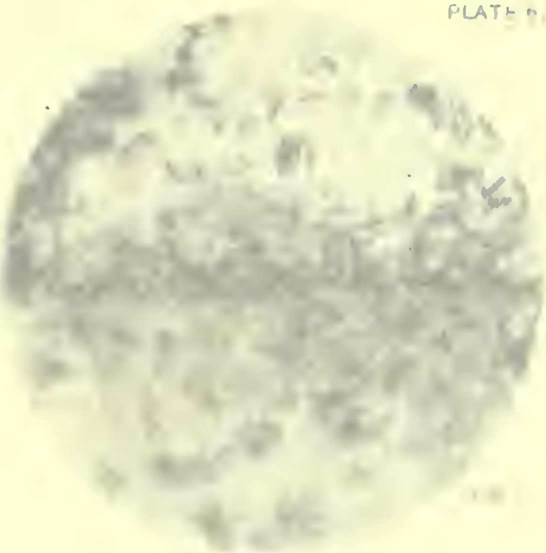
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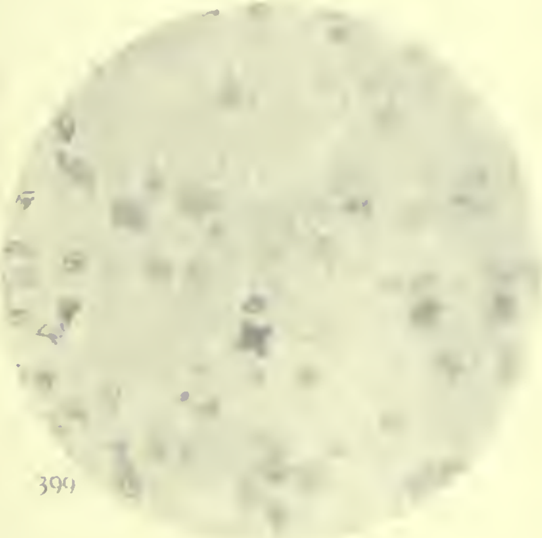

399


400


401


402

397 [illegible]
[illegible]
shown in [illegible]

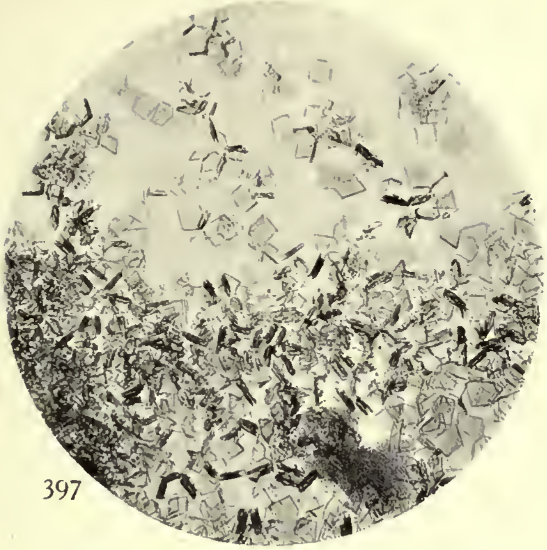
398. Same, type [illegible]

399. Same, type *a*, showing [illegible]
the [illegible]

400. Same, showing crystals of [illegible]
of oxalate.

401 Same, showing [illegible]

402. Same, showing [illegible]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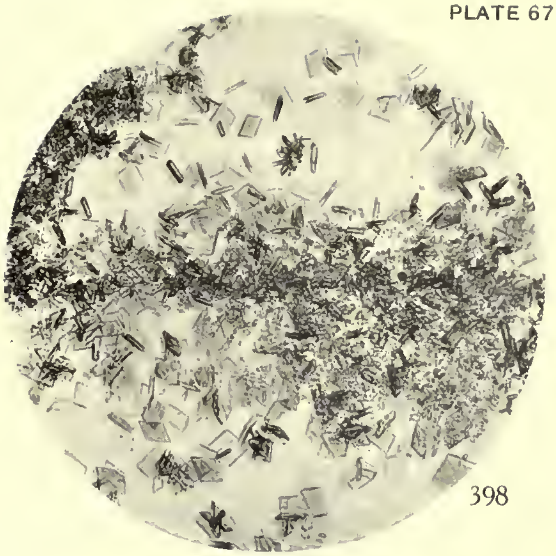
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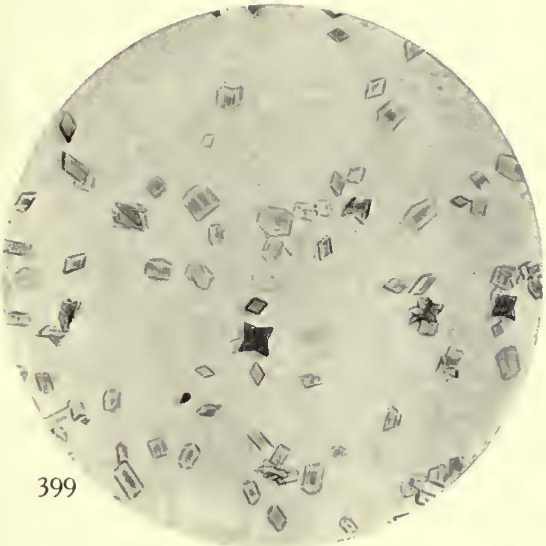
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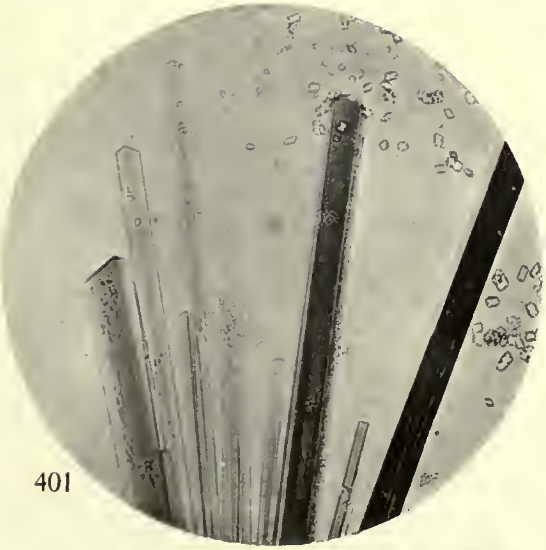
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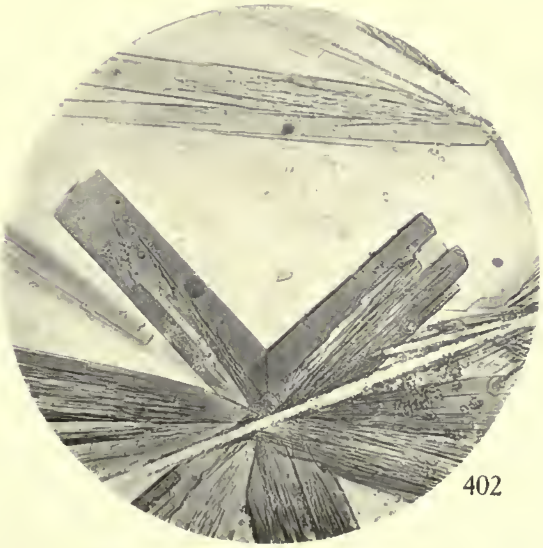
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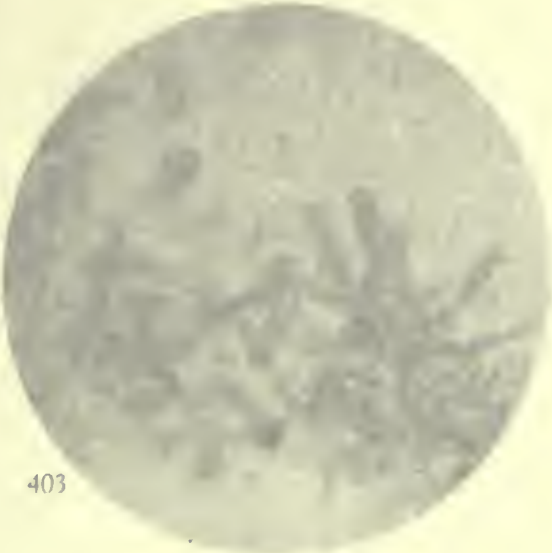
397. Oxyhemoglobin of the Badger (*Taxidea americana*), type *a*, showing small, first-formed tabular crystals, five-sided, and consisting of unit prism and base with one face of hemiorthodome. Badger twin shown in many of these crystals.
398. Same, type *a*, showing tabular crystals more symmetrically developed and four-sided.
399. Same, type *a*, showing more prismatic development of crystal consisting of prism, base, and hemiorthodome; also penetration twins.
400. Same, showing crystals of type *a* in short prismatic form and long prisms of type *b* growing from crystals of oxalate.
401. Same, showing long prisms of type *b* growing in a tuft.
402. Same, showing radiating group of type *b* crystals growing from an oxalate crystal.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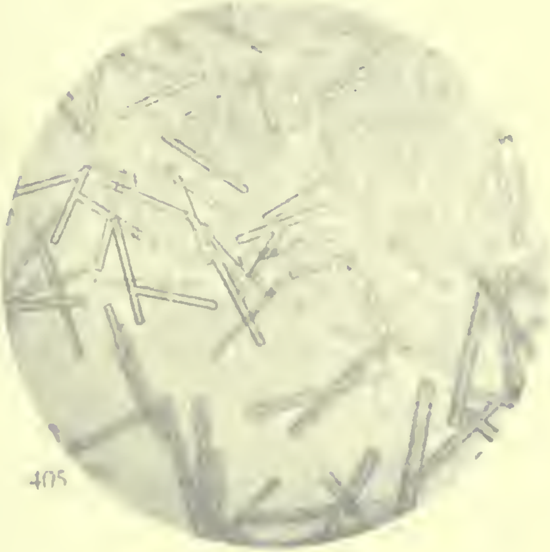
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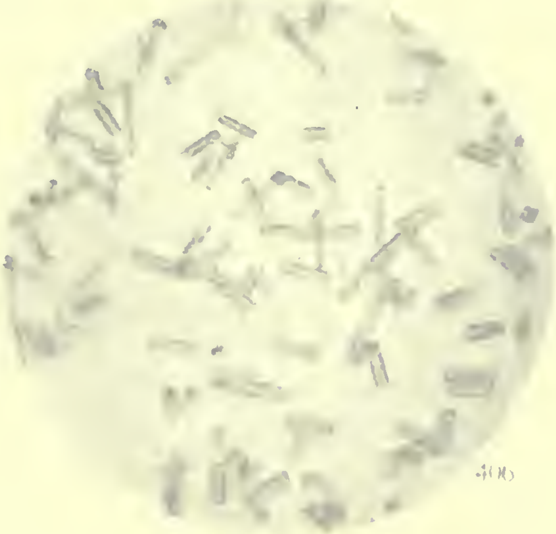
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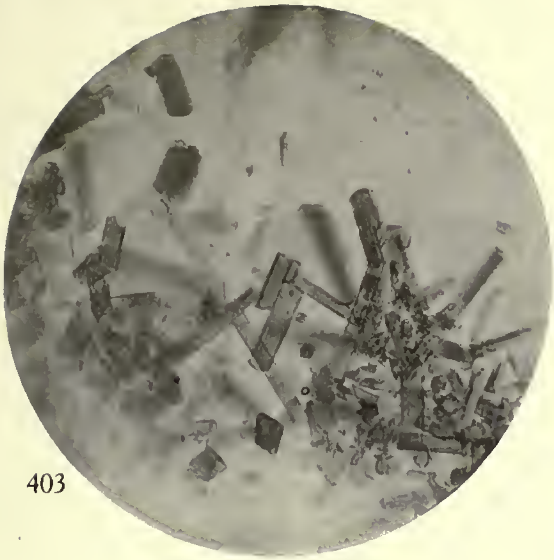
408

403, 404. Oxyhemoglobin of the Kinkajou (*Cercoleptes caudivolvulus*), showing prismatic crystals of type *a* in different aspects.

405. Oxyhemoglobin of the Cacomyxl (*Bassariscus astuta*), showing longer type of prismatic crystal, consisting of unit prism and brachydome.

406. Same, shorter type of crystal showing same combination as 405.

407, 408. Same larger crystals of type of 405, but showing brachypinacoid in addition to unit prism and brachydome. Some are in twin position, and in 408 cross-sections show brachypinacoid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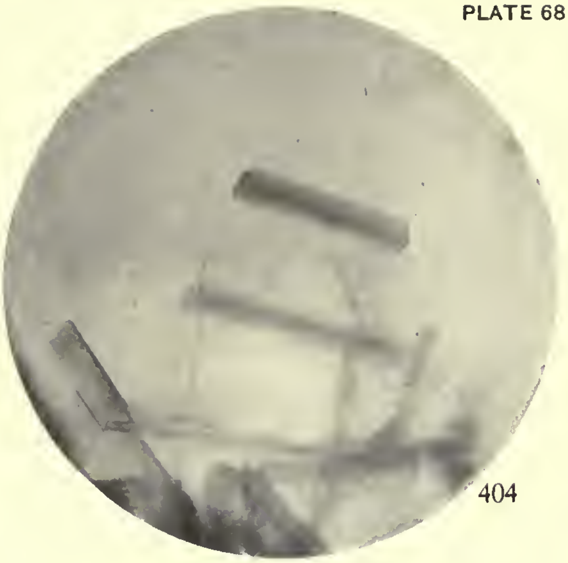
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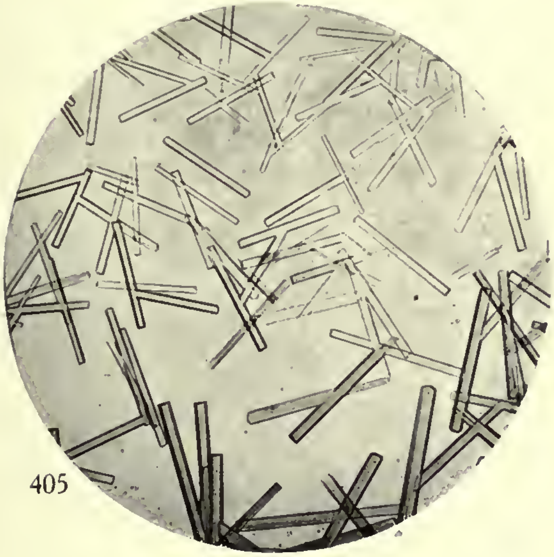
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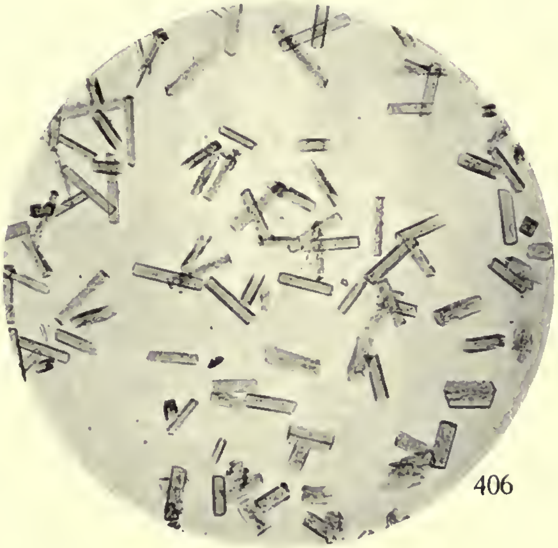
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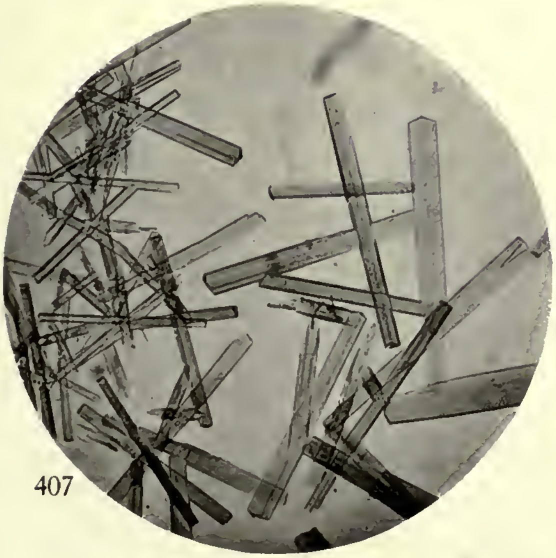
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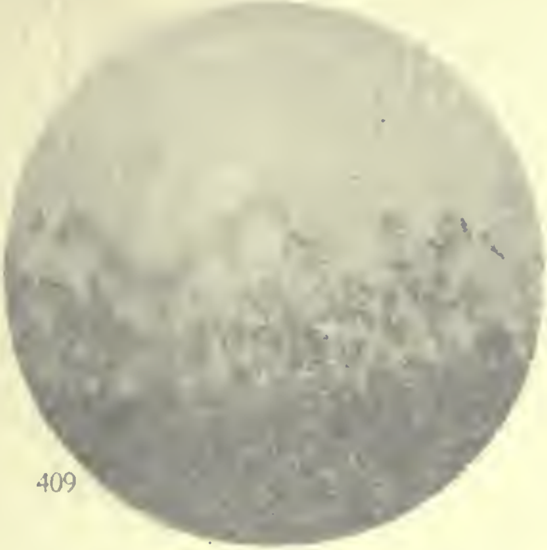
408

403, 404. Oxyhemoglobin of the Kinkajou (*Cercoleptes caudivolvulus*), showing prismatic crystals of type *a* in different aspects.

405. Oxyhemoglobin of the Cacomyxl (*Bassariscus astuta*), showing longer type of prismatic crystal, consisting of unit prism and brachydome.

406. Same, shorter type of crystal showing same combination as 405.

407, 408. Same, larger crystals of type of 405, but showing brachypinacoid in addition to unit prism and brachydome. Some are in twin position, and in 408 cross-sections show brachypinacoid.



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414

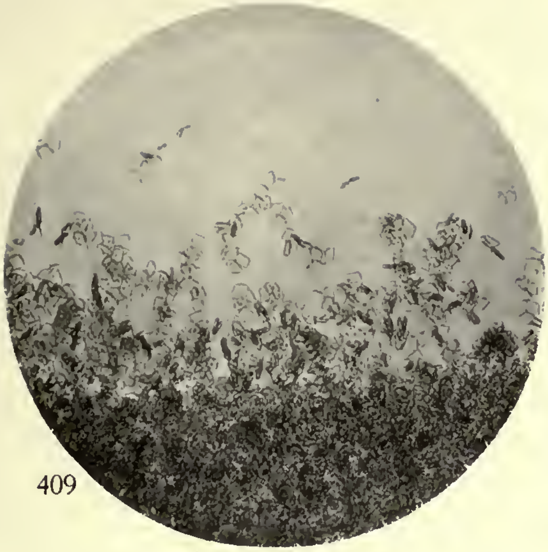
409. Oxyhemoglobin of the Black Bear (*Ursus americanus*), showing small, first-formed crystals in trillings, the bear-type twin.

410. Same, large, single crystals showing parallel growth. Combination is base cut at one end of ortho-axis by unit prism and at opposite end by unit pyramid.

411. Same, showing larger crystals in same combination as 410 with clinopinacoid cutting unit pyramid at one end of axis. Small section of crystals above, to the right shows hemimorphic form.

412. Same, showing crystals in various orientations and in section.

413, 414. Same, showing aggregation of crystals in parallel growths and radiating groups along cover edge.



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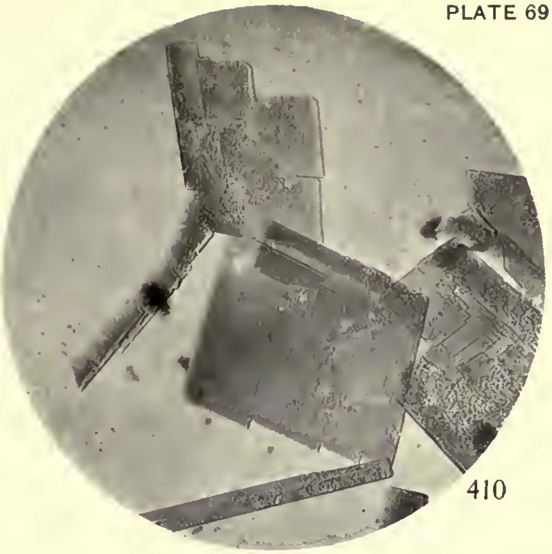

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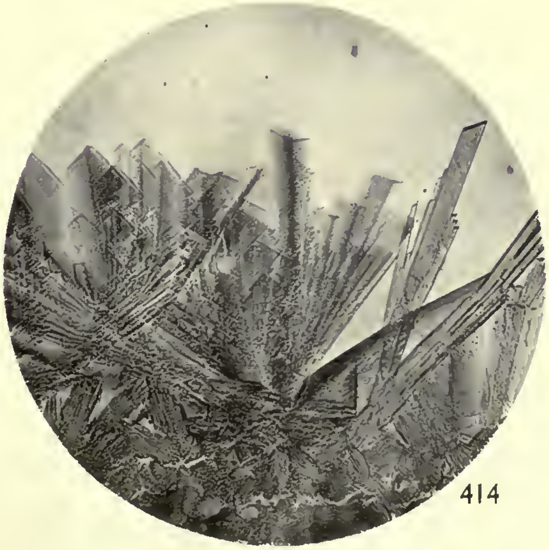


414

409. Oxyhemoglobin of the Black Bear (*Ursus americanus*), showing small, first-formed crystals in trillings, the bear-type twin.

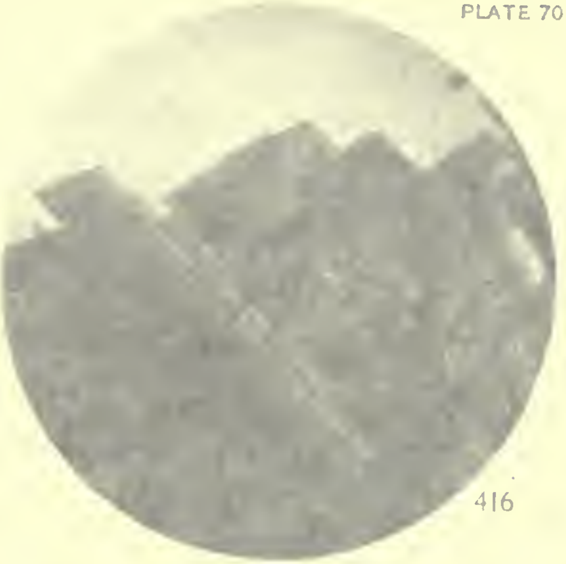
410. Same, large, single crystals showing parallel growth. Combination is base cut at one end of ortho-axis by unit prism and at opposite end by unit pyramid.

411. Same, showing larger crystals in same combination as 410 with clinopinacoid cutting unit pyramid at one end of axis. Small section of crystals above, to the right shows hemimorphic form.

412. Same, showing crystals in various orientations and in section.

413, 414. Same, showing aggregation of crystals in parallel growths and radiating groups along cover edge.


415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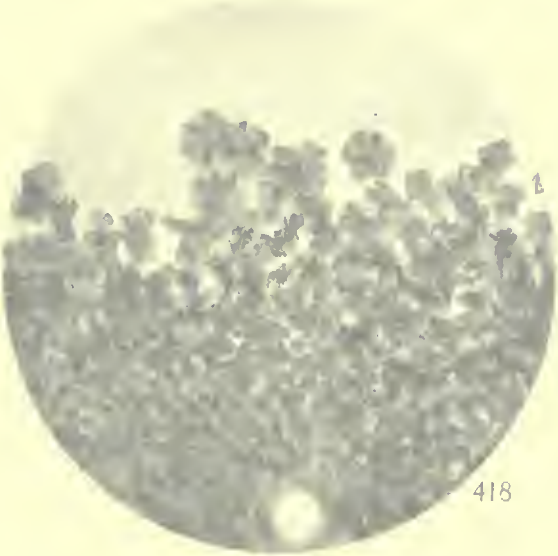
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419


420

415. Oxyhemoglobin of the Black Bear (*Ursus americanus*), showing large crystals along cover edge, mostly in section.

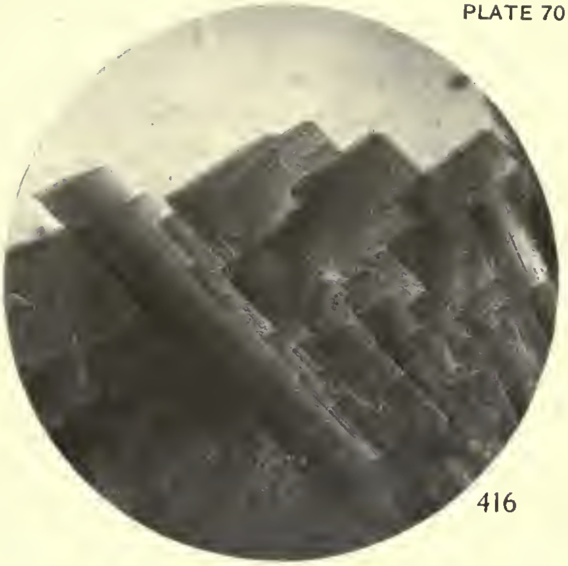
416. Same, showing two large groups of crystals along cover edge in parallel orientation, divided by large crystals in oblique section.

417, 418. Oxyhemoglobin of the Polar Bear (*Ursus maritimus*), showing small, first-formed crystals twinned in bear-type twin, growing in protein ring.

419. Same, single crystals showing hemimorphism and bear-type twins near cover edge.

420. Same, larger tabular crystals along cover edge, showing parallel growth.


415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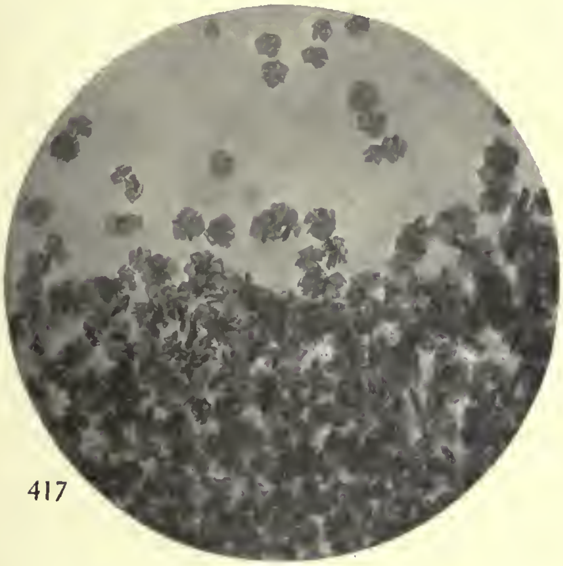
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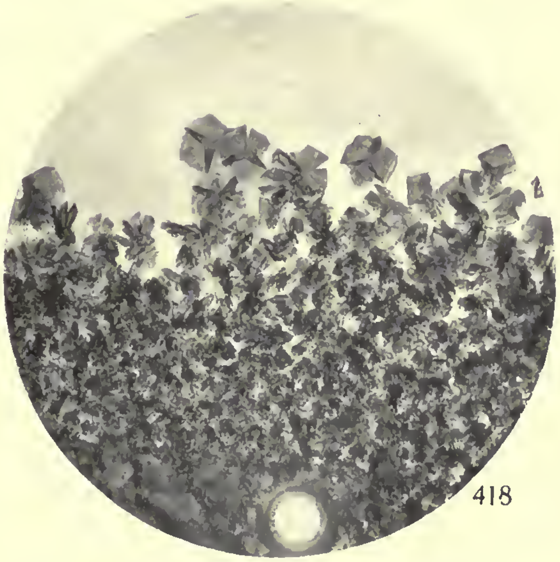
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420

415. Oxyhemoglobin of the Black Bear (*Ursus americanus*), showing large crystals along cover edge, mostly in section.

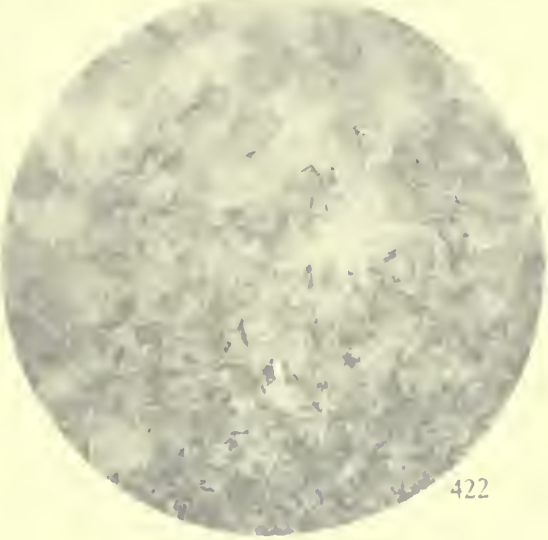
416. Same, showing two large groups of crystals along cover edge in parallel orientation, divided by large crystals in oblique section.

417, 418. Oxyhemoglobin of the Polar Bear (*Ursus maritimus*), showing small, first-formed crystals twinned in bear-type twin, growing in protein ring.

419. Same, single crystals showing hemimorphism and bear-type twins, near cover edge.

420. Same, larger tabular crystals along cover edge, showing parallel growth.


421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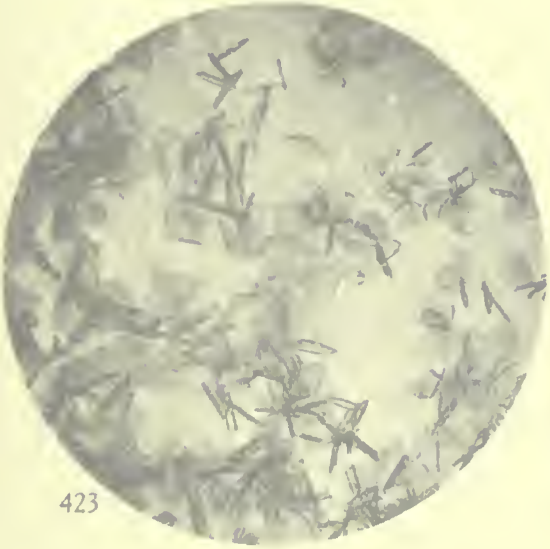
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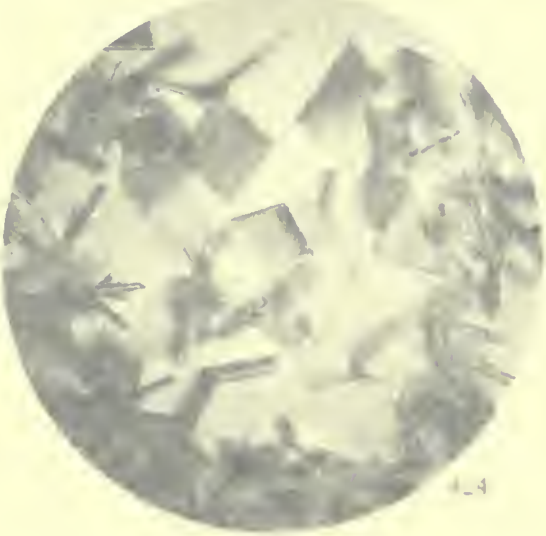
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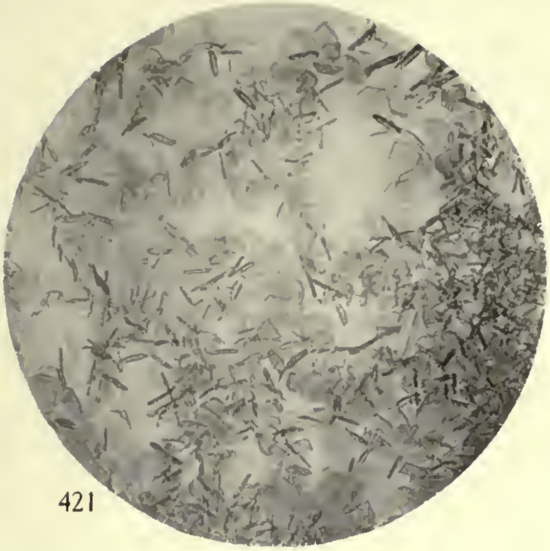
426

421, 422. Oxyhemoglobin of the [illegible] and bear-type twins. [illegible] and by unit [illegible]
423. Same, larger crystals, [illegible]
424, 425. Same, larger [illegible]
426. Same, group of larger crystals [illegible] of crystals.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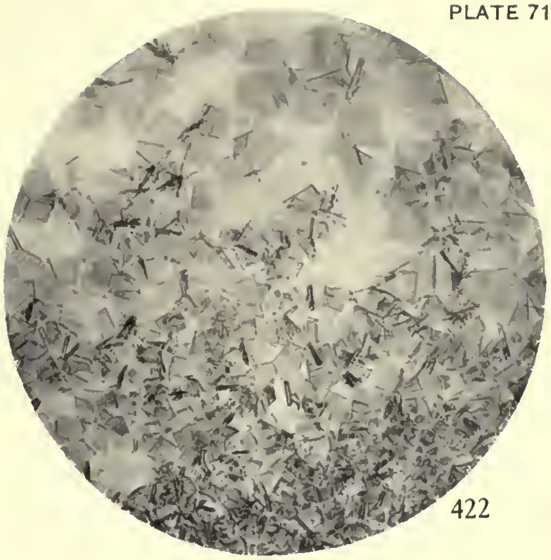
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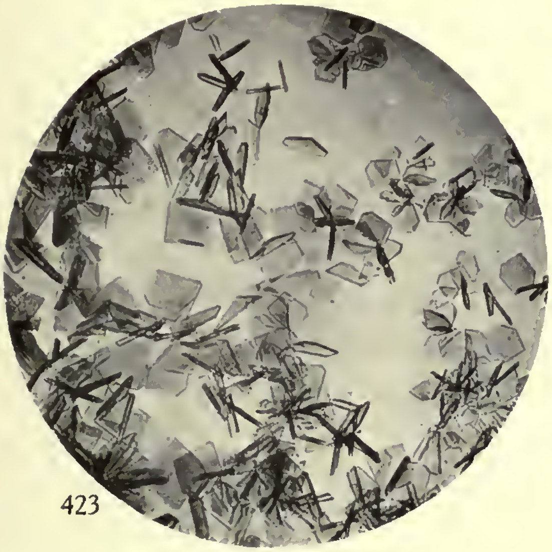
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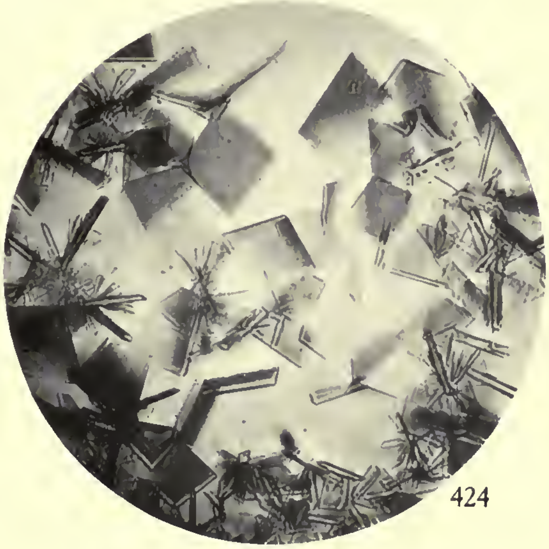
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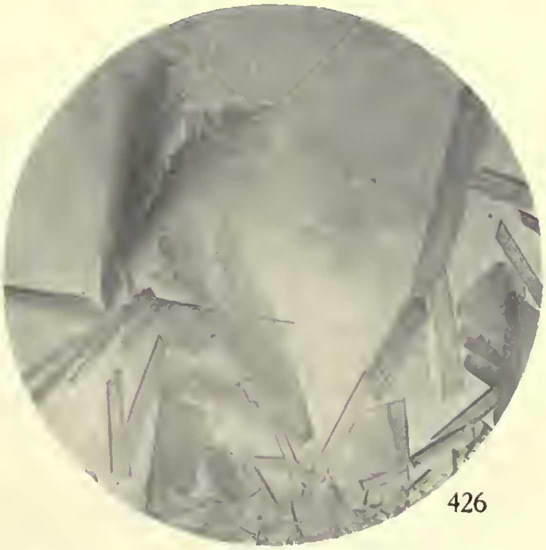
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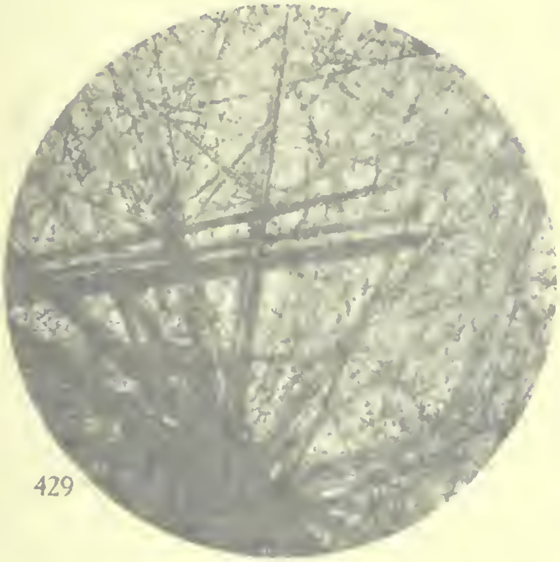
421, 422. Oxyhemoglobin of the Sloth Bear (*Melursus ursinus*), showing small, first-formed single crystals and bear-type twins. Combination is base cut by the unit prism at one end of ortho-axis and by unit pyramid at the other end, and with or without orthopinacoid.
423. Same, larger crystals, most of them showing orthopinacoid.
424, 425. Same, larger second-crop crystals from along cover edge, a few showing bear-type twin.
426. Same, group of larger crystals from near cover edge, showing parallel growth and cross-sections of crystals.

427

428



429

430

431

432

427. Oxyhemoglobin of the Sloth Bear (*Melursus ursinus*), showing group of crystals in parallel growth along ortho-axis. The white crystals are oxalate.

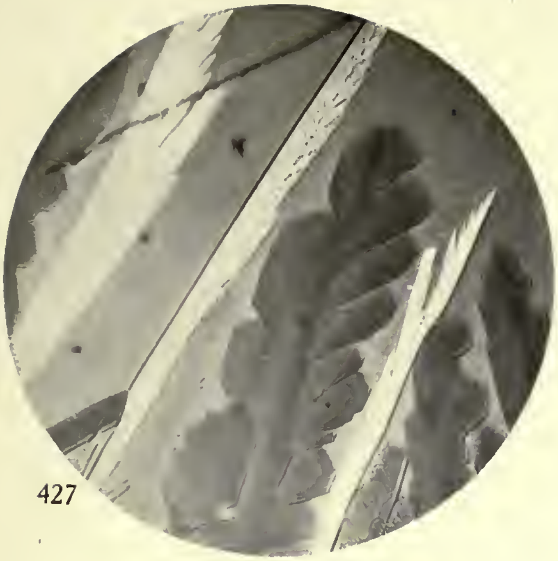
428. Same, showing irregular groups in parallel growth orientation.

429. *a*-Oxyhemoglobin of the Dog (*Canis familiaris*), showing mass of capillary crystals produced in thick slides.

430. Same, showing striated prismatic crystals growing from protein ring

431, 432. Same, showing divergent groups of long prismatic crystals.

PLATE 72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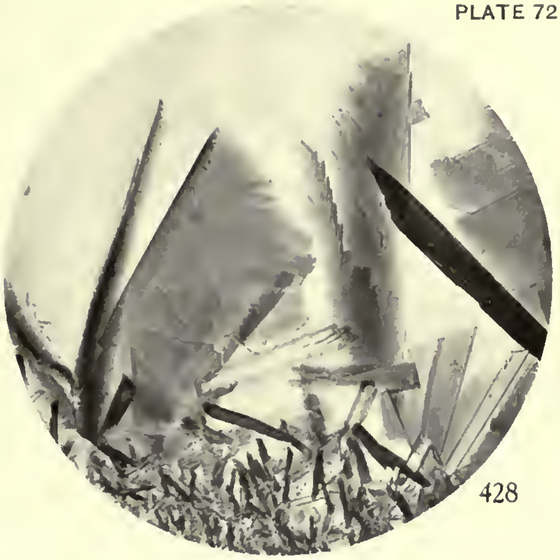
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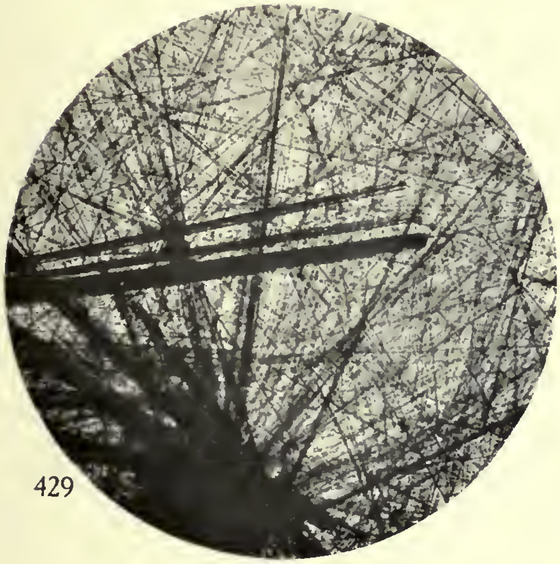
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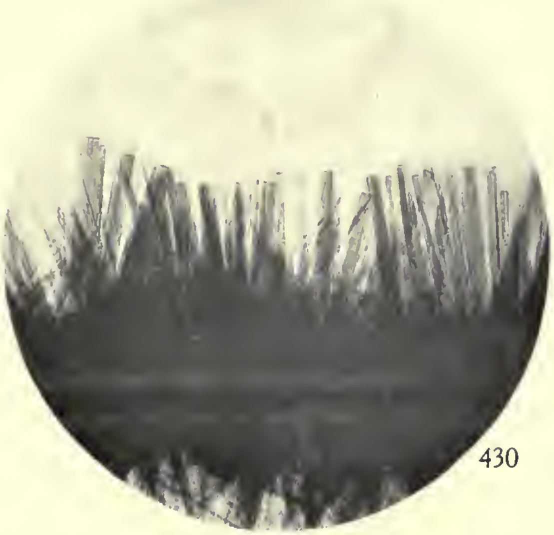
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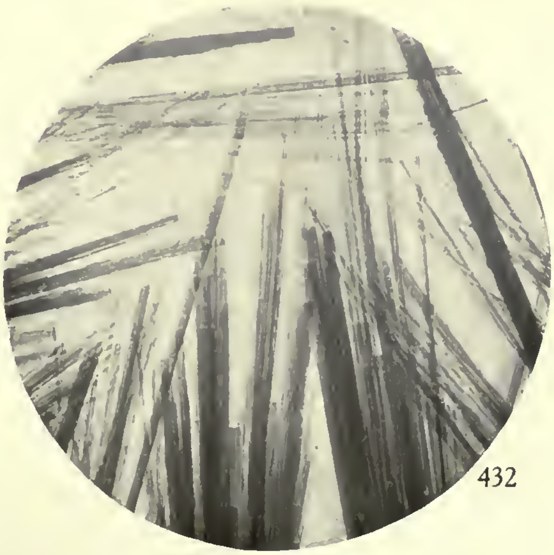
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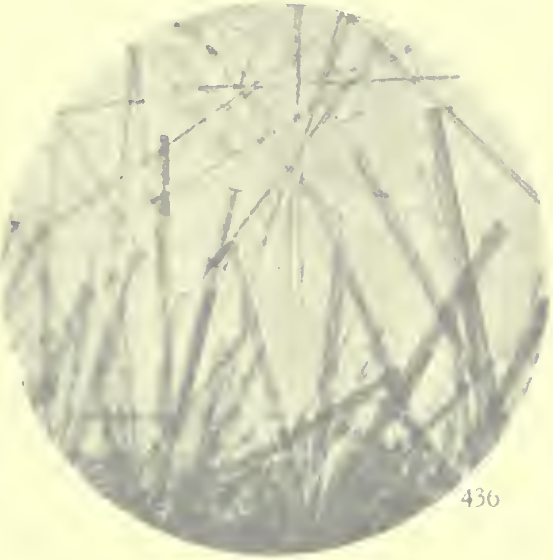
427. Oxyhemoglobin of the Sloth Bear (*Melursus ursinus*), showing group of crystals in parallel growth along ortho-axis. The white crystals are oxalate.
428. Same, showing irregular groups in parallel growth orientation.
429. *a*-Oxyhemoglobin of the Dog (*Canis familiaris*), showing mass of capillary crystals produced in thick slides.
430. Same, showing striated prismatic crystals growing from protein ring.
431, 432. Same, showing divergent groups of long prismatic crystals.

433

434

435



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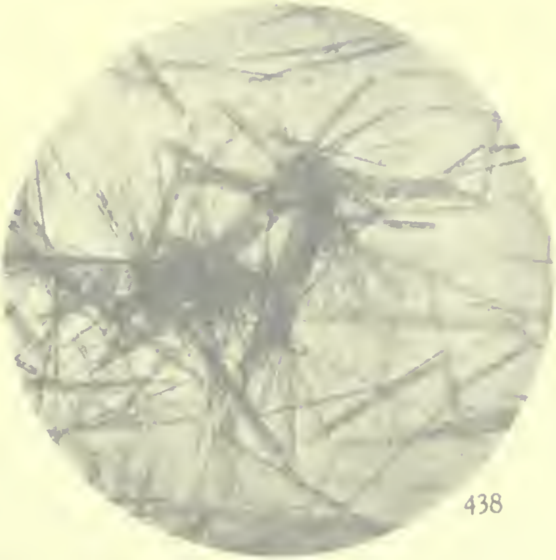
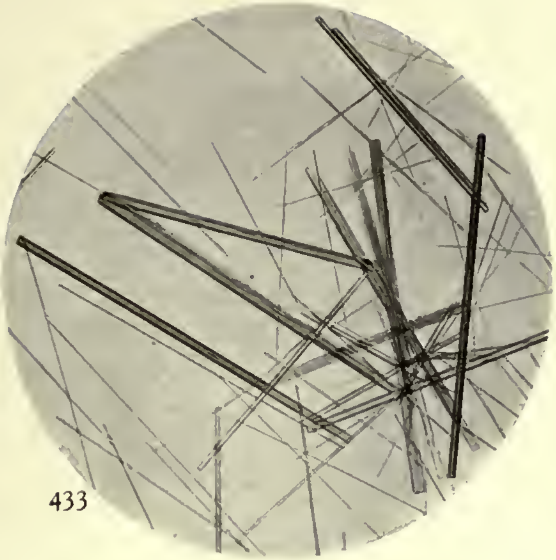


438

433. [illegible] of the Chow Dog (*Canis familiaris* var.), showing capillary and long prismatic crystals [illegible] terminated.
434. Same, showing shorter prismatic crystals in divergent tufts and overgrown by sphenelitic groups of [illegible] prisms.
435. Same, showing radiating cluster of larger prisms growing from protein ring.
436. Same, showing large crystals growing from protein ring.
437. [illegible]globin of the cross between Collie (*Canis familiaris*) and Coyote (*Canis latrans*), showing capillary and [illegible], several being the group of two and others more composite.
438. Same, showing grouped crystals, some probably in twin orientation.



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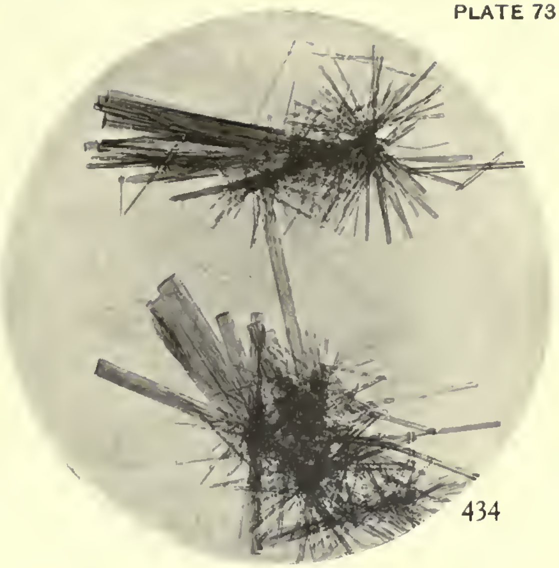

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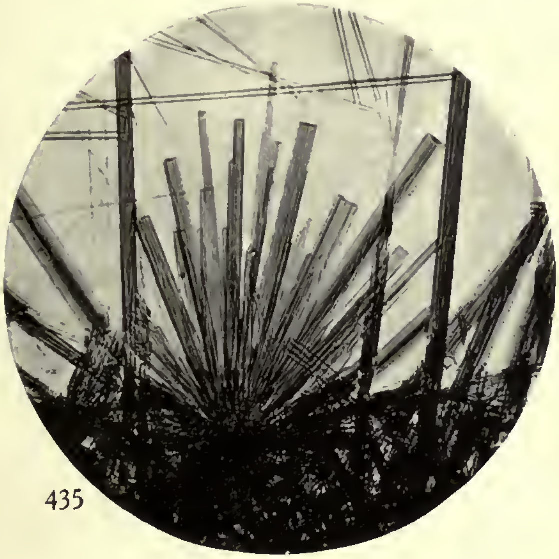

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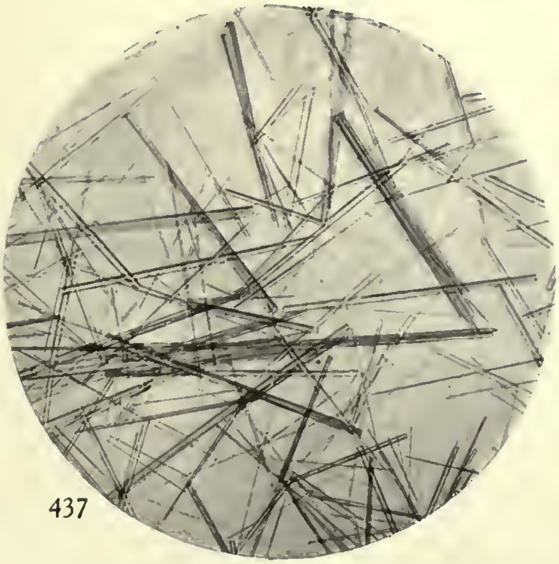

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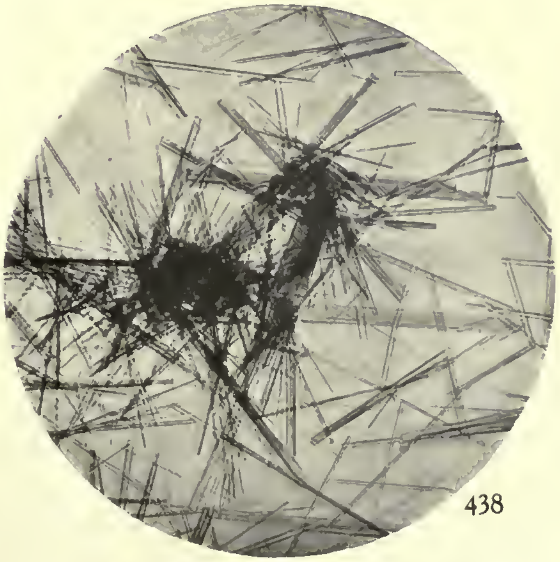

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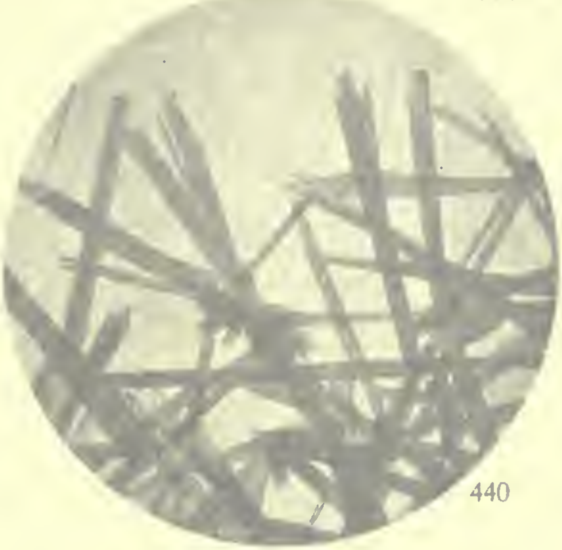
433. Oxyhemoglobin of the Chow Dog (*Canis familiaris* var.), showing capillary and long prismatic crystals doubly terminated.
434. Same, showing shorter prismatic crystals in divergent tufts and overgrown by sphenelitic groups of smaller prisms.
435. Same, showing radiating cluster of larger prisms growing from protein ring.
436. Same, showing large crystals growing from protein ring.
437. Oxyhemoglobin of the cross between Collie (*Canis familiaris*) and Coyote (*Canis latrans*), showing capillary and thicker prisms, several being the group of two and others more composite.
438. Same, showing groups of crystals, some probably in twin orientation.

439



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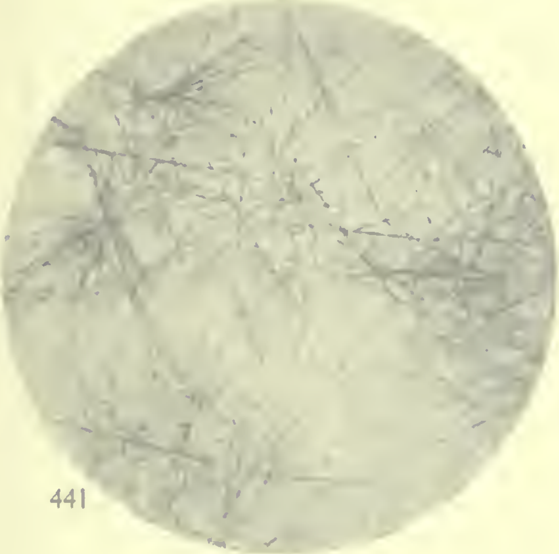

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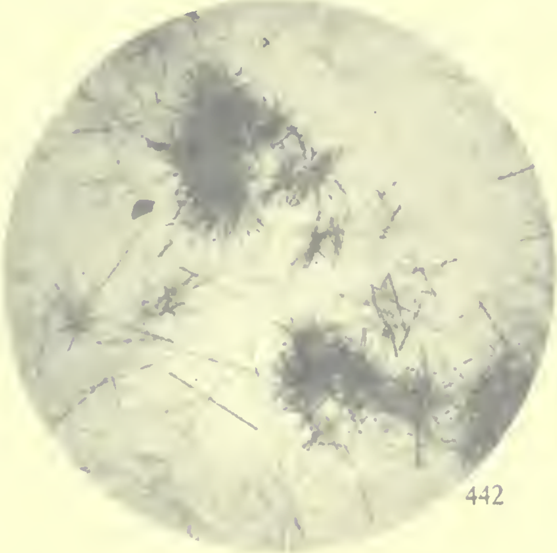

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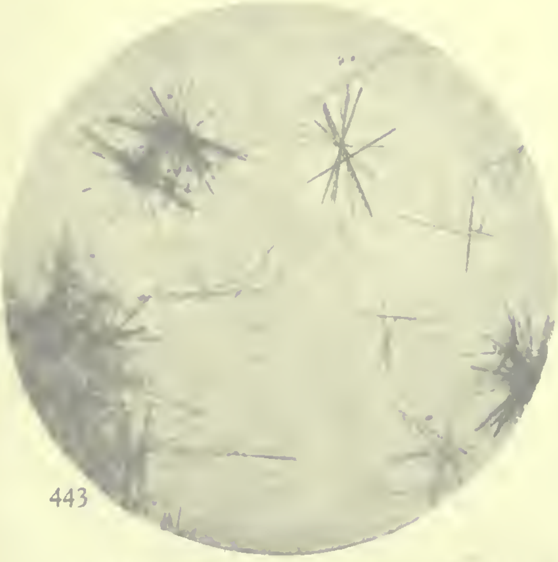

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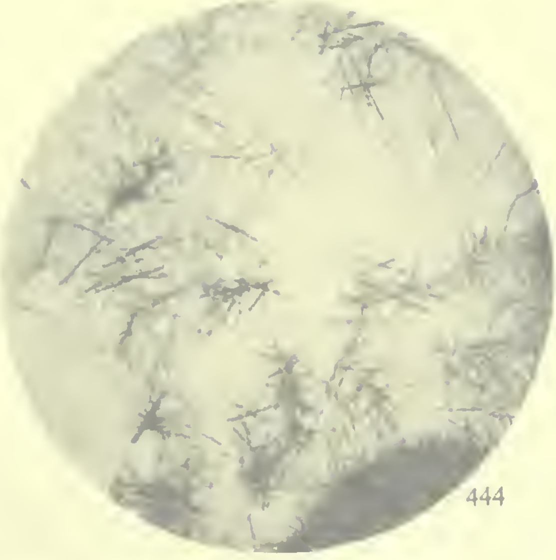
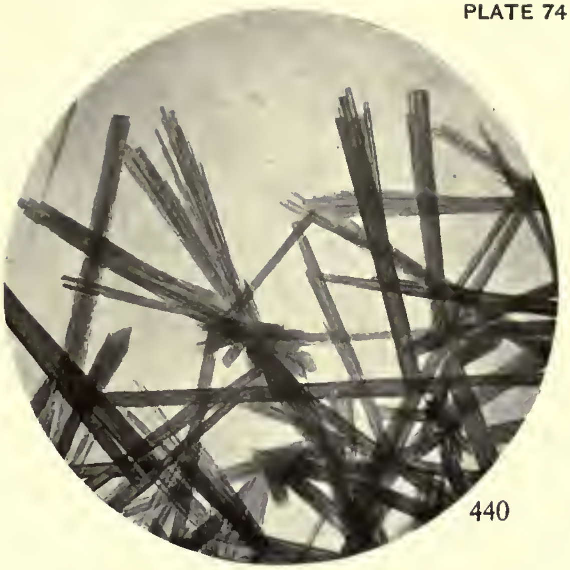


444

439. Metoxyhemoglobin of the Wolf (*Canis lupus mexicanus*), showing radiating groups of crystals.
440. Same, showing brush-like ends on some crystals.
441. Oxyhemoglobin of the Coyote (*Canis latrans*), showing mass of capillary crystals.
442. Same, showing short capillary crystals and single shorter composite crystal.
443. Same, showing radiating groups of crystals.
444. Same, showing mass of short needle-like crystals.

439



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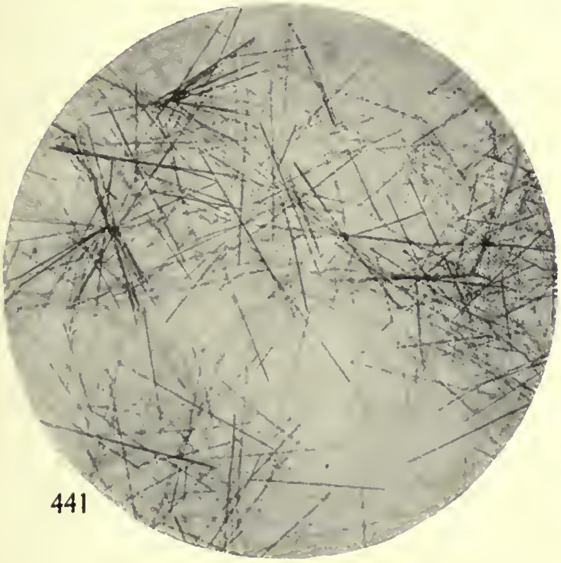

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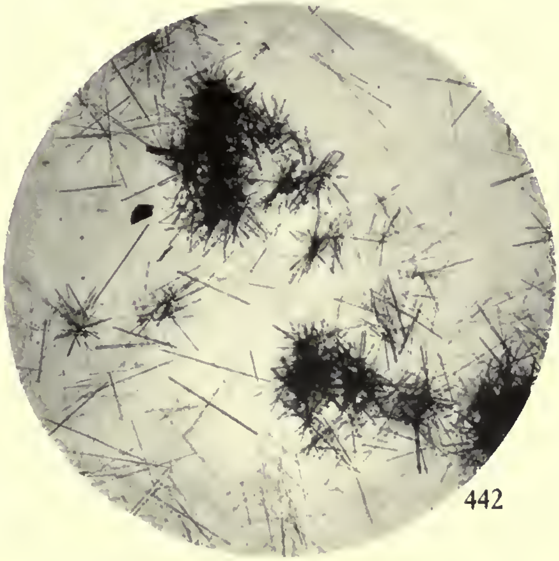

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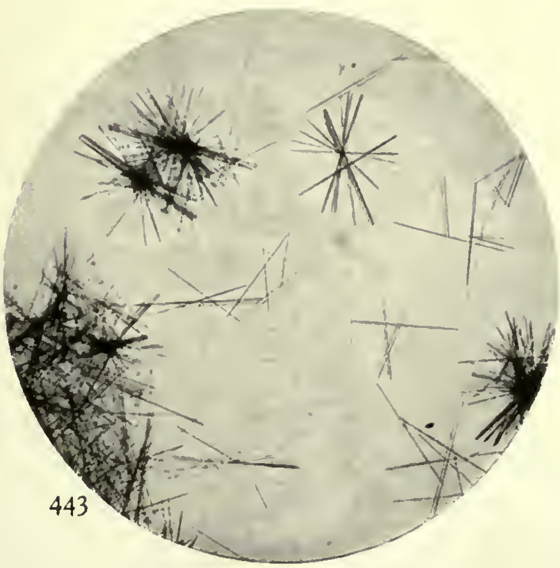

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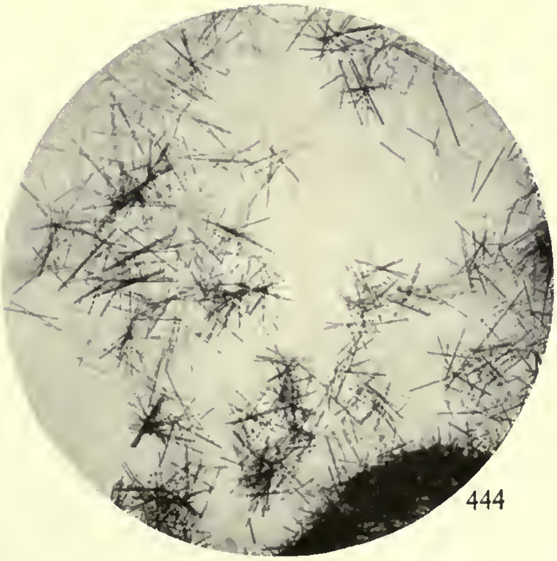


444

439. Metoxyhemoglobin of the Wolf (*Canis lupus mexicanus*), showing radiating groups of crystals.
440. Same, showing brush-like ends on some crystals.
441. Oxyhemoglobin of the Coyote (*Canis latrans*), showing mass of capillary crystals.
442. Same, showing short capillary crystals and single shorter composite crystal.
443. Same, showing radiating groups of crystals.
444. Same, showing mass of short needle-like crystals.

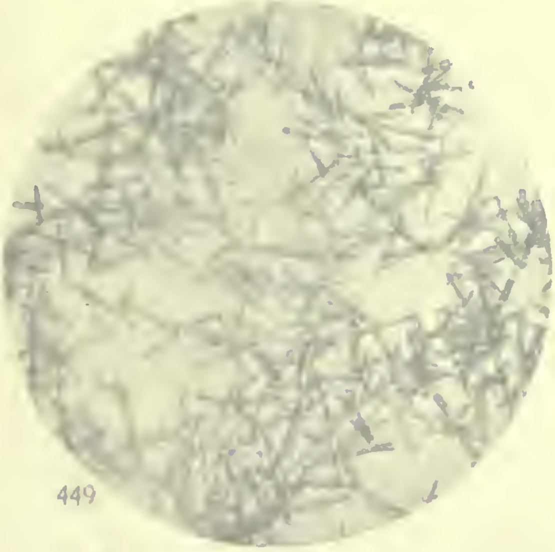
PLATE 7

445

446

447

448



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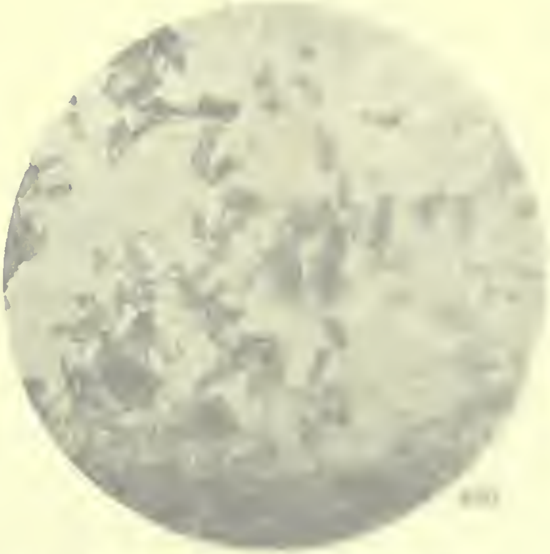
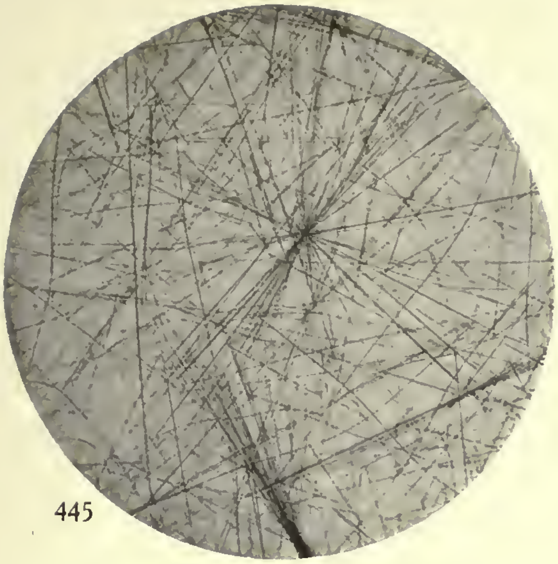


450

445. [illegible] globin of the Jackal (*Canis aureus*), showing divergent [illegible]. Flexibility of crystals shown by curvature.
446. Same showing capillary and shorter prismatic crystals.
447. Same, showing shorter prismatic crystals along protein ring.
448. Same, showing part of the protein ring that has developed [illegible]
449. [illegible] of the Dingo (*Canis dingo*), [illegible] in twin position as they occur through [illegible]
450. Same, showing short composite prisms that [illegible]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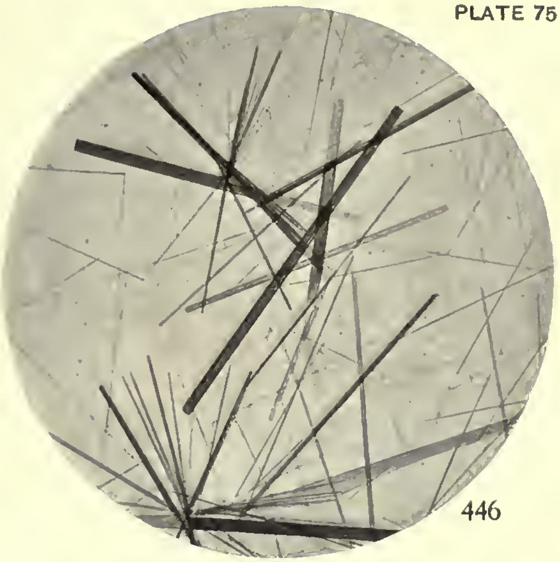
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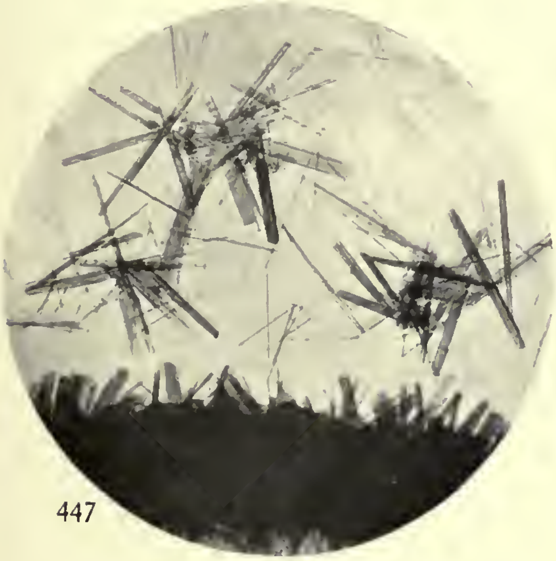
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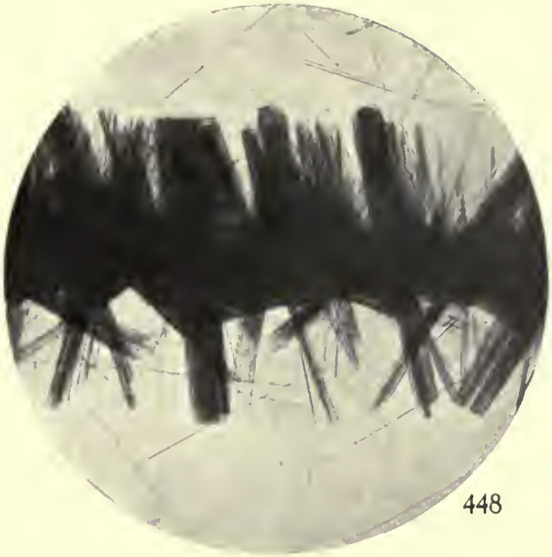
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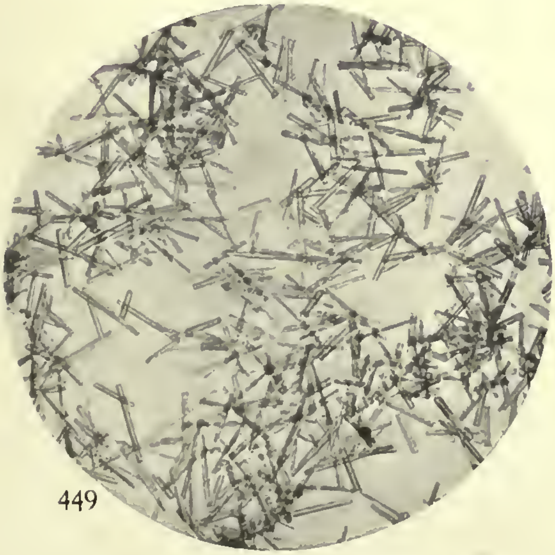
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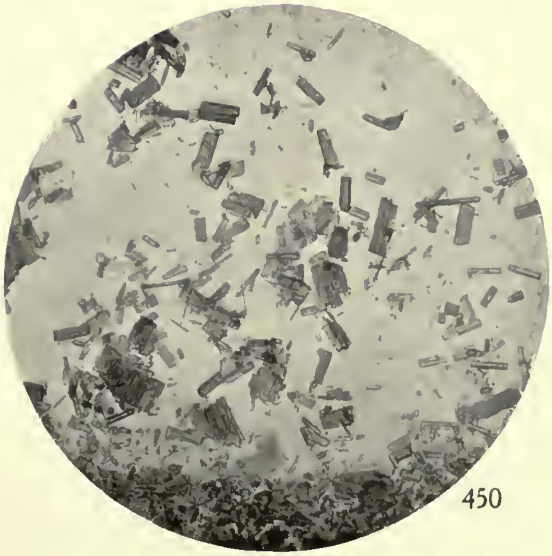
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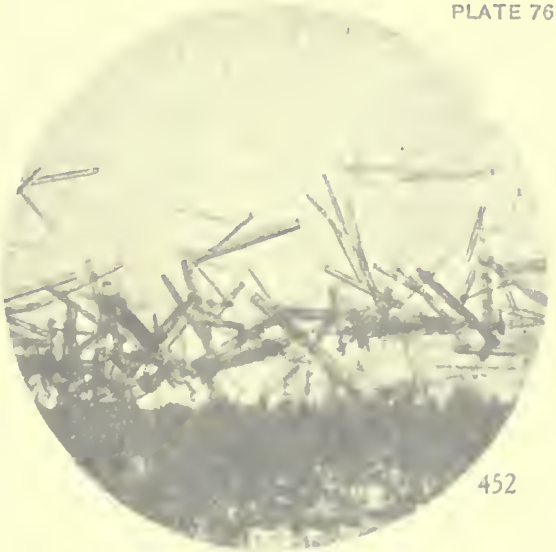
445. Oxyhemoglobin of the Jackal (*Canis aureus*), showing divergent and radiating aggregates of capillary crystals. Flexibility of crystals shown by curvatures.
446. Same, showing capillary and shorter prismatic crystals.
447. Same, showing shorter prismatic crystals along protein ring.
448. Same, showing part of the protein ring that has developed into doubly terminated, composite crystals.
449. Oxyhemoglobin of the Dingo (*Canis dingo*), showing short prismatic doubly terminated crystals, some in twin position as they occur through body of slide.
450. Same, showing short composite prisms that develop near protein ring.

451



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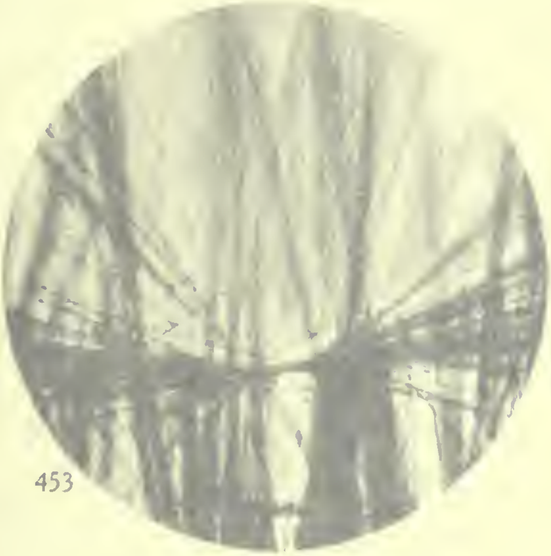

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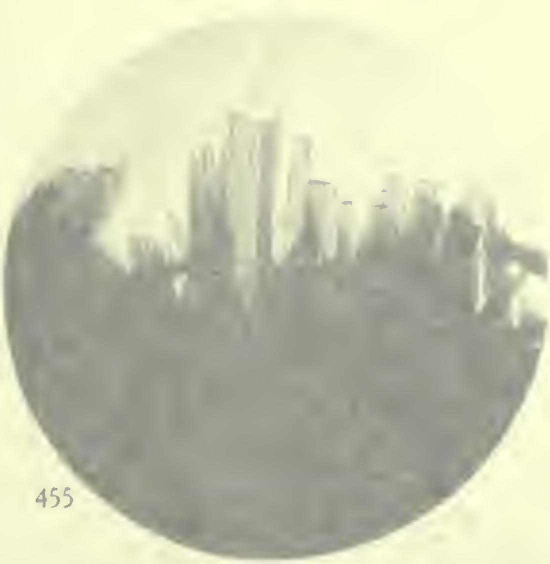

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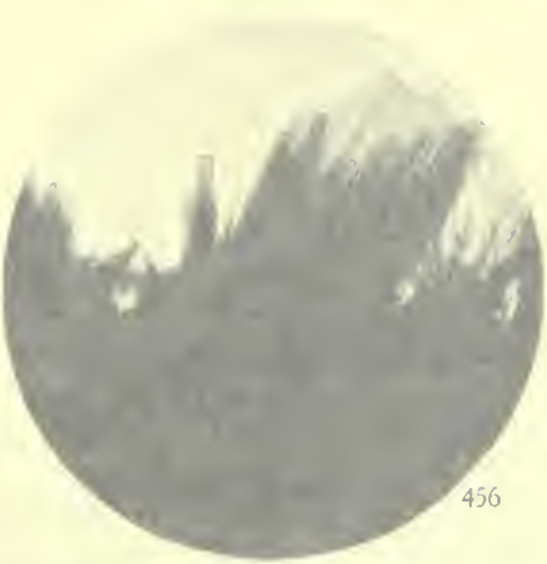
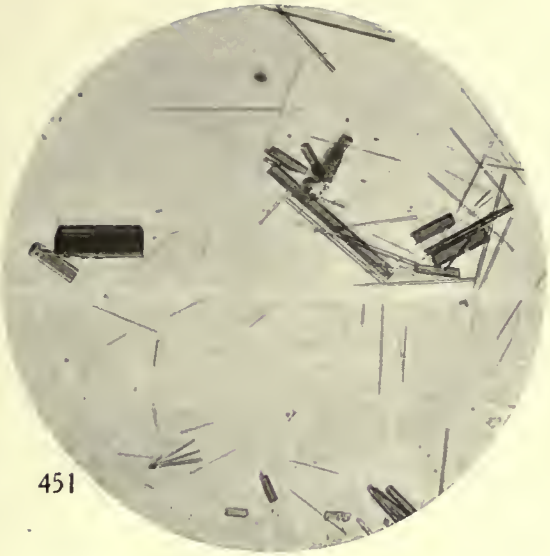


456

451. Oxyhemoglobin of the Dingo (*Canis dingo*), showing stout and thin prisms.
452. Same, showing medium thick prisms along protein ring.
453. Oxyhemoglobin of Azara's Dog (*Canis azaræ*), showing divergent tufts of long capillary crystals.
454-456. Same, showing masses of crystals aggregated in approximately parallel growth along protein ring or cover edge.



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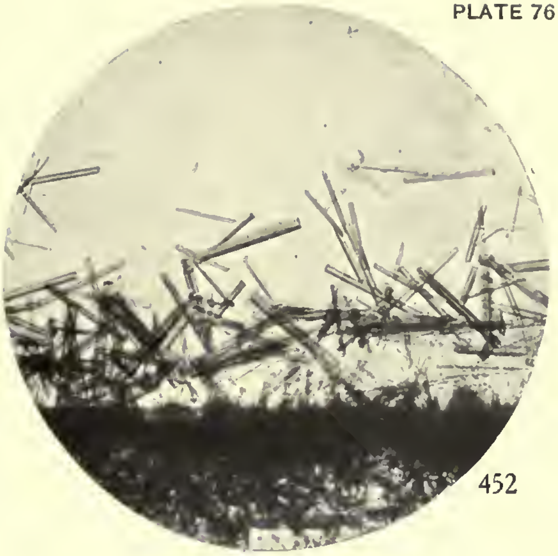

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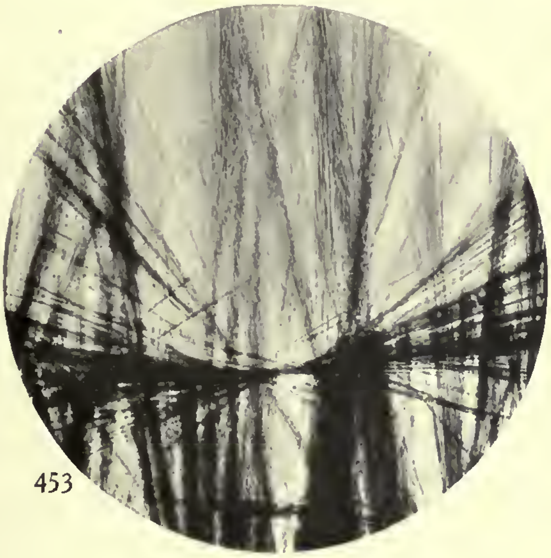

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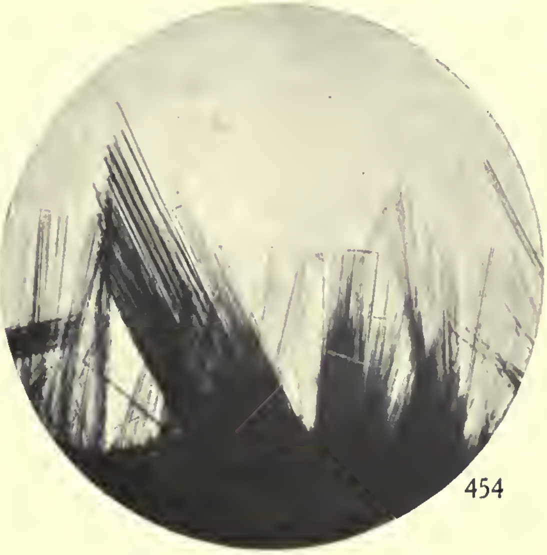

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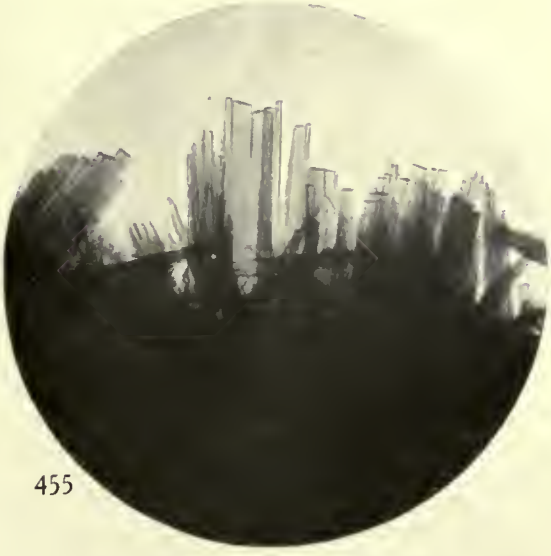

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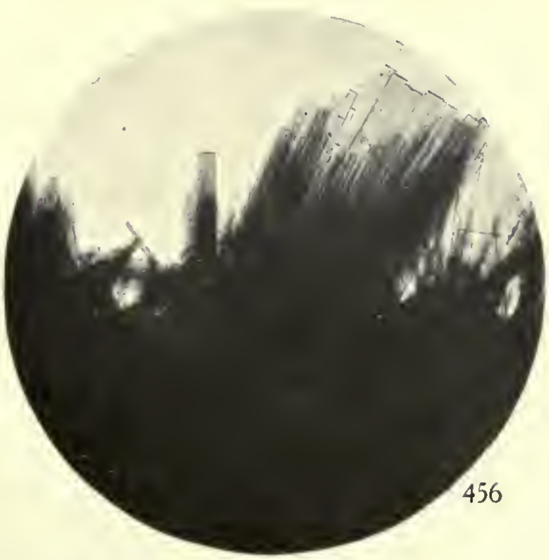

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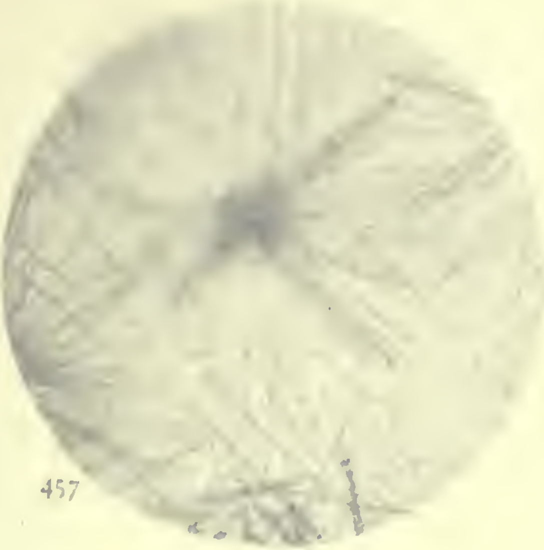
451. Oxyhemoglobin of the Dingo (*Canis dingo*), showing stout and thin prisms.
452. Same, showing medium thick prisms along protein ring.
453. Oxyhemoglobin of Azara's Dog (*Canis azaræ*), showing divergent tufts of long capillary crystals.
454–456. Same, showing masses of crystals aggregated in approximately parallel growth along protein ring or cover edge.



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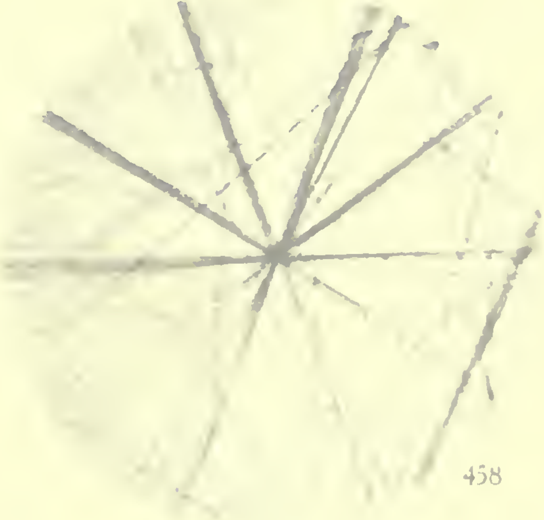

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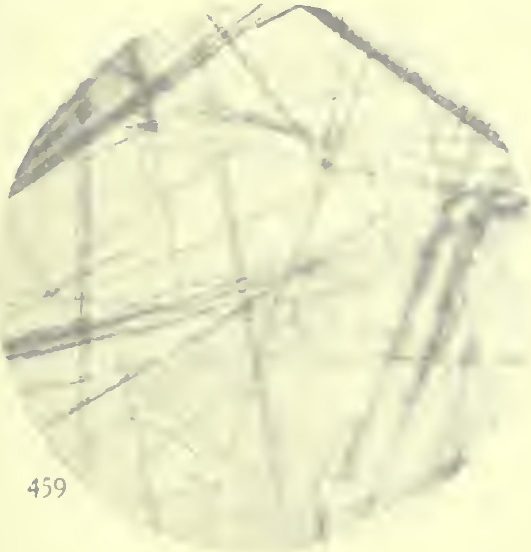


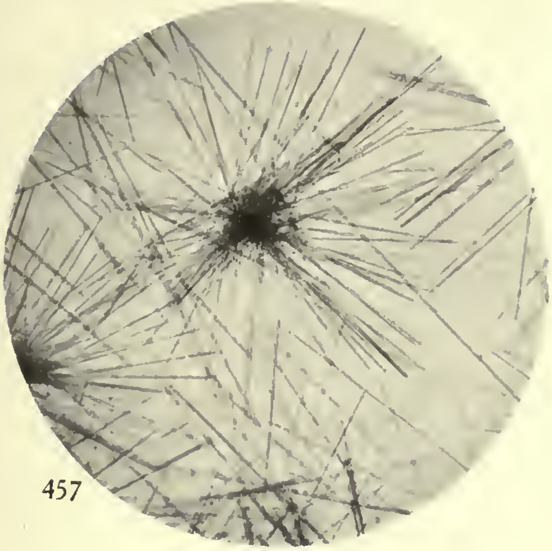
459

460

461

462

[illegible] (*[illegible] fulvus*), showing radiating groups of thin prismatic crystals.
[illegible] bundles of crystals, probably in twin orientation.
[illegible] crystals.
[illegible] divergent crystals.
[illegible] of a large group of the crystals.
[illegible] in approximately parallel growth.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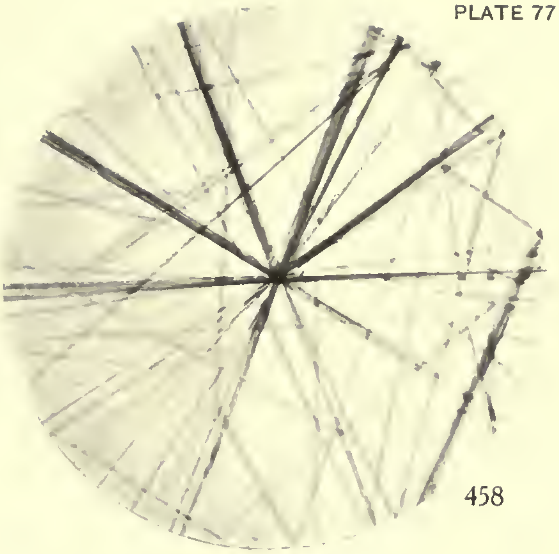
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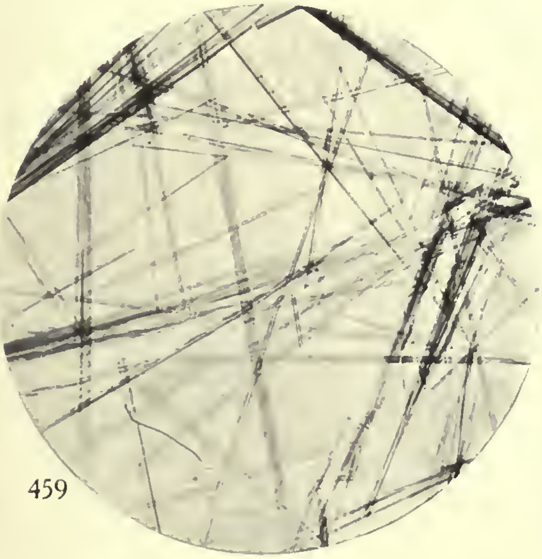
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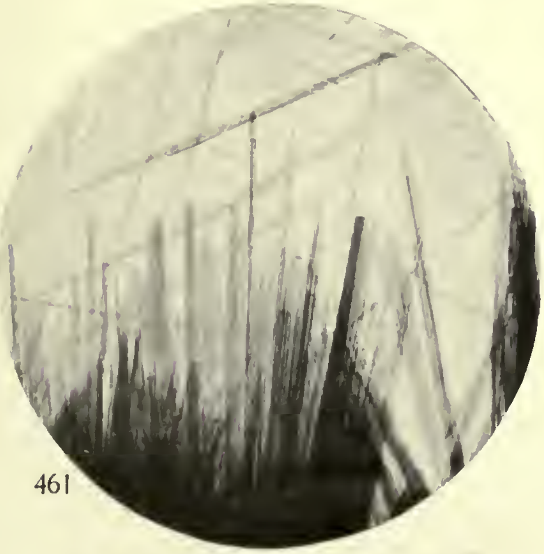
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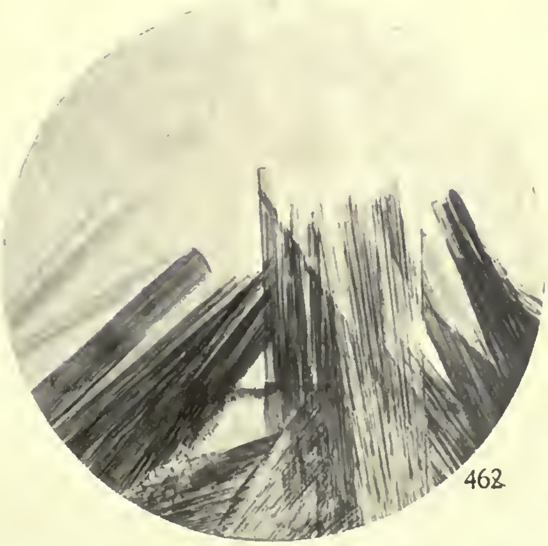
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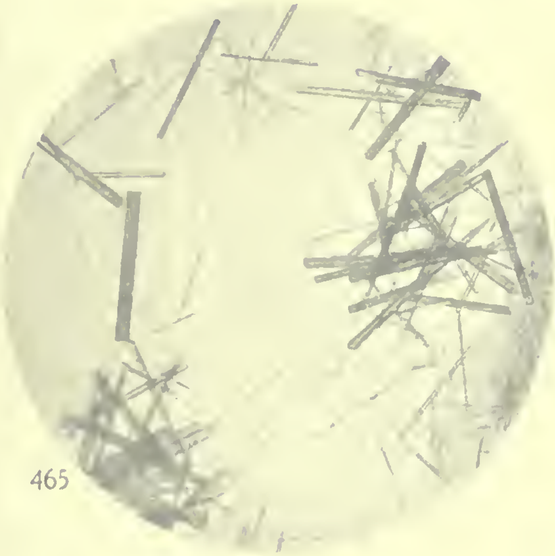
457. Oxyhemoglobin of the Red Fox (*Vulpes fulvus*), showing radiating groups of thin prismatic crystals.
458. Same, showing radiating groups of bundles of crystals, probably in twin orientation.
459. Same, showing mass of long prismatic crystals.
460. Same, showing bundles of slightly divergent crystals.
461. Same, showing approximate parallelism of a large group of the crystals.
462. Same, showing groups of crystals in approximately parallel growth.


463


464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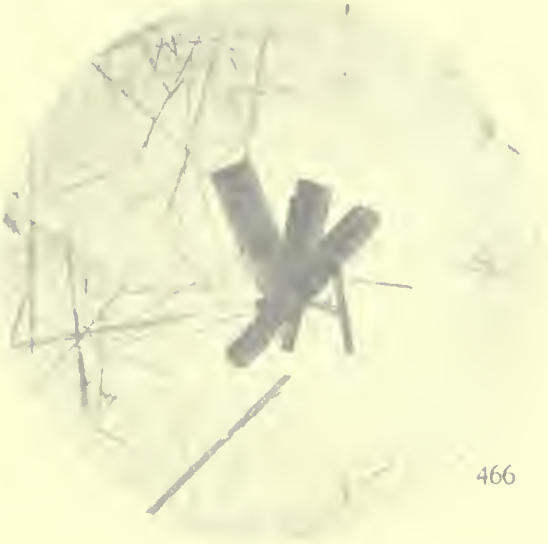
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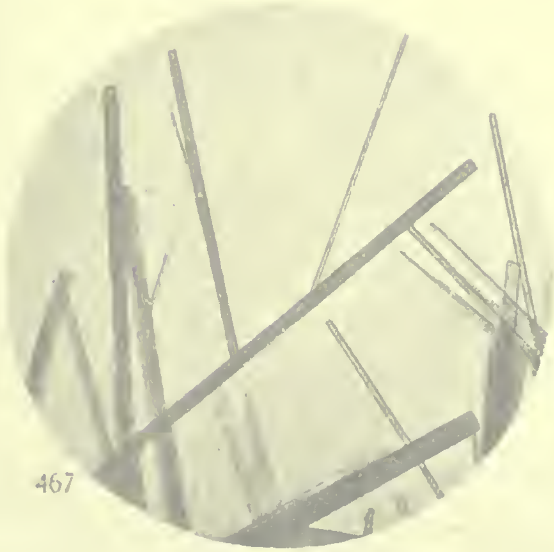
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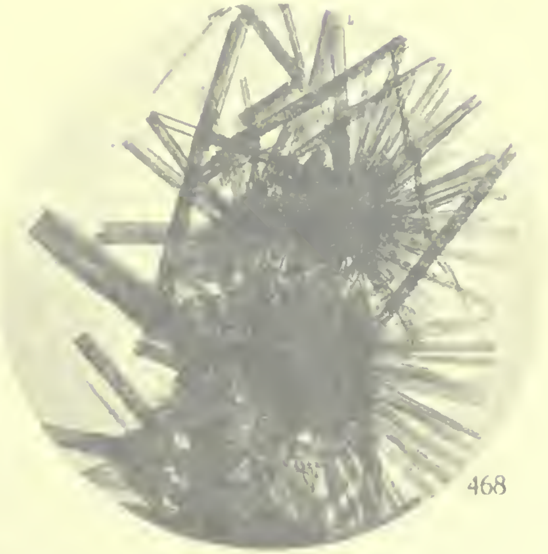
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463, 464. Oxyhemoglobin of the Swiss Fox (*Vulpes vulpes*), showing long striated prisms and capillary crystals along protein ring.

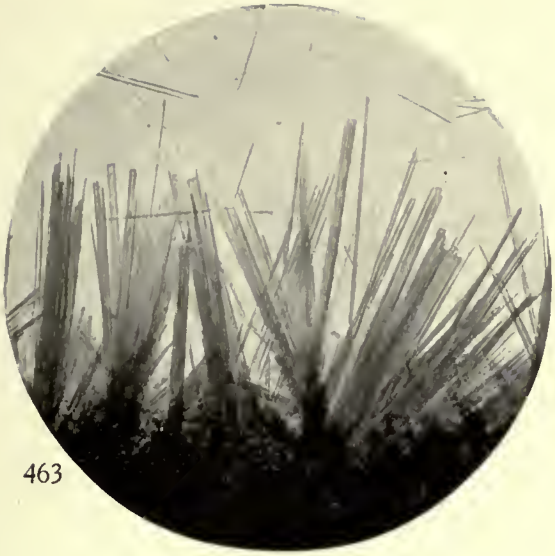
465. Oxyhemoglobin of the Blue Fox (*Vulpes lagopus*), showing capillary and stout prismatic crystals.

466. Same, showing group of short stout prisms.

467. Same, showing long square-ended prisms.

468. Metoxyhemoglobin of the Blue Fox, showing radiating groups of crystals in protein ring.

PLATE 78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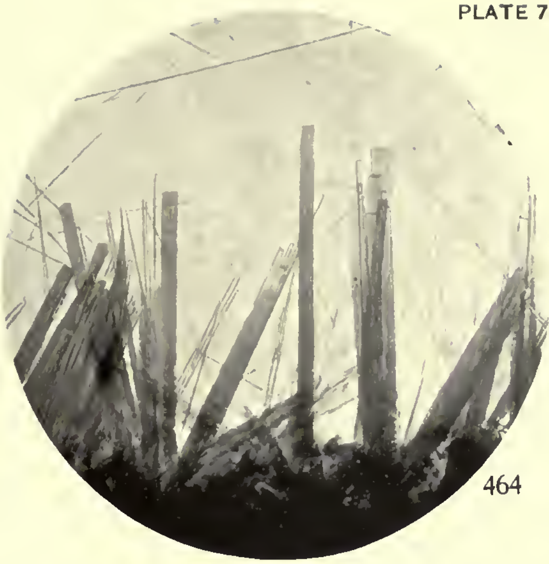
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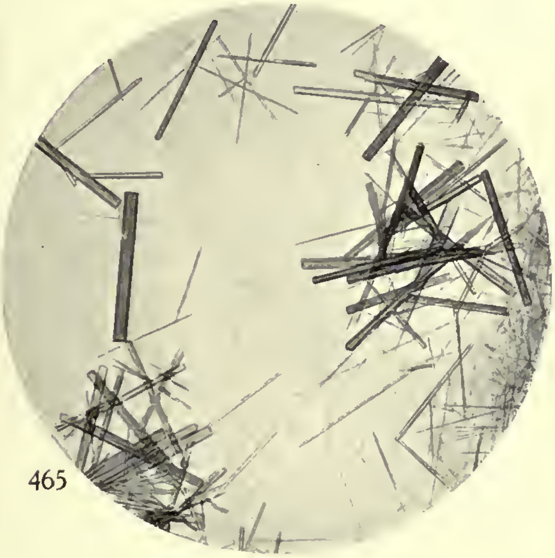
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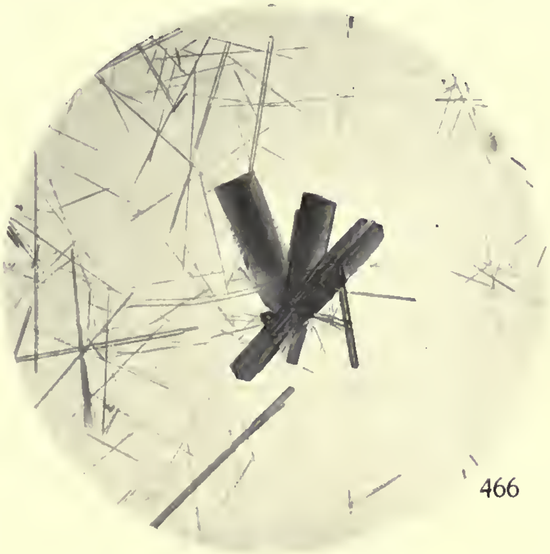
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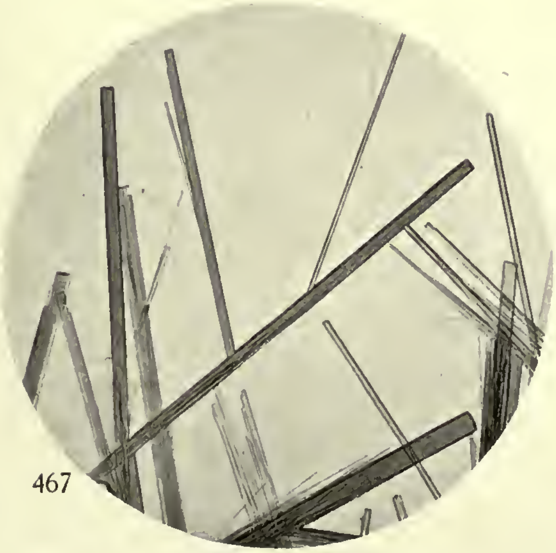
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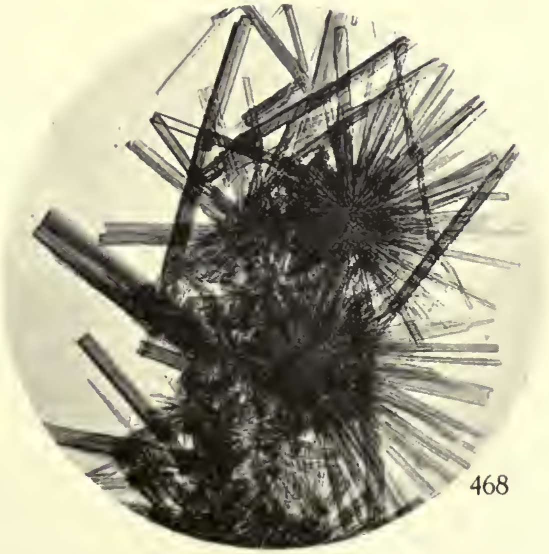
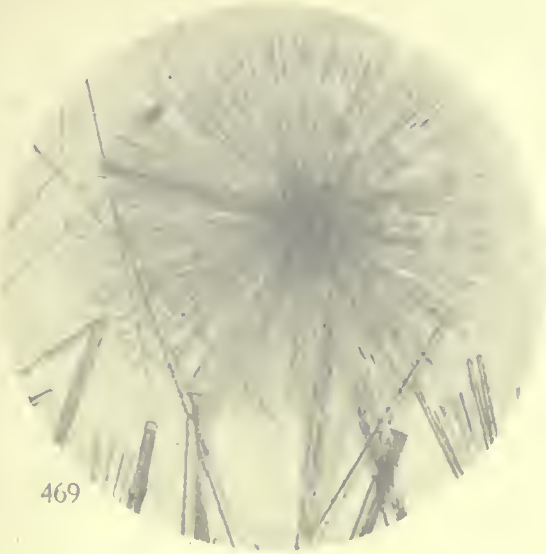

468

463, 464. Oxyhemoglobin of the Swiss Fox (*Vulpes vulpes*), showing long striated prisms and capillary crystals along protein ring.
465. Oxyhemoglobin of the Blue Fox (*Vulpes lagopus*), showing capillary and stout prismatic crystals.
466. Same, showing group of short stout prisms.
467. Same, showing long square-ended prisms.
468. Metoxyhemoglobin of the Blue Fox, showing radiating groups of crystals in protein ring.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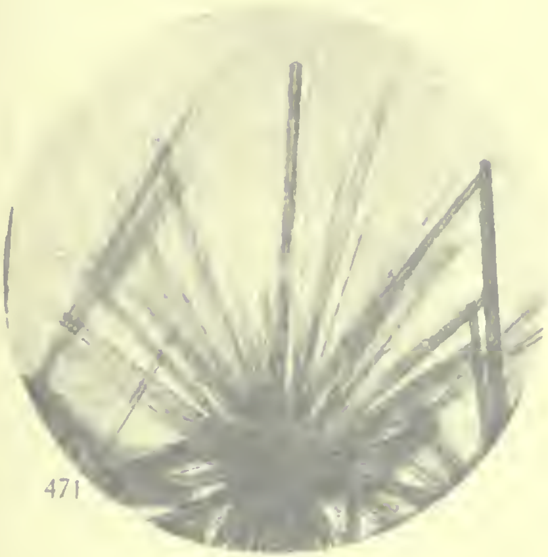
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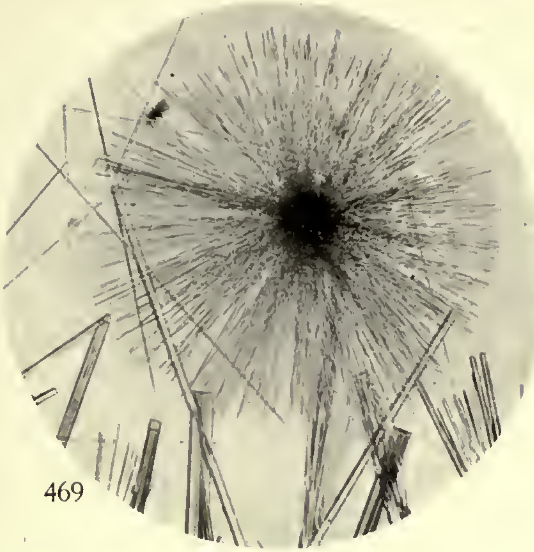
474

469. Oxyhemoglobin of the Gray Fox (*Urocyon cinereo-argenteus*), showing sphenelitic groups of thin prismatic crystals, an occurrence rarely seen.

470. Same, showing large masses of crystals in parallel growth, a characteristic aggregate in this species.

471, 472. Same, showing radiating tufts that develop in retarded crystallization.

473, 474. Same, showing shorter thicker crystals developed in undiluted preparations. These crystals formed in protein ring, and in 474 one of the small rare sphenelitic groups is seen.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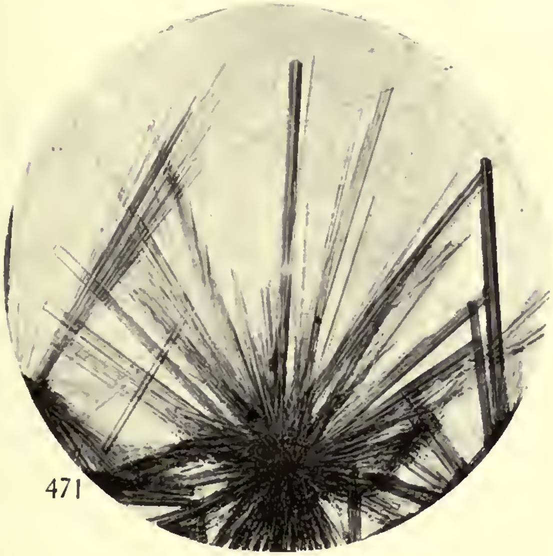
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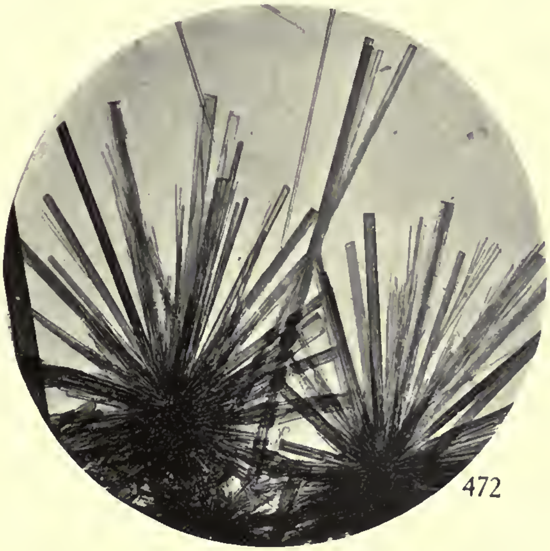
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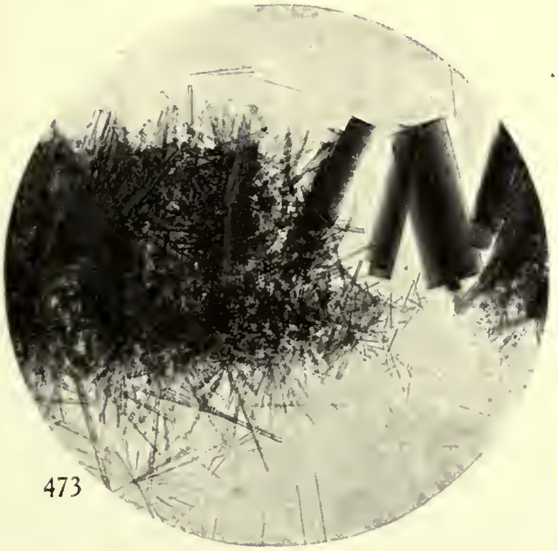
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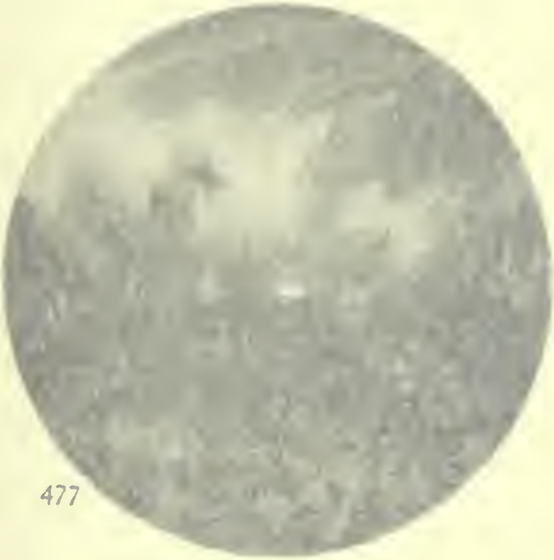
474

469. Oxyhemoglobin of the Gray Fox (*Urocyon cinereo-argenteus*), showing sphenelitic groups of thin prismatic crystals, an occurrence rarely seen.
470. Same, showing large masses of crystals in parallel growth, a characteristic aggregate in this species.
471, 472. Same, showing radiating tufts that develop in retarded crystallizations.
473, 474. Same, showing shorter thicker crystals developed in undiluted preparations. These crystals formed in protein ring, and in 474 one of the small rare sphenelitic groups is seen.


475


476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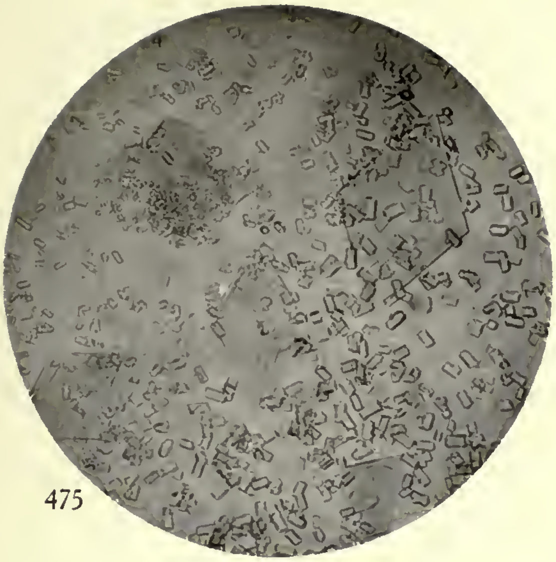
475. [illegible] of the Lion (*Felis leo*), showing [illegible] growth. Short prismatic crystals [illegible]
476. [illegible] an irregular aggregate of [illegible] crystals.
477–480. [illegible] crystals of type *a* and [illegible] crystals may be seen to lower left of field in figure [illegible] crystals.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480

475. Oxyhemoglobin of the Lion (*Felis leo*), showing type *a* crystals occurring singly and exhibiting parallel growth. Short prismatic crystals in this plate are reduced hemoglobin of first crop.
476. Same, showing an irregular aggregate of type *a* crystals.
477–480. Same, showing crystals of type *a* and type *b* along cover edge. Characteristic form of type *b* crystals may be seen to lower left of field in figure 480. All figures show reduced hemoglobin crystals.

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481. Oxyhemoglobin of the Lion (*Felis leo*), showing group of type *a*, rhombic crystals, some exhibiting parallel growth.

482. Oxyhemoglobin and Reduced Hemoglobin of the Lion, showing rhombic plates of oxyhemoglobin and brush-like aggregates of hemoglobin needles of second crop

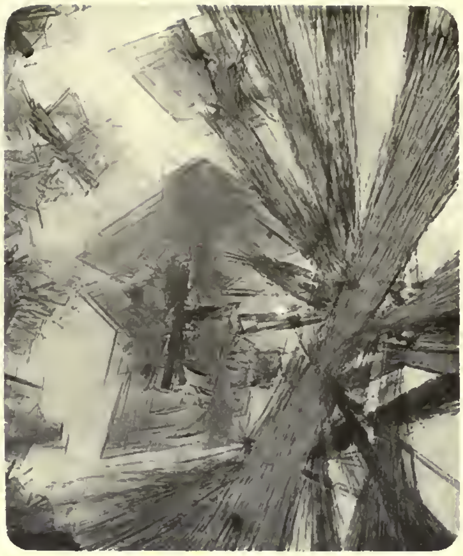
483. Same, showing large crystals of oxyhemoglobin showing parallel growth, embedded in tuft of crystals of reduced hemoglobin of second crop.

484. Oxyhemoglobin of Lion, showing large type *a* crystal with smaller crystals on it in parallel growth.

485. Oxyhemoglobin and Reduced Hemoglobin of Lion, showing large type *a* oxyhemoglobin crystals on flat and on edge, and containing small embedded crystals of reduced hemoglobin. Needle-like crystals to left are reduced hemoglobin.

486. Reduced Hemoglobin of Lion, of the short prismatic type; pleochroism very distinct.


481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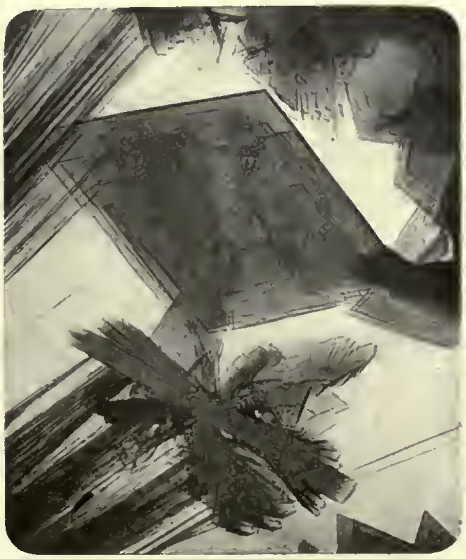
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486

481. Oxyhemoglobin of the Lion (*Felis leo*), showing group of type *a*, rhombic crystals, some exhibiting parallel growth.
482. Oxyhemoglobin and Reduced Hemoglobin of the Lion, showing rhombic plates of oxyhemoglobin and brush-like aggregates of hemoglobin needles of second crop.
483. Same, showing large crystals of oxyhemoglobin showing parallel growth, embedded in tuft of crystals of reduced hemoglobin of second crop.
484. Oxyhemoglobin of Lion, showing large type *a* crystal with smaller crystals on it in parallel growth.
485. Oxyhemoglobin and Reduced Hemoglobin of Lion, showing large type *a* oxyhemoglobin crystals on flat and on edge, and containing small embedded crystals of reduced hemoglobin. Needle-like crystals to left are reduced hemoglobin.
486. Reduced Hemoglobin of Lion, of the short prismatic type; pleochroism very distinct.


487


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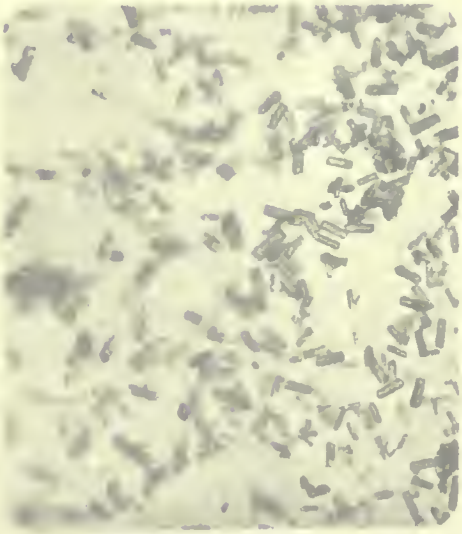
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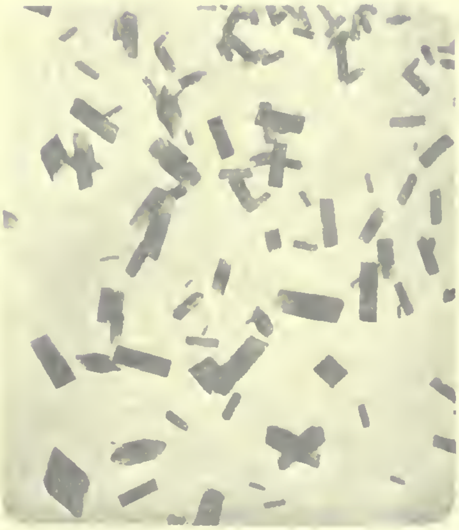
492

487. Reduced Hemoglobin of the Lion (*Felis leo*), showing smaller, more normally developed first-formed crystals of third crop in various orientations.
488. Same, showing larger crystals of third crop. Traces of needles of second-crop hemoglobin crystals may be seen in crystals at top of figure, penetrating the large crystals.
489. Same, showing one of these large, third-crop crystals penetrated by needles of second-crop crystals.
490. Same, various sections of these large, third-crop crystals, showing traces of penetration by needles of second-crop reduced hemoglobin.
491. Reduced Hemoglobin of the Tiger (*Felis tigris*), showing small, first-crop crystals.
492. Same, showing larger crystals of first crop, some distorted by growth or by contact with slide or cover.


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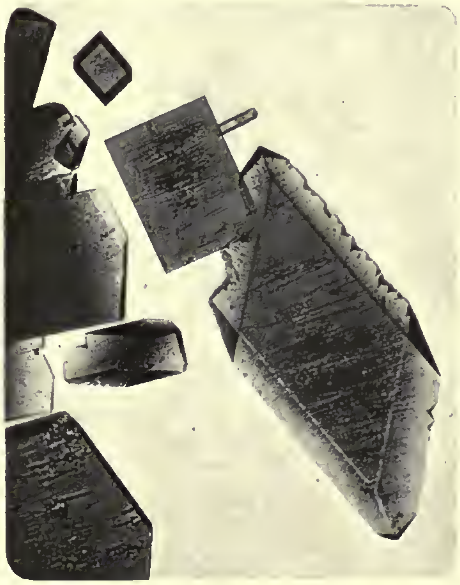
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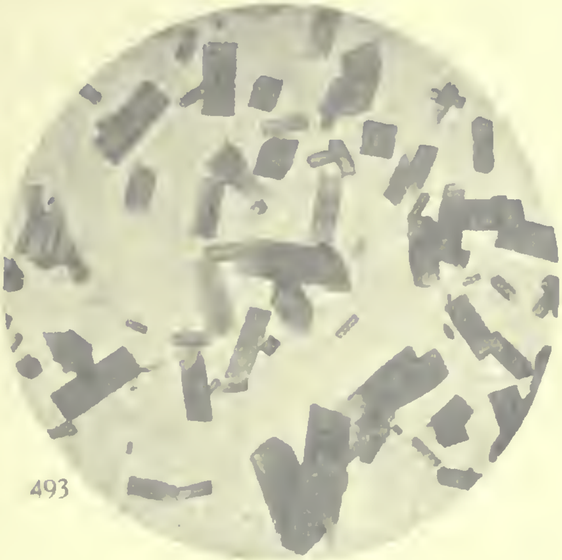
492

487. Reduced Hemoglobin of the Lion (*Felis leo*), showing smaller, more normally developed first-formed crystals of third crop in various orientations.
488. Same, showing larger crystals of third crop. Traces of needles of second-crop hemoglobin crystals may be seen in crystals at top of figure, penetrating the large crystals.
489. Same, showing one of these large, third-crop crystals penetrated by needles of second-crop crystals.
490. Same, various sections of these large, third-crop crystals, showing traces of penetration by needles of second-crop reduced hemoglobin.
491. Reduced Hemoglobin of the Tiger (*Felis tigris*), showing small, first-crop crystals.
492. Same, showing larger crystals of first crop, some distorted by growth or by contact with slide or cover.

PLATE 83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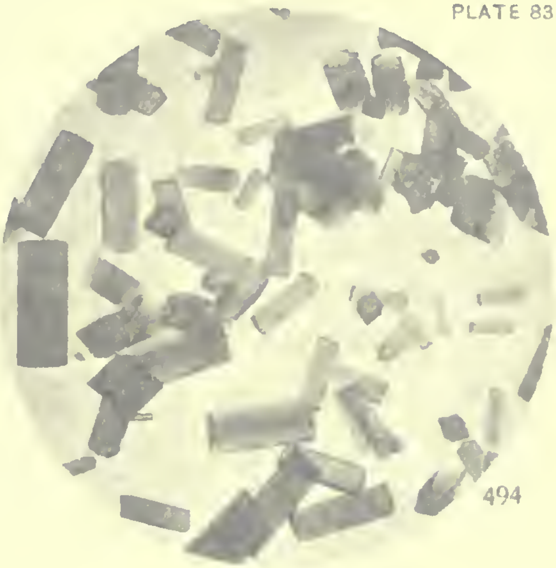
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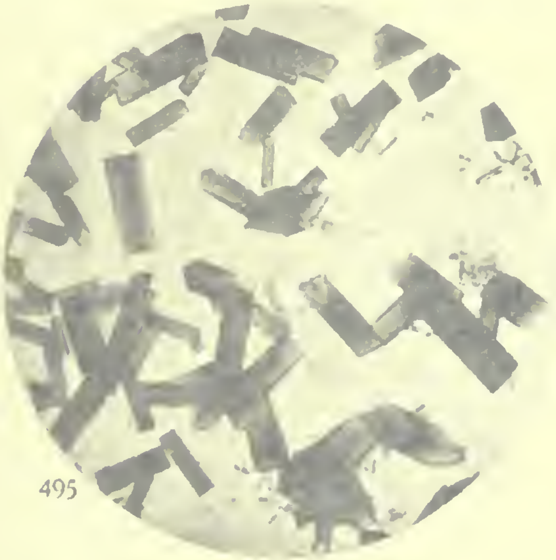
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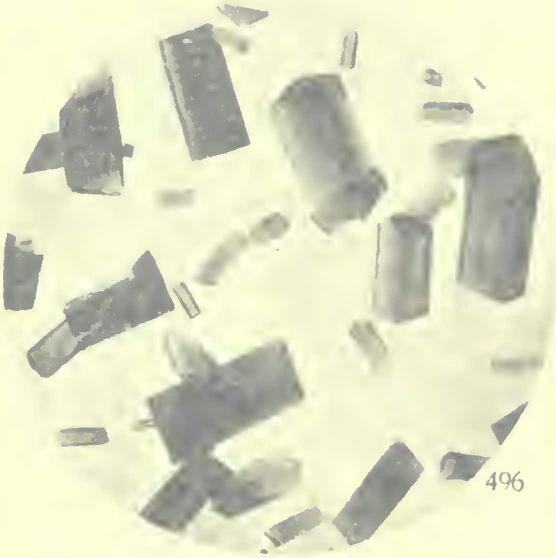
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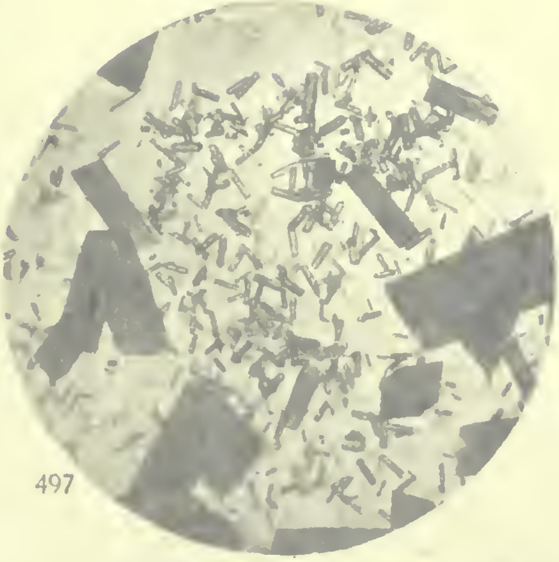
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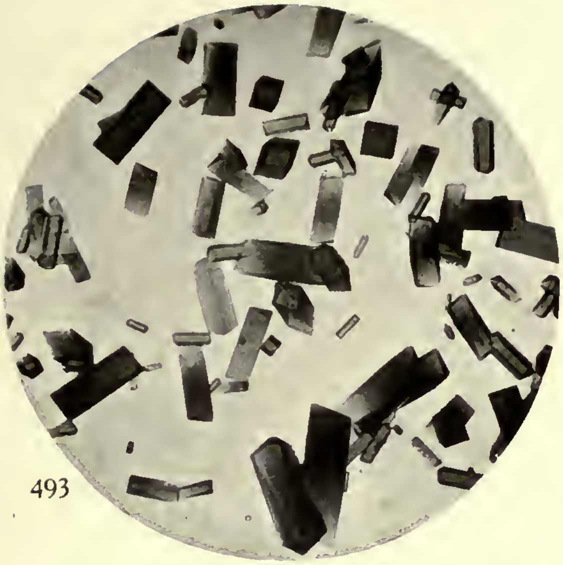
498

493. Reduced Hemoglobin of the Tiger (*Felis tigris*), showing various aspects of medium-sized crystals. Angle of macrodome may be seen in crystal to left of center of field.
494, 495. Same, showing different orientations of medium-sized crystals.
496. Same, showing large crystals.
497, 498. Same, showing large crystals of second crop, along with small crystals of first crop.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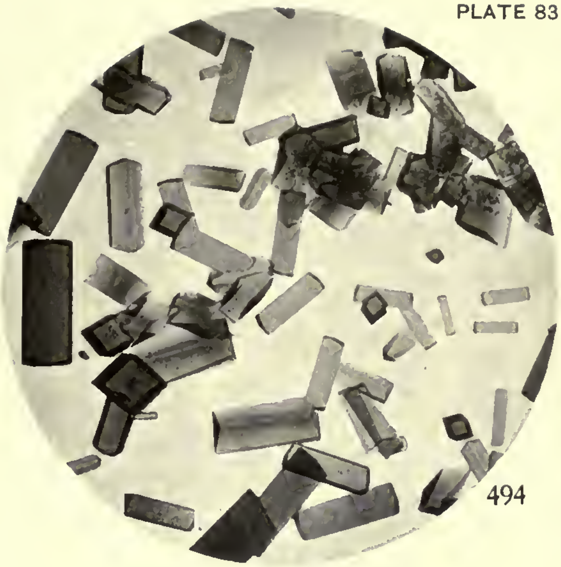
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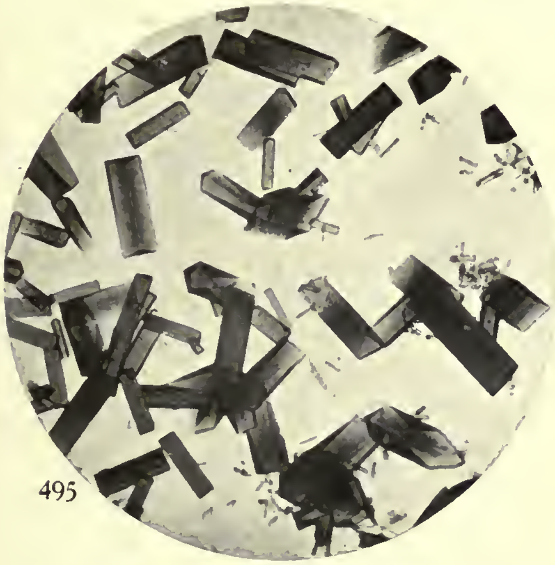
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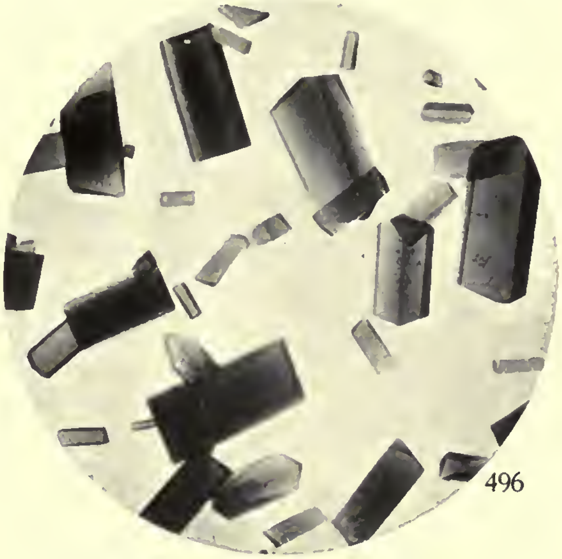
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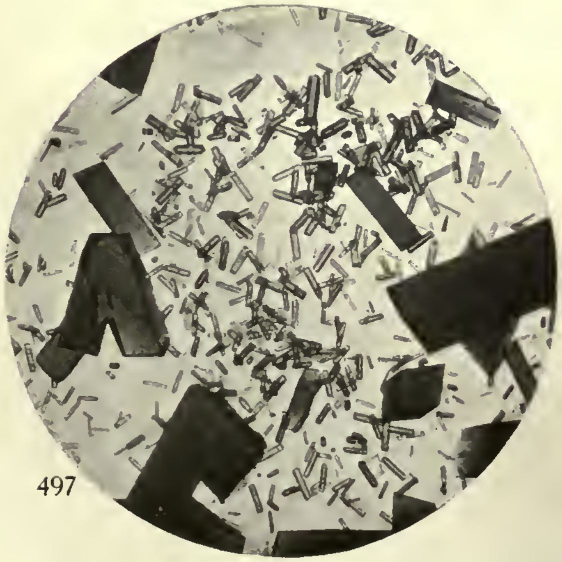
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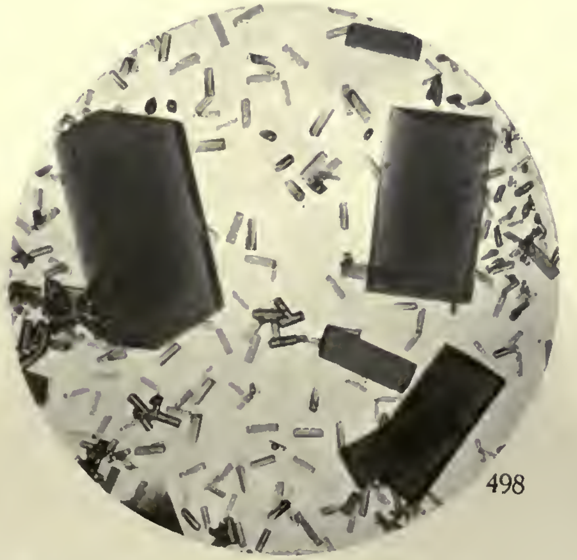
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493. Reduced Hemoglobin of the Tiger (*Felis tigris*), showing various aspects of medium-sized crystals. Angle of macrodome may be seen in crystal to left of center of field.

494, 495. Same, showing different orientations of medium-sized crystals.

496. Same, showing large crystals.

497, 498. Same, showing large crystals of second crop, along with small crystals of first crop.

PLATE 84


499


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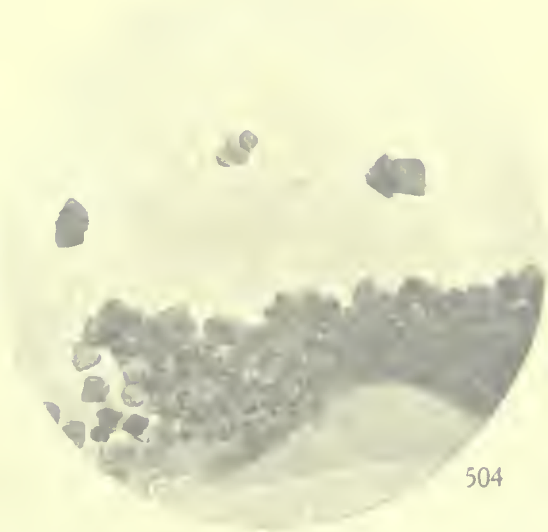
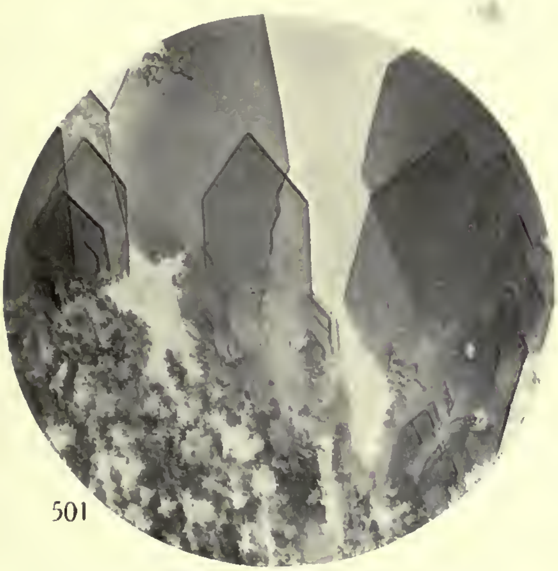

504

499. α-Oxyhemoglobin of the Jaguar (*Felis onca*), two groups of tabular crystals showing parallel growth.
500. Same, showing large group of crystals all in parallel growth orientation. Rod-like crystals are reduced hemoglobin.
501. Same, showing three groups of tabular crystals, each in parallel growth orientation.
502. Same, showing large group in parallel growth orientation.
503. β-Oxyhemoglobin of the Jaguar, showing dodecahedral crystals.
504. Same, showing octahedral crystals.


499


500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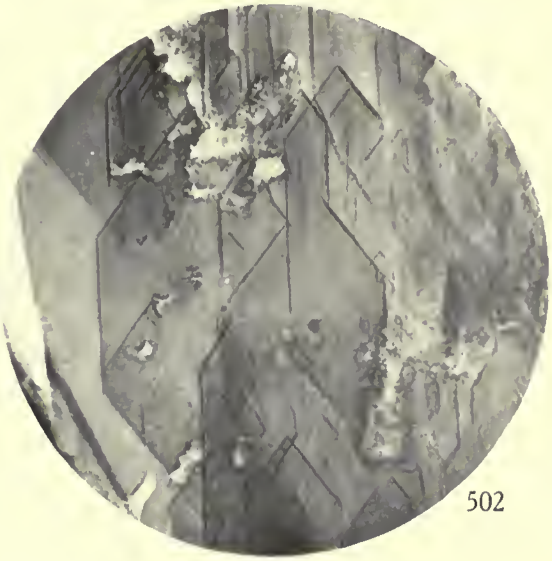
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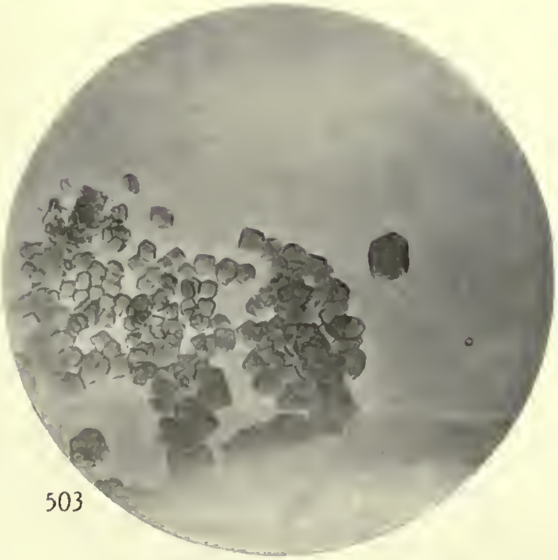
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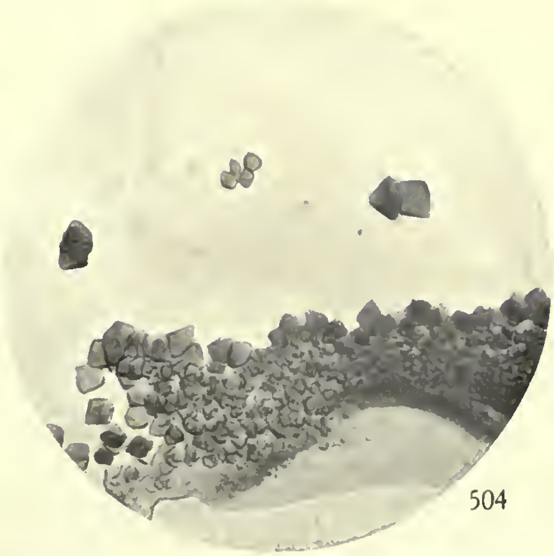
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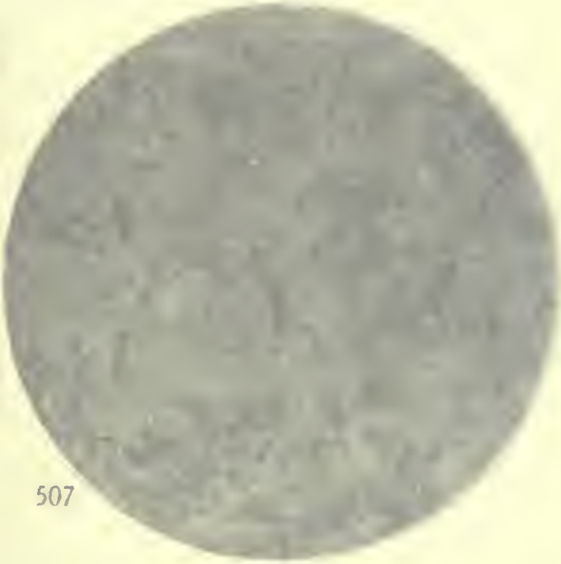
499. α-Oxyhemoglobin of the Jaguar (*Felis onca*), two groups of tabular crystals showing parallel growth.
500. Same, showing large group of crystals all in parallel growth orientation. Rod-like crystals are reduced hemoglobin.
501. Same, showing three groups of tabular crystals, each in parallel growth orientation.
502. Same, showing large group in parallel growth orientation.
503. β-Oxyhemoglobin of the Jaguar, showing dodecahedral crystals.
504. Same, showing octahedral crystals.

505

506



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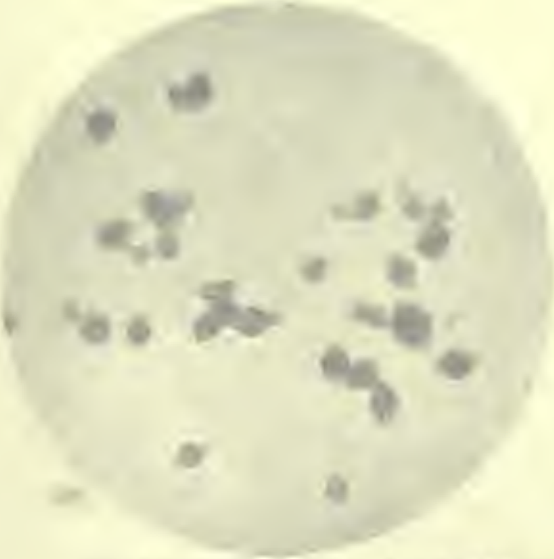

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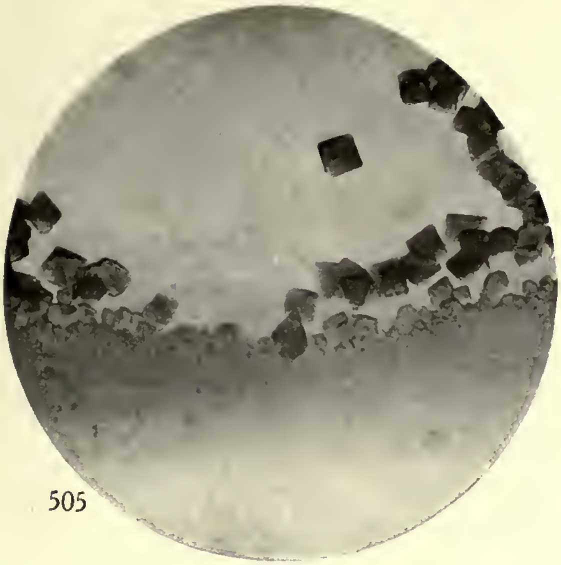
510

[illegible] n of the Jaguar (*Felis onca*), [illegible]
[illegible] n of the Jaguar [illegible]
[illegible] of the Mountain Lion (*Felis concolor*), [illegible]
[illegible]
[illegible]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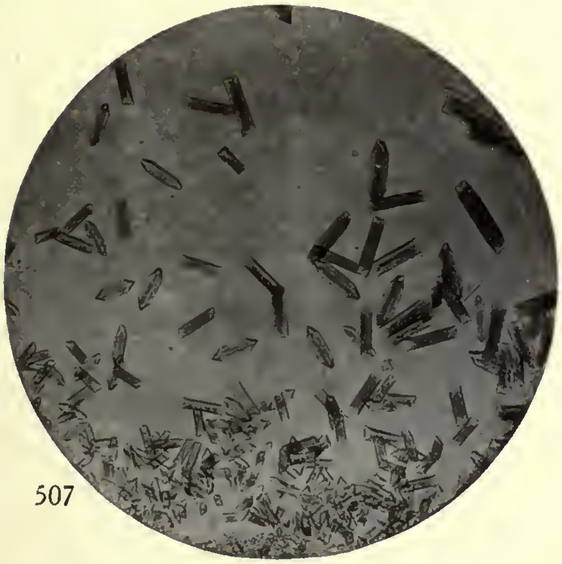
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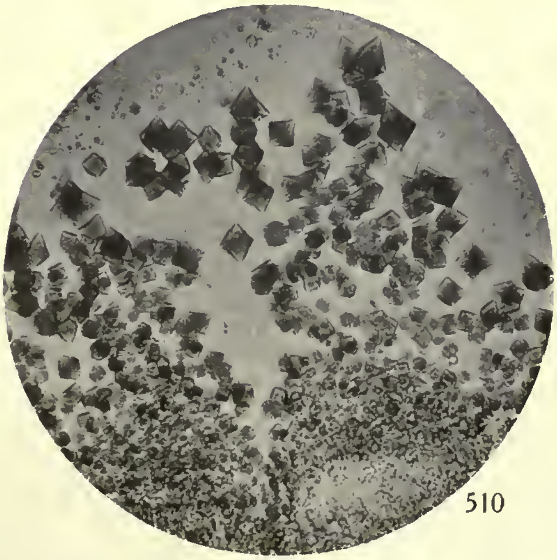
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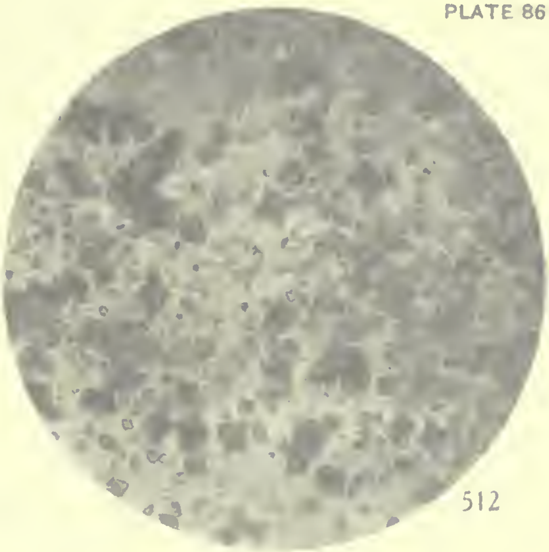
505. $\beta$-Oxyhemoglobin of the Jaguar (*Felis onca*), showing octahedral crystals.
506. Reduced Hemoglobin of the Jaguar, showing long, square, four-sided prisms ending in a brush of fibers.
507, 508. $\alpha$-Oxyhemoglobin of the Mountain Lion (*Felis concolor*), showing type *a* crystal, consisting of unit prism and brachydome.
509. Same, showing type *b* crystal.
510. Same, showing type *b* crystal, some passing into $\beta$-Oxyhemoglobin.


511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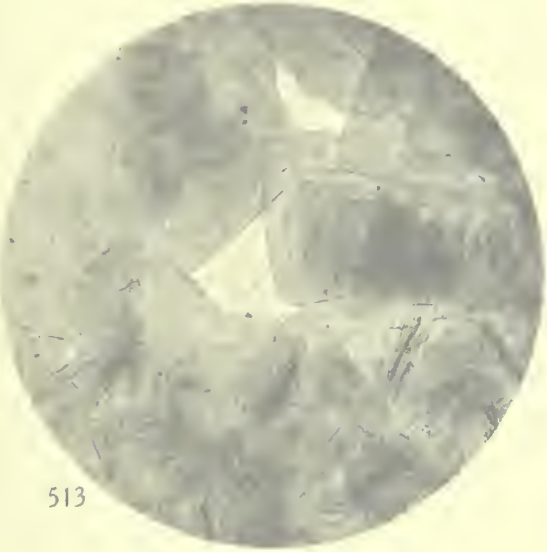
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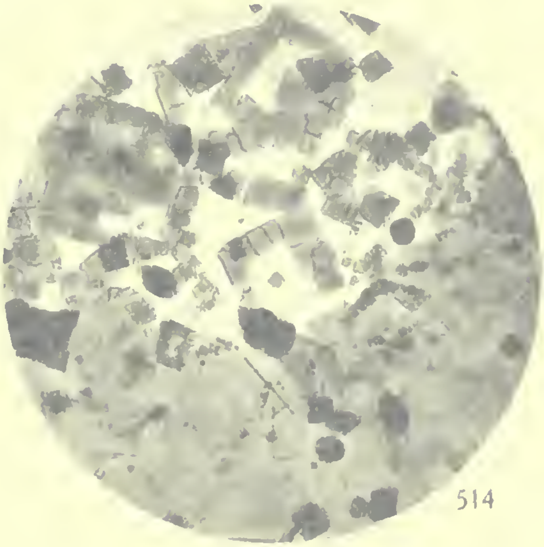
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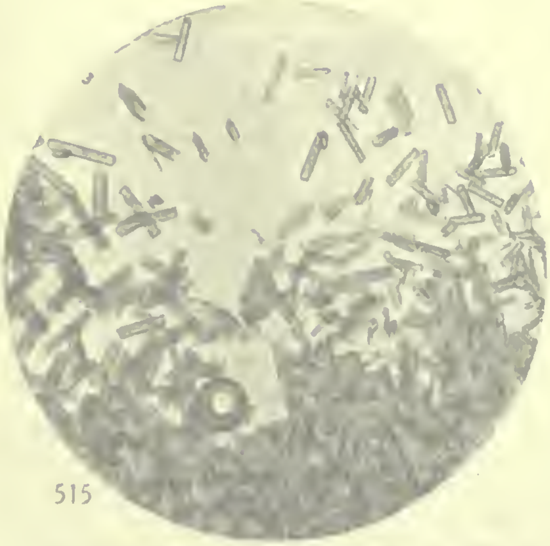
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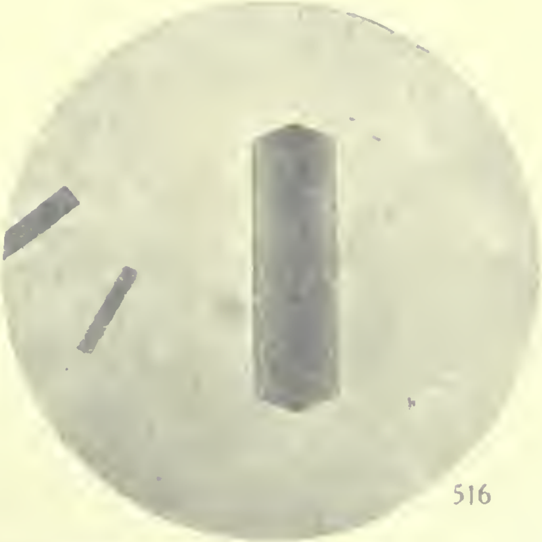
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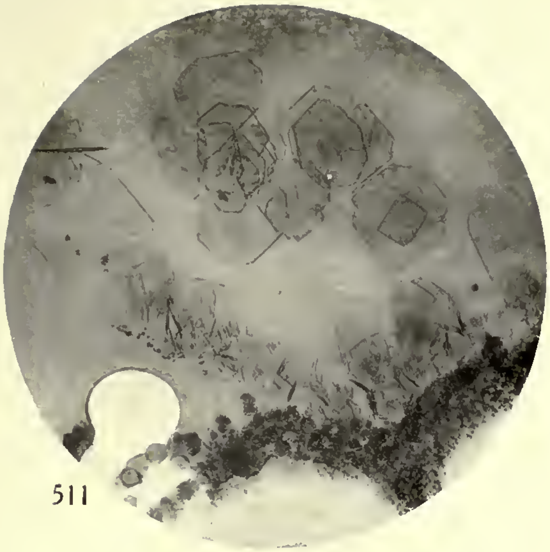
511. Oxyhemoglobin of the Mountain Lion (*Felis concolor*), showing type *c* crystal.
512. β-Oxyhemoglobin and α-Oxyhemoglobin of the Mountain Lion, showing type *b* crystal of α-Oxyhemoglobin twinned and passing into β-Oxyhemoglobin octahedron
513. α-CO-Hemoglobin of the Mountain Lion, showing type *c* crystal.
514. CO-Hemoglobin of the Mountain Lion, showing tabular type *c* crystals of α-CO-Hemoglobin and crystals of β-CO-Hemoglobin that have grown on α-CO-Hemoglobin crystals and gradually absorbed them.
515. Reduced Hemoglobin of the Leopard-cat (*Felis bengalensis*), showing small, first-formed crystals.
516. Same, showing symmetrical crystal consisting of unit prism and macrodome.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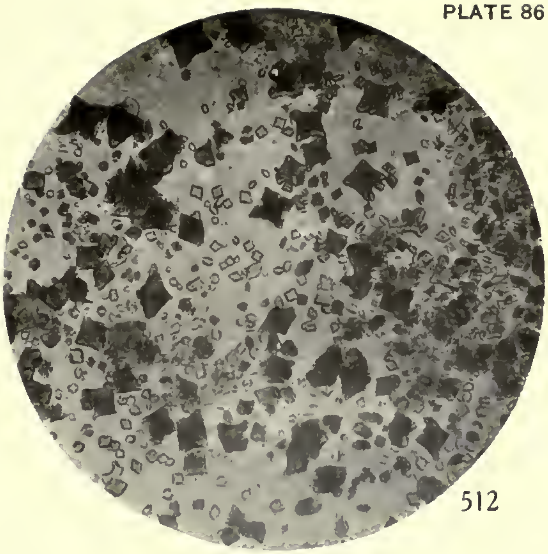
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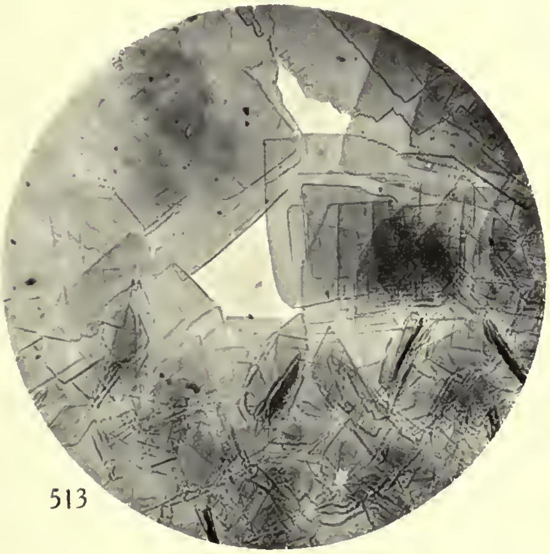
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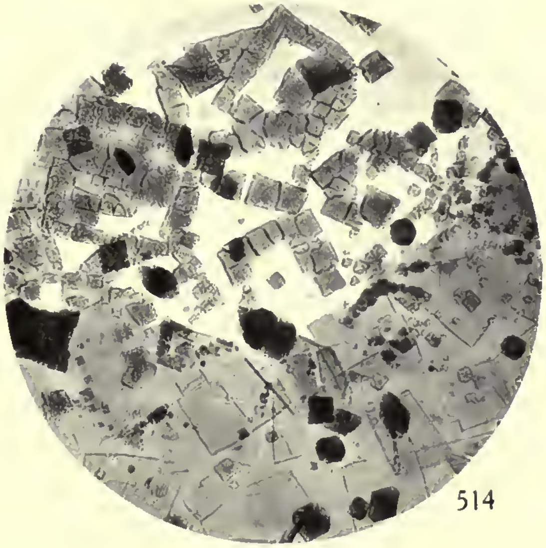
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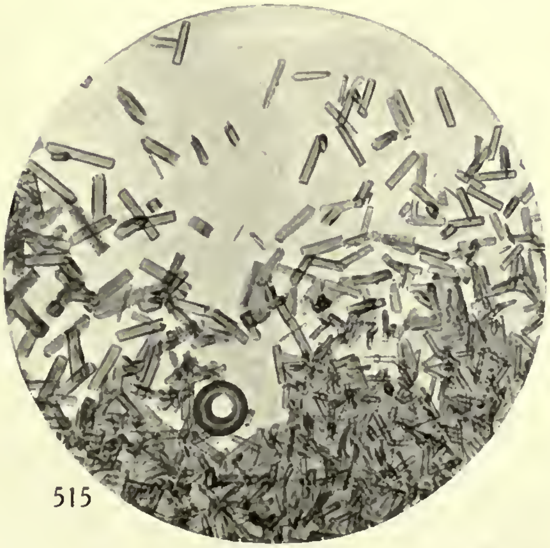
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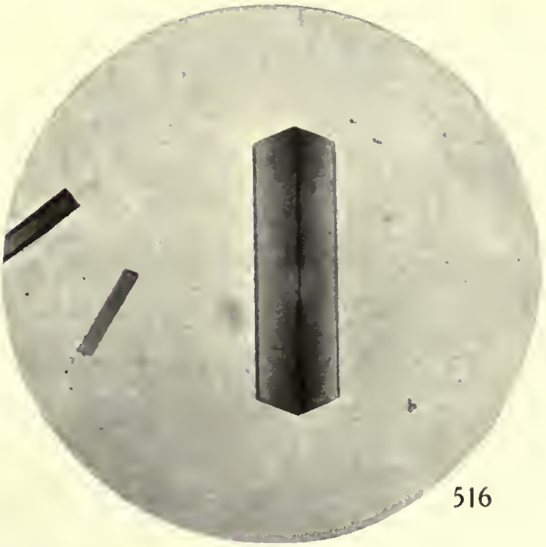
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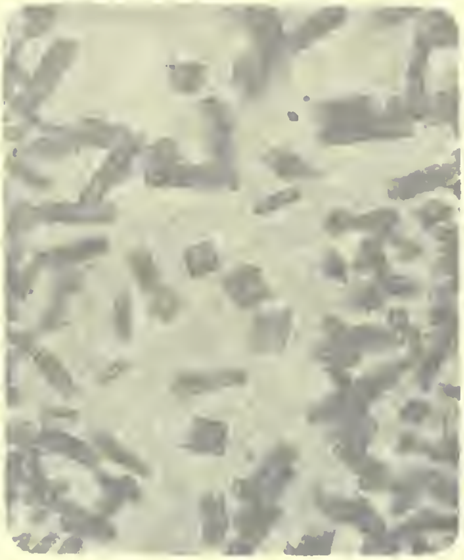
511. Oxyhemoglobin of the Mountain Lion (*Felis concolor*), showing type *c* crystal.
512. β-Oxyhemoglobin and α-Oxyhemoglobin of the Mountain Lion, showing type *b* crystal of α-Oxyhemoglobin twinned and passing into β-Oxyhemoglobin octahedron.
513. α-CO-Hemoglobin of the Mountain Lion, showing type *c* crystal.
514. CO-Hemoglobin of the Mountain Lion, showing tabular type *c* crystals of α-CO-Hemoglobin and crystals of β-CO-Hemoglobin that have grown on α-CO-Hemoglobin crystals and gradually absorbed them.
515. Reduced Hemoglobin of the Leopard-cat (*Felis bengalensis*), showing small, first-formed crystals.
516. Same, showing symmetrical crystal consisting of unit prism and macrodome.

517



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522

517, 518. Reduced Hemoglobin of the Leopard-cat (*Felis bengalensis*), showing single crystals in various orientations. In 518 a number of different sections of the prism are shown.

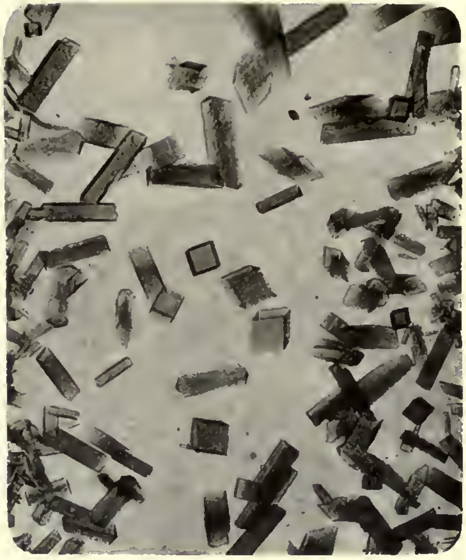
519. Same, showing two skeleton groups in parallel growth orientation.

520. Same, showing group of large crystals growing from a square cross-section of a prism as a nucleus. Cross-section of a prism seen near bottom of figure.

521. Same, showing group similar to 520, with single large prism and oblique section of another prism that resembles an acute rhombohedron.

522. Same, showing single large crystal with outgrowth of smaller crystals.


517


518


519


520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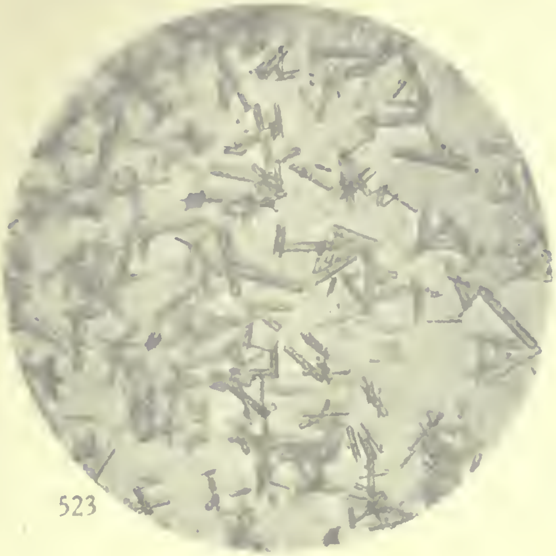
522

517, 518. Reduced Hemoglobin of the Leopard-cat (*Felis bengalensis*), showing single crystals in various orientations. In 518 a number of different sections of the prism are shown.
519. Same, showing two skeleton groups in parallel growth orientation.
520. Same, showing group of large crystals growing from a square cross-section of a prism as a nucleus. Cross-section of a prism seen near bottom of figure.
521. Same, showing group similar to 520, with single large prism and oblique section of another prism that resembles an acute rhombohedron.
522. Same, showing single large crystal with outgrowth of smaller crystals.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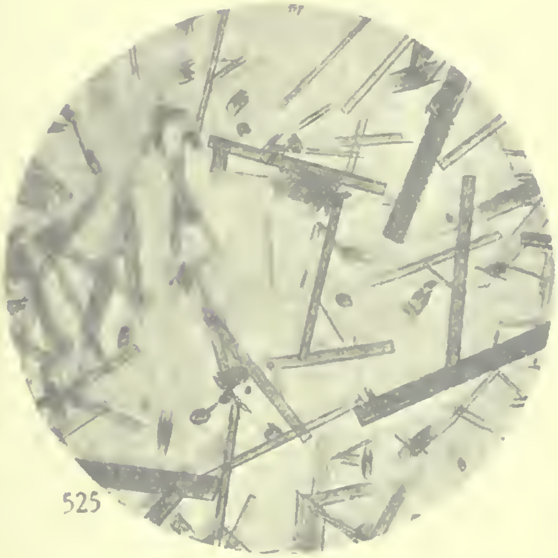
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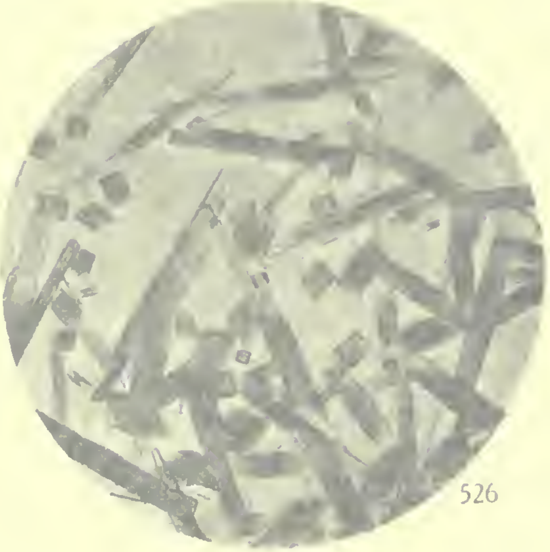
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527


528

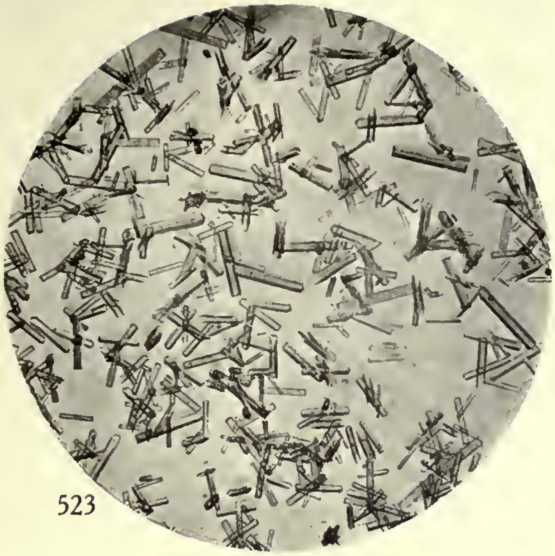
523. Reduced Hemoglobin of the Ocelot (*Felis pardalis*), showing small, first-formed crystals, growing singly or twinned.

524. Same, showing large crystals covered with radiating tufts of smaller crystals.

525, 526. Same, large crystals, many in cross-section, showing [illegible]

527. Reduced Hemoglobin of the Cat (*Felis domestica*), [illegible] parallel growth aggregates of reduced hemoglobin [illegible] as symmetrical crystals develop.

528. Same, showing network of long prismatic crystals [illegible]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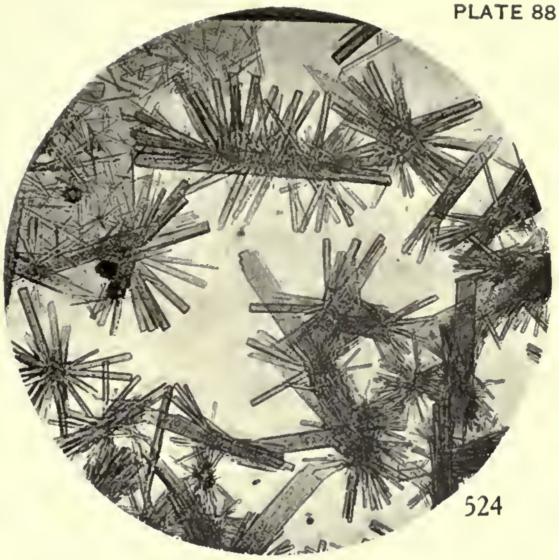
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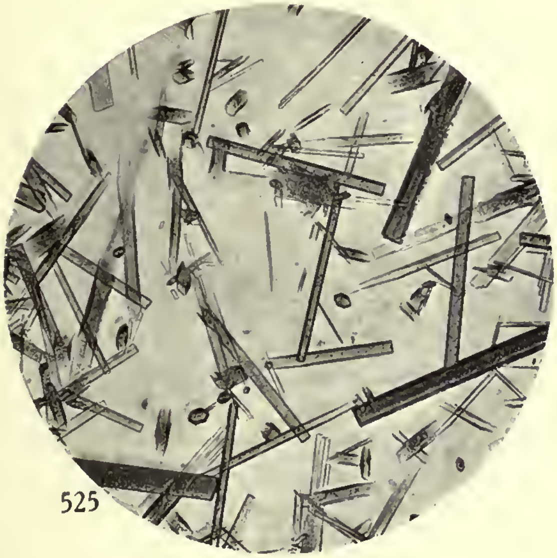
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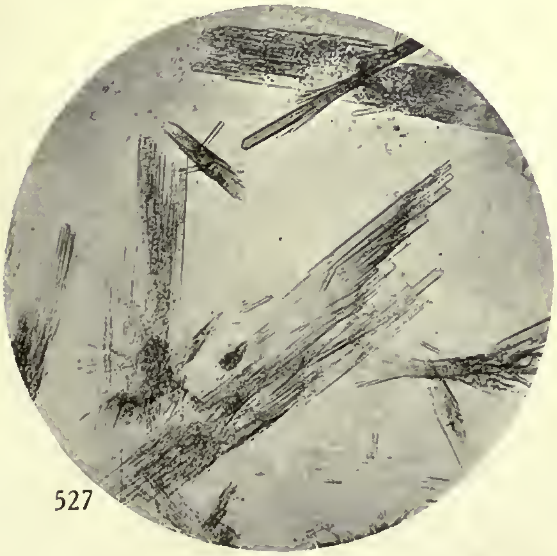
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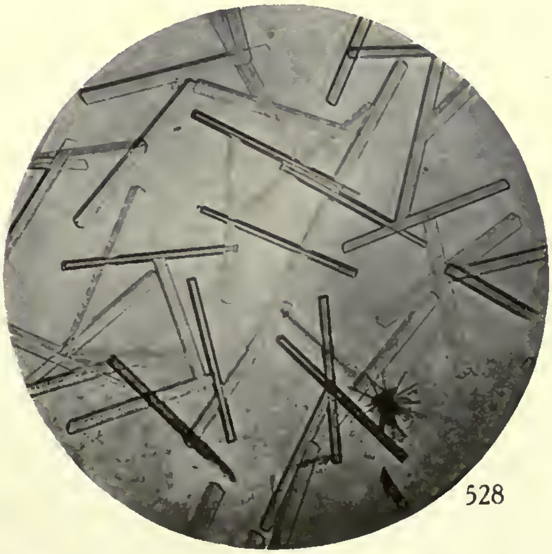
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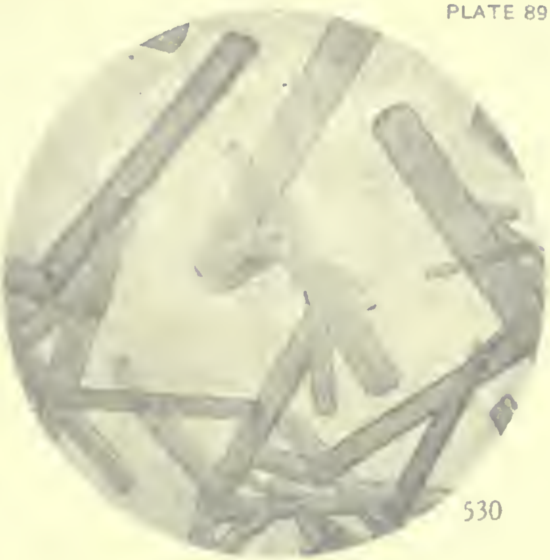
523. Reduced Hemoglobin of the Ocelot (*Felis pardalis*), showing small, first-formed crystals, growing singly or twinned.
524. Same, showing large crystals covered with radiating tufts of smaller crystals.
525, 526. Same, large crystals, many in cross-section, showing macropinacoid.
527. Reduced Hemoglobin of the Cat (*Felis domestica*), showing first definite crystals to form and parallel growth aggregates of reduced hemoglobin rods. These groups are later absorbed as symmetrical crystals develop.
528. Same, showing network of long prismatic crystals of second crop.

529



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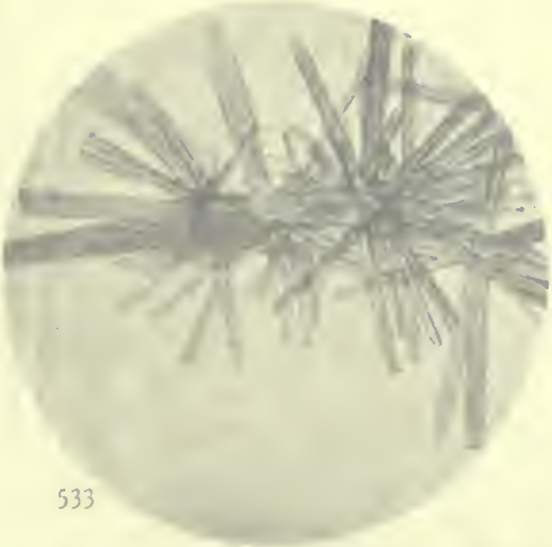
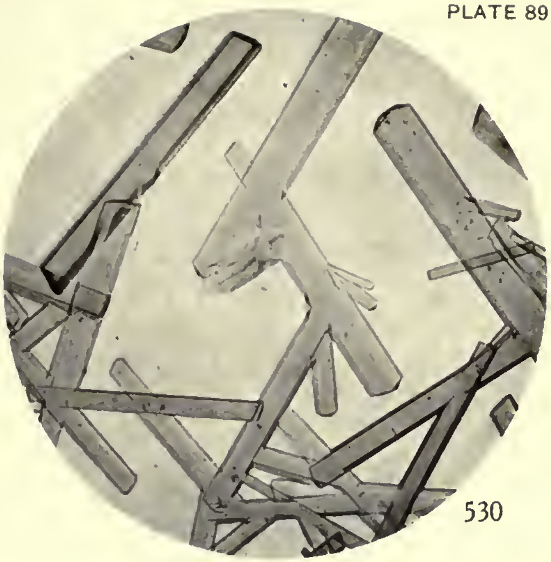


533

534

529. Reduced Hemoglobin of the Cat (*Felis domestica*), showing rough parallel growth aggregates like those of first-formed crystals.
530. Same, showing network of large prismatic crystals.
531. Same, showing single crystal lying on face of prism.
532. Same, showing smaller, later-crop crystals, growing from cross-section of large crystal.
533. Same, showing smaller crystals growing in radiating form from large crystals.
534. Same, showing them symmetrically and unsymmetrically developed, and oxyhemoglobin of Wild Cat (*Lynx rufus*).

529



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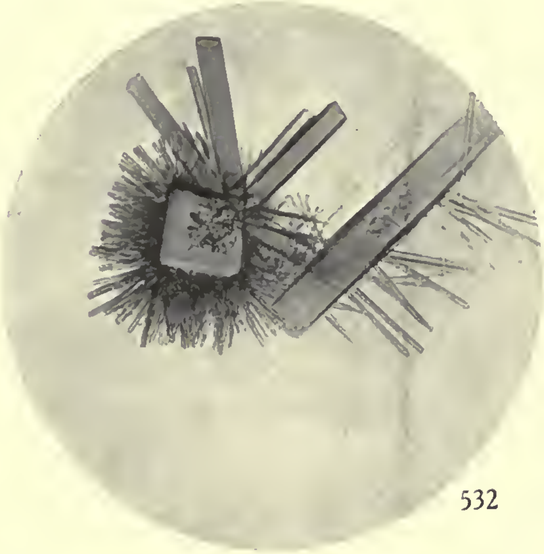

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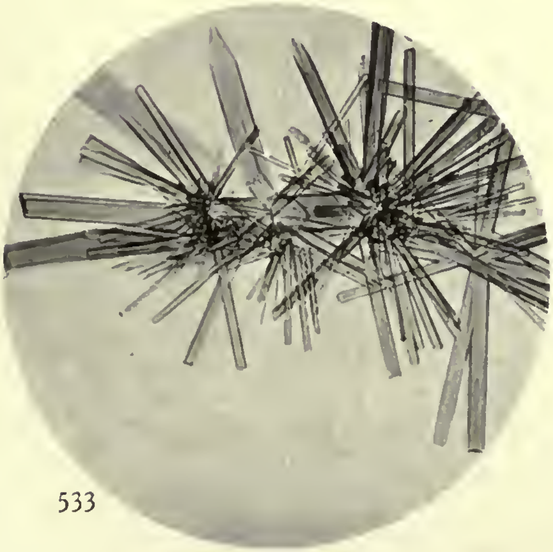

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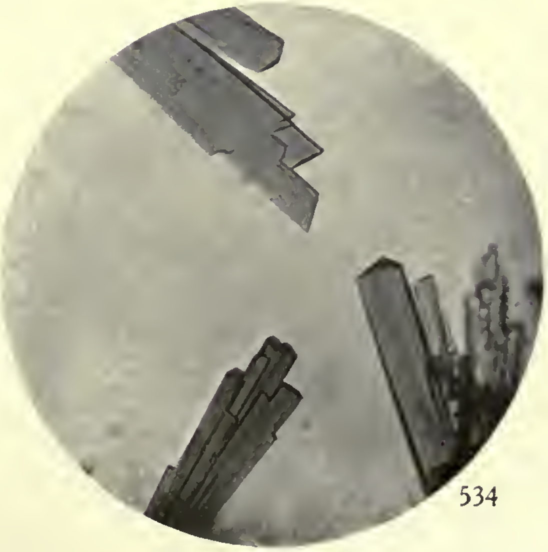

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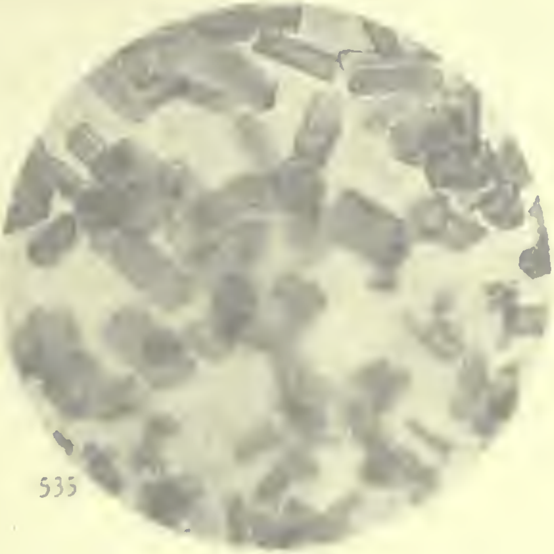
529. Reduced Hemoglobin of the Cat (*Felis domestica*), showing rough parallel growth aggregates like those of first-formed crystals.
530. Same, showing network of large prismatic crystals.
531. Same, showing single crystal lying on face of prism.
532. Same, showing smaller, later-crop crystals, growing from cross-section of large crystal.
533. Same, showing smaller crystals growing in radiating form from large crystals.
534. Same, showing dome symmetrically and unsymmetrically developed; and oxyhemoglobin of Wild Cat (*Lynx rufus*).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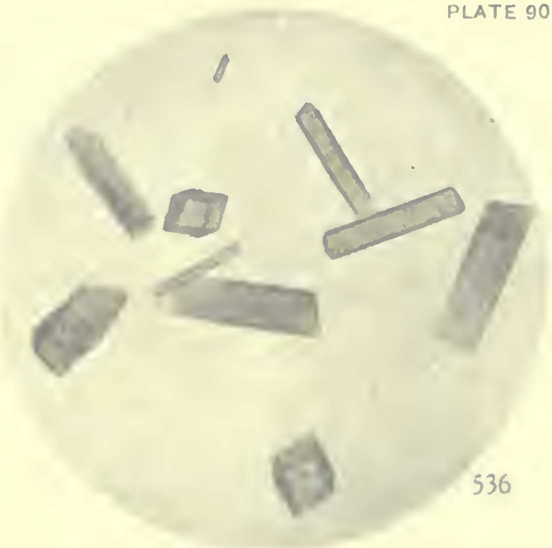
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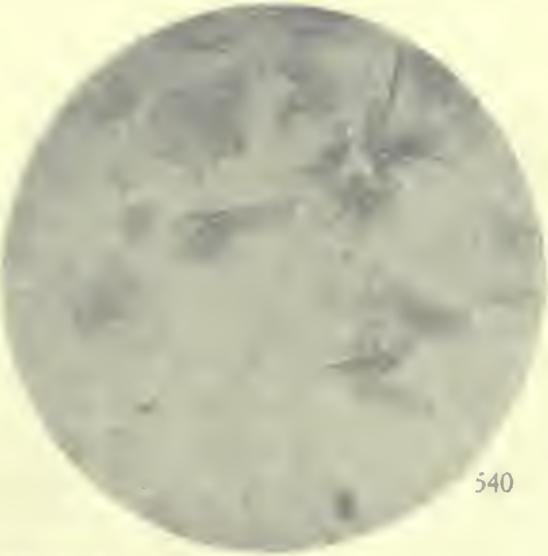
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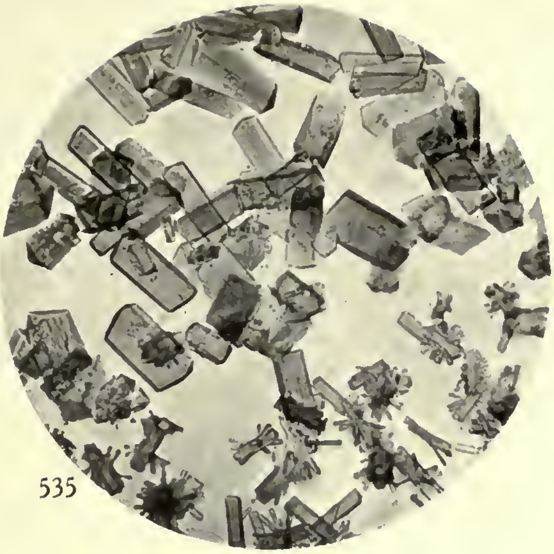
535. Reduced Hemoglobin of the Cat (*Felis domestica*) showing short prismatic type of crystal.
536. Same, showing cross-section of prism.
537. Same, showing group of short type of crystals growing on an oblique cross-section of prism; also prism with one dome face developed making a monoclinic-looking crystal.
538. Same, showing radiating groups of smaller crystals growing on larger prisms.
539. Same, showing prismatic type of crystal.
540. Same, showing bladed type of crystal.

PLATE 90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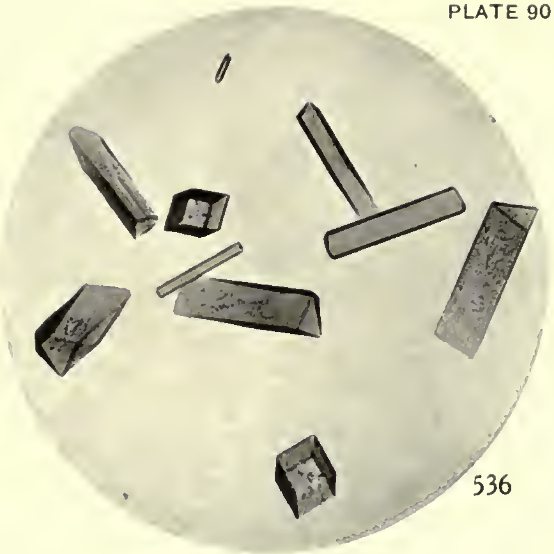
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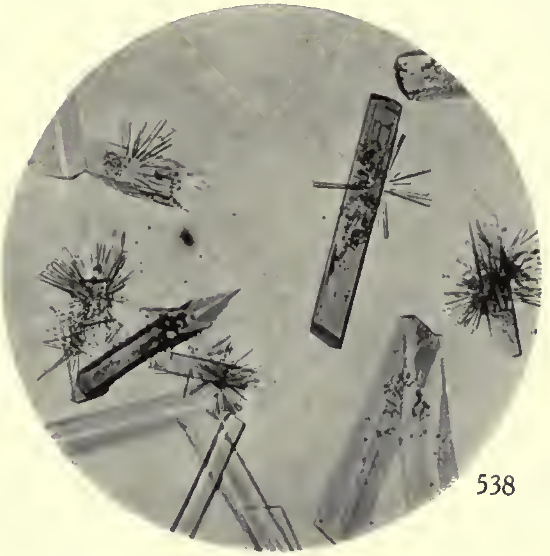
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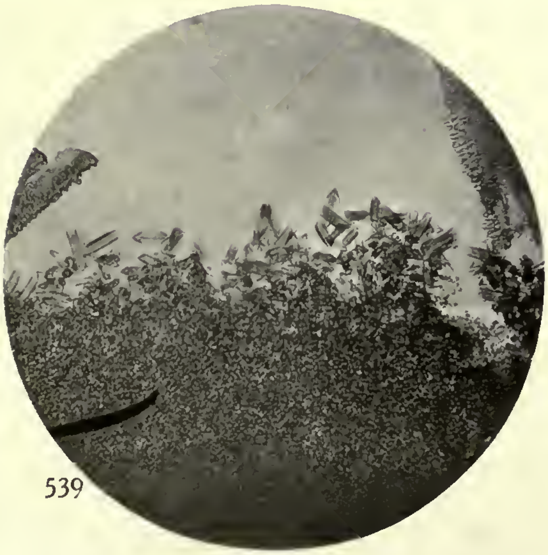
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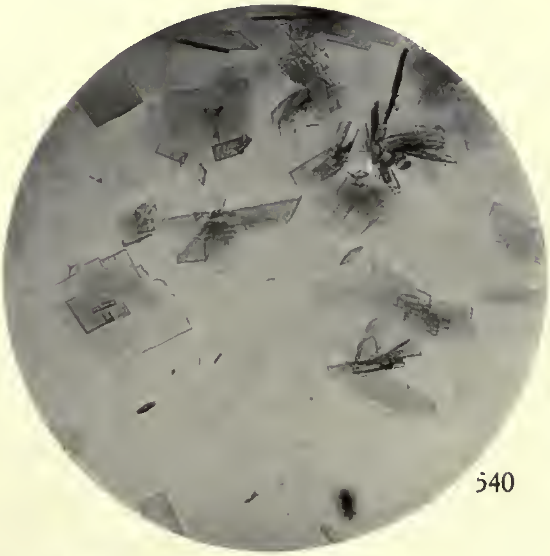
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535. Reduced Hemoglobin of the Cat (*Felis domestica*), showing short prismatic type of crystal.
536. Same, showing cross-section of prism.
537. Same, showing group of short type of crystals growing on an oblique cross-section of prism; also prism with one dome face developed, making a monoclinic-looking crystal.
538. Same, showing radiating groups of smaller crystals growing on larger prisms.
539. Same, showing prismatic type of crystal.
540. Same, showing tabular type of crystal.


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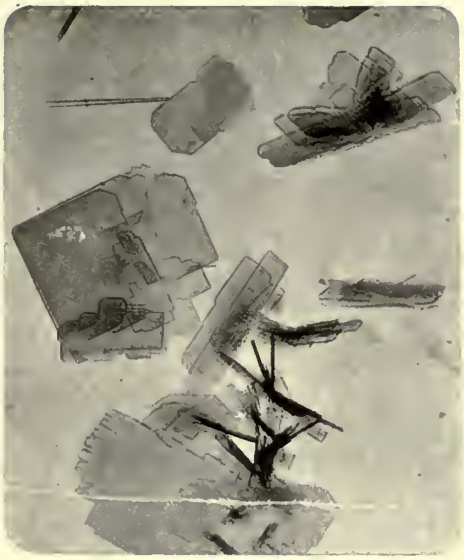
541, 542. Oxyhemoglobin of the Wild Cat (*Lynx rufus*), showing tabular type of crystals, the base bounded by unit prism and two pinacoids.

543. Same, showing large prismatic crystals covered with a secondary growth. Unsymmetrical dome termination seen in large crystal below middle of plate.

544. Same, showing larger prismatic crystals covered by growth of smaller crystals.

545, 546. Reduced Hemoglobin of the Wild Cat, showing long prismatic type of crystals and (in 546) forming network. Many of these [illegible]



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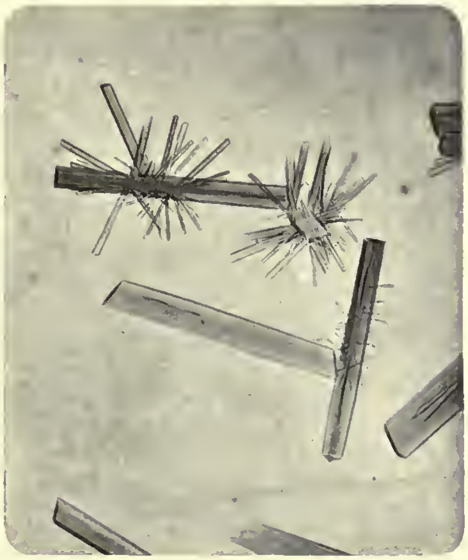

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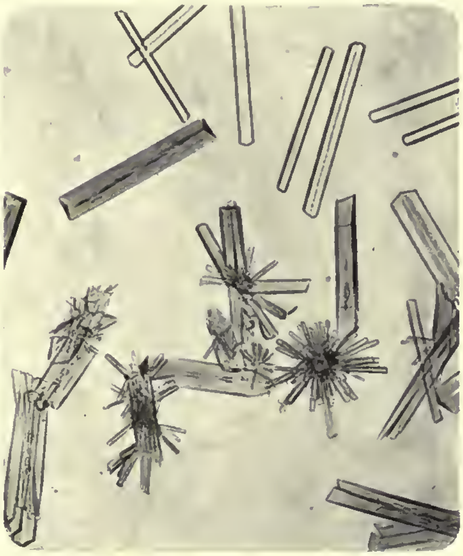

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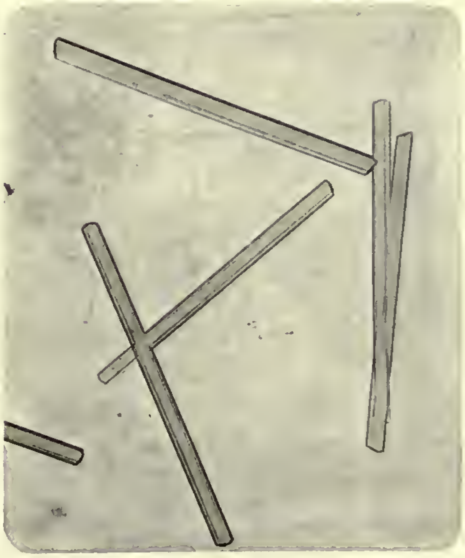

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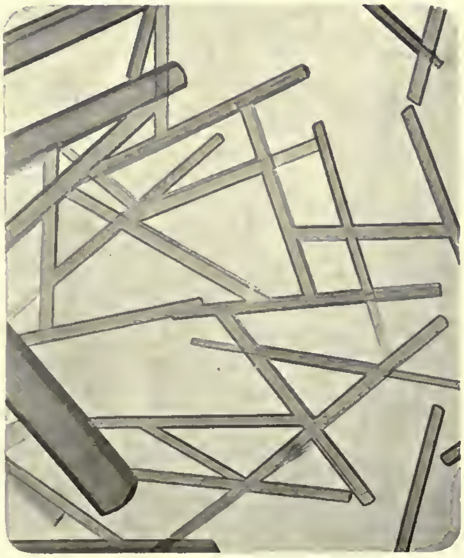

546

541, 542. Oxyhemoglobin of the Wild Cat (*Lynx rufus*), showing tabular type of crystal, the base bounded by unit prism and two pinacoids.

543. Same, showing large prismatic crystals covered with a secondary growth. Unsymmetrical dome termination seen in large crystal below middle of plate.

544. Same, showing larger prismatic crystals covered by growth of smaller crystals.

545, 546. Reduced Hemoglobin of the Wild Cat, showing long prismatic type of crystal and (in 544) forming network. Many of these are in twin positions.


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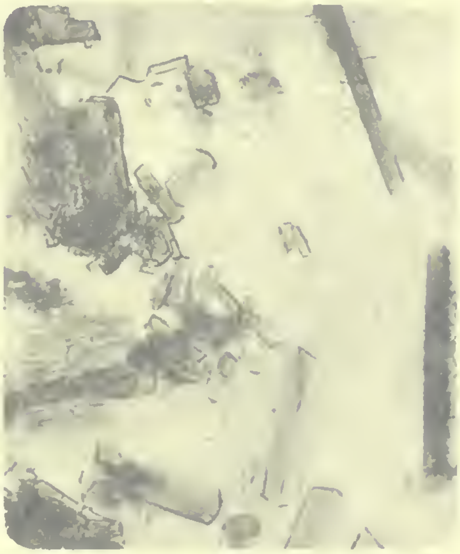
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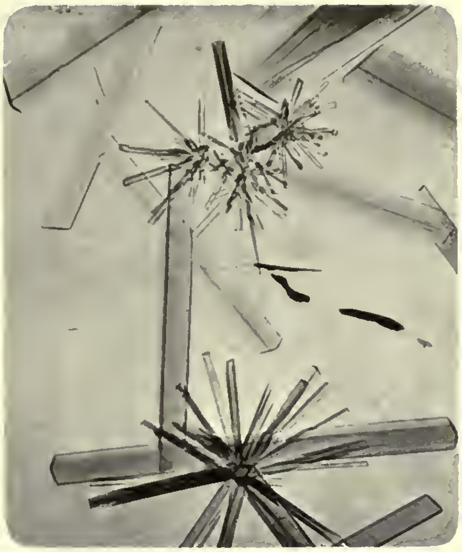
552

547. Reduced Hemoglobin of the Wild Cat (*Lynx rufus*), showing long prismatic crystals, some with unsymmetrical ends, some with an overgrowth of radiating smaller crystals. The two crystals to upper left are in twinned position.
548. Same, showing larger long prismatic crystals with attached overgrowth of smaller crystals.
549. Same, showing short type of prismatic crystal.
550. Same, showing twin on brachydome in upper left and immediately below it a parallel growth showing group extending in direction of macro-axis. Crystals on either side of middle individual have unsymmetrical development of brachydome, but this is arranged symmetrically with respect to middle individual, one right-handed and the other left-handed.
551, 552. Oxyhemoglobin of the Lynx (*Lynx canadensis* var.), showing thin tabular crystals, some in parallel growth orientation and some in divergent tufts.



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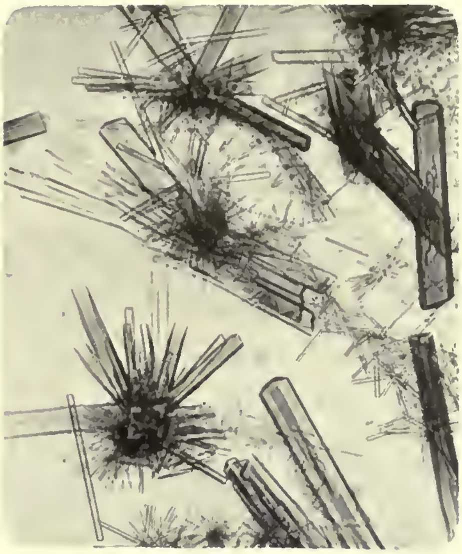

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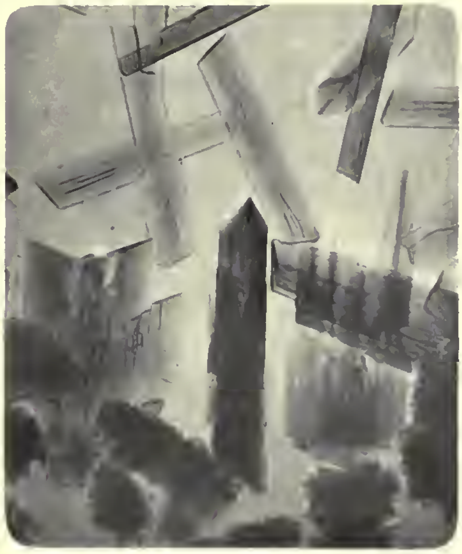

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547. Reduced Hemoglobin of the Wild Cat (*Lynx rufus*), showing long prismatic crystals, some with unsymmetrical ends, some with an overgrowth of radiating smaller crystals. The two crystals to upper left are in twinned position.
548. Same, showing larger long prismatic crystals with attached overgrowth of smaller crystals.
549. Same, showing short type of prismatic crystal.
550. Same, showing twin on brachydome in upper left and immediately below it a parallel growth showing group extending in direction of macro-axis. Crystals on either side of middle individual have unsymmetrical development of brachydome, but this is arranged symmetrically with respect to middle individual, one right-handed and the other left-handed.
551, 552. Oxyhemoglobin of the Lynx (*Lynx canadensis* var.), showing thin tabular crystals, some in parallel growth orientation and some in divergent tufts.

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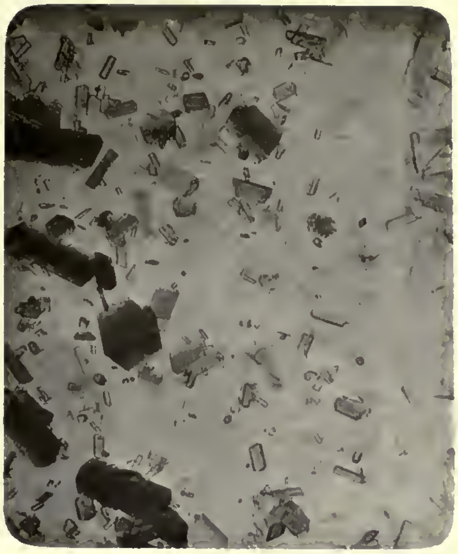
558

[illegible]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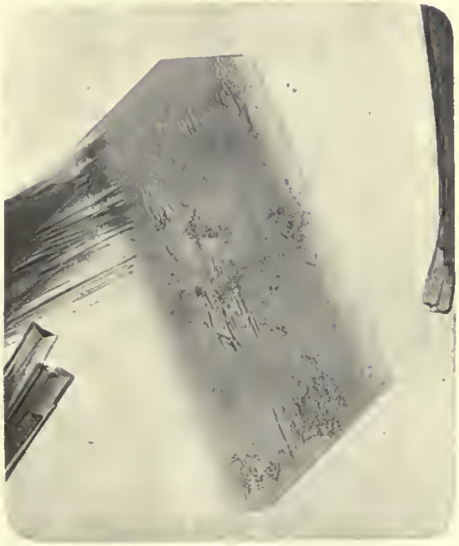
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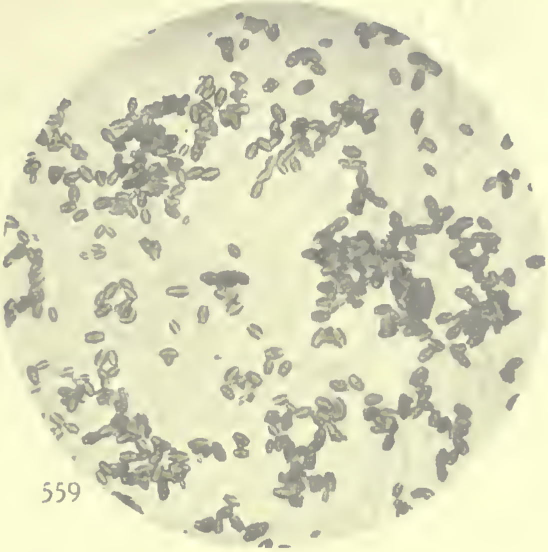
553. Reduced Hemoglobin of the Lynx (*Lynx canadensis* var.), showing type *a* crystals, consisting of brachyprism and macrodome; in some the prism is very short.
554. Same, showing type *a* crystals, some showing macropinacoid in addition to brachyprism and macrodome. Distorted crystal with unequally developed dome faces seen on left.
555. Same, showing type *b* crystal with long type *a* prisms growing out from it. Several smaller type *b* crystals are seen in this figure.
556. Same, showing parallel growth in groups of type *a* crystals.
557. Same, showing large type *b* crystal with a parallel growth group of type *a* crystals.
558. Same, showing large type *a* crystal.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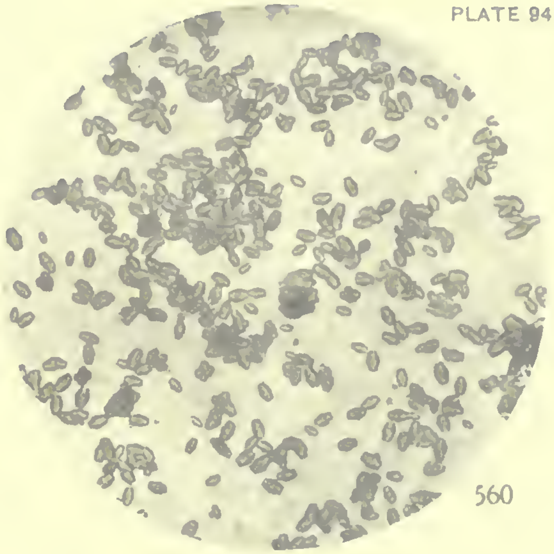
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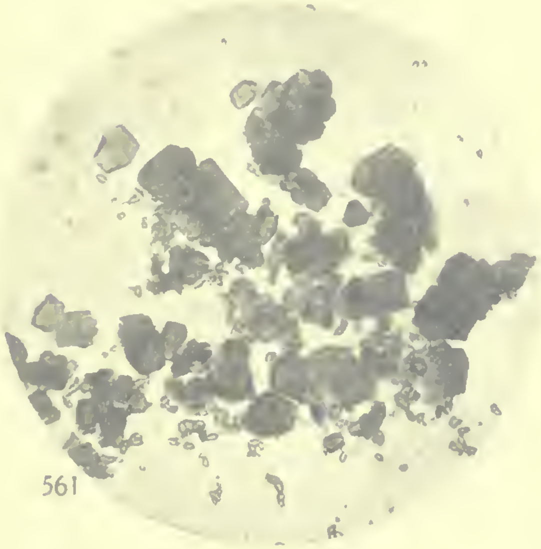
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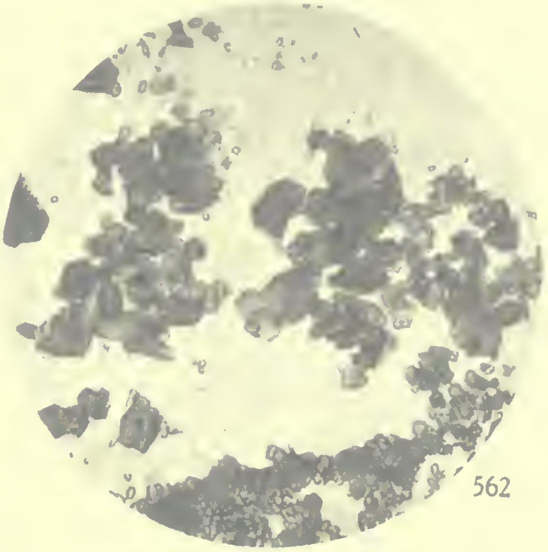
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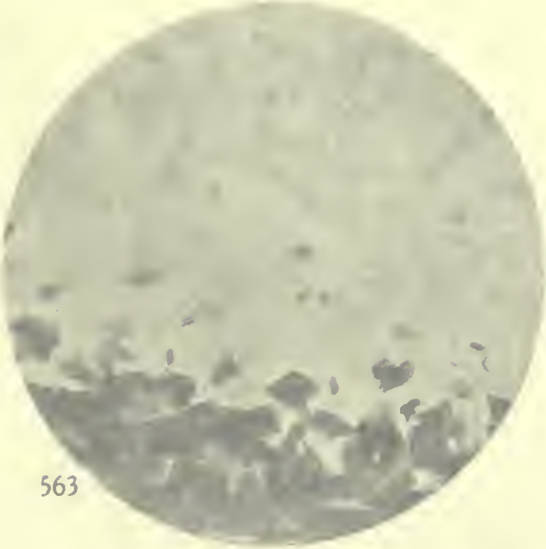
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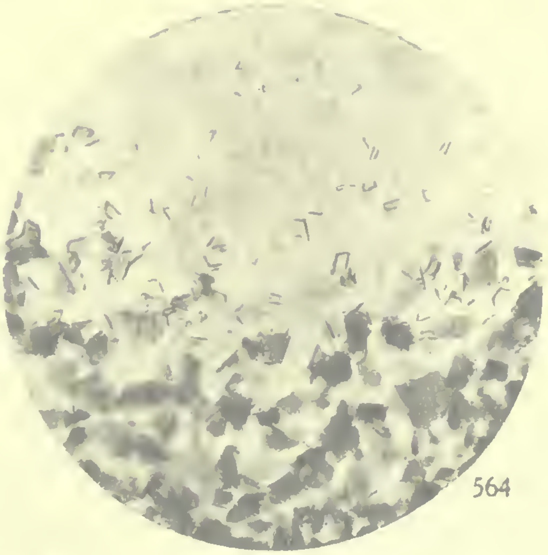
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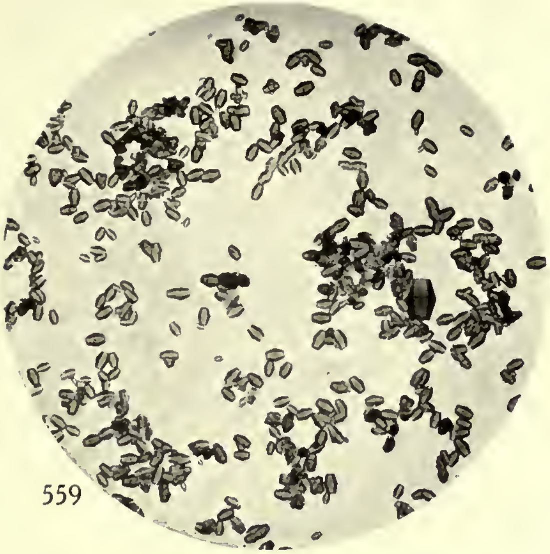
559, 560. Oxyhemoglobin of the Mole (*Scalops aquaticus*), showing small barrel-shaped crystals, some in twinned position.

561, 562. Same, showing large crystals along with crystals of first crop.

563, 564. Oxyhemoglobin of the Fox-bat (*Pteropus medius*), showing small tabular crystals.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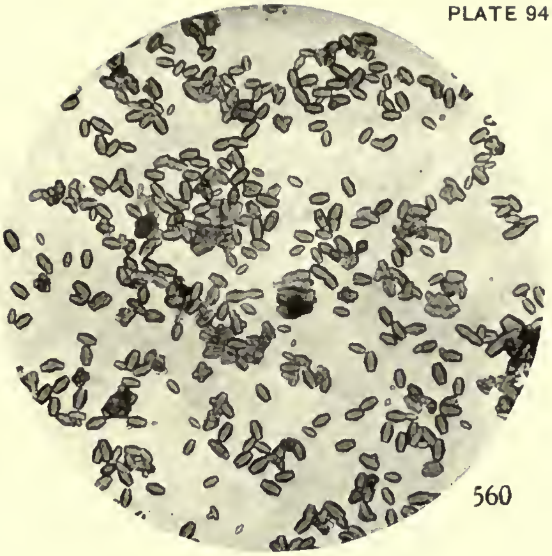
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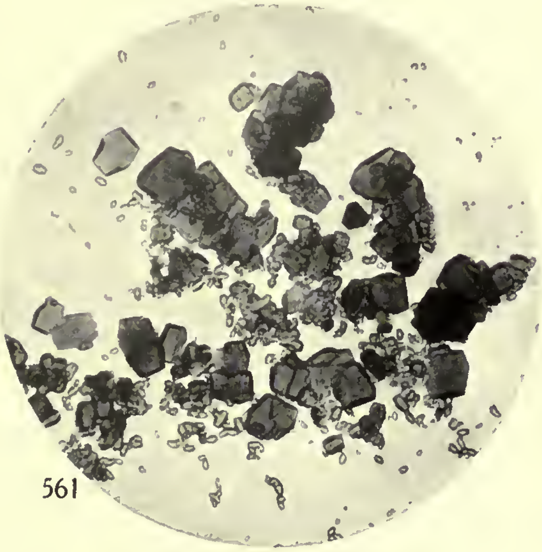
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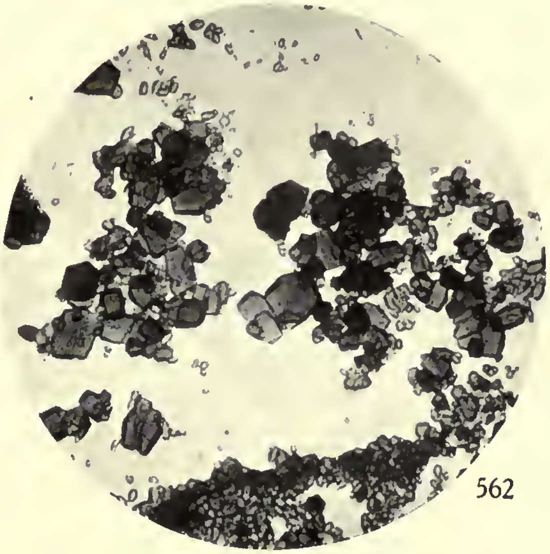
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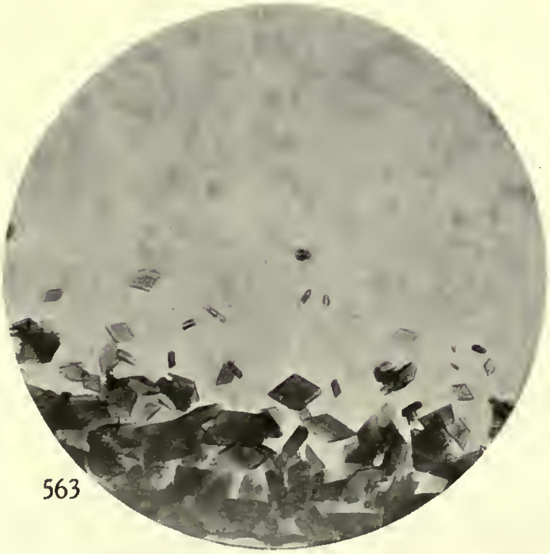
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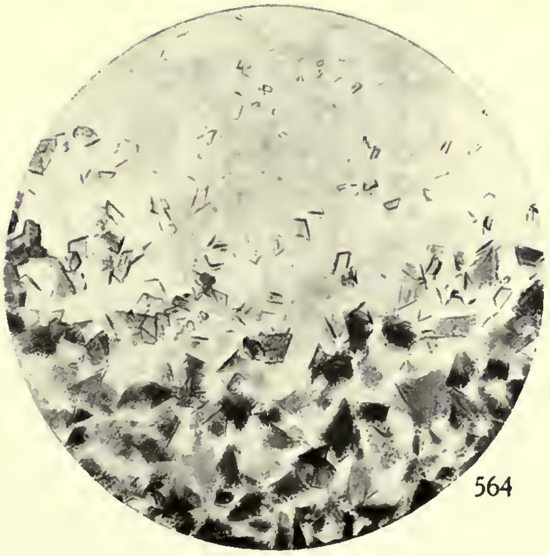
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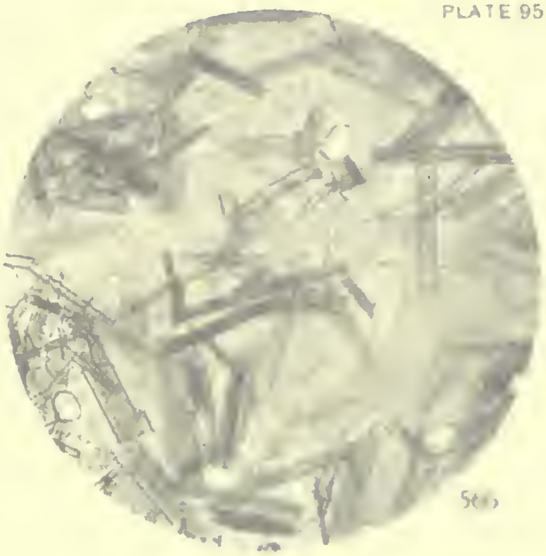
559, 560. Oxyhemoglobin of the Mole (*Scalops aquaticus*), showing small barrel-shaped crystals, some in twinned position.
561, 562. Same, showing large crystals along with crystals of first crop.
563, 564. Oxyhemoglobin of the Fox-bat (*Pteropus medius*), showing small tabular crystals.


565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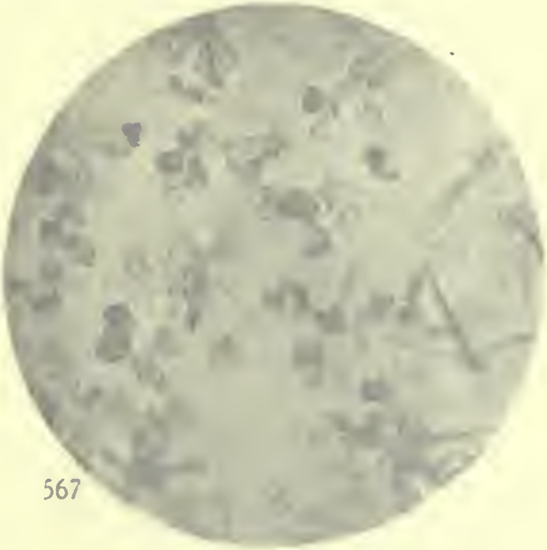
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565, 566. Oxyhemoglobin of the Brown Bat (*Vespertilio fuscus*), showing broad lath-shaped crystals, tabular on base.

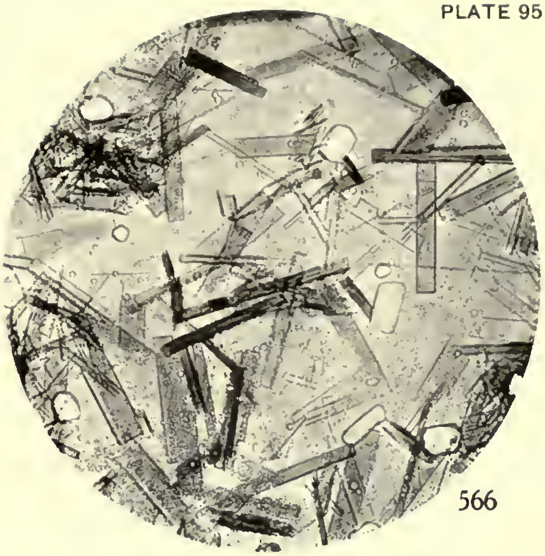
567. Oxyhemoglobin of the Ring-tailed Lemur (*Lemur catta*), showing the crystals to form imperfect prisms consisting of bundles of fibers crossing each other at definite angles and also small imperfect tabular crystals.

568. Same, showing hexagonal plate produced by twinning. Other crystals are seen growing on base of main plate, not all in exact orientation.

569. Same, showing mimetic hexagonal tabular crystals.

570. Same, showing rosette-shaped groups of crystals.

565



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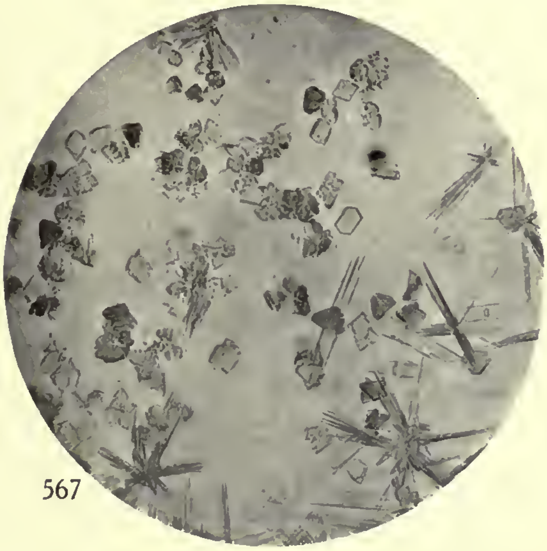

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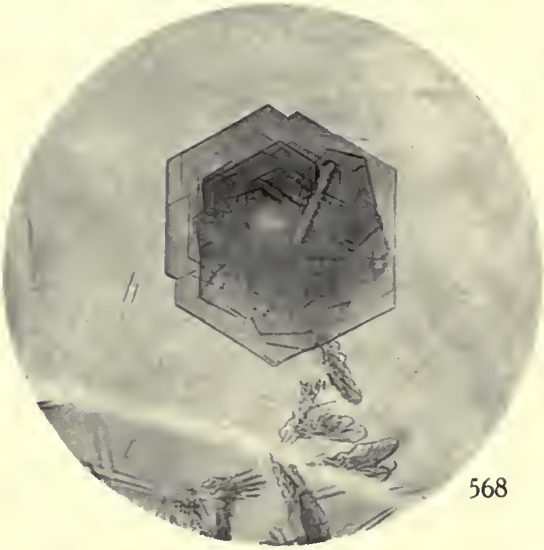

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570

565, 566. Oxyhemoglobin of the Brown Bat (*Vespertilio fuscus*), showing broad lath-shaped crystals, tabular on base.

567. Oxyhemoglobin of the Ring-tailed Lemur (*Lemur catta*), showing first crystals to form imperfect prisms consisting of bundles of fibers crossing each other at definite angles and also small imperfect tabular crystals.

568. Same, showing hexagonal plate produced by twinning. Other crystals are seen growing on base of main plate, not all in exact orientation.

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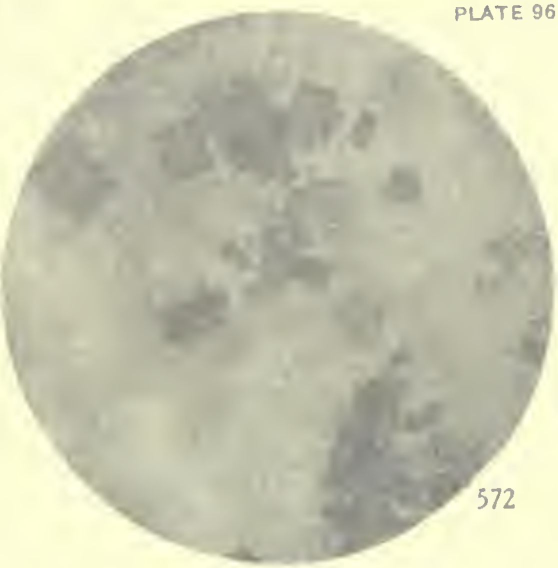
570. Same, showing rosette-shaped groups of crystals.


571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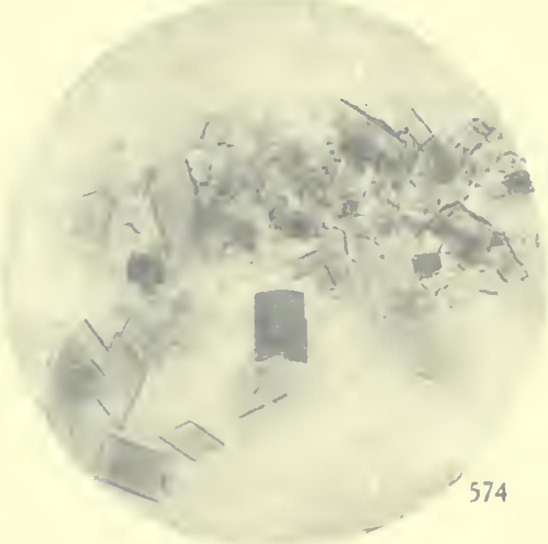
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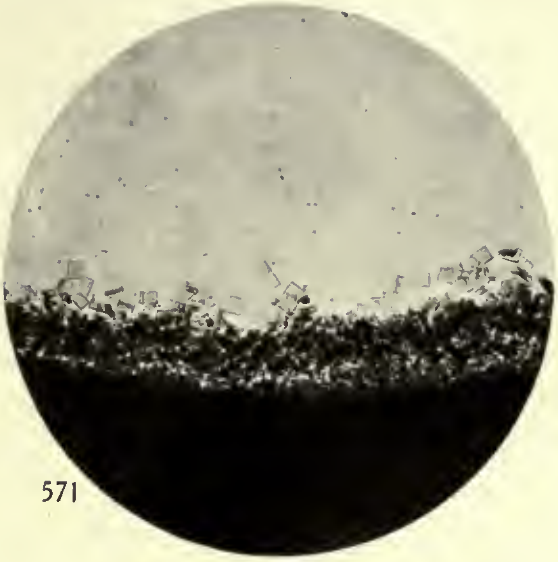
571. α-Oxyhemoglobin of the Yellow Baboon (*Papio babuin*), showing small tabular crystals along protein ring.
572. Same, showing thicker tabular, nearly cubical crystals.
573, 574. β-Oxyhemoglobin of the Yellow Baboon, showing tabular and prismatic types of crystals.
575, 576. Oxyhemoglobin of the Drill (*Papio leucophæus*), showing long rod-like crystals growing in divergent tufts and irregular aggregates.

PLATE 96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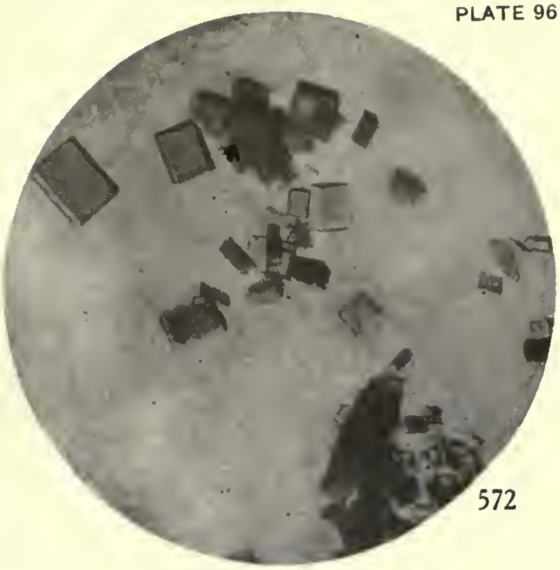
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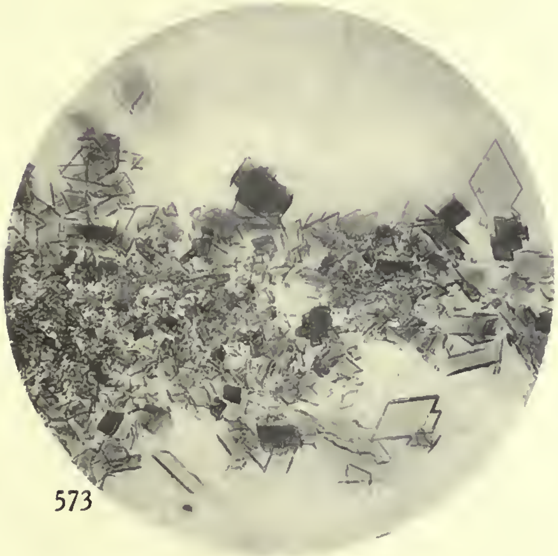
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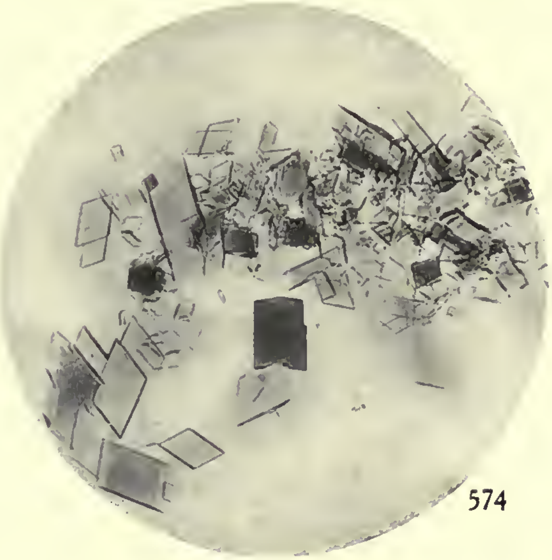
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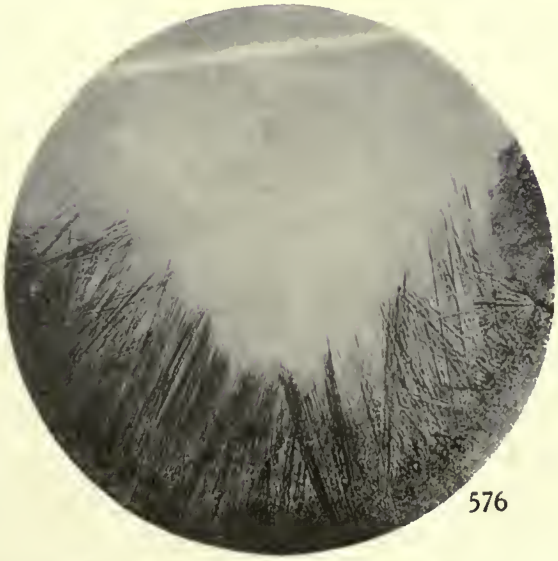
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571. α-Oxyhemoglobin of the Yellow Baboon (*Papio babuin*), showing small tabular crystals along protein ring.
572. Same, showing thicker tabular, nearly cubical crystals.
573, 574. β-Oxyhemoglobin of the Yellow Baboon, showing tabular and prismatic types of crystals.
575, 576. Oxyhemoglobin of the Drill (*Papio leucophæus*), showing long rod-like crystals growing in divergent tufts and irregular aggregates.

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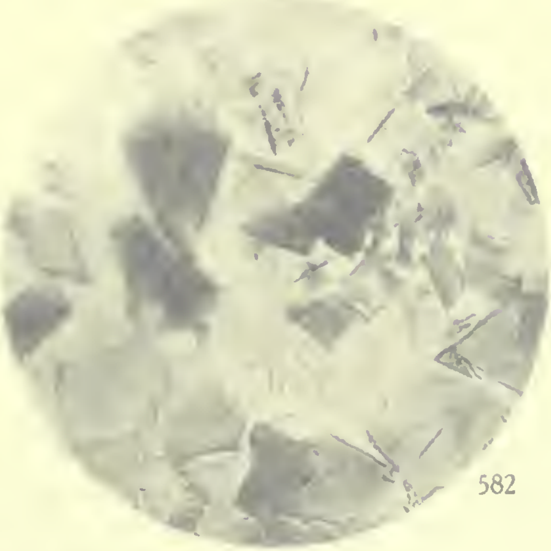


582

577, 578. [illegible] of the [illegible] (*Papio sphinx*), showing long diamond-shaped tabular crystals, [illegible] and piling up in approximately parallel growth.

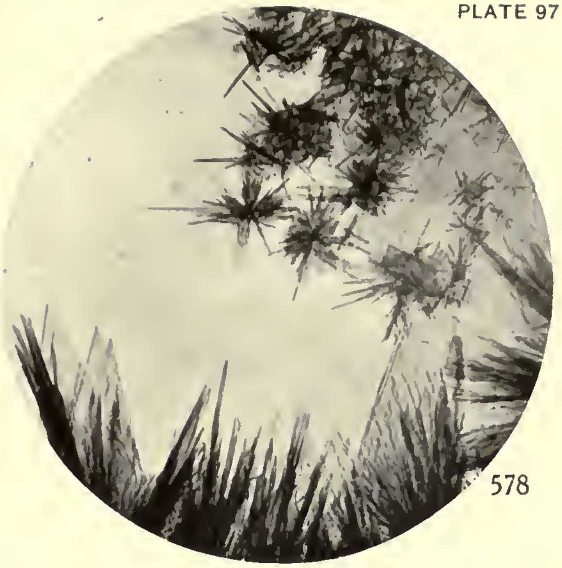
579. [illegible] of the Long-armed Baboon (*Papio langheldi*), showing [illegible] crystals of α-Oxyhemoglobin and [illegible] tabular crystals of β-Oxyhemoglobin.

580. [illegible] of the Long-armed Baboon, showing [illegible] crystals.

581. [illegible] twin on brachydome.

582. [illegible] of the Long-armed Baboon, showing [illegible] tabular and prismatic crystals. [illegible] depth of shading is due to [illegible]


577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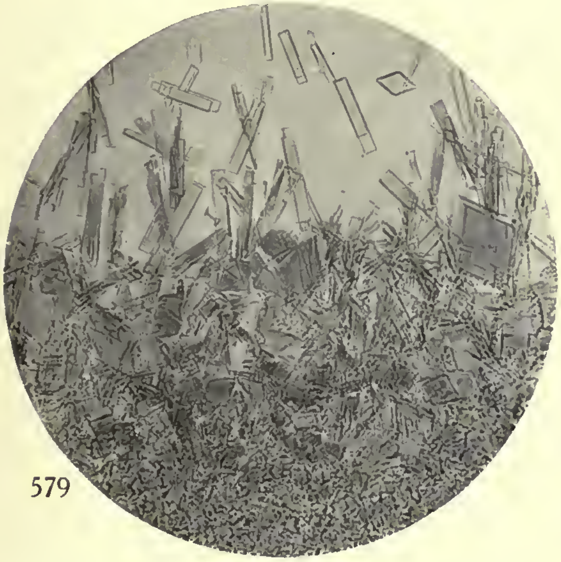
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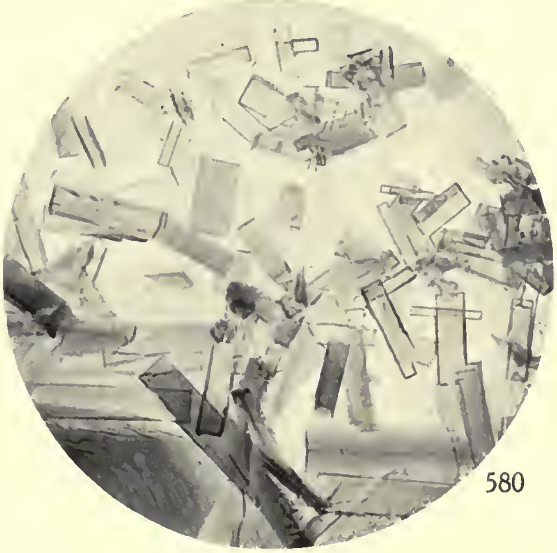
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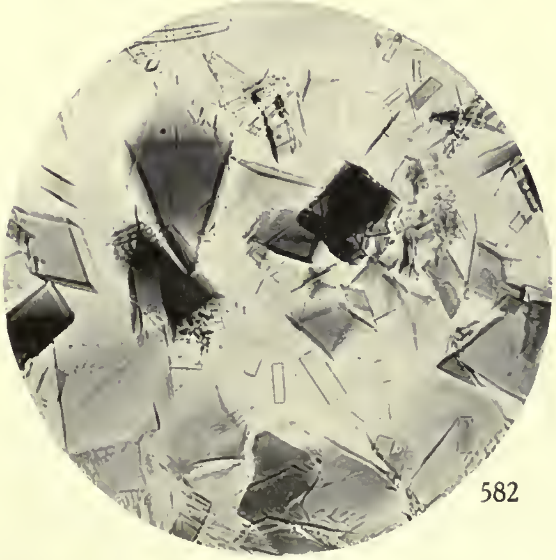
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577, 578. γ-Oxyhemoglobin of the Guinea Baboon (*Papio sphinx*), showing long diamond-shaped tabular crystals, growing in divergent tufts and piling up in approximately parallel growth.

579. α-Oxyhemoglobin and β-Oxyhemoglobin of the Long-armed Baboon (*Papio langheldi*), showing short lath-shaped crystals of α-Oxyhemoglobin and rhombic tabular crystals of β-Oxyhemoglobin.

580. α-Oxyhemoglobin of the Long-armed Baboon, showing thicker tabular crystals.

581. Same, showing twin on brachydome.

582. β-Oxyhemoglobin of the Long-armed Baboon, showing thick tabular and prismatic crystals. Different depth of shading is due to pleochroism.


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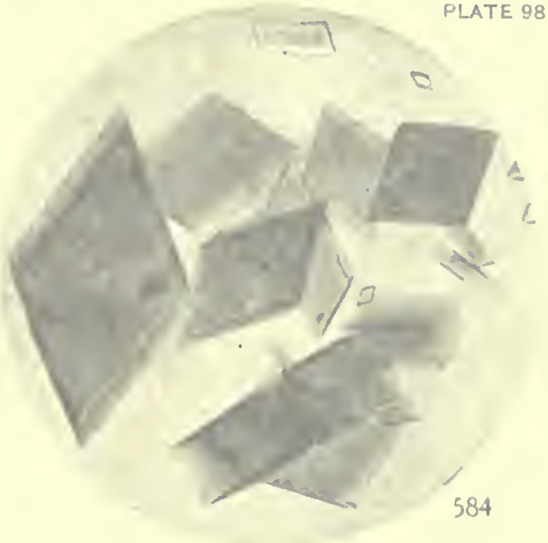
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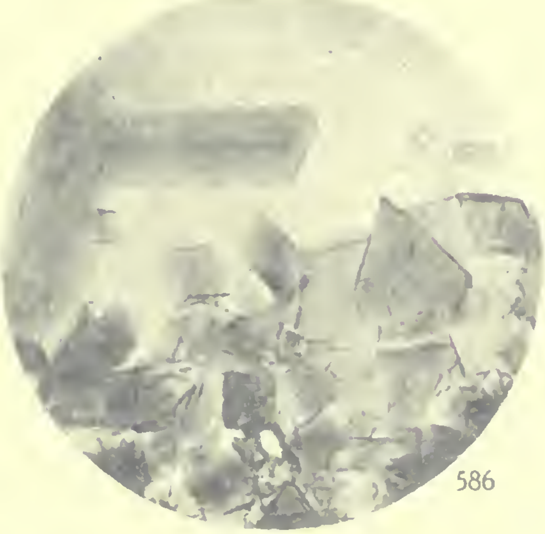
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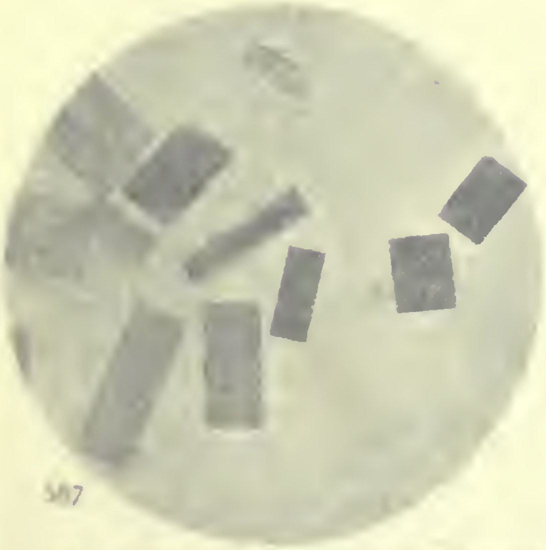
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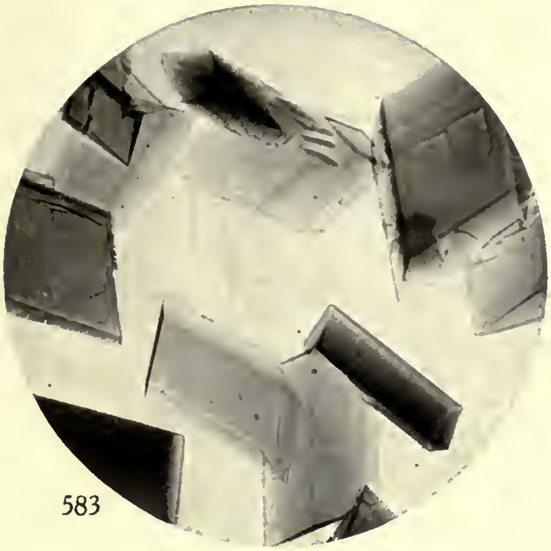
583. β-Oxyhemoglobin of the Long-armed Baboon (*Papio [illegible]*), showing tabular and prismatic types of crystals.
584. Same, showing tabular and prismatic types of crystals. Horse-type twin on edge is seen in lower part of field.
585. Same, showing horse-type twin on right of field.
586. Same, showing long and short prismatic crystals.
587. α-Oxyhemoglobin of the Chacma (*Papio porcarius*), showing tabular crystals. Differences in tint is due to pleochroism.
588. Same, showing partial parallel growth and fan-shaped appearance of groups when seen on edge.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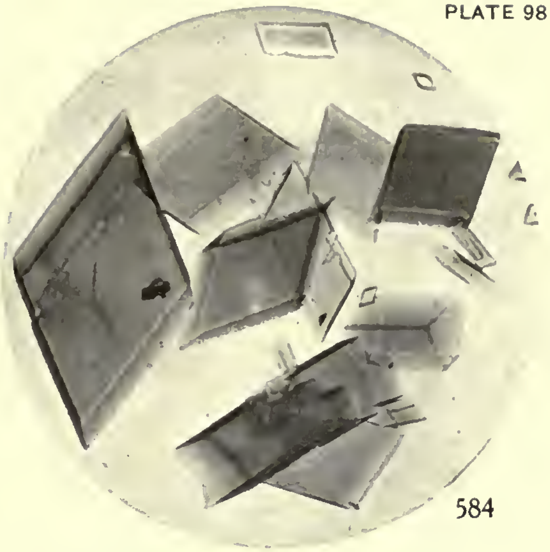
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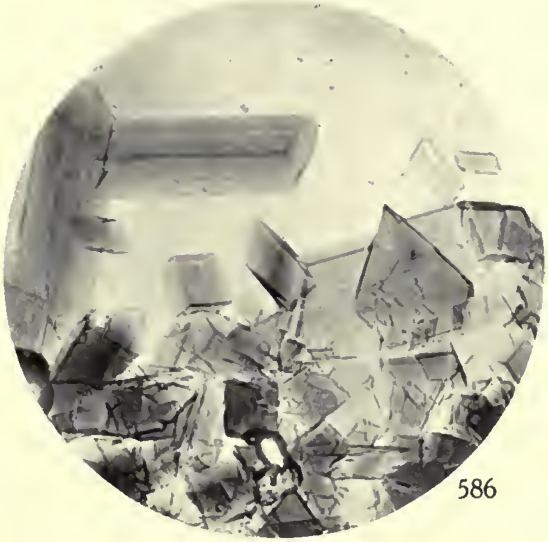
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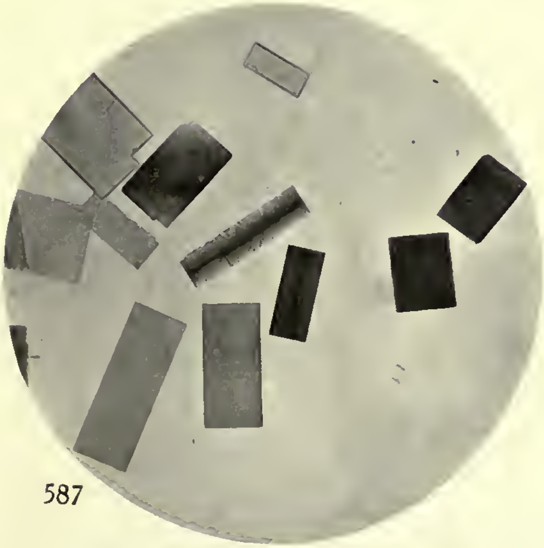
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583. β-Oxyhemoglobin of the Long-armed Baboon (*Papio langheldi*), showing tabular and prismatic types of crystals.
584. Same, showing tabular and prismatic types of crystals. Horse-type twin on edge is seen near lower part of field.
585. Same, showing horse-type twin on right of field.
586. Same, showing long and short prismatic crystals.
587. α-Oxyhemoglobin of the Chacma (*Papio porcarius*), showing tabular crystals. Difference in tint is due to pleochroism.
588. Same, showing partial parallel growth and fan-shaped appearance of group when seen on edge.

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[illegible]


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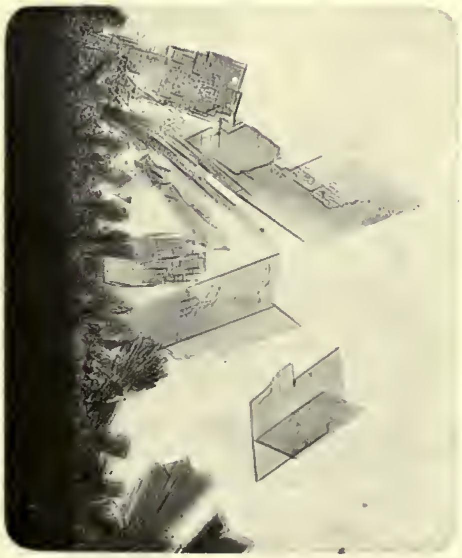
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589. α-Oxyhemoglobin of the Chacma (*Papio porcarius*), showing thick tabular crystals in various orientations and illustrating pleochroism as crystals are viewed in different positions.
590. Same, showing parallel growth and fan-shaped aggregates.
591. β-Oxyhemoglobin of the Chacma, showing side view of horse-type twin.
592. Same, showing parallel growth in direction of ortho-axis.
593. α-Oxyhemoglobin and β-Oxyhemoglobin of the Chacma, showing horse-type twins of β-Oxyhemoglobin.
594. α-Oxyhemoglobin and β-Oxyhemoglobin of the Chacma, showing parallel growth and horse-type twinning.


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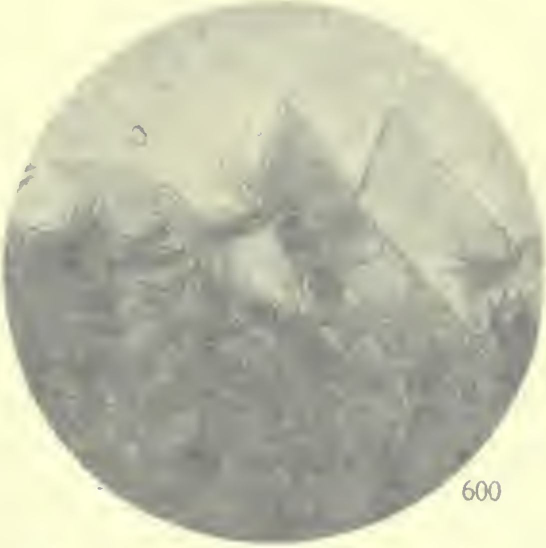
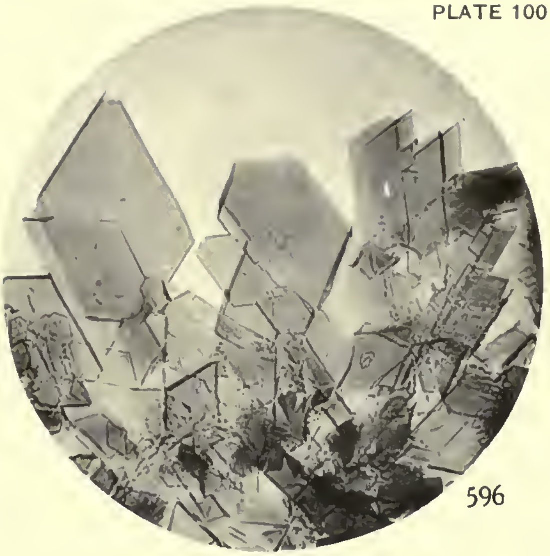

600

595. α-Oxyhemoglobin of the Anubis Baboon (*Papio anubis*), showing tabular crystals with unequal development of orthopinacoid faces.
596. Same, showing tabular crystals in horse-type twins, on edge and on flat.
597. Same, showing single tabular crystals.
598. Same, showing prismatic type of crystal.
599. Same, showing large crystals with tufted groups of thin crystals of β-Oxyhemoglobin.
600. α-Oxyhemoglobin and β-Oxyhemoglobin of the Anubis.


595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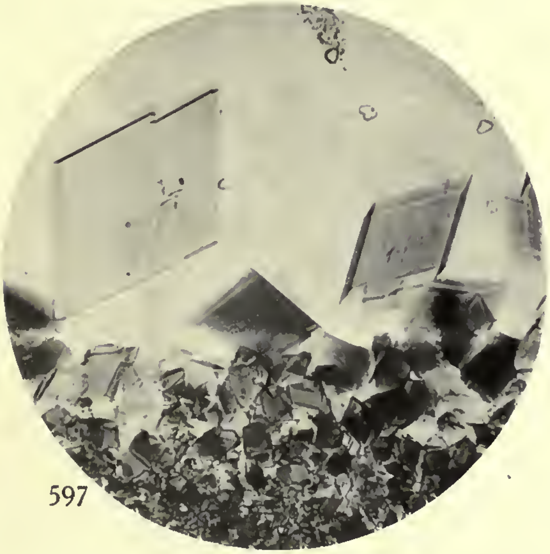
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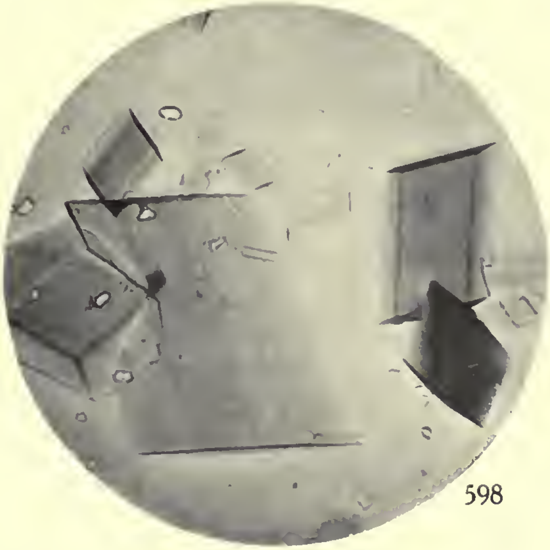
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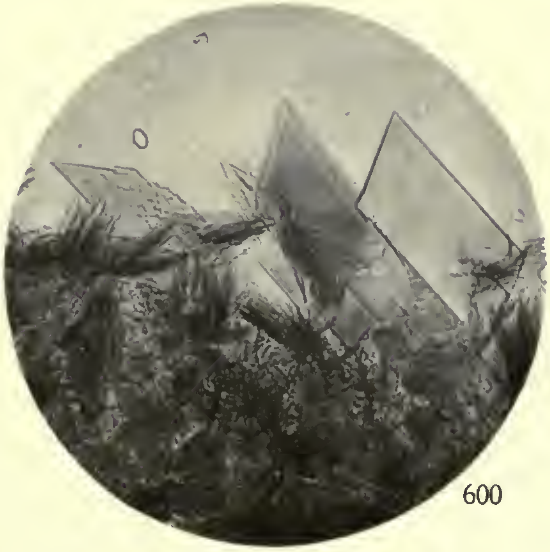
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595. $\beta$-Oxyhemoglobin of the Anubis Baboon (*Papio anubis*), showing tabular crystals with unequal development of orthopinacoid faces.
596. Same, showing tabular crystals in horse-type twins, on edge and on flat.
597. Same, showing single tabular crystals.
598. Same, showing prismatic type of crystal.
599. Same, showing large crystals with tufted groups of thin crystals of $\gamma$-Oxyhemoglobin.
600. $\beta$-Oxyhemoglobin and $\gamma$-Oxyhemoglobin of the Anubis.