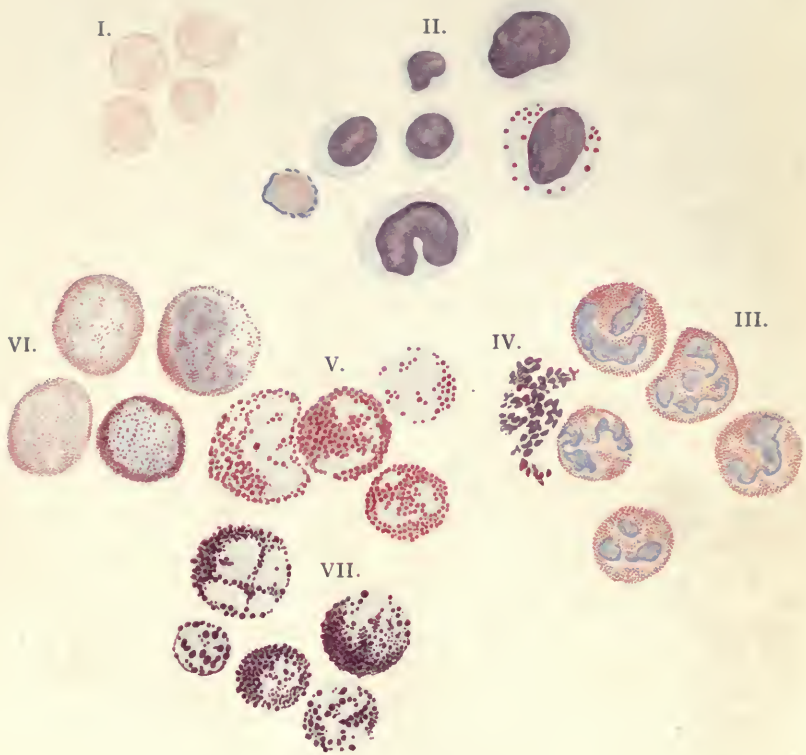
# CLINICAL HEMATOLOGY.

DaCOSTA.




THE LEUCOCYTES.
(WRIGHT'S STAIN.)

I. Normal erythrocytes.
II. Large and small lymphocytes and transitional forms.
III. Polynuclear neutrophiles.
IV. Blood plaques.
V. Eosinophiles
VI. Myelocytes.
VII. Mast cells.

(*E. F. Faber, fec.*)

# CLINICAL HEMATOLOGY

## A PRACTICAL GUIDE TO THE EXAMINATION OF THE BLOOD WITH REFERENCE TO DIAGNOSIS

BY

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**Second Edition, Revised and Enlarged**

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TO

MY FATHER,

**John C. DaCosta, M.D.,**

THESE PAGES ARE

AFFECTIONATELY DEDICATED.

# PREFACE TO THE SECOND EDITION.

Three noteworthy lines of advance have developed from recent work in hematology: the identification of new clinical entities, the correlation of blood pictures with a number of diseases hitherto unstudied or imperfectly investigated hematologically, and the proof of the septic nature of many of the specific infections. The first has established upon a clinical basis trypanosomiasis, kala-azar, spotted (Montana) fever, and cyanotic polycythemia; the second has shown in detail the blood changes incident to chloroma, hydatid infection, multiple periostitis, pancreatitis, variola, arthritis deformans, sprue, leukanemia, burns, and *x*-ray therapy; and the last has proved the bacteriemic character of enteric fever, Malta fever, pneumonia, scarlet fever, and, possibly, rheumatic fever. Progress of great practical value has been made in the technic of blood examinations, notably in staining methods, in serum diagnosis, and in blood culturing. These advances, all made during the past two years, have been incorporated in this edition, together with much other new material, gleaned by the consultation of more than nine hundred late references to hematological literature.

The original data of the book are based upon the records of about ten thousand blood examinations, made chiefly at the German, the Jefferson, and the Philadelphia General Hospitals. This new matter relates more especially to the primary anemias, malignant disease, cholelithiasis, icterus, pancreatitis, gastric ulcer, pneumonia, septicemia, and suppurative lesions. For the sake of clearness, tabulations have been avoided as far as possible, and the data analyzed and presented as summaries. Among the improvements in technic described are Wright's stain, Milian's method of estimating the coagulation time, Reudiger's serum test, medico-legal tests for blood, and cryoscopy. A brief account is given of Ehrlich's side-chain theory and its relation to immunity and to hemolysis. The detailed revision of the text has been supplemented by the addition of a new colored plate and numerous other illustrations.

In this revision the plan of the first edition has been adhered to—the interpretation of the blood report as a rational aid to

diagnosis. The author has profited by the views of his critics whenever they could be consistently adopted, and he begs to acknowledge his appreciation of the many suggestions from this source.

1022 Spruce Street, Philadelphia,
*November* 1, 1904.

# PREFACE TO THE FIRST EDITION.

This book, designed as a practical guide to the examination of the blood by methods adapted to routine clinical work, represents an endeavor to recount the salient facts of hematology as they are understood at the present time, to correlate certain of these facts with familiar pictures of disease, and to apply them to medical and surgical diagnosis. The purpose has been to interpret the blood report according to its true value as a clinical sign, neither exploiting it as a panacea for every diagnostic ill, nor belittling it because of its failure consistently to give the sought-for clue in every instance.

A minimum amount of theoretical discussion has been introduced in the sections dealing with the physiology and pathology of the whole blood and of the cellular elements—only sufficient, in the author's judgment, to add clearness to the number of the mooted points of this science, which in its present transitional stage must still be regarded as one from which more or less hypothesis and conjecture are inseparable. Intimate familiarity with technic being an essential qualification for the comprehensive study of the blood, a somewhat lengthy consideration of this subject is given. The methods of examination likely to prove useful in everyday practice have been described in detail, perhaps somewhat at the risk of prolixity, in the hope of thus simplifying for the novice the minutiæ of blood counting, staining, and other means of investigation. In the discussion of the primary anemias and of the anemias peculiar to infancy, prominent clinical features other than those referable to the blood have been briefly mentioned, in order to add clearness to the differential diagnosis. For convenience in reference, the various diseases included in the section on general hematology are arranged alphabetically, rather than grouped according to a traditional classification.

The greater part of the original data referred to in the text is taken from the records of the Pathological Institute of the German Hospital, where a systematic account of all blood examinations has been kept for the past six years. The remaining data represent the writer's personal examinations in hospital and private practice and in the Army Medical Service, these sources of statistics together including about four thousand blood reports in various pathological conditions.

Hematological literature has been freely consulted in the preparation of this volume, special acknowledgment being due to Hayem, Ehrlich and Lazarus, von Limbeck, Rieder, Löwit, Türk, Grawitz, Cabot, Stengel, Thayer, Ewing, Taylor, and Coles for the profitable information gleaned from their writings. Due credit in the text has been given to these as well as to the other authors of whose labors use has been made.

The colored plates and other histological illustrations, the originals of which were made by Mr. E. F. Faber from fresh and stained specimens, bear evidence of the artist's technical skill and faithful attention to structural detail. Mr. S. Trenner has kindly furnished the engravings of several of the special instruments.

The author takes pleasure in acknowledging the assistance of his wife and critic in revising the proofs of these pages; in crediting Dr. G. P. Müller for collecting and verifying much statistical matter relating to hospital cases; and in thanking Dr. J. Chalmers Da Costa and Dr. T. G. Ashton for helpful suggestions.

313 South Thirteenth Street, Philadelphia,
*November* 1901

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# INTRODUCTION.

The rapid growth and development of hematology during recent years and the practical application of many of its teachings to the diagnosis of various diseases have made this science one which no progressive medical man can afford to disregard. Examination of the blood gives definite clinical information which may be profitable both to the practitioner of internal medicine and to the surgeon, and the procedure is capable of throwing light upon the diagnosis in so wide a range of pathological conditions that it is difficult to single out any disease in which it may not be of some utility, either as positive or as negative evidence.

In the light of our present knowledge of the subject, clinical information of two kinds may be derived from hematology, namely, findings which are pathognomonic of certain diseases; and auxiliary data which, if considered in connection with other clinical manifestations, may prove either essential or helpful in establishing the precise nature of a disease.

The list of diseases in which pathognomonic blood findings are met with includes leukemia, the malarial fevers, relapsing fever, filariasis, trypanosomiasis, and piroplasmiasis. In pernicious anemia a typical picture is also found, if two conditions capable of exciting identical blood changes are excepted, the profound secondary anemias due to certain intestinal parasites and to nitrobenzene poisoning.

The blood examination affords data which, although not pathognomonic, are nevertheless essential for the diagnosis of chlorosis, Hodgkin's disease, splenic anemia, chloroma, Osler's disease, multiple periostitis, kala-azar, and secondary anemias dependent upon various causes. For example, in chlorosis a definite group of blood changes must exist in order to justify an unconditional diagnosis, although the occurrence of these changes, unassociated with other equally definite clinical signs, is insufficient evidence of this disease. In Hodgkin's disease, a condition indistinguishable from leukemia by an ordinary physical examination, the absence of a leukemic state of the blood at once excludes the latter disease. In the secondary anemias, it is obvious that the blood count alone can give the

exact clue to the condition, by determining the degree and character of the blood impoverishment, and by tracing from time to time its progress. In this connection it is important to remember that pallor may go hand in hand with a normal hemoglobin percentage and erythrocyte count, and that, on the other hand, a high color by no means invariably signifies that the individual is not anemic. In addition to the diseases just named, hematology gives information which is often of great assistance in, although not essential for, the diagnosis of such conditions as enteric fever, sepsis, pneumonia, pertussis, appendicitis, diabetes, rabies, syphilis, gastric ulcer, malignant disease, helminthiasis, the exanthemata, the hemorrhagic diseases, and suppurative processes. Clinical experience has repeatedly illustrated the value of the serum reaction in enteric and Malta fevers and in dysentery; of Williamson's test in diabetes mellitus; of eosinophilia in trichiniasis, in echinococcus disease, and in other forms of helminthiasis; of mononucleosis in variola and in pertussis; and of leucocytosis and iodophilia in sepsis, in suppurative lesions, and in many of the acute infections. The forensic use of Bordet's test for the identification of blood stains, and the application of cryoscopy to the diagnosis of renal disease are also of practical utility.

Negative results from a blood examination also possess diagnostic value within certain limits, but too great reliance upon evidence of this sort more often proves delusive than helpful. In a patient whose waxy, yellowish facies suggests with equal force pernicious anemia, chronic nephritis, and, perhaps, liver cirrhosis, the absence of characteristic blood changes is sufficient to exclude the first-named condition. But failure to detect the malarial parasite does not necessarily exclude malarial fever; a negative serum test does not absolutely rule out enteric fever; and an absence of leucocytosis cannot be regarded as an infallible sign that a suppurative focus does not exist, nor does it always indicate the benignity of a neoplasm. Negative evidence, then, is usually to be considered merely suggestive, the real pertinence of the hint thus obtained depending upon its correlation with other physical signs and symptoms.

The significance of positive findings in bacteriological investigations of the blood is patent, and recent improvements in technic have made this means of research simple, dependable, and certain. Blood cultures furnish conclusive information in general septicemia, pneumonia, enteric fever, Malta fever, plague, scarlet fever, malignant endocarditis, and similar conditions in which bacteria invade the blood stream.

At the present time the most useful information furnished by

hematology has been derived from study of the cellular elements of the blood, but closer familiarity with the chemistry of this tissue, still an undeveloped science, will undoubtedly in the near future afford not only more tangible clues to the etiology and pathology of the blood diseases, but also will bring to light additional facts which may be applied to the diagnosis of these and other maladies. The study of the coagulation time of the blood is of practical utility in the study of purpura, hemophilia, jaundice, and other conditions characterized by slow clotting and by a tendency toward hemorrhage.

The technic of blood examinations, such as described in the following pages, is neither elaborate nor difficult to master. Necessarily, it must be rigidly exact, but no more so than any other branch of physical diagnosis, if the worker is content only with the best results. To acquire a good working knowledge of hematology takes but a fraction of the time and application that one must spend in familiarizing one's self with the most common heart murmurs or chest signs, and the time thus spent equips the physician with an additional diagnostic agent of the greatest value. If the newly graduated physician would provide himself with a microscope and a set of blood instruments, and systematically study the blood in the various general diseases which he encounters in practice, many a slip-shod diagnosis might be avoided, and a great stride forward made in popularizing this practical branch of clinical diagnosis.

*"L'avenir appartient à l'hématologie. C'est elle qui nous apportera la solution des grands problèmes nosologiques. Elle doit nous apparaître comme une vaste science puisant ses matériaux dans toutes les branches des connaissances biologiques et recueillant les diverses notions de l'humorisme ancien pour les rajeunir et les compléter à la lumière des découvertes modernes en anatomie, en physiologie, en chimie biologique et en pathologie."*

GEORGES HAYEM.

SECTION I.

# EXAMINATION OF THE BLOOD BY CLINICAL METHODS.

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General Schema. A systematic examination of the blood by clinical methods of established utility includes the following different processes:

I. Microscopical examination of the fresh blood.
II. Estimation of the percentage of hemoglobin.
III. Counting the erythrocytes and the leucocytes.
IV. Microscopical examination of the stained specimen.

These four procedures, which should invariably be included in every clinical blood report, furnish the most important information to be derived from hematological study, and are sufficient for routine clinical work. In certain instances in which more detailed investigation of special points is sought, it may be thought advisable to supplement the above plan by employing one or more of these remaining procedures:

V. Counting the blood plaques.
VI. Estimation of the relative volumes of corpuscles and plasma.
VII. Estimation of the specific gravity.
VIII. Estimation of the alkalinity.
IX. Determination of the rapidity of coagulation.
X. Cryoscopical examination.
XI. Estimation of the resistance of the erythrocytes.
XII. Spectroscopical examination.
XIII. Bacteriological examination.
XIV. Determination of the serum reaction.
XV. Medico-legal tests for blood.

### I. MICROSCOPICAL EXAMINATION OF THE FRESH BLOOD.

Obtaining the Specimen. The finger-tip or the lobe of the ear is the part usually selected from which to obtain the blood, by puncture, for examination. The former site is preferable in most instances, owing to its con-

venient situation and ease of manipulation; but in nervous individuals and in children the ear-lobe may be chosen, because of its limited sensibility and on account of the patient's inability to watch the operation.

The puncture may be made with one of the special blood lancets devised for this purpose, or, in lieu of such an instrument, a Hagedorn or spear-pointed surgical needle or a new sharp-pointed steel pen from which one nib has been twisted off, will answer the purpose equally as well. The author is accustomed to use a small steel trocar blade, mounted on a metal shaft which screws into an outer barrel by means of a thread. By the use of a threaded locking-nut, any desired length of the trocar may be exposed, so that the depth of the wound may be controlled at will, irrespective of the force used to drive the point of the instrument through the skin. It is not necessary to sterilize the puncture-needle: wiping it with a towel wet with alcohol is all that is required in ordinary examinations. Of course, should the patient happen to be syphilitic or septic, it is safer to pass the blade through an alcohol flame after having used it.

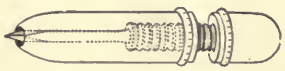

FIG. 1.—BLOOD LANCET.

Having chosen, say, the patient's middle or ring-finger, the part is first thoroughly cleansed with alcohol or ether and then with water, and wiped perfectly dry with a clean, lint-free towel, which may then be folded into a pad and slipped behind the finger to isolate it from the neighboring digits, and to serve as a cushion for the back of the hand. The operator, holding the patient's hand in a firm, steady position, makes the puncture with a rapid motion of the wrist, such as one is accustomed to use in percussing the thorax, the depth of the wound being just sufficient to cause a free flow of blood in good-sized drops, unaided by the slightest pressure on the finger other than that necessary to start the initial oozing. The needle should be aimed so as to strike a point in the center of the flexor surface of the finger, just back of the extreme tip. The blood drop to be used for the examination should under no circumstance be squeezed from the finger, for blood secured in this manner is certain to be more or less highly diluted with lymph from the surrounding tissues—a condition which will lead to erroneous results, especially to lower hemoglobin, specific gravity, and corpuscular estimations than actually exist. In severe anemias, especially in those of the pernicious type, the bloodless condition of the superficial vessels is sometimes so marked that it may be impossible to obtain

enough blood for the examination by an ordinary puncture, even from the ear-lobe, which, as a rule, is highly vascular. Relatively deep incisions are unavoidable in such instances. On the contrary, in most cases of leukemia, unless the coëxisting anemia is of striking intensity, the blood usually flows very freely, and may even spurt from the wound in a fine jet several inches in height.

Most writers on hematology utter an emphatic warning against hemophilics, in whom the slightest prick of a needle may cause troublesome bleeding. The writer has never had the misfortune to meet with this accident, but recognizes the wisdom of observing the precaution to question every patient concerning an abnormal tendency toward hemorrhage.

The observer's attention should be directed to the color and the density of the blood drop as it flows from the puncture, and a note taken of the various macroscopical changes which may occur, such as the pale, hydremic condition of the blood found in severe anemias, the deep blue color in cyanosis, and the milky appearance in leukemia and in diabetes. These and other alterations in the naked-eye appearance of the fresh blood have been discussed in another section.

PREPARING THE SLIDE.

The first few drops of blood which follow the puncture are wiped away, and the site of the incision freed from every trace of moisture, after which a perfectly clean cover-glass, held edgewise between the thumb and forefinger, is lightly touched to the *summit* of the next drop as it oozes from the puncture, and is then immediately placed, blood side downward, upon the surface of a clean glass slide. If the cover-glass and the slide are perfectly clean and dry, and if the drop is of the proper size, the blood will at once spread out in a thin film consisting of a single layer of corpuscles (Fig. 2), surrounded by an outer zone in which the cells are heaped up in masses and rouleaux; this thicker area of the specimen is unsuited for examination (Fig. 3). Gently heating the slide over an alcohol flame just before use will insure a thin, even spread. If prolonged study of the specimen is intended, it is advisable to exclude air from the film, by ringing the margins of the cover-glass with a thin layer of cedar oil or vaselin, but ordinarily this precaution is unnecessary. In order to prevent distortion of the corpuscles, pressure must be avoided while adjusting the cover-glass. If the blood does not spread of itself, without the aid of pressure, it is usually owing to the presence of particles of dust or grease between the opposed surfaces of the slide and cover-glass.

Absolute cleanliness of the covers and slides is an essential detail to which too great attention cannot be paid, for neglect of this precaution is responsible for the majority of failures to secure good specimens. Perhaps the most useful cleansing agent is the

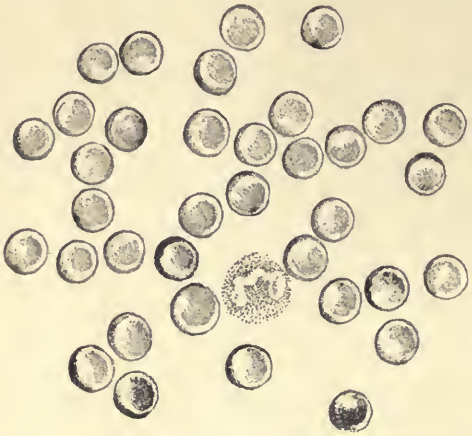

FIG. 2.—PROPER DISTRIBUTION OF THE CORPUSCLES IN A FRESH BLOOD FILM PREPARED FOR MICROSCOPICAL EXAMINATION.

solution popularly known as "acid alcohol" (hydrochloric acid, 1 part; absolute alcohol, 29 parts; water, 70 parts), which quickly and effectually removes all traces of grease and dirt from the glasses, so that their preliminary soaking in soap-suds or in a strong mineral acid, as some recommend, may be dispensed with. The slides and covers may be conveniently kept in closed glass receptacles containing this solution, from which they are removed as the occasion demands, being then dried and polished with a bit of clean linen or with tissue-paper. Ordinary soft "toilet-paper" is excellent for this purpose. Oblong cover-glasses, measuring $\frac{3}{4} \times 1\frac{1}{4}$ inches and of "No. 1" thickness, are more easily handled without forceps than smaller square or circular slips, and also have a much larger surface than the latter, which is often decidedly advantageous.

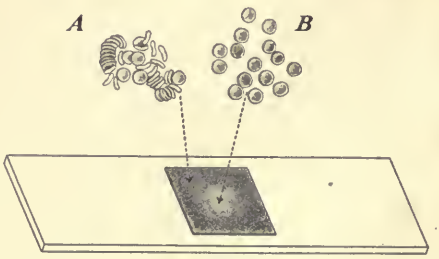


FIG. 3.—ZONES OF ROULEAUX (*A*) AND OF ISOLATED CELLS (*B*) IN A FRESH BLOOD FILM.

The use of forceps is unnecessary if care is observed to hold the cover-glass in the manner already directed, so that only its edges come in contact with the thumb and finger.

MICROSCOPICAL EXAMINATION.

The specimen, prepared in the manner just described, is examined under the microscope with both low and high powers, a $\frac{1}{5}$ or $\frac{1}{6}$ inch dry, and a $\frac{1}{12}$ inch oil-immersion, objective being the most satisfactory lenses for the purpose. The substage condenser and diaphragm should be adjusted so that the field is but moderately illuminated, rather than flooded with a glare of white light. Microscopical examination of the fresh blood film furnishes information about the following points:

*Changes Affecting the Erythrocytes.*—With a little practice one soon becomes able to detect, with a tolerable degree of accuracy, any conspicuous decrease in the number of erythrocytes, by the relatively small number of cells in the field in comparison with their number in a similar field of normal blood. With less confidence it is also possible to decide whether or not the number of erythrocytes is much in excess of the normal standard.

Deficiency in hemoglobin produces unmistakable changes in the appearance of the cells, those in which this change is well defined appearing as pale, washed-out bodies which stand in striking contrast to the darker, yellowish-green color of the normal erythrocytes.

Abnormal viscosity of the erythrocytes, their tendency toward rouleaux formation, the presence of deformities of size and of shape, and the occurrence of structural degenerative changes may also be distinguished in the fresh, unstained blood film. Nucleated erythrocytes are not demonstrable in the fresh specimen.

*Changes Affecting the Leucocytes.*—A glance is usually sufficient to determine whether or not the number of leucocytes is markedly in excess of normal, but too great dependence should not be placed on such a method of detecting the presence or absence of a leucocyte increase, since it is at the best approximate, and sometimes erroneous. As will be explained elsewhere, any marked decrease in the number of erythrocytes, the leucocytes remaining normal, may so increase the ratio of the latter to the former, that the leucocytes may be apparently increased.

Having tentatively determined that an increase in the total number of leucocytes is present, it is furthermore possible for one familiar with the morphology of the unstained leucocyte to make a fairly accurate differential count of these cells, and thus to decide whether the increase is due to a pure leucocytosis

or to some form of leukemia. This distinction is not at all difficult in most instances, when one recalls the characteristics of the several forms of leucocytes in the fresh blood, viz.: small lymphocytes, large lymphocytes, and transitional forms, appearing as cells having a single spherical or indented nucleus, and a clear, shining, non-granular protoplasm; polynuclear neutrophiles, as cells with polymorphous or multiple nuclei, and a protoplasm crowded with very fine, moderately refractive granules; eosinophiles, as cells with a single polymorphous nucleus or multiple nuclei, and a protoplasm containing coarse, spherical, highly refractive, fat-like granules; and myelocytes, as cells with a single spherical or ovoid nucleus, and a protoplasm crowded with very fine, moderately refractive granules. It is, of course, obviously impossible to distinguish basophile cells in the fresh blood, as well as some of the cells containing fine eosinophile granules, but the characteristics noted above are sufficiently plain to justify at least a provisional diagnosis of either of the conditions in question, which, in every instance, should be verified by a careful examination of the stained specimen.

While most of the degenerative changes which affect the leucocytes are clearly demonstrable only in the stained specimen, it is still possible to recognize some of the grosser examples of such a process by a study of the fresh film. Vacuolation of both nucleus and protoplasm, extrusion of portions of the cell substance, and the various stages of nuclear disintegration and of apparent solution of the protoplasm are the alterations most commonly observed. In certain specimens "fractured" leucocytes are seen with more or less frequency, a cell thus affected being drawn out into a diffuse, irregularly shaped body with indistinct and ragged margins, about which the cell granules, which have escaped from the protoplasm, are scattered in the form of a nebulous mass. The eosinophile leucocytes seem especially prone to undergo this disintegration. The exact significance of this phenomenon is not clear, but it probably represents a degenerative change in which the cells have become abnormally vulnerable, and thus highly susceptible to mechanical injury from the pressure of the cover-glass.

Ameboid activity of the leucocytes and pigmentation of these cells are among the other changes to be observed in a histological examination of the unstained blood film.

*Increase in Fibrin, Blood Plaques, and Hemokonia.*—The density of the fibrin network and the rapidity with which it forms may be studied as coagulation of the blood film progresses. Unless the blood plaques are very greatly increased in number,

they are not usually noticeable in the specimen prepared in the ordinary manner. The presence of hemokonia, or "blood dust," is at once rendered conspicuous by the rapid and incessant molecular motion with which these bodies are endowed.

*Blood Parasites.*—The hematozoa of the malarial fevers, the spirilla of relapsing fever, the organisms of trypanosomiasis, and the embryonic forms of the parasite of filarial disease should be studied in the fresh blood whenever this is possible, rather than in the fixed and stained film, since in the latter the characteristic morphology of these parasites is greatly altered and their motility lost. The stained specimen is more useful in studying the finer structure of these organisms than for diagnostic examinations.

The distoma of bilharzia disease, although, strictly speaking, a blood parasite, is not found in the general circulation, since this worm resides solely in the portal vein and branches, the vena cava, and certain veins of the lower pelvis. Leishman-Donovan bodies are obtained by puncture of the spleen, but they do not enter the peripheral blood.

*Foreign bodies,* such as free fat droplets, collections of extracellular pigment, and, very rarely, the crystalline bodies of Charcot may also be observed in the fresh specimen during the course of certain diseases.

Microscopical examination of the fresh specimen should form the initial step taken in every systematic examination of the blood, since it may be the means of determining whether or not a more elaborate investigation is necessary. By this simple procedure an immediate diagnosis may be made in a number of instances, while in others the findings, although not pathognomonic, are of distinct clinical value. Close familiarity with the normal histology of the blood is, of course, essential for the appreciation of the various pathological changes which have been outlined above. Fuller reference to these changes has been made in other parts of this book. (See Sections III and IV.)

## II. ESTIMATION OF THE PERCENTAGE OF HEMOGLOBIN.

Methods. No fewer than half a dozen different hemoglobinometers, or instruments for estimating the amount of hemoglobin in the blood, are in vogue at the present time, of which the most reliable for general clinical use are those devised by Dare, by von Fleischl, by Oliver, and by Gowers. The hemometer of von Fleischl has been the general

favorite for a number of years, both in this country and on the Continent, but in America, at least, Dare's hemoglobinometer is rapidly supplanting it; in England it has been supplanted to some extent, first by Gowers' hemoglobinometer, and in recent years by the hemoglobinometer lately invented by Oliver. The instruments of von Fleischl, Gowers, and Oliver are based upon a similar principle, that of measuring the depth of color of the diluted blood by a standard color scale of varying intensity, the gradations of which correspond to different hemoglobin values; that of Dare uses undiluted blood.

DARE'S HEMOGLOBINOMETER.

With this instrument a thin film of *undiluted* blood is brought into direct comparison with a standard semicircular wedge of tinted glass ranging in color from a claret red at the thickest part to a pale pink at the thinnest. The instrument consists of the following parts: (1) A *capillary blood chamber*, constructed of two rectangular plates of polished glass, the opposed surfaces of which are so ground that, when clamped together in a metal bracket, a shallow compartment holding a thin film of blood is formed. One plate is made of transparent, the other of opaque, glass, the latter being next to the source of light, in order to soften its glare, when the instrument is in use. The metal bracket of the blood chamber is adjusted to the stage of the instrument in such a manner that the blood film fits over an aperture communicating with a camera tube screwed to the opposite side of the rubber case. (2) A *graduated color standard* made of a semicircle of glass tinted with Cassius' "golden purple," and thinning out like a wedge with various depths of color corresponding to the tints of fresh blood containing different

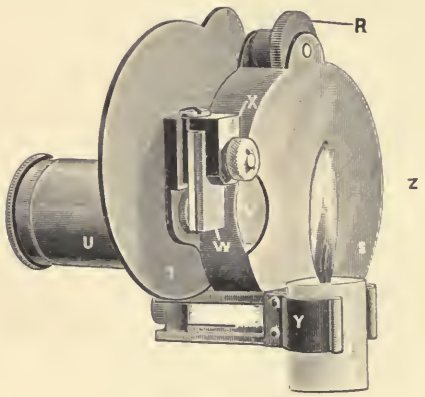


FIG. 4.—DARE'S HEMOGLOBINOMETER.

R, Milled wheel acting by a friction bearing on the rim of the color disc; S, case inclosing color disc, and provided with a stage to which the blood chamber is fitted; T, movable wing which is swung outward during the observation, to serve as a screen for the observer's eyes, and which acts as a cover to inclose the color disc when the instrument is not in use; U, telescoping camera tube, in position for examination; V, aperture admitting light for illumination of the color disc; X, capillary blood chamber adjusted to stage of instrument, the slip of opaque glass, W, being nearest to the source of light; Y, detachable candle-holder; Z, rectangular slot through which the hemoglobin scale indicated on the rim of the color disc is read.

percentages of hemoglobin. It is mounted upon a disc adjusted in the frame of the instrument, so that it may be revolved to bring various portions of its surface over an aperture directly alongside of the one through which the blood film is visible. A scale, read from the outside of the instrument, indicates in units the hemoglobin percentages from 10 to 120. (3) A *hard rubber case* incloses the color standard when the instrument is in use, the disc upon which the standard is mounted being revolved by turning a small milled wheel acting upon the rim of the disc by a friction bearing. To one side of the case a telescopic camera tube, fitted with an eye-piece, is attached, while on the opposite side a stage furnishes support for the blood chamber, back of which a candle, held between a pair of spring clips, is adjusted. Two apertures of equal diameter, placed side by side on the same level, transmit the light of the candle through the blood film and the color standard to the field of vision inclosed by the camera tube. By reference to the accompanying diagram (Fig. 5) it will be seen that the light of the candle, J, equally illuminates the blood film inclosed between the two rectangular glass plates, O and P, and the edge of the color standard, L, mounted upon the glass disc, K. The differences in the two colors are visible through the two apertures, M and M′, communicating with the camera tube, N. By revolving the disc the tint of the color standard may be altered until it matches that of the blood film.

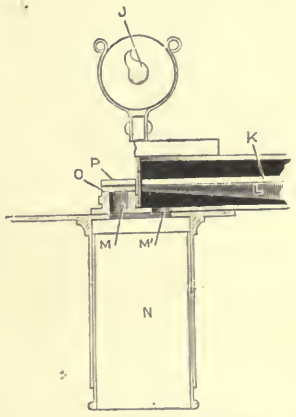


FIG. 5.—HORIZONTAL SECTION OF DARE'S HEMOGLOBINOMETER.

*Method of Use.*—The instrument is prepared for use by first swinging outward the movable screen which serves as a cover for the case. The two apertures overlying the blood film and the color scale are thus brought into view, the direct light from the candle being shaded from the observer's eyes by the intervening screen. The camera tube and the candle-holder are then fitted to their attachments on opposite sides of the instrument, and a candle adjusted so that the surface of its "wick end" is just on a level with the top of the spring clips.

The blood chamber is filled by touching its edge to the side of a rather large drop of blood as the latter flows from the puncture, so that the blood at once flows into and fills, by capillary force, the shallow compartment between the pair of glass plates.

As soon as this occurs, any excess of fluid which may have adhered to the outer surface of the blood chamber is carefully wiped away, and the latter is slipped into the tongue, which holds it in position on the stage of the instrument.

The candle having been lighted, the observer holds the instrument as a field glass, and compares with one eye the colors of the blood film and the standard disc which are seen side by side in the field of vision limited by the camera tube. The disc is made to revolve by making short, quick turns with the milled wheel until the two colors are identical, and the hemoglobin percentage indicated by the scale is then noted.

The color comparisons need not be made in a darkened room, although the observer should avoid facing the direct sunlight, and, in order to exclude reflected light, should hold the instrument against a dark surface, such as a black coat-sleeve. When the observation is completed, the two glass plates of the blood chamber are removed from the bracket by loosening the screw which holds them in position. They are then cleaned with water and with acid alcohol, dried, polished, and replaced in the bracket. The various parts of the instrument, when detached, fit into a small leather carrying-case.

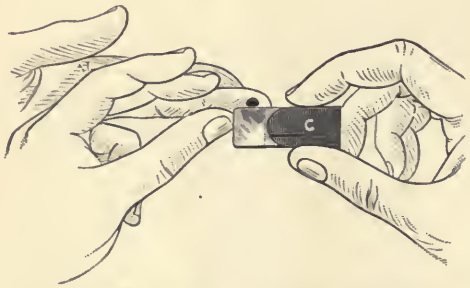


FIG. 6.—MANNER OF FILLING BLOOD CHAMBER.

The chief advantage of Dare's instrument lies in the fact that dilution of the blood is not required, and therefore errors due to incorrect measuring and dilution of the blood, which must be carefully guarded against in the older hemoglobinometers, are entirely eliminated. A film of whole blood also gives a relatively deep and definite color, which may be judged with greater ease and accuracy than the paler and more indefinite tint of a blood solution. It is also obvious that errors due to the turbidity of an aqueous solution of leukemic blood are avoidable by the use of an undiluted film. Coagulation of the film does not occur with sufficient rapidity to constitute a source of error, since the test may be completed within a few seconds after the blood has been drawn.

Three years' constant use of this hemoglobinometer in the

Jefferson Medical Clinic has proved it the most accurate, simple, and convenient instrument for clinical purposes. Its readings closely correspond to those of Oliver's hemoglobinometer, and average somewhat higher than those of the von Fleischl instrument. The color standard of Dare's apparatus, being wedge-shaped and therefore gradually blending the tints, is open to the same criticisms which have been urged against the scale of the hemometer.

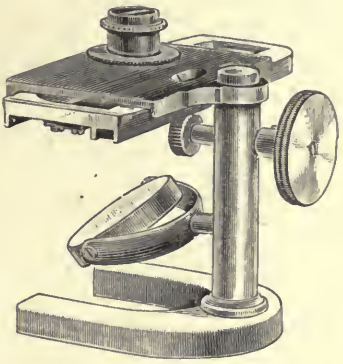

Fig. 7.—Von Fleischl's Hemometer.

**Von Fleischl's Hemometer.**

With this instrument the color of a fixed volume of blood in an aqueous solution of a definite strength is compared with the color of a movable glass wedge, tinted with Cassius' "golden purple." The hemometer consists of the following parts:

(1) A *tinted glass wedge*, the thickest portion of which is of a deep pink color, and the thinnest portion almost colorless, with every intermediate color gradation between the two extremes. It is mounted in a metal frame provided with a scale, graduated at every five degrees from 0 to 120, the former corresponding to the thinnest, and the latter to the thickest, part of the wedge. The metal frame is grooved so that it fits beneath (2) a *small*

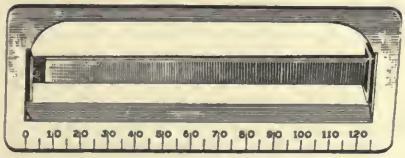


Fig. 8.—Tinted Glass Wedge of the von Fleischl Hemometer.


Fig. 9.—Capillary Pipette of von Fleischl's Hemometer.

*stage*, in which it may be moved backward and forward by turning a milled wheel. In the center of this stage there is a circular opening through which the light of a candle is reflected by a disc of calcium sulphate, mounted on the pillar supporting the stage, like the mirror of a microscope. Back of this opening there is a small oval slot through which the scale of the underlying

tinted wedge is visible, when the latter is adjusted to the stage. (3) A *mixing chamber*, consisting of a short metal tube closed at the bottom by a disc of glass and divided into two equal compartments by a vertical partition, fits accurately over the circular opening in the stage. When properly adjusted to the latter, the vertical partition exactly coincides with the upper edge of the underlying tinted wedge, so that the upper compartment of the chamber is illuminated by the dull white light from the reflector, while the lower compartment receives the color of the tinted wedge. (4) A *capillary pipette* mounted in a short metal handle, used for making the blood dilution. A single pipetteful of normal blood mixed with sufficient distilled water to fill exactly one of the compartments of the mixing chamber gives a solution which matches the color of the tinted wedge opposite the mark 100. (5) A small, fine-pointed *glass dropper*, used for filling the compartments with water.

*Method of Use.*—As a preliminary step, each compartment of the mixing chamber is filled about one-quarter full of distilled water by means of the glass dropper, to one end of which a rubber cap has been fitted. A puncture having been made, as previously directed, a measured volume of blood is collected by bringing one end of the capillary pipette lightly in contact with the blood drop as it oozes from the wound, so that the tube is instantly filled with blood, by capillary force. No difficulty will be experienced in quickly filling the tube if it is applied horizontally to the side of the blood drop, rather than vertically to its summit, care being observed not to immerse the end too deeply. It is needless to add that the interior of the tube must be absolutely clean and dry, to insure which a very fine needle and thread may be passed through it just before using. As soon as the pipette is filled, every trace of blood must be removed from its outer surface and the precaution taken to see that the column of blood is exactly flush with the ends of the tube, being neither bulged out nor depressed. The blood is then washed into one of the compartments of the mixing chamber, by forcing a stream of distilled water through the pipette by means of the glass dropper, this rinsing being repeated until it is certain that every trace of blood has been removed. The preceding steps must be carried out quickly, in order to avoid errors arising from coagulation of the blood. The blood and water in the compartment are now thoroughly mixed by stirring with the handle of the pipette until the color of the solution is diffused uniformly, after which water is added, drop by drop, to each compartment until they are both filled exactly to their brims. In doing this, no water must be spilled on the

thin edge of the vertical partition, for should this occur, it may cause an overflow of the liquid from one compartment to the other, and thus alter the strength of the blood solution. If the latter should appear turbid or muddy, as it sometimes does with leukemic blood, a few drops of a weak aqueous solution of potassium hydrate may be added to the diluent as a preventive of this change. The addition of a little ether will clear the solution if the turbidity is due to the presence of fat.

Having carried out the preceding steps, the mixing chamber is adjusted over the circular opening in the stage of the instrument, so that the compartment containing the blood solution is uppermost, overlying the semicircle illuminated by the clear white light; while the compartment filled with water fits over the semicircle, which receives the tint of the underlying glass wedge. The remainder of the test, the comparison of the color of the two compartments, must be completed by artificial light, preferably by candle-light. Moderately bright illumination is better than a strong glare, for the latter interferes seriously with the accurate determination of delicate color differences. By means of the milled wheel the tinted glass wedge is moved backward and forward until its color precisely corresponds to that of the diluted blood. When this occurs, the percentage of hemoglobin is read off from the scale visible through the oval slot in the stage of the instrument.

While making the color comparison the observer should stand facing one end of the glass wedge (*not* the milled wheel), so that the partition between the two compartments of the mixing chamber is on a line with the vertical axis of his eyes, the distance from the latter to the top of the stage of the instrument being about ten or twelve inches. Gross errors may be avoided if the observation is made with one eye and if the same eye is habitually used, since the two eyes may differ radically in their sensitiveness to color impressions. It is important to decide the color differences as quickly as possible, for prolonged examination rapidly dulls one's color perception, and creates uncertainty as to the proper reading. It is a good plan first to bring into the field of vision the darkest portions of the wedge between the figures 100 and 120 of the scale, and then, by short, sudden turns of the milled wheel, to produce abrupt color contrasts of from 5 to 10 degrees at each turn, until the two tints approximately correspond.[1] When this point is reached, the eye should be rested for a few

[1] It is important to bear in mind the fact that the judgment of color differences is much easier if marked contrasts in color value are made, than if a gradual blending of the two tints is attempted, by slowly moving the wedge past the visual field.

moments, and then, by a succession of shorter turns, the wedge is again swept to and fro until the colors appear identical. In the average instance an error of about 5 degrees must be anticipated in spite of every precaution to insure accuracy.

FIG. 10.—METHOD OF USING THE VON FLEISCHL HEMOMETER.

Note that the septum between the two halves of the blood compartment is at right angles to the horizontal axis of the observer's eyes. A cylinder of paper may be fitted over the blood compartment, to serve as a camera tube.

In cases in which low hemoglobin percentages (30 per cent. or less) are suspected, it is essential to use two or three pipettefuls of blood in making the dilution, dividing the percentage indicated by the instrument by two or three, as the case may be.

This precaution effectually removes the objection which has been urged against this instrument on account of its inaccuracies in the determination of low hemoglobin percentages. Another criticism of the von Fleischl instrument has been made on the ground that, since the length of the tinted wedge visible through the compartment of the mixing chamber includes a color range of 20 per cent., it is impossible for one to select a single point in the center of this color for comparison with the even, diffuse tint of the blood solution. This objection may be overcome to a great extent by using a metal diaphragm, provided with a slit one-eighth of an inch in width, which is placed over the glass disc at the bottom of the compartments, to limit the field of vision. Adjusted so that the slit crosses at right angles the partition separating the two colors, the use of this device cuts down the field of observation to a portion of the glass wedge corresponding to about 2.5 degrees on the scale.

The hemoglobin percentages indicated by this instrument appear to be low for the blood of the average healthy American, since it is more common to obtain readings of from 90 to 95 than of the arbitrary standard 100, in persons in whom there is no good reason to suspect subnormal hemoglobin values. In instruments of recent manufacture, however, this fault is largely corrected.

In order to exclude the light of the candle from the field of vision while making the color comparison, it is customary to use a tube of cardboard or stiff paper, which is slipped over the mixing chamber and rests upon the platform of the instrument. This sort of a device answers very well when the examination is made in a darkened room, as, for example, at a patient's residence. In hospital work, however, the inconvenience, sometimes considerable, of being compelled to carry the diluted blood some distance from the bedside to a dark room may be avoided by the use of a light-proof box, which may be conveniently carried from ward to ward, so that the test may be completed at the bedside (Fig. 11). A box of this kind should measure sixteen inches in height by twelve inches in length and in width, being fitted with a hinged door which may be fastened by a simple catch, and provided with a circular opening through which the milled wheel of the hemometer projects when the door is closed. A metal camera tube, flanged at the upper extremity for the observer's eye, pierces the top of the box and communicates inside with the mixing chamber of the hemometer. The tube fits loosely in a circular opening in the top of the box, so that it may readily be raised and lowered; its diameter is a trifle greater

than that of the mixing chamber, around which it should fit snugly when lowered into position; and its length is governed by a fixed collar outside the box, which prevents it from slipping and jarring the instrument. Wooden guides, such as are used for securing a microscope in its box, are provided to receive the

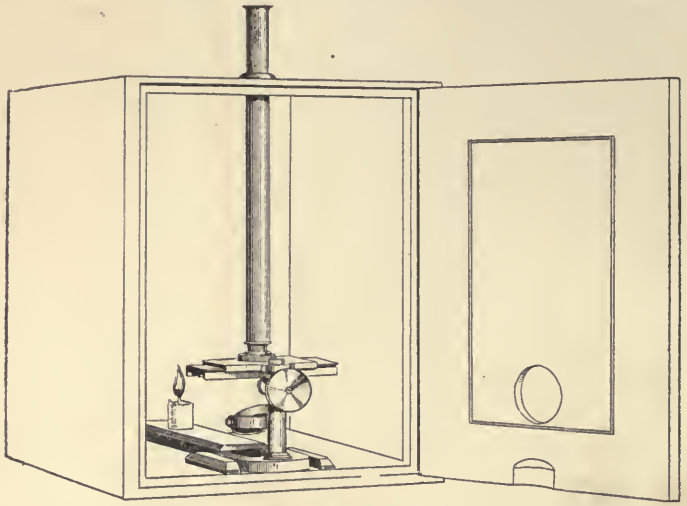

FIG. 11.—LIGHT-PROOF BOX FOR THE VON FLEISCHL HEMOMETER.
The door of the box is closed and the color comparison made through the camera tube.

*Note.*—Reichert, at the suggestion of Miescher, has introduced a modification of the original von Fleischl hemometer, designed to increase the accuracy of the test, by making it possible, by definite dilution of the blood, to select that part of the tinted wedge which is best adapted for the examination of any particular sample. This innovation was prompted by the discovery that the intermediate portions of the wedge are better adapted for obtaining accurate readings than the terminal parts. The principal modification of the new hemometer consists in the substitution, for the original capillary blood pipette, of a special mixing pipette, similar to a *mélangeur*, graduated so that the blood may be diluted 1 : 200, 1 : 300, and 1 : 400 times. A table supplied with the instrument translates the combined results of the dilutions and the figures indicated by the scale on the wedge into absolute hemoglobin percentages. The instrument is also supplied with mixing chambers of different depths, and with a diaphragm designed to limit the field of vision. The writer has had no practical experience with the Miescher-Fleischl hemometer, but an examination of the instrument justifies the belief that its elaborateness renders it undesirable for general clinical work. Its cost ($50.00) is also a bar to many.

horseshoe base of the hemometer, holding it firmly in such a position that when the camera tube is lowered into place the milled wheel of the instrument projects through the opening in the closed door. The interior of the tube and of the box is painted a dull black. A candle is placed in position on the floor of the

box, on a line with the "mirror" of the instrument. In using this device first the candle within the box is lighted, and the hemometer base is slipped into place between the wooden guides. The blood dilution having been made in the usual manner, the mixing chamber is then set upon the platform of the instrument, and the camera tube, which has been raised to allow this to be done, is lowered until it telescopes around the mixing chamber and rests firmly upon its collar. The door of the box is now closed, and the two compartments are brought into their proper positions over the glass wedge by turning the camera tube from the outside of the box, the observer meanwhile noting the result by looking through the flanged extremity of the tube. This accomplished, the projecting wheel of the instrument is turned to and fro until the colors of the two compartments are the same, when the door is opened and the percentage read off from the scale of the hemometer. Care must be observed to see that the exterior of the mixing chamber is perfectly dry, for if any moisture collects between its outer surface and the inner surface of the camera tube, the contents of the compartments may be disturbed and serious errors result. As the opening in the door of the box is covered by the hand with which the milled wheel is turned, sufficient light to interfere with the test cannot leak in at this situation.

With a light-proof box of this sort it is possible accurately to carry on hemoglobin estimations in the brightest daylight, which may be entirely excluded from the instrument, while the observer's field of vision is limited to the two semicircles illuminated by the candle burning within the box.

OLIVER'S HEMOGLOBINOMETER.

With this instrument the principles of Lovibond's tintometer are applied to the quantitative estimation of hemoglobin, the color of a blood solution of a definite strength being compared, by light reflected from a dead white surface, with a series of tinted glass standards which constitute a progressive color scale. Thus, a series of fixed, definite tints is provided, each of which accurately corresponds to the specific color curve of progressive dilutions of normal blood, this having been determined individually by means of the tintometer. Two sets of color standards have been devised: one for daylight readings and one for observations by candle-light, the latter being preferable on account of the greater delicacy of its readings. Oliver's complete apparatus consists of: (1) A *capillary blood measure*, made of heavy glass tubing, and having a capacity of 5 c.mm. The end to be presented to the blood drop, in filling the

measure, is tapered to a blunt point and highly polished. (2) A *mixing pipette*, provided with a short rubber tube which fits over the tapered end of the blood measure, while rinsing out the blood from the latter into the (3) standard *blood cell*, which, when filled exactly to the brim with distilled water in which one measureful of blood has been dissolved, yields a blood solution of approximately one per cent. When filled, the cell is covered with a glass slip provided for this purpose. (4) A standard *color scale*, consisting of 12 tinted glass discs, mounted in two series, and corresponding to hemoglobin percentages ranging from 10 to 120. (5) A set of *riders*, or squares of tinted glass, used for determining the intermediate degrees of color between the decimals indicated by the fixed tints of the scale. For ordinary clinical work two riders are sufficient, which, when laid over the discs of the standard scale, read 2.5 and 5 degrees respectively on its upper half, but double this amount on the lower half. For physiological observations requiring readings in units a set of nine riders is supplied. (6) A collapsible *camera tube* through which the color comparisons are made.

*Method of Use.*—In making hemoglobin estimations with Oliver's apparatus, first the capillary measure is filled with blood by the method directed for filling the pipette of the von Fleischl instrument. The rubber nozle of the mixing pipette, previously filled with distilled water, is then adjusted over the polished end of the blood measure, and the blood washed into the standard cell by forcing through the water, drop by drop. As soon as all the blood contained in the bore of the measure has been thus washed out into the cell, the rubber nozle of the pipette is removed, and the handle of the measure used as a stirrer to mix the blood solution, more water being added in single drops, from time to time, until the cell is accurately filled. The blue cover-glass is then adjusted, with the result that, if the cell has not been overfilled, a small air-bubble forms on the surface of the liquid. The blood cell, filled in this manner with a blood solution of definite strength, is now placed by the side of the standard scale, opposite the tinted disc to which it corresponds most closely, the eye readily recognizing its approximate position. More accurate matching of the two colors is made with the aid of the camera-tube, the cell being moved from disc to disc in an endeavor to match exactly the color of the blood solution by one of the standard tints of the scale. If this is successful, the hemoglobin percentage indicated by the disc is read off, and the observation is completed. But if it happens that the tint of the blood solution is obviously deeper than a certain disc, but paler than the

one immediately above, the cell is kept alongside the lower of the two, over which a rider is adjusted in order to deepen its color, while the square of white glass is placed over the cell, so as to compensate for the thickness of the rider. If, now, the colors correspond, the final reading is ascertained by taking the percentage of the disc plus the value of the superimposed rider. If the color of the blood solution is darker than that of any one of the standard discs, but paler than the disc plus a rider, the mean average of the two is taken as the final reading; similarly, if the

FIG. 12.—METHOD OF USING OLIVER'S HEMOGLOBINOMETER.

color of the blood solution is darker than a certain disc plus a rider, but paler than the disc immediately above, the values of the two must be averaged. An error of two per cent. is unavoidable, even in the hands of a skilful observer.

During the observation the candle should be placed three or four inches from the end of the color scale, being adjusted so that the flame is on a line with the opposed sides of the cell and of the scale, thus illuminating both with equal intensity. The positions of the candle and of the apparatus are shown in the accompanying illustration (Fig. 12). Small-sized candles, such as are used

for decorating Christmas trees, furnish a flame of the proper degree of brilliancy, the candle of ordinary size giving too intense a light. Total exclusion of daylight is not necessary, so that the observation may be made in the corner of a partly darkened room, as, for example, behind a closet door or some other similar shield against direct rays of light.

Oliver's hemoglobinometer is a trial to the patience of one who has habitually used the Dare or the von Fleischl, and it takes some time to become accustomed to it after having worked with the comparatively simple color comparisons of other instruments. Its accuracy is undeniable, although for clinical work a simpler apparatus is to be preferred.

FIG. 13.—GOWERS' HEMOGLOBINOMETER.

GOWERS' HEMOGLOBINOMETER.

This instrument, which for many years has been popular in England and is used to some extent in this country, consists essentially of *two small flattened tubes* of equal diameter, which, when in use, are fixed upright and parallel to each other in a small wooden support furnished for this purpose. One tube contains glycerin jelly colored with picrocarmin to correspond to the tint of a 1 : 100 solution of normal blood (or 20 c.mm. of blood in 2 c.c. of water), this being taken as the standard with which the blood solution contained in the second tube is compared. The second tube is provided with a scale graduated in units from 5 to 120, each degree of which equals the volume of blood required for the test. Twenty c.mm. of normal blood, dissolved in sufficient distilled water to fill this tube to the 100 mark on the scale, give a solution which corresponds to the tint of the standard tube. The special *capillary pipette* used for measuring the blood is graduated at 10 and at 20 c.mm., and fitted with a bit of rubber tubing and mouthpiece for filling it by suction.

*Method of Use.*—The technic of hemoglobin estimations with Gowers' apparatus is extremely simple. Having made the puncture in the usual manner, the blood is sucked up the caliber of the capillary pipette until the mark 20 is reached, and then immediately blown out into the graduated tube, into which a few drops of distilled water have previously been placed, in order to insure instantaneous solution of the measured amount of blood. All traces of blood which may have adhered to the bore of the capillary pipette are removed by filling it several times with water,

the rinsings being added to the mixture of blood and water in the tube. During the preceding steps the usual precautions must be observed to wipe all surplus blood from the outside of the pipette before expelling its contents, and to measure the blood rapidly, so as to guard against errors arising from rapid clotting. Distilled water is now added, drop by drop, to the mixture in the tube until the color of the blood solution exactly corresponds to that of the picrocarmin standard, the contents of the tube being mixed between each addition by rapidly reversing it two or three times, with its open end closed by the thumb. The drop or two of liquid adhering to the thumb should be wiped off against the wall of the tube, so that it may drain back into the liquid. When the tints of both tubes are precisely similar, the division of the scale to which the diluted blood reaches is read off, to express the percentage of hemoglobin in the specimen under consideration.

In comparing the colors, which is done by daylight, the tubes should be held against a sheet of white paper, or, as suggested by Gowers, between the eye and a window, and viewed at such an angle that their adjoining edges appear to overlap, thus cutting off the vertical streak of white light visible between them should this precaution be neglected. Owing to the diagonal position in which the two tubes are adjusted in their support, the proper angle to produce this effect may readily be determined.

The chief drawback to the use of this instrument is the likelihood of overdiluting the blood after it has been mixed in the graduated tube, the occurrence of this accident necessitating, of course, a repetition of the entire operation. It is not always easy to decide just when sufficient water has been added to the blood to bring its color *down* to that of the standard tint, since one must depend solely upon a gradual weakening of the tint of the blood solution, and this is much more difficult than to compare a definite blood color with a sliding scale or with a series of discs. The instrument may be regarded as accurate within two or three per cent. for hemoglobin percentages above ten, below which figure it is impossible to distinguish a difference between the tints of the two tubes. This source of error, however, is too remote a possibility to detract from the instrument's practical value. A real source of error in Gowers' instrument is the deterioration with age of the picrocarmin color standard, with a consequent change in its tint.[1]

[1] Haldane (Jour. Physiol., 1901, vol. xxvi, p. 497) has modified Gowers' instrument by substituting for the picrocarmin jelly a one per cent, solution of carbonic oxid hemoglobin, which remains stable indefinitely. Using Gowers' technic, the blood is first partly diluted and then charged with illuminating gas,

TALLQUIST'S METHOD.

A simple method of approximately determining hemoglobin percentages without the aid of a special instrument has recently been devised by Tallquist,[1] the procedure consisting, in brief, in allowing a drop of blood to soak into a bit of filter-paper and comparing with the naked eye the color strength of the stain with a series of printed standard tints of known value. The latter are arranged as a scale of ten different colors, corresponding to the colors of stains produced by bloods having hemoglobin values ranging from 10 to 100 per cent., the latter being regarded as the normal. A lithographed copy of the color standard accompanies Tallquist's original article. Dealers in laboratory supplies also furnish a small book containing the color scale and a supply of standard absorbent paper. The test is made in the following manner: A drop of blood, large enough to make a stain about 5 or 6 mm. in diameter, is caught in the center of a piece of white filter-paper, care being taken in collecting it to apply the paper to the exuding drop in such a manner that the blood soaks in very slowly, and thus produces a stain which is evenly colored throughout. Perfectly white filter-paper, having a smooth surface and of a thickness corresponding to about 55 leaves to the centimeter, should be used for the test. The blood stain thus made is pressed lightly against a pad of filter-paper, and then compared, by direct daylight, with the series of standard tints, the figure opposite to the tint which the stain most accurately matches being read off, to indicate the percentage of hemoglobin in the specimen under examination. The comparison must be made immediately after the stain loses its humid gloss, since blood soon changes its color after exposure to the air.

This direct method of hemoglobin testing is, of course, only approximate, at the best, and cannot be expected to furnish results comparable in point of accuracy with those to be obtained by any of the instruments just described. It may, however, be employed to excellent advantage when a hemoglobinometer is

by means of a special fitting to be attached to a gas-burner. The oxyhemoglobin of the blood is thus converted into carbonic oxid hemoglobin, the color of which is comparable to that of the standard solution. After having been charged with gas, the blood is mixed by repeatedly inverting the tube, the open end of which is closed by the thumb, after which the dilution is proceeded with until the colors match, when the final reading is made. The inventor's claims for the accuracy of his instrument have been substantiated by Horder (Lancet, 1903, vol. i, p. 1305). In ten estimates by different observers it was determined that the possible errors with Haldane's instrument averaged 1.9 per cent., and with the hemometer, 4.25 per cent. Haldane's device, despite its accuracy, obviously is better adapted to physiological research work than to clinical hematology.

[1] St. Paul Med. Jour., 1900, vol. ii, p. 291.

not at hand, or in certain cases in which only a rough estimate of the amount of coloring matter of the blood is sought. Tallquist, who has tested his method, under the control of the hemometer, in his clinic at Helsingfors, claims that the limit of error generally does not exceed ten per cent.

Here may be mentioned *Haig's blood decimal card*, devised for roughly estimating the color index of the blood. It consists of a series of four different colors, scaled 0.80, 0.60, 0.40, and 0.20, respectively. By matching one of these colors with the color of the patient's gums or tongue an approximate idea of the individual's color index is obtained. Haig's device may serve for a hurried examination, but it is obviously too crude to give accurate results.

## III. COUNTING THE ERYTHROCYTES AND THE LEUCOCYTES.

Methods.

Of the various instruments used for counting the blood corpuscles, the hemocytometers devised by Thoma and by Gowers are most generally employed at the present time, the former being used almost to the exclusion of the latter everywhere except in England, where Gowers' apparatus has many firm adherents. Durham, by adapting and modifying a number of the details of the older instruments, has succeeded in devising an improved form of hemocytometer which possesses many advantages over the original models, being of simple construction, accurate, and comparatively inexpensive. The method of making the estimate, which is essentially the same with all three of these instruments, consists, briefly, in first diluting the fresh blood in definite proportions with some indifferent preservative fluid, and in then counting, under the microscope, the number of corpuscles in a drop of the diluted blood, the latter being contained in a small glass cell on the floor of which is ruled a series of micrometer squares of certain dimensions. The cubic contents of the cell and the degree of the blood dilution being known, the number of corpuscles counted in any given number of these squares may be taken as a basis for calculating the total count of corpuscles to the cubic millimeter of blood.

Strong and Seligmann dispense with a special counting chamber, and enumerate the cells in a measured quantity of blood diluted in definite proportions with a diluent-stain and mounted as a permanent dry specimen.

Oliver has devised an instrument with which the number of

erythrocytes may be estimated by means of their optical effect, without the use of the microscope.

DILUTING FLUIDS.

Diluting fluids for use with the hemocytometers of Thoma, Gowers, and Durham should be of such a composition that when mixed with the fresh blood they preserve unaltered the form of the corpuscles. This requirement being met, the examiner may choose from the numerous formulas in current use the one which best suits his individual preference. Among the most satisfactory solutions used for this purpose the following may be mentioned:

TOISSON'S SOLUTION.

| | |
|---|---|
| Methyl-violet, 5 B | 0.025 |
| Sodium chlorid | 1.0 |
| Sodium sulphate | 8.0 |
| Neutral glycerin | 30.0 |
| Distilled water | 160.0 |

SHERRINGTON'S SOLUTION.

| | |
|---|---|
| Methylene-blue | 0.1 |
| Sodium chlorid | 1.2 |
| Neutral potassium oxalate | 1.2 |
| Distilled water | 300.0 |

For general clinical work no better formulas have ever been suggested than the preceding two. Both solutions act as excellent preservative fluids, and each contains just sufficient quantity of a basic anilin dye to stain the leucocytes with great distinctness, so that they may readily be differentiated from the erythrocytes, which remain uncolored.

HAYEM'S SOLUTION.

| | |
|---|---|
| Mercuric chlorid | 0.25 |
| Sodium chlorid | 0.5 |
| Sodium sulphate | 2.5 |
| Distilled water | 100.0 |

Oliver specifies this solution as the diluent invariably to be employed with his instrument, but it may be used also with the other forms of hemocytometers, although with less satisfaction than the formulas first mentioned.

Among the simpler diluting fluids, all of which are dependable, are solutions in distilled water of common salt (0.7 per cent.), of potassium bichromate (2.5 per cent.), and of sodium sulphate (5 per cent.), to any of which about 0.5 per cent. of an alcoholic solution of methyl-violet may be added, in order to stain the leucocytes, and thus to facilitate the counting.

An aqueous solution of acetic acid, varying in strength from 0.3 to 0.5 per cent., which destroys the erythrocytes and at the same time renders more conspicuous the leucocytes, has been recommended by Thoma as a diluent in counting the latter cells, by means of his special pipette.

Türk recommends the use of a one per cent. solution of acetic acid containing one per cent. of gentian-violet, in order to recognize the different forms of leucocytes, as well as to count their total number. This method is, however, only approximate, and should not replace the examination of the dried stained film. (See p. 75.)

As spores are liable to develop and precipitates to form in all the above-mentioned solutions, they should always be filtered before using and kept in tightly corked bottles.

THE THOMA-ZEISS HEMOCYTOMETER.

The Thoma-Zeiss hemocytometer, which is to-day regarded as the standard instrument for blood counting, consists of two graduated capillary pipettes for diluting and mixing the blood, and a counting chamber in which a measured volume of diluted blood is placed for the purpose of counting the corpuscles under the microscope. One of the pipettes is intended for counting the erythrocytes, and, for convenience sake, may be termed the erythrocytometer; while the other pipette, which is used for counting the leucocytes, may be called the leucocytometer. It is not, however, necessary to purchase both pipettes, as supplied with the complete apparatus, since both erythrocytes and leucocytes may be counted accurately with the erythrocytometer.

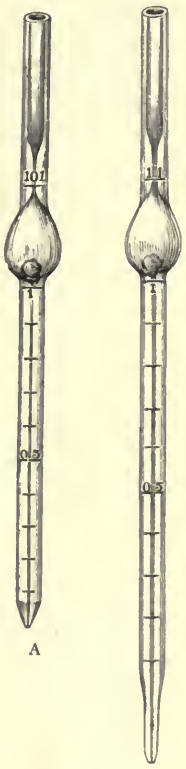


FIG. 14. — THE THOMA-ZEISS HEMOCYTOMETER. A, ERYTHROCYTOMETER; B, LEUCOCYTOMETER.

The *erythrocytometer* consists of a heavy glass capillary tube, the lumen of which is expanded near the upper end into a bulb containing a small cubical glass bead, which serves as a stirrer. The lower end of the tube is ground to a blunt point, and to the upper end is fitted a short bit of rubber tubing capped by a bone mouthpiece for filling the tube by suction. A scale is enameled into the glass wall of the pipette, the three main divisions of which are indicated by the figures 0.5, 1, and 101, the first two grada-

tions being below, and the latter one above, the bulb; the lower portion of the tube is further graduated in tenths, by cross lines, from 0.1 to 1. If blood is drawn up in the pipette to the mark 1, and the diluent added until the mark 101 is reached, the blood is thus diluted one hundred times; or if the blood is drawn up only to the mark 0.5, and the diluent added as before, a two-hundred-fold dilution is obtained.

The *leucocytometer* is a capillary tube similar to the former, but having a larger lumen, so that lower dilutions are obtained with it. If blood is drawn up to the mark 1, and the diluent added until the mixture reaches the mark 11, the blood is diluted ten times; or if the blood column reaches the mark 0.5, with the same addition of diluent, the dilution thus made is twenty-fold. In the latest model of this pipette the lower end tapers to a fine point, the diameter of the lumen thus gradually decreasing as the extreme tip is approached. The chief object of this

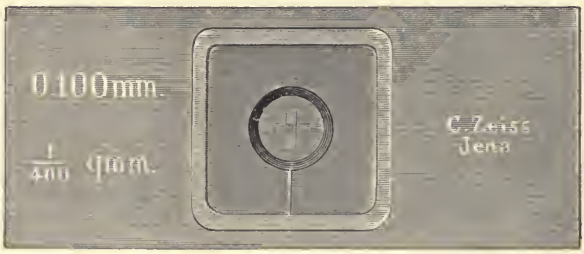


FIG. 15.—THOMA-ZEISS COUNTING CHAMBER.

modification is to prevent accidental leaking out of the column of blood when the tube is held vertically, while sucking up the diluting fluid—an accident difficult to avoid with the old-style pipette having a large lumen from tip to bulb.

The *counting chamber* (Fig. 15) consists of a heavy glass slide in the center of which is cemented a square glass plate provided with a circular opening which fits around a ruled disc; the diameter of the latter being slightly less than that of the opening in the surrounding plate, a shallow, narrow gutter is thus formed between the two. The surface of the ruled disc is exactly $\frac{1}{10}$ mm. below the level of the glass plate by which it is inclosed, so that a chamber of this depth is formed when both are superimposed by a cover-glass having an absolutely plane surface, two such covers being furnished with each instrument. An ordinary cover-glass should never be used, for, owing to the unevenness of its surface, a deviation from the standard in the depth of the under-

lying chamber must necessarily result. When an objective having an extremely close "working distance" is employed, the special hollow cell cover-glass made by Zeiss will prove useful.

The central part of the disc's surface is divided, by microscopical diamond-rulings, into 400 small squares, each of which has an area of $\frac{1}{400}$ sq. mm., these small squares being grouped into sets of sixteen by a series of vertical and horizontal double rulings bisecting each fifth column of squares (Fig. 16). The cubic contents of each small square, when the cover-glass is adjusted, is $\frac{1}{4000}$ c.mm., since they measure individually $\frac{1}{10}$ by $\frac{1}{20}$ by $\frac{1}{20}$ mm. In Zappert's modified ruling of the Thoma-Zeiss counting chamber extra lines have been added so as to

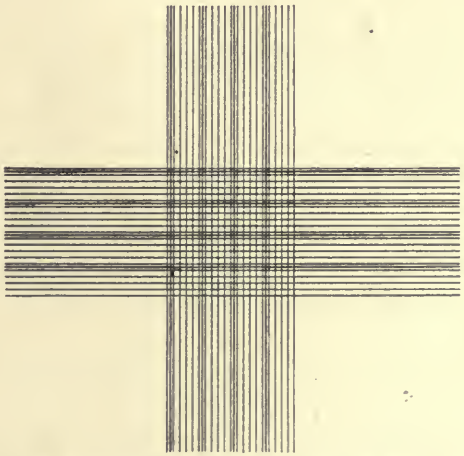

FIG. 16.—RULED AREA OF THE THOMA-ZEISS COUNTING CHAMBER (ORDINARY RULING).

increase the ruled area of the disc to nine times its original size. As illustrated in the accompanying diagram (Fig. 17), the surface of the disc is thus divided, by heavy cross-rulings, into nine large squares, each equal in area to the central group of 400 small squares, the whole ruled surface therefore equaling an area covered by 3600 of the latter. To simplify the counting, the peripheral squares are subdivided into four, each of the latter being of the same area as 100 of the small central squares. This improved form of counting chamber is an invaluable convenience in leucocyte counting, and should be chosen in preference to the older model.[1]

[1] Other counting chambers, ruled so as to provide a larger number of squares in the field, have been devised by Türk, Breuer, Elzholz, and others, but none of them possesses any real advantages over Zappert's slide.

If any difficulty should be experienced in distinguishing the ruled lines under the microscope, they may be made more conspicuous by blackening them with a little soft lead-pencil dust placed on the surface of the disc and thoroughly rubbed in with the ball of the finger, the excess being wiped off and the disc polished with a bit of lens-paper or a soft handkerchief.

*Counting the Erythrocytes.*—Having made the puncture, as already described, the point of the erythrocytometer is plunged into the blood drop as it flows from the wound, and, by making gentle, uniform suction, a column of blood is drawn up the capillary tube exactly to the mark 0.5. The point of the instrument is then wiped perfectly dry, and immediately dipped into the dilut-

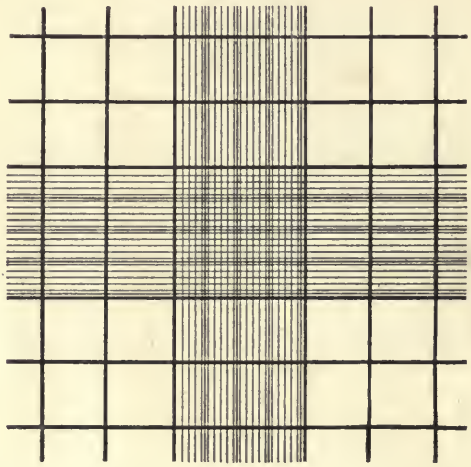

FIG. 17.—ZAPPERT'S MODIFIED RULING OF THE THOMA-ZEISS COUNTING CHAMBER.

ing fluid, which is drawn up the tube in the same manner until the mixture of blood and diluent reaches the mark 101 above the bulb. While adding the diluent, the pipette should be twisted to and fro between the thumb and forefinger, in order that the blood and diluent may be mixed, by the whipping about of the glass bead, as they fill the bulb; if this precaution is neglected, a portion of the blood will rise in a distinct layer above the diluent as the latter flows into the bulb, and may be drawn, unmixed, into the capillary constriction beyond. A more thorough mixture of the blood and diluent is now made, the rubber tubing being slipped from the instrument, which is then grasped so that its ends are closed by the thumb and middle finger, and rapidly

shaken for about half a minute. By the above steps a mixture is made in which the proportion of blood to diluent is 1 : 200, a degree of dilution with which it is most convenient to work in the great majority of instances. For two reasons a 1 : 200, rather than a 1 : 100, dilution is to be preferred in routine work: (1) If, as not infrequently happens, the blood column is accidentally drawn up the tube beyond the mark 0.5 in an attempt exactly to reach this gradation, it is a simple matter to correct the error by gently blowing or shaking the blood column down to the

FIG. 18.—METHOD OF FILLING THE CAPILLARY TUBE OF THE THOMA-ZEISS HEMOCYTOMETER WITH BLOOD.

proper level; whereas, in attempting to make a 1 : 100 dilution, should the mark 1 be exceeded, the blood column will almost surely escape into the bulb, whence it cannot be blown back again into the capillary tube, thus necessitating a repetition of the whole operation with a fresh drop after having cleaned and dried the erythrocytometer. (2) It is easy to count the corpuscles in a 1 : 200 dilution, since the surface of each ruled square of the counting chamber is, as a rule, occupied by not more than half a dozen cells; on the contrary, in a 1 : 100 dilution, except in an occasional instance in which there is a striking paucity of cells,

the field may be so overcrowded with corpuscles that their enumeration is difficult and often inaccurate.

The next step is to place a drop of the diluted blood in the counting chamber, preparatory to counting the corpuscles under the microscope. The unmixed diluting fluid in the lower portion of the capillary tube is first expelled, by blowing out four or five drops, after which the point of the pipette is dried with a soft cloth and a small drop of the blood mixture is allowed to fall, by force of gravity, exactly in the center of the surface of the ruled disc. The cover-glass is then immediately placed in position, and the slide left undisturbed for several minutes, so that the corpuscles may settle. The drop placed on the disc should be of sufficient size to occupy only its central portion, the object being to use just enough of the blood mixture to cover the ruled area and exactly to fill in the vertical space between the surfaces of the disc and cover-glass when the latter is placed in position. If the drop contains air-bubbles, or if it is so large that it overflows into the gutter and perhaps finds its way between the cover-glass and the glass plate beneath, errors will result, so that in the event of either of these accidents the procedure must be repeated with another drop, after having cleaned and dried the cover-glass and the counting chamber. Water, and not alcohol or xylol, is to be used for this purpose, since the repeated use of chemicals will soon dissolve the cement which fixes the disc to the counting chamber. In repeating the operation the original technic must be rigidly followed—*i. e.*, the erythrocytometer must be briskly shaken for half a minute or so, and the contents of its capillary stem blown out, before placing the new drop in the counting chamber.

In a properly prepared slide concentric rings of color—Newton's rings—may be seen at the points of contact between the cover-glass and the underlying glass plate. If these rings are invisible, or if they do not appear when pressure is made upon the cover-glass, it is a sign that the contact between the two glass surfaces is not true, this being due to the presence of particles of dust or of moisture beneath the cover-glass. Inasmuch as this may seriously affect the correctness of the count, it is a safe rule invariably to reject a slide in which these color rings are not visible.

As soon as sufficient time has elapsed for the corpuscles to sink to the bottom of the counting chamber—about five minutes—the slide is transferred to the stage of the microscope, which should not be inclined, for fear of disturbing the uniform distribution of the cells. The field is first brought into focus with a low-power objective (a No. 3 objective of Leitz, for example), and the slide moved

across the stage until the extreme upper left-hand corner of the group of small ruled squares is brought into view, when a higher power, to be used in counting, is substituted. For this purpose the writer is accustomed to use a Leitz No. 6 objective and No. 4 ocular, which lenses, with a tube length of 155 mm., cut off a field occupied by a block of 25 small squares.

As a basis for the final calculation, the erythrocytes in 400 small squares, or the entire ruled surface of the old-style disc, should be counted, preferably by going over two groups of 200 squares each in two different drops, rather than by taking the entire 400 squares in a single specimen. By following this plan the

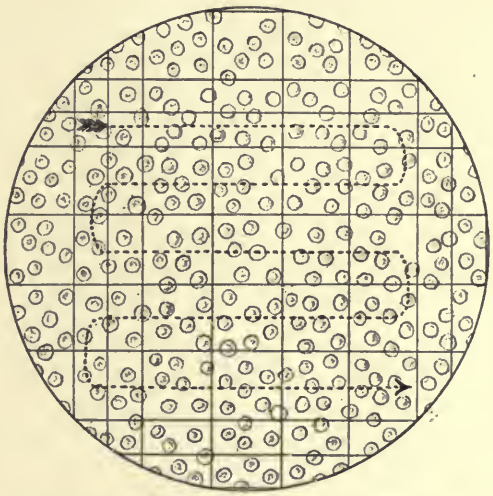

FIG. 19.—PLAN OF COUNTING THE ERYTHROCYTES.
The small squares are examined in the order indicated by the arrow, successive blocks of 25 squares being covered until the required number of cells has been counted.

count of one drop may be controlled by the count of the other, and any discrepancy between the two discovered, for if the difference in the counts is striking, a third group of 200 squares must be examined in an additional drop, and an average taken of the two counts which most closely correspond.

In order to simplify the process of counting, some routine method of examining the ruled area, such as the following, should be adopted: Beginning at the upper left-hand corner of the ruled disc, the corpuscles in the first 100 small squares are counted, the slide being moved from above downward, preferably by the aid of a mechanical stage, as the successive groups of squares are

covered. By employing the magnification to which reference has just been made, three shifts of the slide are sufficient to bring into the field the requisite number of squares in blocks of 25 each. Examining each small square in succession, proceed from left to right along one row of five, then drop to the next row and count from right to left, and continue in the manner illustrated by the diagram (Fig. 19) until all the erythrocytes in the first group of 100 squares have been counted, the totals of each block of 25 squares being noted as they are completed. To avoid repetition in counting it is necessary to include in the total all the corpuscles which touch the upper and left boundary lines, and to disregard those which touch the lower and right boundaries. A second group of 100 squares, not immediately adjacent to the first, is then inspected in a similar manner, after which the cover-glass and counting chamber are washed with water and dried, and the operation repeated with a second drop. Thus the 400 squares are covered by examining 16 blocks of 25 squares each—8 in the first and 8 in the second drop of diluted blood. In a 1:200 dilution of normal blood this involves the counting of approximately from 2400 to 2800 erythrocytes, and gives results which are accurate within one and one-half per cent.

To calculate the number of erythrocytes to the cubic millimeter of blood the following formula is employed:

$$\frac{\textit{Number of erythrocytes counted} \times \textit{Degree of dilution (200)} \times \textit{Cubic contents of each square (4000)}}{\textit{Number of squares counted (400)}} = \textit{Total number of erythrocytes per c.mm.}$$

For example, supposing that in the 400 squares of a 1 : 200 blood dilution a total of 2500 erythrocytes is counted, the calculation is made thus:

$$(a)\ \frac{2500 \times 200 \times 4000}{400} = 5{,}000{,}000\ \textit{erythrocytes per c.mm.}$$

$$(b)\quad 2500 \times 2000 = 5{,}000{,}000\ \textit{erythrocytes per c.mm.}$$

*Counting the Leucocytes.*—The leucocytes may be counted by two different methods: (*a*) With the erythrocytometer, in the same drop of diluted blood in which the erythrocytes are estimated; or (*b*) with the special leucocytometer, as a separate procedure. Of the two methods, the former is greatly to be preferred, since it is fully as accurate and much more convenient and time-saving than the latter. Furthermore, there is an undoubted advantage in counting both the red and the white corpuscles in the same drop of the blood dilution.

(*a*) If the leucocytes are counted with the erythrocytometer, the same technic is followed as in determining the number of

erythrocytes, except that a much larger area of the counting chamber must be examined, owing to the comparatively small number of leucocytes contained in the 1 : 200 blood mixture. It is necessary, for the sake of accuracy, to count the leucocytes in the entire space inclosed by Zappert's ruling, *and to repeat the count in a second drop*, making an area equal to eighteen times the ruled space of the old-style counting chamber to be examined. If the totals of both counts are approximately the same, their combined figures, representing the corpuscles found in a space corresponding to 7200 of the small ruled squares, are taken as a basis for the final estimate; but if these totals differ widely, a third drop is to be examined in the same manner, and, as in counting the erythrocytes, an average taken of the two totals which are nearest alike. Since in normal blood, in a 1 : 200 dilution, each block of 400 small squares contains from 3 to 6 leucocytes, the examination of the above-mentioned area of the counting chamber involves the counting of approximately from 54 to 108 of these cells—an operation which, practically, is not nearly so laborious as it appears from the description, being easily completed within ten or fifteen minutes in most cases.[1]

As an example of the method of calculating the final estimate, supposing that 90 leucocytes have been counted in the area equal to 7200 small squares, the blood dilution being 1 : 200, this formula is employed:

$$90 \times 200 \times 4000 \div 7200 = 10{,}000 \textit{ leucocytes per c.mm.}$$

If the old-style counting chamber is used, the leucocytes in the unruled portion of the disc outside of the central block of 400 squares may be counted with the aid of an eye-piece diaphragm, which, when adjusted inside the tube of the ocular, cuts off a field exactly the size of 100 small squares (Fig. 20). A black metal or cardboard disc having a central aperture of the proper size will answer just as well for this purpose as the more expensive and elaborate mechanical eye-piece devised by Ehrlich, which is provided with a diaphragm having a square opening the size of which is regulated by a small lever. Having first counted all the leucocytes in the 400 small squares, the cells are then counted in 32 of the diaphragm-fields outside the latter, in order to cover

FIG. 20.—OCULAR DIAPHRAGM.

[1] Should the leucocytes be decidedly increased, it is unnecessary to cover so large a number of squares. One hundred cells taken as a basis for the calculation will give an accurate estimate.

an area of the disc corresponding to the entire ruled surface of the Zappert counting chamber. This operation having been repeated in a second drop, the totals of both counts are taken as the basis for the final calculation, which is made in the manner already described.

If one happens to have neither an eye-piece diaphragm nor a Zappert counting chamber, the following method of calculating the cubic contents of the portions of the disc outside the ruled area may be adopted, as advised by Stengel.[1] Using, for example, a $\frac{1}{5}$-inch objective and a 1-inch ocular, the ruled lines are brought into focus, and the tube of the microscope drawn out until one of the parallel lines of the ruled disc exactly coincides with either boundary of the field of vision. Assuming that 8 of these parallel columns, each $\frac{1}{20}$ mm. in width, are included in the visual field, the diameter of the latter is therefore $\frac{8}{20}$, or $\frac{2}{5}$ mm., and the radius one-half of this figure, $\frac{8}{40}$, or $\frac{1}{5}$ mm. The area of the field may then be readily determined by multiplying the square of its radius by 3.1416. Its cubic contents are obtained by also multiplying by $\frac{1}{10}$ mm., the formula being:

$$\tfrac{1}{5} \times \tfrac{1}{5} \times \tfrac{1}{10} \times 3.1416 = 0.0125664, \textit{ cubic contents of the visual field.}$$

Having in this manner ascertained the cubic contents of each field of vision, the final calculation of the number of leucocytes to the c.mm. of undiluted blood is made by multiplying the total number of these cells found in a definite number of fields (for instance, 50) by the degree of dilution (usually 1 : 200), and then by dividing the cubic contents of each field (0.0125664) multiplied by the number of fields examined. The formula for this calculation is:

*Total number of leucocytes counted* × *Degree of dilution* ÷ *Cubic contents of visual field* × *Number of fields examined* = *Total number of leucocytes per c.mm.*

For example, in a 1 : 200 blood dilution a total of 30 leucocytes is noted in fifty fields, each having a cubic contents of 0.0125664, since they individually include 8 parallel columns of the ruled disc:

$$(30 \times 200) \div (0.0125664 \times 50) = 9550 \textit{ leucocytes per c.mm.}$$

(*b*) If the special leucocytometer is used for counting the leucocytes, a 0.3 per cent. aqueous solution of glacial acetic acid must be employed as a diluent, in order to render invisible the erythrocytes and at the same time to make the leucocytes appear

[1] "Twentieth Century Practice of Medicine," New York, 1896, vol. vii, p. 271.

more conspicuously in the field. A 1 : 10 dilution is made by drawing the blood up the capillary tube of the instrument until the mark 1 is reached, and by then adding the diluent until the mixture reaches the mark 11. The leucocytes are then counted in an area of the counting chamber equal to 800 of the small squares (preferably by examining 400 squares in two separate drops), and the calculation made according to the method previously described. For instance, if in a given case 130 leucocytes were counted in 800 squares, the estimate would be made as follows:

$$130 \times 4000 \times 10 \div 800 = 6500 \textit{ leucocytes per c.mm.}$$

The chief objection to this method of leucocyte counting lies in the difficulty in distinguishing the cells, owing to the unavoid-

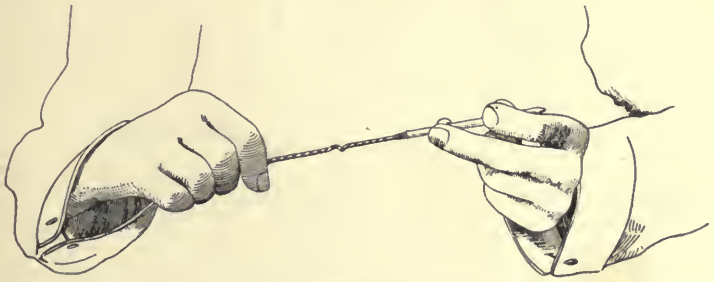

FIG. 21.—EXPELLING CONTENTS OF ERYTHROCYTOMETER.
By twisting the rubber suction tube into a tight spiral rope the fluid in the bore of the pipette may be forcibly expelled in a fine jet.

able presence in the field of masses of granular débris resulting from the action of the acetic acid solution upon the erythrocytes. For this reason, if for no other, it seems advisable to dispense with the leucocytometer, and to make the count of both red and white corpuscles with the erythrocytometer in the same drop.

*Cleaning the Pipette.*—As soon as the count has been finished, the pipette should be carefully cleaned and dried. Having first expelled what remains of the blood dilution, the instrument is rinsed out, first with distilled water and then with a mixture of equal parts of absolute alcohol and ether, the latter being used to remove all traces of the dye, in case either Toisson's or Sherrington's solution has been employed as a diluent, as well as to dry the interior of the tube. The pipette, while it may be filled with a fluid by suction, should not be emptied by blowing through it, for if this is done, a certain amount of moisture from the breath unavoidably becomes deposited in its lumen. Its contents may be

expelled in the form of a fine jet, simply by twisting the rubber suction tube into a tight spiral rope, as shown in the illustration (Fig. 21). When the interior of the instrument is perfectly clean, it is dried by forcing through it a current of air by means of a rubber atomizer bulb, or an ordinary bicycle-pump, until the glass bead no longer clings to the wall of the bulbous expansion, as it will as long as the slightest trace of moisture remains.[1]

DURHAM'S HEMOCYTOMETER.

A new form of hemocytometer has been recently designed by Durham, who has embodied in this device the principles of the older instruments, together with the substitution of a self-measuring pipette designed to overcome the sources of error which may occur in making blood dilutions with a suction pipette. Durham's instrument, which appears to be a valuable improvement over other forms of blood-counting apparatus, consists of the following parts:

1. Several *capillary pipettes* of the Oliver type, each mounted

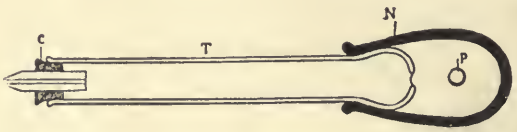


FIG. 22.—CROSS-SECTION OF DURHAM'S BLOOD PIPETTE.
T, Glass tube; N, rubber nipple; p, lateral perforation in nipple; c, cork in which a capillary pipette is fitted.

in a glass tube, provided with a rubber nipple having a lateral perforation. The capacity of the pipettes is 5 and 10 c.mm.

2. A number of *mixing vessels*, each consisting of a small glass test-tube, graduated from 1 and for 0.5 c.c. of fluid. The tubes holding 1 c.c. measure $2\frac{3}{8} \times \frac{7}{16}$ in., and those holding 0.5 c.c., $2\frac{3}{8} \times \frac{3}{8}$ in. One or more glass beads are shaken about in the tube to mix the blood and the diluting fluid.

3. A number of *graduated pipettes* for measuring the diluting fluid, of 1 and 0.5 c.c. capacity, marked at 995 and 990 c.mm. and at 495 and 490 c.mm., respectively. Used with the appropriate capillary pipette, dilutions of 1:200, 1:100, and 1:50 may be obtained.

4. A *counting chamber* of the Thoma-Zeiss pattern.

*Method of Use.*—Having placed in one of the mixing vessels some of the diluting fluid, the quantity of which is measured with

[1] A 0.1 per cent. solution of pepsin in one per cent. hydrochloric acid is useful for removing any bits of clotted blood which may adhere to the caliber of the instrument.

one of the graduated pipettes according to the dilution desired, the capillary pipette is filled with blood by touching it lightly to the blood drop as it flows from the puncture. All traces of blood are then removed from the outside of the pipette, the contents of which are now expelled into the fluid contained in the mixing vessel. This is accomplished by inserting the pipette into the latter, keeping its point about half an inch above the level of the diluting fluid, and by then rotating it between the thumb and forefinger so that the lateral perforation is brought under the ball of the thumb; the nipple is now squeezed gently, and, continuing the pressure, the pipette is rotated back so that the perforation is free again. In this manner the blood is forced from the pipette but is not sucked back. The blood remaining in the pipette is now completely washed away by thrusting its point into the diluting fluid, this at once filling its caliber, by capillarity. Withdrawing the pipette from the fluid, the rotation and pressure of the nipple are repeated, the capillary tube being thus rinsed out several times in order to remove completely all the blood clinging to its interior.

The blood and the diluting fluid are now mixed by briskly rotating the mixing vessel between the opposed hands, so that the tumbling about of the glass beads in the vessel may thoroughly distribute the cellular elements through the fluid. When the mixing is completed, a drop of the fluid is transferred to the counting chamber, and the corpuscles counted under the microscope in the usual manner.

Durham's device makes it possible for the unskilled to measure accurately the desired volumes of blood and diluting fluid, and largely eliminates the errors which are likely to occur in sucking up the blood and the diluent with either the Thoma-Zeiss or the Gowers hemocytometer. The ease and thoroughness with which the capillary blood pipette may be cleaned are also advantages, this being done by passing through its caliber a piece of darning-cotton, dry or soaked in ether, by means of a needle. Comparative observations made with the Thoma-Zeiss hemocytometer have shown that the readings of the two instruments are practically identical.

GOWERS' HEMOCYTOMETER.

In this form of hemocytometer the blood and the diluting fluid are each measured in a separate pipette, and deposited in a small receptacle, in which they are mixed, a small portion of the mixture then being placed in a counting chamber and the number of corpuscles counted under the microscope. Gowers prefers to use as a diluent an aqueous solution of sodium sulphate having a specific gravity of 1.025, but Toisson's solution, or any of the

other diluting fluids previously mentioned, will prove satisfactory. The instrument comprises five working parts, as follows:

1. A *pipette*, graduated to hold a volume of 995 c.mm., for measuring the diluting fluid.

2. A *capillary pipette*, graduated to hold a volume of 5 c.mm., for measuring the blood.

3. A small *glass mixing jar*, in which the dilution of the blood is made.

4. A *glass stirring rod*, for mixing the blood and the diluent in the jar.

5. A *counting chamber*, consisting of a glass slide mounted on a brass plate, and containing a cell $\frac{1}{5}$ mm. in depth, the floor of the cell being divided by cross-rulings into squares the sides of which measure $\frac{1}{10}$ mm. When a cover-glass is fitted over this cell, being retained in position by means of a pair of clips attached to either end of the brass plate, the cubic contents of the space overlying each square measure $\frac{1}{5\,0\,0}$ c.mm.

*Method of Use.*—In using the instrument 995 c.mm. of the diluting solution are first measured by means of the larger pipette and blown out into the mixing jar. The latter must be perfectly clean and absolutely free from moisture before it is used, in order to avoid errors in the count. Now, using the capillary blood pipette, 5 c.mm. of blood are secured from the puncture, and immediately added to the diluent contained in the jar. The blood and the diluent are then thoroughly mixed, by rapidly stirring the solution with the glass rod. The dilution thus made is in the proportion of 1:200 of blood to diluent. As soon as the mixture is completed, a small drop of the solution is transferred to the center of the cell in the middle of the counting chamber, the small end of the glass rod being used for this purpose, after which the cover-glass is gently placed in position, and the clips adjusted so as to hold it in place. The counting chamber may then be placed upon the stage of the microscope, and the corpuscles overlying the ruled portion of the cell brought into focus with a low-power objective.

It is necessary to use a small drop of the diluted blood, and to place it exactly in the center of the block of ruled squares, otherwise the fluid may flow toward the walls of the cell, altering its volume and making it necessary to reject the specimen and to prepare a new drop, after thoroughly cleaning and drying the cell, and again stirring the blood solution.

The corpuscles having settled to the bottom of the cell, their number in a given number of squares is noted, and the final calculation made according to the formula:

$$\frac{\text{Number of corpuscles counted}}{\text{Number of squares counted}} \times 200 \times 500 = \text{Total number of corpuscles per c.mm.}$$

In counting the erythrocytes at least 20 squares of the counting chamber should be inspected, in different drops, a procedure involving the enumeration of about 1000 cells, in normal blood. Except in high leucocytoses, the number of leucocytes is usually estimated indirectly, by determining their ratio to the erythrocytes, and basing their actual number upon this figure. This plan (the necessity for which is a serious drawback to the use of this instrument) is followed so as to dispense with the tedious filling and refilling of the counting chamber, in an endeavor to find a sufficient number of leucocytes to serve as a basis for the calculation, should the latter be direct. Ordinarily, not more than two of these cells are contained in an area including 20 squares. Gowers [1] claims that the limit of error with his instrument is less than 3 per cent.

After use, the different parts of the instrument are to be thoroughly cleaned and dried, in the manner already described.

OLIVER'S HEMOCYTOMETER.

For making rapid numerical estimates of the erythrocytes Oliver has designed an instrument based upon the following principles: When a candle-flame is viewed through a flat glass test-tube filled with water, a bright transverse line is visible, composed of densely packed, minute images of the flame produced by the longitudinal corrugations of the glass. If for the water a mixture of blood and Hayem's solution [2] is substituted, a more or less opaque fluid results, so that, in low dilutions, this illuminated line is invisible, reappearing only when a definite degree of higher dilution is reached, by the gradual addition of the diluent; when this point has been obtained, the line is again detected as a bright, delicate streak horizontally crossing the tube. Experiments having proved that the development of such a line, by gradual dilution of the blood with Hayem's fluid, is an accurate gage of the percentage of erythrocytes in the specimen tested, it remained for Oliver to devise a hemocytometer consisting of the following essential parts:

1. A *capillary pipette* for measuring the blood.

2. A *glass dropper*, one end of which is capped by a rubber nipple, the other by a short rubber nozle which fits over the blunt end of the pipette.

3. A *standard graduated tube*, in which the blood and the diluent are mixed.

[1] Lancet, 1877, vol. ii, p. 797.

[2] For formula see page 56.

The four walls of the tube are flattened so that it is rectangular on cross-section, one wall being provided with an etched scale indicating units from 10 to 120. Each of these divisions is equivalent to 50,000 erythrocytes, the point marked 100 degrees representing the arbitrary normal number, 5,000,000.

Small-sized wax candles, known as "Christmas candles," are to be preferred for the illumination, as they give the small flame requisite to obtain a sharply defined line, but the flame from a gas-jet turned low may also be used with satisfaction.

*Method of Use.*—In making the observation the pipette, which has been previously cleaned and dried, is filled with blood in the usual manner, and any excess of blood on the outside carefully removed. The rubber nozle of the dropper, filled with Hayem's fluid, is then slipped over the blunt end of the pipette, and the blood washed out into the graduated tube by squeezing the nipple. This preliminary dilution is continued until the column in the tube rises to within 10 or 15 degrees below the figure for the hemoglobin percentage of the same blood, this having been previously determined. For instance, if the hemoglobin percentage was found to be 70, the diluting fluid is added in large quantities until the mixture in the tube reaches to about the mark 60, after which it is added more cautiously and in smaller quantities at a time, careful search for the bright line being made after each addition. In cases of chlorosis and of pernicious anemia, in which parallelism between the hemoglobin and corpuscular loss is lacking, it is, of course, impossible to depend upon the hemoglobin percentage as an index to the amount of diluent required, so that in instances of this kind the line must be developed more slowly, by making a smaller primary dilution and by adding the requisite volume of liquid more deliberately.

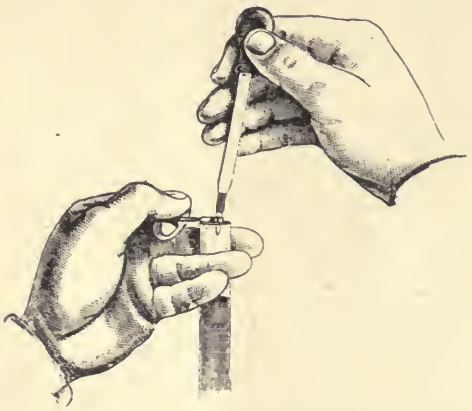

FIG. 23.—METHOD OF USING OLIVER'S HEMOCYTOMETER. Showing manner in which the blood is washed from the capillary pipette into the tube containing Hayem's solution.

After the first dropperful of diluent has been added to the contents of the tube, the latter are mixed by inverting the tube a

number of times with the thumb held over its mouth, the precaution being taken also to remove the thumb by drawing it over the mouth of the tube, in order to restore to its contents any liquid which may have adhered to the skin. The tube should be inverted thus after each addition of the diluting fluid.

The steps of the observation succeeding the measuring of the blood and its primary dilution are to be made in a dark room, free from cross lights, the candle being placed about ten feet distant from the observer. In order to shut out the diffused light of the candle the tube should be held vertically in the concavity formed between the thumb and forefinger, being kept close to the eye while searching for the bright line. Oliver states that the earliest indications of this line are obtained by turning the tube on its axis, when it will become visible at the sides.

Apart from the "personal equation," the serious drawback to this test is its failure to indicate the number of leucocytes, this fact alone being sufficient to curtail its use for routine clinical work. It is also apparent that in cases of marked leucocytosis and of leukemia the optical principles of the test must necessarily fail because of the enormous number of leucocytes in the blood. Furthermore, the instrument gives false results with blood in which conspicuous deformities of the erythrocytes exist, for the reason that the standard tube is corrected for normally shaped corpuscles, so that blood composed largely of microcytes, megalocytes, or poikilocytes will give different readings from blood in which the cells are of unaltered biconcave shape and of normal size. The discrepancies between the Oliver and the Thoma-Zeiss instruments have been carefully worked out by Emerson[1] and by Baumgarten.[2]

Dry Film Method.

This method, devised by Strong and Seligmann,[3] aims to eliminate errors due to variations in the depth of the Thoma-Zeiss counting chamber, and at the same time to furnish permanent specimens which may be examined at any subsequent time. It is practically a simplification of the method suggested by Einhorn and Laporte.[4] The principle involved consists in diluting a measured volume of fresh blood with a measured volume of a diluent with which a suitable differential stain is combined, a definite quantity of the mixture then being spread upon a glass slide, allowed to evaporate, mounted in the ordinary manner, and examined microscopically.

[1] Johns Hopkins Hosp. Bull., 1903, vol. xiv, p. 9.
[2] *Ibid.*, 1902, vol. xiii, p. 176.
[3] Brit. Med. Jour., 1903, vol. ii, p. 74. [4] Med. News, 1902, vol. lxxx, p. 741.

Two different diluting fluids are used, one for the leucocytes and one for the erythrocytes. Tabloids (made by Parke, Davis and Company) containing 0.25 gm. of sodium chlorid combined with 0.004 gm. of methyl-violet (for leucocytes) and with 0.0025 gm. of eosin (for erythrocytes) are employed for making the diluents, instead of stock solutions, which become unstable after a short time. To prepare the diluents one of each of these tabloids is dissolved in 30 c.c. of distilled water to which 0.5 c.c. of formalin is added, the mixture then being filtered.

*Counting the Leucocytes.*—Five c.mm. of blood are sucked up into a graduated pipette, and then blown out into a small vessel containing 495 c.mm. of the methyl-violet diluent. After stirring, 5 c.mm. of this 1:100 mixture are drawn up in a pipette and deposited upon the surface of a glass slide so as to form a film about 10 or 12 mm. in diameter. The film thus made is allowed to evaporate and then mounted with balsam and a cover-glass.

The count is made by going over the entire area of the film with a $\frac{1}{6}$-inch dry objective, and noting the number of violet-stained cells. In order to facilitate this procedure, either a square or an oblong ocular diaphragm, made of black paper or of metal, should be used, together with a mechanical stage. The blood dilution being 1 : 100 and the volume of this mixture spread upon the slide being 5 c.mm., the number of leucocytes per c.mm. of undiluted blood is therefore $100 \div 5$, or 20 times the total number counted in the entire film. For example, having noted 400 leucocytes in the latter, the simple formula $400 \times 20 = 8000$ leucocytes per c.mm., gives the final calculation. An error of 50 cells in the count alters the final result by only 1000 cells—a trivial matter.

*Counting the Erythrocytes.*—In this instance a 1:20,000 dilution is made, by mixing 5 c.mm. of the above 1:100 blood and methyl-violet diluent with 995 c.mm. of the eosin diluent. After allowing the erythrocytes to stain for a few minutes, 5 c.mm. of this mixture are spread over a slide, as described above, similarly mounted, and examined microscopically, the erythrocytes being recognized by their rose-red color. The number of erythrocytes per c.mm. of undiluted blood is 4000 times the number counted in the dry eosined film, since 5 c.mm. of a 1:20,000 dilution of the blood has been used; thus, $20{,}000 \div 5 = 4000$. For instance, having counted 1200 erythrocytes in the 5 c.mm. film, the formula $1200 \times 4000 = 4{,}800{,}000$ erythrocytes per c.mm., gives the final result.

The originators of the dry film method of blood counting insist that its results are more accurate than are possible with a

hemocytometer. In three Thoma-Zeiss instruments, three years old, they found figures consistently ten per cent. higher than those obtained by new instruments of the same design and make, an error which could be explained by an increase of 0.01 mm. in the depth of the counting chamber. Counts made by the dry method averaged 1.1 per cent. lower than those in which the hemocytometer was used. Several sources of error, however, must be guarded against. For example, in making the dilutions, unless every trace of the measured 5 c.mm. of whole blood is blown from the pipette into the diluting fluid, the dilution will be too high; and if, in making the film, the diluted blood is blown out too forcibly, the film will be scattered and ragged and stippled with air-bubbles. The erythrocytes may stain violet instead of rose should the diluting solution not be perfectly fresh. The leucocytes, which may stain violet, usually take the color of eosin, with the eosin dilution for erythrocytes. Thus, the former are unconsciously counted with the latter, but under ordinary circumstances this is an unimportant error, since the number of leucocytes is comparatively too small appreciably to affect the erythrocyte count. In leukemia, however, the error may be sufficiently great to need correction. In this disease, therefore, all the blood cells, red and white, should be counted, multiplied by 4000, and from this total is to be subtracted the total leucocyte estimate previously determined, the result being obviously the total number of erythrocytes per c.mm. Unfortunately, the specimens prepared by Strong and Seligmann's technic are marred by deposits of salt crystals and by masses of amorphous eosin, both at the edges of the film and scattered throughout it. This defect, if it does not render an accurate count impossible, may at least make it difficult and tedious. In a passably good specimen the counts of erythrocytes and leucocytes together should not take longer than half an hour.

## IV. MICROSCOPICAL EXAMINATION OF THE STAINED SPECIMEN.

Objects of Staining.

The microscopical study of the dried and stained blood film, which should supplement the methods of investigation just described, is for many reasons the most important step in the clinical examination of the blood. By means of this method of "color analysis" it is possible to differentiate easily and with absolute certainty the various forms of leucocytes, and, by differential counting, to calculate the relative percentages of each

variety of these cells; to distinguish the several structural degenerative changes affecting chiefly the erythrocytes, and to a less extent the leucocytes; and to recognize and classify according to their histological character the nucleated forms of the erythrocytes. To sum up, in the words of Ehrlich,[1] to whom we owe this rational means of investigation: "Everything that is to be seen in the fresh specimens—apart from the quite unimportant rouleaux formation and ameboid movements—can be seen equally well, and indeed much better, in a stained preparation; and there are several important details which are made visible only in the latter, and never in wet preparations."

THE ANILIN DYES.

According to the classification introduced many years ago by Ehrlich,[2] the anilin dyes are divided into three different groups: acid, basic, and neutral. Acid dyes, or compounds in which the coloring principle acts or exists as an acid, possess a special affinity for cell protoplasm, and, therefore, are generally employed as plasma stains; in hematological work acid fuchsin, eosin, and orange G are the principal dyes used for this purpose. Basic dyes, or compounds in which the coloring principle exists chemically as a base in combination with a colorless acid, are especially useful as nuclear stains, since they exhibit a special affinity for chromatin structures; members of this group of dyes commonly used in blood staining are methylene-blue, toluidin-blue, methyl-green, methyl-violet, thionin, and hematoxylin. Neutral dyes are the coloring principles which result from the mixture of solutions of an acid and a basic dye; they are used for the demonstration of the so-called neutrophile granules of the leucocytes, for which they show a selective affinity.

PREPARING THE FILMS.

*Cover-glass Films.*—For the preparation of the dried blood films it is advisable to have at hand at least half a dozen perfectly clean, polished cover-glasses, which may be arranged in pairs on a sheet of white paper within convenient reach of the examiner. After having wiped away the blood which immediately follows the puncture, a minute portion of the next drop is collected, by lightly touching the center of one of the cover-glasses to its summit, care being taken to avoid bringing the polished surface of the glass in contact with the skin of the patient's finger. The charged cover-glass is then at once dropped, blood side downward, upon the

[1] Ehrlich and Lazarus, "Die Anaemie," Vienna, 1900 (Nothnagel's "Spec. Path. u. Therap.," vol. viii, No. 2).

[2] Zeitschr. f. klin. Med., 1880, vol. i, p. 555.

surface of the second glass (Fig. 24), with the result that the blood quickly spreads out in a thin film between the two, and extends to their peripheries, provided that the proper quantity of blood has been used (Fig. 25). As soon as the film has reached the margins of both cover-glasses, they are rapidly drawn apart in a horizontal direction, so that the surface of each, when thus separated, is covered with a thin layer of blood (Fig. 26), which should be rapidly dried, either by blowing briskly upon its surface or by holding the glass for a few seconds high over the flame of an alcohol lamp. If care is taken to use but a very small drop of blood, to avoid pressure in opposing the surfaces of the two cover-glasses, and to separate them in their true horizontal planes, the films will consist of a single layer of corpuscles, most of which will be sufficiently isolated to allow the study of their individual morphology and other characteristics. The beginner should persistently practise the technic of film-making until he is able to obtain a satisfactory percentage of good specimens from every batch of spreads. Thick, uneven spreads, in which the corpuscles are heaped up and glued together in dense masses, are of no value for histological study of the cells, although they are often useful when searching for parasites in blood containing very few organisms. In such instances Ross[1] advises smearing the blood thickly on the slide, and, after it has dried, washing out the hemoglobin with water. The film thus dehemoglobinized, unfixed, and still wet, is then stained with aqueous solutions of eosin and methylene-blue, washed with water, and mounted. The parasites stain the color of the basic dye, but the erythrocytes, since they contain no hemoglobin, are practically colorless. This method, while, of course, unsuitable for differential counting, may be of value in certain cases of malarial fever, filariasis, and trypanosomiasis.

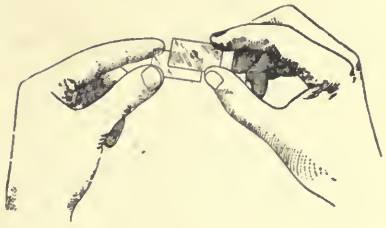

FIG. 24.—SUPERIMPOSING THE CHARGED COVER-GLASS.

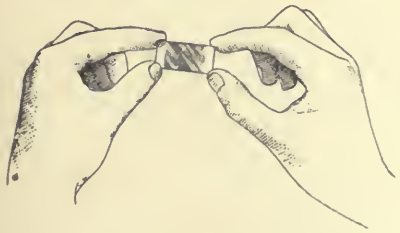

FIG. 25.—DRAWING APART THE COVER-GLASSES.

[1] Thompson Yates and Johnston, Lab. Rep., 1903, vol. v, p. 117.

The films, after having been dried, may be placed in a pill box and labeled, to await fixation and staining at the examiner's convenience. Dried specimens will keep for an indefinite period if not exposed to dust or to moisture. Unfixed cover-glass specimens of leukemic blood have been "triple-stained" by the writer, with perfect results, more than three years after they were spread. With the Romanowsky method, however, the fresher the specimen, the sharper the stained film.

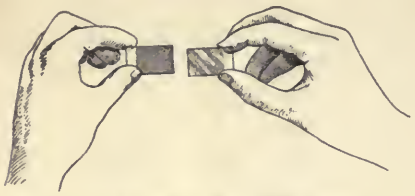

FIG. 26.—THE COVER-GLASSES AFTER SEPARATION.

Many histologists recommend the use of special forceps for holding the cover-glasses while making the spreads, claiming thus to avoid the injurious effects upon the blood corpuscles which may be caused by the moisture of the fingers if they come in con-

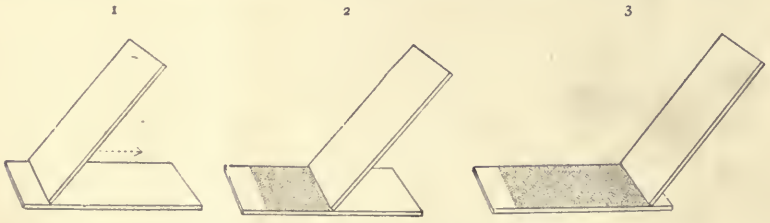


FIG. 27.—SPREADING A FILM WITH TWO GLASS SLIDES.

tact with the films. The careful worker need have no fear on this score, for if the covers are held in the manner shown in the illustrations, this accident will not occur. A pair of light thumb forceps is useful for picking up the cover-glasses from a flat surface, but the employment of special spreading forceps is quite unnecessary.

FIG. 28.—SPREADING A FILM WITH CIGARETTE PAPER.

*Glass Slide Films.*—Some prefer to make the spread upon an ordinary glass slide, but this method rarely yields as thin and even a film as the one just described, though it is easier to learn. A small-sized drop of blood is distributed along the edge of a glass slide,[1] which is then held at an angle of 45 degrees against the surface of another slide,

[1] Waldstein's smearing slip (Med. Rec., 1896, vol. l, p. 385), made of crown glass, with its "spreading edge" ground smooth and round, answers much better than an ordinary slide.

over which it is rapidly drawn, with moderate pressure, thus depositing a thin film of blood upon the surface of the latter (Fig. 27). Instead of a glass slide, a leaf of cigarette paper or a slip of thin tissue-paper, trimmed to a narrower width than that of the slide, may be used as a spreader (Fig. 28). A fair spread may also be made by depositing a drop of blood upon a slide, near one end, and then distributing it by means of a needle or a glass rod, the shaft of which is applied to the drop with even pressure. The film thus made is immediately dried in air and treated as a cover-glass spread.

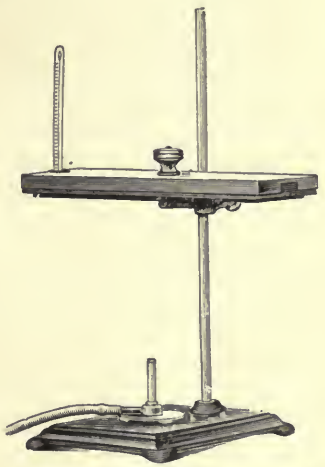

Fig. 29.—Oven for Fixing Blood Films.

Fixation Methods. As a step preliminary to staining, the albuminoid principles of the blood must be fixed, by exposing the dried film either to a high degree of dry heat or to various chemical hardening agents, the choice between these two methods being determined by the character of the staining solution to be used subsequently.

*Heat Fixation.*—This method may be employed with any of the stains described in the following pages, except with Wright's solution; it *must* be used with Ehrlich's triple stain, in preference to fixation by chemicals, in order to obtain crisp, clean-cut pictures.

The author is accustomed to use an oven, such as is illustrated above (Fig. 29), consisting of a copper box with a heavy bottom and hinged cover, mounted on an ordinary iron burette stand, by means of a thumb-screw. A small "baby" Bunsen lamp placed underneath the box furnishes the requisite degree of heat, the temperature being indicated by a thermometer mounted at one end and resting upon the floor of the oven. By sliding the latter up and down the vertical rod to which it is attached the desired degree of temperature may be obtained at will. The blood films are inclosed in the copper box, and the latter fixed at a point eight inches above the summit of the burner, after which the gas is lighted and allowed to burn until the temperature, as indicated by the thermometer, has gradually crept up to 160° C. As soon as this degree of heat has been reached the gas is extinguished, the cover of the oven thrown back, and, after the temperature has fallen to 30° C., the films

removed, being now thoroughly fixed and ready for staining. Fifteen minutes suffice for the whole operation, from the time the gas is lighted until the films have been removed and cooled, for staining.

A less satisfactory method of heat fixation is by the use of a copper plate upon which the films are kept at a temperature of from 100° to 110° C. for from one-half to one hour. The apparatus used for this purpose consists of a rectangular plate of sheet copper, about fifteen inches long by four inches wide by one-sixth of an inch thick. An alcohol or a Bunsen lamp burns under one end of the plate, which is elevated about six inches above the flame by four metal legs. After having heated the plate for ten or fifteen minutes, until a relatively constant temperature becomes established, water is dropped upon its surface, beginning with the end farthest from the flame, until a point is reached at which the water boils. This part of the plate is considered to have a temperature of 100° C., and at this point the blood films are placed, "spread" side downward, and heated for the required time. No one with much blood staining to do will choose this method of prolonged heating at a relatively low, approximate temperature in preference to brief heating at a high, definite temperature in an oven. The use of the latter, aside from its convenience as a time-saver, insures constant and certain results, for overheating and underheating of the blood film may be avoided, since the degree of heat is exactly indicated and easily controllable. In triple-stained specimens the blood cells are much more brilliantly colored and sharply differentiated when the films are fixed at a temperature of 160° C. than at a lower degree.

Should nothing but a Bunsen or an alcohol lamp be available, the cover-glass film, held with a pair of forceps, may be fixed by passing it rapidly through the flame thirty or forty times and then holding it twelve or fifteen inches above the flame for a minute or so. This makeshift method, which is often sufficient for a hurried clinical examination, usually gives fair, and sometimes very good, results, but the fixation is generally uneven, and the specimen is frequently scorched in some places and underfixed in others.

*Chemical Fixation.*—Immersion of the dried films in ether, in absolute alcohol, or in a mixture of equal parts of the two (Nikiforoff's method) gives satisfactory results with specimens stained by any of the single basic dyes, or with the simpler double stains, such as eosin and methylene-blue or hematoxylin. The time of fixation varies from five to fifteen minutes with any of these agents, the specimen then being dried without using heat and stained

without previously washing. If time is an object, the specimens may be boiled for one minute in a test-tube containing absolute alcohol, as advised by Ehrlich.[1] Some workers employ one minute's fixation by a one per cent. alcoholic solution of formalin (Benario's method), while others prefer to expose the films to the vapors of this chemical for the same length of time. Five to ten minutes' immersion in a concentrated aqueous solution of mercuric chlorid is one of the older, but useful, methods of fixation. Solley[2] has recently suggested that the film be flooded with a two per cent. aqueous solution of chromic acid, which is washed off after exactly thirty seconds, the specimen being then stained while still wet; he recommends this procedure as a substitute for heat in fixing specimens for triple staining, but the method, while fairly good, cannot be regarded as entirely satisfactory. In the author's hands both Merck's methyl alcohol (five minutes) and a two per cent. aqueous solution of osmic acid (half a minute) have been found to be fair substitutes for heat fixation.

METHODS OF STAINING

In hematological as in other histological work the choice of a staining method is determined by the character of the investigation to be undertaken. For general clinical purposes it is advantageous habitually to employ some routine method by means of which the greatest possible number of elements may be demonstrated in a single blood film, this procedure being known as panoptic staining. Thus, by using a solution containing several of the anilin dyes, the stroma of the erythrocytes, the cell granules, the cell nuclei, and the various blood parasites may be simultaneously stained each in a characteristic manner, owing to the selective affinity displayed by the different coloring principles of the mixture toward these several histological elements. The most useful solutions which have been devised for this purpose are Wright's alkaline eosinate of methylene-blue[3] and Ehrlich's triacid mixture.[4] Practically all the information that it is possible to derive from the study of the stained dry blood film may be obtained with the aid of these two solutions.

Simple combinations of an acid and a basic dye, such as eosin and methylene-blue, eosin and hematoxylin, and orange and hematoxylin, are used by many investigators, chiefly for the purpose of staining the cell stroma and the nuclear structures; but, as a general rule, such mixtures are not adapted for clinical work,

[1] *Loc. cit.*

[2] Med. and Surg. Reports of the Presbyterian Hospital, New York, 1900, vol. iv, p. 169.

[3] Jour. Med. Research, 1902, vol. vii, p. 138.

[4] *Loc. cit.*

since with none of them is it possible to differentiate the neutrophile granules. Solutions of a single dye are but seldom used except for the demonstration of special elements, such, for instance, as the staining of the malarial parasite by thionin, the mast cells by dahlia, and certain bacteria by the basic dyes, such as methylene-blue and gentian-violet. Since by this method of staining only the particular elements toward which the dye reacts are differentiated, the employment of single stains is inadequate for the study of the general morphology of the blood cells.

The following formulas will be found sufficient for all purposes of clinical investigation:

WRIGHT'S STAIN.—This excellent stain is an improvement on Leishman's modification[1] of the late Louis Jenner's solution,[2] and is so prepared that its basic constituent (methylene-blue) acquires the polychromatic properties of the Romanowsky stain,[3] so valuable in differentiating chromatin and cytoplasm. Wright's stain gives sharp pictures of cell protoplasm, chromatin, and nuclei, and is especially useful in studying the lymphocytes, the mast cells, the blood plaques, and the finer structure of the malarial parasite. It is prepared according to the following somewhat complicated formula:

1. To a 0.5 per cent. aqueous solution of sodium bicarbonate contained in an Erlenmeyer flask add one per cent. of Grübler's methylene-blue ("medicinal"), and steam the mixture in a sterilizer for one hour, counting from the time "steam is up."[4] This step not only serves to develop the polychromatic property of the alkaline methylene-blue, but increases its power as a nuclear and granular stain.

2. Cool the bicarbonized methylene-blue solution after steaming, and then, without filtering, add to it, meanwhile stirring with a glass rod, sufficient of a 1:1000 aqueous solution of Grübler's yellowish eosin ("water soluble") to change the color of the solution from blue to purple, with a surface scum of a yellowish, metallic luster. About 500 c.c. of the methylene-blue solution to 100 c.c. of the eosin solution are required to produce this change.

[1] Brit. Med. Jour., 1901, vol. ii, p. 757. [2] Lancet, 1899, vol. i, p. 370.
[3] Centralbl. f. Bakt., 1899, vol. xxv, p. 764.
[4] When a steam sterilizer is not available, polychrome blue may be developed by heating the methylene-blue solution with freshly precipitated silver oxid. Daniels ("Studies in Laboratory Work," London, 1903, p. 63) directs that a solution of sodium hydrate be added to one of silver nitrate until no more precipitate forms, the precipitate, silver oxid, being then washed until the washings are neutral to litmus-paper. This neutral precipitate is added to the methylene-blue solution and allowed to stand for twenty-four hours, when the supernatant fluid is decanted off from the sediment and filtered before using. Polychrome blue thus prepared is treated according to the above directions for making Wright's solution.

3. Collect, by filtration, this scum (which consists of a granular black precipitate), and dry, without washing. When dry, make of it a saturated solution in methyl alcohol. Three-tenths of a gram of the dry precipitate saturates 100 c.c. of methyl alcohol.

4. Filter the alcoholic solution of the precipitate and add to the filtrate 25 per cent. of methyl alcohol. For 80 c.c. of the filtrate, the amount usually available, 20 c.c. of methyl alcohol are required. This alcoholic solution is used for staining. It does not deteriorate with age if kept in a well-corked bottle, and is sufficiently dilute to prevent precipitation during staining, an accident which was the chief drawback to Jenner's original stain.

*Technic of Staining.*—Owing to the methyl alcohol which it contains, Wright's solution fixes and stains the blood film simultaneously, so that preliminary fixation may be omitted.

The *unfixed* film is stained for one minute, as much of the solution being used as the cover-glass will hold without spilling. Next, to the staining fluid upon the specimen are added, drop by drop, eight or ten drops of distilled water—sufficient to develop a reddish tint at the margins of the cover-glass and a semitranslucency in the stain, with a metallic scum on the surface. The stain, thus diluted, is allowed to act for two or three minutes, when it is rinsed off with water, showing that the film has become stained deep blue or purplish. The final step in the process is the decolorization of the overstained specimen and the differentiation of its histological elements. This is accomplished by washing with water until the color of the film changes to yellowish or pink. From one to three minutes' washing, depending upon the intensity of the staining, is required to reach the desired tint. The specimen is then dried between filter-paper (never by heat), and mounted in balsam.

Wright's stain gives the following results: erythrocytes, orange or pink; nuclei of the leucocytes, blue or dark lilac; neutrophile granules, lilac; eosinophile granules, pink; fine basophile granules, deep blue; and coarse, mast cell granules, deep royal purple. The nuclei of the erythroblasts and bacteria stain various shades of blue, and the blood plaques purplish, flecked with red.

The body of the malarial parasite stains blue, and its chromatin varies from lilac to red to almost black.

Aside from its obvious value as a panoptic staining fluid, this solution will often prove of great convenience for the reason that it does not require special fixation of the blood film.

Ehrlich's Triacid Stain.—This "triple stain," containing one basic and two acid dyes (methyl-green, orange G, and acid fuchsin), is peculiar in that a chemical combination is formed by

its acid and basic components, which may be regarded as a neutral coloring principle, serving the purpose of selectively staining the so-called neutrophile elements for which the primary components of the mixture have no affinity. With this stain histological structures having an affinity for the acid dyes are stained the color of one of its acid constituents, basic structures the color of its basic dye, and structures having an equal affinity for acid and basic dyes the color of the neutral compound.

Saturated aqueous solutions of the three dyes are first prepared, and allowed to stand for several days until they have become thoroughly cleared. It is essential that the anilin dyes used for making these "stock" solutions should be chemically pure, to insure which the products of Grübler or of the Berlin Anilin Dye Company should invariably be chosen. From these saturated solutions the following mixture is made:

| | |
|---|---|
| Acid fuchsin solution | 6–7 c.c. |
| Orange G solution | 13–14 c.c. |
| Distilled water | 15 c.c. |
| Absolute alcohol | 15 c.c. |

Mix the above thoroughly and add, drop by drop, with continuous agitation, in the following order:

| | |
|---|---|
| Methyl-green solution | 12.5 c.c. |
| Absolute alcohol | 10.0 c.c. |
| Glycerin | 10.0 c.c. |

The mixture should under no circumstance be filtered, but allowed to stand for about twenty-four hours in order that a slight precipitate may form. As soon as this occurs the stain is ready for use, the necessary quantity being pipetted from the supernatant fluid without disturbing the precipitate.

*Technic of Staining.*—The heat-fixed film, held preferably with a pair of Stewart's staining forceps, is flooded with the stain, which is washed off in running water after the lapse of from five to eight minutes, the specimen then being dried by gentle heat and mounted in xylol balsam or in cedar oil.

In the specimen thus prepared the stroma of the erythrocytes is stained orange, the nuclei of the leucocytes greenish-blue, the neutrophile granules violet or lavender, and the eosinophile granules copper red. The nuclei of the erythroblasts react with varying degrees of intensity toward the basic component of the mixture, those of the normoblasts staining deep purple or black, and those of the megaloblasts pale green or greenish-blue. The basophile granules remain unstained, appearing as dull white, coarse, stippled areas in the cell protoplasm—"negative staining." In order to stain these granules, as well as the basic protoplasm of

the lymphocytes, Hewes[1] suggests that the triple-stained film, after having been washed, be subjected for from one-half second to ten seconds to Löffler's[2] methylene-blue solution, after which it is again washed, and mounted as above directed. This modification is of undoubted value, chiefly because it usually enables one to differentiate the larger forms of lymphocytes from the large mononuclear leucocytes. Malarial and other parasites are also distinctly stained by this method.

Unsatisfactory results with the triple stain, provided that the latter is properly made, can almost always be attributed to faulty fixation. As already remarked, heat is the only method of fixation which will insure faultless differentiation in the specimen stained with this mixture. The perfect specimen is of a deep, rich orange tint to the naked eye; if underheated, the film reacts too strongly toward the acid fuchsin of the mixture, and, consequently, is the color of this dye; if overheated, the plasma stain, orange G, is but feebly displayed, so that the color of the film is pale lemon yellow.[3] As a stain for general clinical work Ehrlich's is inferior to Wright's. Although a sharper neutrophile stain, it reacts feebly toward basophile structures, and requires careful and skilful heat fixation of the films.

Prince's Stain.—This mixture, which consists of an aqueous solution of one basic and two acid dyes, is an excellent stain for the differentiation of both nuclei and granules, and may be employed as a fair substitute for either of the two preceding solutions. It should be made in this manner:

| | |
|---|---|
| Saturated aqueous solution of toluidin blue | 24 c.c. |
| Saturated aqueous solution of acid fuchsin | 1 c.c. |
| Two per cent. aqueous solution of eosin | 2 c.c. |

These solutions are mixed in the order named, and shaken briskly for several minutes, so as to secure complete precipitation of the basic toluidin blue by the acid dyes. The solution, which should not be filtered, is ready for use as soon as made. Only the supernatant fluid should be employed, care being taken not to disturb the sediment.

*Technic of Staining.*—If a newly made solution is used, the films are stained for from one-half to one minute, after which they are rinsed in water, dried in air, and mounted; but if the solution has stood for several weeks, its basic constituent becomes less

[1] Boston Med. and Surg. Jour., 1899, vol. cxli, p. 39.

[2] Saturated alcoholic solution of methylene-blue, 30 c.c.; 1 : 10,000 aqueous solution of potassium hydrate, 100 c.c.

[3] A reliable triacid stain, made according to Ehrlich's formula, is sold by Messrs. Shinn and Kirk, Philadelphia.

active, so that the specimen requires to be stained for from five to ten minutes. Either chemical or heat fixation of the blood film may be used with this stain, both methods giving equally sharp differentiation. Prince's solution colors the erythrocytes rose-red, the nuclei of the leucocytes and erythroblasts blue, the neutrophile granules pink, the eosinophile granules maroon, and the fine and coarse basophile granules blue. Blood parasites are also stained the color of the basic dye.

Double Staining with Eosin and Methylene-blue.—Crisp, clear pictures of nuclear and stroma structures, of the malarial parasites, and of the basophile granules may be obtained by the use of these two dyes, and to investigations of this nature should this staining method be restricted. It is impossible, for example, accurately to distinguish a large lymphocyte from a myelocyte in a specimen stained in this manner, so that for differential counting a more elaborate stain is essential. In films stained by this method the stroma of the erythrocytes and the eosinophile granules react toward the acid dye, staining the color of eosin; while the nuclei of the leucocytes and erythrocytes, the basophile granules, and all blood parasites show an affinity for the basic dye, being colored various shades of blue. The protoplasm of the polynuclear neutrophiles is either colorless or tinged a delicate pink, the granules of these cells remaining unstained.

The author has always found the following simple formula dependable:

| | |
|---|---|
| Eosin (aqueous), to which sufficient water has been added for solution | 0.5 gm. |
| Absolute alcohol | 0.5 c.c. |
| Saturated aqueous solution of methylene-blue | 96.0 c.c. |

*Technic of Staining.*—Films are fixed by immersion for ten minutes in absolute alcohol or in equal parts of absolute alcohol and ether. The cover-glass is flooded with the stain, gently heated for one minute over a Bunsen flame, allowed to stain without heat for two or three minutes longer, and then thoroughly washed in running water, dried in air, and mounted.

Another method of staining with eosin and methylene-blue, slower than the above, but as a rule giving sharper differentiation, is to stain without heat for five minutes with a 0.5 per cent. solution of eosin in absolute alcohol to which an equal quantity of water is added. Then, after having washed off the eosin solution and dried the film in air, the specimen is counterstained for one minute or less with a saturated aqueous solution of methylene-blue, after which it is rinsed again in water, dried in air, and mounted.

Among the many other methods of staining with eosin and methylene-blue those suggested by Chenzinsky,[1] by Plehn,[2] by Holmes,[3] by Laporte,[4] and by Hastings[5] will be found the most useful.

Double Staining with Eosin and Hematoxylin.—By the employment of these two dyes the erythrocytes and the eosinophile granules are stained the color of eosin, and all nuclei and parasites, the color of hematoxylin. This method, which is decidedly inferior to staining with the eosin and methylene-blue mixtures just described, is useful for little else than the study of nuclear structures. It should not be used for differential counting, since in films stained in this manner the neutrophile granules are invisible. Ehrlich[6] recommends this formula:

| | |
|---|---|
| Eosin (cryst.) | 0.5 gm. |
| Hematoxylin | 2.0 gm. |
| Absolute alcohol | 100.0 c.c. |
| Distilled water | 100.0 c.c. |
| Glycerin | 100.0 c.c. |
| Glacial acetic acid | 10.0 c.c. |
| Alum in excess. | |

This mixture must "age" for several weeks before it can be used for staining.

*Technic of Staining.*—Specimens, fixed either chemically or by heat, are stained for from one-half hour to two hours, thoroughly washed in water, dried, and mounted. In order to obtain the best results, it is advisable to filter the solution before using, and to wash the films very thoroughly after staining.

If time is an object, the following rapid method may be substituted for the above: The film is first stained for about five minutes with a 0.5 per cent. solution of aqueous eosin in 50 per cent. alcohol, washed, and dried in air; it is then counterstained for about one-half minute with Delafield's hematoxylin,[7] washed a second time, and mounted in the usual manner.

Staining with Thionin.—Thionin (also known as the "violet of Hoyer" and the "violet of Lauth") is an excellent stain for

[1] Zeitschr. f. wiss. Mik., 1894, vol. xi, p. 260.
[2] "Aetiologische und klinische Malaria Studien," Berlin, 1890.
[3] Jour. Amer. Med. Assoc., 1898, vol. xxx, p. 303.
[4] Med. Rec., 1903, vol. lxiii, p. 1017.
[5] Johns Hopkins Hosp. Bull., 1904, vol. xv, p. 122.
[6] *Loc. cit.*
[7] This solution is made by first adding 4 gm. of hematoxylin crystals, dissolved in 25 c.c. of alcohol, to 400 c.c. of a saturated aqueous solution of ammonia-alum. The mixture is left exposed to the sunlight and air in an uncorked bottle for four days, at the end of which time it is filtered, and mixed with 100 c.c. each of methyl alcohol and glycerin. This solution is allowed to stand until it becomes dark colored, when it is filtered and placed in a tightly corked bottle to "age" for at least two months before it can be used successfully for staining. Owing to the complicated manner in which Delafield's hematoxylin must be prepared, it is usually preferable to purchase it ready-made, from a dealer in microscopical supplies, Grübler's make being entirely reliable.

blood parasites in general, being especially useful for the demonstration of the malarial parasites and the filarial embryos. Thionin should not be used as a stain for films in which the general morphology of the blood cells is to be studied, since the basophile granules and the nuclei are the only histological elements for which it displays any decided affinity. Structures reacting toward the dye are stained violet of varying degrees of intensity. The following recipe, suggested by Futcher and Lazear,[1] will prove satisfactory:

| | |
|---|---|
| Thionin | 0.3 gm. |
| Absolute alcohol | 10.0 c.c. |
| One per cent. solution of carbolic acid q. s. ad | 100.0 c.c. |

*Technic of Staining.*—Films which have been fixed either chemically or by heat are stained in the above solution for from one to three minutes, being then washed in water, dried, and mounted as usual. The best results are obtained by using the French thionin, made by Cogit et Cie, of Paris.

Staining with Polychrome Methylene-blue.—Goldhorn's solution of methylene-blue and lithium carbonate affords a rapidly acting stain, excellent for the demonstration of the finer structure of the malarial parasite in every phase of its development. In addition to giving crisp, clear-cut pictures of the chromatin of this organism, the solution also brings out distinctly the granular degeneration of the erythrocytes, the nuclear characteristics of the erythroblasts and leucocytes, the basophile granules, and all ordinary bacteria.

*Technic of Staining.*—The films are fixed for fifteen seconds in methyl alcohol, rinsed in water, and then stained, unheated, for from one to two minutes, after which they are thoroughly washed in running water, dried without the use of heat, and mounted in balsam. Preliminary staining for ten or fifteen seconds with a 0.1 per cent. aqueous solution of eosin, followed by washing, gives a picture in which the contrast between the plasma and the basic elements of the cells is clearly differentiated. Polychrome methylene-blue, prepared according to Goldhorn's formula,[2] is sold by dealers in laboratory supplies, or it may be made in this manner:

Two grams of methylene-blue are dissolved in 300 c.c. of warm water and 4 gm. of lithium carbonate are added, with constant agitation. The mixture is poured into an uncovered porcelain capsule, which is heated over a shallow water-bath for ten or

[1] Johns Hopkins Hosp. Bull., 1899, vol. x, p. 70.

[2] *Ibid.*, 1899, vol. x, p. 70; also N. Y. Univ. Bull. of Med. Sci., 1901, vol. i, p. 57.

fifteen minutes, being frequently stirred with a glass rod. After removal from the water-bath the fluid is bottled, without filtering, and set aside for several days, after which its reaction is corrected by the cautious addition of a 5 per cent. acetic acid solution until the dye is but very faintly alkaline. Should the solution become too alkaline after having been kept for some time, its reaction may be corrected by adding a small quantity of acetic acid, as in the preparation of the original mixture.

DIFFERENTIAL COUNTING.

A differential count of the leucocytes consists in determining, by microscopical examination of the stained specimen, the relative percentages of the different varieties of these cells, the estimate being based upon a count of several hundred cells, which are classified according to the several forms described in a following section (Section IV). This procedure, by means of which qualitative changes affecting the leucocytes may be detected, is obviously a most important step in every blood examination, and one which should not be regarded as of secondary importance to the numerical estimate with the hemocytometer.

The technic of differential counting consists simply in examining successive microscopical fields until at least 500 leucocytes have been counted, the cells in each field of vision being identified as they appear, and jotted down on a piece of paper by the observer under their appropriate class. As soon as the requisite number of cells has been counted, the percentages of the different forms are calculated, to express the final result. For the examination a $\frac{1}{12}$-inch oil-immersion objective is practically indispensable, for to any but the skilled worker it is difficult, if not sometimes impossible, to distinguish the various forms of leucocytes with a lower magnification than this lens provides. In order to be certain that each field is taken in accurate succession to its neighbor, the slide should be moved across the visual field by the aid of a mechanical stage; systematic examination of any given area of the specimen is well-nigh an impossibility if the slide is simply laid on, or clipped to, the stage of the microscope, and pushed across it with the fingers alone.

If nucleated erythrocytes are found in the specimen, it is equally important to include them also in the differential count, classifying them into two histological divisions, normoblasts and megaloblasts. In calculating the number of these cells, it is obviously impossible to employ any direct method, so that the estimate must of necessity be more or less approximate, since it is based upon the ratio of erythroblasts to a given number of leucocytes. Having first counted the latter with the hemocytom-

eter, the number of nucleated erythrocytes is noted in an area of the stained specimen in which a fixed number of leucocytes is contained, and having ascertained these data, the estimate is made according to the formula:

$$\frac{\textit{Number of erythroblasts counted in the stained film} \times \textit{Number of leucocytes per c.mm.}}{\textit{Number of leucocytes counted in the stained film}} = \textit{Number of erythroblasts per c.mm.}$$

For example, in a case of pernicious anemia in which the leucocytes number 4000 per c.mm., and a total of 35 erythroblasts is noted while counting 1000 leucocytes in the stained film, the calculation is as follows :

$$35 \times 4000 \div 1000 = 140 \textit{ erythroblasts per c.mm.}$$

Whenever erythroblasts are found, it is important to determine their number to the c.mm. of blood, and should normoblasts and megaloblasts both occur, to estimate the ratio between these two types of cells.

## V. COUNTING THE BLOOD PLAQUES.

Determann's method[1] of indirectly estimating the number of plaques to the c.mm. of blood is both simple and accurate. It consists, briefly, in first determining the ratio of these elements to the erythrocytes, which are then counted, to furnish the basis for the final calculation.

In obtaining the blood, a drop of the diluting fluid is placed upon the patient's finger and the puncture made through it, in order that the blood, as it flows from the puncture, will instantly mix with the diluent without coming in contact with the air. The blood and diluent are then thoroughly mixed for a few moments by the aid of a cover-glass, after which a small portion of the mixture is transferred to a Thoma-Zeiss counting chamber, and the ratio of plaques to erythrocytes determined under the microscope. In the healthy adult this ratio, according to Determann, ranges from 1 to 18 to 1 to 30, averaging about 1 to 22. With another drop of blood the erythrocyte count is then made by the usual method, and the actual number of plaques to the c.mm. of undiluted blood calculated from the figure thus obtained. For example, in a given specimen of blood in which the ratio of plaques to erythrocytes is found to be 1 to 25, the count of the latter cells being 5,000,000, the actual number of plaques is therefore 200,000 per c.mm.

[1] Deutsch. Arch. f. klin. Med., 1899, vol. lxi, p. 365.

The diluents for which Determann expresses a preference are either a 9 per cent. aqueous solution of sodium chlorid to which a little methyl-violet has been added, or an aqueous solution containing one per cent. of sodium chlorid and 5 per cent. of potassium bichromate. Brodie and Russell[1] recommend equal parts of dahlia-glycerin and a two per cent. aqueous solution of sodium chlorid.

Any of the diluting fluids already mentioned are also suitable for the purpose.

## VI. ESTIMATION OF THE RELATIVE VOLUMES OF CORPUSCLES AND PLASMA.

The use of centrifugal force for the purpose of determining the relative volumes of blood corpuscles and plasma was first applied in a practical manner by Hedin,[2] who embodied the earlier ideas of Blix in an instrument known as the hematocrit. More recently Daland,[3] by improving the mechanical construction of the original instrument and by simplifying the technic of using it, has made centrifugalization of the blood a method of investigation adapted to general clinical work. By the use of the hematocrit a pair of capillary glass tubes filled with undiluted blood are rotated in their horizontal axes at a high rate of speed until, as the result of the centrifugal force thus applied, the corpuscular and liquid portions of the blood become separated, the former being distinguishable in the lumen of the tube as a column the length of which is dependent upon the volume which the corpuscles constitute in relation to the rest of the blood mass.

DALAND'S HEMATOCRIT.

This instrument (Fig. 30) is composed of a set of cog-wheels inclosed in a metal box and geared in such a manner as to cause 10,000 revolutions a minute of a vertical spindle, by turning a handle at a definite, uniform rate of speed. A metal frame, which may be securely fastened to the spindle by a modified bayonet-lock, carries a pair of capillary glass tubes, each of which fits into two cup-like, rubber-lined depressions, and is adjusted and held in place by a spring. Each tube measures 50 mm. in length with a lumen of 0.5 mm., and has engraved upon its outer surface a scale representing 100 equal divisions, the glass

[1] Jour. Physiol., 1897, vol. xxi, p. 390.

[2] Skandinavisch. Arch. f. Physiol., 1890, vol. ii, p. 134.

[3] University Med. Mag., 1891, vol. iv, p. 85; also Edwards' supplement to Keating's "Cyclopedia of the Diseases of Children," Philadelphia, 1899, vol. v, p. 537.

immediately above the scale being molded so as to form a lens-front, to magnify the column of blood and to facilitate the reading of the divisions. A bit of rubber tubing fitted with a mouthpiece is used for filling the capillary tube, in the same manner in which the blood is measured with the hemocytometer. While in use, the instrument is securely attached to the projecting edge of a table or shelf by means of a clamp operated by a thumb-screw.

*Method of Use.*—Having cleaned and punctured the patient's finger in the usual manner, the beveled end of one of the capillary tubes is immersed in the drop of blood, which is sucked up the lumen of the tube until it is exactly filled. The forefinger, smeared with a little vaselin, is then applied to the beveled end of the tube, while the rubber suction tube is carefully removed by twisting it free—not by forcibly pulling it off, since this may accidentally cause removal of a portion of the blood column by suction. The tube thus charged with blood is at once adjusted to one arm of the frame, and the empty tube similarly fixed in the other arm, to equalize the balance, this step being completed as rapidly as possible, in order to anticipate coagulation. When the tubes have been thus adjusted, and the frame securely locked in the spindle, the handle of the instrument is turned for three minutes[1] at the rate of 77 revolutions a minute, this rate of speed securing 10,000 rotations a minute of the frame, since the latter revolves 130 times with each complete turn of the handle. The centrifugalization having been finished, the charged tube is carefully removed from the frame, and held against a piece of dull white paper, so that the height of the blood column may be easily determined. In order to make the reading with accuracy, it is sometimes necessary to use a small magnifying glass, for the divisions on the scale of the tube are but 0.5 mm. apart—a distance too small to judge easily with the naked eye. On examination, three distinct divisions of the lumen of the tube containing the centrifugalized blood may be distinguished : first,

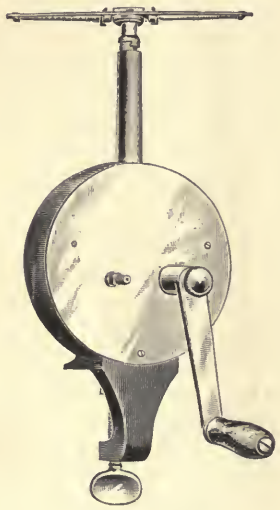

Fig. 30.—Daland's Hematocrit.

[1] In a recent personal communication Dr. Daland advises that, in order to insure the most accurate results with his instrument, the centrifugalization be continued for three, instead of for two, minutes, as he formerly recommended.

a dark-colored column consisting of erythrocytes, reaching, in normal blood, to a point between the divisions marked 50 and 51 ; second, a thin layer of leucocytes, showing, in blood in which these cells are not largely increased, as an indistinct, milky stratum overlying the erythrocytes ; and, third, a layer of clear plasma occupying the remainder of the lumen. The normal volume of erythrocytes being arbitrarily regarded as 100 per cent., to compute this result the figure of the scale to which these cells rise is multiplied by two. Unless the leucocytes are greatly increased in number, the layer formed by these cells is too delicate and too dully defined to be read with any degree of accuracy ; but in cases of high leucocytosis and of leukemia it is quite possible to estimate roughly the relative proportions of leucocytes to erythrocytes.

The capillary tube which has been filled with blood should be cleaned as soon after use as possible, water, followed by alcohol and ether, being used for this purpose. A fine wire should be passed through its lumen, to dislodge any obstruction which may result from drying of the column of closely packed corpuscles.

The hematocrit, if its clinical application is limited to the determination simply of the relative volumes of the blood corpuscles and plasma, may be relied upon to furnish, on the whole, dependable information, the necessary errors attending its use probably being within two per cent. It is also useful in the study of hemoglobinemia, cholemia, and lipemia. If employed in the rôle of a hemocytometer, however, its results must needs be highly inaccurate, just in those instances in which exact methods of investigation are all important. It is true that in normal blood, in which the size of the corpuscles ranges within the physiological limits, it is correct to consider each percentage volume as representing approximately a count of 100,000 erythrocytes per c.mm. In blood characterized by any considerable deformity in the size and shape of these cells, as in high-grade anemia or in leukemia, it is perfectly obvious that no such correspondence between the count and the percentage volume can be expected—blood in which microcytosis is pronounced is certain to show a lower percentage volume of erythrocytes than blood in which megalocytosis prevails, or than blood containing normal-sized cells, although the counts of all three may be identical. Similarly, a given number of lymphocytes should indicate a lower percentage volume than an equal number of myelocytes, or even polynuclear neutrophiles. On account of these sources of fallacy, if for no other reason, the hematocrit estimate should never be taken as a basis for calculating the count in pathological condi-

tions, in lieu of the more accurate, if more laborious, method of counting the corpuscles.

Capps[1] considers that the hematocrit may be used to advantage in conjunction with the hemocytometer, in determining the actual size or volume of the individual erythrocyte, and he regards this method as far more reliable than the use of the micrometer, since with the latter only the transverse diameter of the cells, and not their depth, can be measured. The formula for calculating this "volume index" has been given elsewhere. (See p. 173.)

## VII. ESTIMATION OF THE SPECIFIC GRAVITY.

This method of investigation is used as an indirect means of computing the percentage of hemoglobin, owing to the more or less constant parallelism maintained between it and the specific gravity of the whole blood. The correspondence between the two, together with the sources of error inseparable from the test, has been pointed out in another section. (See p. 133.)

HAMMERSCHLAG'S METHOD.

Hammerschlag's modification[2] of Roy's method[3] of determining the specific gravity of the blood best serves the purpose of those who choose this roundabout means of approximating the hemoglobin percentage. It consists in first making a mixture of benzol and chloroform of such a specific gravity that a small drop of blood deposited in the liquid remains suspended, after which the specific gravity of the mixture is determined with a hydrometer, the figure thus obtained representing the density of the blood used in the test. The hemoglobin percentage corresponding to this figure is then selected from a table giving the various degrees of blood densities and the percentages of hemoglobin to which they are equivalent.

The apparatus required for making the test includes a *hydrometer* provided with a scale graduated to 1.070, a *hydrometer jar* having a wide, substantial base, a *glass capillary tube*, and a *glass stirring rod*. An ordinary urinometer may be used instead of a special hydrometer, since specific gravities in excess of 1.060 (the highest gradation on the scale of most urinometers) are not often encountered. More accurate results, however, are possible with a standardized instrument. Levy[4] has shown that with an

[1] Jour. Amer. Med. Assoc., 1900, vol. xxxvi, p. 464.
[2] Zeitschr. f. klin. Med., 1892, vol. xx, p. 444.
[3] Cited by Devoto, Zeitschr. f. Heilk., 1889, vol. xi, p. 175.
[4] Lancet, 1903, vol. i, p. 1302

ordinary hydrometer an excessive reading, ranging from 3 to 10 degrees, always occurs, owing to the disturbing effect upon the instrument of the low surface tension of the chloroform-benzol mixture. To obtain accurate figures it is necessary to use a hydrometer which has been standardized to these reagents. Either a Thoma-Zeiss leucocytometer or a medicine dropper, the free end of which should be heated in a flame and bent into an obtuse angle, will serve as a capillary pipette.

Benzol and chloroform are mixed together in the hydrometer jar in such proportions that the specific gravity of the liquid is approximately equal to that of normal blood, 1.060. This mixture having been made and its specific gravity taken, the point of the capillary pipette, charged with blood, is plunged beneath the surface of the liquid and a small bead of blood gently expelled. If the blood drop rises to the surface of the mixture, a few drops of benzol are added, while if it sinks to the bottom of the jar, chloroform is used, the addition of the appropriate reagent being continued until the drop neither rises nor sinks, but remains stationary, suspended in the mixture. When this point has been determined, the specific gravity of the liquid is taken by means of the hydrometer, this figure obviously representing the specific gravity of the blood drop itself. To convert the specific gravity into its hemoglobin equivalent the figure obtained by the above procedure is compared with one of the tables given on page 133. After each addition of benzol or of chloroform the contents of the jar must be thoroughly mixed by stirring with the glass rod, in order to secure uniformity in the density of the liquid. The latter, if it is filtered free from blood and preserved in a tightly stoppered bottle, may be used again in subsequent tests.

In spite of the enthusiasm evinced by certain authors for this method of obtaining hemoglobin values, considerable experience with the test has convinced the writer that it is both crude and untrustworthy—it is useful, no doubt, when a hemometer cannot be obtained, but in no sense is it an efficient substitute for colorimetric methods. The liability of the blood drop to split up into numerous fine particles, to adhere to the inside of the jar, and to become altered in composition from the influence of the reagents, as well as the tedious attempts which must usually be made to add just the proper quantities of benzol and chloroform to secure a mixture in which the drop neither sinks nor rises, are a few of the drawbacks which must make the test unpopular with busy clinicians. For a critical review of the clinical value of Hammerschlag's test Baumann's article[1] should be consulted.

[1] Brit. Med. Jour., 1904, vol. i, p. 473.

## VIII. ESTIMATION OF THE ALKALINITY.

ENGEL'S ALKALIMETER.

A convenient clinical method of determining the alkalinity of the blood is by the use of Engel's alkalimeter (Fig. 31). By means of this instrument a measured quantity of fresh blood is diluted with distilled water in the proportion of one to ten, and then titrated with a $\frac{1}{75}$ normal solution of tartaric acid until the mixture reacts with lacmoid paper, the total alkalinity being calculated from the amount of the tartaric acid used. The methods of alkalinity estimation devised by Landois,[1] by Liebreich,[2] by Haycraft and Williamson,[3] by Wright,[4] and by Kraus[5] are not well adapted to routine blood work, being either too complicated and elaborate for such a purpose or too inaccurate.

The apparatus which Engel has devised consists of the following parts: a *diluting and mixing pipette,* resembling a large-sized Thoma-Zeiss erythrocytometer; a *graduated burette* by means of which the amount of tartaric acid solution used in the test is measured; a *glass cylinder* in which the titration is made; and a *glass stirring rod.* The mixing pipette is graduated in three principal divisions marked 0.025, 0.05, and 5.0 respectively, the first two divisions being further scaled in tenths by fine horizontal markings; otherwise the instrument is modeled like a blood-counting pipette. The burette has a capacity of 5 c.c., and is provided with a scale indicating 100 equal divisions; when in use, it is clamped upright, by means of a special attachment, to a vertical brass support which screws into a fitting in the box containing the apparatus.

*Method of Use.*—The technic of using the alkalimeter is simple and time saving in comparison with that required by other well-known methods of alkalinity testing. Finger-blood, obtained by a rather deep puncture, so as to afford a good-sized drop, is sucked up in the pipette until it reaches the mark 0.05, immediately after which distilled water is similarly drawn up the lumen of the tube until the mixture of blood and water fills the bulbous expansion and reaches the mark 5.0 in the constricted portion beyond. While sucking up the water the pipette should be rapidly twisted to and fro between the thumb and forefinger, to insure thorough mixing of the blood and water as they together fill the expanded portion of the instrument. As soon as the dilution has been made, the pipette should be shaken for a minute or so, until the

[1] Real-Encyclop., 1885, vol. iii, p. 161.
[2] Berichte d. deutsch. chem. Gesellsch., 1868, vol. i, p. 48.
[3] Proc. Roy. Soc., Edinburgh, June 18, 1888.
[4] Lancet, 1897, vol. ii, p. 719.
[5] Zeitschr. f. Heilk., 1889, vol. x, p. 106.

mixture becomes of a uniform "laky" tint, which indicates that all the hemoglobin has been dissolved from the corpuscular stroma. The contents of the pipette are blown out into the glass cylinder, which is placed beneath the faucet of the burette, the latter having been previously filled to the mark o with a $\frac{1}{15}$ normal solution of tartaric acid. By turning the stop-cock of the burette the test solution is now added, drop by drop, stirring between each addition, to the measured amount of diluted blood in the cylinder. From time to time a drop of the mixture is re-

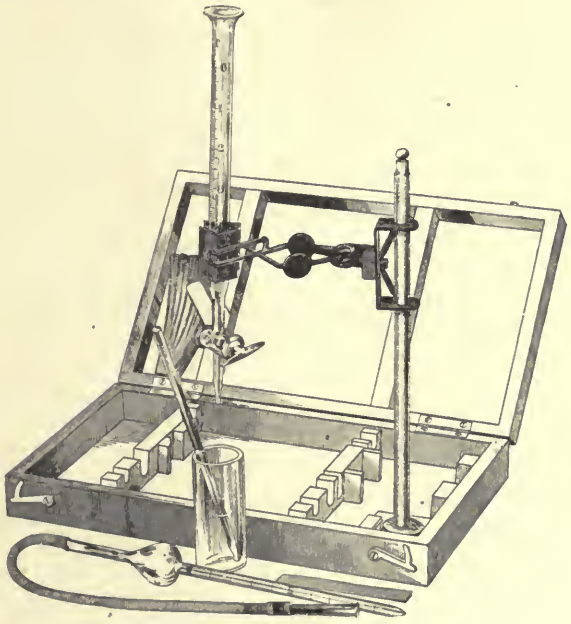

FIG. 31.—ENGEL'S ALKALIMETER.

moved by means of the glass rod and tested with the lacmoid paper, the titration being continued until the reaction, recognized as a bright-red halo which forms around the edge of the drop, is obtained. The titration is then stopped, and a note made of the number of drops of the test solution which have been used. In normal blood the writer finds that from 9 to 11 drops are required to give the reaction. The estimate of the total alkalinity of the blood is made by multiplying by the figure 53.3 the number of drops of the tartaric acid solution used, according to

the formula, 10 : $a$ :: 533.0 : $x$, $a$ representing the drops of the reagent.[1] The result thus obtained is expressed in milligrams of NaOH per 100 c.c. of blood. The following table may be useful for reference in determining the various degrees of alkalinity:

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| If | 6 | drops of the solution are used, the alkalinity equals | | | | | | | | 319 | mgm. | NaOH |
| " | 7 | " | " | " | " | " | " | " | " | 373 | " | " |
| " | 8 | " | " | " | " | " | " | " | " | 436 | " | " |
| " | 9 | " | " | " | " | " | " | " | " | 479 | " | " |
| " | 10 | " | " | " | " | " | " | " | " | 533 | " | " |
| " | 11 | " | " | " | " | " | " | " | " | 586 | " | " |
| " | 12 | " | " | " | " | " | " | " | " | 639 | " | " |
| " | 13 | " | " | " | " | " | " | " | " | 692 | " | " |
| " | 14 | " | " | " | " | " | " | " | " | 746 | " | " |

After use the pipette should be thoroughly washed out with water, alcohol, and ether, and then dried, in the manner already directed for cleaning the Thoma-Zeiss instrument.

DARE'S HEMO-ALKALIMETER.

This ingenious instrument[2] is used in connection with a spectroscope, its principle depending upon the dissipation of the absorption bands of oxyhemoglobin when exact neutralization of the blood ensues, after the addition of a $\frac{1}{2000}$ normal solution of tartaric acid. The working parts of the apparatus are explained by the accompanying illustration (Fig. 32). The test solution to be employed is made up as follows:

| | |
|---|---|
| Acid. tartaric. (Merck's reagent) | gr. j (0.075 gm.). |
| Alcohol | ℥ v (20 c.c.). |
| Aqua destil. | q. s. ℥ vj (200 c.c.). |

*Method of Use.*—The alkalimeter tube (Fig. 32, A), fitted with its blood pipette, B, is held horizontally, and the pipette filled with blood by presenting its exposed end to the drop as it flows from the puncture. With an ordinary medicine dropper containing distilled water and coupled to the blood pipette by a bit of rubber tubing, the measured blood is washed into the alkalimeter tube until the point 0 is reached. Now, with the thumb closing the opening, C, in its bulb, the alkalimeter tube is inverted several times, in order to mix the blood and water. The reagent pipette, D, is filled with the test solution, connected by tubing with the free end

[1] Assuming that 0.5 c.c. of tartaric acid is used to neutralize 0.05 c.c. of blood, therefore for every 100 c.c. of blood 1000 c.c., or one liter, of a $\frac{1}{75}$ normal solution of tartaric acid are required. As the alkalinity of the blood is not expressed by the amount of acid necessary to saturate it, but in milligrams of an alkali, sodium hydrate, the calculation is made thus: as the equivalent weight of tartaric acid is 75, and that of sodium hydrate 40, one liter of water dissolving 75 gm. of the former saturates 40 gm. of the latter—that is, one liter of a $\frac{1}{75}$ normal tartaric acid solution saturates $\frac{40}{75}$ gm., or, in other words, 533 mgm., of sodium hydrate, this figure being taken by Engel as the degree of normal alkalinity of the blood.

[2] Phila. Med. Jour., 1903, vol. xi, p. 137.

of the blood pipette, and compressed so as to force the reagent into the diluted blood within the alkalimeter tube. In doing this it is necessary to thumb the opening in the bulb of the latter, to avoid the mixture of the blood and reagent. The tube and reagent pipette, still connected, are now grasped and inverted, to mix thoroughly the contents of the former, after which a preliminary observation is made by adjusting the lower part of the tube (below the mark o) to the cleft of a Browning pocket spectroscope. If the oxyhemoglobin absorption bands persist, more of the reagent is added, drop by drop, inverting the tube between, and examining with the spectroscope after, each drop. When the bands disappear, the figure to which the neutralized blood solution reaches is noted, and compared with the table of equivalents given below, to obtain the final result, which is expressed in milligrams of NaOH per 100 c.c. of blood. The figure for normal blood with this instrument is 266.

TABLE OF EQUIVALENTS.

| Cubic Centimeters of Reagent. | Mgm. of NaOH to 100 c.c. of Blood. |
|---|---|
| 2.6 | 345.0 |
| 2.4 | 319.0 |
| 2.2 | 292.0 |
| 2.0 | 266.0 |
| 1.8 | 239.0 |
| 1.6 | 212.0 |
| 1.4 | 176.0 |
| 1.2 | 159.0 |
| 1.0 | 133.0 |
| 0.8 | 96.0 |
| 0.6 | 79.0 |
| 0.4 | 53.0 |
| 0.2 | 26.6 |

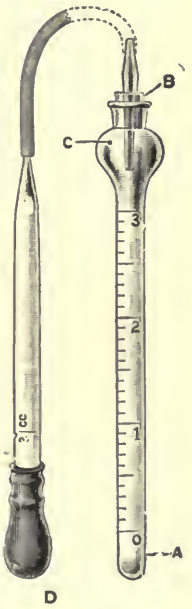


Fig. 32.—Dare's Hemo-alkalimeter.

A, Alkalimeter tube; B, automatic blood pipette; C, opening for the admission of air; D, reagent pipette.

While, up to the present time, it cannot be claimed that information of any real diagnostic pertinence has been obtained from the study of the alkalinity of the blood, this procedure should prove of value in the systematic investigation of many cases, especially those of high-grade anemia. As elsewhere mentioned, the degree of normal blood alkalinity varies greatly according to the particular method by which this figure is ascertained, so that it follows that the results obtained by means of one apparatus cannot be compared with those based upon investigation with another instrument of different design.

## IX. DETERMINATION OF THE RAPIDITY OF COAGULATION.

This procedure is useful in the study of such conditions as icterus, purpura, hemophilia, scurvy, various specific infections, and other diseases characterized by abnormalities in the clotting time of the blood. Knowledge of how long it takes a given blood to clot not only adds completeness to the clinical history, but allows the physician to judge of the effect of remedies administered with a view to promoting coagulation. No careful surgeon today neglects to test the patient's blood coagulability before operating for the relief of obstructive lesions of the biliary passages. No physician should omit to make repeated coagulation tests in treating one of the hemorrhagic diatheses or a primary anemia.


FIG. 33.—A. INCOMPLETE COAGULATION. Tear-shaped drop. B. COMPLETE COAGULATION. Convex drop.

GLASS SLIDE METHOD.

The coagulation time may be determined approximately by collecting several individual drops of blood of the same size upon the surface of a perfectly clean, slightly warmed glass slide. At regular intervals of about one minute a straw of a whisk-broom is lightly trailed through each drop in succession, until sooner or later a delicate thread of fibrin may be observed clinging to the straw. The period which has elapsed between the deposit of the blood on the slide and the appearance of this indication of clotting is expressed in minutes, to represent the coagulation time of the specimen under investigation. Normal blood thus treated coagulates in from two and one-half to five minutes.

The method described by Milian[1] is perhaps even simpler. A rather large drop of blood is collected upon the center of a clean, dry glass slide, which, after the lapse of a minute or so, is carefully tilted to a vertical plane. With the slide held in this position the profile of the coagulated drop forms a symmetrical, mound-like convexity, while that of the incompletely clotted drop is tear-shaped (Fig. 33). The time elapsing between the collection of the blood drop and the first-named change is considered the coagulation time. Five minutes is the average period for normal blood.

[1] Presse méd., 1904, vol. i, p. 202.

WRIGHT'S COAGULOMETER.

The latest model of this instrument[1] consists of a tin reservoir fitted with a removable rack holding a thermometer and a set of twelve calibrated glass coagulation tubes. For filling the latter an aspirator tube with a rubber connection is employed. The thermometer indicates degrees of the Centigrade scale, and is

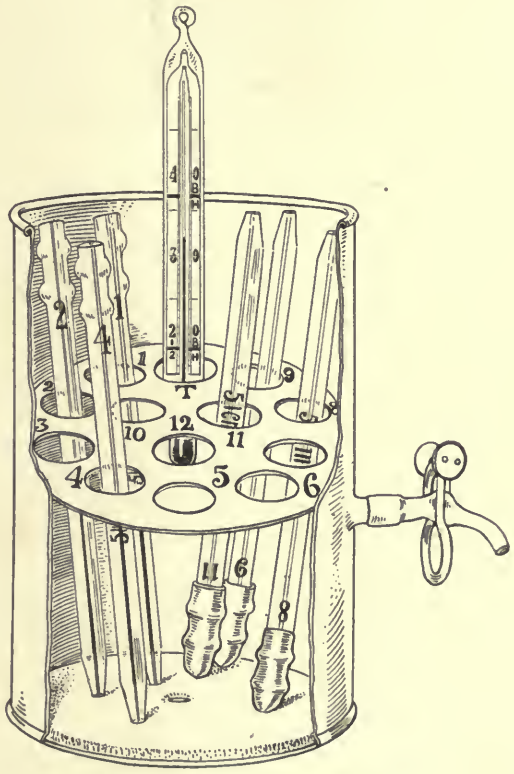

FIG. 34.—WRIGHT'S COAGULOMETER.

also graduated at 18.5° and at 37°, the temperature of "half blood heat" and of "blood heat," respectively. The coagulation tubes, made of stout glass, are furnished with tight rubber caps, used to seal their blunt ends when immersed; they have an internal diameter of 0.25 mm., and are marked to indicate a blood column of 5 c.c. Each tube is numbered to correspond with its appropriate place in the rack of the reservoir.

[1] Lancet, 1902, vol. ii, p. 15.

*Method of Use.*—The reservoir is filled with water having a temperature of 18.5° C.,[1] and the tubes, each fitted with a rubber cap, are placed in the rack, sealed end downward, where they are allowed to remain immersed for a few minutes until they acquire approximately the same temperature as that of the water. They are then removed, dried, and stripped of their rubber caps.

Having then pricked the patient's finger, 5 c.c. of blood are sucked up each tube, at successive intervals of one minute, a tube as soon as filled being returned to its appropriate place in the reservoir. It is not necessary to replace the caps—the tubes are simply placed point downward in the water, the temperature of which is from time to time readjusted to the standard by adding hot water to the reservoir as its contents cool. After the lapse of an appropriate interval the tube first filled is removed from the reservoir and tested by attempting to blow out its contents upon the surface of a piece of white filter or blotting paper. The other tubes are similarly tested in rotation, at intervals of one minute or less, until, after having thus tried a variable number one is found from which the blood cannot be expelled.

Coagulation may then be considered to have occurred, the time required for this process being expressed by the number of minutes elapsing between the filling of the tube in question and the evidence of clotting thus demonstrated. With normal blood the coagulation time, as determined by this instrument, generally ranges from about three to six minutes. In rare instances Wright[2] found that in the healthy male adult clotting may be prolonged for fifteen minutes.

After use, a fine wire should be forced through the lumen of the tubes to dislodge the clots, after which the remaining traces of blood are to be removed by thorough washing with distilled water, alcohol, and ether, in the order named.

## X. CRYOSCOPICAL EXAMINATION.

Cryoscopy. Cryoscopy is the method of determining the freezing-point of a given liquid and the comparison of this figure with the freezing-point of distilled water. This method of investigation is based upon the principle that the freezing-point of any liquid is proportionate to the number of its contained molecules. The greater the molecular concentration, the lower the point at which the fluid freezes.

[1] In slowly coagulating bloods the temperature should be about that of blood heat, 37° C.

[2] Brit. Med. Jour., 1902, vol. ii, p. 1706.

Cryoscopy of the blood and urine was first applied to clinical medicine by von Korányi,[1] who thus was able to determine the status of the renal function. The average freezing-point of normal blood is —0.57° C., ranging between —0.56° and —0.58° C. Urine in the healthy individual freezes between —0.9° and —2° C. Venous blood freezes at a slightly lower temperature than arterial. In *renal disease* with decided insufficiency of the kidneys it is found that the freezing-point of the blood is lowered, while that of the urine is correspondingly raised, owing to the retention in the blood of matter which the crippled kidneys are unable to excrete. Excision of one kidney does not disturb the normal freezing-points of the blood and urine, but double nephrectomy promptly lowers that of the former and raises that of the latter.

A freezing-point of —0.6° C. or lower for blood and of 1° C. or higher for urine indicates a sufficient degree of renal impairment to contraindicate surgical interference in lesions of the kidneys. This dictum was first expressed by Kümmel,[2] who bases it upon an experience of 170 cases of *renal surgery* in which cryoscopy was practised, including chronic nephritis, nephrolithiasis, tuberculosis, cysts, neoplasms, pyonephrosis, hydronephrosis, and post-operative anuria. Rumpel's extensive researches[3] in 300 similar cases showed that in normal individuals and in those with unilateral renal lesions the blood freezing-point did not vary from the figures given above (—0.56° to —0.58° C.), while in bilateral lesions of the kidneys it ranged between —0.55° and —0.81° C. In renal disease unaccompanied by *uremia* Lindemann[4] found no variations from the normal freezing-point, but with the onset of this complication the blood froze at a point as low as —0.7° C.

Koeppe's data[5] in conditions other than those involving the kidneys are of interest. He found, in a series of cases including *functional neuroses*, *carcinoma*, *diabetes*, *pleurisy*, and *pneumonia*, a blood freezing-point varying from —0.5° to —0.63° C. Similar figures, of theoretical rather than practical interest, have also been determined in various general diseases by Ogston,[6] Tinker,[7] Tieken,[8]

[1] Zeitschr. f. klin. Med., 1897, vol. xxxiii, p. 45; *ibid.*, 1899, vol. xxxiv, p. 1; Berlin. klin. Wochenschr., 1901, vol. xxxviii, p. 424.
[2] Centralbl. f. Chir., 1902, vol. xxix, p. 121.
[3] Münch. med. Wochenschr., 1903, vol. l, pp. 19, 67, and 117.
[4] Deutsch. Arch. f. klin. Med., 1899, vol. lxv, p. 1.
[5] Cited by Cattell, Internat. Clinics, 1904, vol. i, p. 6.
[6] Lancet, 1901, vol. ii, p. 1253.
[7] Johns Hopkins Hosp. Bull., 1903, vol. xiv, p. 162.
[8] Med. News, 1904, vol. lxxxiv, p. 416.

Ceconi,[1] Sollmann,[2] and others.[3] It is of interest to note that the *pregnant woman's* blood freezes at a higher temperature than normal, but that the latter figure is reached after delivery, as the molecular concentration of her blood rises. In *diabetes mellitus* the freezing-point is low, but in *pernicious anemia* and in various forms of *hydremia* it is high. Inhalations of *oxygen* lower the figure, but in conditions of *cyanosis* with an excess of carbonic acid in the blood the freezing-point rises. It is of value also to observe that *cystitis* and *pyelitis* do not affect the blood's freezing-points.

Carrara[4] and Revenstorf[5] found that in cases of *drowning* the resulting dilution of the blood causes radical deviations from the normal freezing-point, which rises in the case of drowning in fresh water and falls after death by submersion in salt water. In this connection the general statement applies, that the freezing-point of the diluted blood approaches that of the fluid with which the body is waterlogged. Whether the deviation be plus or minus, it is generally more marked in the blood of the left than of the right heart, because of the greater dilution of the venous blood, owing to the water drawn into the lungs and entering the pulmonary capillaries. Exceptionally this difference between the halves of the heart is not apparent, as in the case of bodies remaining submerged for a long period and in those in which the circulation persists for a few moments after the blood dilution, in either of which instances the entire blood mass tends to become equally diluted. The utility of cryoscopy as a forensic test of death by drowning is obvious from these experiments, although its value is to some extent restricted by the fact that advanced putrefaction and prolonged submersion interfere with its reliability.

Cryoscopy of the blood and urine is of value in determining the condition of renal adequacy, but it should be supplemented by other laboratory tests devised for this purpose. Forensically, the test may yield reliable evidence not only in cases of death by drowning, but possibly also in the identification of blood stains from various sources.

FONTAINE'S CRYOSCOPE.

The cryoscope made by Fontaine, of Paris (Fig. 35), is simply constructed, durable, and thoroughly satisfactory for clinical use. It consists of a stout glass freezing-jar, A, provided with a large test-tube, B, passing to its center and kept in position by a

[1] Rif. med., 1901, vol. iii, p. 109. [2] Amer. Med., 1902, vol. iii, p. 656.

[3] For an excellent resumé of cryoscopy in all its phases see Cattell, Proc. Phila. Path. Soc., 1904, vol. vi, p. 244.

[4] Arch. Ital. de Biol., 1901, vol. xxxv, p. 349.

[5] Münch. med. Wochenschr., 1902, vol. xlix, p. 1880.

metal support, C. At the base of the jar there is a drain, D, for the liquid which accumulates as the ice-salt mixture melts. A small test-tube, E, having a lateral vent, F, fits within the larger tube, being adjusted by means of a rubber collar, G,[1] so that between the two tubes an air chamber is formed. A thermometer, H, encircled by a metal spiral stirrer, I, is let down into the smaller test-tube and adjusted so that it touches neither the walls nor the bottom of the latter, being kept in this position by means of a vertical standard fitted with an adjustable horizontal arm. The thermometer registers from —3° C. to +3° C., being graduated in $\frac{1}{200}$ of a degree, and is provided with a pear-shaped bulb at the top, to allow for the expansion of the mercury column. The thermometer is an extremely delicate and expensive bit of apparatus, and must be handled with care, for fear of breakage. It should be tested with distilled water, so that any deviation may be taken into account in subsequent observations.

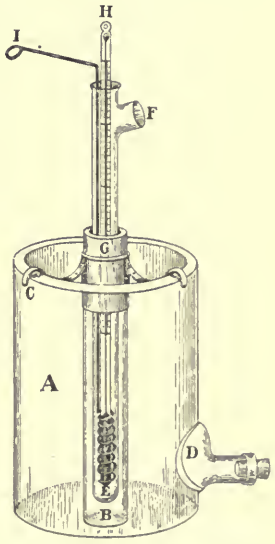

FIG. 35.—FONTAINE'S CRYOSCOPE.

*Method of Use.*—The freezing-jar, with its large test-tube adjusted, is filled to the brim with a mixture of cracked ice and rock-salt, packed in alternate layers, the whole being covered, at the level of the mouth of the jar, with a layer of salt an inch in depth. The size of the bits of ice should be large enough to insure gradual thawing, for finely crushed ice rapidly turns to slush. Ten c.c. of the blood or urine[2] are placed in the small test-tube, which is laid against a block of ice, to cool, while the freezing-jar is being packed. By the time this is accomplished (about five minutes) the fluid to be tested will have cooled sufficiently, and the next step in the test may

[1] In the original model of the instrument this collar, as well as the metal band supporting the large test-tube, interferes with the reading of the thermometer scale, but this defect may be easily remedied by cutting in each a small window, so as to allow a clear view of the mercury column. The apparatus is made by G. Fontaine, 16, Rue Monsieur le Prince, Paris; it costs, duty free, 90 francs. The A. H. Thomas Company, Philadelphia, makes an excellent cryoscope of the Fontaine model, the cost of which is considerably less than that of the French instrument.

[2] The patient's blood and urine should be collected at the same time: the former by aspirating a superficial vein, the latter by catheterizing each ureter if the test involves a determination of the integrity of each kidney.

proceed. Great care must be taken that both the test-tubes and the thermometer are absolutely dry, for the slightest trace of moisture so alters the freezing-point that gross inaccuracies in the final reading may result. The small test-tube is now fitted within the larger one, after which the thermometer, with the stirrer in place, is carefully lowered into position, resting free from contact with the walls and bottom of the tube, with its mercury bulb immersed in the test fluid. The thermometer, when correctly adjusted, is hung from the arm of the standard, placed alongside the freezing-jar. The handle of the stirrer is now constantly moved up and down, so as to equalize the temperature of the test fluid as it congeals, and this mixing is to be continued intermittently during the rest of the observation. After a wait of about five minutes the column of mercury begins to fall, first very slowly, then rapidly, to approximately two degrees below zero, at which point it remains for a few moments, and then, because of the heat evolved, rises to the true freezing-point, where it remains stationary for about two minutes, after which it falls to the temperature of the outside mixture of ice and salt. When the point of stability is attained, the degree registered by the mercury column is noted to obtain the freezing-point of the specimen. In making this end-observation the eyes should be on a level with the top of the mercury column. It may be hastened somewhat by the insertion of a pellet of ice in the vent of the small test-tube just before the freezing-point is reached.

## XI. ESTIMATION OF THE RESISTANCE OF THE ERYTHROCYTES.

This method of examination is of more than mere theoretical value in the study of diseases associated with hemoglobinemia, in which conditions it indicates, and with great accuracy, the vulnerability of the erythrocytes, as expressed by their resistance to the action of salt solutions of different strengths. Among the pathological conditions in which the method is useful may be named the severe anemias, the specific infections, such as malarial fever, yellow fever, and sepsis, all forms of icterus, the different cachectic states, paroxysmal hemoglobinemia, and toxemias due to snake venom and to other hemolytic agents.

HAMBURGER'S METHOD.

Hamburger's method, as modified by von Limbeck,[1] requires the use of twelve small glass receptacles, about the size of a Gowers' hemocytometer mixing cell, each of which contains a small glass

[1] "Einer klinische Pathologie des Blutes," 2d ed., Jena, 1896; also New Sydenham Soc. trans. by Arthur Latham, London, 1901.

bead. Into these vessels is placed 1 c.c. of salt solutions of different strengths, each differing by 0.02 per cent., the minimum being 0.3 per cent., the next 0.32 per cent. (or 0.02 per cent. stronger), and so on. A drop of blood as it drips from the puncture is allowed to fall into each of the vessels, which are then shaken briskly for a minute or so, in order to cause defibrination by the whipping about of the glass beads. When this is accomplished, the blood-charged solutions are allowed to stand for six hours, when it will be noted that some of them are tinged with hemoglobin, while others remain clear. The first tube showing no solution of hemoglobin indicates the isotonicity of the cells under examination. For normal blood this index ranges between 0.46 and 0.48 NaCl.

## XII. SPECTROSCOPICAL EXAMINATION.

For clinical work the Sorby-Beck microspectroscope, to be used in connection with the microscope, is an excellent instrument, being both accurate and, comparatively speaking, easy to manipulate. Other very perfect instruments for the spectroscopical examination of the blood, differing but little from the original Sorby model, are also made by Zeiss, by Leitz, and by Browning.

THE SORBY-BECK MICROSPECTROSCOPE.

This instrument (Fig. 36) when in use fits into the tube of the microscope like an ordinary ocular, for which it is substituted. Its essential part consists of a tube, *A*, in which a series of five prisms, two of flint and three of crown glass, is arranged in such a manner that the emergent rays, which are separated by dispersion, leave the prisms in practically the same direction as that taken by the entering immergent ray. At one side of the tube is fixed a right-angle reflecting prism, so that the spectrum of a solution of normal blood may be thrown alongside that of the specimen under investigation, the two spectra thus being comparable. The adjustment of the spectra is effected by means of the two small screws, *B*, *B'*. The receptacle containing the control solution of blood is clamped to the stage, *C*, by a spring clip, *D*, light being reflected through the liquid and into the rectangular aperture, *E*,

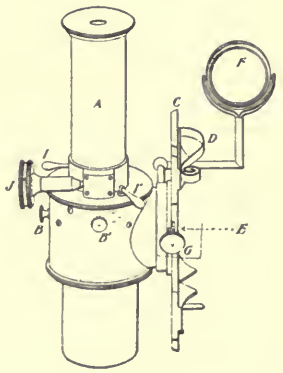


FIG. 36.—SORBY-BECK MICROSPECTROSCOPE.

by the swinging mirror, *F*. The width of this aperture is controlled by the screw, *G*. The receptacle containing the blood solution to be examined is placed upon the stage of the microscope, being brought into focus with a low-power ($\frac{2}{3}$ or 1 inch) dry objective. Beneath the tube inclosing the series of prisms is mounted an achromatic ocular, below which a narrow, slit-like diaphragm is situated, the vertical size of this opening being regulated by a milled screw, not shown in the illustration, and its breadth by the two small levers, *I*, *I'*. Both ocular and prisms may be moved simultaneously toward and away from the diaphragm, by a rack-and-pinion mechanism controlled by the wheel, *J*, so that any part of the spectrum may be brought into focus.

The liquids to be examined should be placed in Sorby's tubular cells, and cover-glasses superimposed. These cells (Fig. 37) are narrow-lumened glass receptacles made of barometer tubing, both ends of which are accurately ground to parallel surfaces, one end being cemented to a small polished glass plate.

*Method of Examination.*—The specimen of blood, obtained in the usual manner, by puncture, is first diluted with distilled water 100 times by means of the Thoma-Zeiss erythrocytometer, and sufficient of this laked blood dropped into a Sorby cell to fill it exactly to the brim. A cover-glass is then carefully laid over the open end of the cell, the precaution being taken to prevent the formation of air-bubbles upon the surface of the column of liquid thus inclosed. A second cell, to be used as the control, is filled with normal blood, similarly diluted, and both are then adjusted in their respective positions, as already explained.

FIG. 37.—SORBY TUBULAR CELL.

In making the examination a ray of artificial light (that from a Welsbach incandescent burner being most suitable) is projected by the microscope mirror through the lumen of the cell containing the suspected blood, and the surface of the liquid focused with an ordinary ocular. The latter is then removed from the microscope tube and replaced by the spectroscope ocular, and the second spectrum, that of the normal blood, is brought into proper position alongside that of the first, so that any differences between the two may be contrasted by the observer.

The appearance of the spectra of normal and of pathological blood, together with the circumstances under which the latter occur, has been described in another section. (See p. 168.)

## XIII. BACTERIOLOGICAL EXAMINATION.

The demonstration of bacteria in the circulating blood, provided that faultless technic is employed, furnishes in some instances a diagnostic sign of the greatest importance. The pathological significance of such a finding is much greater than that of a similar result obtained postmortem, since with the latter there is no means of determining whether the bacterial invasion of the blood current took place during the active stages of the disease, or whether it occurred as either a preagonal or a postagonal process.

METHODS. Cultural methods with blood aspirated directly from a superficial vein should invariably be used whenever such a procedure is practicable, for blood obtained simply by pricking the skin is most likely to be contaminated with various bacteria which have their normal habitat in the epidermis and its appendages, notably by the *Staphylococcus epidermidis albus*. Welch,[1] who first drew attention to this source of error, emphasizes the fact that no diagnostic significance should be attached to the demonstration of this bacterium in blood obtained by puncture of the skin.

Direct examination of stained cover-glass specimens prepared from finger blood gives either negative or erroneous results in the great majority of instances. In certain overwhelming infections, notably in some of the severer forms of bubonic plague, it may often be possible to detect the specific micro-organism in the stained film, but the method must be regarded as too crude and unreliable to furnish accurate findings in the average case.

*Blood Cultures.*—In order to secure the most reliable information from blood culturing, the systematic observance of three precautions is essential. First, contamination by the skin bacteria above referred to must be carefully avoided, by the thorough sterilization of the patient's skin at and adjacent to the site from which the blood is aspirated. Second, not less than 0.5 c.c. of blood should be used for each culture, since only in rare instances are bacteria so numerous in the peripheral circulation as to be demonstrable in a single drop of blood. Third, fluid, rather than solid, culture media should be used, in sufficiently large quantities to dilute the blood freely,—about 100 parts of medium to each part of blood,—the object of this precaution being to secure attenuation of the bactericidal properties of the blood, which otherwise might prove strong enough to prevent all bacterial development.

[1] Dennis' "System of Surgery," Philadelphia, 1895, vol. i, p. 251.

For aspirating the blood the author prefers to use a tube of about 10 c.c. capacity, like that illustrated below (Fig. 38). One end of the tube is ground to fit a No. 42 hypodermic needle, while over the other end (plugged with a small bit of cotton) is slipped a piece of rubber tubing for aspirating. The apparatus, minus the rubber tubing, is inclosed in a larger glass tube, both open ends of which are also plugged with cotton, and sterilized by dry heat, the aspirating tube being adjusted at the time the blood is to be collected. This instrument is far superior to an antitoxin or a hypodermic syringe for the purpose intended, being simple, inexpensive, easily sterilized, and readily cleaned after use. It is especially well adapted for making cultures at a distance from a laboratory, where the sterilization of an ordinary piston-syringe is difficult, if not impossible.

At least one hour before the aspiration of the blood the skin of the patient's arm at and for some distance on all sides of the bend of the elbow should be scrubbed thoroughly for several

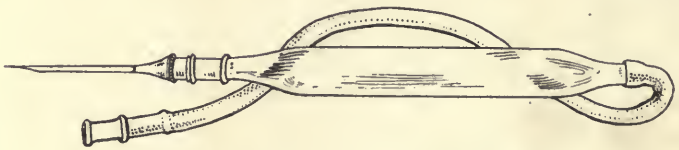

FIG. 38.—ASPIRATING TUBE FOR BLOOD CULTURING.

minutes either with a strong ethereal soap or with tincture of green soap, after which the part is well rinsed with hot sterile water, and finally washed with alcohol and ether. A moist hot 1:500 bichlorid compress is then applied over the site thus cleaned, being left in place until the time of the withdrawal of the blood. As a preliminary to this operation the dressing is removed, and the part freely douched and scrubbed with hot sterile water, in order to remove every trace of the bichlorid. A rubber drainage tube, previously sterilized, is twisted tightly around the patient's arm above the bend of the elbow, so as to cause distention of the superficial veins in this situation, and the point of the needle is then thrust *obliquely* into the most prominent of these vessels, with the result that the blood immediately begins to flow into the bore of the instrument. If, for any reason, the force of the blood flow should fail to fill the caliber of the tube, sufficient blood may easily be obtained by making gentle suction through the rubber tubing. While introducing the needle it should be held almost parallel to the long axis of the vein, for should it be simply

plunged into the vessel at right angles, there is danger that the point will pass completely through the vessel from wall to wall and penetrate the surrounding tissues—an accident which may explain the cause of many a "dry tap." The site of the aspiration may be made anesthetic by preliminary freezing with a spray of ethyl chlorid, but to most patients the operation is not painful enough to necessitate this.

Having thus collected, say, 10 c.c. of blood, the contents of the tube are divided equally among five Pasteur flasks, each containing at least 200 c.c. of broth or other suitable fluid culture medium. The flasks are then shaken for a few moments, in order to mix the blood and medium and to dilute thoroughly the former, after which they are placed in an incubator. The identity of the growths, should any occur, remains to be determined by secondary culturing and microscopical examination, for descriptions of which the student should consult text-books on bacteriology. Cultures made by this technic, suggested by Adami,[1] are much more favorable to the growth of any bacteria which may be in the blood stream than the older methods of using solid media, except under special circumstances, such as the cultivation of the gonococcus, the influenza bacillus, and other germs which grow most luxuriantly on special forms of media.

*Staining Methods.*—In the limited number of instances to which such methods are applicable the technic described below will be found useful.

An attempt should be made to sterilize the skin of the finger from which the blood is obtained by thoroughly scrubbing the part first with ethereal or green soap, and then with a 1:500 bichlorid solution, alcohol, and ether, in the order named, this being followed by sponging with sterile water. A deep puncture having been made with a needle which has been sterilized by the naked flame, and the first few drops of blood escaping from the wound allowed to drip away, one of the succeeding drops is transferred by means of a sterile platinum needle to the surface of a cover-glass upon which a second cover-glass is at once laid, the two being drawn apart, in order to secure a pair of spreads. The latter are immediately dried by gentle heat and then passed several times through a Bunsen flame. It is needless to add that the cover-glasses used for making the films must be sterilized by heat, and handled by means of a pair of sterile forceps. Films thus prepared may be stained with any of the basic anilin dyes (thionin, methylene-blue, and methyl- or

[1] Jour. Amer. Med. Assoc., 1899, vol. xxxiii, p. 1514.

gentian-violet being most useful for this purpose), after which they are washed in water, dried, and mounted in Canada balsam or in cedar oil. Should a double-stained specimen be desired, one of the eosin and methylene-blue solutions referred to previously may be depended upon to give satisfactory results.

Günther's method[1] will be found useful if the object is to destroy the color of the erythrocytes, so as to leave a freer field of vision for any bacteria which may be present in the film. According to this method, the specimen is first immersed for ten seconds in a 5 per cent. aqueous solution of acetic acid, until the tint of the hemoglobin has entirely faded away, after which the reagent is removed by briskly blowing upon the surface of the cover-glass; the latter is then held, face downward, over the open mouth of a bottle containing strong ammonia water, so as to neutralize all remaining traces of the acid. The film is now stained for twenty-four hours with the Ehrlich-Weigert fluid (contained in a covered staining dish), at the end of which time it will be found to be colored a deep blue. It is then decolorized by a few seconds' immersion in a 1 : 14 aqueous solution of nitric acid, until the color fades to a light green; rinsed in alcohol; dried in air; and mounted in balsam.

The Ehrlich-Weigert fluid is prepared by adding from 10 to 15 drops of anilin oil to 6 c.c. of distilled water, held in a test-tube. The fluid is thoroughly mixed by shaking, and then filtered. To the filtrate a few drops of a concentrated alcoholic solution of methyl- or gentian-violet are added—just sufficient of the dye to produce a slight turbidity of the liquid, which clears up in a few minutes. The mixture prepared in this manner is employed as the staining agent.

## XIV. DETERMINATION OF THE SERUM REACTION.

WIDAL'S TEST.

In 1894 Pfeiffer[2] noticed that the vibrios of Asiatic cholera, if injected into the peritoneal cavity of a guinea-pig immunized against this disease, rapidly lost their characteristic motility, and tended to become granular, broken up, and dissolved, while in the healthy, non-immune animal they developed normally and abundantly, and failed to show any such changes in their morphology. He claimed that this reaction, known as "Pfeiffer's

[1] Fortschr. d. Med., 1885, vol. iii, p. 775.

[2] Zeitschr. f. Hyg., 1894, vol. xviii, p. 1; *ibid.*, 1895, vol. xix, p. 75; also Centralbl. f. Bakt. u. Parasitenk., 1896, vol. xix, p. 191; also Deutsch. med. Wochenschr., 1896, vol. xxii, p. 97.

phenomenon," was specific, and emphasized its value as a means of laboratory differentiation. Two years later Pfeiffer and Kolle[1] found that the same changes occurred in experiments with the bacillus of Eberth and animals rendered immune to enteric fever, and, furthermore, discovered that the test could be conducted *in vitro*, by mixing in a test-tube typhoid cultures and immune serum. It is of interest to note that results somewhat analogous to those of Pfeiffer had been observed in 1891 by Metschnikoff,[2] and in 1889 by Bordet,[3] and by Charrin and Roger,[4] although none of these workers appeared to recognize the significance of their observations.

In 1896 Grüber and Durham[5] applied the principles of Pfeiffer's phenomenon to many other motile as well as non-motile bacteria, deduced new facts regarding its utility as a means of differentiating various species of germs, improved the technic of the test, and made the important announcement that agglutination and immobility of typhoid bacillus cultures were produced by the action of blood serum from a patient having recently recovered from an attack of enteric fever. It remained, however, for Widal,[6] in 1896, first to apply the reaction clinically, and to announce that enteric fever could be diagnosed by noting the clumping and immobilization of the typhoid bacillus when mixed in definite proportions with blood serum from a patient suffering from typhoid. This reaction, Widal insisted, was one of infection, and was demonstrable not only during convalescence, but during the incipiency and the height of the disease.

The serum reaction is to-day recognized as an important sign in the diagnosis not only of enteric fever, but also of Asiatic cholera, Malta fever, relapsing fever, paracolon infections, and bacillary dysentery, while its value still remains less certainly established in many other conditions, such as, for example, leprosy, tuberculosis, bubonic plague, sepsis, and pneumococcus infections. The technic of the test and its diagnostic significance under various circumstances will be described under the headings of the diseases in which it occurs. (See Section VII, "General Hematology.")

[1] Zeitschr. f. Hyg., 1896, vol. xxi, p. 203; also Deutsch. med. Wochenschr., 1896, vol. xxii, p. 735.

[2] Annal. de l'Institut Pasteur, 1891, vol. v, p. 473; *ibid.*, 1894, vol. viii, p. 714; *ibid.*, 1895, vol. ix, p. 433.

[3] *Ibid.*, 1895, vol. ix, p. 462; *ibid.*, 1896, vol. x, p. 191.

[4] Compt. rend. Soc. biol., Paris, 1889, vol. i, p. 667.

[5] Münch. med. Wochenschr., 1896, vol. xliii, p. 285.

[6] Bull. méd., 1896, vol. x, pp. 618 and 766; Sem. méd., 1896, vol. xvi, p. 259; *ibid.*, 1897, vol. xvii, p. 69; Lancet, 1896, vol. ii, p. 1371; Münch. med. Wochenschr., 1897, vol. xliv, p. 202.

## XV. MEDICO-LEGAL TESTS FOR BLOOD.

The examination of suspected blood stains for forensic purposes includes microscopical search for blood corpuscles, spectroscopy, the hemin and guaiacum tests, and the biological serum test of Bordet. The first three of these procedures are used merely to determine whether a given stain is or is not composed of blood, but they failed conclusively to prove the source of the latter. By the biological reaction it is possible to supplement these tests by proving the precise origin of the blood, whether human or derived from one of the lower animals. It need scarcely be added that in medico-legal work it is essential to use all four tests in the investigation of the material submitted for study.

Microscopical Examination.

In undertaking the demonstration of erythrocytes in a dried clot, the latter must be treated with an agent which will macerate and dissolve the cells without laking them and thus removing their hemoglobin and altering their shape. Virchow's fluid—a 30 per cent. aqueous solution of potassium hydrate—is suitable for this purpose; or Ranvier's solution—a saturated aqueous solution of iodin containing 2 per cent. of potassium iodid—may be used; this stains various starchy cells which might otherwise counterfeit blood cells. The solution having been effected, a minute portion of the dissolved stain is either mounted as a wet specimen or spread as a dry film between cover-glasses, subsequently stained with appropriate dyes, and mounted in balsam as a permanent specimen. For the microscopical examination a $\frac{1}{12}$-inch oil-immersion objective is essential, and, if measurements are to be attempted, an ocular micrometer.

If corpuscles resembling those of blood are detected in the specimen, especial attention should be directed to their average size, their shape, and to the presence or absence of nuclei. The diameter of the normal human erythrocyte ($\frac{1}{3200}$ in.) is almost equaled by that of the dog's corpuscles, which averages approximately $\frac{1}{3500}$ in. If the cells are disc-shaped and measure $\frac{1}{4000}$ in. or less in diameter, it is safe to consider them non-human, and belonging to some one of the common domestic animals, such as the cat, horse, cow, ox, pig, sheep, or goat. If the corpuscles are of oval shape and nucleated, they are certainly not mammalian, but are derived from a fowl, a fish, or a reptile. A possible, though highly improbable, contradiction to these general premises must be recalled, namely, the structural alterations of human erythrocytes occurring in anemic bloods—particularly the tendency toward microcytosis in chlorosis, and toward megalo-

cytosis, poikilocytosis (often of oval character), and nucleation in intense anemia, such as that of the primary pernicious type. Leukemic blood, however, is characteristically hall-marked. Here may be noted Dresbach's case,[1] unique of its kind, of a healthy young mulatto 90 per cent. of whose erythrocytes were of oval or elliptical shape.

In a considerable proportion of cases microscopical examination of a dried blood clot avails nothing, owing to the destructive changes which have taken place in the cells. Even under the most favorable conditions all one can usually accomplish with the aid of microscopy is to hazard an opinion that a given specimen of blood is either mammalian or derived from a fowl, a fish, or a reptile. For the medico-legal aspects of such examinations, together with the rigid technical precautions demanded, the reader is referred to the standard works on forensic medicine.[2]

SPECTROSCOPY.

This method of examination has already been described. (See p. 107.) It is to be employed whenever a sufficient quantity of a blood solution is available, the purpose being to determine the presence or absence of blood pigment, and further to identify its particular variety. If the blood clot is tolerably fresh, it may yield, in aqueous solution, the absorption bands of oxyhemoglobin, which, on the addition of ammonium sulphid, characteristically change to the spectrum of reduced hemoglobin. Old stains, especially those whose hemoglobin has been altered by exposure to the air and sunlight, show a spectrum of methemoglobin, while blood which has undergone putrefaction, in addition to yielding this spectrum, shows that of hematin. Recent stains can usually be dissolved in distilled water, but old clots require a more active solvent, such as acetic acid or sodium hydroxid. When such reagents are used, the spectra either of acid hematin or of alkaline hematin result, according to the reaction of the solvent employed. If the stain has been subjected to a high degree of heat, with the consequent formation of hematoporphyrin, it should be dissolved with concentrated sulphuric acid, the resulting solution producing the spectrum of hematoporphyrin in acid solution.

TEICHMANN'S HEMIN TEST.

If positive, Teichmann's hemin test is certain proof of blood, although it does not, of course, indicate its source. The test is of extreme delicacy, and may be relied upon to show the presence of the slightest trace of blood in the material examined,

[1] Jour. Amer. Med. Assoc., 1904, vol. xlii, p. 837.

[2] Peterson and Haines, "Text-book of Legal Medicine and Toxicology," Philadelphia, 1904; Tidy, "Legal Medicine," London, 1882.

provided that the composition of the blood is not too materially altered. Negative results are therefore not conclusive, since the reaction may fail in stains exposed to a high temperature or to the prolonged action of the sun's rays, and in those contaminated by certain substances, such as naphtha, iron rust, lime, lead, and animal charcoal.

The hemin test is carried out as follows: A small particle of the suspected material, reduced to a fine powder, is mixed with a drop of normal salt solution upon the surface of a glass slide, the mixture then being evaporated slowly by moderate heat until a dry film forms. Care should be observed not to use too great heat in the evaporation, for fear of spoiling the reaction by causing decomposition of the hematin. The dry film is now covered with a cover-glass, under which a drop of glacial acetic acid is allowed to flow, after which the slide is again heated until minute bubbles begin to form. At this instant the heating should cease, and the preparation be allowed to cool. Active boiling at this stage of the test may drive off all the free hydrochloric acid evolved by the addition of acetic acid, and thus prevent the formation of the sought-for crystals. When cool, the specimen is examined microscopically with a low power ($\frac{1}{5}$ inch) dry objective, which shows, if the material contained blood, distinctive crystals of hemin or hematin hydrochlorid, consisting of yellow or brown rhombohedral plates, lying singly and arranged as crosses or as stellate designs.

**The Guaiacum Test.**

Van Deen's guaiacum test is sufficiently delicate to show the presence of blood pigment in a solution as dilute as 1 : 5000, but unfortunately it responds to so many other substances that its only value is as a negative sign. A bit of the suspected clot or a shred of the stained fabric is moistened with distilled water, in order to dissolve the blood pigment, and to this solution are added a few drops of freshly prepared tincture of guaiacum. To this a drop or two of hydrogen peroxid is added, with the result that a blue color immediately develops in the presence of even minute traces of blood. Or the test may be carried out just as satisfactorily simply by pressing against the dry stain a piece of wet filter-paper, and then adding to the moist daub thus made the guaiacum and peroxid. According to Peterson and Haines,[1] the following substances produce the same reaction with this test as given by blood pigment: potato skin, casein, glue, iron and copper compounds, the double chlorid of gold and sodium, manganese dioxid, potassium permanganate, and indigo; all of which means

[1] *Loc. cit.*

that a positive reaction indicates absolutely nothing definite, although, on the other hand, a negative result proves with great certainty the absence of blood pigment in the material examined.

THE BIOLOGICAL TEST FOR BLOOD.

Originally Bordet,[1] later Uhlenhuth and Tchistovitch,[2] Wassermann and Schutze,[3] and others demonstrated the important fact that the blood serum of an animal into which has been injected the blood of another animal of different species develops the property of agglutinating and dissolving erythrocytes similar to those injected, but has no such effect upon blood derived from another source. The principle of this biological reaction is well expressed by Valée's law: If an animal, A, be inoculated repeatedly with an albuminoid material from an animal of a different species, B, the blood serum of A acquires the specific property of precipitating *in vitro* albuminoid fluids derived from animals belonging to the species B. Thus, in the blood of the inoculated animal are developed antibodies selectively hostile to the toxic principles of substances identical with those injected, and the serum containing such antibodies is known as an antiserum. Lysins, which dissolve; precipitins, which precipitate; and agglutinins, which clump, the poisonous substances and antidote their toxicity, are examples of the antibodies evolved in this manner. On this principle it is possible to produce antisera not only for homologous bloods, but also for different animal albuminous fluids and cells and for vegetable albumins. For instance, such sera have been developed which react specifically with cow's milk, with horse-meat, with semen, with various epithelial cells, and with a number of other substances homologous to those injected.

In the case of antisera for various bloods, while the test is specific with homologous blood, it is also true that feeble reactions may occur with the blood of closely related species. Thus, rabbits treated with human blood yield a serum reacting not only with the blood of man, but also with that of certain species of monkeys. Nuttall and Dinkelspiel[4] and Grünbaum[5] showed that it is the blood of the anthropoid apes (*Simiadæ*), especially the gorilla, the chimpanzee, and the orang, which reacts most

[1] Annal. de l'Institut Pasteur, 1898, vol. xii, p. 688; *ibid*, 1899, vol. xiii, p. 273.

[2] Deutsch. med. Wochenschr., 1901, vol. xxvii, p. 82; *ibid*., 1902, vol. xxviii, pp. 659 and 679.

[3] Berlin. klin. Wochenschr., 1901, vol. xxxviii, p. 187; also Wassermann, "Immune Sera" (Eng. trans. by Chas. Bolduan), New York, 1904.

[4] Brit. Med. Jour., 1901, vol. i, p. 1141; also Nuttall, "Blood Immunity and Blood Relationship," Cambridge, 1904.

[5] Lancet, 1902, vol. i, p. 143.

decidedly with human antiserum; while the lower orders of monkeys, represented by the *Hapalidæ*, the *Cercopithecidæ*, and the *Cebidæ*, give less positive results. Practically, this startling biological confirmation of Darwin's views is scarcely a source of error, since in high dilutions (500 or more) human antiserum does not show a precipitate even with the highest order of primates' blood (Layton[1]).

Antisera for horses, cattle, sheep, pigs, dogs, birds, and other animals and fowls all react typically with homologous bloods, but also sometimes, although always atypically, with alien bloods. Pig antiserum, for example, reacts faintly with the blood of the wild boar; horse antiserum with donkeys' blood; fox antiserum with wolves' and dogs' blood; and sheep antiserum with goats' blood. It is a notable fact that these are reactions between *biologically related* species of animals, and that they are feeble and atypical, in comparison with the reactions occurring between homologous antisera and bloods. It is also true that the more remote the biological relation of the animal from the one whose blood activates the antiserum, the feebler the reaction becomes. As explained subsequently, these pseudo-reactions do not occur if the blood to be tested is adequately diluted before examination.

Technic.—*Preparation of the Antiserum.*—The antiserum is prepared by injecting healthy rabbits with from 5 to 10 c.c. of human defibrinated blood, at intervals of about four days, until a total of between 50 and 80 c.c. has been administered. One or two weeks after the last injection the animal is bled, and the serum obtained is collected in sterile test-tubes, which are sealed and stored for future use. Belgian hares are excellent antiserum producers, and are preferable to ordinary rabbits, being more resistant to the toxic effects of the injections, and yielding highly potent antiserum. Goats, sheep, and dogs have also been used, but not with wholly satisfactory results. Ewing[2] obtained excellent antiserum from a hen, which, being biologically far removed from man, should theoretically furnish a highly selective human antiserum.

The blood used for the immunization may be conveniently secured from the placenta and umbilical cord. It is collected in a sterile flask containing several small glass beads, which defibrinate the blood when the flask is agitated for a few minutes. The blood thus defibrinated may be injected immediately into the animal, or placed in a refrigerator for subsequent use. The fresher the blood injected, however, the more powerful the antiserum which it produces. The injections may be subcutaneous, intravenous, or

[1] Amer. Med., 1903, vol. v, p. 913. [2] Med. News, 1903, vol. lxxxiii, p. 925.

intraperitoneal, the last being the simplest and least dangerous to the animal if properly carried out. One-half of the rabbit's lower abdomen having been shaved and disinfected with a sublimate solution, the needle of the syringe is firmly thrust through the tissues at a point within the prepared area, and the injection made when the abdominal cavity is entered. Experience will determine the amount of pressure and manipulation necessary to penetrate the abdomen to a sufficient depth and to avoid wounding the gut. While making the injection an assistant should hold the animal in such a position that its abdominal wall is kept tense. Since the injection of human blood not infrequently causes toxic symptoms in the treated animals, they should be kept in the best possible hygienic surroundings, with ample runways, an abundance of air and light, and plenty of food and water. It is a good plan temporarily to discontinue the injections in animals which show marked loss of weight and other evidences of severe reaction.

The blood of the animal under treatment should be tested from time to time, and when found to be sufficiently potent with human blood, the injections are stopped. A week or two later the antiserum will be sufficiently powerful, and it is then collected by bleeding the rabbit either from an ear vein or from the carotid artery. In neither instance is it necessary to exsanguinate the animal. The blood is collected, under aseptic precautions, in a dish, from which the serum, after coagulation has occurred, is pipetted into small sterile test-tubes measuring 10 cm. in height by 0.5 cm. in diameter. The filled tubes are then plugged with cotton and set upright in a refrigerator until required for the test. As a preservative a few drops of chloroform may be added to each tubeful of antiserum. If this is done, however, the antiserum must be incubated for half an hour before being used, in order to remove, by volatilization, all traces of the chloroform, which otherwise might cause a pseudo-reaction with alien blood. Other preservatives, such as lysol, lysoform, carbolic acid, and thymol, may also cause clouding of antiserum, and therefore should not be used. Solutions of mercuric chlorid strong enough to be antiseptic destroy the antiserum.

The test antiserum may be preserved in dry form by pouring it into Petri dishes and drying in a cool place, the film thus obtained being powdered and kept in a tightly stoppered bottle until required for use. To prepare the antiserum, this powder is simply dissolved in normal salt solution in definite proportions.

*Testing the Suspected Stain.*—In all cases the suspected stain must be proved to be blood by the hemin crystal test, the spectroscope, and the other older methods described above. This is

highly essential in forensic work, because body albumins other than blood (sputum, saliva, pus, feces, exudates, and albuminous urine) are capable of giving positive reactions with human antiserum. Contamination by such materials is, however, readily detectable by other tests.

The stain to be examined is dissolved with a few drops of a 0.6 per cent. aqueous solution of chemically pure sodium chlorid,[1] and the resulting cloudy mixture cleared by filtration through either asbestos or Schleicher's "blue ribbon" filter-paper, or by centrifugalization. The solution of the stain thus rendered perfectly clear is then added to the antiserum in the proportion of at least 100 to 1, and the mixture incubated at 37° C. If the test be positive, a distinctly flocculent precipitate will form in the test-tube within three hours, this reaction being preceded by the formation of a more or less marked turbidity immediately or very shortly after having mixed the antiserum and the blood solution. Slight turbidities do not constitute positive reactions—they may occur with heterologous bloods in dilutions less than the above. Several controls are also to be prepared and incubated simultaneously: one of the salt solution used as a solvent; a second of the pure dissolved and cleared stain; a third of the pure test antiserum; a fourth of human blood and normal rabbit's serum; and a fifth of human blood and the test antiserum. All but the last of these controls should remain clear.

*Sources of Error.*—Aside from pseudo-reactions due to under-dilution of the antiserum, accidental clouding and precipitation may arise from the following group of factors:

1. *Bacterial Growths.*—Antiserum clouded by bacterial contamination may be rendered clear by filtration through a Pasteur-Chamberland filter or by centrifugalization. Clouding from this cause does not occur within the arbitrary three-hour time limit of the test.

2. *Preservatives and Solvents.*—As stated above, various chemicals used for preserving the antiserum and for dissolving the suspected stains may lead to wrong inferences.

3. *Hyperacidity and Hyperalkalinity.*—The solution of the stain should be neutral in reaction, feebly acid, or feebly alkaline.

[1] In the case of old, difficultly soluble stains Uhlenhuth uses a one per cent. aqueous solution of sodium hydrate, while Ziemke prefers a concentrated solution of potassium cyanid, with subsequent neutralization with tartaric acid. These chemicals, though active solvents for old blood stains, are not dependable, because of their likelihood to cause false reactions with various antisera. Graham-Smith and Sanger obtained precipitates with solutions of potassium cyanid (one per cent.), tartaric acid (0.1 per cent.), and sodium hydrate (0.1 per cent.). In medico-legal work it is safe to be guided by Nuttall's advice, and refuse to submit to the test bloods which are insoluble in normal salt solution

If too highly acid or alkaline, positive reactions may occur with unrelated blood.

4. *Contamination of the Specimen.*—Human albumins and various chemicals may be mixed with the suspected blood stain or may resemble it, and in forensic work their presence must be absolutely excluded by appropriate tests. Nuttall[1] found that tannin especially causes decided clouding even in 1 : 1000 dilutions, and that solutions contaminated with yellow polished leather acted similarly. He found that human blood mixed with shoe polish, and blood allowed to dry upon black leather, wall-paper, various dress fabrics, rubber, oil-cloth, silver and copper coins, coal, wood, and other substances, reacted typically with human antiserum. The clouding due to the admixture of earth (referable to the presence of lime salts) Nuttall obviates by saturating with carbon dioxid and subsequent filtration.

Old blood stains, except that they develop the precipitate slowly, react like fresh blood. Ziemke[2] examined stains known to be twenty-five years old and obtained positive findings. According to J. Meyer,[3] even solutions of the muscular tissues of five-thousand-year-old mummies react positively! Uhlenhuth[4] found that specimens could be frozen for two weeks at a temperature of —10° C. without in any manner affecting the sensitiveness of the reaction, and that blood mixed with soapy water, menstrual urine, and other contaminating fluids responds typically and promptly. Nuttall and Dinkelspiel[5] demonstrated that human blood mixed with the blood of different animals (sheep, oxen, horses, and dogs) reacts characteristically with human antiserum. Dried blood crusts subjected to an hour's heating at 130° C. do not respond to the test.

*Value of the Test.*—The Bordet reaction has already figured in three murder trials in this country,[6] and in these cases it has been accepted by the Court as valid evidence. In order to insure infallibility, faultless technic, bred only of long and intelligent experience, is one of the first essentials. This acquired, the examiner must exclude every source of error outlined above, and consider as positive only those reactions which, with proper dilution, afford a turbidity and distinct precipitate within the prescribed time limit, the behavior of the several controls being consistent with that of the main test. Under these conditions a solution

[1] *Loc. cit.*
[2] Deutsch. med. Wochenschr., 1901, vol. xxvii, pp. 424 and 731.
[3] Münch. med. Wochenschr., 1904, vol. li, p. 663.
[4] *Loc. cit.* [5] Brit. Med. Jour., 1901, vol. i, p. 1141.
[6] 1901, State of Maine *vs.* Lambert (Whittier); 1903, State of Del. *vs.* Collins (Robin); 1903, Commonwealth of Penna. *vs.* Bechtel (Lear).

proved to be blood which reacts typically with human antiserum may surely be pronounced of human origin.

HANGING-DROP TEST.

The agglutination of human erythrocytes after their admixture with alien serum and the absence of this change on the addition of homologous serum may be observed under the microscope. This fact was first noted by Marx and Ehrnrooth,[1] who use the principle as the basis for the following simple test for identifying blood stains.

The suspected blood stain is dissolved with a small quantity of normal salt solution mixed with an equal amount of fresh human blood, and of this solution a hanging-drop preparation is made and examined under the microscope with a $\frac{1}{6}$-inch objective. If the stain tested is human blood, the erythrocytes of the added fresh blood remain unclumped at the end of fifteen minutes' time, while if it is alien blood, they become distinctly agglutinated within this period. Monkeys' blood, although it fails to clump the erythrocytes, causes them to shrink and to become polygonal in shape. The writer can confirm this test, so far as human blood is concerned, but its medico-legal value must remain undetermined until further studies have been made. The reaction should be controlled by Bordet's test, for which it is in no sense a substitute.

## OTHER METHODS OF BLOOD EXAMINATION.

Numerous methods of blood analysis other than those described in this section have also been devised, more especially for scientific investigation than for clinical use. Their detailed description not being germane to the plan of this book, the reader is referred to the original articles for exact data. Among these non-clinical methods of research are included the estimations of the *total volume* of blood (Haldane and Smith[2]); of the amount of *solids* (Stintzing[3]); of the percentage of *blood iron* (Jolles[4]); of the quantity of *fat* and *fatty acids* in the blood (Engelhardt[5]); of the *blood viscosity* (Hirsch and Beck[6]); of the *osmotic tension* of the plasma (Hamburger[7]; de Vries[8]); and of the *resistance of the erythrocytes* to the action of electricity, heat, and mechanical injury (Laker[9]; Maragliano[10]).

[1] Münch. med. Wochenschr., 1904, vol. li, p. 293.
[2] Jour. Physiol., 1900, vol. xxv, p. 311.
[3] Verhandl. d. XII. Cong. f. inn. Med., 1893.
[4] Arch. f. med. Exp., vol. xiv, p. 73.
[5] Deutsch. Arch. f. klin. Med., 1901, vol. lxx, p. 182.
[6] *Ibid.*, 1901, vol. lxix, p. 503. [7] Centralbl. f. Physiol., 1893, vol. vii, p. 656.
[8] Jahrb. f. w. Botanik, 1884, vol. xiv, p. 427.
[9] Wien. med. Presse, 1890, vol. xxxi, p. 1375.
[10] Berlin. klin. Wochenschr., 1887, vol. xxiv, p. 797.

## SECTION II.

# THE BLOOD AS A WHOLE.

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## THE BLOOD AS A WHOLE.

### I. GENERAL COMPOSITION.

PLASMA, SERUM, AND CELLS.

Blood is a tissue consisting of fluid and corpuscular elements, the former constituting about three-fifths, and the latter two-fifths, of its total volume. It has been approximated that the total quantity of blood in the normal individual is from one-twelfth to one-fourteenth of the body-weight, the proportion being somewhat less in the infant than in the adult. Much lower proportions than these were determined by Haldane and Smith,[1] who found that in health the bulk of blood ranged from one-thirtieth to one-sixteenth of the body-weight (3.34 to 6.27 per cent.), these figures having been calculated by a method based upon the capacity of the blood to absorb $CO_2$. The fluid element of the blood, known as the *plasma* or *liquor sanguinis*, is an alkaline, yellowish liquid, of a specific gravity ranging from about 1.026 to 1.030, and containing approximately 10 per cent. of solid matter, of which three-fourths are proteids, consisting of fibrinogen, serum albumin, and serum globulin. Coagulation of the blood results in its separation into a densely reticulated, somewhat granular substance, *fibrin*, and into a clear, straw-colored, alkaline fluid, *serum*. Fibrin is a sparingly soluble, highly elastic, proteid body, which incloses and imprisons within its multitude of delicate fibrils the corpuscular elements, the whole forming the *blood clot* or *crassamentum*. Serum is a clear, straw-colored, alkaline fluid, having a specific gravity of about 1.026 and containing practically the same amount of solids and relative proportion of proteids as are found in the plasma ; its proteid constituents are fibrin ferment, which replaces the fibrinogen of the plasma, serum albumin, and serum globulin.

The corpuscular elements of the blood are free cellular bodies suspended in the plasma. They are of two varieties : the *erythrocytes* or *red corpuscles*, and the *leucocytes* or *white corpuscles*.

[1] Jour. Physiol., 1900, vol. xxv, p. 33.

In addition to these cells, two other elements are also found, namely, the *blood plaques* and the *hemokonia*, although these bodies, while they may be conveniently grouped with the red and white cells, are not to be regarded as definite corpuscular entities.

The salts of the blood include sodium chlorid, potassium chlorid, sodium carbonate, sodium phosphate, magnesium phosphate, calcium phosphate, and sulphates ; of these salts, sodium chlorid is the most abundant, constituting from 60 to 90 per cent. of the total amount of mineral matter.

Certain extractives are also found, among which are urea and uric acid, creatin, creatinin, xanthin, hypoxanthin, sugar, fats, soaps, and cholesterin.

The gases of the blood consist of oxygen, nitrogen, and carbon dioxid, the oxygen existing chiefly in combination with hemoglobin in the erythrocytes, and the carbon dioxid as carbonates ; the nitrogen is held in simple solution. About 60 volumes of gas are contained in each 100 volumes of blood. Arterial blood contains roughly 20 volumes of oxygen and 40 of carbon dioxid, while venous blood contains less than 10 volumes of oxygen and almost 50 of carbon dioxid ; the quantity of nitrogen in both arterial and venous blood is from 1 to 2 volumes.

## II. COLOR.

NORMAL VARIATIONS.

The distinctive color of the blood is due to the presence of the hemoglobin contained in the erythrocytes, and alterations in the chemical composition of this pigment produce corresponding changes in the color of these cells, and, consequently, in the naked-eye appearance of the whole blood. The color of arterial blood is bright scarlet, inasmuch as it contains a large amount of oxygen in chemical combination with the hemoglobin ; while venous blood, on the other hand, is of a dark, purplish-blue tint, owing to its deficiency in oxygen and to the presence of more or less uneliminated carbon dioxid. This difference in color is so obvious that a cursory glance suffices to distinguish arterial and venous bloods.

DENSITY AND OPACITY.

The presence of immense numbers of hemoglobin-containing elements accounts for the varying degree of density and opacity which the blood possesses, distinguishing it from a mere transparent, colored fluid. If, for any reason, the hemoglobin escapes from the erythrocytes into the surrounding plasma, this characteristic opacity is quickly lost, and the blood

becomes transparent and of a " laky " color. The cells are laked by the influence of heat, water, fat solvents, and hydrogen and hydroxyl ions, all of which act upon the outer semipermeable layer of the cells' stroma and thus favor hemoglobin dissolution. The density and the opacity, and, consequently, the color, of the blood increase and diminish according to the fluctuations which occur in the relative amounts of plasma and erythrocytes, and also according to the cells' richness in hemoglobin, irrespective of their numerical variation.

PATHOLOGICAL VARIATIONS.

In anemic conditions the blood is usually pale in color, somewhat transparent, and thin and watery-looking. This is the case particularly in *primary pernicious anemia*, in *chlorosis*, and in *leukemia;* in the first-named disease it is sometimes difficult to believe that the watery, pale fluid which flows from the puncture is anything but pure serum ; in leukemia the blood drop may have a peculiar light, mottled, streaked appearance, or a uniform milky-white tint may predominate over the normal red hue. In cases of *dyspnea*, arterial blood, because of its inadequate oxygenation, may be dark blue, closely resembling blood from the veins. This similarity has also been noted in cases of poisoning by *sulphuretted hydrogen*, in which condition the blood may even be changed to a dark greenish tint. In some cases of *diabetes mellitus* the presence of large quantities of free fat in the circulation seemingly divides the blood drop into two distinct layers—an upper, light-colored portion, containing supernatant fat droplets, and a lower, darker layer of pure blood ; at first glance diabetic blood may be somewhat pinkish or salmon-colored.

In poisoning by *anilin*, *nitrobenzol*, *hydrocyanic acid*, and *potassium chlorate* the blood is chocolate- or dun-colored ; and in poisoning by *carbon monoxid*, bright cherry-red. In severe *icterus* a yellowish-red tint of the blood has been observed.

## III. ODOR AND VISCOSITY.

Owing to the presence of certain volatile fatty acids blood possesses a peculiar and characteristic odor or *halitus*, which may be intensified by the addition of concentrated sulphuric acid, and which rapidly disappears after the withdrawal of the blood from the body. The slippery, greasy feeling of freshly drawn blood is quickly lost after its exposure to the atmosphere, and is replaced by a *viscosity*, or stickiness, as coagulation progresses. The viscosity of the whole blood is apparently influenced to a large extent by the cellular elements, chiefly by the erythrocytes, although the

viscosity of the serum must also be regarded as a determining factor of more or less importance.

Hirsch and Beck[1] have determined that the "viscosity value," as they term it, of human blood is about five times that of distilled water—*i. e.*, the viscosity of blood having a specific gravity ranging between 1.045 and 1.055 is expressed by the figure 5.1, in comparison with that of water, which equals 1, the temperature of both fluids being the same, 38° C. Although no close relationship can be distinguished between the degree of viscosity and the specific gravity of the blood, these experimenters have apparently proved that the lower the density of the blood, the less marked its adhesiveness. This quality is exaggerated in individuals living upon a largely nitrogenous diet, and it is greatly modified by starvation. S. Weir Mitchell[2] has observed that hyperviscosity develops when blood is subjected to the direct action of snake venom, while Stengel[3] has noted a similar condition resulting from contaminating fresh blood with the serum of patients suffering from chlorosis, pernicious anemia, and leukemia. Any one who has done much blood work is familiar with the marked fluidity of the fresh specimen in the high-grade anemias, and with the diminished viscosity of the erythrocytes and their disinclination to form rouleaux under such circumstances.

## IV. REACTION.

Reaction in Health.

Under normal conditions the reaction of the blood is alkaline, owing chiefly to the presence of sodium carbonate and disodium phosphate. Clinically, the degree of alkalinity is determined by ascertaining the amount of sodium hydroxid which is exactly neutralized by 100 c.c. of blood, the result being usually expressed in milligrams of NaOH per 100 c.c. of blood. The figures given by different investigators as representing the normal alkalinity range within the widest limits, chiefly in consequence of the many different methods by which such data were obtained. In view of these marked discrepancies the alkalinity figures of different workers are in no sense comparable unless they are based upon precisely similar methods of investigation pursued with identical technic. The following table, compiled from reliable data, illus-

[1] Deutsch. Arch. f. klin. Med., 1901, vol. lxix, p. 503.

[2] Mitchell and Stewart, "A Contribution to the Study of the Effect of the Venom of Crotalus adamanteus upon the Blood," Washington, 1898.

[3] "Twentieth Century Practice of Medicine," New York, 1896.

trates the range of the normal blood alkalinity as estimated by various observers:

| Observer. | Degree of Alkalinity. |
|---|---|
| Kraus | 162–232 mgm. NaOH per 100 c.c. of blood. |
| Burmin | 182–218 " " " " " " " |
| Rumpff | 182–218 " " " " " " " |
| Jeffries | 200 " " " " " " " |
| Freudberg | 200–240 " " " " " " " |
| Lépine | 203 " " " " " " " |
| Canard | 203–276 " " " " " " " |
| Drouin | 206 " " " " " " " |
| Von Limbeck | 218 " " " " " " " |
| Zuntz and Lehmann | 240 " " " " " " " |
| Orlowsky | 240–267 " " " " " " " |
| Von Jaksch | 260–300 " " " " " " " |
| Schultz-Schultzenstein | 260–300 " " " " " " " |
| Dare | 266 " " " " " " " |
| Strauss | 300–350 " " " " " " " |
| Brandenburg | 330–370 " " " " " " " |
| Löwy | 449 " " " " " " " |
| Berend | 450–500 " " " " " " " |
| Engel | 479–533 " " " " " " " |
| Mya and Tassinari | 616 " " " " " " " |

With the titration method, now generally admitted to furnish fairly accurate results, appreciably higher figures are obtained with laked whole blood than with serum alone, since by the former method the alkalinity of all the plasma and cellular elements is estimated, while by the latter the influence of the corpuscles is entirely eliminated.

Physiological Variations.

The alkalinity of the blood is slightly higher, as a general rule, in *men* than in *women* and *children*, and is somewhat influenced by the *time of day*, being at its minimum during the early morning hours, gradually rising during the afternoon, and falling again during the evening. Some observers maintain that it is increased during the *period of digestion*, but this fact is disputed by others. It is temporarily diminished by the effects of *muscular exercise* and by a diet deficient in *nitrogenous substances;* on the contrary, *richly nitrogenous food* eaten during the performance of muscular work overcomes the effect of such exertion in lowering the alkalinity. The effects of *cold baths* are said to increase the alkalinity of the blood. Orlowsky,[1] from a study of 63 cases of various maladies, concludes that the degree of blood alkalinity is proportional to the erythrocyte count, but that it bears no relation to the number of leucocytes.

In health, by the perfect mechanism of the emunctory organs of the body, the normal balance of blood alkalinity is constantly

[1] Deutsch. med. Wochenschr., 1903, vol. xxix, p. 601.

maintained, in spite of the entrance of acids into the blood, whether by the ingestion of acid substances or by their production within the system, for the hyperacidity from such causes is promptly removed from the blood by the action of the kidneys, the skin, and the lungs. Thus, the ingestion of acids is quickly followed by increased acidity of the urine and sweat, while at the same time an increased quantity of carbon dioxid is given off by the lungs. It is probable that the tendency to acidity is partly neutralized by the ammonium salts generated from proteid foods, and also by the action of the liver. The blood alkalinity may be transiently increased by administering an alkali internally or by enema, the latter method having the more pronounced effect, according to Orlowsky.[1]

Increased alkalinity goes hand in hand with *increased antidotal action of the blood* against bacterial infection, as experiments have shown that animals whose blood had been artificially rendered highly alkaline by the administration of sodium salts, showed much greater resistance to the effects of virulent micro-organisms than untreated animals. Therefore, it is believed that the power of immunity against infections may, to a certain degree, be measured by the alkalinity of the blood, for, in animal experimentation, the fact is evident that the greatest degree of blood alkalinity is found in animals whose immunity is absolute. The rat, which is naturally immune to anthrax, has excessively alkaline blood and other body fluids. This hyperalkalinity, however, does not protect this rodent against plague. An excess of alkali in the blood is probably antidotal to invading bacteria, not *per se*, but rather because of its power to dissolve and liberate cell nucleins, which, as alexins, are directly bactericidal.

Pathological Variations.

Unfortunately, the question of alteration in the alkalinity of the blood in various pathological conditions is at the present time one about which the opinions of different observers conflict, so that conclusions concerning this subject must be accepted with more or less reserve.

It is of interest, however, to note that most observers agree that, as a rule, the alkalinity of the blood is perceptibly lowered in those diseases associated with a *febrile movement*, but no definite relation between the intensity of the pyrexia and the degree of lessened alkalinity has been established. Auerbach,[2] having noted that a temperature of 108° F. renders alkaline culture media bactericidal, argues that in acute infections the pyrexia, although it diminishes the alkalinity of the blood, at the same time may be

[1] *Loc. cit.* [2] Abst. in Jour. Amer. Med. Assoc., 1903, vol. xli, p. 1122.

beneficial, in that it also increases its bactericidal action. Subnormal alkalinity figures have also been met with in the *primary* and *secondary anemias*, with the exception of *chlorosis*, in which condition the blood alkalinity usually is either normal or perhaps slightly increased. Desevres[1] has drawn attention to the fact that in the early stages of *acute diseases* the alkalinity is either normal or somewhat increased, and in the majority of instances it becomes perceptibly diminished during convalescence. In *chronic diseases* it is usually decreased if the duration of the disease has been of long standing.

Drouin[2] found a lessened alkalinity of the blood in *enteric fever*, *pneumonia*, *malarial fever*, *diphtheria*, *rheumatic fever*, *erysipelas*, *appendicitis*, and in many other acute infections. Cantani[3] maintains that in the algid stage of *Asiatic cholera* the reaction of the blood during life in some cases may be even acid, and that the alkalinity is always markedly reduced. Von Jaksch,[4] Peiper,[5] Kraus,[6] and others state that the alkalinity is generally diminished in *uremia*, *diabetes*, *osteomalacia*, *organic diseases of the liver*, and in *poisoning by carbon monoxid* and by *phosphorus*, especially by the latter. Decreased alkalinity has also been noted in *cholemia*, *Addison's disease*, *Hodgkin's disease*, *poisoning by mineral acids*, the late stages of *malignant neoplasms*, and in various long-standing *cachectic conditions*. Thomas[7] found the alkalinity reduced in *acute alcoholism* and as the result of *chloroform narcosis*. Tchlenorff[8] found a diminished alkalinity in a wide variety of skin diseases, among which are named *psoriasis*, *eczema*, *pemphigus*, *purpura hæmorrhagica*, *erythema multiforme*, *lichen rubra*, and *elephantiasis*. Since it was also found that the administration of arsenic failed to increase the blood alkalinity, the action of this drug upon dermatoses is evidently not due to its influence upon the blood.

In *organic diseases of the heart* unassociated with pyrexia and in *nervous diseases* the alkalinity of the blood has been found to be increased. In *chronic rheumatism* and in *renal lesions* unaccompanied by uremic symptoms the reaction of the blood is usually found to be unaltered.

[1] Thèse de Lyon, 1897–98.
[2] "Hémo-alcalimétrie et Hémo-acidimétrie," Thèse de Paris, 1892, No. 83.
[3] Centralbl. f. d. med. Wissensch., 1884, vol. xxii, p. 785.
[4] Deutsch. med. Wochenschr., 1893, vol. xix, p. 10.
[5] Arch. f. pathol. Anat., 1889, vol. cxvi, p. 337.
[6] Zeitschr. f. Heilk., 1889, vol. x, p. 106.
[7] Arch. f. exper. Pathol. u. Pharm., 1898, vol. xli, p. 1.
[8] Russkiy Vrach, 1898, vol. xix, p. 248; abst. in Jour. Cutan. and Genito-Urin. Dis., 1898, vol. xvi, p. 544.

## V. SPECIFIC GRAVITY.

NORMAL RANGE.

In the majority of healthy *male adults* the specific gravity of the blood varies from 1.055 to 1.065, the average being in the neighborhood of 1.060. In *women* the average is somewhat less—about 1.056; in *children* it is about 1.051; and in *new-born infants* 1.066 is considered normal. *Diurnal variations* in the specific gravity have been noted, but these fluctuations are slight and unimportant. The blood density of habitual dwellers in *high altitudes* is distinctly increased. Lloyd Jones[1] and Schmaltz[2] found that *muscular exercise*, if moderate, lowers the blood density, but if prolonged and attended by free sweating, distinctly increases it. *Venous* blood is said to be of slightly higher specific gravity than *arterial*. The average specific gravity of the blood of the two sexes, as determined by the principal observers, is as follows:

| AUTHORITY. | MALES. | FEMALES. |
|---|---|---|
| Askanazy | 1060.1 | 1056.4 |
| Schmidt | 1060.0 | 1050.0 |
| Hammerschlag | 1061.5 | 1057.5 |
| Lloyd Jones | 1058.5 | 1051.5 |
| Landois | 1057.5 | 1056.0 |
| Becker | 1057.0 | 1056.5 |
| Schmaltz | 1057.0 | 1056.0 |
| Peiper | 1055.0 | 1053.0 |

PATHOLOGICAL VARIATIONS.

From a clinical standpoint the specific gravity of the blood may be regarded, within certain limits, as a tolerably accurate index to the corpuscular richness of this tissue and to the hemoglobin equivalent of the erythrocytes, since fluctuations in these constituents immediately give rise to corresponding alterations in the density of the blood mass. It follows, then, that in the various conditions of anemia, characterized by corpuscular and hemoglobin losses, low specific gravities are encountered; on the other hand, it is also obvious that in conditions of polycythemia the cellular increase and the high hemoglobin equivalent are mirrored by the corresponding rise in the density of the blood. An increase promptly follows any sudden drain upon the fluids of the system sufficient to cause inspissation of the blood, such as may result from copious diarrhea, free sweating, or hyperemesis; while the density is at once lowered as the result of sudden dilution of the blood, following, for example, the injection of a large quantity of saline solution or even the inges-

[1] Jour. Physiol., 1887, vol. viii, p. 1.
[2] Deutsch. Arch. f. klin. Med., 1890, vol. xlvii, p. 145.

tion of a large volume of liquid. Fluctuations in the specific gravity of the blood under such circumstances, which are purely physiological in character, are invariably of transient duration, for the normal relation between the relative volumes of corpuscles and plasma becomes quickly reëstablished by means of the liquid interchange between the tissues and the blood vessels.

Owing to the fact that in most instances a close relationship exists between the amount of hemoglobin and the specific gravity, some investigators are accustomed to take this parallelism as a basis for calculating the percentage of hemoglobin in the blood. Thus, by determining the specific gravity and by comparing the figure thus obtained with a table giving the hemoglobin equivalents corresponding to varying degrees of blood density, fairly accurate results have been obtained. The following hemoglobin equivalents of different specific gravities of the blood have been determined by Hammerschlag[1] and by Lichty.[2]

| Hammerschlag. | |
|---|---|
| *Specific gravity.* | *Hemoglobin equivalent.* |
| 1033–1035 | 25–30 per cent. |
| 1035–1038 | 30–35 " " |
| 1038–1040 | 35–40 " " |
| 1040–1045 | 40–45 " " |
| 1045–1048 | 45–55 " " |
| 1048–1050 | 55–65 " " |
| 1050–1053 | 65–70 " " |
| 1053–1055 | 70–75 " " |
| 1055–1057 | 75–85 " " |
| 1057–1060 | 85–95 " " |

| Lichty. | |
|---|---|
| *Specific gravity.* | *Hemoglobin equivalent.* |
| 1035–1038 | 25–30 per cent. |
| 1038–1043 | 30–40 " " |
| 1043–1045 | 40–45 " " |
| 1045–1047 | 45–50 " " |
| 1047–1049 | 50–55 " " |
| 1049–1052 | 55–65 " " |
| 1052–1054 | 65–70 " " |
| 1054–1056 | 70–75 " " |
| 1056–1060 | 75–85 " " |
| 1060–1063 | 85–95 " " |
| 1063–1065 | 95–100 " " |

It will be noted that in both these tables the variations in density are somewhat greater in high than in low hemoglobin percentages. It has been stated by Diabella[3] that, on the average, a difference of 10 per cent. in hemoglobin corresponds to 4.46 parts per thousand in specific gravity, and that differences amounting to from 3 to 5 parts per thousand in the specific gravity may arise from the influence of the stroma of the erythrocytes, in blood characterized by a striking disturbance in the parallelism which normally exists between these cells and the hemoglobin.

In the clinical application of this indirect method of computing hemoglobin percentages several conditions, in which factors other than the presence of hemoglobin in the erythrocytes influence the specific gravity, must be excluded. In *leukemia*, for example, it will be found that hemoglobin percentages based on the above tables are much higher than actually exist, the cause of this fal-

[1] *Loc. cit.* [2] Phila. Med. Jour., 1898, vol. ii, p. 242.
[3] Deutsch. Arch. f. klin. Med., 1896, vol. lvii, p. 302.

lacy being the presence of enormous numbers of leucocytes in the blood; in *pernicious anemia* the hemoglobin is frequently higher than the specific gravity indicates, for in this disease the individual corpuscles are much richer in hemoglobin than normally; and in conditions associated with extensive *dropsy* the hemoglobin percentage does not parallel the specific gravity, owing to the abnormally high proportion of fluids in the blood mass.

These three sources of error, aside from the rather trying technic by which one must first determine the specific gravity of the blood drop (see p. 94), are sufficient to make most workers reluctant to adopt this method as a substitute for the hemometer.

## VI. FIBRIN AND COAGULATION.

The essential factor of coagulation of the blood is the formation of fibrin, a proteid substance, produced in the plasma after the withdrawal of the blood from the body, by complex chemical changes occurring between the soluble calcium salts and the nucleoproteids of the blood, with the consequent production of a fibrin ferment. The theories regarding coagulation are numerous, conflicting, and unsatisfactory, and must necessarily remain disputed points until our present uncertain knowledge of the chemistry of the blood proteids becomes fuller and more definite.[1] Coagulation is delayed and imperfect in *hemoglobinemia*, in *asphyxia*, in *jaundice*, in conditions of *general dropsy*, and in individuals who are prone to *bleed freely* from trivial wounds. A similar effect is produced by the administration of *alcohol*. Lamb[2] has found that the *venom* of certain poisonous snakes conspicuously affects clotting. Cobra venom diminishes coagulation to a marked degree, and in some instances may even wholly prevent it. Intoxication with daboia poison (the venom of Russell's viper) acts variously, according to the quantity injected. In small doses it hinders coagulation and causes the formation of soft, loose clots; but in large doses this venom so increases the coagulability of the blood as to lead to fatal intravascular clotting. Small doses of the *calcium salts*, especially the chlorid, promote coagulation, but the opposite effect ensues if the dose is too large. The administration of *gelatin* usually acts in the same manner, because, so Gley[3] and others of the French school believe, of the fact

[1] Schäfer's "Text Book of Physiology," vol. i, Edinburgh and London, 1898, contains a complete exposition of the various theories of coagulation of the blood existing up to the present time.

[2] Glasgow Med. Jour., 1903, vol. lix, p. 80.

[3] Sem. méd., 1903, vol. xxiii, p. 113.

that it always contains from 2 to 5 per cent. of calcium chlorid. Brat[1] explains the therapeutic action of gelatin in promoting intravascular clotting by assuming that it contains substances which favor the deposit of plastic material, presumably derived from the blood cells, at the site of the clot. The studies of Boggs[2] show that gelatin is a much less active clotting agent than calcium chlorid, and that it may entirely fail to act in some cases. Blood coagulability as a factor of intestinal hemorrhage and of venous thrombosis in *enteric fever* is referred to elsewhere. (See Section VII.)

Carstairs Douglas[3] found the following coagulation figures in healthy women and in normal and complicated pregnancies:

| Group. | Average Coagulation Time in Minutes. | Minimum. | Maximum. |
|---|---|---|---|
| Albuminurics (16 cases): | | | |
| (*a*) Pregnant | 5.60 | 4.70 | 7.5 |
| (*b*) Puerperal | 7.00 | 5.60 | 10.0 |
| Eclampsias (22 cases): | | | |
| (*a*) Pregnant | 7.40 | 4.50 | 9.0 |
| (*b*) Puerperal | 7.00 | 4.50 | 9.5 |
| Healthy pregnant women (7 cases) | 7.40 | 5.00 | 9.0 |
| Healthy non-pregnant women (7 cases) | 7.75 | 5.00 | 10.0 |

From these findings Douglas concludes that the thrombi found in various organs in fatal cases of eclampsia are not due to increased intravascular clotting.

In the microscopical examination of a slide of fresh blood fibrin appears as extremely delicate, straight, filamentous lines which cross and recross the field in every direction. It forms a network of fine, interlacing, fibrillary bands, in the clear areas of the serum intervening between the masses of corpuscles, some of the fibrin threads apparently radiating from centers consisting of small irregular masses of blood plaques. The relation of these islands of blood plaques to coagulation and fibrin formation, if, indeed, any exists, is undetermined.

**Hyperinosis and Hypinosis.**

In normal blood the formation of the fibrin network becomes apparent within two or three minutes after exposure of the blood to the air, and the process is completed within about six minutes. In certain pathological conditions, however, both the length of time required for its formation

[1] Berlin. klin. Wochenschr., 1902, vol. xxxix, pp. 1146 and 1170.
[2] Med. News, 1904, vol. lxxxiv., p. 182. [3] Brit. Med. Jour., 1904, vol. i, p. 709.

and the density of the network vary. An increase in the amount of the fibrin network is spoken of as *hyperinosis*, while a decrease in fibrin is termed *hypinosis*.

Pathological Variations.

In general terms it may be stated that fibrin is increased in acute inflammatory and infectious diseases, especially in those attended by an active febrile movement and by exudative processes, the amount of fibrin roughly corresponding to the intensity of the process. This statement is made with certain reservations, for the rule does not hold true in all such instances, as is

Fig. 39.—Normal Blood.
Showing rouleaux formation and fibrin network.

noted below. All febrile states do not imply a fibrin increase, for none is found in the fevers associated with grave cases of chlorosis and of pernicious anemia. Hayem [1] suggests that the density of the fibrin network may be taken as an indication of the individual's resisting powers against disease, inasmuch as it appears to be more marked in the blood of the vigorous than of the feeble. In acute inflammations accompanied by serous and purulent exudates a dense fibrin reticulum is observed, the extent of the exudation being in a degree measured by the density

[1] "Du Sang," Paris, 1889.

of the network. Fibrin is increased to a slighter extent in parenchymatous inflammations, in inflammations of the mucous membranes and skin, and in the febrile stages of chronic suppurations. Among the diseases which are associated with an increase in fibrin are the following: *abscess*, *pneumonia*, *rheumatic fever*, *erysipelas*, *acute gout*, *severe angina*, *bronchitis*, *influenza*, *diphtheria*, *pleurisy*, *peritonitis*, *pericarditis*, *hepatitis*, *meningitis*, *acute gastritis*, *enteritis*, *cystitis*, *vaginitis*, *pustular stage of variola*, and *suppurating tuberculous cavities*. Fibrin is not increased in *malignant neoplasms*, *enteric fever*, *malarial fever*, *tuberculosis*, *pernicious anemia*, *leukemia*, *chlorosis*, and *purpura*. In

FIG. 40.—HYPERINOSIS.
Showing marked increase in the density of the fibrin network.

*parenchymatous nephritis* it is but slightly, if at all, increased, while in *interstitial nephritis* the increase may be notable.

Pfeiffer[1] declares, as the result of his investigations, that in all diseases in which an increase of fibrin exists inflammatory leucocytosis is also present, and that he has never been able to demonstrate hyperinosis without coëxisting increase in the number of leucocytes. But leucocytosis does not invariably imply hyperinosis, although the two conditions almost always go hand in hand. Leucocytosis may occur in purpura and in malignant disease unattended by fibrin increase; on the other hand, in in-

[1] Zeitschr. f. klin. Med., 1897, vol. xxxiii, p. 215.

fluenza the fibrin network is denser than normal, while the number of leucocytes is not increased.

## VII. OLIGEMIA.

The term *oligemia* signifies a reduction in the total volume of the blood, involving a diminution of both the liquid and the cellular portions. It occurs most conspicuously after hemorrhage, and probably after this accident only. Sometimes, the hemorrhage having been profuse, the oligemia proves rapidly fatal; but in other instances, where the hemorrhage has been less extensive, the decreased volume of blood is slowly made up, first by a rapid osmosis of serum into the depleted capillaries from the neighboring lymph spaces, and later by a slower numerical increase of the cellular elements, the products of an actual manufacture of erythrocytes by the blood-making organs.

In some of the advanced cachectic states, in which profound adynamia and poor nourishment of the body are prominent clinical manifestations, there is seemingly good reason for believing in the existence of a true oligemia; but in the absence of confirmatory evidence, reduction of the blood volume in this class of cases must remain rather a suspicion than an accepted fact. The term oligemia is, therefore, in the light of our present understanding, applicable only to blood losses resulting from hemorrhage.

## VIII. PLETHORA.

The term *plethora* is currently used to express a condition characterized by an actual excess in the total volume of the blood, affecting both the liquid and the cellular elements. According to the views of many of the older and a few of the modern pathologists, a true *polyemia*, or an increase in the blood volume, unaccompanied by any qualitative changes, is thought to exist in certain individuals whose mode of life and luxurious habits are supposed to predispose and give rise to excessive blood-formation. The compatibility between a real full-bloodedness and a "high-liver" was formerly much more generally credited than at the present time, and the association of such signs as a rich, ruddy complexion, enlargement of the superficial blood vessels, and a full, bounding pulse was depended upon for the recognition of this condition. Of late, however, the drift of opinion is against the probability of any such permanent increase in blood volume, but until an accurate method of estimating the total quantity of

blood in the body has been devised, the presence or absence of a real plethora must obviously remain conjectural.

True plethora may occur as a transitory condition, as the result of the direct transfusion of blood, or the mechanical forcing back into the general circulation of a quantity of blood from a part to be removed from the body, as by the use of an Esmarch rubber bandage previous to the amputation of a limb; in a similar manner a new-born infant may become temporarily plethoric by a complete emptying of the placenta before tying the umbilical cord. Plethora resulting from any of these influences is invariably of a transient character, for the physiological balance of the organism rapidly disposes of the surplus amount of blood by destruction of the excess of cellular elements and by the elimination of the liquid portions.

*Serous plethora* may be defined as an increase in the volume of blood due to excessive quantities of its liquid and saline constituents, without augmentation in the number of its cellular elements. A condition of this sort may be dependent upon the ingestion of large amounts of liquids, upon the transfusion of saline solutions, or upon vasomotor dilatation, whereby the transfer of an unduly large amount of liquids from the tissues to the blood vessels is promoted. In organic lesions of the *kidneys* and of the *heart*, with diminished elimination of water from the system, a serous plethora of more or less chronicity may develop. The condition, however, is usually of transient duration, as the surplus liquids in the circulatory system are quickly disposed of and the blood volume reduced to normal by intracapillary transudation. J. L. Smith[1] believes that in *chlorosis* a true excess in the volume of blood exists, though Lloyd Jones[2] maintains that the condition is one of hydremia (see below) rather than of actual serous plethora.

*Cellular plethora* is a term which may appropriately be applied to the condition, also known as *polycythemia*, consisting in an increase in excess of the normal standard in the number of erythrocytes. The circumstances under which this change occurs will be discussed later. (See p. 197.)

## IX. HYDREMIA.

A relative increase in the quantity of the liquid constituents of the blood is known as *hydremia*. This condition must not be confused with serous plethora, which is characterized by both a relative and an absolute increase in the liquids of the blood. The

[1] Jour. Physiol., 1900, vol. xxv, p. 6. [2] "Chlorosis," London, 1897.

specific gravity of the blood is observed to fall in relation to the degree to which the change develops.

Hydremia may be produced by any factors which disturb the normal relations between the cellular and the liquid elements of the blood, so that the latter are unduly increased. In other words, the blood is diluted, in consequence of which a given drop of such blood shows an apparent decrease in the number of cellular elements, although the latter are in reality unaffected by the change. Hydremia is observed after extensive *hemorrhages*, in which the primary effect of the oligemia is the taking up by the capillaries of an excess of tissue fluids to replace the blood loss; later, as blood formation gradually makes up for the cellular deficiency, the normal ratio between the corpuscles and the plasma is reëstablished. In the *acute febrile infections* hydremia may develop in consequence of excessive destruction of the blood albumins by the pyrexia. Hydremia may also occur as the result of the *ingestion of large amounts of liquids*, after the *injection of normal saline solution*, and as a consequence of *vasomotor dilatation*. The watery constituents of the blood are relatively increased in certain of the severe *anemias*, owing to the deficiency of corpuscular elements, which is compensated by fluids derived from the tissues. In some *dropsical conditions*, notably those associated with renal and cardiac lesions, hydremia may also be said to exist, either with or without anemia. Hydremia, while it does not necessarily imply the coëxistence of anemia, is naturally often an accompaniment of the latter condition.

Hydremia dependent upon such physiological factors as ingestion of fluids and vasomotor dilatation is a transient condition, for the excess of fluid is promptly eliminated, and the normal relations restored. In other conditions the duration of the change obviously depends upon the nature and permanency of the etiological factor or factors.

## X. ANHYDREMIA.

*Anhydremia* is a condition in which a relative diminution in the liquid constituents of the blood occurs, as the result of rapid osmosis from the capillaries into the surrounding tissues. Inasmuch as the cellular elements do not share in this draining-away, their number is necessarily increased in a given drop of such concentrated blood. The specific gravity of the blood increases in relation to the extent of the fluid drain.

Conditions which cause the sudden dissipation of large quantities of liquids from the body, in consequence of hyperactivity of

the mucous and serous surfaces, are the most prominent factors in producing anhydremia. Thus, after profuse *diarrheas*, *urinary crises*, free *sweating*, excessive *vomiting*, and sudden and extensive *pleural* and *peritoneal effusions* the blood becomes concentrated from a temporary loss of its fluid elements, which pass from the vessels into the tissues to replace the liquids lost in consequence of the drain. Ewing[1] illustrates this principle of anhydremia by his observations on patients in whom a prolonged attack of malarial fever with severe anemia was followed by enteric fever or by acute dysentery. In such instances the thin, watery blood of the malarial infection promptly became thicker and darker in color as the inspissating effects of the complicating illness supervened.

Oliver[2] has shown also that a moderate degree of anhydremia may arise as the result of various influences which cause an increase in arterial tension and a consequent acceleration in the transfer of water from the vessels into the tissues. For example, the change has been brought about by the influence of *local* and *general exercise*, *faradism*, *massage*, *cold bathing*, and the administration of *suprarenal extract*.

From the nature of the drain, which is rapidly compensated by the constant interchange which goes on between the vessel and the tissue fluids, anhydremia is a temporary condition. A perfect physiological balance limits its duration to brief periods of time. (See "Polycythemia," p. 197.)

## XI. LIPEMIA.

Fat is present in normal blood in the form of an exceedingly fine emulsion, the amount varying in man from 1.00 to 3.25 parts per thousand of blood, the mean amount being 1.6, according to the analyses of Becquerel and Rodier.[3]

By the term *lipemia* is meant the presence of an excess of free fat in the circulating blood, a phenomenon which is observed in a number of conditions, both physiological and pathological. In addition to fat globules, the blood in lipemia may also contain fine granular particles failing to respond to the usual tests for fat. The nature of these granules is still in dispute, but it is generally believed that they are either a proteid substance precipitated in the presence of free fat, or that they represent the

[1] "Clinical Pathology of the Blood," 2d ed., Philadelphia and New York, 1903.
[2] Croonian Lectures, Lancet, 1896, vol. i, pp. 1541, 1621, 1699, and 1778.
[3] Cited by Futcher, Jour. Amer. Med. Assoc., 1899, vol. xxxiii, p. 1006.

albuminous envelop surrounding certain of the fat globules.[1] During the *period of digestion*, especially after a meal rich in fats, the blood may contain a sufficient amount of fat to give rise to temporary lipemia; the condition may also be met with in the *breast-fed infant*, in the *pregnant woman*, and in the *obese*. *Menstrual suppression* is also capable of overloading the blood with fat.

The existence of lipemia is of little clinical importance, for it has been observed in a number of diseases, so that it cannot be considered characteristic of any particular lesion. It has been noted in the following conditions: *arteriosclerosis*, *chronic alcoholism*, *diabetes mellitus*, *gout*, certain diseases of the *liver*, *heart*, and *pancreas*, *chronic nephritis*, *tuberculosis*, *splenitis*, *malarial fever*, *typhus fever*, *Asiatic cholera*, *peritonitis*, *pneumonia*, and *poisoning* by *phosphorus* and by *carbon monoxid*. Lipemia commonly occurs as the result of *lacerated wounds of the blood vessels* situated in fatty tissues, and after *fractures of the long bones* involving injury of the fatty marrow.

The degree of lipemia may be so marked that the macroscopical appearance of the fresh blood is altered, the presence of large quantities of free fat rendering it salmon-colored, turbid, and milky. This is especially conspicuous in the specimen of blood serum obtained by centrifugalization, which has a distinct grayish, opaque appearance, not unlike that of chyle.

Macroscopically, the presence of lipemia may be determined by mixing with ether in a test-tube a portion of the turbid blood serum, the excess of fat promptly dissolving, so that the serum becomes clear. Heyl,[2] W. Hale White,[3] and others have been able to distinguish lipemic blood in the retinal vessels by means of the ophthalmoscope.

Microscopically, lipemia may be recognized by the presence of large numbers of glistening fat droplets, about 0.5 to 2 $\mu$ in diameter, which lie in the plasma between the groups of corpuscles, often exhibiting very lively Brownian movements. These droplets respond to the usual tests for fat, dissolving in ether, and staining black with osmic acid and brick-red with Sudan III.

## XII. MELANEMIA.

The occurrence in the circulating blood of minute particles of melanin or pigment, derived usually from the hemoglobin of the

[1] See Futcher, *loc. cit.;* also Cole (cited by White), Lancet, 1903, vol. ii, p. 1007.

[2] Trans. Amer. Ophthal. Soc., 1880, p. 54. [3] Lancet, 1903, vol. ii, p. 1007.

erythrocytes destroyed by blood parasites, is known as *melanemia*. These melanin particles appear as fine bits of granular matter, black or of a reddish-yellow color, either lying free in the blood plasma or embedded in the protoplasm of the leucocytes. In some instances the granules are extremely small-sized and few in number, and again the amount may be considerable, large numbers of pigment particles being apparently fused into masses.

Melanemia is frequently present in *malarial fever*, especially of the severer types, both in the form of free pigment and as pigmented leucocytes. Particles of pigment in the bodies of the leucocytes have also been seen in cases of *insolation*, in *relapsing fever*, in *melanotic sarcoma*, and in *Addison's disease*.

## XIII. GLYCEMIA.

*Glycemia*, or the presence of grape-sugar in the blood, occurs in perfectly normal blood to a very slight degree, the quantity of sugar found under physiological circumstances not exceeding 1.5 parts per thousand.[1] The presence of sugar in excess of this figure, which may be termed *hyperglycemia*, is met with in *diabetes mellitus*, in which disease as high as 9 parts per thousand have been detected.[2] The investigations of Freund[3] and of Trinkler[4] apparently show that the blood in *carcinoma*, especially of visceral involvement, contains an excess of some reducing agent, to all intents and purposes identical with sugar. The former author, in consequence of this fact, lays stress on the finding as a means of differentiating between carcinoma and sarcoma, since no such increase has been observed as an accompaniment of the latter type of neoplasm. Lépine[5] finds that the sugar content of the blood appreciably increases after *extirpation of the pancreas*, a hyperglycemia developing within twenty-four hours after the ablation of this organ. He also finds hyperglycemia after *ligation of the duct of Wirsung*.[6]

The most accurate method of detecting small quantities of sugar in the blood is by the phenylhydrazin hydrochlorid test, conducted by von Jaksch[7] as follows: A small amount of blood,

[1] The term "potential sugar" (Lépine) is applied to the sugar produced in normal blood after having been kept outside the body for half an hour, at a temperature of 58° C. This sugar is believed to be evolved from one or more carbohydrate molecules of the blood proteids.

[2] Hoppe-Seyler, Virchow's Arch., 1858, vol. xiii, p. 104.

[3] Wien. med. Blätter, 1885, vol. vii, pp. 268 and 873.

[4] Centralbl. f. d. med. Wissensch., 1890, vol. xxviii, p. 498.

[5] Cited by Flexner, Univ. of Penna. Med. Bull., 1902, vol. xiv, p. 391.

[6] Sem. méd., 1903, vol. xxiii, p. 385. [7] Zeitschr. f. klin. Med., 1886, vol. xi, p. 20.

obtained by wet-cupping, is first freed from proteids by adding an equivalent weight of sodium sulphate and then boiling and filtering, the filtrate thus obtained being used for the test. A solution is now made in a test-tube, by mixing 2 parts of phenyl-hydrazin hydrochlorid and 4 parts of sodium acetate with about 6 c.c. of water, and gently heating the fluid, if necessary, to effect solution. Five c.c. of the proteid-free filtrate, while still warm, are added to an equal volume of the test solution. This mixture is then placed in a test-tube half filled with water, heated for half an hour in a water-bath, and allowed to stand until cool. When cooling of the mixture has occurred, it shows under the microscope the presence of the characteristic yellowish crystals of phenyl-glucosazon, either detached or in clusters, together with colorless crystals of sodium sulphate.

## XIV. URICACIDEMIA.

The presence in the blood of a demonstrable amount of uric acid has been designated as *uricacidemia*. The blood of the normal individual does not contain this substance in amounts sufficiently large to be detected by ordinary clinical tests, but it is found in appreciable quantities in a number of pathological conditions. Garrod,[1] many years ago, recognized that excessive accumulation of uric acid in the blood was associated with *gout*, and he attached to this sign much diagnostic significance. Later investigations, however, have proved the utter unreliability of this finding as a pathognomonic sign of this disease, for in recent years a large number of other conditions has been found to be more or less constantly accompanied by relatively large amounts of uric acid in the circulating blood. Notable examples of such diseases are *pneumonia*, *hepatic cirrhosis*, *acute* and *chronic nephritis*, *uremia*, *chronic gastritis*, *leukemia*, *severe anemia*, and those conditions in which deficient blood aëration constitutes a prominent clinical symptom, such as *organic cardiac disease*, *exudative pleurisy*, and *emphysema*. Uric acid is not found in the blood in *enteric fever* nor in *rheumatic fever*. *Pyrexia*, of itself, evidently has no influence in producing uricacidemia, nor is it at all probable that this condition goes hand in hand with an excessive elimination of uric acid in the urine.

Garrod's test is well adapted clinically for detecting the presence of appreciable quantities of uric acid in the blood. Slightly modified, it may be applied in the following manner: Two

[1] Med. and Chirurg. Trans., 1854, vol. xxxvii, p. 49; *ibid.*, 1848, vol. xxxi, p. 183.

and one-half c.c. of blood serum, obtained by blistering, are placed in a shallow watch-glass and acidulated by the addition of about 4 drops of a 30 per cent. aqueous solution of acetic acid. A linen thread is then immersed in the acidulated blood, which is slowly evaporated at a temperature not exceeding 70° F. At the expiration of from twenty-four to forty-eight hours, if the sample of blood contains uric acid, characteristic crystals of this substance are deposited upon the thread, their identity being readily detected by microscopical examination and by the murexid test.

## XV. CHOLEMIA.

The presence in the blood of bile or bile-pigments has been termed *cholemia*, a condition which accompanies various forms of *icterus*. Bilious blood may have, as already stated, a yellowish-red color, and may yield, on agitation, an abundant foam, tinged with yellow. Hypertonicity of the serum and a tendency toward hemoglobin dissociation are characteristic of cholemic blood. (See "Icterus.") Bilirubin may be detected in the blood even when urine tests for this substance have proved negative, according to von Jaksch,[1] who employs this procedure to demonstrate its presence: About 10 c.c. of blood, obtained by wet cupping, are allowed to clot, after which the serum is pipetted off, filtered through asbestos, and coagulated at a temperature of 80° C. Thus treated, the presence of bilirubin is betrayed by a greenish discoloration of the serum, which, if bile-free, remains a pale straw color. Should a brownish color develop by this test, the presence of hemoglobin in the serum is indicated.

## XVI. ACETONEMIA AND LIPACIDEMIA.

The occurrence in the blood of demonstrable amounts of acetone and of fatty acids is referred to as *acetonemia* and *lipacidemia*, respectively. Acetonemia has been found in association with numerous pathological conditions, chiefly in those characterized by *pyrexia*, while fatty acids in the blood have been detected in *diabetic coma*, in *malignant jaundice*, in *leukemia*, and in various *acute infections*.

For the recognition of acetone Simon[2] recommends Denigè's test, to be applied as follows: About 3 c.c. of blood are treated with 30 c.c. of Denigè's reagent (20 gm. of concentrated sulphuric acid mixed with 100 c.c. of distilled water, to which 5

[1] *Loc. cit.*

[2] "Clinical Diagnosis," 5th ed., Philadelphia, 1904.

gm. of yellow oxid of mercury are then added), and allowed to stand until a dark-brown precipitate has formed, after which the supernatant fluid is filtered off and treated with more of the reagent, so as to effect complete precipitation. It is then acidified by the addition of about 3 c.c. of a 30 per cent. solution of sulphuric acid, and boiled for one or two minutes. The appearance of a white precipitate on boiling indicates the presence of acetone. This precipitate may be almost wholly dissolved by the addition of hydrochloric acid in excess.

Fatty acids may be detected by boiling equal parts, by weight, of blood and sodium sulphate, filtering, evaporating the filtrate to dryness, and then extracting the residue with absolute alcohol. Microscopical examination of the residue will reveal crystals of fatty acids if lipacidemia exists.

## XVII. BACTERIEMIA.

OCCURRENCE.

*Bacteriemia*, or the presence of bacteria in the circulating blood, is a condition associated with a number of infectious diseases, in which instances it is frequently, but by no means constantly, possible to discover the specific micro-organism of the disease in question by careful bacteriological examination of the blood. The demonstration in the blood of such bacteria as *pyogenic cocci* in general septicemia, of the *Streptococcus pyogenes* and other pyogenic organisms in malignant endocarditis, of the *bacillus of Eberth* in enteric fever, of the *gonococcus* in gonorrheal arthritis, of the *pneumococcus* in pneumonia, and of the *Bacillus tuberculosis* in severe cases of acute miliary tuberculosis, is sufficient proof, without citing other instances, of the diagnostic value of bacteriological blood examinations. Such examinations are warranted in every case of severe infection the nature of which appears doubtful, since by their aid alone it is often possible to derive diagnostic clues of the greatest practical value.

LATENT INFECTION.

From the clinician's viewpoint, normal blood is absolutely sterile, since no cultural method has yet been devised by which it is possible to demonstrate the presence of bacteria in the circulation of the healthy individual. From the pathologist's standpoint, however, such a statement must be accepted guardedly, in the light of recent investigations. Adami,[1] in a comprehensive résumé of the whole field of bacterial infection, cites a series of apparently

[1] Jour. Amer. Med. Assoc., 1899, vol. xxxiii, pp. 1509 and 1572; also Ford, Jour. of Hygiene, 1901, vol. i, p. 277.

conclusive experiments by his assistants, Nicholls and Ford, who found that the kidneys and livers of healthy animals, removed aseptically immediately after death and placed in agar-agar kept at the temperature of the body, showed, after a few days, a relatively abundant growth of bacteria. This observer concludes that under normal conditions the leucocytes pass out through the mucosa on to the free surface of, more especially, the alimentary tract, some of these cells then undergoing destruction, while others, now laden with various foreign matters, including bacteria, pass back again into the submucosa and find their way either into the lymphatic channels or into the portal venules. In both of these sites there exists a decided tendency toward bacterial disintegration and destruction. Such isolated bacteria as may have escaped leucocytal destruction, or removal by the lymphatic glands or by the endothelium of the portal system, may pass either through the thoracic duct or through the liver, and enter the systemic circulation, from which they are eliminated chiefly by the kidneys. Such a condition as this, known as "latent infection" or "latent microbism," appears to be compatible with perfect health, for the number of bacteria which thus gain access to the blood stream and tissues is so small that unless their virulence is especially striking and the susceptibility of the individual peculiarly marked, the resisting powers of the tissues remain sufficiently strong to prevent bacterial proliferation. It is also obvious that the presence in the blood of so limited a number of bacteria cannot be demonstrated by culturing.

Blood Cultures.

If, on the other hand, the conditions are such that bacteria multiply in the blood to any decided extent, then their development in artificial media outside the body may be successfully obtained in many instances, provided that proper technic is employed. That this has not been more successfully accomplished is no doubt due to the powerful bactericidal action of the shed blood, whereas this influence in the circulating blood is but trifling. As Adami remarks, "Because certain observers have failed to discover bacteria in the blood from cases of infectious diseases, it by no means follows that the blood when shed has been free from bacteria." In modern methods of examination precautions are taken to attenuate the bactericidal properties of the shed blood by freely diluting it with a large quantity of *fluid* media, instead of using relatively small amounts of solid culture, as has been done largely in the past, and as the result of this improved technic blood culturing now yields a much higher percentage of positive

findings, and gives more uniform results than were formerly obtained. (See "Bacteriological Examination," p. 109.)

BACTERIA FOUND IN THE BLOOD.

Among the various bacteria which different observers have succeeded in isolating from the circulating blood are included many micro-organisms, the identity of which, as etiological factors of disease, is generally recognized, and also a number to which pathogenicity cannot be convincingly attributed. The following list gives the most important examples of the former class:

*B. aërogenes capsulatus.*
*B. anthracis.*
*B. coli communis.*
*B. influenzæ.*
*B. lepræ.*
*B. mallei.*
*B. œdematis maligni.*
*B. pestis bubonicæ.*
*B. pneumoniæ.*
*B. proteus vulgaris.*
*B. tetani.*
*B. tuberculosis.*
*B. typhosus.*
*Diplococcus intracellularis meningitidis.*
*Gonococcus.*
*Micrococcus tetragenus.*
*Pneumococcus.*
*Pyogenic staphylococci.*
*Pyogenic streptococci.*

In addition to this list, a certain amount of interest attaches to the discovery in the blood of certain *bacilli* (Achalme), *micrococci* (Walker), and *diplococci* (Triboulet) in rheumatic fever; of peculiar *bacilli* (Afanasiew) in relapsing fever, in addition to the specific spirillum of this infection; of *diplobacilli* (Craig) in mumps; and of *diplococci* (Class) in scarlet fever and in typhus fever (Balfour and Potter). The presence of the *Bacillus icteroides* (Sanarelli) in the blood of yellow fever patients is now known to mirror a secondary infection.

The conditions in which the above-named bacteria occur in the blood will be discussed in a later section, under the diseases in question. (See "General Hematology," Section VII.)

## XVIII. ANEMIA.

DEFINITION.

In a clinical sense the term anemia refers to any deterioration in the quality of the blood, affecting the erythrocytes, the hemoglobin, or both of these elements. Thus, in pernicious anemia the most conspicuous deterioration in the quality of the blood is a diminution in the number of erythrocytes, or an *oligocythemia;* in chlorosis the most marked change is usually a loss of hemoglobin,

or an *oligochromemia;* while in many other anemic conditions the erythrocytes and hemoglobin are decreased more or less proportionately. While it is true that, strictly speaking, the word anemia may also be used to designate a reduction in the blood volume, this condition is better defined by the use of the term *oligemia. Ischemia* is a form of local anemia resulting from some mechanical interference with the blood supply of the affected area.

PSEUDO-ANEMIA.

In certain individuals with such decided pallor of the skin and mucous membranes that their appearance at once leads one to infer that they are suffering from a well-defined anemia, no signs of this condition can be discovered, for even after the most careful examination of the blood the number of erythrocytes and the percentage of hemoglobin may be found to be normal. Such instances of apparent blood deterioration have been called *pseudo-anemia;* they are often explained by hereditary peculiarities, by vasomotor disturbances affecting the superficial capillaries, and by deficiencies in the pigment and in the development of the capillary network of the skin. In *pseudo-chlorosis* the patient shows the typical objective signs of chlorosis, yet her hemoglobin and corpuscular values are normal. (See "Chlorosis.") Vermehren[1] has described, under the term *angiospastic pseudo-anemia*, the transient periods of almost cadaveric pallor which occur in some individuals with normal blood, as the result of vasomotor spasm provoked by cold, fatigue, emotion, and like influences. Dwellers in tropical countries are especially prone to a spurious form of anemia, to which the misnomer *tropical anemia* is occasionally applied. Every medical clinic can furnish patients suffering from neurasthenia, tuberculosis, and advanced Bright's disease, whose pallid countenances are a striking contrast to their normal blood counts. "Prison pallor" suggests lack of fresh air and sunshine rather than severe blood deterioration. It does not follow, therefore, that pallor of the skin and mucous membranes is invariably an indication of anemia, although this sign is not misleading in the majority of instances. On the other hand, it should not be forgotten that persons of good color and robust appearance sometimes suffer from decided anemias without the fact becoming evident at first glance. In *chlorosis florida*, for example, red cheeks are not incompatible with a low hemoglobin percentage. In view of these sources of error, in order to diagnose anemia with absolute accuracy, an examination of the blood is essential, for no matter how valuable other clinical signs may appear, the changes in the blood are often the real key to the situation.

[1] Sem. méd., 1903, vol. xxiii, p. 167.

CLASSIFICATION.

An entirely satisfactory classification of the various forms of anemia still remains to be devised, in spite of the numerous attempts which not a few eminent authorities have made to group these conditions according to sound pathological considerations. Therefore, largely for the sake of convenience, all anemias may be broadly grouped into two theoretical classes: *primary* and *secondary*.

According to this tentative classification, *primary anemias* may be considered those in which a lesion of the hematopoietic organs is essentially accountable for the production of the disease. In anemias of this sort, the etiological factors are either entirely undiscoverable, or, if they are to be detected, too trivial to explain the intensity of the disease. Here the predominant clinical manifestations are to be found in the changes occurring in the composition of the blood, the other symptoms being considered secondary to, and dependent upon, these alterations.

Under the term *secondary anemia* are included those cases of anemia which are apparently secondary to, and symptomatic of, certain definite pathological lesions not primarily affecting the blood-making organs, such as, for example, enteric fever, syphilis, septicemia, malignant disease, malarial fever, and hemorrhage. In such anemias the other clinical symptoms are, as a rule, much more conspicuous than the blood changes, which are thought to be secondary. An exception to this general rule must be taken, however, in regard to the anemia caused by the presence of the *Bothriocephalus latus* in the intestinal canal, for in this infection the blood picture is by all odds the most striking clinical manifestation. It is, furthermore, true that in some instances a secondary anemia may apparently merge into one of the primary type, should the protracted duration of the former in course of time cause such profound systemic effects that finally the blood-making organs become exhausted, and refuse adequately to supply the constant demand for corpuscles, with the result that the most prominent clinical signs are now found in the blood, and not in the original symptoms of the disease in question. The high grade anemia which sometimes follows enteric fever, becoming of such intensity that it counterfeits a primary anemia, may be cited as an example of this change.

Until further progress has been made in the study of the physiology and pathology of the blood-making organs the following provisional classification of the anemias may be used for clinical purposes:

I. PRIMARY ANEMIA.—*Chlorosis, pernicious anemia, splenic*

*anemia, lymphatic leukemia, myelogenous leukemia, Hodgkin's disease.*

II. SECONDARY ANEMIA.—Dependent upon causes such as *hemorrhage, intestinal parasites, prolonged lactation, unfavorable hygiene, metal poisoning, malignant disease, acute infections,* and *chronic diseases* producing long-standing drains on the albumin of the blood.

PATHOGENESIS.

Excluding the effects of hemorrhage, deficient blood formation, excessive blood destruction, and a combination of these two processes are generally regarded as the three possible essential factors in the production of anemia. Deficient hemogenesis is to be attributed to a large number of different causes, among the most prominent of which may be mentioned the influence of unhygienic surroundings and insufficient nourishment from improper food and from inadequate powers of assimilation. It is also probable that congenital and acquired failure of the blood-making organs and the presence of growths which intercept the material for blood formation are to be considered as the origin of defective hemogenesis in some instances.[1] Excessive blood destruction may be due to acute febrile and infectious conditions, or to the presence in the blood of certain toxins which destroy the corpuscles. It is characterized during life by an excess of urobilin and iron in the urine, and by the development of hematogenous jaundice.

## XIX. HEMOLYSIS.

EHRLICH'S SIDE-CHAIN THEORY.

In order to understand the process of hemolysis it is necessary briefly to refer to Ehrlich's side-chain theory of immunity,[2] the hypothesis which furnishes the best explanation of the organism's reaction against bacteria, toxins, and other noxious agencies. According to this theory, it is assumed that the body cell consists of a central group of molecules by virtue of which the inherent characteristics of the cell are determined and maintained, and a second, subsidiary molecular group, which, by means of its unsatisfied affinities, is capable of combining with nutrient materials, toxins, and other substances, which are thus brought into intimate relationship with the cell

[1] Mackenzie, Lancet, 1891, vol. i, p. 73.

[2] Proc. Roy. Soc., London, 1900, vol. lxvi, p. 424; Nothnagel's "Spec. Path. u. Ther.," 1901, vol. viii, p. 1; Klin. Jahrb., 1898, vol. vi, p. 299; also Ehrlich and Morgenroth, Berlin. klin. Wochenschr., 1899, vol. xxxvi, pp. 6 and 481; *ibid.*, 1900, vol. xxxvii, pp. 453 and 681; *ibid.*, 1901, vol. xxxviii, pp. 251, 569, and 598.

structure. These subsidiary molecules, known as *side-chains* or *receptors*, are simply links between assimilable substances and the cell, which can neither be nourished nor poisoned except by the intermediation of its receptors. Just as receptors possess specific affinities for linking with certain food stuffs and with no others, so there is believed to be a congenial reaction between receptors and toxins, certain varieties of receptors combining with one kind of toxin and other varieties with another. The receptor and the toxin, therefore, must be homologous before the two can combine. The receptors concerned in the process of toxin immunity belong to Ehrlich's "first order"; they have but a single uniting bond for combining with the toxin, and for this reason are also termed *uniceptors*.

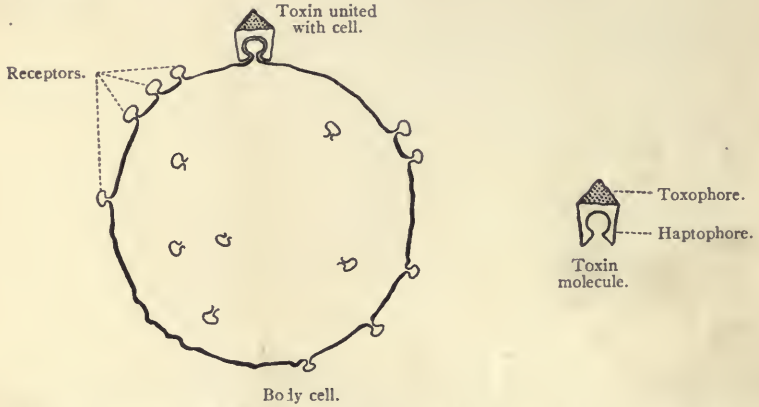


FIG. 41.—ILLUSTRATING THE MECHANISM OF THE TOXIN-CELL UNION BY THE INTERMEDIATION OF RECEPTORS.

A toxin molecule consists of two atomic groups, each with different affinities and with separate functions. One, termed the *haptophore group*, serves to combine the toxin unit with the cell receptor for which it has a selective affinity; the second, known as *toxophore group*, serves to injure the cell when the haptophore-receptor combination is formed. The mechanism of this anchoring of the toxin to the vulnerable cell may be represented by the above diagram (Fig. 41).

The union of toxin and cell leaves the latter more or less crippled, either so severely that it dies, or but slightly, so that it still lives. If the latter be the case, the cell commences to elaborate more receptors in its combat against the toxin—elaborates them in great excess of its needs, so that in spite of the fact that many of these new-born receptors are promptly seized by other toxin molecules,

some of them are thrown off by the cell and float off free in the blood and other body fluids. These liberated receptors, or *haptins*, constitute *antitoxin*. In their free state they can combine with homologous toxin molecules just as readily as when still attached to the cell, and such a combination obviously renders inert the toxin, since its haptophore group is thus satisfied and cannot now become anchored to the cell. According to Welch's hypothesis, these antidotal substances may act not only in their primary function as toxin neutralizers, but, under certain conditions, may irritate the invading bacteria to elaborate similar substance for their own protection. In other words, this theory of reciprocity in infection assumes that if bacteria irritate the body cells, the latter in turn similarly affect the bacteria.

Toxins deprived of their toxophore groups, but retaining their haptophore group, are designated *toxoids*, and such bodies, though capable of becoming attached to the cells by their haptophore group, are inert, because they contain no toxic element. Toxoids can also unite with antitoxin (free receptors or haptins) by means of their haptophore link. Toxins incompletely combined with antitoxins, and therefore still capable of causing modified poisonous effects, are known as *toxones*. The following diagram illustrates the production of antitoxin and the union of toxins and toxoids with the cell and its liberated receptors:

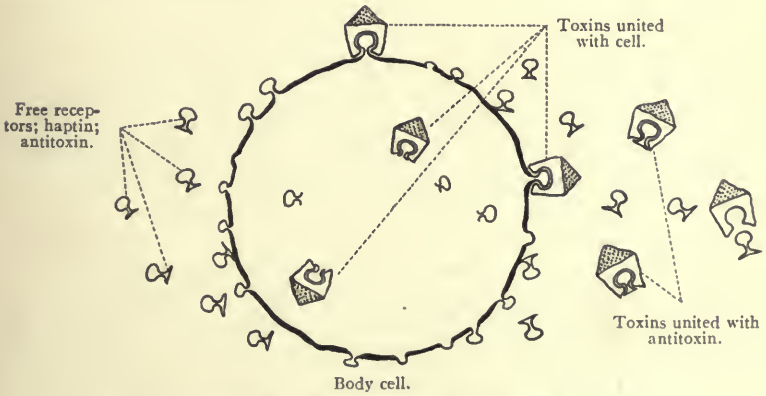


FIG. 42.—ILLUSTRATING THE ELABORATION AND ACTION OF ANTITOXIN.

HEMOLYSIS. The injection into animals of bacteria, various body cells, and the products of these substances in time gives rise to the development of specific "antibodies" in the blood serum of the treated animal, as it ac-

quires immunity. When the latter is complete, the animal's blood will be found to have acquired a lytic or destructive action upon cells similar to those injected. For example, the injection of erythrocytes gives rise to hemolytic serum; of bacteria, to bacteriolytic serum for the specific organism introduced; of bone marrow and lymphatic tissue, to leucolytic serum; of spermatozoa, hepatic cells, and epithelial cells, to spermolytic, hepatolytic, and epitheliolytic sera, respectively. The adaptive changes thus produced in the animal injected are absolutely specific—the injection of erythrocytes produces a serum acting only upon the erythrocytes, not upon the leucocytes, bacteria, or body cells.[1]

*Isolysis* is a term used to denote a hemolytic action due to the injection of one animal with cells from another animal of the same species, the active factors of this process being known as *isolysins*. The destructive influence of a normal individual's blood upon the erythrocytes of another person illustrates this phase of hemolysis. *Autolysis* should result, theoretically at least, by the immunization of an animal against injections of his own cells, with the consequent evolvement of substances termed *autolysins*. The occurrence of hemolysis in icterus, in Winckel's disease, and after internal hemorrhage is suggestive of autolysis.

The lytic property of the blood Ehrlich attributes to the interdependent action of two distinct elements of the plasma, the combined influences of which are essential to produce the change. In the process of hemolysis the receptors of the erythrocytes serve to connect these cells, by the interposition of an intermediary body, or *amboceptor*, with a complementary toxic body, or *complement*, which, when thus united to the cells, exerts its destructive influence. The receptors concerned in hemolysis are said to belong to the "third order"; they are provided with two haptophore groups, one for combining with the vulnerable cell and the other with the complement, which then can act upon the cell attached to the first haptophore group.

The amboceptor is formed within the body, as the result of a cellular hyperactivity excited by the organism's adaptation to alien blood or to other irritant and toxic material. Amboceptors are thought to represent liberated "third order" receptors, evolved and cast off by the cells during the process of adaptation. They are stable substances, capable of progressive increase, and act as connecting links between the complement and the cell. This function

[1] For a complete account of these reactions see: (1) Vaughan and Novy, "Cellular Toxins," Philadelphia, 1902; (2) Welch, Huxley Lecture, Lancet, 1902, vol. ii, p. 977; (3) Prudden, Med. Rec., 1903, vol. lxiii, p. 241; (4) Aschoff, Zeitschr. f. allg. Physiol., 1902, vol. i, p. 69; (5) Wassermann, "Immune Sera," Eng. transl. by Chas. Bolduan, New York, 1904.

of anchoring the complement to the cell is performed by means of two groups of atoms (hence the term, amboceptor), one with an affinity for the corpuscle (*cytophilic group*), and the other with an affinity for the complement (*complementophilic group*). The complement, which acts upon the erythrocyte partly as an enzyme and partly as a toxin, is normally present in the blood, being probably derived largely from the leucocytes. It is provided with a *haptophore group* of atoms, which link it to the amboceptor, and with a *zymophore group* (corresponding to a toxin's toxophore group) upon the action of which depends the cellular destruction. The complement is of unstable nature, being destroyed at a temperature of 55° C., and possesses many of the characteristics of the enzyme. Its toxic action upon the erythrocytes is exhibited only when it is anchored to them by the intervention of the amboceptor, so that unless this link is formed, the complement can exert no deleterious effect upon these cells. The accompanying diagram (Fig. 43) shows the mechanism by which an erythrocyte succumbs to the zymotoxic action of the complement:

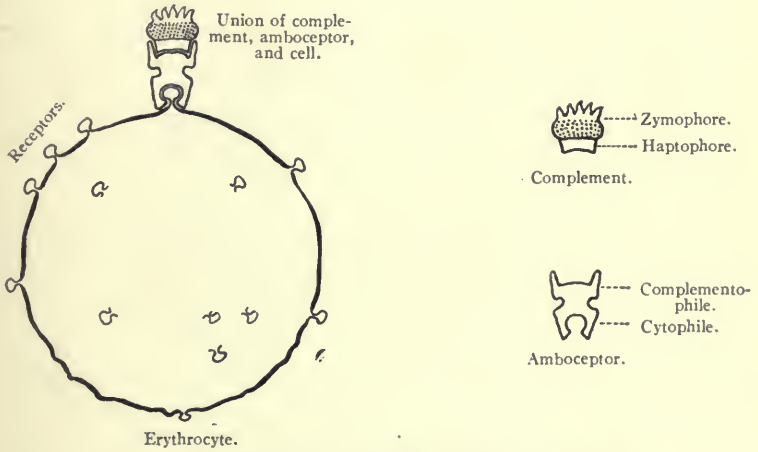


FIG. 43.—ILLUSTRATING THE MECHANISM OF HEMOLYSIS.

ANTIHEMOLYSIS. Resistance of the erythrocytes to hemolysis is attributed to the protective influences of substances known as *antihemolysins*, which in their action correspond to antitoxins. Antihemolysins are formed within the blood plasma after inoculation with hemolysins, and are of two kinds: *anticomplements* and *antiamboceptors*. The former combine with the haptophore group of the complement,

and the latter unite with the cytophilic group of the amboceptor—combinations which in either instance break the continuity of the complement-amboceptor-erythrocyte chain essential for the cell's destruction. The action of these two forms of antihemolysins may be illustrated thus:

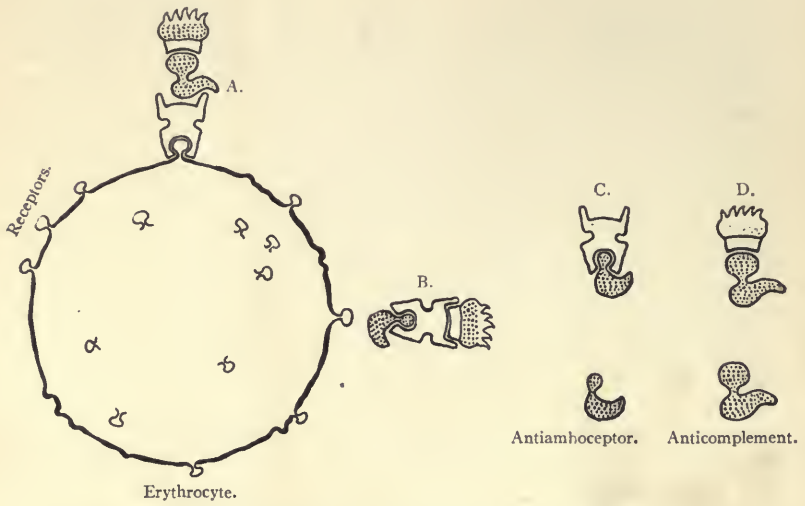


FIG. 44.—ILLUSTRATING THE MECHANISM OF ANTIHEMOLYSIS.

A. Interference of anticomplement with complement-amboceptor union. B. Interference of antiamboceptor with amboceptor-cell union. C. Antiamboceptor-amboceptor union. D. Anti-complement-complement union.

AGGLUTINATION AND PRECIPITATION.

Hemolysis, although generally associated with more or less agglutination and precipitation of the erythrocytes, is not always part and parcel of these phenomena. Serum may clump and precipitate the cells without exerting the slightest hemolytic effect, while, on the other hand, dissolution of the cells may progress without their becoming clumped or precipitated. The principles of agglutination and precipitation have been applied clinically in the diagnosis of enteric fever and other specific infections, and in the biological test for the detection of human blood (*q. v.*).

The injection of bacteria, alien erythrocytes, and other foreign cells produces in the blood serum of the animal thus treated the property of agglutinating and precipitating, *in vitro*, the bacteria or cells injected. These phenomena are attributed to the presence, in the animal's serum, of substances known as *agglutinins* and *precipitins*, developed during the process of adaptation or im-

munization. According to Ehrlich's theory, *agglutinins* are liberated receptors elaborated by the body cells concerned in the process, such receptors having a haptophore group with which the bacterium or cell, as it may be, combines, and a zymophore or agglutinophore group, which, when this link is formed, exhibits its clumping properties. It will be noted that agglutination, unlike hemolysis and bacteriolysis, does not involve the action of a complementary toxic body—the receptor at once anchors and clumps the homologous cell or bacterium through the combined offices of its haptophore and zymophore atomic groups. Receptors of this sort belong to Ehrlich's "second order." An *agglutinoid* is the term used to designate a receptor deprived of its zymophore, agglutinating group, but retaining its haptophore, combining group. It is analogous to a toxoid, and, like this substance, is developed by heat.

*Precipitins* are elaborated by the injection of albuminous body fluids, such as defibrinated blood, into certain animals whose blood serum, in consequence, acquires the property of precipitating, *in vitro*, the albumins against which the adaptation is directed. The serum of a rabbit adapted to human blood precipitates the blood of man but none other, except, in low dilutions, the blood of certain monkeys! (See p. 117.) Similarly, the injection of milk from one animal develops in the serum of the animal treated a precipitin which is specific for the milk used in the adaptation, but for milk of no other species. "Antisera," as they are termed, can also be prepared for various warm- and cold-blooded animals, and such fluids precipitate only the blood of the animals for which adaptation is sought, save in the case of closely related species, in which a partial, incomplete reaction may occur in a low dilution. Other albuminous body fluids (pus, albuminous urine, inflammatory exudates, and saliva) are capable of evolving, after injection, sera which precipitate the blood of the species from which they were derived. The mechanism of precipitation is analogous to that of agglutination, the precipitins consisting of liberated "second order" receptors provided with a haptophore, combining group, and a zymophore, precipitating group of atoms which together act upon the susceptible cell.

## SECTION III.

# HEMOGLOBIN, ERYTHROCYTES, BLOOD PLAQUES, AND HEMOKONIA.

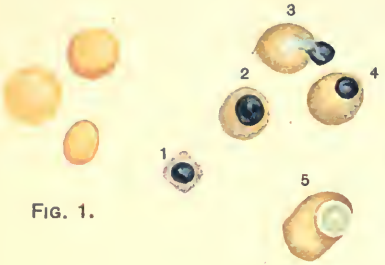


FIG. 1.

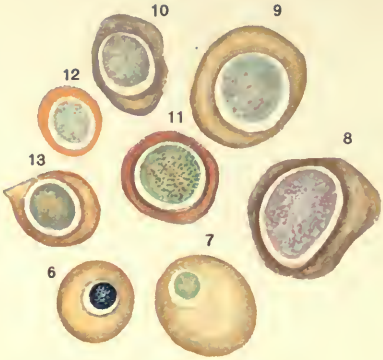


FIG. 2.

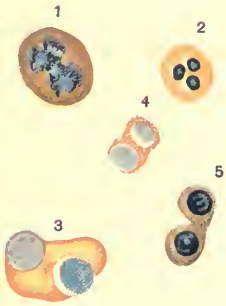


FIG. 3.

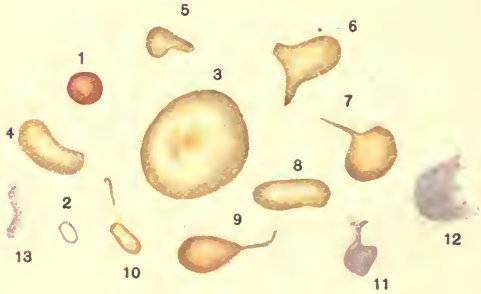


FIG. 4.

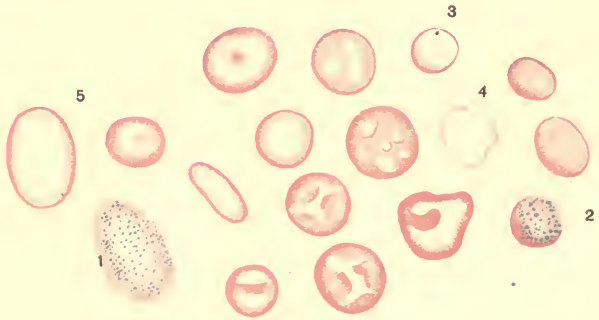


FIG. 5.

THE ERYTHROCYTES.

(Figs. 1, 2, 3, and 4, *Triacid Stain;* Fig. 5, *Eosin and Methylene-blue.*)

(E. F. FABER, *fec.*)

(*Triacid Stain.*)

**Fig. 1. Normal Erythrocytes.**

**Fig. 2. Erythroblasts.**

1. Microblast. Note the dense, glistening nucleus, and the scanty, ragged zone of protoplasm.

2, 3, 4, 5, 6. Normoblasts. The process of partial nuclear extrusion is apparently shown in 3 and 5; in the latter cell the basic affinity of the nucleus is singularly slight. The cell, 6, while as large as many megaloblasts, retains the nuclear characteristics of the normoblast, of which it represents perhaps a hydropic form. Some writers regard such erythroblasts as megaloblasts, on account of their large size.

7, 8, 9, 10, 11, 12, 13. Megaloblasts. In 7 the nucleus, while normoblastic in size, is megaloblastic in structure and in staining affinity. Note the variation in the size of these cells, their delicate nuclear chromatin, and their decided tendency toward polychromatophilia. In all the nucleus and protoplasm are separated by a conspicuous hyaline ring.

**Fig. 3. Erythroblasts with Multiple Nuclei.**

1. Cell with a constricted, convoluted nucleus, apparently undergoing solution in the protoplasm.
2. Normoblast with three nuclei arranged somewhat in the form of a clover-leaf.
3. Cell with two large nuclei, each apparently in an early stage of extrusion. Note the affinity for fuchsin displayed by the protoplasm and by the upper nucleus, and the distinct hyaline zone encircling the lower one.

4, 5. Normoblasts in karyokinesis.

**Fig. 4. Erythrocytes Deformed in Shape and Size.**

1, 2. Microcytes.

3. Megalocyte.

4, 5, 6, 7, 8, 9, 10, 11, 12, 13. Poikilocytes. Many of these cells are highly polychromatophilic, especially 11, 12, and 13.

(*Eosin and Methylene-blue.*)

**Fig. 5. Erythrocytes Showing Degenerative Stroma Changes.**

Granular basophilia is shown by 1 and 2; extreme decolorization by 3, 4, and 5. The other cells represent various stages of hemoglobin loss and protoplasmic degeneration.

## SECTION III.

## HEMOGLOBIN, ERYTHROCYTES, BLOOD PLAQUES, AND HEMOKONIA.

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### I. HEMOGLOBIN.

General Properties.

*Hemoglobin*, which occurs in the circulating blood in chemical union with oxygen as *oxyhemoglobin*, is an extremely complex ferruginous and albuminoid substance contained within the stroma of the erythrocytes. It constitutes approximately nine-tenths of their total bulk, and a trifle less than 14 per cent. of the whole blood. Hemoglobin displays a striking avidity for combining with oxygen to form a peculiarly unstable, but definite, chemical compound, and a similar tendency to yield up to the tissues much of its oxygen during its passage through the capillary circulation. Under the influence of deoxidizing agents oxyhemoglobin may be deprived of its loosely combined oxygen molecule, the resulting oxygen-free constituent being known as *reduced hemoglobin*. Rhombic crystals of oxyhemoglobin, scarlet or reddish-green in color, are rapidly formed if, for any reason, separation of this substance from the corpuscular stroma takes place. These crystals may be easily demonstrated by Reichert's method[1] of laking a small quantity of blood with ether and then adding from one to five per cent. of ammonium oxalate. *Methemoglobin* is an oxygen compound of hemoglobin containing the same quantity of combined oxygen as the latter, but differing from it in holding its oxygen constituent in a more intimate union. The dingy brown color which develops in a solution of oxyhemoglobin after prolonged exposure to the atmosphere evidences the production of this variety of blood pigment. (See "Methemoglobinemia," p. 167.)

The amount of iron (in the form of *hemochromogen*) which hemoglobin contains is considerable—somewhat in excess of four per cent. It has been shown, clinically, by estimates made with the ferrometer and the hemometer, that no fixed parallelism

[1] Amer. Jour. Physiol., 1903, vol. ix, p. 97.

is maintained between the percentage of hemoglobin and the iron contained in the blood.[1]

Under the action of acids, strong alkalis, or heat, hemoglobin may be readily decomposed into two constituents: *hematin*, or an iron-containing principle; and an albuminous residue of unknown character, but somewhat resembling *globulin*. In combination with hydrochloric acid hematin forms a crystalline hydrochlorid of hematin termed *hemin*, or *Teichmann's crystals*. Under the microscope these crystals appear as black or dark brown, elongated, rhombic prisms belonging to the triclinic system, which are insoluble in water, alcohol, ether, chloroform, and dilute acids. They may be demonstrated by preparing a slide of blood (or of any dried substance containing blood pigment) to which a small quantity of common salt has been added; a drop of glacial acetic acid is then run beneath the cover-glass, so that it mixes with the blood and salt, and the specimen thus prepared is heated to just below the boiling point over a Bunsen flame. On cooling, Teichmann's crystals may be seen under the microscope with a low-power dry objective. (See p. 116.)

Iron-free hematin, or *hematoporphyrin*, may be derived from blood by the admixture of concentrated sulphuric acid. This substance is closely related chemically to urobilin, and occurs occasionally as a pigment in nature and in normal and pathological urines. *Hematoidin*, which is also free from iron, occurs in the form of reddish, rhombohedral crystals, only in old clots resulting from blood extravasations, such as cerebral hemorrhages and splenic infarcts. It is derived from hematin, and is probably identical with bilirubin.

ORIGIN.

The chief source of hemoglobin is the iron contained in various food products, about 10 mgm. daily representing the amount of this metal ingested in an ordinary diet, according to the analyses of Stockman.[2] In the event of a stoppage of this source of an iron supply, the formation of hemoglobin may proceed from the supply of iron stored up in the various organs of the body, notably in the liver. Bunge[3] has shown that in the young infant, whose natural food, milk, contains but a slight trace of iron, this source of hemoglobin manufacture is most potent.

The recent experiments of Aporti[4] regarding the origin of hemoglobin and the erythrocytes have shown that animals sub-

[1] Rosin and Jellinek, Zeitschr. f. klin. Med., 1900, vol. xxxix, p. 109.
[2] Jour. Physiol., 1897, vol. xxi, p. 55; *ibid.*, 1895, vol. xviii, p. 484.
[3] Zeitschr. f. physiol. Chem., 1892, vol. xvi, p. 177.
[4] Centralbl. f. inn. Med., 1900, vol. xxi, p. 41.

jected to repeated bleedings and kept on an iron-free diet are able, up to a certain point, to utilize the supply of body iron for hemoglobin manufacture; but that when such a demand became so great that this supply was exhausted, the red corpuscles became progressively paler and paler, and the animal finally died. During the course of these experiments, if the animal received injections of iron, a prompt and striking increase in hemoglobin occurred, the gain ranging from 50 to 95 per cent. within a week's time. The injection of arsenic, on the contrary, produced no effect upon the hemoglobin percentage, although it caused an increase in the number of erythrocytes. The investigations of Stockman and Charteris[1] show that arsenic does not stimulate hemogenesis in the bone marrow, and that its favorable action in malarial fever and diseases of this class is probably due to its parasiticidal effect. In animals injected with small doses of arsenic the marrow changes consisted of hyperemia, decrease in the number of giant cells and fat cells, increase in the leucoblasts, and slight, if any, proliferation of erythroblasts.

Baumann,[2] studying the effects of iron carbonate, iron albuminate, and arsenic upon regeneration of the blood in dogs after hemorrhage, arrived at these conclusions: that the administration of either Blaud's pill or an albuminate of iron causes a rapid gain in hemoglobin, even to a higher figure than that originally found before the blood loss, the effects of each of these preparations of iron being similar; that arsenic and iron combined, although stimulating hemoglobin formation less than the above drugs, are better general hemogenetic agents, so far as regeneration of the corpuscles, proteids, and plasma is concerned; and that arsenic, when given alone, is but an indifferent blood-builder. The practical import of these experiments is patent.

VARIATIONS IN AMOUNT.

Diminution in the amount of hemoglobin, as indicated by the hemometer, is known as *oligochromemia*, or *achroiocythemia*. It is a condition usually, but not invariably, associated with a corresponding decrease in the number of erythrocytes. An apparent increase in the hemoglobin percentage may result from the concentration of the blood caused by a reduction in the quantity of blood plasma consequent to excessive drains upon the liquids of the body. By a similar physical mechanism factors producing a dilution of the blood are capable of causing an apparent diminution in the hemoglobin. Marked oligochromemia is commonly observed in chlorosis, pernicious anemia, and leukemia;

[1] Jour. Path. and Bacteriol., 1903, vol. viii, p. 443.
[2] Jour. Physiol., 1903, vol. xxix, p. 18.

and in the secondary anemias dependent upon such factors as hemorrhage, mineral poisoning, acute and chronic infections, malignant neoplasms, and constitutional diseases. The behavior of the hemoglobin under such conditions is more fully alluded to in connection with the lesions in question. Poggi,[1] from a series of experiments upon normal women, has shown that the hemoglobin is slightly lowered (10 or 15 per cent.) for a few days before menstruation, but with the establishment of the flow the oligochromemia soon disappears. The primary loss he attributes to retarded hemogenesis consequent to the lessened consumption of albumin occurring in menstruating women, while the subsequent gain he explains by the increased functional activity of the hematopoietic organs. Double oöphorectomy in sexually active bitches is followed by a distinct oligochromemia and by a less marked oligocythemia, persisting for from two to six weeks, but in old dogs the operation has no such effect. This experimental evidence is advanced by Breuer and von Seiller[2] in corroboration of the ovarian theory of chlorosis.

In passing, it may be of interest to compare the degree of hemoglobin loss in the various forms of anemia, as illustrated by the following averages determined by the writer:

| | | | | | | |
|---|---|---|---|---|---|---|
| Average of 50 estimates in | | | | pernicious anemia | 25.5 | per cent. |
| " | " | " | " | " chlorosis | 43.2 | " |
| " | " | " | " | " leukemia | 39.4 | " |
| " | " | " | " | " secondary anemia | 55.2 | " |

Bierfreund's investigations[3] in Mikulicz's clinic have led to the current impression among surgeons that it is highly dangerous to give a general anesthetic to a patient whose hemoglobin percentage is below 30; some operators regard 40 per cent. as the lowest limit of safety, and refuse to employ any but a local anesthetic in cases with an oligochromemia exceeding this figure, except under circumstances of imperative necessity. Any one, however, who has attempted to verify the correctness of this general belief must accept it with a shrug of the shoulders. The writer knows of numerous patients whose hemoglobin percentages all were below 30 in whom operations under general anesthesia with ether were followed by uneventful recovery; in one instance (a pan-hysterectomy lasting more than an hour and a half) the hemoglobin was but 21 per cent., yet no ill effects were observed.

Patients with hemoglobin percentages of from 15 to 30 have

[1] Policlin. Roma, 1899, vol. vi, p. 1.
[2] Wien. klin. Wochenschr., 1903, vol. xvi, p. 869.
[3] Langenbeck's Arch., 1890–91, vol. xli, p. 1.

been successfully operated by Girvin, Shober, Le Conte, Noble, Baldy, J. C. Da Costa and others.[1]

ABSOLUTE AMOUNT.

Assuming that in the normal adult 14 gm. represent the average amount of hemoglobin in 100 gm. of blood, the absolute amount of hemoglobin may be readily calculated thus:

*Hemoglobin percentage* × 14 ÷ 100 = *Grams of hemoglobin in 100 gm. of blood.*

For example, in blood in which the percentage of hemoglobin, as determined by the hemometer, is found to be 40, the calculation (40 × 0.14) gives the absolute amount of hemoglobin as 5.6 gm.

COLOR INDEX.

The proportionate amount of hemoglobin contained in each erythrocyte, or its corpuscular richness in hemoglobin, is known as the *color index*, or *blood quotient*, or *valeur globulaire*. In normal blood the color index is theoretically expressed by the figure 1, although, practically, it varies from 0.95 to 1.05 in men, and from 0.9 to 1 in women.[2]

In those anemias in which the decrease in the amount of hemoglobin in the blood is coincident with a proportionate decrease in the number of erythrocytes, the color index remains practically at the normal figure. If, however, the cellular decrease happens to be relatively greater than the hemoglobin loss, then the index will naturally be found to rise above normal; thus, in pernicious anemia, in which condition the loss of cells is proportionately much greater than the loss of hemoglobin, high color indices, approaching or even exceeding 1.25, are frequently observed. On the contrary, if the hemoglobin loss is relatively more excessive than the corpuscular decrease, the color index falls below normal; for example, in chlorosis, in which, as a rule, the decrease affects the hemoglobin much more strikingly than the erythrocytes, low indices, such as 0.50 or less, are common.

To calculate the color index, the percentage of hemoglobin is divided by the percentage of erythrocytes, the result being expressed in decimals. In order to simplify this procedure 5,000,000 erythrocytes per c.mm. must be arbitrarily considered as normal, or 100 per cent. To obtain the percentage of corpuscles the actual number counted in one c.mm. of blood is simply multiplied by two, and two or three decimals pointed off from the left, depending upon whether the count is below or

[1] Trans. Coll. of Phys. of Phila. (Sect. on Gynecology), 1902, vol. viii, p. 26; also Amer. Jour. Obstet., 1902, vol. xlv, pp. 666 and 701.

[2] Oliver, *loc. cit.*

above the normal 5,000,000. The following examples serve to illustrate the calculation in several conditions:

*Normal Adult.*

Erythrocytes: 5,000,000 per c.mm. (100 per cent.).
Hemoglobin: 100 per cent.
100 ÷ 100 = 1: Color index.

*Secondary Anemia.*

Erythrocytes: 2,650,000 per c.mm. (53 per cent).
Hemoglobin: 40 per cent.
40 ÷ 53 = 0.75: Color index.

*Pernicious Anemia.*

Erythrocytes: 840,000 per c.mm. (16.8 per cent.).
Hemoglobin: 18 per cent.
18 ÷ 16.8 = 1.07: Color index.

*Chlorosis.*

Erythrocytes: 4,100,000 per c.mm. (82 per cent.).
Hemoglobin: 32 per cent.
32 ÷ 82 = 0.39: Color index.

These examples, of course, refer only to the usual blood findings, for the color index is by no means *always* high in pernicious anemia, nor *always* low in chlorosis. The color index shows simply the relative relations of the hemoglobin and the corpuscular percentages. It is only suggestive, not diagnostic, of a specific blood disease.

HEMOGLOBINEMIA.

The term *hemoglobinemia* is used to designate a condition in which the hemoglobin is dissolved from the corpuscular stroma as the result of some pathological factor, and is held in solution by the blood plasma. In extreme instances this condition is sooner or later succeeded by hemoglobinuria.

Among the most potent causal factors of hemoglobinemia are certain drugs which act as blood poisons when administered in toxic doses, of which the following are examples: *arseniuretted hydrogen*, *sulphuretted hydrogen*, *potassium chlorate*, *carbolic acid*, *hydrochloric acid*, *sulphuric acid*, *pyrogallic acid*, *nitrobenzol*, *antimony sulphid*, *iodin*, *naphthol*, and many of the coal-tar derivatives, such as *acetanilid*, *antipyrin*, and *phenacetin*. A similar liberation of the hemoglobin may be observed as the result of poisoning by certain varieties of *mushrooms*, by some *snake-venoms*, by the bite of *scorpions*, and by a number of *vegetable glucosids*. *Sunstroke*, *extensive burns*, and *exposure to excessive cold* are also capable of giving rise to hemoglobinemia. Experimentally, hemoglobinemia may be produced by the *transfusion of blood* from one animal into the circulation of another belonging to a different species.

Hemoglobinemia is observed with more or less constancy in a number of acute infectious diseases, such as grave cases of *septicemia, diphtheria, malignant jaundice, syphilis, malarial fever, enteric fever, scarlet fever, yellow fever, typhus fever,* and *variola.* It also may occur in *scurvy* and in *Raynaud's disease,* and is a prominent blood finding in those two obscure conditions known as *epidemic hemoglobinuria* of the new-born and *paroxysmal hemoglobinuria.*

Hemoglobinemia may be readily detected by the following method, recommended by von Jaksch:[1] A small amount of blood, drawn from the patient by means of a cupping-glass, is immediately placed in a refrigerator, in which it is allowed to remain for twenty-four hours. In normal blood the serum which separates at the expiration of this period is of a perfectly clear straw-color, whereas if hemoglobinemia exists, the serum is colored a beautiful ruby-red. If this hemoglobinemic serum is examined with the spectroscope, the two characteristic absorption bands of oxyhemoglobin may be observed. If it is coagulated by heat, a deep brown color is imparted to the coagulum.

METHEMOGLOBINEMIA.

*Methemoglobinemia,* or the presence in the circulating erythrocytes of methemoglobin, is produced by the action of a number of toxic substances, which, if given in sufficiently massive doses, may seriously or fatally cripple the oxygenating functions of the blood. Among the agencies which cause this conversion of oxyhemoglobin into methemoglobin are *potassium chlorate, anilin, iodin, bromin, ether, turpentine, acetanilid, potassium permanganate, hydrochinon, kairin, thallin,* and *pyrocatechin.* The inhalation of *amyl nitrite* and the intravenous injection of *sodium nitrite* also act in a similar manner. Henri and Mayer[2] found that methemoglobinemia could be produced by the influence of *radium rays.*

Spectroscopical examination of the blood is essential for the detection of methemoglobinemia. The spectrum of methemoglobin in alkaline solution shows three absorption bands: one well-marked band between *C* and *D* of Fraunhofer's lines and two others of much less distinct appearance, lying between *D* and *E,* each immediately adjacent to the lines. In acid and neutral solutions the spectrum of methemoglobin shows four absorption bands: a decided one between *C* and *D,* two between *D* and *E,* and one closely adjacent to *F.* This spectrum, it is true, is identical with that produced by an acid solution of hematin, but it may be easily distinguished from the latter by the fact that

[1] "Clinical Diagnosis," 3d ed., London, 1897, p. 75.
[2] Sem. méd., 1904, vol. xxiv, p. 68.

the spectrum of methemoglobin, when acted upon by ammonium sulphid, changes first to that of oxyhemoglobin, and later to that of reduced hemoglobin, while when hematin is thus treated, a spectrum which shows two bands between *D* and *E* is produced.

CARBON MONOXID HEMOGLOBIN.

Aside from the bright, cherry-red color of the blood in coal-gas poisoning the presence of *carbon monoxid hemoglobin* may be determined by spectroscopical examination and by a number of distinctive chemical reactions.

Recalling the characteristic spectrum of oxyhemoglobin (two distinct absorption bands between *D* and *E*, the one nearest *D*

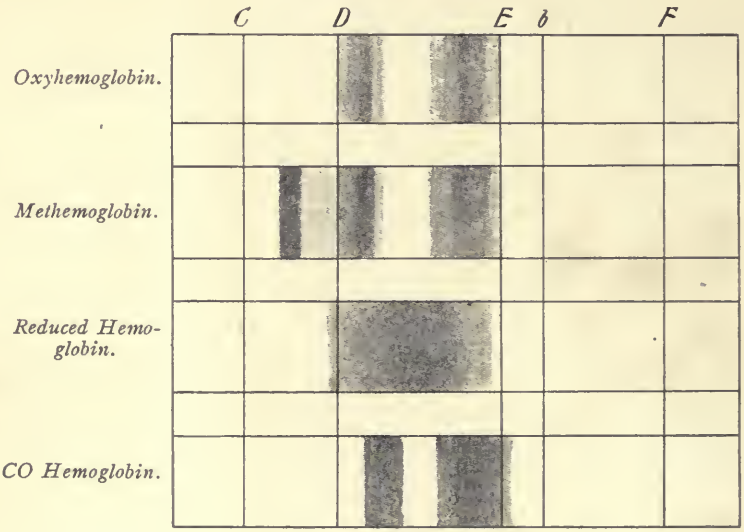



FIG. 45.—PRINCIPAL BLOOD SPECTRA.

being darker, narrower, and more sharply defined), it is found that in the spectrum of carbon monoxid hemoglobin these bands are replaced by two others, also between *D* and *E*, but nearer together, and somewhat closer to the violet end of the spectrum. This distinction, which may be so slight as to appear confusing, is at once emphasized by the fact that the addition of ammonium sulphid has absolutely no effect upon the carbon monoxid spectrum, while it transforms the spectrum of oxyhemoglobin into that of reduced hemoglobin.

Carbon monoxid hemoglobin in the blood is also demonstra-

ble by the following simple test devised by Hoppe-Seyler[1]: A small quantity of blood, removed from the patient by means of a wet-cup, is mixed with twice its volume of a 10 per cent. solution of potassium hydrate. Thus treated, blood containing carbon monoxid hemoglobin changes the color of the mixture to a rich cinnabar-red, while with normal blood the solution turns brownish-green.

## II. THE ERYTHROCYTES.

Appearance in Fresh Blood.

The *erythrocytes* or *red corpuscles* are thin, flattened, biconcave discs, of sharply defined, regular outline and of smooth, even surface. That they are neither bell-shaped nor globular, as is also maintained, is obvious on careful microscopical examination. In the blood of the normal individual the erythrocyte does not possess a nucleus. When the corpuscle is examined microscopically as it rests upon its flat surface, its central concavity is plainly indicated by a dark central area surrounded by a narrower, lighter rim as the periphery of the cell is brought into sharp focus, changing to a pale, white center encircled by a darker periphery as the objective is brought closer to the corpuscle. When viewed in profile, it is shaped somewhat like a slim dumb-bell, with regularly rounded poles tapering from either end toward a shallow central concavity on either surface. The color of the cells, when examined singly under the microscope, is a pale greenish-yellow, but when they are collected in masses, a more or less marked reddish tint becomes apparent. The erythrocytes possess a peculiar tendency of collecting and adhering in more or less regularly arranged piles, like rolls of coins stacked up face to face, this being known as *rouleaux formation*.

After withdrawal of the blood from the body various structural changes in the erythrocytes, commonly known as *crenation*, may be observed. In normal blood the rapidity with which these changes progress depends upon the quantity of air which leaks in between the slide and the cover-glass, and thus causes degeneration of the corpuscular stroma. The development of one or more small, bright, highly refractive spots in the body of the corpuscle, and a slight indentation of the cell's periphery are the the most conspicuous indications of beginning crenation. As the process goes on, more and more of these hyaline points develop,

[1] *Loc. cit.*

until finally the whole surface of the corpuscle becomes thickly studded with glistening, bead-like spines. As the stroma becomes drier and drier, its typical biconcavity and sharply-cut outline are lost, contracting strands of the stroma are seen to extend from point to point among the beaded projections, the periphery of the cell changes to a cogged rim, and finally the cell becomes shrunken and shriveled up into a small, many-starred asterisk. Some of the erythrocytes become fragmented, and small bits of their stroma are observed to break off and float through the plasma. Others become progressively paler and paler as the hemoglobin is dissolved out, until complete decoloration occurs. Still others become distorted into designs of every conceivable shape, so that their resemblance to the normal cell becomes most remote. These changes, which never occur in normal blood until the cells have been exposed to prolonged atmospheric influence, must not be confused with similar alterations in the structure of the erythrocytes occurring as the result of pathological states of the blood. The latter changes are described more fully in another place. (See p. 184.)

Histological Structure.

The finer structure of the erythrocyte is still a moot point among different histologists, the view most generally accepted regarding it as a homogeneous cell composed of an insoluble spongy network, the *stroma of Rollet*, in the interstices or trabeculæ of which is embedded a soluble, finely granular substance, the hemoglobin, existing probably as a compound with some unknown constituent of the cell. In lieu of a distinct limiting membrane the portions of the stroma nearest to the surface of the corpuscle are condensed, to protect it from injury during its movement through the blood stream. This outer layer, according to Peskind,[1] is composed of a proteid substance, lecithin, and cholesterin, but contains no hemoglobin. The corpuscles are highly elastic and contractile, to permit of the rapid and marked temporary distortions of shape which they constantly undergo in the circulating blood.

Other authorities, notably Schäfer,[2] disagree with Rollet's view, inclining rather to consider the erythrocytes as vesicular masses, consisting of an external envelop inclosing a fluid contents. Thus, Schäfer believes that the cell consists of two distinct portions, a colored and a colorless, the former being a solution of hemoglobin, while the latter, or so-called stroma, consists chiefly of lecithin and cholesterin, together with a small amount of cell

[1] Amer. Jour. Physiol., 1903, vol. viii, p. 404.
[2] Quain's "Anatomy," Philadelphia, 1891, pt. 2, p. 210.

globulin. Without attempting to discuss the correctness of either of these two views, a single tangible reason for regarding the corpuscle according to Rollet's opinion may be stated, viz.: the fact that exposure of blood to destructive temperatures results in fragmentation of the corpuscles into numerous minute portions, each one of which consists of a bit of hemoglobin-containing stroma. This obviously seems to disprove the existence of a limiting membrane, without further investigation.

ORIGIN AND LIFE HISTORY.

In the human body an active manufacture of erythrocytes constantly goes on during health, in order to compensate for the continuous drain on their number by the destruction of those cells which have become incapable of function and useless, their life cycle being run. That this reproduction is the direct answer to a call for new cells is proved by the prompt and rapid increase of corpuscles following the loss of blood from hemorrhage. That such a manufacture is attempted in severe pathological conditions, although the attempts are sometimes abortive, is evinced by the large numbers of immature and misshapen erythrocytes which appear in the blood in certain of the grave anemias.

In the adult it is generally conceded that the erythrocytes are reproduced in the red bone marrow, being developed from their direct antecedents, the nucleated erythrocytes or erythroblasts, which exist in this tissue in large numbers. The erythroblasts appear to multiply in the thin-walled capillaries and veins of the red marrow, and, having lost their nuclei, become transformed into normally developed erythrocytes, which pass from the blood channels of the marrow into the general circulation. Some authorities have attributed to the spleen and lymphatic glands a share in the formation of the red cells, while others have maintained that they may be transformed from the leucocytes in the circulating blood, but none of these theories has been associated with convincing evidence, so that it is fair to consider the red bone marrow the chief, if not the only, seat of production, in the light of our present knowledge of the subject. Hayem's ingenious theory, that the erythrocytes arise from the hematoblasts, does not enjoy the confidence of modern investigators.

When finally the erythrocyte, after having executed its function for a certain length of time, becomes useless in its primary office as an oxygen carrier, its death ensues, the destruction of the cell probably taking place largely in the liver and to a less degree in the spleen. Bain[1] has shown that this hemolytic power resides in both of these viscera, the liver acting chiefly upon the

[1] Jour. Physiol., 1903, vol. xxix, p. 352.

erythrocytes and the spleen affecting mainly the leucocytes. After the passage of blood through the liver it was proved that the hemoglobin-deficient cells were most prone to destruction, that the hemoglobin content of the invulnerable cells was distinctly increased, and that the perfusion appreciably augmented the quantity of iron in the liver and was accompanied by a considerable output of highly pigmented bile. The perfused spleen was also found to contain an increase in the amount of iron, indicative of a relatively slighter destruction of erythrocytes. Splenectomy in animals does not to any great extent interfere with erythrocytic destruction (Lapicque).[1] Warthin's[2] studies show that destruction of the erythrocytes also occurs in the splenolymph glands, minute vascular sinuses situated chiefly in the retroperitoneal and mediastinal tissues, and in the thyroid and thymus regions. The possibility that certain of the partly degenerate cells also undergo a certain form of repair, first in the spleen and then in the liver, rendering them still capable of function, is an interesting but obviously unproved conjecture.

SIZE.

The average diameter of the erythrocyte is about 7.5 $\mu$,[3] its average thickness being about 1.8 $\mu$. According to Gram,[4] the diameter appears to vary somewhat with the geographical and climatic conditions surrounding the individual, being considerably larger in inhabitants of northern countries than in southerners, as the following average measurements of this observer attest:

| COUNTRY. | AVERAGE DIAMETER. |
|---|---|
| Italy | 7 to 7.5 $\mu$ |
| France | 7.5 to 7.6 $\mu$ |
| Germany | 7.8 $\mu$ |
| Norway | 8.5 $\mu$ |

Hayem[5] distinguishes three different sizes: large, averaging 8.5 $\mu$ in diameter; medium, averaging 7.5 $\mu$ in diameter; and small, averaging 6.5 $\mu$ in diameter. Of these three classes, approximately 75 per cent. are of the medium size, while 12.5 per cent. each are large and small. The diameter varies within somewhat wider limits in the infant and in the young child than in the adult. It is, however, not materially influenced by sex. The pathological increase and decrease in the diameter of the erythrocytes occurring in certain anemias are discussed in another place.

[1] Med. News, 1903, vol. lxxxii, p. 311.

[2] Jour. Boston Soc. Med. Sci., 1901, vol. v, p. 414.

[3] The Greek letter $\mu$ is used to represent a micromillimeter, or $\frac{1}{1000}$ of a millimeter, which is a standard unit of measurement used in microscopy.

[4] Fortschr. d. Med., 1884, vol. ii, p. 33.

[5] *Loc. cit.*

NORMAL NUMBER. The normal number of erythrocytes in the healthy male adult may be approximated at 5,000,000 to the c.mm. of blood. Higher counts than this are frequently observed, however, especially in healthy, well-developed men, so that this figure should be taken to represent a rather low average, subject to an upward fluctuation of half a million cells, and occasionally even more. In females a count of about 4,500,000 erythrocytes per c.mm. may be regarded as normal.

Arterial and venous blood contain practically the same number of corpuscles, the apparent slight increase in favor of the latter, mentioned by some observers, being within the limits of technical error. For a like reason, under normal conditions, peripheral blood may be taken as representative of the blood of the entire body. Blood derived from dependent parts of the body contains a diminished proportion of corpuscular elements. Oliver's[1] studies of this question have shown that blood from the finger invariably gives a higher count of erythrocytes than blood from the toe, this disparity being explained by the fact that the larger quantity of lymph gravitating to the more dependent parts of the body causes a dilution of the blood in these areas.

VOLUME INDEX. This term has been applied by Capps[2] to the figure representing the percentage volume of the individual erythrocyte, in contradistinction to the color index, which expresses the amount of hemoglobin in the single cell. It is calculated by dividing the percentage volume of the erythrocytes as a whole, obtained by centrifugalization of the blood, by the percentage number of erythrocytes, as determined by the actual count with the hemocytometer, the normal volume index being taken as 1.00. For example, the erythrocyte column, after centrifugalization with the hematokrit, reaches to the mark 40 on the capillary tube, indicating a total volume of 80 per cent.; while the count with the hemocytometer gives 3,000,000 cells per c.mm., or 60 per cent. of the normal number. Then, $80 \div 60$, or 1.33, equals the volume index, a figure which in this instance shows an increase of 33 per cent. in the volume of each corpuscle. As a general rule, it may be stated that the volume index and the color index rise and fall together, although the parallelism between the two is not always closely maintained. The volume index is generally lowered in *chlorosis*, in *leukemia*, and in most of the *secondary anemias*, while in *pernicious anemia* it tends to rise above the normal standard.

[1] *Loc. cit.*

[2] Jour. Amer. Med. Assoc., 1900, vol. xxxvi, p. 464; also Jour. Med. Research, 1903, vol. v, p. 367.

The cell's volume is influenced mainly by factors affecting cell degeneration, and is altered but slightly by osmotic influences. During blood regeneration the volume of the cell is restored before the color becomes normal.

## III. INFLUENCE OF PHYSIOLOGICAL FACTORS ON THE ERYTHROCYTES.

Age and Sex.

Polycythemia, associated with a proportionately high percentage of hemoglobin, is found in the blood of the new-born infant immediately after birth, the maximum counts being observed some time during the first twenty-four hours of life, after which period they progressively diminish until, at the end of eight or ten days, about 1,000,000 cells per c.mm. have been lost. Each period of nursing is generally followed by a prompt temporary decrease in the count, and a similar change has been observed as the effect of premature ligation of the cord at birth. Hayem[1] found an average of 5,368,000 erythrocytes per c.mm. in 17 infants at birth, the highest count being 6,262,000, and the lowest, 4,340,000. Fehrsen[2] regards 6,000,000 per c.mm. as the average count at birth. The cause of this polycythemia is attributed to concentration of the blood from the abstraction of water by the tissues to replace the fluids of the body lost during the first few days of life. As soon as this loss is made up by the ingestion of a sufficient amount of liquids by the child, the normal relation between the liquid and the solid portions of the blood is reëstablished, so that the polycythemia disappears.

During the growth of the adult the average number of erythrocytes continues to rise, until the maximum number is attained at some time between the third and fifth decades, after which a decrease is observed, usually becoming more marked as the decline of life progresses. Schwinge[3] and others have shown that during the period of sexual activity the counts in females are generally lower than in males, but that after the climacteric the number of cells in the two sexes is practically identical.

The influence of age and sex upon the number of erythrocytes is well illustrated in the following table prepared by Sörensen[4]:

| Age. | Males. | Age. | Females. |
|---|---|---|---|
| 5 to 8 days | 5,769,500 | 1 to 14 days | 5,560,800 |
| 5 years | 4,950,000 | 2 to 20 years | 5,120,000 |
| 19.5 to 22 years | 5,600,000 | 15 to 28 years | 4,820,000 |
| 25 to 30 years | 5,340,000 | 41 to 61 years | 5,010,000 |
| 50 to 52 years | 5,137,000 | | |
| 82 years | 4,174,700 | | |

[1] *Loc. cit.*
[2] Jour. Physiol., 1903, vol. xxx, p. 322.
[3] Pflüger's Arch., 1898, vol. lxxiii, p. 299.
[4] Cited by von Limbeck, *loc. cit.*

PREGNANCY, MENSTRUATION, AND LACTATION.

There are no conspicuous changes in the number of erythrocytes in any of these conditions. In primiparæ there is often a slight decrease in the number of corpuscles, particularly in the later months of pregnancy, but in multiparæ this change is rarely observed. Distinct anemia during gestation is invariably pathological, although a moderate degree of hydremia may be physiological. J. Henderson[1] found an average hemoglobin percentage of 68.2 in 38 cases at term, and an average erythrocyte count of 3,975,348; by the eleventh day after delivery these values increased to 74 and 4,020,000, respectively. Postpartum oligocythemia commonly develops, the intensity of which depends largely upon the amount of blood lost and upon the general health of the woman; this loss of cells is gradually made up, and unless convalescence is delayed, reaches the normal by the second or third week after delivery. Bar and Daunay[2] have determined that the density of the blood progressively declines toward the end of pregnancy, but rapidly increases after delivery.

During *menstruation* there may be a trifling reduction, caused by the physiological hemorrhage of the phenomenon, but the loss is rapidly made up in a few days' time. Sfameni[3] found that a transient polycythemia usually occurs shortly before the establishment of the menstrual flow, and that the average loss of hemoglobin and corpuscles during the flow does not exceed 4.5 per cent., the decrease being in direct proportion to the actual volume of blood lost.

In healthy, robust women *lactation* is accompanied by a normal count, but in weak, young girl-mothers, particularly those of the "chlorotic age," a moderate reduction is sometimes observed.

Here may be noted O. Schäffer's observation,[4] thus far unconfirmed, that pregnancy can be detected by an increased affinity of the erythrocytes for iodin in blood obtained by puncture of the cervix. Using a reagent containing 1 gm. of iodin, 2 gm. of potassium iodid, and 300 c.c. of water, Schäffer found that the number of iodin-stained erythrocytes began to increase immediately after conception, until just before delivery they became at least twenty times more numerous than the unstained cells, which in the non-pregnant woman they outnumber only between two and five to one.

[1] Amer. Jour. Obstet., 1902, vol. xlv, p. 745.
[2] Sem. méd., 1904, vol. xxiv, p. 28.
[3] Centralbl. f. Gynäkol., 1899, vol. xxiii, p. 1311.
[4] *Ibid.*, 1901, vol. xxv, p. 1375.

CONSTITUTION AND NUTRITION.

Well-developed, robust individuals average a larger percentage of erythrocytes than the poorly nourished and weakly. In the former, counts much in excess of 5,000,000, and in the latter counts of less than 5,000,000, are the rule.

*Fasting*, inasmuch as it causes a drain upon the liquid elements of the vascular system, may rapidly bring about an apparent polycythemia due to concentration of the blood, this increase in cells being in direct relation to the length of abstinence from food. Hayem[1] states that a twenty-four hours' fast will cause a gain of between 400,000 and 500,000 cells; while the experiments of Reyne[2] on a dog, starved to death after a twenty-four days' fast, showed an increase of 2,500,000 corpuscles at the expiration of this period.

MUSCULAR EXERCISE.

Active *muscular exercise* (gymnasium work, walking, running, swimming, and so forth) provokes transient increase in the peripheral erythrocyte count, due primarily to increased blood pressure, which not only inspissates the blood, but also disseminates peripherally many cells which hitherto lay inactive in the deeper circulation. From the studies of Willebrand[3] and of Zuntz and Schumberg[4] it seems that the duration of this increase stands in inverse ratio to the length of the period of exercise. Hawke[5] found that the average gain in erythrocytes was 21 per cent. after swimming, 16.6 per cent. after sprinting, 12.8 per cent. after walking, and 12 per cent. after bicycling.

FATIGUE.

*Physical labor* prolonged to the point of fatigue appreciably diminishes the number of erythrocytes. Cadet's[6] investigations of the blood of a number of peasants, examined after two months of hard field labor during the summer, showed a moderate oligocythemia—in one instance a loss of over 1,000,000 cells, and in the others diminutions averaging about one-half of this figure. Cadet believes that this anemia is referable to a true blood destruction, and notes as a rather mythical support of this view that the blood plaques were increased in these cases.

Among other factors increasing the erythrocyte count may be noted *cold tubbing* (Winternitz;[7] Thayer[8]), *warm baths* (Knöpfelmacher[9]), and *general massage* (J. K. Mitchell[10]).

[1] *Loc. cit.* [2] Cited by Hayem, *loc. cit.*
[3] Skandin. Arch. f. Physiol., 1903, vol. xiv, p. 176.
[4] "Studien zu einer Physiologie des Märsches," Berlin, 1901.
[5] Amer. Jour. Physiol., 1904, vol. x, p. 384.
[6] Cited by Hayem, *loc. cit.* [7] Centralbl. f. klin. Med., 1893, vol. xiv, p. 177.
[8] Johns Hopkins Hosp. Bull., 1893, vol. iv, p. 37.
[9] Wien. klin. Wochenschr., 1893, vol. vi, p. 810.
[10] Amer. Jour. Med. Sci., 1894, vol. cvii, p. 502.

DIGESTION. FOOD.

Within an hour after a meal there is a slight, transitory increase in the number of erythrocytes, amounting on the average to a gain of one-quarter of a million cells to the c.mm. This acme is soon followed by a gradual decline, corresponding to the period of digestion, the normal standard being regained within two or three hours after the preliminary rise began. Corresponding fluctuations in the hemoglobin and in the density of the blood also occur.

Oliver[1] states that these variations are not affected by the taking of liquids with meals, for he has noticed that they were quite as pronounced when water was withheld. The same observer has shown[2] that these fluctuations correspond accurately to what he terms the "digestive lymph wave," or the to-and-fro intermediary circulation between the capillaries and the lymph spaces excited by the ingestion of food. Immediately after a meal this wave begins to rise, and coincidentally with this leakage of fluid from the blood into the tissues the hemoglobin and erythrocyte values begin to rise, as the blood thus becomes more and more concentrated. The acme is reached within an hour, and at this time the blood count and the percentage of lymph in the tissues are at their maximum. The wave then begins to decline slowly, and as the effused fluid gradually is restored to the blood vessels, the hemoglobin and corpuscle figures correspondingly decline, until they reach normal, after the lapse of two or three hours after their initial rise. The following table, from von Limbeck,[3] illustrates the variations in the red and white cells caused by taking food.

| TIME. | ERYTHROCYTES. | LEUCOCYTES. | HEMOGLOBIN. |
|---|---|---|---|
| 11 : 15 A. M. | 5,553,000 | 7,666 | 98 per cent. |
| 12 M. | Dinner of meat and farinaceous food. | | |
| 12 : 15 P. M. | 5,320,000 | 6,166 | |
| 1 : 15 P. M. | 5,480,000 | 8,500 | |
| 2 : 15 P. M. | 4,733,000 | 12,000 | |
| 3 : 15 P. M. | 4,872,000 | 14,000 | 89 per cent. |
| 4 : 15 P. M. | 4,720,000 | 10,830 | |

Hayem[4] believes that meat eaters average a higher percentage of erythrocytes than vegetarians, on account of the more nitrogenous character of their food, and that a diet of fats and albuminoids is most favorable for the increase of the cellular elements of the blood.

[1] *Loc. cit.*
[2] Lancet, 1903, vol. ii, p. 940.
[3] *Loc. cit.*
[4] *Loc. cit.*

HIGH ALTITUDES.

The habitual polycythemia of individuals living in high altitudes is an interesting and inadequately explained fact in hematology. Viault,[1] Wölff and Koeppe,[2] Egger,[3] and other observers have shown the invariable occurrence of this polycythemia both in inhabitants of elevated districts and in the occasional visitor. In the case of the latter, as the individual ascends from the sea-level to the mountainous district, a rapid increase in corpuscles and in hemoglobin develops, this increase bearing a certain relation to the height ascended, and becoming apparent usually within twenty-four or forty-eight hours after his arrival in the highland. Campbell and Hoagland[4] computed the increase at the rate of 50,000 cells to the c.mm. per 1000 feet of elevation. Ten hours' stay in a balloon, at a height of over 15,000 feet, produced no morphological changes in the blood of two aëronauts, Schroetter and Zuntz,[5] who undertook this experiment. Viault counted 8,000,000 erythrocytes to the c.mm. in the residents of the Cordilleras, at an elevation of 14,274 feet above the sea-level; Cazeaux[6] found 7,100,000, at a height of 5904 feet. Egger counted 7,000,000 at Arosa, at a height of 6100 feet; and Wölff and Koeppe found an average of 5,970,000 in dwellers at Reiboldsgrün, at a height of 2257 feet. Oliver[7] relates the interesting experience of finding in his own blood, during a stay at Davos Platz, at an elevation of 5200 feet, an increase of corpuscles within twenty-four hours after his arrival, the maximum count, 5,550,000, being attained within seven days, and the number declining within five days after his return to London.

The following table, by Marie,[8] illustrates the fact that the higher the altitude the higher is the count of erythrocytes:

| PLACE. | HEIGHT ABOVE SEA-LEVEL. | COUNT OF ERYTHROCYTES. | AUTHOR. |
|---|---|---|---|
| Christiania..... | 0 | 4,974,000 | Laache. |
| Göttingen ..... | 148 meters. | 5,225,000 | Schäfer. |
| Tübingen ..... | 314 " | 5,322,000 | Reinert. |
| Zürich ..... | 414 " | 5,752,000 | Stierlin. |
| Auerbach ..... | 425 " | 5,748,000 | Koeppe. |
| Reiboldsgrün..... | 700 " | 5,900,000 | Koeppe. |
| Arosa .....1,800 | " | 7,000,000 | Egger. |
| The Cordilleras....4,392 | " | 8,000,000 | Viault. |

[1] Compt. rend. Soc. biol., Paris, 1890, vol. iii, p. 917.
[2] Münch. med. Wochenschr., 1893, vol. xl, p. 904.
[3] XII. Cong. f. inn. Med., Wiesbaden, 1893.
[4] Amer. Jour. Med. Sci., 1901, vol. cxxii, p. 654.
[5] Pflüger's Arch., 1902, vol. xcii, p. 615.
[6] Cited by Tissier, Cohen's "System of Physiological Therapeutics," Philadelphia, 1903, vol. x, p. 182.
[7] *Loc. cit.*
[8] "Leçons de Clinique Médicale," Paris, 1896, p. 237.

Foa[1] reports that animals taken to a height of 10,000 feet develop polycythemia within eight hours after their arrival, and that the increase disappears within thirty-six hours after their return to a normal level. On the contrary, Armand-Delille and Meyer[2] were unable to detect any definite changes either in the peripheral and heart blood or in the hematopoietic organs of animals kept for from two to seven weeks at an altitude of 6000 feet.

The hemoglobin changes which accompany these cellular alterations are never so marked as the latter, both the rise and the fall being less rapid; consequently it is common to find a low color index at first, whereas later, inasmuch as the rapidity of the cellular loss is greater than the fall in hemoglobin, a high color index is likely to persist for some time after return to the lowland. Curry[3] could determine no consistent changes in hemoglobin, the volume of the cells, or the specific gravity of the whole blood at an elevation of 6000 feet. The blood changes, as a rule, are more conspicuous in normal than in anemic persons. In phthisics living in high altitudes, Meissner and Schröder[4] found that the hemoglobin value rose and fell in relation to the patient's improvement and decline in health. Kemp[5] calls attention to the fact that the morning erythrocyte count is always higher by from 500,000 to 1,000,000 than the evening estimate. He also noted that a large increase in the number of blood plaques developed as the patient ascended to a high elevation.

Concentration of the blood doubtless explains the polycythemia of high altitudes, this change being due largely to the great loss of body fluids (Grawitz), and partly to the increased arterial tension (Oliver) arising from a rarefied atmosphere. Koeppe's ingenious theory that the process mirrors an actual manufacture of new cells is scarcely tenable, for although this observer has found numerous microcytes and poikilocytes coincidentally with the appearance of the polycythemia, normoblasts were not detected, as an evidence of rapid hemogenesis, nor did such signs of excessive blood destruction as icterus and hemoglobinuria develop, as the increased count rapidly declined on the individual's descent to a lower level.

It has been recently urged that in high elevations the effect upon the hemocytometer of atmospheric pressure and temperature may be the real secret of the cellular increase, but how such influences act, if, indeed, they are active, is unknown.

[1] Brit. Med. Jour., 1904, vol. i, p. 1097.
[2] Sem. méd., 1903, vol. xxiii, p. 371. [3] Amer. Med., 1902, vol. iv, p. 367.
[4] Cohen's "System of Physiological Therapeutics," Phila., 1903, vol. x, p. 191.
[5] Med. News, 1904, vol. lxxxiv, p. 383; also Johns Hopkins Hosp. Bull., 1904, vol. xv, p. 177.

CLIMATE.

A *sea climate* apparently causes a moderate increase in the number and hemoglobin value of the erythrocytes. Marestang's studies[1] of the blood of several sailors during a sea voyage are the only available proof of this change.

A *tropical climate* of itself probably does not affect the blood, although it offers fruitful factors of anemia in predisposing to such infections as the malarial fevers, ankylostomiasis, and filariasis. So-called *tropical anemia* is more often apparent than actual.

## IV. PATHOLOGICAL CHANGES IN THE ERYTHROCYTES.

AMEBOID MOTILITY.

True ameboid movements of the erythrocytes are sometimes observed, as the result of the effect of globulicidal agents, or of some pathological state of the blood, such as a severe, high-grade anemia. The inherent elastic and contractile qualities shown by the cells, by virtue of which they undergo various changes in shape while floating about in the plasma, must not be confounded with the actual ameboid motility which they exhibit in disease. The oscillatory dancing movements of bits of fragmental corpuscles, and the characteristic motility of the intracellular hyaline malarial parasite, also must be distinguished from the progressive, deliberate characteristics of the truly ameboid red blood cell.

ALTERATIONS IN ISOTONICITY.

Within the body the hemoglobin and other constituents of the erythrocytes are preserved intact within the corpuscular stroma by the composition of the blood plasma, which is such that a perfect osmotic balance is constantly maintained. Outside of the body, if this relationship is disturbed by the addition of distilled water to a specimen of blood, thus lowering the concentration of the plasma, the corpuscles swell, and a rapid discharge of hemoglobin into the surrounding tissue ensues, but the addition of saline solutions of a definite strength prevent such a change. Solutions of salts of just sufficient concentration to preserve the corpuscles and to prevent removal of their elements are known as *isotonic*, solutions of greater strength are termed *hypertonic*, and those of lesser strength, *hypotonic*. In normal blood it has been determined that the isotonicity of the erythrocyte usually ranges from about 0.48 to 0.46 per cent. NaCl; that is, salt solutions of this concentration are just sufficient to prevent

[1] Cited by von Limbeck, *loc. cit.*

the discharge of hemoglobin by the cell, although it may swell by taking up water. A 0.9 per cent. or "normal" salt solution not only preserves the hemoglobin within the cell, but also prevents alterations in its size and contour. Hamburger[1] and others have shown that alterations in isotonicity depend not only upon changes in the plasma, but also upon the constitution of the erythrocytes themselves. Fluctuations in the amount of the cells' diffusible albumins, chlorids, and phosphates are attended by corresponding osmotic variations.

Owing to the conflicting results obtained by different investigators, the isotonicity of the erythrocytes in different diseases is of little clinical value. Stengel[2] found the percentage 0.52 and 0.6 in two cases of *pernicious anemia*, yet in other cases the figures were normal; in other diseases marked by anemia, such as *carcinoma*, *hepatic cirrhosis*, *renal disease*, and *tuberculosis*, he found that the variations were trivial. Von Limbeck[3] found that the isotonicity was usually, but not invariably, increased in high-grade *secondary anemias*, in *leukemia*, and in many of the *acute infections*, while it was decreased in *chlorosis* and in *catarrhal icterus*. A decidedly increased isotonicity was found by Vicarelli[4] in *pregnant* and *nursing women*. As a general rule, it is believed that degenerative changes in the erythrocytes, whatever their nature, predispose to dissociation of hemoglobin from the stroma, and that in such instances the isotonic percentages are higher than normal.

HYPERVISCOSITY.

In the fresh specimen of blood, exaggeration of the adhesive properties of the erythrocytes may be observed in a number of conditions, but up to the present time no special clinical significance has been assigned to the phenomenon. It occurs to some extent in most *inflammatory diseases*, and, according to Hayem,[5] is often seen in the anemias associated with marked *cachexia*. Striking examples of hyperviscosity result when the erythrocytes are subjected to the action of various poisons, notably *snake-venom*, and of *heterogeneous pathological blood serum*. (See p. 128.) From the effect of such influences the erythrocytes, instead of forming normal rouleaux, tend to adhere in large, irregular masses in which the distinctive characteristics of the cells are masked or lost. The individual cells, unattached to such a mass, may exhibit every possible variety of distortion, losing their typical biconcavity and regular disc-like appearance, and becoming

[1] Arch. f. Anat. u. Physiol., 1886, p. 476; *ibid.*, 1887, p. 31.
[2] *Loc. cit.*
[3] *Loc. cit.*
[4] Cited by von Limbeck, *loc. cit.*
[5] *Loc. cit.*

converted into elongated, misshapen bodies. It frequently happens that the cell is provided with one or more long, delicate processes several times the length of its diameter, this being due to the adhesion of a bit of the stroma to the cover-glass while preparing the specimen; in the spread film it will be noted that these processes all point in the same direction.

DEFORMITIES OF SHAPE AND SIZE.

Changes in the shape and size of the erythrocytes are common in all anemias which reach a severe grade, the degree of such deformities corresponding closely to the intensity of the anemic process. The diameter of the cells may be more or less uniformly increased or decreased, and such pronounced alterations in their shape may occur that many of them bear but slight resemblance to the typical discs of normal blood (Plate I; also Fig. 46). Abnormal inequality in the size of the erythrocytes is expressed by the term *anisocytosis*.

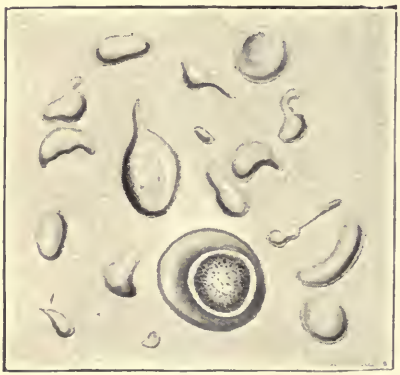


FIG. 46.—DEFORMITIES OF SHAPE AND SIZE.

Illustrating various grades of cell deformity associated with severe anemia. The large nucleated erythrocyte is a typical megaloblast. (Ehrlich's triacid stain.)

When the corpuscle becomes greatly enlarged in diameter it is known as a *megalocyte* or *macrocyte*, the presence of large numbers of such cells being known as *megalocytosis* or *macrocytosis*. The diameter of a megalocyte generally varies from 9 to 12 $\mu$, but sometimes much larger forms are seen, measuring as much as 20 $\mu$. They are present in the severer anemias, especially in the pernicious form, in which they constantly occur in large numbers. The megalocyte found in this disease is usually characterized by an excess of hemoglobin, while in the secondary anemias such cells are generally deficient in their hemoglobin content.

The smaller forms, the *microcytes*, illustrate the extreme decrease in size of the red cell under pathological conditions. The microcyte is an extremely small globular body, measuring from about 3 to 5 $\mu$ in diameter. It is found in all the varieties of anemia, but is most commonly associated with chlorosis and with the moderately developed second forms. An abundance of microcytes in the blood is known as *microcytosis*.

*Eichhorst's corpuscles* are deeply colored, highly refractive microcytes, about 3 $\mu$ in diameter, and usually of regularly spherical shape. They were once regarded as pathognomonic of pernicious anemia, but are now considered diagnostic of no especial condition, being frequently found in severe anemias of any type, and often being absent in pernicious anemia.

It seems reasonable to infer that deformities in the size of the erythrocyte are referable chiefly to two different factors: to faulty hemogenesis and to degenerative changes of the corpuscle which lead to alterations in its histological structure. Megalocytes, for example, may in some instances represent an actual giantism of the cell, bred in the marrow from correspondingly large-sized nucleated antecedents; in other instances (of which those exceedingly pale, "washed-out" forms are examples) their abnormal size may be attributed to hydropic enlargement, resulting from their imbibition of fluids from the surrounding plasma. Microcytes may enter the circulating blood as such, or, as is frequently the case, they may be the products of corpuscular budding and fragmentation.

In severe forms of anemia, characterized by excessive cellular loss, there appears also to be a tendency toward a compensatory hypertrophy of many of the erythrocytes, in order thus to increase the oxygen-carrying capacity of the blood, which, were it not for these numerous megalocytes, might in some instances be too limited to sustain life.

*Poikilocytes* are erythrocytes deformed in shape as the result of some pathological condition of the blood. *Poikilocytosis*, the name by which this condition is designated, is akin to crenation in so far as in both conditions the cells may be similarly distorted and misshapen. But it is unlike crenation for the reason that poikilocytosis is a pathological condition, and demonstrable the moment the blood is withdrawn from the body; while crenation is a physiological phenomenon depending upon external influences for its production, and never occurring until the blood has remained exposed to the air for some time. Poikilocytes may be of large or small size, the varieties of deformities being infinite, and the degree marked or slight in relation to the nature of the blood disease. Some of the cells may resemble the shape of a gourd or a horseshoe; others may be drawn out at both ends until they form a spindle-shaped or oval body, while others appear sharply beaked at one or more points, or shaped like a dagger or the blade of a tomahawk. Occasionally very minute, rapidly oscillating, rod-shaped forms are seen, morphologically not unlike large, unstained bacilli—*pseudo-bacilli of Hayem*. These rod-

shaped forms are products of corpuscular fragmentation, and indicate lowered vitality and feeble powers of resistance to the pathological influences affecting the cells.

Poikilocytosis is not characteristic of any single disease of the blood, but it is generally most marked in the grave forms of primary anemia, such as leukemia and pernicious anemia. Oval-shaped red cells are considered by Cabot [1] as particularly abundant in the latter disease.

The conditions of deformity affecting the shape and size of the erythrocytes are nearly always associated. As a general rule, it may be stated that in the milder types of anemia small-sized, slightly deformed poikilocytes and microcytes are most common; and that in the severe forms, large-sized, conspicuously distorted poikilocytes and megalocytes predominate.

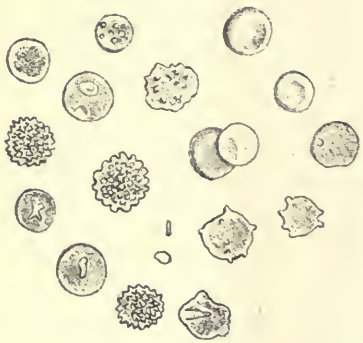


FIG. 47.—DEGENERATIVE CHANGES IN THE ERYTHROCYTES. (FRESH BLOOD FILM.)

ENDOGLOBULAR DEGENERATION.

Loss of color by the erythrocytes, which progresses hand in hand with alterations in their size and shape and other structural changes, is regarded as a degenerative process of purely endoglobular nature. It is observed in the fresh specimen of blood in many severe anemic conditions, especially in the anemias associated with infectious diseases, such as variola, typhus fever, and grave septicemia and pyemia (Fig. 47).

The decoloration may commence in one or more spots, or it may equally involve the whole surface of the corpuscle, beginning at its center and spreading progressively toward its periphery. Clear, hyaline areas of oval, round, or elongated shape appear within the stroma, in some instances sharply contrasting with the relatively dark color of the hemoglobin, but in other instances imperceptibly blending with the tint of the surrounding cell body. The active motility of these decolorized spots must be carefully distinguished from the ameboid movements of the young malarial parasite. Complete decoloration transforms the cell into a mere colorless shell or "phantom," which would be practically invisible

[1] "A Guide to the Clinical Examination of the Blood," 5th ed., New York, 1904, p. 131.

were it not for its faintly colored periphery. Such cells are known as *Ponfick's shadow corpuscles* or as *Hayem's achromacytes.*

Maragliano and Castellino[1] have minutely described this process of decoloration, along with certain other alterations in the structure of the erythrocyte, which they have termed *endoglobular necrosis.* This process first becomes apparent by a visible enlargement of the central concavity of the corpuscle, together with a simultaneous fading away of the hemoglobin in this situation. This central area of pallor gradually spreads toward the periphery of the cell, until finally the latter alone shows evidence of containing coloring matter. Such a corpuscle, when examined on cross-section, appears to be shaped like the figure 8. Fragmentation of this delicate rim of coloring matter may occur, in the event of which numerous independent, rod-like bits of stroma are formed. The decolorized area is not always symmetrical, so that frequently various strikingly *bizarre* designs, widely differing in shape and appearance, may be observed, It has been determined that in the dried blood film these areas of decoloration show a decided affinity for basic stains, such as methylene-blue and thionin.

TOTAL NECROSIS.

Total cellular necrosis, also described by the authors mentioned above, represents a phase of structural degeneration in the erythrocyte of more advanced development than the endoglobular changes. This process begins with the development of several small elevations or corrugations in the stroma of the corpuscle, which gradually multiply, increase in size, and change in shape until the larger portion of the cell's surface is thus deformed. Ameboid movements are seen to begin, as if the entire cell as a whole were involved, the final stage of the process resulting in the formation of a poikilocyte, from which body points and small fragments are observed to break off and to float free in the plasma. Decoloration, starting usually from a single point and in time affecting the whole stroma, also accompanies this necrotic alteration. On cross-section the cell appears as an elongated, thin rod with rounded poles. This fragmentation of the cells is spoken of as *schistocytosis.* The erythrocytes are much more resistant than the leucocytes, which succumb much more readily to necrobiotic influences.

Endoglobular degeneration and total necrosis of the erythrocytes may be observed both in normal and in pathological blood. In normal blood they occur as the result of prolonged contact with the air, the endoglobular phase becoming first apparent

[1] XI. Cong. f. inn. Med., Leipsic, 1892.

within from thirty to seventy minutes, and the total necrosis in from three to four hours, after the preparation of the specimen. In pathological blood the changes are thought to be due chiefly to increased globulicidal properties of the plasma, whereby intravascular necrosis is excited, and partly to decreased resistance of the erythrocytes, in consequence of which their degeneration is abnormally hastened by contact with normal plasma and by exposure to extraneous influences. In disease it follows that they are demonstrable immediately or very shortly after the blood has been withdrawn, and that the development of the changes occurs with much greater rapidity than in normal blood. The endoglobular changes are regarded as a more favorable prognostic sign than the total necrosis, being usually associated with anemias of less severe character than those in which the latter process prevails.

ATYPICAL STAINING REACTION.

The normal erythrocyte, when fixed and stained with anilin dyes, according to one of the methods described in another section, possesses a strong affinity for a single, acid stain; it is therefore termed *monochromatophilic*. When solutions are used containing both acid and basic dyes, such as eosin and methylene-blue or eosin and hematoxylin, the normal erythrocyte is always stained by the eosin; and with Ehrlich's triple mixture, which is so formulated that acid, basic, or so-called neutral principle may be selected by the elements subjected to its action, according to their affinities, the erythrocyte invariably is colored by the orange G of the mixture (Plate I).

In certain morbid conditions some of the corpuscles lose their affinity for the acid stain, and with mixtures of both acid and basic dyes are stained atypically by either or both elements. Such corpuscles are said to be *polychromatophilic*. Thus, when stained with an eosin and methylene-blue mixture, they are tinged a dirty grayish-purple or violet, instead of the rose color of eosin; and with the triple mixture they may be stained purple, reddish-brown, or pale yellowish-pink, flecked here and there with shadings of a darker red (Plate I).

In polychromatophilic corpuscles the staining is likely to be very unevenly shaded, often being quite dark in spots, especially around the periphery of the cell and the margin of the nucleus, if the cell be nucleated. These color changes affect not only the protoplasm, but the nucleus as well, and are strongly emphasized in megaloblasts, the nuclei of which may show every sort of color combination. The more deficient the corpuscle in hemoglobin, the more decided its polychromatophilic tendency; and

the more strikingly the latter is developed, the more intense the cell's affinity toward the basic element of the stain.

Polychromatophilia may occur in severe forms of anemia due to any cause, and it is especially noted in two of the primary varieties—pernicious anemia and myelogenous leukemia—in both of which conditions the process is a prominent characteristic of the blood picture. *Corpuscles of Poggi*, or erythrocytes which stain with basic dyes in the fresh, unfixed specimen, are also found in various anemias. They probably represent immature elements whose presence in the circulating blood reflects stimulated hemogenesis.

NUCLEATION.

Nucleated erythrocytes, or *erythroblasts*, are found in the blood of the adult only during the existence of pathological conditions, but occur in large numbers in the blood of the fetus, and occasionally in the infant during the first few days of life. Being invisible in the fresh blood, they must be studied in the dried, stained specimen. In such preparations the finer structure of their nucleus, which bears a special affinity for the basic anilin dyes, may be beautifully illustrated by the use of solutions containing methylene-blue, methyl-green, and hematoxylin.

According to their size and nuclear characteristics the erythroblasts are designated as *normoblasts*, *megaloblasts*, and *microblasts*. Certain intermediate forms are also common, sometimes termed *mesoblasts*, such cells being atypical, and sharing characteristics of both the normoblast and the megaloblast.

*Normoblasts* (Plate I).—The normoblast is a nucleated erythrocyte of about the general size and shape of the normal erythrocyte. In the typical mature cell the nucleus is round or ovoid in shape, very deeply stained, and situated rather toward the periphery of the cell than in the exact center, its diameter approximating more than one-half that of the corpuscle which it occupies. In the normoblast of an earlier developmental stage the nucleus is relatively larger and is composed of delicate, faintly basic chromatin —hall-marks of histological youth. In some of the typical cells the nucleus appears to have become partly or completely extruded from the protoplasm, lying either somewhat over the periphery of the cell or, being completely detached from it, free in the plasma (Fig. 49, III). The nucleus may be single, or partly divided by constricting bands of chromatin into a figure like a dumb-bell or a clover-leaf, or completely divided into several small, round sections. More rarely, karyokinesis may be observed, the diaster and early convolution stages, with an intact but plainly constricted cell body, being the phases ordinarily found. (Plate I,

Fig. 3; also Fig. 49, II.) In carefully stained films it will be noted that the nuclear framework of the typical normoblast consists of a rather sharply defined network of chromatin having relatively wide intervening open spaces, so that the general appearance of the nucleus is not unlike that of a coarse net.

The protoplasm of this cell is usually of regular outline along the periphery, stains somewhat more intensely than that of the normal erythrocyte, and may show distinct evidences of polychromatophilia, this characteristic being especially marked in forms with dividing nuclei.

The normoblast is regarded as the immediate antecedent of the normal erythrocyte or normocyte, into which it becomes transformed by the loss of its nuclear structure. The exact manner in which the nucleus is disposed of has long been a bone of contention among histologists, and even at the present time views on this question should be held but tentatively, notwithstanding many exhaustive investigations, especially those of the German school. According to the views of Rindfleisch,[1] it is lost by extrusion from the cell body, which thus becomes a normal erythrocyte, while the free nucleus, to which a small fringe of protoplasm still remains adherent, collects from the plasma material by virtue of which it ultimately develops into a new erythroblast. Ehrlich[2] believes that in blood rich in normoblasts a series of connected pictures may be observed, showing that the normoblast becomes transformed into the erythrocyte by the extrusion or emigration of the nucleus. The later investigations of Neumann and Köl-liker,[3] however, tend to prove that the nucleus is disposed of by its destruction and absorption within the cell, and that its apparent extrusion from the stroma is simply the result of mechanical influences. Pappenheim and Israel[4] also believe that the normoblast's nucleus disappears by decay and solution within the body of the corpuscle, and that the apparently extruded nuclei are to be taken as an evidence of plasmolysis, or a solution of the protoplasm of the cells once containing nuclei. To attempt a reconciliation of these diametrically opposed views is a task for future workers to undertake. Meanwhile, the general trend of opinion inclines toward the theory of nuclear solution within the corpuscle, and regards the so-called free nuclei of the normoblasts simply as artefacts (Fig. 49, I and III).

Normoblasts exist in the red bone marrow of the normal indi-

[1] Arch. f. mik. Anat., 1880, vol. xvii, p. 1.
[2] *Loc. cit.*
[3] Zeitschr. f. klin. Med., 1881, vol. iii, p. 411.
[4] Virchow's Arch., 1896, vol. cxlv, p. 587; also Pappenheim, Inaug. Dissert., Berlin, 1895.

vidual, but are found in the circulating blood only when the marrow, in consequence of pressing demands made upon it for the rapid manufacture of new erythrocytes, becomes unable to furnish an adequate supply of perfectly developed cells, so that some of these immature, nucleated forms prematurely leave their birthplace in the marrow, and pass into the blood stream in company with large numbers of mature, non-nucleated discs. Normoblasts are associated with lesions in which active hemogenesis of the normal type is stimulated, being the prevailing type of erythroblast in the anemias resulting from hemorrhage and in other severe anemias of a secondary type. They sometimes appear in the blood in successive crops of large numbers during the course of certain severe anemias, this phenomenon having been termed by von Noorden[1] a *blood crisis*. Blood crises, which are of abrupt onset and of brief duration, lasting but a few hours, are usually the direct precursors of an increase in the erythrocyte count and in the hemoglobin percentage, being therefore a favorable sign, indicating regeneration of the blood. They occur with especial frequency after loss of blood from hemorrhage and in chlorosis, and are not uncommon in long-standing cases of myelogenous leukemia and primary pernicious anemia, in which diseases periods of temporary improvement are likely to take place from time to time.

*Megaloblasts* (Plate I; also Fig. 48).—The typical megaloblast is much larger in size than the normoblast, and contains a single, large, pale-staining nucleus which occupies the greater part of the cell body. Both cell and nucleus are round or ovoid in shape, the diameter of the former being from about 11 to 20 $\mu$, and that of the latter from 6 to 10 $\mu$. The greatest extremes of these measurements apply to those forms which are seen with relative infrequency, for the megaloblast most commonly observed does not usually measure more than 12 $\mu$ in diameter, with a nucleus of proportionate size. The nucleus, which may be situated either in or away from the center of the cell, is composed of a chromatin network having relatively small intervening open spaces, so that the nuclear structure is decidedly more delicate and less well-defined than that of the normoblast. With the triacid solution and with the Romanowsky stain it is tinted pale green or blue, or it may show every sort of irregular tinctorial reaction to the anilin dyes, certain portions being deeply stained, while other parts are but faintly colored; the undertone of green or blue is frequently stippled with fine dots of purple or of brilliant crimson, especially about the periphery; or it may be mottled and splotched here and there with areas of purple

[1] Charité-Annalen, 1891, vol. xvi, p. 217.

or of dark blue. The nucleus, in the triple-stained film, usually is sharply differentiated from the body of the cell by a distinct white margin which encircles it and is thrown out in bold relief by the deep staining of the nuclear and cell bodies on either side. Occasionally a megaloblast shows a coarse, small nucleus of very basic affinity, resembling that of the normoblast—characteristics of nuclear senility.

The protoplasm of the megaloblast often seems swollen and enlarged, and appears to contain areas of depression and elevation at different points; it is sometimes quite round or oval in contour, and sometimes more or less deformed. It is usually polychromatophilic, and, like the nucleus, may show the greatest variety of color combinations. Some cells stain, with the triacid mixture, a dull brownish-yellow color with deeper shadings of a burnt-sienna tint in the neighborhood of the nucleus and of the periphery; others have an undertone of crimson, as if the stain contained an excess of fuchsin, and are streaked and dotted with yellow and tan-colored patches; still others stain a diffuse purple, blending in spots into a light pink. With Wright's stain the color varies from greenish-blue to purple to dull yellow. Mitotic megaloblasts, with figures similar to those exhibited by normoblasts thus dividing, are met with occasionally in anemias of great severity. (Plate I, Fig. 3; also Fig. 49, II.)

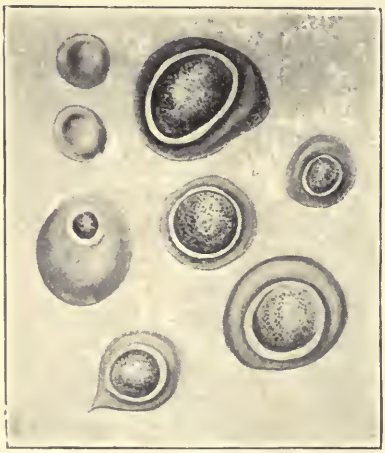


FIG. 48.—MEGALOBLASTS.

Common types of megaloblasts, showing variations in size and shape and peculiarities of the nuclear structure. (Ehrlich's triacid stain.)

The megaloblast is an element of the bone marrow of the young fetus, and is totally foreign both to the marrow and to the blood of the normal adult. According to the views of Ehrlich, it represents the immediate antecedent of the megalocyte, into which it develops by the absorption of its nucleus. Apparent extrusion of megaloblastic nuclei is never observed. Megaloblasts are found in the circulating blood only under conditions in which the blood-making organs have reverted more or less to the fetal type, so that their presence in the circulation is considered to indicate that a sluggish hemogenesis of embryonal character exists. The

significance of megaloblasts, therefore, is diametrically opposed to that of normoblasts, for, while the latter are regarded as an expression of blood *regeneration* and are considered to be of favorable prognostic significance, the former must be looked on as an evidence of *degeneration* of the hematopoietic organs, and, consequently, are of grave prognosis.

In the following table the principal points of distinction between the typical normoblast and the megaloblast are emphasized:

| | Normoblast. | Megaloblast. |
|---|---|---|
| *Size.* | 7.5 to 10 μ. | 11 to 20 μ. |
| *Nucleus.* | Sharply defined.<br>Intensely basic.<br>Coarsely meshed.<br>Occupies about one-half of cell body. | Dully defined.<br>Feebly basic.<br>Delicately meshed.<br>Occupies greater part of cell body. |
| *Protoplasm.* | Sometimes very scanty and of ragged outline. Occasionally polychromatophilic. | Frequently appears swollen; outline fairly regular, but surface undulating in many cells.<br>Striking tendency toward polychromatophilia. |
| *Histological Significance.* | Typical of active, adult hemogenesis. | Typical of sluggish, embryonal hemogenesis. |
| *Occurrence.* | Prevailing type of erythroblast in anemias with active blood regeneration. | Prevailing type of erythroblast in anemias with megaloblastic degeneration of the bone marrow. |

Megaloblasts are found in the blood, almost invariably in association with normoblasts, in various anemias of marked severity, but in only three conditions, viz., primary pernicious anemia, certain cases of anemia due to *Bothriocephalus latus* infection, and nitrobenzol poisoning, have these cells been found to constitute the *prevailing type* of erythroblast.

In pernicious anemia the prevalence of megaloblasts is generally admitted to be a sign that in this disease the bone marrow, in consequence of its reversion to a fetal type, throws into the blood stream large numbers of these blood cells of embryonal character, these degenerative changes, the presence of megaloblasts, overshadowing the regenerative changes, or the presence of normoblasts. In bothriocephalus anemia, in which also the megaloblasts may outnumber the normoblasts, it is believed that the toxins produced by the parasite cause changes in the hematopoietic organs precisely similar to those found in pernicious anemia. Rosenquist's[1] elaborate studies show that the excessive

[1] Berlin. klin. Wochenschr., 1901, vol. xxxviii, p. 666.

albumin disintegration caused by the bothriocephalus toxin is essentially like that occurring in typical pernicious anemia. In a single case of nitrobenzol poisoning, reported by Ehrlich and Lindenthal,[1] large numbers of erythroblasts were noted; normoblasts predominated at first, but in the later stages of the intoxication they were outnumbered by megaloblasts. In other grave anemias, notably in leukemia, the regenerative signs appear to be more active than the degenerative, for, while in these conditions megaloblasts are frequently found, they are never so numerous as the normoblasts.

*Microblasts.*—The microblast, which is the rarest form of nucleated erythrocyte, is a cell usually not larger than 5 or 6 $\mu$ in diameter, and often of smaller size. It consists of a deeply stained, round nucleus like that of the normoblast, encircled by a fragment of ragged protoplasm of a dull brownish-yellow tint, in films stained with the triacid solution. Wright's stain frequently colors this stroma blue. Microblasts are thought to be simply forms of the normoblast in a more or less advanced stage of protoplasm degeneration, this process accounting for the characteristic scantiness and frayed-out appearance of their cell body. Their clinical significance, naturally, is identical with that of the normoblast.

From what has been stated, it may be concluded that normoblasts and megaloblasts constitute two distinct classes of nucleated erythrocytes, each evidencing a separate type of blood-formation, and each carrying a different clinical meaning. Normoblasts, being an adult type of cell, have sharply defined, dense, deeply stained nuclei; megaloblasts, being an embryonal type of cell, have poorly defined, delicate, feebly stained nuclei.

*Atypical Erythroblasts.*—In some of the severer anemias, notably in myelogenous leukemia and in pernicious anemia, various atypical erythroblasts are frequently found, corresponding partly to one and partly to the other of the first two species of cells described above. These so-called "mesoblasts," which may be regarded either as normoblasts with immature nuclei or as megaloblasts with mature nuclei, are in some instances almost as numerous as the typical forms of erythroblasts. It is sometimes impossible accurately to determine to which type such cells belong, but usually they may be classified by taking size as a criterion for differentiation. Those approximating the normocyte in size may safely be classed as normoblasts; those of larger size, as megaloblasts—regardless of their nuclear peculiarities. The two fol-

[1] Zeitschr. f. klin. Med., 1896, vol. xxx, p. 427.

lowing commoner forms of atypical erythroblasts may be recognized:

1. Corpuscles about 8 or 10 $\mu$ in diameter, containing a relatively large, round or ovoid nucleus, composed of a finely meshed chromatin framework. The nucleus is pale, and is often filled with

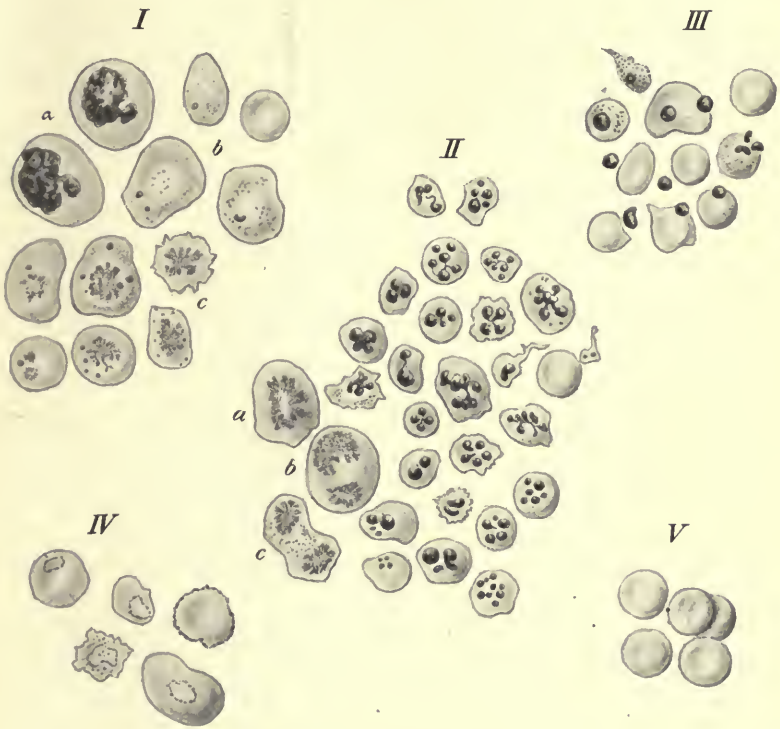



FIG. 49.—ATYPICAL FORMS OF ERYTHROBLASTS.

*I*, Megaloblasts and normoblasts showing nuclear solution; the two cells (*a*) show early, and the three (*b*) late, stages of karyolysis. Five of the six cells (*c*) contain nuclear remains consisting of both coarse and delicate chromatin masses. Note the granular basophilia in the groups of cells at *I*, *II*, *III*, and *IV*. *II*, Erythroblasts with multiple and dividing nuclei; the megaloblast (*a*) represents the wreath-shaped (monaster) stage of karyokinesis, the megaloblast (*b*) the double star-shaped (diaster) stage, and the megaloblast (*c*) the convolution (daughter cell) stage. The other cells illustrate the kinds of erythroblasts with convoluted and multiple nuclei ordinarily found in high-grade anemias. *III*, Erythroblasts showing so-called nuclear extrusion. *IV*, Erythrocytes containing ring bodies. *V*, Normal erythrocytes. (Wright's stain.)

finely stippled areas of acid affinity. The cell body is usually of regular outline, and, as a rule, is decidedly polychromatophilic. Such cells may be regarded as immature forms of normoblasts, with which they may properly be classed in the differential count (Plate I; also Fig. 48).

2. Corpuscles about 12 to 15 $\mu$ in diameter, having a small, coarsely meshed nucleus not exceeding 2 or 3 $\mu$ in diameter, and, as a rule, situated eccentrically. The nucleus stains very basically, and may or may not be separated from the protoplasm by a colorless zone. The body of the cell is round or ovoid, and stains faintly. This form of cell appears to carry the same clinical significance as the megaloblast, of which it is probably a late developmental phase (Plate I; also Fig. 48).

*Ring Bodies.*—In severe anemia Wright's stain often brings out peculiar intra- and extra-cellular *ring-shaped bodies* whose outlines resemble those of the nuclear figures of erythroblasts (Fig. 49, IV). Cabot,[1] who described these bodies, suggests that they may represent nuclear remnants, or portions of the erythrocytes' nuclei which are especially resistant to whatever forces ultimately destroy the cells and their nuclei. Basic (blue) ring bodies within the erythrocytes were described and pictured, in 1901, by Strauss and Rohnstein,[2] who interpreted them as a variety of granular basophilia. The bodies have been found, in association with frankly nucleated erythrocytes, polychromatophiles, and basically stippled cells, in *lead poisoning*, in *pernicious anemia*, and in *lymphatic leukemia*. The writer has noted them in high-grade anemia secondary to *sepsis*. With Wright's solution the ring-shaped bodies stain red and, rarely, blue; they are variously shaped—circles, rude rosettes, clover leaves, figures-of-eight, and forms with twisted threads and with netted structures. These designs, it will be noted, correspond accurately to the nuclear figures of various erythroblasts.

GRANULAR BASOPHILIA.

In certain of the severe anemias, staining with methylene-blue shows a peculiar granular condition of the protoplasm in some of the erythrocytes, attention first having been called to this fact by von Noorden,[3] who demonstrated the basophilic characters of such granules, and described their occurrence in various pathological states. Many of the corpuscles thus affected are of the nucleated form, but non-nucleated cells may be similarly granulated; as a rule, such corpuscles are also strikingly polychromatophilic.

The granules appear either as fine or as coarse, stippled areas, staining intensely with the basic stain, and distributed through the body of the cell either quite uniformly or in localized patches

[1] Jour. Med. Research, 1903, vol. iv, p. 15.

[2] "Die Blutzusammensetzung bei den verschiedenen Anämienen," Berlin, 1901, p. 224.

[3] Charité-Annalen, 1892, vol. xvii, p. 202.

at one or at several points. In some cells they are exceedingly fine and closely packed together, so that at first glance the whole protoplasm appears to be a homogeneous mass of purplish discoloration; in others the protoplasm is dotted here and there with coarse granules, not more than five or six being found in the whole cell; still others may contain both fine and coarse granules irregularly sprinkled over the surface (Plate I; also Figs. 49 and 50).

The occurrence of somewhat similar granulations in the erythrocytes of the embryo has been noted by Engel,[1] Pappenheim,[2] and others, who regard them as nuclear débris, the product of nuclear disintegration. Such an origin in embryonic blood is

FIG. 50.—GRANULAR BASOPHILIA.
Erythrocytes showing various degrees of basophilia, with fine, coarse, spherical, ovoid, and spiculate granules. Note the basophilic normoblast. (Wright's stain.)

probably physiological. In post-uterine life, however, this process is to be regarded as a sign of stroma degeneration, arising in all likelihood through the influence of various blood poisons. In some instances the change precedes all other recognizable alterations in the blood, and appears as the first, and, indeed, sometimes the only, distinct sign of anemia.

Granular basophilia of the erythrocytes has been noted with more or less constancy in these conditions: *pernicious anemia, leukemia, Hodgkin's disease,* so-called *tropical anemia, bothriocephalus anemia, malarial fever, sepsis, carcinoma, long-standing suppurative lesions,* and *chronic lead poisoning.* In *chlorosis,* if

[1] Verhandl. d. Vereins f. inn. Med. z. Berlin, 1898–99, vol. xviii, p. 216.
[2] *Loc. cit.*

uncomplicated by symptoms of intestinal auto-intoxication, the erythrocytes do not exhibit this alteration; granule cells are also absent in *syphilis*, in *acute infectious diseases*, in *chronic lesions of the kidney* and the *liver*, and in *diabetes*, according to Grawitz.[1] Regarding the occurrence of this change in pernicious anemia, Ehrlich[2] believes that the number of granule cells in the blood bears a certain relation to the severity of the disease, stating that they decrease and often disappear during the periods of remission, reappearing as the other blood changes again become evident. On the other hand, Litten,[3] who asserts that he has found these basophilic granulations in one-tenth of all cases of anemia, has been unable to determine their clinical significance from either a diagnostic or a prognostic point of view. The studies of Grawitz and Hamel[4] show that granular degeneration of the erythrocytes occurs with great regularity in *saturnism*, both in obscure and in well-marked cases, and these authors attach considerable diagnostic value to this fact, concluding that the sign is important in the diagnosis of lead poisoning in patients in whom the intoxication is merely suspected, being evidenced by no other definite symptoms. Experimentally, basophilia has been produced by the administration of lead salts, tin chlorid, copper, pyrodin, atropin, toluylendiamin, and phenylhydrazin. A dose of any of the proprietary preparations of hemoglobin may also promptly excite the change, as may the ingestion of whole blood—facts which lead Grawitz[5] to assume that blood in the gastro-intestinal canal elaborates toxic substances the absorption of which acts deleteriously upon the erythrocytes. This and other phases of basophilia have been reviewed at length by the writer elsewhere.[6]

Here may be mentioned certain areas of reddish stippling (*Schüffner's granules*) demonstrated by polychrome methylene-blue in the erythrocytes of tertian malarial fever (*q. v.*).

OLIGOCYTHEMIA.

*Oligocythemia*, or diminution in the number of erythrocytes below the normal standard, is present to a more or less marked degree in all forms of anemia, being associated, naturally, with an oligochromemia, or diminution in the percentage of hemoglobin, but not necessarily with an oligemia, or reduction in the volume of the blood mass.

The loss of corpuscles may be slight or it may be marked, according to the nature of the anemia of which it is symptomatic.

[1] Amer. Jour. Med. Sci., 1900, vol. cxx, p. 277.
[2] *Loc. cit.*
[3] Deutsch. med. Wochenschr., 1899, vol. xxv, p. 717.
[4] Deutsch. Arch. f. klin. Med., 1900, vol. lxvii, p. 357.
[5] Deutsch. med. Wochenschr., 1901, vol. xxvii, p. 908.
[6] Amer. Med., 1903, vol. v, p. 571.

The most striking examples of oligocythemia are encountered after hemorrhages involving the loss of a large amount of blood and in pernicious anemia; while in chlorosis and in the majority of the secondary anemias the decrease is relatively less marked. The following summary of the averages of fifty consecutive counts each in cases of primary and secondary anemia illustrates the various degrees of cellular loss which ordinarily accompany these conditions:

| Average of 50 Counts. | Erythrocytes per c.mm. |
|---|---|
| In pernicious anemia | 1,152,470 |
| " leukemia | 2,729,763 |
| " secondary anemia | 3,642,900 |
| " chlorosis | 4,111,000 |

It is impossible to designate the degree of oligocythemia which may exist without a fatal outcome, although a number of authorities have attempted to set fixed limits beyond which reduction in the number of erythrocytes is supposed to cause death. The effects of a blood loss are so diverse in different individuals that all such arbitrary rules must, of necessity, prove practically valueless. It should be remembered that while in some persons a comparatively moderate decrease may prove fatal, in others a most astonishing loss is compatible with life. It may be stated in general terms that few individuals recover in whom a count of less than 500,000 erythrocytes to the c.mm. is found, although occasional exceptions to this rule have been reported.

Polycythemia.

Whether or not an actual, permanent *polycythemia*, or an increase in the number of erythrocytes above the normal standard, exists is still an unsettled question, but the majority of authorities maintain that such a condition is due merely to some physical change producing concentration of the blood, or unequal distribution of the corpuscles, in favor of the peripheral blood vessels. In health, it would not seem unreasonable to suppose that a moderate degree of polycythemia may be habitual in the strong, overdeveloped adult, whose blood-making organs are possibly developed proportionately to the other parts of his system. In pathological conditions there is nothing tangible upon which to base the belief that an actual and permanent overproduction of the erythrocytes ever takes place, the polycythemia associated with certain diseases being satisfactorily accounted for by coëxisting physical conditions, in no way peculiar to the lesion in question. While it is true that in some conditions it is not always possible to explain the increase by purely physical causes, still there is no posi-

tive proof, in these instances, that the change is pathological. There seems, therefore, no evidence to warrant an arbitrary classification of polycythemia into two divisions, actual and relative, as some authors have suggested.

The cause of polycythemia, then, may be attributed to physiological factors such as concentration of the blood, increased blood pressure, peripheral stasis, increased viscidity of the erythrocytes, and their unequal distribution through the circulatory system.

The polycythemia associated with various physiological and pathological conditions will be considered under their appropriate headings. Briefly, an increase of erythrocytes over the normal number is found in the following conditions:

1. In the new-born.
2. After taking food.
3. In starvation.
4. During residence in high altitudes.
5. From the effect of cold and hot baths, muscular exercise, massage, and electricity.
6. From the administration of lymphagogues, emetics, purgatives, and thyroid extract.
7. During active blood regeneration.
8. During reformation of an exudate after aspiration.
9. After urinary crises, diaphoresis, emesis.
10. In poisoning by illuminating gas and by phosphorus.
11. In Asiatic cholera, dysentery, and diarrhea.
12. In acute yellow atrophy of the liver and myxedema.
13. In conditions of cyanosis and peripheral stasis, for example, uncompensated organic heart disease, emphysema, asphyxia, and Osler's disease.
14. After the transfusion of blood.

## V. BLOOD PLAQUES.

If a drop of fresh blood is examined microscopically immediately after it has been taken from the body, a few pale, somewhat spherical bodies, much smaller in size than the erythrocytes, may usually be observed. These bodies are known as the *blood plaques* or *blood platelets*. They are of homogeneous structure, either almost colorless or of a pale yellowish tint, spherical or irregularly ovoid in shape, and measure from 1 to 3 or 4 $\mu$ in diameter. They are non-nucleated, and react toward both basic and acid anilin dyes, having an amphophilic affinity. Deetjen[1] has

[1] Virchow's Arch., 1901, vol. clxiv, p. 239.

shown that the plaques exhibit definite ameboid movement—a property denied these bodies by the earlier investigators—and that they are apparently nucleated.

The plaques exist as free bodies in the general circulation, but directly after the withdrawal of the blood from the vessels they show a remarkable degree of viscosity, by virtue of which they tend to adhere in racemose masses, the occurrence of which at or near the radiating points of the fibrin network has already been described. Zeri and Amalgia[1] found that in malarial fever this agglutination of the plaques did not occur, although it was regularly observed in other infections, such as pneumonia, pleurisy, tuberculosis, enteric fever, and the exanthemata.

The belief of Bizzozero[2] and of Hayem,[3] that the plaques represented a so-called "third corpuscle" of the blood, is not justified, for it has been proved that these bodies are not distinct cellular entities, but rather débris, derived either from the blood corpuscles or from the plasma. It is evident, from the work of Arnold,[4] Engel,[5] Klebs,[6] and others, that at least a large proportion of the plaques are simply bits of globular matter extruded from the erythrocytes, and in eosin-methylene-blue films the apparent eruption of plaques from the stroma of the erythrocytes can be readily demonstrated. It is possible that some of the plaques are derived by the disintegration of the nuclei of the leucocytes (Lilienfeld;[7] Howell;[8] Gibson[9]); and that still others are masses of precipitated globulin (Löwit[10]). Heim[11] believes that the plaques are nuclear formations of the erythrocytes, and claims that regeneration of the latter and increase in the number of blood plaques progress *pari passu*.

Ducchesi's method of macroscopically demonstrating the plaques is of clinical interest: A few drops of blood are collected in a watch-glass, which is gently rocked from side to side for a few minutes, and then held up to the light, showing the plaques as groups of delicate white granules in the stratum of blood next to the glass. These granular masses appear within from forty seconds to two minutes after withdrawal of the blood, and disappear as coagulation commences.

[1] Il Policlin., 1903, vol. ix, p. 485. [2] Virchow's Arch., 1882, vol. xc, p. 261.
[3] Compt. rend. Soc. biol., Paris, 1877, vol. ii, p. 85.
[4] Centralbl. f. allg. Path., 1897, vol. viii, p. 289.
[5] "Leitfaden zur klinischen Untersuchung des Blutes," 2d ed., Berlin, 1902, p. 51.
[6] Ziegler's Beitr., 1889, vol. iv, p. 528.
[7] Zeitschr. f. physiol. Chem., 1895, vol. xx, p. 155.
[8] Jour. Morph., 1891, vol. iv, p. 57.
[9] Jour. Anat. and Physiol., 1886, vol. xx, p. 100.
[10] Arch. f. mik. Anat., 1891, vol. lviii, p. 598.
[11] Deutsch. med. Wochenschr., 1903, vol. xxix, p. 588.

Exposure to the air appears to cause an almost immediate disappearance of the plaques from the blood, and, therefore, they are but seldom noticed in the blood film prepared by the ordinary methods. With Wright's stain, however, they are readily demonstrable in the dry film. In studying the plaques in their fresh state the blood may be drawn directly through a drop of Hayem's solution or a weak solution of osmic acid, the mixture of the blood and fixative being then placed upon a slide and examined in the usual manner. (See p. 90.)

The number of plaques in normal blood varies within wide limits, according to the statements of different authorities, but about 300,000 to the c.mm. is generally considered the normal average, and from 180,000 to 500,000 the range under physiological circumstances.

The plaques generally are increased in *pernicious anemia*, *severe secondary anemias*, *leukemia*, *pneumonia*, *arthritis deformans*, *myelitis*, *tuberculosis*, *bubonic plague*, and as the effect of residence in *high altitudes*. They are diminished in *hemophilia*, *purpura*, and acute febrile diseases, such as *erysipelas*, *typhus fever*, and the *malarial fevers*.

## VI. HEMOKONIA.

Müller[1] has called attention to the constant presence in normal and pathological blood of small, colorless, refractive bodies, of spheroidal or dumb-bell shape, not larger than 1 $\mu$ in diameter. These bodies, to which the terms *hemokonia* and *blood dust* have been applied, are highly refractive, and possess active, limited molecular motility, but not true ameboid motion. They have been compared in appearance to fine fat droplets, to micrococci, and to granules derived from the protoplasm of the leucocytes. Nothing is known of their histological character and significance beyond the facts that they are not concerned in the process of fibrin formation, and that they are not fatty bodies, since they are neither stained by osmic acid nor dissolved by ether. Both Stokes and Wegefarth,[2] and Nicholls[3] regard them as free granules of the neutrophile and eosinophile leucocytes, and believe that they are probably concerned in the protective properties of the blood in immunity. Stengel[4] suggests that they may be simply the products of fragmentation of the erythrocytes, such as may

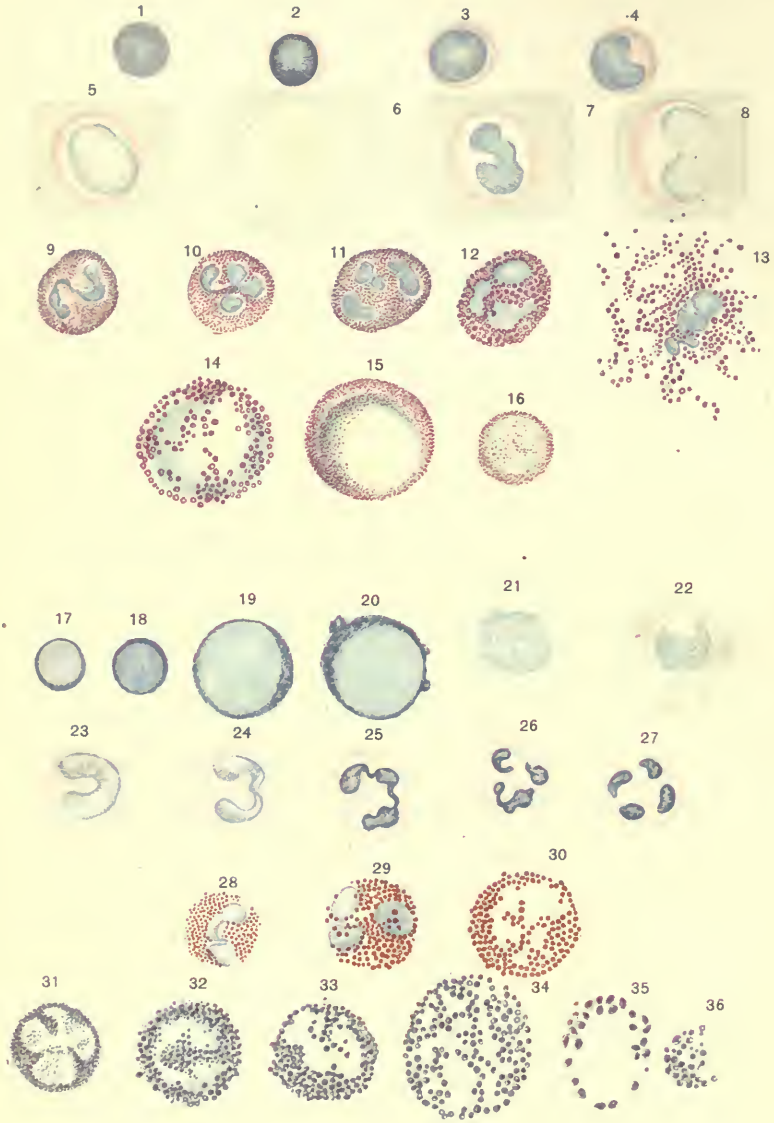
[1] Centralbl. f. Path. u. Bakteriol., 1896, vol. xxv, p. 529.
[2] Johns Hopkins Hosp. Bull., 1897, vol. viii, p. 246.
[3] Phila. Med. Jour., 1898, vol. i, p. 387.
[4] "Text-book of Pathology," 4th ed., Philadelphia, 1903, p. 335.

be produced by heating fresh blood to destructive temperatures, when bits of the corpuscles are seen to bud out, break off, and float free in the plasma, endowed with pseudo-ameboid motility.

Müller found large numbers of hemokonia in a case of *Addison's disease*, but these bodies were very scanty in a number of markedly *cachectic conditions*. Their occurrence in the blood appears to carry no definite clinical significance.

# SECTION IV.

# THE LEUCOCYTES.




THE LEUCOCYTES.

(1–16, *Triacid Stain*; 17–36, *Eosin and Methylene-blue.*)

(E. F. FABER, *fec.*)

(*Triacid Stain.*)

1, 2, 3, 4. **Small Lymphocytes.**
Contrast the faintly colored protoplasm of these cells in the triple stained specimen with their intensely basic protoplasm in the film stained with eosin and methylene-blue, 17 and 18. The cell body of 1 is invisible. Note the kidney-shaped nucleus in 4.

5, 6. **Large Lymphocytes.**
With this stain the nucleus reacts more strongly than the protoplasm; with eosin and methylene-blue (19, 20), on the contrary, the protoplasm is so deeply stained that the nucleus appears pale by contrast. This peculiarity is also observed in the smaller forms of lymphocytes.

7, 8. **Transitional Forms.**
Note the moderately basic and indented nucleus, and the almost hyaline non-granular protoplasm. Compare 8 with the myelocyte, 7, Plate IV, these cells differing chiefly in that the myelocyte contains neutrophile granules.

9, 10, 11. **Polynuclear Neutrophiles.**
These cells are characterized by a polymorphous or polynuclear nucleus, surrounded by a cell body filled with fine neutrophile granules. In 11 the nuclear structure is obviously separated into four parts; in 9 it is moderately, and in 10 markedly, polymorphous.

12, 13. **Eosinophiles.**
The nuclei are not unlike those of the polynuclear neutrophile, except that they are somewhat less convoluted, and poorer in chromatin, staining less intensely. The protoplasm is filled with coarse eosinophile granules, the characteristics of which are clearly illustrated by 13, a "fractured" eosinophile.

14. **Eosinophilic Myelocyte.**
Compare with 15.

15, 16. **Myelocytes.** (*Neutrophilic.*)
These cells are morphologically similar to 14, except that they contain neutrophile instead of eosinophile granules. Note that the granules of the myelocyte are identical with those of the polynuclear neutrophile. A dwarf form of myelocyte is represented by 16.

(*Eosin and Methylene-blue.*)

17, 18. **Small Lymphocytes.**
Note the narrow rim of pseudo-granular basic protoplasm surrounding the nucleus, and the pale appearance of the latter.

19, 20. **Large Lymphocytes.**
Budding of the basic zone of protoplasm is represented by 20. Both of these cells belong to the same type as 5 and 6.

21, 22. **Large Mononuclear Leucocytes.**
Compared with 19 and 20, these cells have a decidedly less basic protoplasm, but a somewhat more basic nucleus. In the triple stained film these differences cannot be detected, so that they must be classed as large lymphocytes.

23. **Transitional Form.**
The distinction between this cell and 24 is not marked; the nucleus of the latter simply being somewhat more basic and convoluted.

24, 25, 26, 27. **Polynuclear Neutrophiles.**
With this stain these cells show a feebly acid protoplasm, and lack granules. Note that the more twisted the nucleus the deeper it is stained. Compare with 9, 10, and 11.

28, 29. **Eosinophiles.**
Compare with 12 and 13.

30. **Eosinophilic Myelocyte.**
Compare with 14.

31. **Basophile.** (*Finely granular.*)
This cell is characterized by the presence of exceedingly fine δ-granules, staining the *pure* color of the basic dye. The nucleus is markedly convoluted and deficient in chromatin. The cell here shown was found in normal blood.

32, 33, 34, 35, 36. **Mast Cells.**
The granules take a *modified* basic color, as shown by their royal-purple tint in this illustration. Note their unusually large size and ovoid shape in 35, their peculiar distribution in 35 and 36, and their irregularity in size in 32 and 36. With the triacid mixture these granules, as well as those of the finely granular basophile, 31, remain unstained, showing as dull-white stippled areas in the cell body. The nuclear chromatin of the mast cell is so delicate and so feebly stained that it is barely visible. These cells were found in the blood of a case of spleno-medullary leukemia.

# SECTION IV.

## THE LEUCOCYTES.

### I. GENERAL CHARACTERISTICS.

Appearance in Fresh Blood.

In the fresh, unstained blood film the leucocytes are recognized as pale nucleated cells, the majority of which are larger in size than the erythrocytes, by which they are greatly outnumbered, the proportion of the former to the latter ranging approximately between 1 : 450 and 1 : 1200 in normal blood. The size of the normal white corpuscles varies from about 7 $\mu$ to 10 or 12 $\mu$ in diameter, and their shape, while in the resting stage, is irregularly round or oval.

By careful examination four different varieties of these cells may be distinguished, the distinction between these forms being made more striking by the addition of a small quantity of a one per cent. acetic acid solution to the fresh film. These varieties, which are essentially the same as those first described by Schultze, in 1865,[1] are as follows: (1) Non-ameboid cells about the size of the normal erythrocyte, consisting of a pale, compact, spherical nucleus encircled by a narrow zone of homogeneous protoplasm. (2) Ameboid cells almost twice the size of the erythrocyte, consisting of a rather coarsely meshed nucleus, spherical, ovoid, or indented in form, surrounded by a relatively large amount of clear protoplasm. The latter is highly opaque, for although it forms an exceedingly thin layer when spread out flat, it effectually obscures the outlines of objects over which it lies—as an explanation for which characteristic Kanthack and Hardy[2] presume that the cell matter is composed of a colorless basis embedding immense numbers of minute vacuoles filled with a substance of a different refractive index. (3) Ameboid cells of slightly smaller size than the second variety, consisting of a single twisted nucleus, or of two or more separate round or ovoid nuclei, embedded in a body of protoplasm crowded with exceedingly delicate, moderately refractive granules. The nuclear network is

[1] Arch. f. mik. Anat., 1865, vol. i, p. 1. [2] Jour. Physiol., 1894–95, vol. xvii, p. 81.

composed of chromatin threads closely united to form a compact, lobulated structure, and the protoplasm appears to consist of a transparent substance, of gelatinous character, having a refractive index but slightly below that of the granules which it contains. (4) Ameboid cells containing a convoluted nucleus, or several spherical nuclei, embedded in a protoplasm filled with coarse, highly refractive, fat-like granules. The nuclear structure consists of a coarsely meshed, knotted network, and the protoplasm is much less refractive than its granules, being clear and structureless in appearance.

AMEBOID MOVEMENT.

Spontaneous changes in the shape of the larger varieties of leucocytes may be observed if the slide is placed upon a warm stage having a temperature of about 98.5° F. During these ameboid movements the shape of the cells constantly undergoes alteration by the alternate contraction and expansion of the protoplasm. Tentacular processes reach out from various portions of the cell body, while at other points its surface becomes retracted, so that it may appear as an irregular nucleated mass provided with one or more long, snake-like arms projecting from a central body. These ameboid cells are chiefly concerned in the process of *phagocytosis*, or the engulfing and destruction of micro-organisms and other foreign matter which may gain entrance into the circulating blood, and to leucocytes which exert this function the term *phagocyte* has been applied. The well-known experiments of Metschnikoff [1] have shown their propensity for seizing upon and devouring pathogenic bacteria, such as the anthrax bacillus and the erysipelas streptococcus, and further proof of such phagocytic action may frequently be found in the fragments of other foreign matter, such as bits of old blood clots, malarial pigment, and fat droplets inclosed in their protoplasm.

It has also been suggested by Gabritschewsky [2] that it may be possible under some circumstances that phagocytes are capable not only of engulfing solid bodies, but that they may also imbibe liquid substances, which are thus rendered harmless to the organism, and to this property the term *pinocytosis* has been given by this author. Drugs injected hypodermically are taken up by phagocytic cells, which, according to Labbé,[3] not only absorb and assimilate medicaments, but perhaps carry them, by election, to a specific lesion of the organism—mercury to a syphilitic nidus, for instance. Iron, iodin, arsenic, mercury, iodoform,

[1] "L'Inflammation," Paris, 1892.
[2] Annal. de l'Institut Pasteur, 1894, vol. viii, p. 673.
[3] Presse méd., 1903, vol. ii, p. 725.

and the salicylates are among the drugs dealt with in this manner.

The ameboid property of the leucocytes is also responsible for the ease with which these cells escape from the blood vessels into the perivascular tissues in inflammatory lesions, and to a less extent in health. This well-known process of *diapedesis* is facilitated by virtue of the leucocyte's ability to elongate and flatten out so that it may readily emigrate through the spaces between the endothelial cells of the vessel wall.

CELL GRANULES.

The identification of the various forms of leucocytes depends largely upon the presence or absence of granules in their protoplasm, and upon the distinctive manner in which these granules react toward the acid, basic, and so-called neutral solutions of the anilin dyes. By means of this method of "color analysis" Ehrlich has provided a rational means by which the study of the leucocytes may be undertaken.

Five varieties of granules, which are designated by the use of the Greek letters $\alpha$, $\beta$, $\gamma$, $\delta$, and $\varepsilon$, may be recognized in the cell bodies of the leucocytes, as follows:

1. *$\alpha$-granules* (eosinophile, oxyphile, or coarse oxyphile granules): Coarse, spherical or ovoid, highly refractive granules of a peculiar fat-like appearance, showing a striking affinity for acid stains, especially for eosin. In normal blood they occur only in leucocytes with polynuclear or polymorphous nuclei, but in certain pathological conditions they may be found in that variety of the leucocyte known as the eosinophilic myelocyte.

2. *$\beta$-granules* (amphophile granules): Fine granules which are capable of reacting toward both acid and basic dyes, invariably staining with the former and sometimes with the latter, if the stains are used singly, while in a mixture of the two they always react toward the acid dye. These granules never occur in normal blood, but in some pathological conditions a varying proportion of the leucocytes may exhibit amphophilic reactions on the part of some of their granules.

3. *$\gamma$-granules* (mast cell or coarse basophile granules): Very coarse granules, measuring from 0.2 to 0.4 $\mu$ in diameter, and possessing an intense affinity for basic dyes. If stained with carboltoluidin-blue, with thionin, or with alkaline methylene-blue, they are colored a distinctive deep purplish-red. These granules occur in a form of leucocyte known as the mast cell, which is abundant in myelogenous leukemia, and is met with occasionally in other diseases.

4. *$\delta$-granules* (fine basophile granules): Fine granules, stain-

ing with basic dyes, and occurring under normal conditions in leucocytes having polymorphous nuclei. They are most clearly demonstrated with such basic dyes as thionin or methylene-blue, by which they are stained a deep blue color.

5. ε-*granules* (neutrophile or fine oxyphile granules): Exceedingly fine granules, formerly thought to have a selective affinity for the neutral element of a solution composed of acid and basic dyes, but now known to have, in reality, a feeble oxyphilic tendency. They occur abundantly in the normal polynuclear neutrophile cells, and also in several pathological forms of leucocytes: the myelocyte, the small mononuclear neutrophile, and the "small neutrophilic pseudolymphocyte."

But little is known of the real nature and function of the leucocyte granules, in spite of their elaborate study by different investigators. Two leading views, which excite much controversy, to-day command attention: the hypothesis of Ehrlich[1] and the bioblastic theory of Altmann.[2] Ehrlich regards them as an evidence of a specific secretory function on the part of the cells, which under normal conditions contain but a single variety of granules. They are to be considered as products of cellular metabolic activity, and are destined to be given off in the vicinity of the cells, this elimination perhaps constituting one of the most important functions of the latter. Far from representing mere waste-products, as some authors contend, they are in reality elements of decided, although obscurely defined, value to the organism. Altmann, in his bioblastic theory, considers cell granules as definite biological entities, and believes that they "serve as a basis for the explanation of the many phenomena of organic metabolism." In summing up their functions he remarks that "they effect through oxygen-transmission both reductions and oxygenation, and in this manner accomplish the disunions and the syntheses of the economy without sacrificing their own individuality."

As a rule, the cell granules are thought to be relatively simple bodies, although their exact composition is as yet undetermined. It has been proved by Weiss[3] and others that they are of albuminous character. The eosinophile granules, in which iron has been demonstrated by Barker[4] and other observers, are more complex than the other varieties. They are of a higher histological structure, consisting of an external limiting portion which may

[1] *Loc. cit.*

[2] "Ueber die Elementärorganismen und ihre Beziehungen zu den Zellen," 2d ed., Leipsic, 1894.

[3] "Hematologische Untersuchungen," Vienna, 1896.

[4] Johns Hopkins Hosp. Bull., vol. v, p. 93.

be clearly differentiated from the central area. Hankin and Kanthack[1] have determined the fact that increased bactericidal power of the blood is closely correlated with the discharge of both eosinophile and neutrophile granules into the plasma, and the former observer[2] has furthermore shown that in experimental infections there is at the point of the infection an accumulation of cells containing eosinophile granules, together with a discharge of such granules during the conflict of the cells with the invading micro-organisms.

NORMAL NUMBER.

In the normal adult the number of leucocytes in the peripheral circulation averages from about 5000 to 10,000 to the c.mm. of blood. In the majority of instances, in which the influences of physical factors are excluded, a count of 7500 leucocytes per c.mm. may be regarded as the mean normal average. Variations of several thousand cells per c.mm. above and below this number are within physiological limits, and frequently occur because of the extreme susceptibility of the leucocytes to agencies causing such transient fluctuations. The following table, compiled from data given by Hayem,[3] Grawitz,[4] and von Limbeck,[5] shows the average number of leucocytes determined by various authorities:

| | |
|---|---|
| Thoma | 8687 per c.mm. |
| Von Limbeck | 8500 " " |
| Rieder | 7680 " " |
| Boeckman; Halla | 7533 " " |
| Graeber; Reinecke | 7242 " " |
| Tumas | 6200 " " |
| Hayem | 6000 " " |
| Average | 7406 " " |

## II. CLASSIFICATION.

Six distinct varieties of leucocytes may be recognized in the healthy adult's blood stained by the Romanowsky method or by Ehrlich's stain, according to the methods described in a previous section. These varieties, together with their normal relative percentages and absolute number to the c.mm. of blood, are as follows:

| VARIETY. | PER CENT. | NUMBER PER C.MM. |
|---|---|---|
| Small lymphocytes | 20–30 | 1000–3000 |
| Large lymphocytes and transitional forms | 4–8 | 200–800 |
| Polynuclear neutrophiles | 60–75 | 3000–7500 |
| Eosinophiles | 0.5–5 | 25–500 |
| Basophiles, as high as | 0.5 | 25 |

[1] Centralbl. f. Bakt. u. Parasit., 1892, vol. xii, p. 777; *ibid.*, 1893, vol. xiv, p. 852.
[2] Jour. Physiol., 1894–95, vol. xvii, p. 81.
[3] *Loc. cit.*
[4] "Klinische Pathologie des Blutes," Berlin, 1896.
[5] *Loc. cit.*

With the decline of life the proportion of polynuclear neutrophiles rises, the lymphocytic forms becoming correspondingly less numerous. This reversal of the youthful blood picture is, according to Dobrovici's researches,[1] most conspicuous after the sixtieth year of age.

The variations in these numbers and percentages, which depend upon different physiological and pathological influences, are referred to in other sections.

SMALL LYMPHOCYTES.

The lymphocytes, or small lymphocytes, as they are commonly designated in contradistinction to the large mononuclear forms, are non-granular cells which measure from about 5 to 10 $\mu$ in diameter, their average size being that of the normal erythrocyte, or 7.5 $\mu$ in diameter. The typical cell of this class consists of a single round, deeply staining nucleus surrounded by a narrow zone of protoplasm, and sometimes provided with one or two pseudo-nucleoli, situated eccentrically upon the nuclear surface. The nucleus is so relatively large that it almost completely fills the cell, being its most conspicuous part, while the rim of protoplasm is usually so narrow and poorly defined that at first glance it may escape notice. These characteristics—a relatively large nucleus and a relatively scanty amount of protoplasm— are more conspicuously exhibited in the smaller than in the larger forms of these cells. (Frontispiece, II, and Plate II, Figs. 1–4, 17, and 18.)

By Romanowsky's method the nucleus stains purple, in which a red tone prevails, and the protoplasm pure sky-blue. Occasionally the protoplasm is stippled with a few rather coarse purple or red granules.

In films stained with simple eosin and methylene-blue solutions the nucleus shows a decided affinity for the basic dye, usually staining dark blue, or, more rarely, pale green. The protoplasm shows as a relatively narrow encircling area of deep blue color, which has been likened in appearance to the surface of ground glass; it is much more intensely basic than the nucleus, which looks pale by contrast. With Ehrlich's triple stain the nucleus, being rich in chromatin, is colored deep blue or purple, and the protoplasm is either entirely unstained, appearing as a narrow hyaline halo surrounding the nucleus, or it is tinged a delicate shade of pink if it happens to react toward the acid fuchsin of the mixture.

Occasionally small lymphocytes are encountered in which the nucleus is atypical both in morphology and in staining properties.

[1] Sem. méd., 1904, vol. xxxiv, p. 198.

Thus, some cells contain a pale, almost hyaline nucleus, composed of an exceedingly scanty chromatin structure which reacts very feebly to the basic dyes; others contain a deeply stained, indented or kidney-shaped nucleus, similar in shape to that of the so-called "transitional" forms; while still others are provided with a nucleus which has evidently become completely divided, so that such a cell really contains two distinct hemispherical nuclei, rich in chromatin, deeply stained, and situated toward the poles of the cell body. These irregular forms of lymphocytes occur both in normal and in pathological blood, but with much greater frequency in the latter, especially in both forms of leukemia.

The small lymphocyte appears to possess greater powers of resistance than any other variety of leucocyte. In his studies of necrobiosis of the blood corpuscles Bodou[1] determined that the degenerative changes first involved the large mononuclear hyaline cells regarded as myelogenous in type; next, the transitional forms; next, the large lymphocytes of lymphatic origin; next, the polynuclear neutrophiles; and last of all the small lymphocytes.

LARGE LYMPHOCYTES.

Under this term it is convenient to include both the larger forms of the true lymphocyte—those measuring 11 $\mu$ or more in diameter—and also that variety of hyaline cell known as the large mononuclear leucocyte. These two forms of cells, although they are generally considered as distinct histological species, one being a true lymphocyte and the other probably a marrow-bred element, may, for practical purposes, be classed together, since it is impracticable to differentiate one from the other in the specimen prepared for an ordinary clinical examination.[2] (Frontispiece, II, and Plate II, Figs. 5, 6, and 19–22.)

Cells of this type may range in size from 11 to 15 $\mu$ or even larger in diameter, and are usually of round or ovoid shape, except in an occasional cell, where, in consequence of the injury received during the preparation of the blood film, the outline may be exceedingly irregular and deformed. The nucleus, which

[1] Virchow's Arch., 1903, vol. clxxiii, p. 485.

[2] Some authors, Ehrlich himself among them, maintain that a distinction between these two forms of cells may invariably be made in the stained specimen. Thus, in the film stained with methylene-blue, it is held that the true lymphocyte, no matter what its size, always possesses a strongly basic protoplasm and nucleus, the latter staining less deeply than the former; while the large mononuclear leucocyte has a feebly basic protoplasm and nucleus, the latter staining *more* intensely than the former. These points of difference, although they may be distinguished in specimens stained by special methods, seem to be too finely drawn to justify their acceptance as reliable criteria for the identification of these two groups of cells in films prepared by the technic adapted to routine clinical work.

is round, ovoid, or somewhat elongated, is generally situated toward the periphery of the cell body. In most of the cells the amount of protoplasm is relatively greater than that of the small lymphocyte, but occasionally this peculiarity cannot be distinguished.

With Wright's stain this cell stains fainter than, but in other respects like, the small lymphocyte, save that it occasionally shows a number of coarse and fine red granules scattered through its protoplasm.

With simple mixtures of a strong acid and basic dye, such as eosin and methylene-blue, the nuclear chromatin stains a diffuse sky-blue tint, and the protoplasm exhibits a more or less decided affinity for the basic element of the staining fluid. This tendency is very marked in some cells, the protoplasm of which contains an intensely basic pseudo-granular zone staining much deeper blue than the rest of the cell body, paralleling the extreme periphery of the cell, and often apparently separated from the nucleus by a distinct unstained area. In other cells this basic affinity is not so conspicuous, their protoplasm staining a diffuse purplish shade in which a rose-red tone prevails.

The nucleus, being poor in chromatin, stains pale blue with the triple stain, and is usually so delicately tinted that it is almost invisible; the protoplasm is faintly tinged with pink or with grayish-blue, or it may remain practically colorless, showing merely as an indefinite hyaline area surrounding the nucleus.

Apparent extrusion of portions of the cell body is not uncommonly observed, this phenomenon producing a peculiar "frayed-out," ragged appearance around the periphery of the lymphocyte, due to the partial detachment of small bits of the peripheral seam of basic protoplasm, which loosely adhere to the outer margin of the cell. Occasionally these small basic masses become entirely detached, and may be seen lying free in the plasma, alongside the cell of which they were once a part.

Typical forms of the large and small lymphocyte, such as are seen in the great majority of stained blood films, may be distinguished without difficulty, but in some diseases, notably in the lymphatic variety of leukemia, irregular forms of these cells are found, the size and nuclear characteristics of which are so confusingly atypical that it is sometimes futile to attempt the classification of such hybrids into two arbitrary groups, large and small. Thus one may meet with cells the size of the small lymphocyte, but having a feebly basic, eccentric nucleus and a relatively large amount of protoplasm; and with cells identical with the large lymphocyte except that they possess a small, spherical, strongly basic nucleus.

The reddish protoplasmic granules of the large lymphocyte, shown by the Romanowsky stain, serve here as valuable criteria, but these granules, unfortunately, are not always demonstrable in irregular cells. In attempting to differentiate these atypical forms in the triple stained specimen it is safe to be guided by the suggestions given by Thayer,[1] who is inclined to place more emphasis upon the character of the nucleus than upon the size of the cell body as a whole. Thus, in a doubtful mononuclear, non-granular cell in which the nucleus is similar in size and shape to that of the small lymphocyte, regardless of its affinity for the basic element of the stain, the cell is classed as a small lymphocyte, until the size of such a cell exceeds that of the polynuclear neutrophile. Some cells no larger than the smallest lymphocyte may be classed as large lymphocytes if their nuclei are decidedly ovoid in shape and pale in color. In spite of every precaution, however, it must be admitted that in some instances differential counts of these two types of cells must be more or less inaccurate, for the obvious reason that so much depends upon the personal equation.

**Transitional Forms.**

The so-called transitional forms are cells which closely resemble the large lymphocyte in shape and in size, but which differ from the latter variety of cell chiefly in having a nucleus which, instead of being ovoid in shape, is indented and drawn out into the form of a crescent with rounded poles, the concave aspect of the nuclear figure lying toward the center of the cell. In other forms the nucleus may have become molded into a figure resembling an hour-glass, which occupies the central portion of the cell body, not lying in contact with its periphery at any point. (Frontispiece, II, and Plate II, Figs. 7, 8, and 23.)

With eosin and methylene-blue the nucleus shows a moderately strong affinity for the basic dye, being colored much darker blue than the nucleus of the large, but distinctly paler than that of the small, lymphocyte; the protoplasm is stained a diffuse pale blue, in which the pink tinge of the eosin conspicuously prevails. With the triple stain the nucleus of this cell is usually stained somewhat darker blue than that of the large lymphocyte, and the protoplasm is either quite colorless or, perhaps, slightly tinged a grayish-blue.

Inasmuch as the clinical significance of the transitional forms is identical with that of the large lymphocytes, it is customary to class both forms together under a single heading in the percentage table of the different forms of leucocytes.

[1] Johns Hopkins Hosp. Reports, 1894, vol. iv, p. 103.

POLYNUCLEAR NEUTROPHILES.

Polynuclear neutrophiles are cells which, as a general rule, measure about from 10 to 12 $\mu$ in diameter, although their size may vary within wide limits, some being not much larger than the small lymphocytes, while others are nearly twice this size. (Frontispiece, III, and Plate II, Figs. 9–11 and 24–27.) The distinguishing characteristics of these cells are the twisted, polymorphous nature of the nuclei and the so-called "neutrophilic" reaction of the granules embedded in the protoplasm. The nucleus may be of almost any shape—elongated, wreathed, lobulated, horseshoe-shaped, or twisted into designs resembling various letters of the alphabet, such as S, Z, U, or E. It usually consists of several apparently separate masses of irregular shape, connected with each other by delicate filamentous strands of chromatin, which dip beneath the surface of the protoplasm, and, owing to the density of the overlying granules, are invisible or but dimly defined in the triple stained specimen. By the use of the simpler double stains, such as eosin and methylene-blue, the presence of these connecting chromatin threads may be demonstrated with great clearness. Less commonly, a cell contains several small oval or round nuclei, which are actually separated from each other, complete division at the points of constriction having resulted in the production of two or three, and in rarer instances even six or seven, distinct nuclei. The nuclear structure is rich in chromatin, which forms a dense, unevenly staining network possessing a marked affinity for the various basic dyes. It stains dark blue or greenish-blue with the triple stain, and still more intensely blue with eosin and methylene-blue solutions.

The fact that the single, twisted type of nucleus predominates in these cells has led to the current use of the adjective "polymorphonuclear" as a substitute for "polynuclear," but it is perfectly obvious that both terms may be used synonymously, the latter perhaps being preferable, because of its brevity and of its established vogue. The irregularity of the nucleus is regarded as a sign of the ameboid activity of the cell, as first suggested by Arnold,[1] and not as an indication of degeneration, as formerly believed. It has been effectually demonstrated by Sherrington[2] that if such cells are allowed to quiet down before they are killed, their nuclei usually return to a spheroidal form.

The protoplasm of the polynuclear neutrophile is densely packed with exceedingly fine, so-called neutrophile granules, which stain

[1] Arch. f. mik. Anat., 1887, vol. xxx, p. 226.
[2] Proc. Internat. Congress of Physiologists, Liége, 1892.

lavender or purple, or, rarely, pink, with Ehrlich's triacid mixture, but which are not stained by simple solutions of eosin and methylene-blue. With Wright's stain these granules are colored reddish-lilac or pink. Kanthack and Hardy[1] have shown that these granules have "a minimal attraction for acid dyes, or, briefly, a minimal oxyphile reaction," and, furthermore, that Ehrlich's neutral mixture, by which they are intensely stained, is not, chemically speaking, a neutral stain, but, on the contrary, a powerful and exceedingly differential acid dye, intensely staining oxyphile granules of all varieties.[2] Thus, having proved that the granules of the polynuclear "neutrophile" cell of Ehrlich display a distinct, although feeble, affinity for acid dyes, and that they are unstained by basic and neutral dyes, the term "finely granular oxyphile cell" has been adopted by these authors for this variety of leucocyte, the granules being known as "finely granular oxyphile" granules. It is doubtful, however, if the use of these unwieldy terms will receive general approval, except by certain of the British school. To designate a polynuclear leucocyte as a "finely granular oxyphile cell" is even more glaringly inappropriate than the use of Ehrlich's term, "neutrophile," for other varieties of leucocytes—*i. e.*, myelocytes and "neutrophilic pseudolymphocytes"—may be just as fittingly described by the former phrase.

The granules are of very small size and of irregular wedge- or spike-shape, never being spherical or ovoid in contour. They are usually most densely distributed about the periphery of the cell, whence they gradually shade off toward the nucleus, which is frequently found to be encircled by a perfectly hyaline, non-granular zone. The granules are not always confined to the cell protoplasm, being scattered over the nucleus, portions of which may be partly obscured by the overlying granular film.

The jelly-like substance of the protoplasm in which the granules are embedded appears to show a slight affinity for acid dyes, the intensity of this affinity varying greatly in different cells. With the triple stain this reaction is evidenced by the variable depth of fuchsin-colored undertone which may be detected beneath the

[1] Jour. Physiol., 1894, vol. xvii, p. 61.

[2] Reasoning upon the basis that eosin stains with most striking intensity in an aqueous solution, less decidedly in a glycerin solution, and even less strongly when dissolved in strong alcohol, these investigators distinguish three classes of oxyphile granules, according to the intensity of their affinity for acid dyes, thus: (1) Those which stain with eosin only in aqueous solutions or in alcoholic solutions of a percentage below 60; (2) those which stain in both aqueous and glycerin solutions, but not in a strong alcoholic solution (90 to 95 per cent.) of the dye; and (3) those which stain with aqueous, glycerin, and strong alcoholic solutions. They include in the first class the neutrophile and the amphophile granules of Ehrlich.

purplish color of the granules; while in the specimen stained with eosin and methylene-blue the protoplasm is tinted evenly the color of eosin.

**Eosinophiles.** These cells are the most conspicuous of all the leucocytes, and may be at once identified by the presence of a more or less polymorphous nucleus embedded in a protoplasm studded with coarse, highly refractive granules which have a strong affinity for acid dyes, such as eosin and acid fuchsin. (Frontispiece, V, and Plate II, Figs. 12, 13, 28, and 29.) Owing to the large size of their granules and to their striking oxyphilic reaction, these cells are also known by the term "coarsely granular oxyphile cells," in contradistinction to the "finely granular oxyphile cells" or polynuclear neutrophiles (Kanthack and Hardy). The size of the eosinophile varies very greatly, but most of them approximate the size of the polynuclear neutrophile, or are, perhaps, a trifle smaller. Their diameter commonly ranges from 8 to 11 $\mu$, although occasionally forms not larger than the normal erythrocyte are to be observed. Their shape is usually that of an irregular sphere or oval.

The nucleus may be kidney- or horseshoe-shaped, or twisted and drawn out into an irregular mass, but it is rarely as constricted and deformed as that of the polynuclear neutrophile. It is nearly always situated eccentrically, cells of this variety with centrally placed nuclei being very uncommonly seen. Occasionally the eosinophile contains multiple nuclei, consisting of several oval or round masses between which no connecting chromatin threads can be distinguished, but usually such portions of the nucleus are joined together by extensions of chromatin running beneath the protoplasm. The nucleus stains faintly, in comparison with that of the polynuclear neutrophile, although more intensely than that of the large mononuclear cell; it is colored pale blue or greenish-blue by the triple stain, and dark blue by eosin and methylene-blue mixtures.

The granules, which are relatively large in size and quite regularly spherical in shape (in contrast to the delicate, irregularly shaped granules of the polynuclear neutrophile), react strongly toward the acid elements of the triple stain; some are stained a brilliant fuchsin color, some deep red, while others are brownish-yellow or copper color, or even almost black; with mixtures of eosin and methylene-blue they take the brilliant color of eosin. There appears to be a marked tendency on the part of the granules to overrun the nucleus, so that its morphology in some cells is almost indistinguishable. The granules are also prone to become readily detached from the protoplasm, which doubtless ac-

counts for their uneven, blotchy distribution in many cells, in which densely packed granular areas alternate with open spaces merely punctuated here and there with an occasional granule.

Eosinophiles appear to offer but feeble powers of resistance against external influences, so that it is common to find these cells so injured by the process of making the film that the nucleus has escaped from the cell body, and the granules, lying free in the plasma, are scattered about it in a cloud. This instability or "explosive" character of the eosinophile is one of its most striking attributes, for, while observed now and then in a polynuclear neutrophile, it occurs with much greater frequency in eosinophiles than in the latter type of leucocyte.

The protoplasm of the cell may or may not show an affinity for the anilin dyes; usually it does not, so that the granules appear to be embedded in a perfectly hyaline substance; occasionally the protoplasm is faintly stained by fuchsin or by eosin. With Wright's stain it frequently takes the color of the basic dye, methylene-blue.

BASOPHILE CELLS. Finely granular basophile cells, containing Ehrlich's $\delta$-granules, are occasionally encountered in normal blood, but with such rarity that their real significance is not understood. (Plate II, Fig. 31.)

In general morphology and size these cells resemble the polynuclear neutrophiles. The nucleus is invariably twisted, and usually consists of two or three distinct lobes joined by thin chromatin bands; in the stained specimen it is never of round or oval shape, but always shows evidences of polymorphism. The nuclear structure is composed of a delicate, scanty network of chromatin, and has a moderate affinity for basic dyes, staining dull blue with the triple stain and pale sea-green with eosin and methylene-blue mixtures.

The protoplasm of the cell is closely packed with fine, irregularly shaped granules having an intensely basic reaction; they stain deep blue with solutions containing methylene-blue, but are not colored by the triple stain, showing in films stained with this mixture as groups of dull white spots scattered through the cell body. Wright's stain is most useful in bringing out the characteristics of these granules.

MYELOCYTES. Myelocytes, or marrow cells, are relatively large round or oval cells, ranging from 10 to 20$\mu$ or even more in diameter, their average size being somewhat larger than that of the large lymphocyte, which they resemble in general morphology. (Frontispiece, V and VI, and Plate II, Figs. 14–16 and 30.) The nucleus of the typ-

ical myelocyte is of spherical or ovoid shape, and is situated eccentrically, lying distinctly toward one side of the cell, so that the peripheries of both cell and nucleus are often closely adjacent for some little distance—usually for from one-third to one-half of their course. The nucleus reacts feebly toward the basic element of the triple stain, being colored a pale, delicate sky-blue with this solution; it stains a moderately deep blue or purple with eosin and methylene-blue mixtures, and appears to be more coarsely netted and deeply stained than in films prepared by the preceding method.

In the smaller forms of myelocytes the nucleus is frequently found to occupy the center of the cell body, so that it is surrounded on all sides by a protoplasmic zone of even width. In some of the larger forms the nucleus may be indented and molded along one margin of the cell body like that of the so-called "transitional" leucocyte. In rare instances actual division of the nucleus appears to have occurred, so that two separate nuclei, each shaped like a flattened hemisphere and situated at an extreme pole of the cell, may be found. Such cells are often mistaken at first glance for polynuclear neutrophiles, inasmuch as both forms of cells contain multiple nuclei and neutrophile granules; but the nucleus of the polynuclear neutrophile is always more or less twisted and of undulating surface, relatively rich in chromatin and stained with decided intensity, and rarely situated at the poles of the cell, while the nuclear halves of this type of the myelocyte are of regular outline and uniformly close to the surface of the cell, relatively poor in chromatin and faintly stained, and invariably occupy the extreme poles of the cell body.

The protoplasm of the myelocyte is filled with fine neutrophile granules, such as occur in the polynuclear neutrophile; they are most densely distributed at the periphery, and grow appreciably less abundant as they approach the nucleus, which they may overrun, spreading over its surface like a thin veil, so that its structure is more or less hidden.

This one characteristic—the presence of neutrophile granules in the protoplasm—at once serves to distinguish the myelocyte from the large lymphocyte, which it may exactly resemble in size, shape, and nuclear structure; the importance of using a selective neutrophile stain to differentiate these granules in specimens used for differential counting is therefore patent.

With Ehrlich's triple stain the granules stain purple or lavender, exactly like those of the polynuclear neutrophile. With Wright's solution the protoplasm has an undertone of light purple, broken here and there by indistinct, darker granular areas of the same

color, indicating the presence of basophile granules, in addition to those of neutrophile reaction, which show as a delicate lilac or pink stippling.

In certain pathological conditions, notably in myelogenous leukemia, an occasional myelocyte may be observed which contains both fine neutrophile and very coarse basophile granules, the latter being precisely identical in size, shape, and tinctorial qualities with Ehrlich's $\gamma$ or mast cell granules. They are situated both in the protoplasm of the cell and over the nucleus, and are, in the author's experience, seen most clearly in specimens stained in solutions containing polychrome methylene-blue. The basic granules show in such preparations as a coarse, brilliant, purple stippling, contrasting vividly with the paler, eosin-colored neutrophile granules which fill the body of the cell, and with the greenish-blue color of the nucleus.

*Eosinophilic myelocytes*, or myelocytes with a protoplasm filled with coarse eosinophile instead of neutrophile granules, are common to several pathological conditions, but occur with especial frequency in the myelogenous variety of leukemia and also in pernicious anemia, to some extent. Such cells are identical in size and morphology of cell body and nucleus with the commoner neutrophilic myelocytes, from which they differ only in containing eosinophile granules. (Frontispiece, V, and Plate II, Figs. 14 and 30.)

The normal habitat of the myelocyte is in the red bone marrow, and its presence in the circulating blood must always be regarded as pathological. At one time regarded as practically pathognomonic of leukemia, the myelocyte is now known to occur in many other conditions, especially those characterized by profound cachexia, by marked anemia, and by increase in the number of leucocytes. The occurrence of myelocytes in the blood in various diseases and the clinical significance of these cells are discussed in another place. (See "Myelemia," p. 259.)

**Mast Cells.** Cells containing Ehrlich's $\gamma$-granules, known by the term *mast cells*, or *mastzellen*, are occasionally present in the peripheral circulation, as the result of certain pathological influences, but are totally foreign to the normal blood of man. (Frontispiece, VII, and Plate II, Figs. 32–36.) They are very constantly found, generally in considerable numbers, in the myelogenous type of leukemia, and also occur, in small percentages, in many cases of pernicious anemia and in other grave blood disorders.

The cells are of spherical or ovoid shape, and are characterized by a relatively large, structureless nucleus inclosed in an almost

indefinable protoplasm, and by the presence of coarse basophile granules scattered irregularly over the surface of the cell—marks of identification which remain unchanged whatever the size of the cell may be. No variety of cell found in the blood exhibits wider ranges in size. The forms most commonly observed measure approximately from 9 to 12 $\mu$ in diameter; some have a diameter of fully 20 or even 22 $\mu$, but cells of this extremely large size are the exception rather than the rule; others are scarcely larger than the small lymphocyte, being but 7 or 8 $\mu$ in diameter, and these very small forms are also uncommon.

The nucleus is round, oval, or somewhat lobulated, and occupies the greater part of the cell body, in which it is usually situated eccentrically. Owing to the similarity in the appearance of the nucleus and the protoplasm it is frequently impossible to determine the precise point at which the former structure begins and the latter ends, so that, in the stained specimen, many cells are met with which appear to consist simply of irregular groups of granules clinging to a pale nucleus, every definite trace of the cell body being lost. (Plate II, Figs. 35 and 36.) In films stained with Wright's solution (which is, by far, the most satisfactory stain for illustrating the finer morphology of these cells) the nucleus is colored a beautiful, iridescent greenish-blue, the tint of which is so extremely delicate that in many cells it is barely perceptible. The staining, though faint, is even and clear, indicating a structure almost totally devoid of chromatin.

The granules are generally large and coarse, and vary greatly in size and in shape. Some are smaller than the granules of the eosinophile cell, while others approach or even slightly exceed 0.5 $\mu$ in diameter. They may be spherical, egg-shaped, or roughly cuboid, the latter form of granule being exceedingly common. A single type of granules is not always found to the exclusion of the others, for one cell often contains granules of every possible variety of shape and size; this peculiarity is especially striking in some of the smaller forms of cells in which extremely coarse egg-shaped and smaller spherical granules may be distinguished clinging to the periphery of the nucleus, about which no evidence of protoplasm is demonstrable. (Plate II, Fig. 36, and Frontispiece, VII.) In other forms, both large and small, the large spherical or ovoid granules may prevail almost exclusively. (Plate II, Figs. 33, 34, and 35, and Frontispiece, VII.) The distribution of the granules through the cell follows no constant rule, but it is evident that a more or less decided tendency exists toward their collection near the periphery. They are always most densely distributed at this point, sometimes extending in-

ward over the nucleus, which is thus partly hidden, and sometimes crowded into a limited zone, which coincides with the outer boundary of the cell for the greater part of its extent.

The granules of the mast cell show an intense affinity for basic anilin dyes, toward which they react metachromatically in a highly characteristic manner. With Wright's solution they are stained a deep royal purple color in which the red tone is distinctly evident, thus differing from the granules of other basophile cells, which are stained a pure blue with this mixture. Dr. H. F. Harris has called the writer's attention to another distinctive method of identifying these granules, by first staining with carbol-toluidin-blue or with thionin, and then by differentiating with Unna's glycerin-ether mixture. In specimens thus treated the mast cell granules are of a dark red color, while other basophile granules stain blue, so that the former must be regarded as having a *modified* basic reaction. They are stained reddish-violet with Ehrlich's acid dahlia solution, and deep blue with aqueous solution of methylene-blue. They are not stained by the triacid mixture, and appear as coarse, dull white spots through the cell body in films stained with this solution. The distinctive manner in which they react toward selective stains for mucin has been discovered by Harris,[1] who, in view of this fact, suggests that the term *mucinoblast* be applied to the mast cell.

The author questions the identity of these coarsely granular basophilic blood cells with the well-known mast cell of the tissues, although most hematologists consider them identical. Both, it is true, contain granules which tinctorially and morphologically are identical, but it is obviously impossible to determine cell identity by criteria such as these. The mast cell of the tissues differs from that of the blood in having a nucleus which is smaller in relation to the size of the cell body, more centrally situated, and richer in chromatin, hence being more deeply and more unevenly stained. The "explosive" nature of the tissue mast cell is also usually more striking, for while cells with this tendency are met with not infrequently in the blood, they seem to be the rule rather than the exception in the tissues, large numbers of them consisting of a nuclear structure surrounded by dense clusters of granules, which are frequently drawn out in long tentacular extensions. In view of these differences it may be well to be more specific, by designating the mast cell found in the blood as the *hemic* mast cell.

[1] Phila. Med. Jour., 1900, vol. v, p. 757.

MONONUCLEAR NEUTROPHILES.

This term has been applied by Capps[1] to a form of leucocyte which he found in certain cases of general paralysis of the insane, its appearance in the blood having been noted after apoplectiform attacks and preceding death. This cell is as large as, or larger than, the polynuclear neutrophile, contains a round or ovoid nucleus which is deeply stained by basic dyes, and has a protoplasm thickly sprinkled with fine neutrophile granules. Capps suggests that the cell may be a form of leucocyte of slightly more mature development than the large lymphocyte, one in which the development of the granules has preceded the nuclear changes. The close resemblance of these cells to the smaller forms of myelocytes, however, makes it reasonable to class them as such.

NEUTROPHILIC PSEUDOLYMPHOCYTES.

Ehrlich has described[2] as a "small neutrophilic pseudolymphocyte" a cell of the same size as that of the small lymphocyte, and characterized by a relatively large, round, intensely basic nucleus, surrounded by a narrow zone of protoplasm filled with neutrophile granules. This cell, it is maintained, is of very rare occurrence, having been found in the blood only in a case of hemorrhagic small-pox and in the exudate of a recent pleural effusion. Ehrlich differentiates it from a myelocyte by its small size, deeply staining nucleus, and scanty amount of protoplasm, but these points of distinction do not appear conclusive, for many of the smaller, "dwarf" forms of myelocytes have similar characteristics. It does not appear unreasonable, therefore, to regard this cell as an exceedingly small form of myelocyte, in which the nucleus is relatively larger and richer in chromatin than is the rule in the larger, more typical varieties.

REIZUNGSFORMEN.

These cells, first described by Türk[3] as "Reizungsformen" (or, literally, "stimulation forms"), are said to occur in the same pathological conditions in which myelocytes are found, but as yet their exact significance is undetermined. They may be found in any condition provoking decided anemia or leucocytosis and thus causing active stimulation of the bone marrow. The writer has seen such cells in the blood of the post-typhoid anemias of infancy, always in association with lymphocytosis. The size of the cell is usually midway between that of the small and large lymphocyte, more often approximating the size of the former. The cell contains a round nucleus, deficient in chromatin, often eccentrically

[1] Amer. Jour. Med. Sci., 1896, vol. cxi, p. 650.
[2] *Loc. cit.* [3] "Klinische Hämatologie," Vienna and Leipzig, 1904, p. 368.

placed in the cell body, and reacting with moderate intensity toward the basic dyes. The protoplasm is non-granular, and stains purple with Wright's stain and intense brown with the triacid mixture. Ehrlich suggests that this cell may possibly represent an early stage of the erythroblast, but reasons for such an inference do not seem clear.

The chief points of distinction between the different forms of leucocytes, as recognized in specimens stained with Wright's Romanowsky mixture, are tabulated below:

| Form of Cell. | Size. | Nucleus. | Protoplasm. |
|---|---|---|---|
| Small lymphocyte. | 6 to 9 $\mu$. | Single.<br>Round.<br>Relatively large.<br>Dark blue or purple. | Relatively small amount.<br>Occasionally granular.<br>Pale sky-blue. |
| Large mononuclear leucocyte or large lymphocyte. | 10 to 15 $\mu$. | Single.<br>Round or ovoid.<br>Relatively small.<br>Very pale blue. | Relatively large amount.<br>Often granular.<br>Pale blue. |
| Transitional leucocyte. | 10 to 15 $\mu$. | Single.<br>Indented, kidney-shaped, or crescentic.<br>Relatively small.<br>Pale blue. | Relatively large amount.<br>Often granular.<br>Pale blue. |
| Polynuclear neutrophile. | 7.5 to 12 $\mu$. | Polymorphous or polynuclear.<br>Relatively small.<br>Moderately dark blue. | Relatively large amount.<br>Contains fine lilac or pink neutrophile granules.<br>Relatively large amount. |
| Eosinophile. | 7.5 to 12 $\mu$. | Polymorphous or polynuclear.<br>Relatively small.<br>Pale blue. | Contains coarse rose-colored eosinophile granules. |
| Basophile. | 7.5 to 12 $\mu$. | Polymorphous.<br>Relatively small.<br>Dull blue. | Relatively large amount.<br>Contains fine blue basophile granules. |
| Myelocyte. | 10 to 20 $\mu$. | Single.<br>Round or ovoid.<br>Relatively large or small.<br>Very pale blue. | Relatively large or small amount.<br>Contains fine lilac or pink neutrophile granules. |
| Mast cell. | 7 to 22 $\mu$. | Single.<br>Round, ovoid, or slightly lobulated.<br>Relatively large.<br>Very pale blue. | Relatively small amount.<br>Contains coarse royal purple basophile granules. |
| Reizungsform. | 6 to 15 $\mu$. | Single.<br>Round.<br>Relatively small.<br>Deep blue. | Relatively large amount.<br>Non-granular.<br>Intense lilac or purple. |

ORIGIN AND DEVELOPMENT.

Two different views are current at the present time regarding the origin and development of the leucocytes, the first being that of Ehrlich[1] and his followers, and the second that maintained by the Russian school, led by Uskow[2] and his pupils.

According to Ehrlich's teachings, the small lymphocyte and its mother-cell, the large lymphocyte, are developed in the lymphatic tissues in the various parts of the body, wherever such structures exist. The large mononuclear leucocytes and transitional forms are considered probably of myelogenous origin. The polynuclear neutrophiles are thought to develop exclusively in the bone marrow, the great majority being evolved from the neutrophilic myelocytes of this tissue, while a very limited number perhaps arise from the non-granular large mononuclear cells. The eosinophiles develop from the eosinophilic myelocytes in the bone marrow, while the basophilic leucocytes similarly have their origin in basophilic marrow antecedents. Thus, it is maintained that all varieties of leucocytes may be classed in two distinct groups which have separate origins, functions, and relations. The first group consists of the lymphocytes, large and small, which are produced solely by the lymphatic tissues; and the second group includes the mononuclear leucocytes and transitional forms, the polynuclear neutrophiles, the eosinophiles, and the basophiles, all of which cells are produced exclusively by the marrow.[3] Cellular reproduction, except in rare instances, does not take place in the circulating blood stream. Labbé[4] considers that no such distinction between lymphoid and myeloid cells is possible in early life, at which period he believes that all blood-forming tissues are capable of producing both hyaline and granular cells. But as adult life approaches the origin of the lymphocytes may be traced to the lymphatic tissue, and the birthplace of the granular leucocytes to the bone marrow.

The scheme devised by the Russian school contends for the continuous evolution of the leucocyte from its earliest to its most mature stages. Accordingly, all varieties of the leucocyte, except the basophilic cells, of which no account apparently is taken, are but different developmental stages of one and the same cell. The youngest form of leucocyte, the small lymphocyte, originates

[1] *Loc. cit.*

[2] "The Blood as a Tissue," 1890 (Russian); also series of articles by Uskow's pupils in Arch. d. Soc. biol., St. Petersburg, 1893–97.

[3] Muir's article on the relations of the bone marrow to leucocyte formation (Jour. Path. and Bacteriol., 1901, vol. vii, p. 161) admirably discusses the natal differences of the lymphoid and myeloid cells of the blood.

[4] "Le Sang," Paris, 1902.

in the lymph glands, the lymphocytic bone marrow, and the spleen, from which sources of origin it reaches the circulation. The small lymphocyte enlarges until it becomes identical in appearance with the cell recognized as the large lymphocyte, its nucleus at this period of its growth having become somewhat less intensely basic, although the basic affinity shown by the cell protoplasm is unaltered. The large lymphocyte in turn undergoes a simple increase in size, its nucleus meanwhile becoming progressively paler and its protoplasm more feebly basic, until it develops into the large mononuclear form. The nucleus of the latter now becomes indented and molded into a roughly crescentic figure, its nuclear and protoplasmic characteristics remaining unchanged, and the so-called transitional form thus originates—a type of cell which is regarded as the immediate antecedent of the polynuclear neutrophile. During the next stage of development the size of the transitional cell decreases and the whole cell becomes ameboid; the nucleus becomes denser, more basic, and polymorphous or polynuclear; while the protoplasm loses the last trace of its basic tendency and becomes sprinkled with fine neutrophile granules, until finally the mature form of leucocyte, the polynuclear neutrophile, is fully developed. The final, or "over-ripe," stage of the leucocyte is represented by the eosinophile, which is thought to be derived from the polynuclear form by a transformation of the latter's neutrophile into eosinophile granules. It is maintained that these transitions from one form of cell to the other occur partly in the circulating blood and partly in the blood-forming tissues—most largely in the latter.

It is beyond the province of this book to discuss the merits and demerits of these two opposing views, but it may be remarked that Uskow's theory, which up to the advent of Ehrlich's observations commanded general attention among hematologists, has been generally supplanted by the latter. The investigations of Ehrlich in this direction constitute the only dependable means by which many of the pathological changes in the leucocytes may be explained, and his views may be accepted, on the whole, as accurate.

Disintegration of the leucocytes occurs chiefly in the spleen and to a less extent in the liver. Bain's perfusion experiments,[1] of repeatedly passing blood through these organs, indicate that the hemolytic action of the spleen is directed mainly toward the leucocytes and especially toward the polynuclear neutrophile cells. From Lewis' studies[2] it is to be inferred that the hemo-

[1] Jour. Physiol., 1903, vol. xxix, p. 352. [2] *Ibid.*, 1902, vol. xxviii, p. 8.

lymph glands have a similar function, by virtue of their phagocytic endothelium.

IODIN REACTION.

In a number of diseases associated with anemia and with bacterial or chemical toxemia the protoplasm of the polynuclear neutrophile leucocytes, and rarely of the myelocytes and basophiles, shows a more or less marked affinity for iodin, as shown by staining with a weak solution of this metal. Extracellular iodin-stained masses also are found in both normal and pathological blood, but they are without significance unless in association with leucocytes showing a similar reaction. Some of these extracellular iodophile masses are doubtless bits of protoplasm torn from the leucocytes; others may be blood plaques, while some are but artefacts.

Goldberger and Weiss[1] recommend the following reagent for demonstrating the iodin reaction:

| | |
|---|---|
| Iodin | 1 |
| Potassium iodid | 3 |
| Distilled water | 100 |

Mix and add sufficient gum arabic (about 50 parts) to make a syrupy mixture.

With a camel's-hair brush a layer of this solution is painted over the surface of the dried, unfixed blood film, upon which it is allowed to act for from one to five minutes. The excess is then removed by blotting with a bit of filter-paper, and the specimen is mounted in cedar oil. Or, as Wolff[2] advises, Zollikofer's method may be used: placing the fresh film for a few minutes in a stoppered bottle containing crystals of pure iodin.

In films thus treated the iodin reaction is recognized by a slight or intense, diffuse brown coloring of the entire protoplasm, or by the presence throughout the protoplasm of numerous intensely stained, reddish-brown granules, the latter change being the more common. In normal blood the protoplasm of the leucocytes is stained a pale yellow and the nuclei remain almost colorless.

The above reaction, known as *iodophilia*, is constant in all *purulent conditions*, persisting as long as the suppurative focus exists, its intensity appearing to bear no parallelism to the extent of the pus collection. It signifies simply a general toxemia severe enough to cause a form of leucocyte degeneration, and, like its corollary, leucocytosis, is merely a symptomatic sign. Kaminer[3] believes that the reaction depends upon three factors for its pro-

[1] Wien. klin. Wochenschr., 1897, vol. x, p. 601.

[2] Zeitschr. f. klin. Med., 1904, vol. li, p. 407.

[3] Deutsch. med. Wochenschr., 1899, vol. xxv, p. 235; also Berlin. klin. Wochenschr., 1899, vol. xxxvi, p. 119.

duction,—pyrexia, leucocytosis, and toxemia,—and that it is caused by the action of some unknown chemotactic substance. He groups the iodophile cells, according to their grade of reaction, into three stages: diffuse brownish staining, circumscribed granulation, and complete metamorphosis.

The reaction is absent in *pure tuberculous abscesses*. It is present with great constancy in *puerperal sepsis* and in other forms of *septicemia*, frequently in *pneumonia*, *pulmonary tuberculosis*, and *malignant disease*, and occasionally in *marked cachexias*. Hofbauer[1] found iodophile granules in the leucocytes in all cases of *pernicious anemia*, their number being greatest in the gravest cases; they were also present in severe forms of *secondary anemia* and in *leukemia*, but were absent in *chlorosis* and in *pseudoleukemia*. This author also observed numerous iodin-stained extracellular masses in a case of *purpura hæmorrhagica*. Dunn,[2] studying the iodin reaction in children, found it invariably present in *croupous and catarrhal pneumonia*, *influenza*, *cerebrospinal meningitis*, *empyema*, and miscellaneous *purulent conditions;* the reaction was generally present in *enteric fever* and in *acute miliary tuberculosis*. Cabot and Locke[3] obtained uniformly positive reactions in *septicemia*, *pneumonia*, *empyema*, and *suppurative appendicitis;* in *serous pleural effusions* and in *catarrhal appendicitis* the test was negative. In about one-half of the cases of *enteric fever* examined by these writers the test was positive, usually only in those complicated by hemorrhage, perforation, furunculosis, or lung lesions. These studies have been recently substantiated by Gulland.[4]

Experimentally, iodophilia has been caused by Spezia[5] by the subcutaneous injection of *peptone*, *glucose*, and *olive oil*, and the sign has also been noted by him during the *digestive leucocytosis* following a hearty meal. Kaminer[6] produced iodophilia in animals by injecting *cultures* and *toxins* of the pneumococcus, the Klebs-Löffler bacillus, the typhoid bacillus, and various pyogenic bacteria; he failed to cause it by injecting tetanus toxin.

The practical value of this test is considerable if it is properly interpreted merely as a symptom. Its constancy in purulent conditions, however slight in extent the focus of pus, is useful in diagnosing a deep-seated abscess, if other causes which also may give rise to the reaction can be ruled out. The sign is also a valuable aid in

[1] Centralbl. f. inn. Med., 1900, vol. xxi, p. 153.
[2] Boston Med. and Surg. Jour., 1903, vol. cxlix, p. 511.
[3] Jour. Med. Research, 1902, vol. vii, p. 43.
[4] Brit. Med. Jour., 1904, vol. i, p. 880.
[5] Lancet, 1903, vol. i, pp. 655 and 1444.
[6] Brit. Med. Jour., 1902, vol. i, p. 1049.

differentiating serous from purulent effusions and inflammations—serous pleurisy and empyema; catarrhal appendicitis and appendicular abscess. It is also useful, according to Sorochowitsch,[1] in differentiating gonorrheal and rheumatic arthritis, since positive reactions occur in the former and negative in the latter. The absence of the iodin reaction in pure tuberculous abscess and its presence in all other forms of abscess may aid in distinguishing between the two, and of ascertaining whether a mixed infection exists. The persistence of iodophilia for forty-eight hours or so after a pneumonia crisis and after the incision of a pus cavity suggests, in the first instance, delayed resolution or some other post-pneumonic complication, and, in the second, imperfect drainage. Although the writer has followed out rather at length the suggestion made by Hofbauer, that the intensity of the reaction serves as an index to the severity of an anemia, the results from this study have not shown the reliability of such a presumption.

PERINUCLEAR BASOPHILIA.

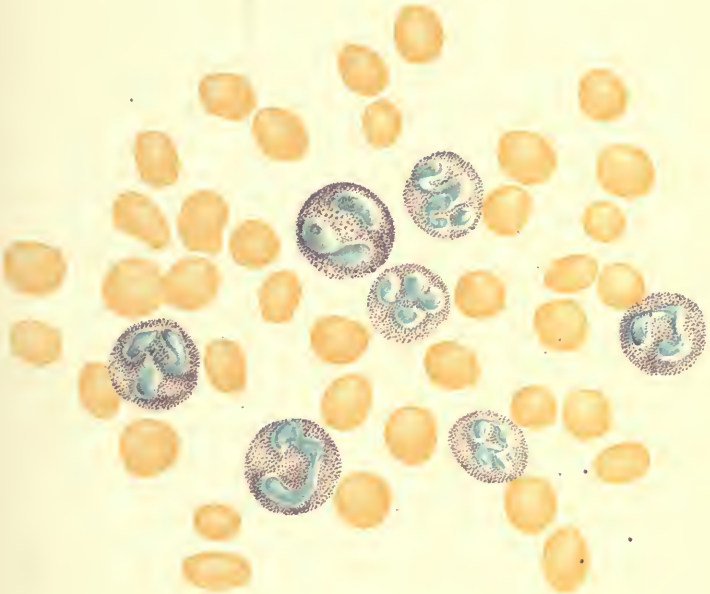
Neusser,[2] in 1894, described certain basic granules about the nuclei of the leucocytes which he regarded as pathognomonic of the *uric acid diathesis*, asserting that this so-called "perinuclear basophilia" could be demonstrated constantly in gout, lithiasis, rheumatism, leukemia, and a number of other diseases. These statements were soon corroborated by Kolisch,[3] and for a time enjoyed more or less general credence. The later researches of Futcher[4] and of Simon,[5] however, have entirely disproved the claims of Neusser and his school, for these investigators, working independently, have proved that perinuclear basophilia is not only quite uncharacteristic of the uric acid diathesis, but that it can be constantly demonstrated in every sort of blood, whether from healthy or from diseased persons. It is now clear that Neusser's granules are simply artefacts, due to some slip in the technic of staining. Ehrlich[6] believes that their presence is but rarely noted if perfectly pure crystalline dyes are used in preparing the stain.

## III. LEUCOCYTOSIS.

Leucocytosis may be described as an increase above the normal standard in the number of leucocytes in the peripheral blood, this

[1] Zeitschr. f. klin. Med., 1904, vol. li, p. 245.
[2] Wien. klin. Wochenschr., 1894, vol. vii, p. 71.
[3] *Ibid.*, 1895, vol. viii, p. 797.
[4] Johns Hopkins Hosp. Bull., 1897, vol. viii, p. 85.
[5] Amer. Jour. Med. Sci., 1899, vol. cxvii, p. 139.
[6] *Loc. cit*

PLATE III.



LEUCOCYTOSIS.

(*Triacid Stain.*)

The blood field from a case of croupous pneumonia. The leucocytes are all of the polynuclear neutrophile type. The erythrocytes show no deformity, and stain a normal orange color.

Contrast this illustration with leukemia, Plates IV and V.

(E. F. FABER, *fec.*)

change either—(*a*) *involving both an absolute and a relative increase in the polynuclear neutrophile cells with a consequent relative diminution in the proportion of mononuclear non-granular forms*, or (*b*) *affecting all varieties of leucocytes alike.*

A leucocytosis of the first kind, also termed a polynuclear neutrophile leucocytosis, is by far the more common of the two types: it may be symptomatic either of pathological or of physiological conditions, being found almost invariably in the former and frequently in the latter. A leucocytosis of the second kind, or a general increase unattended by any disturbance in the normal relative proportions of the different forms of cells, is comparatively rare: it is more frequently dependent for its production upon physiological than upon pathological factors, but it may occur under either of these circumstances.

From these facts it is obvious that simply an increase in the total number of leucocytes, without regard to the differential changes involved, does not of necessity constitute a leucocytosis. Nor is it possible to recognize the condition with certainty by any such criterion as a deviation from the ratio of red to white cells maintained in health. To state that a patient's blood contains, say, 50,000 leucocytes to the c.mm. suggests both leucocytosis and leukemia, but to add to such a statement the fact that of these 50,000 leucocytes 90 per cent. are of the polynuclear neutrophile variety, at once stamps the condition as a genuine leucocytosis. In order, therefore, to distinguish leucocytosis with absolute certainty the *character* of the leucocytes involved in the increase must be determined by a differential count of the stained specimen of blood. (Plate III.)

Leucocytosis may be of a more or less transient character, or may persist for a long period, its duration being dependent upon the nature of the underlying cause. In acute diseases it is usually a temporary condition, but in long-continued affections it is prolonged in relation to the chronicity of the lesion by which the increase is excited.

For clinical purposes all forms of leucocytosis may be classed under two main groups, *physiological* and *pathological*, these being further divided as follows:

*Physiological Leucocytosis.*

1. Leucocytosis of the new-born.
2. Digestion leucocytosis.
3. Leucocytosis of pregnancy and parturition.
4. Leucocytosis due to thermal and mechanical influences.
5. Terminal leucocytosis.

*Pathological Leucocytosis.*

1. Inflammatory and infectious leucocytosis.
2. Leucocytosis of malignant disease.
3. Post-hemorrhagic leucocytosis.
4. Toxic leucocytosis.
5. Experimental leucocytosis.

## Physiological Leucocytosis.

Character. The leucocytoses associated with a number of purely physiological conditions are generally of brief duration, and, as a rule, involve a moderate increase in the white corpuscles, the gain in many instances being trifling and never excessive. As noted in a preceding paragraph, the increase sometimes affects equally all forms of leucocytes, so that, although the total number of cells is higher than the normal standard, the relative percentages of the different varieties remain in the ratio observed in normal blood. In other instances the gain is due to an absolute and relative increase in the polynuclear neutrophiles, with a consequent decrease in the percentage of non-granular, mononuclear forms.

Causal Factors. The increase of leucocytes under such conditions is to be regarded usually as a physical phenomenon depending upon temporary concentration of the blood or upon an unequal distribution of the cells in favor of the peripheral vessels. Evidence is wholly lacking to show that it is caused by an actual overproduction of leucocytes by the blood-forming organs, thus producing a general increase through all parts of the body. It is, therefore, reasonable to believe that the high leucocyte counts may be accounted for by such factors as decrease in the total volume of blood plasma, and the transference of cells from the vessels of the deeper tissues to those of the superficial parts of the body.

1. *Leucocytosis of the New-born.*—The blood of the infant at birth contains two or three times the number of leucocytes found in the normal adult, the count usually ranging from 15,000 to 20,000 or higher during the first forty-eight hours of life. After this time the number of cells gradually decreases until, by the end of the first or second week, it has fallen to an average of from 10,000 to 15,000, which figures may be considered normal for children under one year of age. In ten babies examined by Warfield[1] these averages were found: first day, 26,090; third day,

[1] Amer. Med., 1902, vol. iv, p. 457.

13,270; and eleventh day, 15,740 leucocytes per c.mm. In all of these cases it was found that the younger the infant, the higher the count, which during the first five hours after birth commonly ranged between 28,000 and 34,000. Gundobin[1] and Carstanjen[2] have determined, by a series of differential counts, that the increase is due chiefly to an excessive gain in the polynuclear neutrophiles, the proportion of these cells during the first ten days after birth averaging from 60 to 70 per cent. of all forms of leucocytes. The extent of this increase becomes apparent when one recalls the fact that in the infant the relative proportion of these cells to the other varieties is usually not more than 40 per cent. Japha[3] considers 42 per cent. the average of the polynuclear neutrophiles in infants from several weeks to twelve months of age. By the tenth day this polynuclear increase usually subsides, and the percentage of mononuclear forms rises to the figure normal at this period of life. (See Section VI.) In prematurely-born infants a similar increase in the number of leucocytes is present, but the mononuclear forms rise to their normal percentage more rapidly than in the full-term baby; thus, in the case of an eight-months' child, examined by Whitney and Wentworth,[4] the large and small lymphocytes, which averaged together but 26 per cent. at birth, rose to 80 per cent. by the fourth day, remaining at practically this figure through subsequent counts.

The leucocytosis of the new-born is probably attributable partly to concentration of the blood by venous stasis and by the drain on the body-fluids incident to the early days of life, and partly to the influence of digestion leucocytosis, which is especially active at this period. Schiff[5] first noted the influence of the latter factor, and particularly drew attention to the marked gain in cells after the baby's initial feeding.

2. *Digestion Leucocytosis.*—Within an hour after taking food an appreciable increase in the number of leucocytes may be observed in the great majority of healthy individuals, the count reaching its maximum within from two to four hours after the meal, and then gradually declining. Rieder[6] estimates the average increase at about 33 per cent. in excess of the normal figure. Meals rich in albuminoids are followed by a more marked increase than those consisting chiefly of vegetable articles of diet. In individuals whose process of digestion is slow from any cause the appearance of the leucocytosis is also delayed. The following two

[1] Jahrb. f. Kinderheilk., 1893, vol. xxxv, p. 187.
[2] *Ibid.*, 1900, vol. lii, p. 333.
[3] *Ibid.*, 1901, vol. liii, p. 179.
[4] Cited by Rotch, "Pediatrics," Philadelphia, 1896, p. 348.
[5] Zeitschr. f. Heilk., 1890, vol. xi, p. 30.
[6] "Beiträge zur Kenntniss der Leukocytose," Leipsic, 1892.

instances, taken from von Limbeck,[1] illustrate the development of the leucocytosis in the normal adult:

| Time. | Count of Leucocytes. | Time. | Count of Leucocytes. |
|---|---|---|---|
| 11.15 A. M.[2] | 7,600 | 11.30 A. M.[2] | 5,800 |
| 12.15 P. M. | 6,000 | 12.30 P. M. | 10,600 |
| 1.15 P. M. | 8,500 | 1.30 P. M. | 10,600 |
| 3.15 P. M. | 12,000 | 2.30 P. M. | 9,600 |
| 5.15 P. M. | 14,000 | 3.30 P. M. | 6,800 |
| 7.15 P. M. | 10,000 | 6.00 P. M. | 6,600 |

The gain is due usually to a predominance of polynuclear neutrophile forms, with a consequent relative diminution in large and small lymphocytes; but in some instances the differential count remains normal, all forms of cells sharing equally in the increase.

Digestion leucocytosis is not invariably present even in those who apparently enjoy perfect health, its absence in such instances remaining entirely unexplained. It is also absent occasionally in chronic constipation, frequently in chronic gastric catarrh and anemia, and is found in only a small proportion of cases of gastric carcinoma. Other lesions of the gastro-intestinal tract and diseases characterized by high-grade anemia and by marked debility may greatly delay or even entirely prevent the increase. Rieder[3] is authority for the statement that digestion leucocytosis does not occur during pregnancy, and Bohland[4] finds that it fails to develop during the administration of tannic acid. Digestion leucocytosis ordinarily does not occur when the leucocytes are already increased by some pathological factor.

In children, especially in young breast-fed infants, the increase is very decided; in the new-born counts of from 30,000 to 35,000 may follow the first few feedings. (See Section VI.) The leucocytosis is also marked after fasting and in diabetics.

The factors of digestive leucocytosis are, theoretically, twofold: the chemotactic action of the absorbed albumins, which calls from the bone marrow an excess of polynuclear neutrophiles; and the increased lymph flow from the thoracic duct, which accounts for the lymphocyte gain. The older writers, notably Poll and Hofmeister,[5] thought the latter due to hyperactivity of the gastro-intestinal lymphatic tissue, but this view has been controverted by the careful work of Goodall, Gulland, and Paton,[6] who failed to find the slightest sign of adenoid proliferation in the walls

[1] *Loc. cit.*
[2] Meal of nitrogenous and farinaceous food.
[3] *Loc. cit.*
[4] Centralbl. f. inn. Med., 1899, vol. xx, p. 361.
[5] Cited by von Limbeck, *loc. cit.*
[6] Jour. Physiol., 1903, vol. xxx, p. 1.

of the gut, and who, furthermore, found the quantitative and qualitative count of leucocytes identical in the mesenteric veins and arteries and in the general circulation. These investigators also found that in animals digestion leucocytosis is unaffected by removal of the spleen.

3. *Leucocytosis of Pregnancy and Parturition.*—In the majority of primiparæ a moderate leucocytosis, not usually involving an increase in excess of double the normal count, is observed during the later months of pregnancy. The increase is less constant and much less marked in multiparæ, occurring in a smaller percentage of the latter, and amounting to a cellular gain of about one-sixth the original count on the average. The maximum number of cells is usually found immediately before and after delivery, at which time the number of leucocytes commonly rises to about 15,000 per c.mm. During convalescence the leucocytosis gradually declines, and disappears before the end of the first week after delivery, in uncomplicated cases. As a general rule, in both primiparæ and in multiparæ the degree of increase is more decided in young women than in those of middle age. It is also marked in the late rather than in the early stages of gestation and of labor. The leucocytosis of pregnancy is best explained by the unusually active metabolism of this physiological period and by the hyperactivity of the pelvic lymph glands.

The careful blood studies by Hibbard and White[1] in 55 pregnant women (33 primiparæ and 22 multiparæ) furnish the most reliable data concerning the leucocytosis of this condition. These authors found that leucocytosis occurred before delivery in 84 per cent. of primiparæ and in 75 per cent. of multiparæ, the average counts in 32 of the former being 15,021 (50 per cent. above normal) and in 20 of the latter 11,700 (17 per cent. above normal). In normal labor the number of leucocytes fell rapidly after delivery, gradually reached the normal standard by the fourth or fifth day, and then again slowly rose until the seventh day, when a decline to normal was again observed. In 37 cases at full term (including both primiparæ and multiparæ) examined by J. Henderson[2] the leucocyte count averaged 21,365, falling by the tenth day after labor to an average of 12,327.

After version, forceps application, and other operations the leucocytosis persists for a longer period. Post-partum hemorrhage and lacerations of the genital tract may also prolong the leucocytosis. Zangmeister and Wagner[3] found that post-partum pyrexia

[1] Jour. Exper. Med., 1898, vol. iii, p. 639.
[2] Amer. Jour. Obstet., 1902, vol. xlv, p. 745.
[3] Deutsch. med. Wochenschr., 1903, vol. xxviii, p. 549.

with fetid lochia is not a factor of any decided leucocytosis, such as that supervening in genuine sepsis. Pray[1] found in the blood of a woman delivered by Cesarean section the same leucocyte changes which accompany a normal labor.

Differential counts in 19 cases of Hubbard and White's series showed that the leucocytosis was of the polynuclear neutrophile type, a marked relative and absolute increase in these cells being constantly present; as a rule, their percentage was from 85 to 95 of all forms of leucocytes, usually being higher the higher the leucocytosis. Henderson's counts showed similar differential changes. These results are unlike those obtained by Rieder[2] and by Björkman,[3] the former having stated that the various forms of cells remain practically normal, while the latter attributes the increase to a predominance of mononuclear elements.

*Lactation* of itself has no appreciable effect upon the leucocytes, so that a leucocytosis occurring in a nursing woman should be attributed to inflammatory conditions of the breast or nipple—even a mild mastitis or a slight irritation of the nipple may be capable of causing a prompt leucocytosis.

The number of leucocytes is somewhat in excess of normal for a few days preceding and during *menstruation* in the majority of healthy women, according to the investigations of Sfameni,[4] but the increase scarcely ever reaches a degree which may be regarded as a genuine leucocytosis.

4. *Leucocytosis Due to Thermal and Mechanical Influences.*—A transient increase in the number of leucocytes of the peripheral blood, not involving a disturbance of the normal ratio between the different forms of cells, is produced by active local or general *muscular exercise;*[5] by brief exposure to *atmospheric cold;*[6] by *cold baths*, either local or general;[7] and by the application of *electricity*[6] and of *massage.*[8] The number of leucocytes is also increased by the effect of prolonged *dry* or *moist heat.*[9] *Hot tubbing* likewise causes considerable leucocytosis,[10] as does free sweating, whether natural or induced. It occurred in 24 of 29 instances studied by Hannes,[11] the increase amounting to between

[1] Amer. Gyn., 1902, vol. i, p. 337. [2] *Loc. cit.*
[3] Amer. Medico-Surg. Bull., 1894, vol. vii, pp. 17 and 79. [4] *Loc. cit.*
[5] Oliver, *loc. cit.;* Larrabee, Jour. Med. Research, 1902, vol. ii, p. 76; Schultz, Deutsch. Arch. f. klin. Med., 1893, vol. li, p. 234; Willebrand, Skandin. Arch. f. klin. Med., 1903, vol. xiv, p. 176. [8] Oliver, *loc. cit.*
[7] Winternitz, Centralbl. f. klin. Med., 1893, vol. xiv, p. 1017; Thayer, Johns Hopkins Hosp. Bull., 1893, vol. iv, p. 37.
[8] J. K. Mitchell, Amer. Jour. Med. Sci., 1894, vol. cvii, p. 502; Ekgren, Deutsch. med. Wochenschr., 1902, vol. xxviii, p. 519.
[9] Friedländer, Cong. f. inn. Med., Berlin, 1897.
[10] Knäpfelmacher, Wien. klin. Wochenschr., 1893, vol. vi, p. 810.
[11] Centralbl. f. inn. Med., 1901, vol. xxii, p. 823.

3000 and 5000 cells per c.mm. Checking the perspiration resulted in a fall of the leucocytes to normal within about half an hour.

The increase under these circumstances is generally attributed to blood concentration due to the influence of increased vasomotor tension, whereby the liquid elements of the blood are temporarily decreased, and, in addition, many of the cells lodged in the deeper tissues of the body are swept into the peripheral circulation. As a rule, all varieties of leucocytes share equally in the process, no single form being unduly increased at the expense of the others. In the case of long-distance runners, however, a very decided polynuclear neutrophile increase has been found.[1]

5. *Terminal Leucocytosis.*—Terminal or preagonal leucocytosis is the term applied to an increase in the number of leucocytes of the peripheral circulation frequently observed just before death. It occurs during the terminal stages of a number of different diseases, and is especially marked in those conditions in which death comes slowly, being ushered in by a more or less moribund state of the patient, lasting for a considerable length of time. The increase is usually moderate, and the counts do not often exceed 20,000 or 30,000 per c.mm., except in those cases in which decided circulatory embarrassment has existed for some time. Most commonly the blood picture is one of ordinary polynuclear neutrophile leucocytosis, although occasionally the large and small lymphocytes show disproportionately high percentages, and still more rarely all forms of cells may be increased equally. The presence of myelocytes in small numbers is also common, especially when the leucocyte count is high.

In pernicious anemia the increase may be so great as to simulate lymphatic leukemia, according to Cabot,[2] who found the following blood changes on the day of death in this disease: ratio of white to red corpuscles, 1 to 15; and a differential count of 91.7 per cent. of lymphocytes, 7.7 per cent. of polynuclear neutrophiles, and 0.5 per cent. of eosinophiles. Four megaloblasts to 1000 leucocytes were also found.

The following data were obtained by the writer in a case of pernicious anemia eighteen hours before death: hemoglobin, 12 per cent.; erythrocytes, 622,500 per c.mm.; leucocytes, 18,600 per c.mm. The differential count of 1000 leucocytes showed: lymphocytes, 46 per cent.; polynuclear neutrophiles, 49.7 per cent.; eosinophiles, 2.3 per cent.; myelocytes, 1.6 per cent.; and mast cells, 0.4 per cent. Megaloblasts outnumbered normoblasts 3 to 1,

[1] Cabot, Blake, and Hubbard, Annals of Surg., 1901, vol. xxxiv, p. 372.
[2] *Loc. cit.*

24 of the former being found in the count of 1000 leucocytes. The number of leucocytes in four previous counts having ranged from 1000 to 2400 per c.mm., and the proportion of lymphocytes from 42 to 48 per cent., this case illustrates the occurrence of a terminal leucocytosis without a notable change in the relative percentage of different forms peculiar to the case in question.

The principal cause of this form of leucocytosis is thought to be peripheral stasis dependent upon failure of circulatory compensation, but in many instances there is good reason to believe that terminal infections also act as the causal factors.

## Pathological Leucocytosis.

Occurrence.

Increase in the number of leucocytes, involving chiefly the polynuclear neutrophile cells in the great majority of instances, is associated with a wide variety of pathological conditions, mainly inflammatory, infectious, and toxic in character, and in such conditions the underlying cause of the phenomenon is radically different from that which determines the increase in physiological leucocytosis. Prominent examples of pathological lesions in which leucocytoses of this character are observed are pneumonia, diphtheria, scarlet fever, erysipelas, rheumatic fever, variola, and various septic processes. Enteric fever, paratyphoid fever, tuberculosis, typhus fever, Malta fever, the malarial fevers, influenza, measles, and rötheln are notable exceptions, for in these infections leucocytosis occurs only as the result of some complication.

Degree of Increase.

The extent of the leucocytosis, inasmuch as it depends both upon the nature of the exciting cause and upon the individual's reactive powers, varies within wide limits in different cases. It is safe to state, however, that in the great majority of instances the number of leucocytes is rather below than above 20,000 to the c.mm., counts in excess of this figure being noted in only about one-fourth of the cases in which the leucocytes exceed the normal limits of health. A count of 25,000 cells per c.mm. may be regarded as a decided leucocytosis, while an increase of from 40,000 to 50,000 is of extremely rare occurrence. In an analysis of 100 consecutive counts made by the writer in pathological conditions, in which the number of leucocytes reached or exceeded 10,000 per c.mm., it was determined that the counts were below 20,000 in 65 per cent. of cases, and between 20,000 and 30,000 in 28 per cent.; in 4 per cent. the increase was between 30,000 and 40,000; in 2 per cent., between 40,000 and 50,000; and in only one per cent. did it

exceed 50,000. Judging from these figures, which, it should be remembered, are applicable only to the *average* case, it appears to be the rule that in most leucocytoses the increase amounts to about double the maximum normal number.

DIFFERENTIAL CHANGES.

With rare exceptions the increase affects chiefly the polynuclear neutrophile cells, which commonly constitute at least 85 per cent. of the different forms of leucocytes. In many instances the percentage is much higher, as, for example, in a case of suppurative meningitis, reported by Stengel,[1] in which a differential count showed 99.5 per cent. of this variety of cells. The exceptional cases in which these disproportionately high percentages of polynuclear neutrophiles are sometimes wanting are encountered in the leucocytoses of malignant disease, after hemorrhage, in the moribund, and in children. The relative lymphocytosis which is occasionally observed under these circumstances is considered in connection with these conditions. Coincidentally with the increase in polynuclear forms there is a marked decrease in the relative percentages of large and small lymphocytes and of eosinophiles, the latter variety of cells sometimes entirely disappearing from the blood. In cases in which the increase is marked, small numbers of myelocytes usually may be observed, together with an occasional cell whose characteristics at once suggest a stage of development intermediate between that of the myelocyte and the typical polynuclear neutrophile.

Several times the writer has found in typical infectious leucocytoses practically normal differential counts, notwithstanding the high total estimates. Still rarer are those instances in which an inflammatory or infectious lesion excites a high polynuclear neutrophile percentage with no total increase in the leucocytes. The latter blood change has been, on insufficient grounds, interpreted in the same light as a frank general increase.

CAUSAL FACTORS.

The exact manner in which pathological leucocytosis arises is a question about which many conflicting views are held by different authorities, but the general trend of opinion at the present time attributes the increase chiefly to the influence of chemotaxis. According to the chemotactic theory of leucocytosis, the presence in the blood of certain chemical substances, produced by infective principles, is capable of exerting both an attractive and a repellent influence upon the ameboid leucocytes. If the collections of cells are attracted by such substances, the phenomenon is known as *positive chemotaxis*, but if, on the other hand, they are

[1] *Loc. cit.*

repelled, the condition is termed *negative chemotaxis*. This massing and repulsion of the leucocytes may be caused by various agents—by thermal and mechanical irritants, by bits of necrotic tissue which have gained entrance to the circulation, and especially by the presence in the blood of bacteria or of their metabolic products. *Thermotaxis*, or attraction by heat, may also attract the leucocytes to an inflamed area. Mendelson[1] has shown that a local temperature of 39° C. is most active in provoking such a massing of the cells. In the light of our present knowledge it appears that the different varieties of ameboid leucocytes respond to different kinds of chemotactic influences, as an instance of which the behavior of the neutrophiles and eosinophiles to this sort of stimulus may be cited. Certain substances, which for one of these groups of cells are either positively or negatively chemotactic, are, as a rule, indifferent to the other group, and sometimes even antagonistic, for substances which serve to attract one group either fail to influence or in fact repel the other. Clinically, this theory seems to find corroboration, for in the great majority of instances an increase in either variety of these cells is associated with a constant decrease in the other. In infections with certain animal parasites this general rule does not apply—notably in trichiniasis, in ankylostomiasis, and in filariasis, in which it is apparent that substances chemotactically active for both neutrophile and eosinophile cells are at work. Ehrlich[2] has shown that the mast cells are wholly uninfluenced by those substances which exert a strong chemotactic influence upon the neutrophiles and eosinophiles.

Functions.

The intense cellular activity excited by the entrance of bacteria into the organism indicates an attempt on the part of the leucocytes to destroy the invading principle and to counteract its noxious influences. In this endeavor it is probable that in a restricted sense Metschnikoff's hypothesis holds true, and that the immense numbers of phagocytic leucocytes which crowd the blood stream mechanically engulf and destroy many of the invading micro-organisms. But of still greater significance is the faculty which the leucocytes possess of producing, both by secretion and by cellular disintegration, certain chemical substances (*alexins*) acting either as directly bactericidal or as antitoxic agents. The researches of Buchner,[3] Löwy and Richter,[4] Goldscheider and Jacob,[5] and others

[1] Russkiy Vrach, 1903, vol. ii, p. 4; abst. in Phila. Med. Jour., 1903, vol. xi, p. 785.

[2] *Loc. cit.*

[3] Arch. f. Hyg., 1890, vol. xvii, p. 112.

[4] Deutsch. med. Wochenschr., 1895, vol. xxix, p. 240; also Virchow's Arch., 1898, vol. cli, p. 220.

[5] Zeitschr. f. klin. Med., 1894, vol. xxv, p. 373.

tend to show that such substances either actually destroy the infecting micro-organisms, or at least antidote and render innocuous their poisonous products. This joint process of phagocytosis and bactericidal action is most intensely developed at the period of maximum leucocytosis, according to the statements of Gabritschewsky.[1]

Alexin is an unstable nucleo-proteid, acting as an enzyme and corresponding to the complement of Ehrlich. It is, according to Metschnikoff, a product of the leucocytes, and does not exist free in the blood plasma. In the process of bacteriolysis the alexin's activity depends upon the intermediation of the amboceptor or immune body, according to the hypothesis of immunity elaborated by Ehrlich (p. 151). The French school holds that phagocytosis is excited by the action of the amboceptor, the chief source of which is also the leucocytes. Metschnikoff believes that the functions of the different phagocytic cells in immunity are distinctly dissimilar, the action of the polynuclear cells (or *microphages*) being simply bacteriolytic, while that of the large lymphocytes (or *macrophages*) is solely hemolytic.

It has been suggested by Wright and Douglas[2] that phagocytosis is materially aided by the body fluids, which may so influence invading bacteria as to make them easy prey for the phagocytic leucocytes. They attribute this effect to the presence in the blood serum of an unknown body, "opsonin," which is thought to develop as immunity is established. In a number of patients suffering from furunculosis Wright succeeded, by treating them with a sterile antistaphylococcus vaccine, in increasing the phagocytic power of the blood which before this treatment was distinctly below the normal.

HYPOLEUCOCYTOSIS AND HYPERLEUCOCYTOSIS.

In experimental leucocytosis, caused by the injection of such irritants as bacteria and bacterial products, organic extracts, various albumins, and even by simple trauma, it has been found that the first effect of the irritant is to cause a rapid, transitory diminution in the number of leucocytes in the peripheral blood, known as *hypoleucocytosis*, this decrease being succeeded in turn by an increase of these cells in excess of the normal standard, termed *hyperleucocytosis*. Frequently in simple traumatic leucocytoses after the disappearance of the stage of hyperleucocytosis, the duration of which is variable, Sherrington[3] was able to distinguish a secondary stage of hypoleuco-

[1] Centralbl. f. Bakt. u. Parasit., 1898, vol. xxiii, p. 365.
[2] Proc. Roy. Soc., London, 1903, vol. lxxii, p. 357.
[3] *Ibid.*, London, 1893, vol. lv, p. 161.

cytosis during which the leucocyte count again fell below the normal.

Within certain limits the extent of this preliminary decrease and of the subsequent increase varies directly in accordance with the intensity of the irritant. If the irritant is slight, the repellent influence is feeble, and the consequent cellular increase is inconspicuous—in fact, it is the opinion of many that in such instances there may be merely a local accumulation of leucocytes at the site of the injection, without any real increase in the whole mass of cells. If the effects of the irritant are severe, both the repellent and the attractive stages are promptly excited and markedly developed, and a general increase in the number of leucocytes through the whole circulatory system promptly results. If, on the contrary, the effects of the irritant prove to be too intense, the organism suffers a depression so profound that reaction is stifled, and leucocytosis does not develop. It sometimes happens that the attractive influences of the chemotactic principle predominate over its repellent action, in which case the stage of hyperleucocytosis may develop without the initial stage of hypoleucocytosis. Clinically, the preliminary decrease is practically never observed, perhaps partly for the reason last given, but also in a large measure because the repellent action of the irritant has passed off by the time the disease has developed into a clinical picture. In artificially excited leucocytoses, however, its appearance is quite constant, for under such circumstances the irritant is introduced into the organism suddenly and in a relatively massive dose, thus producing a decidedly repellent influence.

The initial stage of decrease was termed the *leucopenic phase* by Löwit,[1] who attributed the change to an actual destruction of the leucocytes, or a *leucocytolysis*. The subsequent increase he spoke of as the *leucocytic phase*, maintaining that for the production of the latter the preliminary development of the former was in some unexplained manner essential. The work of Goldscheider and Jacob[2] definitely proved the error of Löwit's leucocytolytic hypothesis, and demonstrated the fact that the leucopenia was dependent purely upon an altered distribution of the cells in favor of the vessels of the deeper circulation. Extensive investigations carried on by these authors showed that at the time a decided diminution occurred in the number of leucocytes of the peripheral blood there was a simultaneous increase of these cells in the capillaries of the lungs and other internal organs. Furthermore, it was also shown that in some instances a marked

[1] "Studien z. Physiol. u. Pathol. d. Blutes," Jena, 1892. [2] *Loc. cit.*

leucocytosis may occur without the initial decrease, this being the case after the injection of such substances as the glycerin extract of spleen. From these experiments it seems reasonable to attribute the initial stage of decrease to a repellent action of the irritant, and to infer that the stage of hyperleucocytosis is due to an active stimulation of the hemogenic organs which results certainly in an increased cellular output from, and probably in an increased cellular proliferation in, this situation. Muir's investigations[1] tend to strengthen this belief, and to throw additional light on the phenomenon of pathological leucocytosis. This author found that in experimental leucocytosis in animals, produced by the injection of pathogenic bacteria, changes occurred in the bone marrow, consisting of absorption of the marrow fat, together with a corresponding hyperplasia of the cells from which he believes the leucocytes originate, many of these cells undergoing rapid multiplication by mitosis. In inflammatory leucocytosis Muir found the following suggestive changes: first, a local increase in the polynuclear neutrophile cells; second, an increase of the same variety of cells in the circulating blood; and third, a marked increase in the marrow of their direct antecedents. Opie[2] found in experimental bacterial infections an accumulation of eosinophiles at the point of inoculation, where these cells undergo nuclear fragmentation and other degenerative changes and thereby probably repel infection, although, unlike the polynuclear neutrophiles and large mononuclear leucocytes, they rarely if ever act as phagocytes. Coincident with this massing of eosinophiles at the site of the infection these cells are exceedingly scanty in the peripheral blood, but collect in large numbers in the spleen. According to Ehrlich's latest views,[3] leucocytosis involving mainly an increase of the polynuclear neutrophiles ("polynuclear neutrophile leucocytosis") is the expression of an independent chemotactic reaction on the part of these cells, caused by the remote influence of dissolved substances upon the bone marrow, whereby this tissue throws into the blood current excessive numbers of these elements.

Schultz[4] and others, on the contrary, attribute leucocytosis entirely to changes in the distribution of the cells, maintaining that increase in the number of leucocytes in the peripheral vessels goes hand in hand with a decrease in their number in the vessels of the internal organs, and vice versâ. This view, however, has been shown to be untenable.

[1] Brit. Med. Jour., 1898, vol. ii, p. 604; also Trans. Path. Soc., London, 1902, vol. liii, p. 379.

[2] Amer. Jour. Med. Sci., 1904, vol. cxxvii, p. 988.

[3] *Loc. cit.*

[4] Tagebl. der Naturforsch. Vers., Heidelberg, 1889, p. 405.

In summing up the various experimental and clinical data bearing upon the nature of the leucocytoses associated with pathological conditions, the evidence tends to confirm the view that the process is, in all instances, save perhaps those of trivial local infections, a general one throughout the entire circulatory system, and that it is symptomatic of an excessive output and rapid development of leucocytes by the bone marrow, due to the influence of chemotactic principles. It must be remembered that this view is in part based upon hypotheses, but it nevertheless represents the belief current at the present time.

1. *Inflammatory and Infectious Leucocytosis.*—In this class are included the leucocytoses occurring during the course of a number of diseases of inflammatory and infectious character, in which the increase may be attributed either to simple inflammation or to bacterial infection, or to both. The presence of such a leucocytosis is to be regarded as symptomatic of an attempt on the part of the organism to overcome the noxious invading principle, whatever its nature may be, through the protective action of the white corpuscles. Bearing in mind this construction of the phenomenon, it is possible in many instances to derive valuable clinical information from the presence or absence of a cellular increase.

A genuine inflammatory or infectious leucocytosis is a much more potent defensive agent than a leucocytosis excited artificially by the injection of nuclein, for example. (See p. 251.) According to Labbé,[1] an induced leucocytosis, while hypothetically an antidote to disease, practically is of trivial value as a means of defense against a definitely established infection. As a preventive measure, however, an artificially provoked leucocyte increase may confer a certain degree of protection.[2] Bezançon and Labbé[3] maintain that a polynuclear neutrophile leucocytosis follows active, rapid infections, and confers simply a transitory immunity, while an increase affecting chiefly the mononuclear cells attends slow infections and generally gives permanent protection.

The view expressed by von Limbeck,[4] that the height of the leucocytosis is dependent upon the extent of the inflammatory exudate, is not tenable, for processes characterized by insignificant exudates are capable of causing as great an increase as those in which this outpouring is extensive. As a rule, leucocytoses associated with purulent exudates are much more marked than those due to serous effusions. The essential factor in determining

[1] Presse méd., 1903, vol. ii, p. 725.

[2] For an account of the practical value of induced leucocytosis in wound infections (reviewing the studies of Löwy, Richter, Jacob, Hahn, Goldscheider, Hofbauer, Salieri, and Miyake) see Mikulicz-Radecki, Lancet, 1904, vol. ii, p. 1.

[3] Presse méd., 1902, vol. ii, p. 1071; also "Traite d'Hematologie", Paris, 1904.

[4] *Loc. cit.*

the degree of the increase is not the extent of the exudate nor, in all cases, its character, but rather the systemic reaction to which it gives rise.

The degree of leucocytosis may be considered a general index to the intensity of the infection and to the strength of the individual's resisting powers in reacting against it. It follows, therefore, that intense infections occurring in individuals whose resisting powers are strong produce a decided increase; but the presence of an infection of like intensity in one whose resisting powers are greatly crippled fails to cause leucocytosis, for in such an instance the organism is so overpowered by the effects of the morbid process that it is incapable of reacting. The increase is either absent or slight when a trifling infection is associated with vigorous resisting powers, and moderate when a moderately intense infection is linked to fairly well-developed resisting powers.

The clinical inferences to be drawn from these facts are of value chiefly as corroborative of other well-known physical signs, but are obviously untrustworthy when considered apart from the latter. A marked leucocytosis indicates simply an intense infection in a person whose resisting powers are normally developed and actively exerted against the disease, but it is of no prognostic value in itself, for it conveys no idea of the final outcome of the conflict between the disease and the organism. Absence of leucocytosis or a slight increase may be either of very favorable or of very grave significance, inasmuch as these signs occur both in trivial and in overwhelming infections. If the absence is associated with clinical manifestations which point to a severe infection, the sign may be depended upon as being of grave prognosis.

The clinical significance of the leucocytoses associated with various inflammatory and infectious processes will be discussed in Section VII. A more or less decided increase in the number of leucocytes occurs with great constancy in the following groups of diseases of this nature:

I. *General Infectious Diseases.*

Actinomycosis.
Asiatic cholera.
Bubonic plague.
Cerebrospinal meningitis.
Diphtheria.
Dysentery.
Erysipelas.
Filariasis.
Glanders.
Malignant endocarditis.
Multiple neuritis.
Osteomyelitis.
Pertussis.
Pneumonia.
Pyemia.
Relapsing fever.
Rheumatic fever.
Scarlet fever.
Septicemia.
Spotted fever (Montana).
Syphilis (secondary).
Trichiniasis.
Vaccinia.
Varicella.
Variola.
Yellow fever.

II. *Simple and Infective Local Inflammations.*

Acute yellow atrophy of the liver.
Appendicitis, catarrhal.
Arthritis, serous.
Bronchitis, acute.
Burns.
Cholangitis.
Cholecystitis.
Cystitis.
Conjunctivitis, acute.
Dermatitis.
Eczema.
Endocarditis.
Endometritis.
Enteritis.
Epididymitis.
Gangrene:
  Appendicular.
  Cancrum oris
  Hepatic.
  Pancreatic.
  Pulmonary.
Gastritis, acute.
Gastro-enteritis, acute.
Herpes zoster.
Hydatid disease.
Infected wounds.
Mastitis.
Meningitis.
Nephritis, acute.
Orchitis.
Ovaritis.
Pancreatitis.
Pellagra.
Pemphigus.
Pericarditis.
Peritonitis.
Prurigo.
Purulent lesions:
  Appendicular abscess.
  Cerebral abscess.
  Hepatic abscess.
  Ischio-rectal abscess
  Ovarian abscess.
  Pancreatic abscess.
  Pelvic abscess.
  Perinephritic abscess.
  Prostatic abscess.
  Pulmonary abscess.
  Retropharyngeal abscess.
  Splenic abscess.
  Superficial abscess.
  Arthritis, suppurative.
  Carbuncle.
  Empyema.
  Felon.
  Furuncle.
  Gonorrhea.
  Otitis media, suppurative.
  Phlebitis.
  Pyelonephritis.
  Pyonephrosis.
  Pyosalpinx.
  Quinsy.
Splenitis.

Under this heading it is convenient to include *post-operative leucocytosis*, or the increase commonly occurring after a surgical operation, as a symptom of the normal process of wound repair. The reparative process, however, is not always the sole factor of the leucocytosis, since the effects of the anesthetic and of hemorrhage also must be taken into account. In non-septic uncomplicated cases the increase amounts to between 5000 and 10,000 cells per c.mm. in excess of the pre-operative count. The maximum increase, which is transitory, is generally attained within from twelve to twenty-four hours, and the normal standard is again reached, by a progressive decline in the leucocyte count, within two or three, or at the latest four, days. In uncomplicated cases the count falls to normal within twenty-four hours in Bloodgood's experience[1]; within thirty-six hours in Cabot's[2]; within eighty-four hours in Frazier's[3]; and within five days in C. Y. White's.[4] Persistence of a post-operative leucocytosis is signifi-

[1] Med. News, 1901, vol. lxxix, p. 321.
[2] Annals of Surg., 1901, vol. xxxiv, p. 361.
[3] Univ. of Pa. Med. Bull., 1901, vol. xiv, p. 363.
[4] Univ. Med. Mag., 1900, vol. xiii, p. 260.

cant of some such complication as defective drainage, infection, hemorrhage, or extensive inflammation.

King[1] could determine no relation, in non-septic cases, between the height of the leucocytosis and the ranges of the pulse and temperature. Frazier and Holloway[2] believe that the increase corresponds in general with the extent of the operation, but that it is uninfluenced by the degree of the temperature and by the anesthesia.

2. *Leucocytosis of Malignant Disease.*—A moderate leucocytosis is commonly, but by no means constantly, associated with the various forms of carcinomata and sarcomata, but the cases in which no increase is observed are even more numerous than those in which it occurs. It is more common and the increase is usually regarded as more marked in sarcoma than in carcinoma, but in neither condition are excessively high leucocytoses met with frequently. In the writer's experience the increase, when it does occur, is generally moderate in most forms of malignant disease, counts of less than 20,000 leucocytes per c.mm. being the general rule. Cases in which the number of cells exceeds this figure are comparatively rare, but are distinctly more common in sarcoma than in carcinoma; it is especially in rapidly growing neoplasms of the lung, liver, and kidney that the cells rise to 30,000, 40,000, or even 50,000 or more. In a series of 68 consecutive cases of malignant disease in the German and Jefferson hospitals less than one-half were accompanied by a leucocyte count of 10,000 or more, this figure being reached or exceeded in approximately 45 per cent. of cases of carcinoma, while in sarcoma such an increase was noted in almost 65 per cent. Five per cent. of all cases showed a high leucocytosis—that is, counts ranging between 30,000 and 50,000. (For further data concerning the leucocytosis of these conditions see "Malignant Disease," Section VII.)

The behavior of the leucocytes in malignant disease appears less contradictory when we inquire into the actual influence which these growths exert in provoking leucocytosis. It is the current belief that malignant disease *per se* has little if any influence of this sort, and that the increase, if any occurs, is attributable to local inflammatory complications and to secondary septic infections, rather than to the specific toxic effects of the neoplasm itself. In some instances it is reasonable to suppose that the profoundly cachectic state of the patient also is an important determining factor. Clinically, it is observed that tumors of rapid

[1] Amer. Jour. Med. Sci., 1902, vol. cxxiv, p. 450.
[2] Univ. of Pa. Med. Bull., 1901, vol. xiv, p. 363.

development, involving a large area of tissue and complicated by extensive metastases, cause decided, often high, leucocytoses; while localized tumors, of small size and of slow growth, give rise to trifling, if any, increase. Variations from this general rule are the result of differences in the resisting powers of different individuals, for the effects of this factor in causing leucocytosis are potent in this as in other pathological conditions.

Qualitatively, the leucocytes usually show a marked absolute and relative increase in the polynuclear neutrophiles, with a consequent diminution of the mononuclear forms. But in some instances, both of carcinoma and of sarcoma, the polynuclear forms are relatively below the normal percentage, and the lymphocytes increased, so that the blood picture is not one of leucocytosis, but rather one of relative lymphocytosis; such a change seems especially prone to occur in sarcoma of the lymphatic system, in which it may be so marked that it suggests lymphatic leukemia. There are certain cases of malignant disease in which the proportion of polynuclear neutrophiles rises, although the total number of leucocytes is not increased; the polynuclear gain is less than is usually found with high leucocyte counts, and is not to be regarded as of the same significance as a frank leucocytosis involving an increase in the total number of cells. (See p. 237.)

The eosinophiles are variously affected in different cases: sometimes they are greatly diminished, if not, indeed, entirely absent, as in most leucocytoses; sometimes they are normal; and sometimes they are largely increased in number. The increase may be pronounced in sarcoma, this being due probably to involvement of the bone marrow by the growth, either directly or by metastasis.

Small numbers of myelocytes—0.5 to 1 or 2 per cent.—are exceedingly common, being found with great constancy in cases with marked cachexia, especially in carcinoma. In malignant disease involving the bone marrow the percentage of this variety of cells is much higher.

3. *Post-hemorrhagic Leucocytosis.*—A leucocytosis of moderate grade commonly occurs as the result of hemorrhage due to traumatism or to other causes. It has been found that in animals the stage of increase is preceded by a well-defined leucopenia, which develops immediately after the loss of blood. This initial leucopenia, however, has not yet been demonstrated in man, although it probably occurs. In an extensive traumatic hemorrhage the increase sometimes may be recognized in the peripheral blood within an hour after the accident, but usually it is

not distinguishable until after the lapse of a longer period—from five to ten hours, as nearly as can be ascertained. In hemorrhage accompanying various pathological conditions, such, for example, as gastric ulcer, lung tuberculosis, or uterine disease, the appearance of the leucocytosis is less prompt than in hemorrhage from trauma. The maximum increase is usually within moderate limits—approximately two or three times the normal standard. The injection of a salt solution decidedly aggravates the leucocytosis. As an illustration of the degree of leucocytosis which is commonly encountered, Rieder[1] noted in four cases (hematemesis from gastric ulcer, fatal hemophilia, and uterine hemorrhage) an average count of 22,625, the maximum being 32,600, and the minimum, 15,100. Inasmuch as the height of the increase is thought to correspond to the strength of the organism's reaction in compensating the blood loss, it varies in different cases. In two individuals of equally strong regenerative powers a severe hemorrhage will produce a greater leucocytosis than a slight one. The duration of the increase also varies with the individual case, for it depends upon a similar factor; but in the majority of instances it does not last for more than three or four days, according to the investigations of Lyon.[2] Leucocytoses excited by traumatic hemorrhage are prone to persist longer than those due to other causes, and the long-persisting increases which are sometimes associated with other pathological lesions should be attributed to factors other than the actual loss of blood. In an instance of leucocytosis following venesection Rieder[3] found that the increase persisted for twelve days. Head[4] found that in dogs the leucocytosis following extensive hemorrhage lasted for at least seven days.

Usually the qualitative changes involve chiefly the polynuclear neutrophiles, which are greatly increased at the expense of the other forms of cells, but in an occasional instance it will be found that the mononuclear varieties are greatly in excess of their normal percentages, so that a lymphocytosis is observed. Myelocytes may also be found in considerable numbers in many cases.

4. *Toxic Leucocytosis.*—Typical examples of toxic leucocytosis are found in poisoning by *ptomains* and by *coal-gas*, in both of which conditions the predominant influence of a toxic agency in producing the increase is self-evident. For the same reason the leucocytoses occurring as the result of *ether* and *chloroform narcosis*, and in *convulsions* and *acute delirium*, are included in this classification. In certain diseases, notably in the *uric acid diath-*

[1] *Loc. cit.*
[2] Virchow's Arch., 1881, vol. lxxxiv, p. 207.
[3] *Loc. cit.*
[4] Jour. Amer. Med. Assoc., 1901, vol. xxxvii, p. 501.

*esis*, in *cholemia*, and in *uremia*, the presence in the blood of toxic principles is thought to be the underlying factor of the increase; the same probably is true of a number of other diseases, which, for obvious reasons, have been classed with the infectious and inflammatory leucocytoses.

The effect of *gas-poisoning* is well illustrated by the blood examination of a patient admitted to the German Hospital, fatally poisoned by illuminating-gas. The leucocytes were increased to 32,000 per c.mm., the gain being due to an excessive predominance of polynuclear neutrophiles, as determined by the following differential count: small lymphocytes, 3.5 per cent.; large lymphocytes, 2.5 per cent.; polynuclear neutrophiles, 92 per cent.; eosinophiles, 0.5 per cent.; and myelocytes, 1.5 per cent. To what extent this increase depended upon the actual toxic effects of the gas and to what extent it was attributable to peripheral stasis (which was marked in this patient) are conjectural.

The leucocytosis caused by *ether narcosis* has been exhaustively studied by von Lerber[1] and by Chadbourne.[2] The investigations of von Lerber included 101 cases, of which number leucocytosis was found in more than 95 per cent., the increase frequently amounting to two or three times the original count; in the majority of instances the maximum count was observed several hours after the anesthesia was produced. Chadbourne has carefully studied 21 cases, all of which showed a more or less decided leucocytosis, the minimum gain being 6 per cent., the maximum 73 per cent., and the average 37.3 per cent. He found that the leucocytosis developed most rapidly during the early part of the etherization, and that only exceptionally did it persist for more than twenty-four hours. Differential counts in five cases showed that all forms of cells were proportionately increased. This author attributes the increase to the irritating effects of the ether vapor upon the mucous membrane of the respiratory tract. J. Chalmers Da Costa and Kalteyer,[3] in 50 cases, found an average leucocytosis of about 5000 per c.mm. Results similar to the above also have been obtained by Ewing[4] and by Ames,[5] in the experimental etherization of animals.

Ether also causes moderate polycythemia, but this is due in part to inspissation of the blood by the preparatory treatment of

[1] "Ueber die Einwirkung der Aethernarkose auf Blut u. Urin," Inaug. Dissert., Berlin, 1896.

[2] Phila. Med. Jour., 1899, vol. iii, p. 390.

[3] Annals of Surg., 1901, vol. xxxiv, p. 329.

[4] N. Y. Med. Jour., 1895, vol. lxi, p. 257.

[5] Jour. Amer. Med. Assoc., 1897, vol. xxix, p. 472.

the patient. A slight diminution in hemoglobin is commonly, but not invariably, found.

The effects of *chloroform* are similar to those of ether, but are more marked and persist longer. Solimei[1] found that the leucocytosis induced by chloroform narcosis, while sometimes disappearing within a few hours, often continues for several days, the duration of the increase being related to the amount of the anesthetic used and to the rate of its elimination from the system. This observer also detected a loss of hemoglobin and erythrocytes with moderate poikilocytosis, and, after protracted narcosis, delayed coagulability, the presence of a methemoglobin spectrum, and an increase in the carbon dioxid of the blood, with a corresponding diminution of oxygen. Holman[2] warns the operator against the danger from hemolysis by chloroform in patients whose hemoglobin percentages are low.

The leucocytosis associated with *acute delirium* and with *convulsive seizures*, due to a variety of causes, has been studied in detail by Capps[3] and by Burrows.[4] Under such circumstances the increase is usually marked, and the height of the count in a general way is dependent upon the severity of the attack. The polynuclear neutrophiles are chiefly concerned in the increase, with a consequent decline in the proportion of mononuclear forms. The leucocytosis of this class of diseases is discussed more fully in Section VII.

5. *Experimental Leucocytosis.*—Leucocytoses not differing essentially from those associated with various local and general infections may be caused by the administration of many drugs, chemicals, organic principles, bacteria, bacterial proteins, and by the application of intense irritants and revulsives to the surface of the body. No doubt many of these leucocytoses should be classed either as inflammatory or as toxic, owing to the character of their exciting causes, but for the sake of convenience they may be grouped under this heading.

Leucocytoses resulting from the administration, subcutaneously and by the mouth, of various drugs and other substances have been studied chiefly by the Continental investigators, to whom we are indebted for most of our present knowledge of this subject. The manner in which such agencies act in causing the increase is not at all clear in many instances, but, as a rule, the change is thought to be dependent upon chemotactic influences, as in in-

[1] Gaz. degli Ospedali e. d. Clin., 1902, vol. xxiii, p. 108.
[2] St. Paul Med. Jour., 1902, vol. iv, p. 618.
[3] Amer. Jour. Med. Sci., 1896, vol. cxl, p. 650.
[4] *Ibid.*, 1899, vol. cxvii, p. 503.

flammatory and infectious leucocytoses, as well as upon concentration of the blood from vasomotor changes.

Löwit[1] determined that a preliminary leucopenia succeeded by a more or less decided leucocytosis followed the subcutaneous injection of the following substances: *hemialbumose, pepsin, nuclein, nucleic acid, curare, leech extract, tuberculin, filtered yeast cultures, pyocyanin, sodium urate,* and *uric acid.* This change was not observed, however, after the injection of *urea.*

Goldscheider and Jacob,[2] conducting a large number of experiments with various organic animal extracts, obtained results similar to Löwit's from the injection of the *extracts of spleen, thymus,* and *bone marrow,* but found negative results from the use of the *extracts* of *thyroid, pancreas,* and *liver.*

Winternitz[3] studied the effects resulting from the subcutaneous injection of substances causing both transient inflammatory edema and aseptic abscess formation at the site of the injection. In the former class, which includes *neutral salts* and *dilute acids* and *alkalis,* an increase in the number of leucocytes, amounting to from 40 to 75 per cent. of the original count, was noted; and it was furthermore found that even although a local necrosis was produced by the injection of an irritating salt, the increase still did not become excessive. In the second class of more active irritants which produced local abscesses—*turpentine, oil of mustard, carbolic acid, croton oil, sapotoxin, digitoxin, silver nitrate, cupric sulphate,* and *salts of mercury* and of *antimony*—the leucocytosis was much more decided and of less transient duration. As a rule, in these experiments the height of the leucocytosis ran parallel to the intensity of the local irritation provoked.

Pohl[4] noted a moderate leucocytosis following both the ingestion and the injection of *absinthe, acetic ether, extract of gentian, peppermint, piperin,* the *oils of anise* and *fennel, egg albumin,* and *sodium albuminate.* With the last two substances he determined that the increase was greater when they were given by the mouth than when administered subcutaneously; the gain usually ranged from about 5 to 50 per cent. of the normal count. This investigator also found that *quinin, caffein, calomel, sodium bicarbonate, ethyl alcohol,* and *hydrochloric acid* did not cause a leucocytosis, while *bismuth subnitrate* and *oxid of iron* produced irregular results. Many of the above experiments have been substantiated by the later work of von Limbeck.[5] Memmi[6] produced a moderate

[1] *Loc. cit.* [2] *Loc. cit.*
[3] Arch. f. exp. Pathol. u. Pharmak., 1895, vol. xxxv, p. 77.
[4] *Ibid.,* 1889, vol. xxv, p. 51.
[5] *Loc. cit.* [6] Gaz. degli Ospedali e. d. Clin., 1903, vol. xxiv, p. 995.

persistent leucocytosis by the daily intravenous injection of *mercuric bichlorid*, in ordinary doses, the increase first becoming apparent a few days after beginning the treatment and continuing as long as the drug was given. A single injection generally caused no increase unless the dose was excessive. In such an instance the maximum increase occurred within six hours, and the normal count of cells was reached within twenty-four hours after the injection. The intravenous injection of *lecithin* was found by Stassano[1] to increase the number of leucocytes, especially those of the mononuclear variety, the leucocytosis thus produced lasting four or five days.

Wilkinson[2] observed leucocytosis, preceded by leucopenia, after the injection of *potassium iodid, camphor, quinin, antipyrin, salicin, salicylic acid, nuclein*, and *pilocarpin;* by the repeated administration of the latter drug it was found that the granules of the polynuclear neutrophiles disappeared, although no effect was produced upon the granules of the eosinophile cells. Von Jaksch[3] also studied the effects of the injection of *pilocarpin*, and of the administration by the mouth of *nuclein*, and found that by either procedure a temporary and sometimes very marked leucocytosis resulted. Borini[4] found a similar effect was produced by *aleuron*, but that with *digitalis* a more prolonged leucocytosis occurred. The leucocytosis caused by the ingestion of *salicylic acid*, according to Schreiber and Zandy,[5] gradually disappears after the drug has been given for a few days.

The effects of the ingestion of the essential oils of *peppermint, turpentine*, and *cinnamon* have been studied by Meyer,[6] while Hirt[7] has investigated the influences of the *simple bitters* and drugs, such as the *tincture of myrrh*. Such drugs were found to cause a moderate but easily recognized leucocytosis. Krusman,[8] by the injection of *spermin* and of *protalbumose*, and Besredka,[9] by a similar use of *carmin* and of *arsenic trisulphate*, have obtained varying degrees of increase in the number of leucocytes. A marked increase is produced, according to Bohland,[10] by the injection of *morphin, Dover's powder, sodium salicylate, pilocarpin, antipyrin, phenacetin*, and *antifebrin*.

Shaw[11] finds that a marked polynuclear neutrophile leucocytosis

[1] Méd. mod:, 1902, vol. xiii, p. 63. [2] Brit. Med. Jour., 1896, vol. ii, p. 836.
[3] Centralbl. f. inn. Med., 1892, vol. xiii, p. 81.
[4] Centralbl. f. Bakt. u. Parasit., 1902, vol. xxxii, p. 207.
[5] Deutsch. Arch. f. klin. Med., 1899, vol. lxii, p. 242.
[6] Cited by von Limbeck, *loc. cit.*
[7] *Ibid.* [8] Thèse de St. Petersbourg, 1898.
[9] Annal. de l'Institut Pasteur, 1899, vol. xiii, p. 49.
[10] Centralbl. f. inn. Med., 1899, vol. xx, p. 361.
[11] Jour. Path. and Bacteriol., 1902, vol. viii, p. 70.

develops after the administration of *sodium cinnamate*. The favorable action of this drug in tuberculosis is attributed partly to its ability to excite and to maintain leucocytosis.

In addition to the substances already mentioned, the leucocytosis-producing effect of various *purgative drugs*,[1] of the *transfusion* of *blood* and of *normal salt solution*,[2] of the subcutaneous use of *fibrin ferment*,[3] of *hemoglobin*,[4] and of *bacterial cultures*,[5] *bacterial extracts*,[6] and *protein*[7] has also been demonstrated.

*Thymectomy* in animals is followed by a well-marked leucocytosis, associated with an increase in the bactericidal properties of the blood. In a number of experiments upon dogs Cazin[8] determined that after *contusions of the abdomen* the number of leucocytes was tripled or even quadrupled within three or four hours after the injury; the height of the count and the rapidity of the onset of the leucocytosis corresponded in these experiments to the severity of the visceral injuries inflicted. The same author found but a trifling leucocytosis after *bullet wounds* of the abdomen with intestinal perforation.

## IV. LYMPHOCYTOSIS.

An increase, whether relative or absolute, in the lymphocytes above the number normal in health is known as *lymphocytosis*. The word *mononucleosis* also is used to denote this change. Relative lymphocytosis involves simply a gain in the percentage of lymphocytes without a coincident increase in the total leucocyte count. Absolute lymphocytosis, on the other hand, is characterized by an increase above normal both in the percentage of lymphocytes and in the total number of leucocytes. Barring lymphatic leukemia, in which the lymphocytes are both relatively and absolutely in excess, lymphocytosis is almost always a relative condition, or at least it is not accompanied by a decided rise in the total leucocyte count.

The increase in the proportion of lymphocytes is moderate in most instances, the greater number of differential counts showing percentages of these cells ranging from 50 to 70, in comparison with the maximum normal percentage, about 30. These figures, of course, refer to the blood of adults, for in children the increase

[1] De Rienzi and Boeni, Gaz. degli Ospedali e. d. Clin., 1898, vol. xix, p. 1570.
[2] Hand, N. Y. Med. Jour., 1900, vol. lxxi, p. 556.
[3] Birk, "Das Fibrin-Ferment im lebenden Organismus," Dorpat, 1880.
[4] Bojanus, "Exp. Beiträge z. Physiol. u. Pathol. d. Blutes," Dorpat, 1881.
[5] Hankin and Kanthack, Centralbl. f. Bakt. u. Parasit., 1892, vol. xvii, p. 782.
[6] Buchner, Arch. f. Hyg., 1890, vol. x, p. 84.
[7] *Ibid.*
[8] Sem. méd., 1903, vol. xxiii, p. 351.

is generally greater, owing to the higher proportion of lymphocytes normally found at this period of life. A differential count, which shows, for instance, 60 per cent. of lymphocytes, means a decided lymphocytosis in the adult, but is entirely within the normal limits in the young infant. Either type of cells, large or small, may predominate, or the change may not involve any conspicuous deviation from the normal ratio of one form to the other. Frequently it happens that the two varieties possess such similar characteristics that it is impossible to determine which prevails. Occasionally the lymphocytosis depends largely upon unusually large percentages of the so-called "transitional" forms, while in other instances, notably in the lymphocytosis of von Jaksch's anemia, rickets, syphilis, variola, and scarlatina, the increase chiefly involves Ehrlich's large mononuclear forms, reputed to originate in the marrow.

Lymphocytosis may be due either to changes in the distribution of the cells through the circulatory system, or to their increased production and output by the lymphatic tissues. Ehrlich[1] attributes lymphocytosis to the local irritation of certain areas of lymphatic glands which produces an increased circulatory activity in these situations, in consequence of which large numbers of lymph elements are swept mechanically from the lymphatics and enter the general circulation. He does not regard the change as an expression of an active chemotactic reaction, to which the lymphocytes are insensible. It also appears reasonable to presume that the lymphocytosis which often accompanies leucopenia may be traced to still another factor, that of negative chemotaxis, which diminishes the number of polynuclear neutrophiles and thus brings about a relative increase in the lymphatic elements, upon which the repellent action is not exerted.

Lymphocytosis has been observed in a number of pathological conditions, but its presence may be considered physiological in but a single instance—*in the blood of infants and young children*, in whom such a change is entirely normal. During the decline of life, on the other hand, the lymphocytes are relatively diminished. This tendency toward a lymphocytic increase in infantile life, which becomes less notable as the child matures, is prone to become markedly exaggerated in many of the forms of *secondary anemia* from which children suffer, especially the anemias secondary to syphilis, tuberculosis, rachitis, gastro-enteritis, and scurvy; less commonly, it has been observed in the acute infections.

*Lactation*, *conditions of cachexia*, and *great debility* in the adult are in many instances accompanied by abnormally high per-

[1] *Loc. cit.*

centages of lymphocytes in the blood. It is a well-known fact that differential counts show a higher percentage of mononuclear non-granular elements in the blood of the enfeebled and poorly nourished than in that of the active and vigorous individual.

Similar changes are frequently associated with the *terminal stages of a number of diseases*, and may be found *after hemorrhage* from various causes—trauma, hemophilia, purpura, and following *splenectomy*.

Lymphocytosis, sometimes decidedly marked, is common in certain of the *severe anemias*, especially in chlorosis, pernicious anemia, Addison's disease, and in syphilitic and tuberculous secondary anemias; it may be observed in certain of the *infections*, such as enteric fever, malarial fever, Malta fever, scarlet fever, measles, diphtheria, pertussis, variola, phthisis, pneumonia, plague, and trypanosomiasis. A high degree of lymphocytosis has been reported by Labbé and Bernard[1] in *tropical hematochyluria* resulting from filariasis. Roger's contention,[2] that infections characterized by lymphocytosis are of protozoan type, is scarcely tenable, in view of the occurrence of this change in enteric fever, in Malta fever, and in tuberculosis, to name but three bacterial infections in which the lymphocytes are increased.

Diseases involving the *spleen* and *lymphatic glands* are often the cause of a varying degree of increase in the lymphocytes, common examples of such conditions being chronic malarial splenic tumors; kala-azar; simple, syphilitic, and tuberculous adenitis; and malignant neoplasms, especially sarcoma, of the lymph glands. On the contrary, extreme decrease in the lymphocytes develops in consequence of obliteration of the lymph channels by malignant tumors. *Enlargement of the thyroid gland* relatively increases the lymphocytes, as does that systemic taint known as the *constitutio lymphatica*. *Chloroma* may provoke high absolute lymphocytosis.

Distinct lymphocytosis has been observed by Wilkinson[3] after the injection of *quinin hydrochlorate;* and by Perry[4] and Lépine[5] as the result of the administration of *thyroid extract*. It also follows the injection of *tuberculin*, *pilocarpin*, and emulsion of *cancerous tissue*.

From a clinical viewpoint lymphocytosis is of value chiefly in the diagnosis of *lymphatic leukemia*. Marked absolute increase in the number of lymphocytes, associated with enlargement of the lymphatic glands, forms a pathognomonic picture of this disease.

[1] Sem. méd., 1902, vol. xxii, p. 433.

[2] "Infectious Diseases," Eng. trans. by Gabriel, New York and Philadelphia, 1903.

[3] *Loc. cit.*

[4] Med. Record, 1896, vol. l, p. 289.

[5] Sem. méd., 1902, vol. xxii, p. 409.

The recognition of a doubtful case of *syphilis* may be facilitated by the occurrence of lymphocytosis plus eosinophilia.

## V. EOSINOPHILIA.

The term *eosinophilia* is used to denote an increase above the normal standard in the number of eosinophiles in the circulating blood, this change usually, but not necessarily, being associated with a coincident increase in the relative percentage of these cells to the other forms of leucocytes. Thus interpreted, eosinophilia is a condition of *absolute* increase, in contradistinction to a purely *relative* gain in percentage, to which the term is not strictly applicable.

For the sake of uniformity it is customary to speak of the percentage of eosinophiles rather than of their actual number, but in order to determine accurately the presence or absence of eosinophilia it is also essential in every instance to calculate the number of eosinophiles to the c.mm. of blood from data obtained by a numerical estimate and a differential count of the leucocytes, thus:

*Total number of leucocytes per c.mm.* × *Percentage of eosinophiles to other forms of leucocytes.* = *Total number of eosinophiles per c.mm.*

The necessity for such a calculation is forcibly illustrated in myelogenous leukemia, since in this condition the relative percentage of eosinophiles is often well within the normal limits, and yet a striking degree of eosinophilia may exist. For example, in a given case of this form of leukemia, the blood count shows 300,000 leucocytes per c.mm. with 5 per cent. of eosinophiles. This percentage, interpreted into the actual *number* of cells, means an eosinophilia of 15,000 per c.mm., or an increase of *thirty-fold* in excess of the highest normal figure.

On the basis of a variation in the normal number of leucocytes of from 5000 to 10,000 per c.mm., the absolute number of eosinophiles may range from 25 to 500 per c.mm. in the blood of the healthy adult. An increase in excess of this maximum standard, *regardless of the percentage indicated by the differential count*, constitutes eosinophilia.

Granting the accuracy of the current view that the hemic eosinophiles are purely myelogenous elements, their increase in the blood may be attributed to the influence of chemotaxis, probably of a specific and selective character. Under the influence of such a stimulus the eosinophiles are attracted from, and perhaps overproduced by, the bone marrow, and are thrown into the gen-

eral circulation in large numbers. It is also possible that to a slight extent their proliferation from like cells may occur in the blood stream as well.

Increase in the number of eosinophiles occurs as a physiological change in *young infants*, in *women during the menstrual period*, and *after coitus*. With these three exceptions the presence of eosinophilia is always to be regarded as an evidence of some pathological condition.

Once believed to be a pathognomonic sign of leukemia, in the light of more recent investigations eosinophilia is now known to be associated with diseases of almost every conceivable nature; in fact, it has been reported in so large a number of conditions of such widely dissimilar pathogenesis that its value as a clinical sign must be largely restricted. Inasmuch as many of these reported instances of eosinophile increase lack verification, it can only prove confusing to give here a list of the many pathological states in which the change is reputed to have been observed. The following list, based upon the work of Cannon,[1] Zappert,[2] Gollasch,[3] T. R. Brown,[4] von Noorden,[5] and others, includes only those diseases in which eosinophilia is observed with a great degree of constancy. Such conditions are:

I. *Diseases of the Skin.*

Dermatitis herpetiformis.
Eczema.
Erythema multiforme.
Herpes zoster.
Leprosy.
Lupus.
Pellagra.
Pemphigus.
Prurigo.
Psoriasis.
Scleroderma.
Urticaria.

II. *Helminthiasis.*

Ankylostomiasis.
Ascariasis.
Bilharziasis.
Filariasis.
Hydatid disease.
Oxyuridiasis.
Strongyloides intestinalis infection.
Teniasis.
Trichiniasis.

III. *Diseases of the Bones.*

Hypertrophy.
Malignant neoplasms.
Multiple periostitis.
Osteomalacia.

[1] Deutsch. med. Wochenschr., 1892, vol. xviii, p. 206.
[2] Zeitschr. f. klin. Med., 1893, vol. xxiii, p. 227; also Wien. klin. Wochenschr., 1892, vol. v, p. 347.
[3] Fortschr. d. Med., 1889, vol. vii, p. 361.
[4] Johns Hopkins Hosp. Bull., 1897, vol. viii, p. 79.
[5] Zeitschr. f. klin. Med., 1892, vol. xx, p. 98.

IV. *Post-febrile.*

| | |
|---|---|
| Malarial fever. | Scarlet fever. |
| Pneumonia. | Septicemia. |
| Rheumatic fever. | Varicella. |

V. *Bronchial Asthma.*

VI. *Myelogenous Leukemia.*

In addition to the conditions listed above, eosinophilia also occurs, but with less constancy, in some forms of the high-grade *secondary anemia* of childhood, *purpura*, *hemorrhagic effusions*, *gonorrhea*, *syphilis*, *malignant disease*, and in *fibrinous bronchitis*. It is also seen in many cases of *splenomegaly* and *after splenectomy*, its development under the latter circumstance being regarded as a compensatory condition. An increase in the percentage of eosinophiles has also been noted in *conditions of starvation*. In *scarlet fever* the eosinophiles usually persist, in spite of the coëxisting polynuclear leucocytosis, and the same peculiarity may often be found in *trichiniasis*, *filariasis*, *hydatid disease*, and *infection with intestinal parasites*.

Eosinophilia may be produced experimentally by the injection of a number of medicaments, such as *antipyrin*, *camphor*, *nuclein*, *phosphorus*, *pilocarpin*, *tuberculin*, and many of the *iron salts*. T. R. Brown[1] found high eosinophilia (12 per cent. in a leucocyte count of 30,000) in *acetanilid* poisoning. The writer detected eosinophilia in 3 cases of poisoning by *nitrites*.

Neusser[2] and his school have contended that eosinophilia is symptomatic of an extensive group of diseases, chiefly those involving the *sympathetic nervous system*, the *sexual organs*, and a long list of disorders which they attributed to the "*xanthin diathesis*." Most of these views have been unsubstantiated, many are misleading, and a few can be shown to be fanciful. Those who are inclined to investigate some of the remarkable claims made by Neusser as to the diagnostic and prognostic value of eosinophilia are referred to his original communication on the subject.

Diminution in the number of eosinophiles occurs as a physiological process during *digestion* and after active *muscular exercise*. It is observed usually in *lymphatic leukemia*, *tuberculosis*, during the febrile stages of *diphtheria*, *influenza*, *pneumonia*, *enteric fever*, and *septicemia*, frequently *after hemorrhage*, and in the *terminal*

[1] Md. Med. Jour., 1902, vol. xlv, p. 307.
[2] Wien. klin. Wochenschr., 1894, vol. vii, p. 737.

*stages of many diseases.* The number of eosinophiles is said to be diminished *after castration.* The writer has found a decrease or even absence of eosinophiles in the majority of cases of *chlorosis* and *pernicious anemia.*

The chief clinical value attached to eosinophilia relates to its presence in trichiniasis, in filariasis, in intestinal helminthiasis, and in hydatid disease, in which infections it has been shown to be a sign of great reliability. It may, however, be absent in the later stages of these infections.

In the diagnosis of an exanthema which is suggestive either of scarlet fever or of measles, eosinophilia points to the former disease, for it does not occur in the latter.

The association of eosinophilia and lymphocytosis constitutes a blood change which may be helpful in the recognition of an obscure case of syphilis.

High percentages of eosinophiles in chlorosis, in pernicious anemia, and after hemorrhage are generally regarded as an evidence of good regenerative powers of the hemogenic organs, and are therefore of favorable import (Rieder).[1]

## VI. BASOPHILIA.

Increase in the number of basophiles in the circulating blood is of rare occurrence, having been observed in but few diseases except the myelogenous variety of *leukemia,* in which this change is quite constant, and sometimes most striking; the basophiles in this disease may constitute 10, 20, or even 30 per cent. of all forms of leucocytes. The increase may involve the finely granular or the coarsely granular (mast cell) forms, or both.

Until recently but little attention has been paid by hematologists to the general circulatory form of basophilia, although the local increase of the basophiles under various conditions has been well investigated. Canon[2] has reported an increase of the mast cells in a case of *chlorosis* and in *various skin diseases.* Sherrington[3] has observed a similar blood change in patients dying in the reaction stage of *Asiatic cholera.* A. E. Taylor[4] states that he has seen a notable circulatory basophilia in a case of *carcinoma,* with marked cachexia, but without bone metastases; in a case of *gonorrhea;* in a case of *mycosis fungoides;* and in two cases of *septic bone disease.* Basophilia has also been observed in *variola,*

[1] *Loc. cit.* [2] Deutsch. med. Wochenschr., 1892, vol. xviii, p. 206.
[3] Proc. Roy. Soc., London, 1894, vol. lv, p. 189.
[4] "Contributions from the William Pepper Laboratory of Clinical Medicine," Philadelphia, 1900, p. 148.

*splenic anemia*, *Hanot's cirrhosis*, and in *hydatid disease* and other forms of helminthiasis. The writer found a mast cell basophilia of 2 per cent., with a leucocytosis of 13,000, in a case of *plumbism*, and has noted large numbers of mast cells in *suppurative appendicitis*, *pernicious anemia*, *filariasis*, and *trichiniasis*.

Owing to our imperfect understanding of this condition no theory regarding the production of basophilia is as yet generally acceptable. It is possibly due to the influence of a specific chemotactic substance, in response to which the basophiles are attracted from the bone marrow and enter the general circulation.

## VII. MYELEMIA.

The presence in the circulating blood of myelocytes, in small or in large numbers, is known as *myelemia*. As previously remarked, this condition is invariably to be regarded as pathological, since myelocytes are never found in the blood of the normal individual.

The most striking example of myelemia is to be found in the myelogenous form of *leukemia*, in which condition this change constitutes one of the most conspicuous features of the blood picture. Myelocytes occur in the blood in this disease in greater absolute and relative numbers and with greater constancy than in any other condition—a fact which is of the greatest diagnostic value. The degree of increase may be enormous, as illustrated by a case of the author's, in which the actual number of myelocytes was found to be 192,738 per c.mm., or 27.3 per cent. of all forms of cells in a total leucocyte count of 706,000. In instances of *splenomegaly* with anemia and moderate leucocytosis small numbers of myelocytes have been reported as a constant finding by Emile Weil and Clerc.[1] Kurpjuweit,[2] in *malignant disease* with bone metastases, found as high as 17 per cent. of myelocytes, and he regards this myelemia as a sign of gravely altered hemogenesis. (See "Malignant Disease.") Von Jaksch[3] noted marked myelemia in his symptom-complex, termed *multiple periostitis with myelocythemia*.

Less frequently myelocytes are observed in *lymphatic leukemia* and in *Hodgkin's disease*, but in these conditions their occurrence is inconstant and their increase trivial. Small numbers of myelocytes (from 0.5 to 2 or 3 per cent.) are found in

[1] Arch. gén. de Méd., 1902, vol. cxc, p. 560.
[2] Deutsch. Arch. f. klin. Med., 1903, vol. lxxvii, p. 553.
[3] Zeitschr. f. Heilk., 1901, vol. xxii, p. 259.

almost every case of *primary pernicious anemia*, and are not uncommon in marked cases of *chlorosis* and in many of the severe forms of *secondary anemia* due to various causes. They are frequently met with in such conditions as *pneumonia*, *septicemia*, *diphtheria*, *syphilis*, *malignant disease*, *rachitis*, *tuberculosis*, *osteomyelitis*, *osteomalacia*, *Addison's disease*, and the *malarial fevers*. The writer has found them also in the following conditions: *carbon monoxid poisoning*, *hepatic cirrhosis*, *acute gout*, *malignant endocarditis*, *exophthalmic goiter*, as well as in the above-named affections. One is forcibly impressed with the almost constant presence of myelocytes in the estivo-autumnal type of *malarial fever*, in severe *septic infections*, and in *enteric fever* in childhood, both in the early stages of the disease and during the later, postfebrile anemic period. Small numbers of myelocytes have been reported also in many other conditions, chiefly those associated with leucocytosis, with anemia, or with both.

Increased activity of the bone marrow, whereby the myelocytes are forced into the blood stream, is in all probability responsible for the production of myelemia. In response to an increased demand for leucocytes the marrow becomes so overstimulated that many immature forms of leucocytes, or myelocytes, accidentally find their way into the general circulation, their passage from the marrow no doubt being accomplished largely by emigration. It is furthermore now believed that substances which are positively chemotactic for the polynuclear neutrophiles also exert a similar attractive influence upon their immediate precursors, the myelocytes, stimulating their increased proliferation in the bone marrow and exciting their emigration from this tissue into the blood stream.

## VIII. LEUCOPENIA.

Decrease below the normal standard in the number of leucocytes in the peripheral blood is known as *leucopenia* or *hypoleucocytosis*. Such a condition, like its antithesis, leucocytosis, may be the result of either physiological or pathological causes. Owing to the variation in the normal number of leucocytes in different individuals, it is difficult to determine arbitrarily just what degree of decrease may be considered as a leucopenia, but it is safe to apply the term to any leucocyte count decidedly below 5000 cells to the c.mm. The number of leucocytes is rarely reduced to less than 3000, except in certain of the essential anemias, in which their decline to one-tenth the maximum normal figure or even less is occasionally to be observed. The most extreme

instance of leucopenia on record has been reported by Koblanck,[1] who found but a *single* leucocyte in a careful search through twenty stained cover-glass preparations of blood from a man of twenty-five years, suffering from epilepsy; the exact numerical estimate of the leucocytes in this case is not given in detail.

The decrease may be accompanied by no deviation from the normal percentages of the different varieties of leucocytes, or it may involve a more or less decided gain in the lymphocytes, the latter being the more common change of the two.

According to the nature of its underlying causes, leucopenia may be considered clinically as either physiological or pathological.

## Physiological Leucopenia.

The decrease in the number of leucocytes observed in several physiological states is generally attributed to vasomotor influences which produce changes in the distribution of the leucocytes throughout the system. Such changes occur from the effect of prolonged *cold*, and brief *hot baths*.[2] Decastelle[3] has found that a temporary leucopenia may be produced experimentally, by *stimulation of sensory nerves*, this procedure causing a reflex contraction of the abdominal vessels and a consequent retention of large numbers of circulating leucocytes in this part of the vascular system. The variations in the number of cells range from 20 to 30 per cent. of the original count; the maximum decrease occurs usually within three or four minutes, and in most instances does not persist longer than ten or fifteen minutes. *Reduction of blood pressure* is promptly followed by a very transient diminution in the leucocytes of the peripheral blood.

*Malnutrition* and *starvation* are also potent factors in the production of leucopenia, the decrease dependent upon such causes frequently being most pronounced. The much-cited case of the faster, Succi, is a good example of the effects produced upon the leucocytes by abstinence from food. Luciani[4] found in the blood of this individual a decrease in the number of leucocytes from 14,530 to 861 per c.mm. after a seven days' fast; on the eighth day an increase to 1530 occurred, this being the average count noted during the remaining twenty-one days of the fast. The subnormal leucocyte counts which are often met with in many of the infirm and the greatly enfeebled can be traced to the effects of faulty nutrition and to the malassimilation of food.

[1] Inaug. Dissert., Berlin, 1889.
[2] Winternitz, *loc. cit.*
[3] Wien. klin. Wochenschr., 1899, vol. xii, p. 395.
[4] "Das Hungern" (German translation by O. Fränkel), Hamburg and Leipsic, 1890.

## Pathological Leucopenia.

Leucopenia, or at least an absence of leucocytosis, occurs during the course of a number of general infectious diseases, prominent among which are the following: *enteric fever, paratyphoid fever, measles, rötheln, influenza, leprosy, Malta fever,* the *malarial fevers, trypanosomiasis,* and *non-septic tuberculosis.* In acute infections which are ordinarily accompanied by leucocytosis the combined influences of an intense infection and feeble resisting powers on the part of the individual may produce a distinct leucopenia, or may prevent the development of the characteristic increase. This is well illustrated by the low counts which sometimes are found in severe cases of pneumonia and of appendicitis.

Leucopenia, often pronounced, is not uncommon in *chlorosis* and in *pernicious anemia,* being much more frequent and more decided in the latter disease. A well-marked leucopenia may be expected in about one-fourth of all cases of chlorosis, and in quite three-fourths of cases of pernicious anemia. It is also often met with in some high-grade *secondary anemias,* notably in those due to syphilis and to rachitis, and in *splenic anemia.*

D'Orlandi[1] has called attention to the frequency with which leucopenia is observed in certain of the severer forms of *chronic gastro-enteritis* in young infants. In a fatal case of *primary infectious pharyngitis* reported by P. K. Brown,[2] the leucocytes never numbered more than 400 to the c.mm.

In the anemias accompanied by a decrease in the leucocytes, especially in primary pernicious anemia, the rule holds good that the more intense the oligocythemia and oligochromemia, the greater the degree of leucopenia. Ehrlich[3] attributes the decrease in such cases to a lessened proliferative function of the bone marrow, in consequence of which there is a diminution in the output of leucocytes by this organ.

In *leukemia* an acute intercurrent infection may produce an abrupt and marked fall in the number of leucocytes, as in Cabot's remarkable case of lymphatic leukemia,[4] in which, as the consequence of a fatal septicemia, the leucocytes fell in three weeks from 40,000 to 419 per c.mm.

Decrease in the number of leucocytes may be caused experimentally by the administration of various drugs and other substances. Bohland[5] found that it followed the injection of *ergot, sulphonal, tannic acid, camphoric acid, atropin, agaricin,* and

[1] Rev. mensuelle des malad. de l'Enfance, 1899, vol. xvii, p. 300.
[2] Amer. Med., 1902, vol. iii, p. 649.
[3] *Loc. cit.*
[4] *Loc. cit.*
[5] *Loc. cit.*

*picrotoxin.* Delezene's[1] investigations showed that a marked decrease results from the injection of various anticoagulant substances, such as *peptone*, *diastase*, and *eel serum;* he attributes the leucopenia thus produced to two factors—actual destruction in the circulation of some of the leucocytes and dilatation of the blood vessels, in which the undestroyed cells tend to accumulate. The transient leucopenia which precedes an increase in the leucocytes has been discussed elsewhere. (See p. 239.)

O. K. Williamson[2] has shown that an increased destruction of leucocytes is attended by an increased excretion of uric acid in the urine. His studies apparently prove that in cases in which a rise in the phosphoric acid curve follows a fall in the leucocyte curve and in the number of granular cells especially, this rise corresponds with a rise in the uric acid curve.

[1] Nouveaux Montpel. méd., 1898, vol. vii, pp. 694, 733, 765, and 789.
[2] Lancet, 1903, vol. i, p. 657.

# SECTION V.

# DISEASES OF THE BLOOD.

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## DISEASES OF THE BLOOD.

### I. CHLOROSIS.

General Features.

Lorrain Smith,[1] by his carbonic oxid method, estimates that the *total volume* of blood is greatly increased in chlorosis, this excess being due to an increase in the bulk of normal plasma, and being more marked the severer the case. Taking the normal blood volume as 3240 c.c., Smith found in 21 chlorotics an average of 4883 c.c. Although the *oxygen capacity* of a blood unit is diminished about one-half, the total oxygen capacity of the blood remains approximately normal—95 per cent. Lloyd Jones[2] also believes in a plasma increase in chlorosis, but further confirmation of the above experimental work is needed before this presumption can be registered as a fact.

The proportion of *dry residue* of the whole blood is subnormal, approximating in the severe case between one-half and one-third the normal amount, with a corresponding increase in water.

There is a decided loss of blood *albumin*, mainly referable to the oligochromemia, but, as Biernacki[3] has pointed out, also due, at least in severe cases, to a deficiency in the amount of serum albumin. In high-grade chlorosis this author found that the dry residue of the whole blood might contain a normal iron content, the inference being that in such instances the albumin loss was not always confined to the hemoglobin.

Appearance of the Fresh Blood.

The blood drop is exceedingly pale and watery-looking, and flows so abundantly from the puncture that it actually seems as if the whole mass of blood in the body must be increased; a large-sized drop usually follows the slightest prick of the needle, in spite of the obviously anemic appearance of the patient—a marked contrast to the difficulty commonly experienced in pernicious anemia of obtaining enough blood for the

[1] Jour. Physiol., 1900, vol. xxv, p. 6. [2] "Chlorosis," London, 1897, p. 24.
[3] Zeitschr. f. klin. Med., 1894, vol. xxiv, p. 500.

examination. The blood spread out in a film over the finger is transparent rather than opaque, and its fluidity is most striking.

Microscopical examination of the fresh film shows excessive pallor of most of the erythrocytes, together with the presence of a variable number of cells of smaller diameter than normal, in the average case, and of cells decidedly deformed in shape, in severe cases. The resistance of the erythrocytes, as shown by their hypotonicity,[1] is increased. The practised observer can determine at first glance that the number of erythrocytes is not greatly decreased, except in an occasional case in which the oligocythemia may be so marked as to lead him to infer that he is dealing with a well-defined secondary anemia.

COAGULATION.

Coagulation of the blood drop, in spite of the fact that hyperinosis is absent, is generally very rapid in chlorosis—often so rapid as to interfere with the technic of the examination, if one delays during this procedure.

SPECIFIC GRAVITY.

The specific gravity of the whole blood is more or less diminished, the degree of decrease being closely parallel with the loss of hemoglobin. Lloyd Jones,[2] who has made elaborate researches concerning this subject, believes that chlorotic blood exhibits an exaggeration of the fall in specific gravity which occurs in healthy girls at about the age of puberty. In the 36 cases studied by this author the specific gravity ranged from 1.030 to 1.049, these figures corresponding to 17 and 58 per cent. of hemoglobin, respectively, as estimated by the von Fleischl hemometer. In 30 cases Hammerschlag[3] found that the density of the whole blood averaged 1.045, and of the serum, 1.030.

ALKALINITY.

Most observers maintain that in this disease the alkalinity of the whole blood generally remains normal, or suffers but a trifling diminution, this being in direct contrast to the condition found in other forms of anemia, in which the fall in the alkalinity figure is usually pronounced. Burmin,[4] in 18 examinations of 9 cases, found that it ranged between 128 and 200 mgm. NaOH, the normal figures of this investigator being 182 to 218 mgm. In 6 of these cases the administration of iron was followed by a marked increase in the alkalinity of the blood, closely paralleling the gain in hemoglobin and erythrocytes. On the other hand, Graeber[5] states that

[1] Von Limbeck, *loc. cit.*
[2] *Loc. cit.*
[3] Wien. med. Presse, 1894, vol. xliv, p. 1068.
[4] Zeitschr. f. klin. Med., 1900, vol. xxxix, p. 365.
[5] "Zur klin. Diag. d. Blutkrankheit.," Leipsic, 1890, p. 289.

in many cases he discovered abnormally high alkalinity figures, so constantly, indeed, that he regarded them as "specific for this condition." Funke's studies, reported by Dare,[1] show that the alkalinity is diminished, and that it closely corresponds to the color index.

HEMOGLOBIN AND ERYTHROCYTES.

The decrease in the percentage of hemoglobin is usually excessive in comparison with the reduction in the number of erythrocytes, this disproportionate oligochromemia being the most conspicuous and most constant feature of the changes affecting chlorotic blood. Naturally, such a change gives rise to very low color indices. This statement applies only to the majority of cases, for a low color index, while it is the rule in chlorosis, and is, diagnostically, a most important feature of the blood picture, is by no means invariably found—no more invariably than a high color index in pernicious anemia. To illustrate this point, of 106 consecutive cases of chlorosis studied by the author, 49, or more than 46 per cent., showed an index below 0.50, the average for the series being 0.51, the maximum 1.01, and the minimum 0.22.

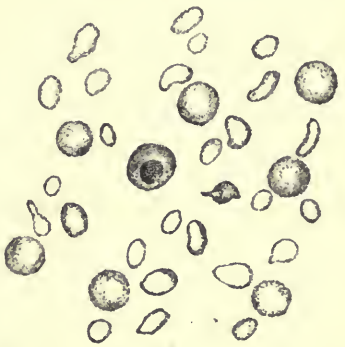


FIG. 51.—CHANGES IN THE ERYTHROCYTES IN CHLOROSIS (TRIACID STAIN).

Showing a general decrease in the diameter of the corpuscles, striking decolorization, and moderate poikilocytosis. The nucleated cell near the center of the field is a normoblast.

The average loss of hemoglobin, as evidenced by the 155 cases tabulated below, amounts to about 53 per cent., in contrast to which stands the mean average erythrocyte decrease, which is equivalent to about 23 per cent., the hemoglobin loss thus averaging about two-and-one-half times that of the corpuscles. Individually, the hemoglobin percentage ranged in these cases from 12 to 87, averaging 46.8, and the count of erythrocytes from 1,720,000 to 5,600,000, averaging 3,816,486. On account of their rather close correspondence, it is interesting to compare with these figures the results obtained by Cabot[2] in 109 cases and those of Thayer[3] for 63 cases. Cabot's cases gave the following mean averages: hemoglobin, 41.2 per cent.; erythro-

[1] Johns Hopkins Hosp. Bull., 1903, vol. xiv, p. 179. [2] *Loc. cit.*

[3] Cited by Osler, "American Text-book of Theory and Practice of Medicine," Philadelphia, 1894, vol. ii, p. 196.

cytes, 4,112,000, with individual counts ranging from 1,932,000 to 7,100,000. In Thayer's series the hemoglobin averaged 42.3 per cent. and the erythrocyte count 4,096,544. Somewhat lower figures are given by Bramwell,[1] who found the following averages in a series of 80 cases: hemoglobin, 34 per cent., or from 10 to 60 per cent.; erythrocytes, 3,437,300, or from 1,425,000 to 5,200,000 per c.mm.; and color index, 0.49, or from 0.20 to 0.96.

While numerous examples may be found of typical cases of chlorosis in which the hemoglobin estimate and erythrocyte count resemble those commonly occurring in pernicious anemia or in the secondary anemias, nothing is more characteristic of chlorosis than the *averages* above mentioned. The great difference is between chlorosis and pernicious anemia, the index usually being high and the corpuscular loss extreme in the latter disease. As compared with the secondary anemias, the difference is too slight and its occurrence too inconstant to enable one to regard it with any degree of certainty from a clinical standpoint. Theoretically, in secondary anemia the hemoglobin loss is fairly proportionate to the erythrocyte decrease, thus producing color indices at or somewhat below the normal, but cases of secondary anemia having a so-called "chlorotic" type of blood are far too common to render any information reliable gained by a simple inquiry into the changes affecting the erythrocytes and their hemoglobin content.

TABLE I.—HEMOGLOBIN AND ERYTHROCYTES IN 155 CASES OF CHLOROSIS.

| Hemoglobin Percentage. | Number of Cases. | Erythrocytes per c.mm. | Number of Cases. |
|---|---|---|---|
| From 80–90 | 5 | Above 5,000,000 | 13 |
| " 70–80 | 11 | From 4,000,000–5,000,000 | 63 |
| " 60–70 | 17 | " 3,000,000–4,000,000 | 49 |
| " 50–60 | 35 | " 2,000,000–3,000,000 | 28 |
| " 40–50 | 24 | " 1,000,000–2,000,000 | 2 |
| " 30–40 | 25 | | |
| " 20–30 | 28 | | |
| " 10–20 | 10 | | |
| Average, 46.8 per cent. | | Average, 3,816,486 per c.mm. | |
| Maximum, 87.0 " " | | Maximum, 5,600,000 " " | |
| Minimum, 12.0 " " | | Minimum, 1,720,000 " " | |

The most conspicuous change to be observed in the stained film of chlorotic blood is the presence of large numbers of undersized, pale erythrocytes, such cells usually being so numerous that one is forcibly impressed with the fact that there must be a general decrease in the average diameter of all the erythrocytes in the field. As a rule, this decrease in size involves a large number of corpuscles moderately, rather than a few to an ex-

[1] "Anemia," London, 1899, p. 35.

treme degree, and therefore, except in severe cases associated with marked oligocythemia, striking examples of microcytosis are wanting. This alteration is just the opposite of what is generally found in pernicious anemia, for in this disease a tendency toward an increase in the average diameter of the erythrocytes, frequently in association with the presence of many extremely small microcytes, is the rule. If well defined, this feature of the blood changes carries a certain amount of diagnostic significance, although it cannot be distinguished in every case of chlorosis, since in some the diameter of the erythrocytes appears to be unaltered, while in others the deformities of size may so affect the cells that the blood picture resembles that of a severe secondary anemia.

The *pallor* of the erythrocytes, shown by their feeble reaction toward the plasma stain, is at once apparent. The great majority of the cells are affected alike, being pale, often quite colorless in the center, and gradually becoming of darker color toward the periphery, in which a certain amount of hemoglobin still remains. This portion of the cell is usually well stained, so that the corpuscles frequently appear as hoops or rings; some, however, do not show even this narrow hemoglobin-filled zone, being practically decolorized throughout. Stroma degeneration, as shown by the changes described by Maragliano, is not demonstrable in the average case of moderate severity, but this process has been observed in occasional cases of high grade. Polychromatophilia, except in cases of the latter class, does not occur.

*Basophilic granulations* in the erythrocytes are not present in this condition, according to Grawitz,[1] a finding which the author has substantiated.[2] Stengel,[3] on the contrary, found them in 11 of 18 chlorotics.

*Deformities of shape* are not noticeable, as a rule, except in the severer types of the disorder. In such cases, in which both the hemoglobin and the cellular losses are excessive, poikilocytosis may be very striking—as great, in fact, as in any blood disease, not excepting pernicious anemia. Poikilocytes, should they occur, are almost invariably of small size.

*Nucleated erythrocytes* are very rare. In the average case they are usually sought for in vain, and even in the severer forms of chlorosis these cells are not numerous. Erythroblasts conforming to the normoblastic type are found almost exclusively; megaloblasts, although they are seen now and then, are extremely uncommon, and have never been found in a large relative or absolute proportion to the other form of nucleated erythrocytes.

[1] *Loc. cit.* [2] Amer. Med., 1903, vol. v, p. 571.
[3] Amer. Jour. Med. Sci., 1902, vol. cxxii, p. 873.

TABLE II.—NUMBER OF LEUCOCYTES IN 155 CASES OF CHLOROSIS.

| Leucocytes per c.mm. | Number of Cases. |
|---|---|
| Above 20,000 | 1 |
| From 15,000–20,000 | 3 |
| " 10,000–15,000 | 14 |
| " 5,000–10,000 | 104 |
| Below 5,000 | 33 |
| Average, 6,457 per c.mm. | |
| Maximum, 21,000 " " | |
| Minimum, 800 " " | |

Leucocytes.

The number of leucocytes per c.mm. is, as a rule, normal in the typical case of chlorosis. If leucocytosis occurs, as it does occasionally, it should be attributed to some hidden or frank complication; if leucopenia exists, as it sometimes does, it may nearly always be regarded as a sign of the severity of the disease, since it is rarely met with except in cases in which the hemoglobin and erythrocyte losses are decidedly marked. The mean average number of leucocytes in the 155 cases of chlorosis to which reference has already been made (Table II) is 6457 per c.mm., or approximately the same as the average count of these cells in normal blood. Counts as low as 800 and as high as 21,000 were made in this series; and in 18 of the cases (or 11.6 per cent.) the increase was sufficiently in excess of the normal standard to justify the application of the term leucocytosis—that is, it was in excess of 10,000. These figures do not differ materially from those of Cabot and of Thayer, alluded to above, Cabot's counts in 104 cases averaging 7400, and Thayer's estimates in 63 cases being but slightly higher—7485.

*Relative lymphocytosis*, usually marked in relation to the severity of the case, is a common, but not a constant, qualitative change. It occurs in both mild and severe cases, but is much more common in the latter. In the author's experience this increase involves chiefly the larger forms of these cells, both the non-granular mononuclear cells with spherical nuclei and the so-called "transitional" forms with indented nuclei; striking increase in the last-named variety of cells was a notable differential change in a large proportion of the 37 cases listed in Table III. In many of the cases in this series the large and small lymphocytes together made up from 45 to as high as 67.5 per cent. of all varieties of leucocytes, the percentage of large forms being repeatedly estimated at 20 or 30, and even 40, in one instance.

Deviations from normal in the relative percentage of *polynuclear neutrophiles* are governed by the behavior of the lymphocytes, low differential counts of the former type of cells accom-

panying high percentages of the latter, and vice versâ. Should leucocytosis exist, it is of the pure polynuclear neutrophile type.

The *eosinophiles* are notably decreased, both absolutely and relatively. The author has never found an increase of these cells in chlorosis, although considerable pains were taken to verify the statements made by some writers that this variety of leucocytes is occasionally observed to be greatly above normal in this condition. Eosinophiles were absent entirely in more than seven-tenths of all the cases collected in Table III, and were never found to exceed two per cent. of all the forms of leucocytes.

TABLE III.—QUALITATIVE CHANGES IN THE LEUCOCYTES IN 37 CASES OF CHLOROSIS AT THE FIRST EXAMINATION.

| Case No. | Small Lymphocytes. | Large Lymphocytes. | Polynuclear Neutrophiles. | Eosinophiles. | Myelocytes. |
|---|---|---|---|---|---|
| 1 | 16.0 | 6.0 | 76.0 | 2.0 | 0 |
| 2 | 11.0 | 3.0 | 85.5 | 0.5 | 0 |
| 3 | 20.0 | 3.5 | 75.0 | 1.5 | 0 |
| 4 | 25.0 | 3.0 | 72.0 | 0.0 | 0 |
| 5 | 19.5 | 20.5 | 60.0 | 0.0 | 0 |
| 6 | 22.5 | 15.0 | 62.5 | 0.0 | 0 |
| 7 | 20.5 | 19.0 | 60.5 | 0.0 | 0 |
| 8 | 32.2 | 17.8 | 50.0 | 0.0 | 0 |
| 9 | 25.5 | 16.0 | 57.5 | 1.0 | 0 |
| 10 | 18.5 | 12.0 | 69.5 | 0.0 | 0 |
| 11 | 18.0 | 10.0 | 72.0 | 0.0 | 0 |
| 12 | 17.0 | 21.5 | 61.5 | 0.0 | 0 |
| 13 | 19.5 | 17.0 | 63.0 | 0.5 | 0 |
| 14 | 22.0 | 14.5 | 63.5 | 0.0 | 0 |
| 15 | 7.5 | 12.4 | 80.1 | 0.0 | 0 |
| 16 | 19.3 | 21.1 | 59.6 | 0.0 | 0 |
| 17 | 18.5 | 16.0 | 65.5 | 0.0 | 0 |
| 18 | 18.0 | 16.0 | 65.0 | 1.0 | 0 |
| 19 | 18.3 | 19.0 | 61.0 | 1.7 | 0 |
| 20 | 15.5 | 24.5 | 60.0 | 0.0 | 0 |
| 21 | 26.0 | 21.0 | 53.0 | 0.0 | 0 |
| 22 | 12.0 | 30.0 | 58.0 | 0.0 | 0 |
| 23 | 13.5 | 12.5 | 73.5 | 0.5 | 0 |
| 24 | 14.0 | 15.9 | 70.1 | 0.0 | 0 |
| 25 | 17.5 | 14.0 | 68.5 | 0.0 | 0 |
| 26 | 15.0 | 17.0 | 68.0 | 0.0 | 0 |
| 27 | 20.5 | 11.5 | 68.0 | 0.0 | 0 |
| 28 | 20.3 | 12.7 | 67.0 | 0.0 | 0 |
| 29 | 31.0 | 9.0 | 60.0 | 0.0 | 0 |
| 30 | 35.9 | 6.0 | 58.0 | 0.1 | 0 |
| 31 | 24.0 | 2.0 | 74.0 | 0.0 | 0 |
| 32 | 27.5 | 40.0 | 32.0 | 0.5 | 0 |
| 33 | 26.0 | 15.0 | 56.0 | 1.0 | 2 |
| 34 | 24.0 | 26.0 | 50.0 | 0.0 | 0 |
| 35 | 22.0 | 6.0 | 72.0 | 0.0 | 0 |
| 36 | 24.5 | 14.5 | 61.0 | 0.0 | 0 |
| 37 | 6.0 | 32.0 | 59.0 | 0.0 | 3 |
| Average: | 20.1 | 15.5 | 64.0 | 0.31 | 0.13 |

Exceptionally, small percentages of *myelocytes* may be encountered, as a rule only in cases of a severe character. These cells are ordinarily present in not more than six per cent. of all cases, and their relative proportion to the other forms of leucocytes is always trifling, being rarely over one or two per cent.

BLOOD PLAQUES. In the great majority of cases it has been generally observed that the number of plaques is considerably in excess of normal. It appears that these elements are especially numerous in blood which clots rapidly.

DIAGNOSIS. The changes in the blood associated with the well-defined case of chlorosis may be summarized as follows :

| | |
|---|---|
| *Color.* | Pale and watery. |
| *Coagulation.* | Usually rapid. |
| *Specific gravity.* | Decreased. |
| *Hemoglobin.* | Marked absolute decrease, in most instances relatively greater than the loss of erythrocytes, thus producing a low color index. |
| *Erythrocytes.* | Moderately decreased, ordinarily to about 4,000,000 per c.mm. Counts of 3,000,000 or lower are common in severe cases. Erythroblasts very rare; if present, cells of the normoblastic type invariably predominate. General decrease in the average diameter of the erythrocytes. In severe cases microcytosis may be marked. |
| | Poikilocytes not numerous, except in severe cases. |
| | Polychromatophilia rare. |
| *Leucocytes.* | Usually normal in number. |
| | Relative lymphocytosis common. |
| | Small percentages of myelocytes, only in severe cases. |
| | Eosinophiles notably decreased. |
| *Plaques.* | Increased in number. |

He who attempts the diagnosis of chlorosis solely by the blood examination is indeed a rash clinician. This point cannot be emphasized too strongly, that there is no blood picture peculiar to this condition, since changes precisely similar to those seen in many a case of typical chlorosis are often observed in the *secondary anemias*, especially in those dependent upon such factors as syphilis, septicemia, malignant disease, and chronic renal lesions. Futhermore, rare cases of chlorosis with typical symptoms

have been reported in which no alterations in the blood were discoverable by ordinary clinical methods. In these cases, mistermed "pseudo-chlorosis," a diminished volume of plasma may mask the blood impoverishment (Lloyd Jones[1]), or this oligoplasmia may be combined with both plasma and cellular hydremia (Biernacki[2]), the dropsical erythrocytes being actually deficient in hemoglobin, although the hemoglobin and erythrocyte estimates of the whole blood remain normal.

The blood changes enumerated above (especially such features as a low color index, the general decrease in the diameter of the erythrocytes, the absence or scantiness of erythroblasts, and the normal number of leucocytes associated with relative lymphocytosis and a decrease of the eosinophiles) are not then, pathognomonic, but simply highly suggestive of the disease under discussion, in view of which fact it becomes essential to seek for other clinical signs and to consider them carefully in connection with the blood findings. One of the most important points which should be borne in mind is the fact that chlorosis is practically confined to females, usually those in early womanhood, at or near the period of puberty. Chlorosis is about as compatible with the male sex as is pregnancy —the so-called "male chlorosis" is nothing more than a diagnostic myth. Osler[3] remarks that in girls in whom the disease occurs early in their teens precocity and almost premature appearance of the menses are likely to exist. A large proportion of those in whom the disease develops later in life complain of scantiness or total suppression of the menstrual flow and of dysmenorrhea, these symptoms being especially common in chlorotics in the early twenties or thereabouts.

The question of heredity also is of some diagnostic value, for it has been frequently noted that the disease exists, for instance, in two or more sisters, the statement being elicited upon further inquiry that their mother suffered from chlorosis at an earlier period. Thus, Allbutt[4] speaks of meeting in his consulting-room the chlorotic daughters of women whom years before he had treated for the same disorder.

Among the other manifestations of the disease to which attention should be paid the following are the more important: a peculiar greenish-yellow color of the complexion and blanching of the mucous membranes (except in those rare instances of *chlorosis florida*, in which the color is high); the occurrence of various gastro-intestinal disturbances, of edema of the face and lower

[1] *Loc. cit.*.
[2] Zeitschr. f. klin. Med., 1894, vol. xxiv, p. 500.
[3] *Loc. cit.*
[4] "System of Medicine," London and New York, 1898, vol. vi, p. 483.

limbs, of vertiginous attacks, and of dyspnea upon physical exertion; and the presence of systolic basic heart murmurs and a venous hum most distinctly audible over the great vessels of the neck. Slight enlargement of the thyroid gland, frequently associated with Joffroy's sign (absence of horizontal wrinkling of the skin of the forehead and of upward curving of the eyebrows when the patient glances suddenly at the ceiling without elevating her head), is a physical sign which should always make one suspicious of chlorosis.

The distinctions between chlorosis and *pernicious anemia*, as shown by the blood examination, will be described under the latter disease. (See p. 289.)

## II. PERNICIOUS ANEMIA.

General Features.

Lorrain Smith's experiments[1] argue a decrease, averaging 48 per cent., in the *total oxygen capacity* of the blood, and also show that the *blood volume* fluctuates greatly in different cases. Any decided increase, however, is at variance with both the clinical and the postmortem findings.

Diminution in the *albumin* of the whole blood, due chiefly to the cellular poverty, is a conspicuous change, the dry residue in extreme instances amounting to but one-half of the normal figure.[2] The albumin of the blood serum is also diminished,[3] but not greatly —a point of difference between Addisonian and secondary anemia, since in severe types of the latter the serum albumin is strikingly subnormal.

Appearance of the Fresh Blood.

In marked cases of pernicious anemia it is sometimes almost impossible to obtain from a puncture of the finger-tip a sufficient quantity of blood for an ordinary clinical examination, owing to the bloodlessness of the superficial vessels. This fact naturally prompts the query, Does an actual reduction in blood volume or oligemia exist in such an instance, or can the dryness of the superficial tissues be attributed to vasomotor disturbances causing an unequal distribution of the blood mass, in favor of the internal viscera and deeper circulation? In a patient in whom puncture of the finger fails to give the requisite amount of blood, the lobe of the ear will generally be found to yield a drop of sufficient size. But even this very vascular part of the

[1] *Loc. cit.*

[2] Gumprecht and Stintzing, Deutsch. Arch. f. klin. Med., 1894, vol. liii, p. 265.

[3] Diabella, *ibid.*, 1896, vol. lvii, p. 302.

body may in extreme cases seem practically bloodless. The writer recalls a case of fatal anemia in which, in order to secure a drop of blood large enough to fill the lumen of an erythrocytometer only to the 0.5 graduation, it was necessary to open a small superficial vessel of the scalp, repeated deep punctures of the fingers, toes, and ear-lobes having given negative results.

The drop as it emerges from the puncture wound is exceedingly pale, thin, and hydræmic, lacking the characteristic opacity of healthy blood, and being of a fluidity and general color which have been likened to those of meat-washings. In an occasional instance the color of the blood may be practically normal, or, rarely, of a brownish-red or chocolate tint; but, as a rule, it resembles a watery, pinkish fluid, deficient both in depth of color and in density. It has been frequently observed that, after having stood for a short length of time, the drop shows a tendency to separate into two more or less distinct parts, consisting of a dark stratum of corpuscles and a clear, watery-looking layer of plasma; or it may be irregularly mottled at different points, as if the corpuscles had become concentrated in isolated, compact groups in various parts of the plasma, thus producing the effect of alternating dark and light areas distributed through the drop.

Microscopical examination of the fresh film shows a great reduction in the number of the erythrocytes, together with the presence of many forms of these cells which exhibit every possible variation in size and in shape. The color of the individual erythrocyte varies, some being normally dark and well colored, while others appear as mere washed-out rings or "phantoms." In some of the cells the hemoglobin appears to be quite evenly distributed throughout the stroma, so that their typical biconcavity is obliterated. The endoglobular degenerative changes and those structural alterations denoting total necrosis of the erythrocytes, previously described, may be demonstrated with great clearness in this condition. Rouleaux formation is either entirely absent, or incomplete and atypical.

The erythrocytes are abnormally vulnerable, as shown by their increased isotonicity[1] and by the readiness with which their contained hemoglobin crystallizes.[2] Von Jaksch's analyses[3] point to a decided relative excess of cellular albumin.

Owing to the extreme oligocythemia common in pernicious anemia, it is advisable in making the films to use a somewhat larger drop of blood than is chosen for making ordinary spreads,

[1] Von Limbeck, *loc. cit.*
[2] Copeman, Brit. Med. Jour., 1901, vol. i, p. 161.
[3] Zeitschr. f. klin. Med., 1893, vol. xxiii, p. 187.

so that the field will not contain such a pronounced scarcity of cellular elements.

Coagulation.

The obvious fluidity of the blood, the deficiency of the fibrin network, and the slowness with which coagulation occurs are marked features of this disease. In fact, in some cases coagulation may be said not to occur at all, according to the experiments of Hayem[1] and others of the French school, as in the case quoted by Lenoble,[2] in which no clotting of a sample of arterial blood was observed even after a lapse of seventy-two hours after its withdrawal from the vessels. Many authors attribute considerable diagnostic value to this absence of clotting, and others go so far as to state that it renders a patient suffering with pernicious anemia especially prone to troublesome hemorrhages, even from a slight finger-prick—an accident which must be extremely rare, however, for it practically never complicates an ordinary clinical examination.

Specific Gravity.

The density of the whole blood is much below the normal standard, specific gravities as low as 1.027 having been reported. It is to be recalled that in cases with a high color index erroneous results may occur from attempting to estimate the hemoglobin percentage by Hammerschlag's table of equivalents, since the hemoglobin, in reality, is somewhat higher than the percentages corresponding to the specific gravity figures. (See p. 133.)

Alkalinity.

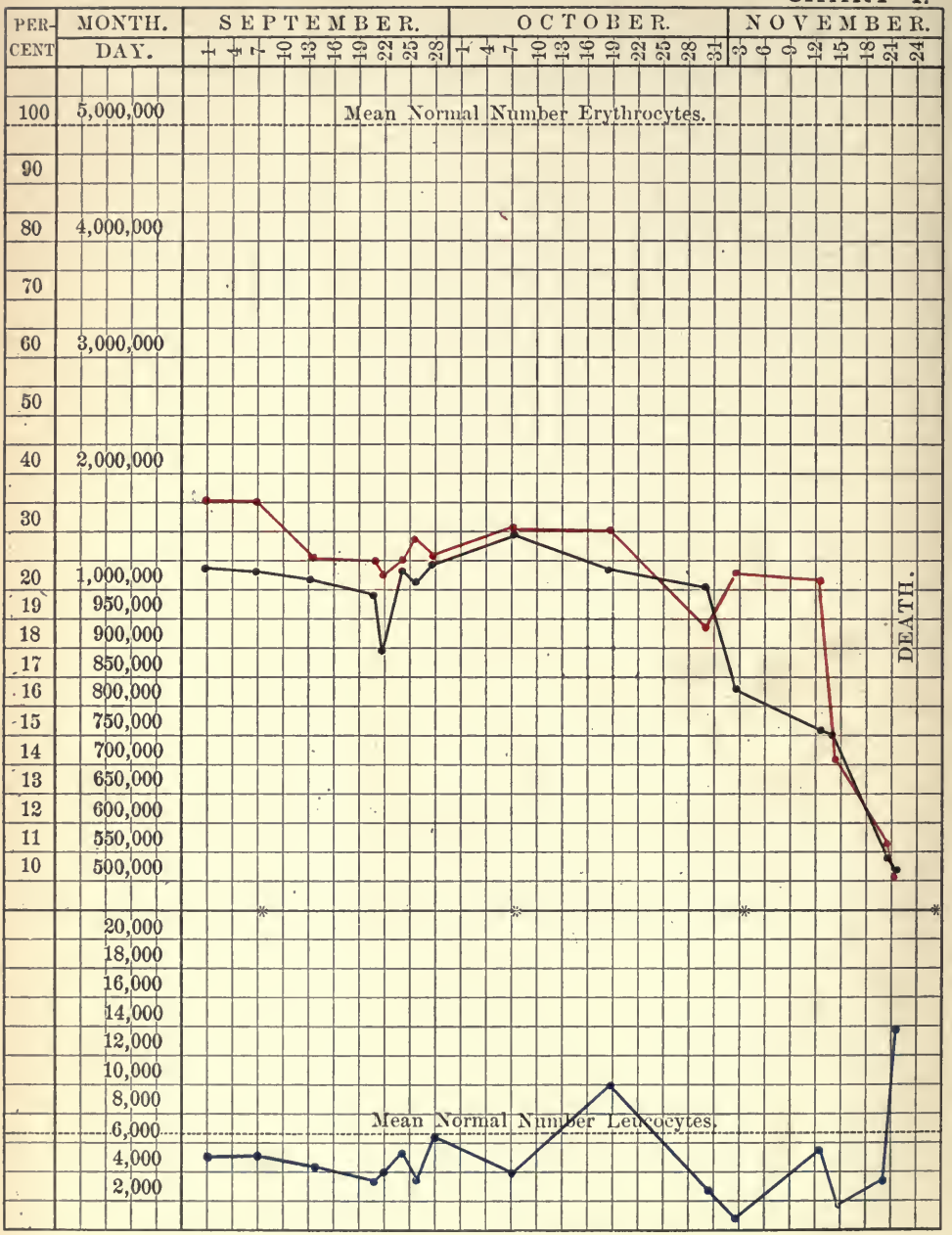
Up to the present time the reaction of the blood in pernicious anemia has not been very thoroughly studied, but the work already accomplished is sufficient to show that the alkalinity is much diminished, as in other severe anemias. That it may be strikingly below normal is shown by a case reported by Waldvogel,[3] who in one case estimated the alkalinity figure at 40 mgm., using Salkowski's method. This author has determined that the normal alkalinity for men ranges from 350 to 400 mgm., and for women from 300 to 350 mgm.

Hemoglobin and Erythrocytes.

Both the percentage of hemoglobin and the number of erythrocytes are greatly diminished, the former, as a general rule, relatively less so than the latter. Thus, inasmuch as the individual corpuscles contain often a normal or even an excessive amount of hemoglobin, it follows that high color indices are common—common but by no means constant, as seems to be

[1] *Loc. cit.* [2] "Charact. séméiol. du caillot et du sérum," Paris, 1898.
[3] Deutsch. med. Wochenschr., 1900, vol. xxvi, p. 685.

CHART I.




PERNICIOUS ANEMIA.

Red, Hemoglobin. Black, Erythrocytes. Blue, Leucocytes.

the current impression among many students, for although it is true that while the average color index is about 1.00 in pernicious anemia cases, the same statement cannot always be applied to the individual case. The author's series of 81 cases (Table IV) showed, at the first examination, hemoglobin percentages varying from a minimum of 10 to a maximum of 70, with a mean average of 27.1; the color index of these cases averaged 0.99. During remissions, as the erythrocytes increase, it is common to find low indices, this peculiarity being especially conspicuous should the improvement in the patient's condition be rapid, since in such instances the corpuscular increase is relatively much more rapid than the gain in hemoglobin. In cases in which improvement takes place more slowly, the color index is likely to remain higher, for here the corpuscles and the hemoglobin are more prone to increase proportionately along parallel lines.

TABLE IV.—HEMOGLOBIN AND ERYTHROCYTES IN 81 CASES OF PERNICIOUS ANEMIA.

| Hemoglobin Percentage | Number of Cases. | Erythrocytes per c.mm. | Number of Cases. |
|---|---|---|---|
| From 60–70 | 1 | Above 3,000,000 | 2 |
| " 50–60 | 2 | From 2,000,000–3,000,000 | 13 |
| " 40–50 | 4 | " 1,000,000–2,000,000 | 41 |
| " 30–40 | 16 | " 500,000–1,000,000 | 23 |
| " 20–30 | 30 | Below 500,000 | 2 |
| " 10–20 | 28 | | |
| Average, 27.1 per cent. | | Average, 1,361,777 per c.mm. | |
| Maximum, 70.0 " " | | Maximum, 3,240,000 " " | |
| Minimum, 10.0 " " | | Minimum, 450,000 " " | |

The oligocythemia is most striking, counts of from 1,000,000 to 2,000,000 erythrocytes per c.mm. being not uncommon when the patient first comes under observation, the number of cells frequently diminishing to about 750,000 or even 500,000 later during the course of the disease. In Quincke's often-quoted case the remarkable count of 143,000 per c.mm. was observed just before the death of the patient, an instance which is almost paralleled by a case recorded by Hills,[1] in which the erythrocyte count fell to 155,760 one day before death. In the series just mentioned (Table IV) the count of erythrocytes per c.mm. averaged 1,361,777, ranging between 450,000 and 3,240,000; this represents an average loss in corpuscular matter of somewhat less than 75 per cent., the greatest decrease amounting to 91 per cent. of normal—a much more striking oligocythemia than is found in any other form of anemia. Thirty per cent. of the cases showed a count of 1,000,000 or lower.

[1] Boston Med. and Surg. Jour., 1898, vol. cxxxix, p. 542.

Periods of temporary increase in the hemoglobin and erythrocytes, followed sooner or later by relapses, are commonly observed, the gain during such periods sometimes being very pronounced. Thus, in one of the cases tabulated above a gain of more than 2,500,000 erythrocytes to the c.mm. was noted during six weeks' time with a subsequent loss of over 1,000,000 cells in the following eight days, the color index during this time ranging from 1.25 to 0.74. Such stages of remission may or may not follow vigorous treatment by arsenic or other medicaments. Elder,[1] by the use of antistreptococcic serum, caused a gain of 4,000,000 cells per c.mm., while DeWitt,[1] by the same means, caused an increase in hemoglobin from 30 to 90 per cent. and in erythrocytes from 1,000,000 to 4,960,000 in three weeks' time.

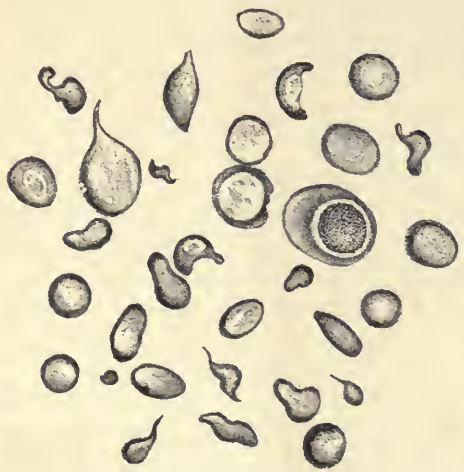


FIG. 52.—CHANGES IN THE ERYTHROCYTES IN PERNICIOUS ANEMIA (EHRLICH'S TRIACID STAIN).

Showing a general increase in the diameter of the corpuscles, and marked poikilocytosis. The nucleated cell in the right of the field is a megaloblast.

These periods of improvement in the condition of the blood are generally associated with an amelioration of the other clinical manifestations of the disease, the patient's general condition improving so substantially that he begins to consider himself on the high road to recovery, but in the course of time the old symptoms return, and the characteristic blood picture again becomes evident. In most cases death is preceded by extreme oligochromemia and oligocythemia, the hemoglobin often falling to 15 or 20 per cent. of normal and the erythrocyte count declining to 750,000 or less; in some cases, however, these losses are not so marked, and the count does not fall below 1,500,000 during the whole course of the disease.

A prominent characteristic of the blood in pernicious anemia is the wide dissimilarity in the size of the erythrocytes, due to the presence of large numbers of megalocytes and microcytes; so

[1] Cited by Packard and Willson, Amer. Jour. Med. Sci., 1902, vol. cxxiv, p. 1015.

striking may this feature of the blood picture be that it is sometimes difficult to find any two cells in the same field of the microscope which are of the same diameter. In the great majority of cases it will be found that the megalocytes distinctly outnumber the microcytes, to such an extent and in so large a proportion of cases that some writers consider this change an almost constant blood finding in this disease. Large erythrocytes, measuring slightly below or above 10 $\mu$ in diameter, are very common, while those measuring in the neighborhood of 15 $\mu$ or even 20 $\mu$ are met with more rarely. Undersized erythrocytes, about 3 or 4 $\mu$ in diameter, are also numerous, but, as remarked above, much less so than those of larger size. The presence of small, dark-colored, spherical microcytes of this size (the so-called "Eichhorst's corpuscles"), once regarded as pathognomonic of pernicious anemia, is neither constant nor diagnostic of this disease, since they are found in many other anemic conditions, and are absent in a large proportion of cases of true pernicious anemia. The fact that, of these alterations in the size of the erythrocytes, megalocytosis predominates, constitutes a sign of valuable diagnostic significance.

*Poikilocytosis*, to a more or less marked degree, is constantly observed, the conspicuousness of the deformities being in some cases extreme, while in others the change is a less notable feature. While marked poikilocytosis usually goes hand in hand with excessive diminution in the number of erythrocytes and in the amount of hemoglobin, the association of these three changes cannot be invariably counted upon, for in some cases, in spite of the fact that both oligocythemia and oligochromemia are marked, deformities in the shape of the corpuscles are but trifling. All varieties of erythrocytes, small, large, nucleated, and non-nucleated, may be deformed, so that the size of the poikilocytes varies from that of the smallest microcyte to that of the largest megalocyte. The kinds of deformity are of infinite variety, but it is still possible to designate certain well-defined forms which are especially common in this disease, these being the horseshoe form (Litten) and the oval form (Cabot), both of which varieties, while by no means peculiar to this condition, are found so frequently and in such abundance in pernicious anemia that their presence in the blood is at least highly suggestive. Of these two forms, the elongated, oval erythrocyte is found more constantly, and has been described in but a few other conditions. The author has been struck with the predominance of cells of this sort in three consecutive cases of purpura hæmorrhagica, in one of which the deformity was so marked that scarcely a

single normally shaped erythrocyte could be found in certain fields of the microscope. Cabot[1] cites Greene as noticing the same change in the blood of two patients in whom the tentative diagnosis of epidemic dropsy had been made. In addition to these well-defined varieties, many cells of other shapes, also met with in other severe anemias, are observed, notably those resembling the form of a sausage, a spindle, or a club. (See Fig. 52, p. 280.)

In the stained specimen the principal point of interest is the presence of *nucleated erythrocytes*, upon the character of which the diagnosis of pernicious anemia must depend. Erythroblasts are always to be found in this disease at some stage of its course.[2] During remissions, however, they may temporarily disappear. Megaloblasts are of much greater clinical significance than normoblasts, and by a differential count will be found always to outnumber them in every genuine case of pernicious anemia at some stage of the disease. This blood picture, which indicates a megaloblastic degeneration of the bone marrow, due in all probability to the influence of some unknown but specific toxic agency, is associated with only two other conditions, namely, nitrobenzol poisoning and some cases of high-grade anemia due to *Bothriocephalus latus* infection. The predominance of megaloblasts over normoblasts in pernicious anemia is well illustrated by Table V, which shows that at the first examination the former type of cells outnumbered the latter in 26 of the 29 cases here collected. The average proportion of megaloblasts to normoblasts in this series is somewhat more than 2 to 1, and in some cases the former were the only kind of erythroblast discovered. The total number of erythroblasts of all varieties averaged 220 per c.mm. of blood, ranging from as low as 3 to as high as 924. Regarding this last statement, it should be remembered that it is not the actual number of nucleated erythrocytes, but their character, which is all important in the diagnosis of this disease. In 52 additional cases of pernicious anemia a predominance of megaloblasts was found in 50, either at the first or by later examination, a total of 76 megaloblastic bloods in the 81 cases studied. Of 139 cases reported by Cabot, megaloblasts predominated in 109 at the first examination, and in all but 3 of the remaining 30 cases at a subsequent period.

[1] *Loc. cit.*

[2] In Ehrlich's "aplastic anemia," a fatal type with profound oligocythemia, leucopenia, and purpura, the marrow so utterly fails to compensate the blood loss that erythroblasts of all varieties are wanting. Such cases are obviously not typical pernicious anemia.

TABLE V.—APPROXIMATE NUMBER OF NUCLEATED ERYTHROCYTES PER C.MM. IN 29 CASES OF PERNICIOUS ANEMIA AT THE FIRST EXAMINATION.

| Number. | Total. | Megaloblasts. | Normoblasts. | Microblasts. |
|---|---|---|---|---|
| 1 | 924 | 693 | 210 | 21 |
| 2 | 840 | 616 | 140 | 84 |
| 3 | 544 | 512 | 16 | 16 |
| 4 | 470 | 320 | 150 | 0 |
| 5 | 368 | 207 | 46 | 115 |
| 6 | 336 | 240 | 90 | 6 |
| 7 | 328 | 0 | 328 | 0 |
| 8 | 260 | 20 | 240 | 0 |
| 9 | 256 | 144 | 80 | 32 |
| 10 | 250 | 180 | 70 | 0 |
| 11 | 235 | 175 | 60 | 0 |
| 12 | 224 | 168 | 56 | 0 |
| 13 | 204 | 148 | 56 | 0 |
| 14 | 200 | 160 | 40 | 0 |
| 15 | 192 | 180 | 12 | 0 |
| 16 | 160 | 96 | 64 | 0 |
| 17 | 101 | 80 | 21 | 0 |
| 18 | 100 | 0 | 100 | 0 |
| 19 | 96 | 73 | 23 | 0 |
| 20 | 67 | 47 | 13 | 7 |
| 21 | 60 | 40 | 20 | 0 |
| 22 | 48 | 32 | 16 | 0 |
| 23 | 48 | 37 | 7 | 4 |
| 24 | 32 | 24 | 8 | 0 |
| 25 | 20 | 20 | 0 | 0 |
| 26 | 15 | 15 | 0 | 0 |
| 27 | 10 | 8 | 0 | 2 |
| 28 | 6 | 6 | 0 | 0 |
| 29 | 3 | 3 | 0 | 0 |
| Average: | 220 + | 146 + | 64 +. | 10 |

Microblasts are rare in comparison to the other forms of nucleated erythrocytes; in some cases they may be relatively numerous, but in the majority they are absent. They were noted in but 9 of the 29 cases tabulated above (Table V), their average number for the series being 10 to the c.mm. In the differential count of nucleated erythrocytes microblasts should be totaled with normoblasts, of which they are simply degenerate forms, more or less stripped of their protoplasm, and hence irregular and ragged in outline.

In addition to the foregoing types of erythroblasts, cells possessing the characteristics of both the normoblast and the megaloblast may be observed in many instances. These atypical forms and their clinical significance have been described in a previous section. (See p. 192.) In certain corpuscles, both of the normoblastic and of the megaloblastic types, division of the nu-

cleus into several parts may have occurred, and in rare instances evidences of true karyokinesis may be seen. Normoblasts showing complete or partial nuclear extrusion and separation of the nucleus into a clover-leaf design are not uncommon, although pictures of this sort are found much more frequently in leukemia. In many cases of pernicious anemia one cannot but be struck with the fact that the majority of these atypical forms appear as cells with a megaloblastic protoplasm and a normoblastic nucleus; they are, in the author's experience, much more numerous in this disease than cells having a normoblastic protoplasm and a megaloblastic nucleus, the latter being more common in leukemia.

TABLE VI.—QUALITATIVE CHANGES IN THE LEUCOCYTES IN 31 CASES OF PERNICIOUS ANEMIA AT THE FIRST EXAMINATION.

| No. | Leucocytes per c.mm. | Percentage of Different Forms. | | | | |
|---|---|---|---|---|---|---|
| | | Small Lymphocytes. | Large Lymphocytes. | Polynuclear Neutrophiles. | Eosinophiles. | Myelocytes. |
| 1 | 13,000 | 43.2 | 2.8 | 49.6 | 2.8 | 1.6 |
| 2 | 8,200 | 7.7 | 2.0 | 86.2 | 1.6 | 2.5 |
| 3 | 7,000 | 14.4 | 1.6 | 81.6 | 2.0 | 0.4 |
| 4 | 7,000 | 13.6 | 5.2 | 77.6 | 2.8 | 0.8 |
| 5 | 6,400 | 34.0 | 4.0 | 60.0 | 1.0 | 1.0 |
| 6 | 6,000 | 11.0 | 2.0 | 84.0 | 1.0 | 2.0 |
| 7 | 5,800 | 14.0 | 3.0 | 73.0 | 0.0 | 10.0 |
| 8 | 5,400 | 22.1 | 7.5 | 67.4 | 2.0 | 1.0 |
| 9 | 5,000 | 32.8 | 3.6 | 55.6 | 5.2 | 2.8 |
| 10 | 4,600 | 56.5 | 6.0 | 34.5 | 0.0 | 3.0 |
| 11 | 4,100 | 53.0 | 6.4 | 39.6 | 0.0 | 1.0 |
| 12 | 4,000 | 30.0 | 14.5 | 52.4 | 2.0 | 1.1 |
| 13 | 4,000 | 26.8 | 15.5 | 56.8 | 0.9 | 0.0 |
| 14 | 4,000 | 23.0 | 16.6 | 58.2 | 1.2 | 1.0 |
| 15 | 4,000 | 15.0 | 5.0 | 77.5 | 0.5 | 2.0 |
| 16 | 4,000 | 34.0 | 9.0 | 45.0 | 8.0 | 4.0 |
| 17 | 4,000 | 16.3 | 8.1 | 72.6 | 2.0 | 1.0 |
| 18 | 3,100 | 19.7 | 23.0 | 54.0 | 1.0 | 2.3 |
| 19 | 3,000 | 10.8 | 20.0 | 60.0 | 7.2 | 2 0 |
| 20 | 2,500 | 45.0 | 12.0 | 40.0 | 1.0 | 2.0 |
| 21 | 2,300 | 22.1 | 16.1 | 60.8 | 0.0 | 1.0 |
| 22 | 2,100 | 32.1 | 21.3 | 40.7 | 1.6 | 4.3 |
| 23 | 2,080 | 45.0 | 14.7 | 38.3 | 1.0 | 1.0 |
| 24 | 2,000 | 65.0 | 20.0 | 14.1 | 0.0 | 0.9 |
| 25 | 2,000 | 14.5 | 14.5 | 69.5 | 1.0 | 0.5 |
| 26 | 1,500 | 25.0 | 14.0 | 61.0 | 0.0 | 0.0 |
| 27 | 1,100 | 19.1 | 12.1 | 67.7 | 0.3 | 0.8 |
| 28 | 1,000 | 17.0 | 21.0 | 58.0 | 2.0 | 2.0 |
| 29 | 1,000 | 13.0 | 11.0 | 72.0 | 1.0 | 3.0 |
| 30 | 1,000 | 20.0 | 9.0 | 70.0 | 0.5 | 0.5 |
| 31 | 500 | 37.5 | 18.5 | 40.8 | 2.3 | 0.9 |
| Average: | 3,925 + | 26 + | 10 + | 58 + | 1 + | 1 + |

Fluctuations in the total number of erythroblasts occur from time to time during the progress of the disease, these changes sometimes taking place with great abruptness, being of wide range and often carrying not the slightest clinical import. A marked increase usually but not invariably precedes and accompanies a gain in the number of erythrocytes and in the percentage of hemoglobin; and a similar increase, usually associated with extreme diminution in the erythrocyte count, is commonly met with as a preagonal sign.

Marked evidences of *polychromatophilia* are found in many of the erythrocytes, both of the nucleated and of the non-nucleated varieties. Such cells, when stained with Ehrlich's triacid mixture, instead of taking the normal orange color of the solution, stain some bastard tint, such as slate color, dull purple, or dirty gray. Others may show a peculiar, streaked appearance, and irregular, pale, unstained areas, while others are scarcely stained at all, the greater part of the protoplasm remaining an indefinite shade of dead white. *Granular degeneration* of the protoplasm is distinctly evidenced in some of the cells, this process being betrayed by the appearance through the stroma of granular areas showing a striking affinity for a basic stain, such as methylene-blue. These basophilic granules, which have already been described, are not peculiar to pernicious anemia, since they have been found in a large number of secondary anemias of severe type due to various causes. (See p. 194.)

The same remarks apply to the presence of erythrocytes containing the "ring bodies" of Cabot. This author[1] reports having found them in 9 of 14 cases of pernicious anemia examined during the active stages, but he failed to detect them in 4 cases in the stages of remission.

TABLE VII.—NUMBER OF LEUCOCYTES IN 81 CASES OF PERNICIOUS ANEMIA

| Leucocytes per c.mm. | Number of Cases. |
|---|---|
| From 10,000–15,000 | 3 |
| " 5,000–10,000 | 25 |
| Below 5,000 | 53 |
| Average, 4527 per c.mm. | |
| Maximum, 15,000 " " | |
| Minimum, 500 " " | |

Leucopenia may be counted on in considerably more than one-half of all cases of pernicious anemia, a fact which stands in direct contrast to the anemias of secondary type, in which an increase in the number

Leucocytes.

[1] Boston Med. and Surg. Jour., 1904, vol. cl, p. 321.

of leucocytes is more common. In an occasional case, especially in one in which the other blood changes are inconspicuous, the number of leucocytes is found to be normal; and, rarely, a moderate leucocytosis, attributable to some complication, exists. In the average case, however, these cells are distinctly below the normal standard, and the degree of leucopenia is sometimes extreme, the number of cells occasionally falling to below 1000 to the c.mm.; in rare instances they may apparently be entirely absent, none being found after prolonged search through both the counting chamber and the stained film. In the 81 cases collected in Table VII the number of leucocytes averaged about the mean low normal count (4527 being the exact figure), and ranged, in the individual case, from 500 to as high as 15,000 to the c.mm. It is interesting to note, in connection with the preceding remarks, that leucopenia was found in 53 or 65.4 per cent. of these cases.

The leucocyte count, except in the event of complications, roughly parallels that of the erythrocytes, falling coincidentally with the oligocythemia and rising again as the erythrocytes increase. (See chart, p. 279.) An exception to this general rule is found in the terminal leucocytosis which not uncommonly develops just before the death of the patient.

*Relative lymphocytosis* is a common, but not a constant, finding in the differential count of the stained film. It seems to be more frequently associated with low than with high counts, although no hard-and-fast rule can be laid down regarding this point. In extremely leucopenic blood a noteworthy finding is the abnormally high percentage of large mononuclear non-granular cells, a change which does not ordinarily take place in connection with leucocyte counts approaching the normal average. The combined percentage of both large and small forms of lymphocytes in Table VI averaged 37.8, individual counts varying from 9.7 to 85 per cent. The writer has noted the frequent occurrence of small lymphocytes containing coarse basic granules. Such cells appear to be especially common in this form of anemia. Preagonal rises in the leucocyte count are sometimes lymphocytic in character, resembling the blood changes seen in lymphatic leukemia, and sometimes purely polynuclear in type. The relative percentage of *polynuclear neutrophiles* averages low (58.6 per cent. in the above series), but isolated counts show a considerable range; their relative proportion to the other forms of leucocytes is largely determined by the fluctuations in the percentage of lymphocytes. The *eosinophiles* are almost invariably decreased, and not infrequently they are wholly wanting, a circumstance which was made note of in more

than 18 per cent. of the cases in the present series, in which the average percentage of these cells was 1.68. In an occasional case their percentage is above normal, as in cases 9, 16, and 19 in Table VI.

In no other disease save the myelogenous form of leukemia are *myelocytes* so constantly found, but almost always in relatively small percentages. In the cases under consideration these cells were absent in only two instances, the average figure for the 31 cases being 1.82 per cent. In a single case (number 7) the remarkably high estimate (for this disease) of 10 per cent. of myelocytes was made, for in the other cases in which myelocytes occurred their percentage ranged from 0.4 to 4.3.

In the stained specimen it is common to find that the leucocytes, particularly the polynuclear neutrophiles and the myelocytes, are of smaller size and more deeply stained than they appear in normal blood. This peculiarity seems to be more constant and more striking in pernicious anemia than in any other disease.

BLOOD PLAQUES. The number of blood plaques is exceedingly variable, so that it is impossible to make definite statements regarding either the increase or the decrease of these bodies. In some cases they apparently are greatly increased, as evidenced by the groups of agglutinated masses of these cells which are sometimes seen (von Limbeck[1]), but in other cases it is evident that their number is appreciably diminished (Hayem[2]). Van Emden[3] supports the latter view. In one case this observer estimated their number at between 32,000 and 64,000 per c.mm.

DIAGNOSIS. In a typical case of pernicious anemia the blood picture upon which the diagnosis rests is as follows:

*Hemoglobin.* Marked absolute decrease, but of relatively higher percentage than that of the erythrocytes, this giving rise to a high color index. Striking de-

*Erythrocytes.* crease, commonly to 1,000,000 or less per c.mm. Counts of about 500,000 are not uncommon during the later stages of the disease. Erythroblasts constant, cells of the megaloblastic type predominating.

Megalocytes and microcytes, the former prevailing.

Poikilocytes, usually numerous and conspicuous.

Polychromatophilia.

[1] *Loc. cit.*

[2] "Leçons sur les Maladies du Sang," Paris, 1900.

[3] "Bijd. t. d. ken. v. h. bloed," Leyden, 1896.

Basophilic stroma degeneration striking in severe cases.

*Leucocytes.* Usually decreased; decided leucopenia common. Relative lymphocytosis in the majority of cases. Small numbers of myelocytes almost invariably present.

Eosinophiles few, sometimes absent.

*Plaques.* Variable.

Usually the diagnosis of pernicious anemia presents no difficulties, and may be made by the examination of the blood alone, the association of marked oligocythemia, a high color index, leucopenia, and erythroblasts, chiefly of the megaloblastic variety, constituting a typical group of blood changes the significance of which is unmistakable.

It should be borne in mind, however, that these changes are not always present in every case when the patient first comes under observation, so that repeated and careful examinations of the blood are sometimes necessary before a diagnosis is possible. Of the above-named changes, the most important, from a clinical viewpoint, is the prevalence of nucleated erythrocytes conforming to the megaloblastic type. With the two exceptions already noted (bothriocephalus anemia and nitrobenzol poisoning) this "megaloblastic blood picture" is seen only in pernicious anemia, and, what is more important, it occurs in every true case of this disease sooner or later during its course. Inability to detect this important characteristic should be regarded rather as a reflection upon the thoroughness of the examiner's technic than as a contradiction of the truth of this statement. Erythroblasts are not always numerous in pernicious anemia, and painstaking and prolonged study of several stained films may be necessary before this important feature is distinguishable.

In those cases of doubtful nature in which the typical blood changes are not at once evident a tentative diagnosis may be made by taking into careful consideration certain other physical signs and symptoms which the patient presents. In such instances attention should be directed to such suspicious points in the clinical history as the existence of a severe anemia arising either idiopathically or without adequate cause, and pursuing a progressively unfavorable course, uninfluenced permanently by treatment; the presence of a light lemon-yellow tint of the skin, of retinal hemorrhages, of a peculiarly soft, smooth, flabby condition of the skin, and sometimes of moderate febrile paroxysms and gastric disturbances; and the remarkable preservation of the patient's general nutrition and body-weight in comparison with the severity of the illness.

The *severe secondary anemias* due to hemorrhage, to advanced syphilis, and to malignant disease, especially of the stomach, sometimes give rise to clinical symptoms which so exactly simulate pernicious anemia that the diagnosis must rest upon the result of the blood findings, which are usually well enough marked to differentiate the conditions. It is true that in these conditions ample proof of sufficient etiological factors for the production of the anemia is generally at hand, and this fact should have important bearing in ruling out anemia of the pernicious type, but it is also equally true that in malignant disease it is sometimes impossible to demonstrate the lesion, and that in syphilis the clinical history may be obscure, so that the blood examination must, after all, often be depended upon for an accurate diagnosis. In secondary anemia from the above causes the oligocythemia is seldom so excessive as it is in pernicious anemia, the erythrocytes rarely falling as low as 1,000,000 per c.mm.; the oligochromemia is likely to be relatively greater than the oligocythemia, so that a lower color index results; leucocytosis is not uncommon; and while deformities of shape and size and nucleation of the erythrocytes are frequently present, in some instances to as great an extent as in pernicious anemia, megalocytes do not predominate, nor do megaloblasts ever outnumber normoblasts.

From *chlorosis*, which sometimes possesses many clinical manifestations in common with pernicious anemia, the diagnosis may usually be readily made by the blood examination, which shows decided differences between the two diseases. In a typical case of chlorosis the deterioration in the quality of the blood affects chiefly the hemoglobin content of the erythrocytes and not the cells themselves. Hence it is common to find in this disease extreme oligochromemia out of all comparison with the more moderate oligocythemia, and consequently a low color index—just the reverse of the condition found in pernicious anemia. Deformities in the shape and size of the erythrocytes are not uncommon in chlorosis, but they are not likely to be conspicuous; the prevalent change affecting their shape is microcytosis of a moderate grade, so that a general decrease in the diameter of these cells is commonly observed. The most important information derived from the blood is, however, of a negative character, consisting in the fact that nucleated erythrocytes, should they be present, are chiefly normoblasts. While it is true that an occasional megaloblast may be encountered in rare instances, no chlorotic blood has ever been known to show a predominance of this type of cells. The behavior of the leuco-

cytes in chlorosis is of no aid in the differentiation of this condition from pernicious anemia, for in both diseases the count is usually low and relative lymphocytosis common; in the former, however, the pronounced leucopenia of the latter condition is not often found. Myelocytes, while they may occur in both diseases, are much less common in chlorosis.

In *bothriocephalus anemia* the expulsion of the parasite by the administration of an appropriate vermifuge is soon followed by a radical change in the blood picture and other symptoms, the megaloblasts disappearing, the hemoglobin and erythrocytes quickly rising to the normal standard, and the patient's health becoming entirely restored. The history of a patient suffering from anemia due to *nitrobenzol poisoning* is sufficiently characteristic to exclude true pernicious anemia. The differential diagnosis between this disease and *splenic anemia* is considered in another place. (See p. 295.)

Several reputed instances of the conversion of pernicious anemia into leukemia have been reported by Leube and Fleischer,[1] Waldstein,[2] and Litten.[3] Some such cases probably belong, as also do many cases of acute leukemia, to Leube's symptom-complex, "leukanemia"[4]—high-grade anemia plus either myelemia or lymphocytosis, with fever, hemorrhage, stomatitis, and hyperplasia of the spleen and lymphatics. That pernicious anemia is ever converted into leukemia is questionable, and it seems reasonable to regard such a change as apparent rather than real. For example, in a case of leukemia, transient disappearance of the myelemia, with persistence of the anemia, such as may occur either spontaneously or from treatment, gives a blood picture not unlike that of true pernicious anemia, and a case of this sort may appear to become converted into leukemia, as the temporarily suppressed leukemic blood changes redevelop in course of time. A marked leucocytosis engrafted upon pernicious anemia may also be mistaken for the transformation of this disease into leukemia, but it is obvious that this counterfeit can be detected by a differential count of the leucocytes.

[1] Virchow's Arch., 1881, vol. lxxxiii, p. 124. [2] *Ibid.*, 1883, vol. xci, p. 12.
[3] Berlin. klin. Wochenschr., 1877, vol. xiv, p. 256.
[4] Münch. med. Wochenschr., 1900, vol. xlvii, p. 1121. See also Arneth, Deutsch. Arch. f. klin. Med., 1901, vol. lxix, p. 331; Luce, *ibid.*, 1903, vol. lxxvii, p. 215; F. B. Weber, Brit. Med. Jour., 1904, vol. i, p. 1416; Kormoczi, Deutsch. med. Wochenschr., 1899, vol. xxv, pp. 238 and 775; Hitschman, Zeitschr. f. Heilk., 1903, vol. xxiv, p. 190.

## III. SPLENIC ANEMIA.

APPEARANCE OF THE FRESH BLOOD.

There is nothing distinctive about the appearance of the drop of freshly drawn blood, the color and density of which vary with the intensity of the anemia present. The author has notes of a case of splenic anemia in which it was remarked that, from its color and general appearance, the blood drop resembled precisely that obtained from a typical case of high-grade pernicious anemia; in a second case the color and opacity were but slightly below normal.

No reliable observations have thus far been made regarding such minor points as the rate of coagulation, the specific gravity, and the reaction of the blood in this form of anemia.

HEMOGLOBIN AND ERYTHROCYTES.

Decided, often extreme, anemia is the general rule in this disease. In the early stages the hemoglobin loss is relatively excessive as compared to the decrease in erythrocytes, so that the color index is consequently low—usually approximating the figures found in many cases of high-grade secondary anemia, but not averaging so low as in chlorosis. As the disease increases in severity, however, the color index tends to rise, as in pernicious anemia, this change being illustrated by the counts tabulated below.

In a series of 15 cases reported by Osler[1] the following results were obtained: the hemoglobin in 13 cases averaged 47 per cent., the lowest estimate being 23 and the highest 60 per cent.; 42 erythrocyte counts averaged 3,336,357 per c.mm., with extremes of 2,000,000 and 5,200,000. These figures are very closely approximated by the averages of 35 cases collected by Lichty[2]: hemoglobin, 47 per cent.; erythrocytes, 3,293,000; and leucocytes, 5594. Similar changes were found in series of cases reported by Rolleston[3] and by Frederick Taylor.[4]

In a case of splenic anemia in Professor Hare's ward at the Jefferson Hospital the writer found the following changes in five consecutive counts:

| DATE. | HEMOGLOBIN. | COLOR INDEX. | ERYTHROCYTES PER C.MM. |
|---|---|---|---|
| March 7, 1898 | 45 per cent. | 0.82 | 2,750,000 |
| March 14, 1898 | 40 " " | 0.73 | 2,725,000 |
| March 22, 1898 | 43 " " | 0.76 | 2,812,000 |
| March 29, 1898 | 45 " " | 0.69 | 3,275,000 |
| April 11, 1898 | 40 " " | 1.00 | 2,000,000 |

[1] Amer. Jour. Med. Sci., 1900, vol. cxix, p. 54; *ibid.*, 1902, vol. cxxiv, p. 781.
[2] Jour. Amer. Med. Assoc., 1904, vol. xlii, p. 528.
[3] "Splenic Anemia," London, 1902.
[4] Lancet, 1904, vol. i, pp. 1477, 1554, and 1636.

*Deformities* affecting the size of the erythrocytes, sometimes tending toward striking megalocytosis, may be met with in cases characterized by great oligocythemia, such an alteration being also associated with a greater or less degree of poikilocytosis, and with signs of stroma degeneration. *Nucleated erythrocytes*, although they occur infrequently, may be present in enormous numbers in severe cases, creating a blood picture which is distinguishable from that of true pernicious anemia only by the fact that normoblasts predominate. Thus, in one of McCrae's counts in a case of Osler's, in which the hemoglobin was reduced to 20 and the erythrocytes to 27.6 per cent., no fewer than 75 erythroblasts (of which 21 were normoblasts, 19 megaloblasts, and 35 "intermediate" forms) were seen while counting 400 leucocytes. In the case above summarized the average number of erythroblasts per 1000 leucocytes was estimated as 67 (the maximum and minimum being 128 and 9, respectively) for the five examinations, 41 of these cells being normoblasts and 26 megaloblasts. The presence of nucleated erythrocytes in such large numbers as were found in these two instances must, however, be regarded as most exceptional. *Polychromatophilia* of many of the erythrocytes may be a striking feature in advanced cases, but in those of a milder grade the phenomenon is absent. Absence of basophilic *granular degeneration* of the cells has been noted by Cohn.[1]

The transition of splenic anemia into lymphatic leukemia has been recorded twice in medical literature, according to Zypkin,[2] who himself encountered such a change.

LEUCOCYTES. Leucopenia, sometimes pronounced, is the characteristic finding, counts of from 2000 to 4000 cells to the c.mm. being common; as in pernicious anemia, the lowest leucocyte counts are generally associated with those cases in which the anemia is most intense. In the adult leucocytosis occurs only as the effect of some complication, and therefore is but occasionally encountered. In Osler's series, above referred to, the number of leucocytes, determined in 14 cases, averaged 4520 per c.mm., ranging from 2000 to 12,497, the latter estimate being the only one exceeding 10,000; in 9 of the cases the count fell below 5000. In the writer's case the five counts averaged 2400, varying from 1000 to 4000. In children, also, leucopenia is the rule, in spite of a child's tendency to develop leucocytosis upon the slightest provocation. (See p. 347.)

[1] Münch. med. Wochenschr., 1900, vol. xlvii, p. 618.
[2] Wien. klin. Wochenschr., 1903, vol. xvi, p. 577.

No constant differential changes have been observed, but *relative lymphocytosis* is not infrequent, sometimes involving chiefly the large, and sometimes the small, forms of these cells. The proportion of both combined may be as high as 50 or 60 per cent., an increase of this kind bringing about a consequent fall in the relative percentage of *polynuclear neutrophiles*. Small numbers of *myelocytes*, rarely in excess of a fraction of one per cent., are to be expected in cases with decided oligocythemia. The *eosinophiles* remain at about the normal standard. Typical coarsely granular *mast cells* are sometimes found in relatively large numbers —as high as 5 or 6 per cent. of all forms of leucocytes.

No special observations concerning the behavior of the *blood plaques* in this disease have been recorded up to the present time. It is evident, however, that they are not notably increased in number.

DIAGNOSIS. To recapitulate, the blood changes which have been most frequently found in splenic anemia may be tabulated as follows:

*Hemoglobin.* Marked diminution; color index variable.

*Erythrocytes.* Usually reduced moderately, sometimes excessively. Counts between 3,000,000 and 4,000,000 cells per c.mm. are most common.
Deformities of shape, especially megalocytosis, and poikilocytosis common in advanced cases.
Erythroblasts rare, except in cases with decided oligocythemia. Normoblasts invariably predominate.
Polychromatophilia in severe cases.

*Leucocytes.* Leucopenia the general rule.
Relative lymphocytosis common.
Small numbers of myelocytes in advanced cases.
Relatively large percentages of mast cells not uncommon.
Eosinophiles normal.

*Plaques.* Not increased.

Many writers still hesitate to assign to splenic anemia the rôle of a definite clinical entity, choosing to regard the condition either as a splenic form of Hodgkin's disease or as high-grade secondary anemia with marked splenic hyperplasia. But of late the majority of observers lean toward at least a tentative recognition of the condition as a distinct although an obscure disease.[1] Osler,[2]

[1] For a critical résumé of the literature on splenic anemia, Sippy's article in the American Journal of the Medical Sciences, 1899, vol. cxviii, p. 570, should be consulted.

[2] *Loc. cit.*

well expresses the consensus of opinion when he remarks that "provisionally, until we have further knowledge, it is useful to group together . . . cases of idiopathic enlargement of the spleen with anemia without lymphatic involvement, and to label the condition splenic anemia." Banti's disease, or the terminal stage of splenic anemia, is characterized by hypertrophic liver cirrhosis with jaundice and by ascites.

It is quite obvious, from a glance at the above synopsis of the blood condition, that splenic anemia presents no characteristic blood picture by which the diagnosis can be made, so that in order to differentiate it from a number of other diseases which it more or less closely simulates, careful study of other clinical features is essential.

The onset of splenic anemia is gradual and insidious, its course is prolonged often for a number of years, and its termination is ultimately fatal. The principal clinical features are the leucopenic anemia, the great splenic tumor, and the absence of enlargement of the superficial lymphatics. In some instances the anemia develops in advance of the splenic tumor, but it is more often the case that the enlargement of the spleen is the earliest demonstrable lesion. The anemia is responsible for such symptoms as dyspnea, vertigo, cardiac palpitation, loss of strength and appetite, and the occurrence of unexplained, irregular periods of fever; and for such signs as hemic heart murmurs, pallor, lemon-yellow discoloration of the skin and mucous membranes, and sometimes pigmentation of the skin. The enlarged spleen may extend as far down as the umbilicus, and sometimes far below this point, even to the iliac crests; the surface of the organ is smooth and free from nodules, its consistence is firm, and its shape is unaltered. It may give rise to no symptoms, but occasionally it is the cause of great pain and of hematemesis, the latter being due to simple mechanical congestion. Epistaxis, purpura, and hematuria have also been observed. Ascites sometimes develops, as the result either of the splenic enlargement or of the anemia. Enlargement of the liver, usually associated with decided icterus, occurs as a terminal symptom, and such gastrointestinal disturbances as anorexia, nausea, vomiting, and both constipation and diarrhea are extremely common. Splenic anemia may prove fatal within six months after the onset of the initial symptoms, or it may drag along for as many years, but as a general rule its duration does not exceed two or three years. In one of Osler's cases the condition probably lasted for at least twelve years, and in a case recently treated in the Jefferson Hospital the splenic tumor and the anemia existed for six years,

if not longer. As in pernicious anemia, periods of remission during which the leading symptoms disappear and the quality of the blood improves are commonly observed in this condition.

The *myelogenous form of leukemia*, *pernicious anemia*, and *Hodgkin's disease* with splenic enlargement all present clinical features counterfeiting more or less faithfully splenic anemia, but the differential diagnosis between these conditions does not involve any great difficulty. The result of the blood examination gives the clue to the two diseases first named, the myelocytic type of blood in leukemia and the predominance of megaloblasts in pernicious anemia being sufficient to fix the identity of these conditions. In Hodgkin's disease with enlargement of the spleen there is more or less marked enlargement of the superficial lymphatic glands, and the splenic tumor rarely attains the size to which it grows in splenic anemia; the blood picture of the two conditions, it must be recalled, may be identical.

Enlargements of the spleen due to such factors as *chronic malarial infection*, *amyloid disease*, *malignant growths*, *echinococcus cysts*, and *hepatic cirrhosis* also occasionally require differentiation from splenic anemia. A history of previous attacks of malarial fever and the detection of the specific parasite or of pigment in the blood will serve to distinguish tumors of the spleen of malarial nature. In amyloid disease a history of long-standing suppuration, of tuberculosis, or of syphilis, and the presence of signs indicating amyloid involvement of other organs, notably the liver, kidneys, and intestines, are the chief differentiating features. In malignant disease of the spleen the tumor is uneven, irregular, and nodular, evidences elsewhere of malignant lesions generally exist, and a well-defined leucocytosis is common. Echinococcus disease of the spleen pursues a protracted course unaccompanied by signs of anemia, but generally shows decided eosinophilia, and, unless secondary infection takes place, is unassociated with rises in temperature; fluctuation of the tumor can frequently be detected, and hooklets can be recognized in the fluid obtained from the organ by aspiration. In splenic enlargements associated with the different varieties of hepatic cirrhosis, the previous history and the cachexia of the patient, the relatively moderate size of the tumor, the signs of portal congestion, the condition of the liver, and the course of the disease should be taken into account.

## IV. SECONDARY ANEMIA.

Appearance of the Fresh Blood.

An approximate idea of the intensity of the anemia may usually be formed by noting the gross appearance of the fresh blood drop, but it must be remembered that it is only when the process has reached a comparatively high grade of development that the fact is betrayed by any marked deviation from normal in the color and density of the blood. In the average case of well-marked secondary anemia the color of the drop is but slightly paler than normal, if, indeed, it is visibly altered; but if the anemia is of decided severity, it may resemble a thin, serum-colored liquid streaked with crimson, similar to the watery blood drop of typical pernicious anemia. In such cases microscopical examination of the fresh film shows that there is little or no tendency toward rouleaux formation.

Coagulation.

In general terms it may be stated that the rapidity of coagulation bears a direct relation to the grade of the anemia, since it has been determined that the greater the oligochromemia and oligocythemia, the more rapid the process of clotting. In secondary anemias with erythrocyte counts under 1,000,000 Lenoble[1] found that coagulation was, as a rule, at least twice as rapid as normal.

Specific Gravity.

The specific gravity of the whole blood is reduced, a change which is dependent chiefly upon the loss of hemoglobin. Sufficient reference has already been made to this subject in a previous section. (See p. 132.)

Alkalinity.

The majority of authors maintain that the alkalinity of the blood is decreased in relation to the degree of the anemia, and a large number of experiments in anemias due to various factors apparently justify this general belief. But several careful investigators, notable among whom is Löwy,[2] have contradicted these reports, having found the alkalinity normal or even above normal in numerous cases. The author quoted, for example, calculated the alkalinity in various cases of secondary anemia at from 360 to 675 mgm. NaOH, as compared with his normal standard, 447 to 508 mgm.

Hemoglobin and Erythrocytes.

Taking the ordinarily well-developed case of secondary anemia as an example, it is found that the hemoglobin percentage and number of erythrocytes are both decidedly, though not strikingly, diminished. As the former usually shows a dis-

[1] *Loc. cit.*

[2] Centralbl. f. d. med. Wissensch., 1894, vol. xxxii, p. 785.

proportionately greater loss than the latter, subnormal color indices are the rule, ranging, say, from about 0.75 to 0.85. In anemias of severer type, such as those due to gastric cancer and to enteric fever, the losses frequently are much more exaggerated, and, in so far as the purely quantitative changes in the erythrocytes and their hemoglobin equivalent are concerned, the blood-picture of true pernicious anemia may be counterfeited. In the anemias of syphilis, of tuberculosis, and of malignant disease in general the disproportionate hemoglobin loss may be so decided that the blood changes cannot be distinguished from those of chlorosis, and to this condition the much-abused term "chloro-anemia" has been applied.

The fact must be emphasized that simply the hemoglobin estimate and erythrocyte count alone are absolutely uncharacteristic in secondary anemias, for they may range in the individual case from slightly subnormal figures to an extreme degree of oligochromemia and oligocythemia. In a patient studied by von Limbeck,[1] for example, at one time the erythrocytes numbered only 306,000 per c.mm., but ultimately perfect recovery ensued and the count rose to 4,280,000. But if averages are used as a basis for conclusions, it becomes evident that the hemoglobin diminution is less marked than in any other blood disease, and that the erythrocyte loss is also less than in any other form of anemia except chlorosis. Data based upon 200 examinations of various types of anemia by the writer give the following results regarding these points:

| Disease. | Average Percentage of Hemoglobin Loss in 50 Consecutive Estimates. | Average Percentage of Erythrocyte Loss in 50 Consecutive Counts. |
|---|---|---|
| Secondary anemia | 44.8 per cent. | 27.1 per cent. |
| Chlorosis | 54.8 " " | 17.8 " " |
| Leukemia | 60.6 " " | 45.4 " " |
| Pernicious anemia | 74.5 " " | 76.9 " " |

Examination of the *stained specimen* shows a variable degree of alteration in the shape, size, and general structure of the cells. In mild cases simple pallor of the erythrocytes and perhaps a few microcytes and moderately misshapen poikilocytes are the only changes to be observed, erythroblasts, polychromatophiles, and cells with basophilic stroma degeneration being entirely wanting. In severe cases, with excessive oligocythemia, a large proportion of the cells are either under- or over-sized, the latter

[1] *Loc. cit.*

forms appearing to prevail in relation to the intensity of the anemic process; poikilocytosis and polychromatophilia are sometimes extreme, and evidences of Grawitz's stroma degeneration are found, together with a more or less abundance of nucleated erythrocytes, the majority of which conform to the normoblastic type. In most instances normoblasts only are present, but rarely an occasional megaloblast, implying a slight tendency toward a fetal type of hemogenesis, is also seen. The significance of erythroblasts in anemia and the circumstances under which they are found have been discussed in a preceding section. (See p. 187.)

LEUCOCYTES. Typical polynuclear neutrophile leucocytosis is common, but by no means constant, in the secondary anemias, independent of their grade, for the cellular increase is provoked by a stimulation of the functional activities of the marrow, which vary according to the individual and to the nature of the exciting cause. The differential changes associated with such a leucocytosis (low percentages of lymphocytes and eosinophiles, with, perhaps, a few myelocytes) have already been referred to in a preceding section. A moderate leucocytosis is especially common in the anemias of children, and in those symptomatic of inflammatory and suppurative conditions and of malignant diseases. While in other anemias, especially those of chronic type, a normal leucocyte count or even leucopenia may be found, often in association with a relative lymphocytosis, as is frequently the case in the anemias of enteric fever and of tertiary syphilis.

BLOOD PLAQUES. The plaques are usually increased, but apparently without any constant relationship to the degree of hemoglobin and erythrocyte loss. In some cases these bodies may number more than double the maximum normal standard, as in a case of anemia in a child with a tumor of the spleen, noted by von Emden,[1] in which an estimate of 829,000 to the c.mm. was made.

DIAGNOSIS. The principal blood changes found in secondary anemia are as follows:

*Hemoglobin.* Variable decrease, usually somewhat more marked than the erythrocyte loss; color index subnormal.

*Erythrocytes.* Variable decrease.

Erythroblasts, in severe cases; normoblasts outnumbering megaloblasts, which are rare.

Deformities of shape and size, polychromatophilia, and basic staining of the stroma in severe cases.

[1] *Loc. cit.*

| | |
|---|---|
| *Leucocytes.* | Commonly increased; rarely leucopenia.<br>Polynuclear neutrophiles usually increased, and lymphocytes and eosinophiles relatively diminished.<br>Lymphocytosis in some cases, usually those of severe type and chronic course.<br>Small numbers of myelocytes sometimes found. |
| *Plaques.* | Usually increased. |

## V. POST-HEMORRHAGIC ANEMIA.

ETIOLOGY. Among the many underlying causes of acute post-hemorrhagic anemias may be mentioned *trauma, abortion, post-partum hemorrhage, epistaxis, pulmonary tuberculosis, peptic ulcer, enteric fever, visceral carcinoma, hemorrhagic pancreatitis,* and the rupture of an *aneurysm,* of a Fallopian tube during *ectopic pregnancy,* and of a mass of extensively *varicose veins.* Chronic hemorrhages, such as those resulting from diseases belonging to the *hemorrhagic diathesis,* from *hemorrhoids,* or from *uterine diseases* usually give rise to a much less decided blood loss than the first-named conditions, but in some instances these factors, if persistent, may be sufficient eventually to provoke anemia of great intensity.

EFFECT UPON THE BLOOD. Reduction in the total volume of blood, or *oligemia,* ensues as the immediate effect of an acute hemorrhage, and a count made immediately after the blood loss may show no reduction in the hemoglobin and corpuscular value, since the oligemia affects the liquid and cellular elements proportionately. As reaction sets in the system attempts to compensate for the loss of blood by the rapid absorption by the capillaries of large amounts of liquids from the tissues, so that the blood soon becomes highly diluted, or hydremic. This is evidenced by a proportionate diminution in the *hemoglobin percentage* and *erythrocyte count,* the degree of this decrease depending upon the extent of the hemorrhage. It is thought that in many instances this fluid transfer from tissue to vessel is inaugurated immediately after or even during the hemorrhage, and that the original volume of blood is restored within a few hours. A further diminution in hemoglobin and erythrocytes occurs after the normal volume of blood has been reëstablished, so that the minimum decrease is not observed until some little time has elapsed after the hemorrhage. As a rule, the minimum count is seen at some period during the

first week after the blood loss—as early as the first or second day in some instances, but as late as the tenth or eleventh day in others. This secondary fall is thought to depend upon the introduction into the circulation of large numbers of immature, feebly resistant erythrocytes, which suffer rapid and premature destruction, and thus bring about a disturbance in the equilibrium between the rate of blood production and blood destruction in favor of the latter. As soon as the marrow is able to meet the drain in an adequate manner, by the increased production of more resistant cells, the anemia ceases, and the hemoglobin and erythrocyte estimates begin to rise.

Other changes consequent to hemorrhage are a diminution in the corpuscular volume in the dry residue of the whole blood, and in the proteids of the serum. The serum solids and fibrin-forming elements are apparently increased. Despite the loss of albumin, Haesslin[1] found a constant fall in the freezing-point of the blood.

Authorities differ as to the degree of blood loss which man is capable of surviving, a difference which is but natural when it is remembered that factors other than the actual amount of blood lost, conspicuous among which are the age, sex, and resisting powers of the patient, are all important in determining the fatality of the hemorrhage. According to Immermann,[2] hemorrhages involving a loss of one-half of the total bulk of blood in the body invariably prove fatal. Hayem[3] is authority for the statement that, as a general rule, recovery is possible when the total volume of blood lost does not exceed one-eighteenth of the individual's body-weight. This author has reported the most astonishing example on record of post-hemorrhagic cellular decrease, in which he observed a diminution in the erythrocytes to 11 per cent. of normal in a case of post-partum hemorrhage, with subsequent recovery of the patient. Béhier[4] has described a case of metrorrhagia in which recovery occurred in spite of a reduction in the erythrocytes to 19 per cent. of normal. Laache[5] has recorded a number of instances in which the corpuscular estimates fell below 50 per cent. of normal—in one case to 32 per cent. These last three examples are sufficient to disprove the former belief that death inevitably ensues when the corpuscular loss, as the result of hemorrhage, falls as low as 50 per cent. of normal.

Increase in the number of *leucocytes*, usually of moderate degree, promptly develops in the great majority of cases and per-

[1] Deutsch. Arch. f. klin. Med., 1902, vol. lxxiv, p. 577.
[2] Cited by Rieder, *loc. cit.*
[3] *Loc. cit.*
[4] Cited by Laache, *loc. cit.*
[5] "Die Anämie," Christiania, 1888.

sists for several days. It usually involves an absolute and relative gain in the polynuclear neutrophile cells, with a consequent decrease in the mononuclear forms, but, rarely, the reverse may be noted. In fatal cases this increase may not occur; nor, according to Baumann,[1] does it occur after hemorrhage during the adminstration of arsenic, the leucocytes in such instances diminishing. This investigator also found that the giving of arsenic and inorganic iron to animals experimentally bled resulted in slighter blood deterioration than when either drug was used alone.

The maximum count is commonly attained within a few hours after the onset of the leucocytosis, the normal being regained within a week or less. (See p. 246.)

The *blood plaques* are strikingly increased after hemorrhage. The coagulability of the blood is abnormally quick, being more rapid in profuse than in moderate hemorrhages.

REGENERATION. Following the reëstablishment of the normal blood volume, regeneration of the erythrocytes and hemoglobin and a consequent dissipation of the hydremia ensue. The time necessary for the completion of this process varies greatly in different individuals, as the rapidity with which blood regeneration occurs depends upon different factors, such as the extent of the original hemorrhage and the age and natural regenerative powers of the patient. The latter are at their maximum during the third and fourth decades of life, at their minimum during infancy and old age, and are regarded as more active in women than in men. The existence of a well-developed cachexia or an infectious disease, as well as the neglect of proper treatment of the hemorrhage, are obstacles which retard the regeneration of the blood to its normal composition. The process appears to be more active if transfusion of a normal saline solution has been practised than in untreated cases, the rapidity of the gain being especially striking during the latter half of the regeneration period. The transfusion of blood hastens regeneration even more decidedly. Otto[2] and Hall and Eubank[3] have shown experimentally in animals, bled and given transfusions of artificial serum, that regeneration once stimulated into activity may carry the blood, quantitatively, considerably beyond the established normal standard.

In uncomplicated cases, according to Bierfreund,[4] regeneration is effected within four weeks if the hemorrhage produces a hemo-

[1] Jour. Physiol., 1903, vol. xxix, p. 18.
[2] Pflüger's Arch., 1885, vol. xxxv, p. 57.
[3] Jour. Exper. Med., 1896, vol. i, p. 656.
[4] Langenbeck's Arch., 1890–91, vol. xli, p. 1.

globin loss of 25 per cent., and in about three weeks if the loss does not exceed 20 per cent. The latter period may be regarded as the average regeneration time in the great majority of instances.

As regeneration proceeds the hemoglobin and corpuscular deficiencies gradually become less conspicuous, but the increase in these two constituents does not occur along parallel lines. The increase in the number of erythrocytes is much more rapid than the gain in the hemoglobin percentage, which usually remains subnormal for some time after the normal number of corpuscles has been reëstablished. Owing to this lagging behind of the hemoglobin low color indices are the rule. Faulty hemogenesis, owing to which the great majority of the erythrocytes are deficient in hemoglobin and many of them of abnormally small size, serves best to explain this slow restitution of the hemoglobin value.

The appearance in the blood of normoblasts is common after hemorrhage, and in rare instances an occasional megaloblast and atypical erythroblast may be observed. According to Ehrlich,[1] if thorough and systematic search is made, normoblasts may be constantly found after the second or third day following the blood loss until the regeneration of the blood is complete. The transient appearance of large numbers of normoblasts, known as "blood crises," has been already described. (See p. 189.) Dawson,[2] who has carefully studied the effects of venous hemorrhage in dogs, found no evidence of any close relation between the number of erythroblasts and the rapidity and character of the regeneration of the hemoglobin and erythrocytes. In severe cases polychromatophilia of the erythrocytes may be noted, this sign first becoming apparent as early as the first day after the hemorrhage, and gradually disappearing as regeneration is effected. Deformities in the size and shape of the erythrocytes are not uncommon, of which microcytes constitute the most frequent example. Large hydropic megalocytes and poikilocytes are met with more rarely.

## VI. LEUKEMIA.

Varieties.

According to the classification in general vogue at the present time two clinical varieties of leukemia, the *myelogenous* or *spleno-medullary* and the *lymphatic*, are recognized. The myelogenous variety, which is almost invariably a chronic process, is associated with a

[1] *Loc. cit.*

[2] Amer. Jour. Physiol., 1900, vol. iv, p. 2.

DIFFERENTIAL TABLE.

| | Chlorosis. | Pernicious Anemia. | Splenic Anemia. | Secondary Anemia. |
|---|---|---|---|---|
| *Hemoglobin.* | Marked decrease, averaging to about 50 per cent.<br>Relatively low to erythrocyte loss. | Striking decrease, averaging to about 25 per cent.<br>Relatively high to erythrocyte loss. | Marked decrease, averaging to about 45 per cent.<br>Relatively low or high to erythrocyte loss. | Moderate or marked decrease.<br>Relatively low or proportionate to erythrocyte loss. |
| *Erythrocytes.* | Moderate decrease, counts averaging about 4,000,000.<br>Pallor conspicuous.<br>Poikilocytosis rarely marked.<br>Erythroblasts rare, normoblasts predominating.<br>Polychromatophilia rare; basophilic stroma degeneration absent.<br>Microcytosis common. | Excessive decrease, counts averaging about 1,250,000.<br>Pallor not marked.<br>Poikilocytosis usually conspicuous.<br>Erythroblasts constant, megaloblasts predominating.<br>Polychromatophilia and basophilic stroma degeneration common.<br>Megalocytosis common. | Decided decrease, counts averaging about 3,500,000.<br>Pallor variable.<br>Poikilocytosis in severe cases.<br>Erythroblasts rare, normoblasts predominating.<br>Polychromatophilia variable; basophilic stroma degeneration absent.<br>Changes in diameter of cells inconstant. | Moderate or excessive decrease.<br>Pallor slight or marked.<br>Poikilocytosis variable.<br>Erythroblasts in severe cases, normoblasts predominating.[1]<br>Polychromatophilia and basophilic stroma degeneration in severe cases.<br>Changes in diameter of cells variable. |
| *Color Index.* | Very low, averaging about 0.50. | High, averaging about 1.00. | Moderately low, averaging about 0.75. | Subnormal, sometimes very low. |
| *Leucocytes.* | Normal or decreased, counts averaging about 7000.<br>Relative lymphocytosis and decrease of polynuclear neutrophiles common.<br>Eosinophiles notably decreased; often absent.<br>Myelocytes very rare.<br>Basophiles not increased. | Decreased, counts averaging about 4000.<br>Relative lymphocytosis and decrease of polynuclear neutrophiles the rule.<br>Eosinophiles often absent, usually much decreased.<br>Myelocytes in small numbers almost constant.<br>Basophiles not increased. | Normal or decreased, counts averaging about 5000.<br>Relative lymphocytosis and decrease of polynuclear neutrophiles frequent.<br>Eosinophiles normal.<br>Myelocytes rare.<br>Basophiles sometimes increased. | Usually increased moderately.<br>Polynuclear neutrophile increase with lymphocyte decrease common.<br>Eosinophiles usually decreased or absent.<br>Small numbers of myelocytes in severe cases.<br>Basophiles not increased. |
| *Plaques.* | Increased. | Variable. | Not increased. | Usually increased. |

[1] Except in anemia due to bothriocephalus infection and to nitrobenzol intoxication.

marked proliferation of myeloid tissue, and is characterized by a striking myelemia and generally by conspicuous enlargement of the spleen, with little or no involvement of the lymphatic glands. The lymphatic form, which may run either an acute or a chronic course, more commonly the latter, is a process associated with a proliferation of lymphoid tissue, and is characterized by a blood picture known as lymphemia, and in the great majority of cases by marked enlargement of the lymphatic glands, with moderate involvement of the spleen. But these two clinical pictures, in so far as they relate to the splenic and lymphatic hypertrophy, are by no means constant, for, although cases of myelogenous leukemia always have enlarged spleens, exceptionally they may also have decided lymphatic hyperplasia. Furthermore, cases of lymphatic leukemia are occasionally encountered in which there is a marked splenic tumor without demonstrable signs of lymphatic enlargement, as well as those in which the bone marrow alone is involved without implication of either the spleen or the lymphatic glands. To this group belong the acute leukemias with myelogenous lesions described by Müller,[1] Walz,[2] Pappenheim,[3] Michälis,[4] Dorothy Reed,[5] A. O. J. Kelly,[6] and others—studies which tend to upset Ehrlich's theory that lymphatic leukemia is essentially a lesion of the lymph glands. Because of such atypical examples, the gross appearance of the spleen and lymphatics must be regarded as a sign of distinctly secondary importance to the blood picture, which is alone the tangible diagnostic clue.

Gerhardt,[7] Fleischer and Penzoldt,[8] and Wey[9] report cases of the apparent transition of myelogenous into lymphatic leukemia and into pernicious anemia. These so-called conversions of type may really represent merely the superposition of a leucocytosis sufficiently great temporarily to dominate the blood picture. If such were the case, it is obvious that with the disappearance of the leucocytosis the true character of the blood changes will become apparent—in the first instance, a lymphocytosis, and in the second, a megaloblastic anemia.

Of the two forms of the disease, the myelogenous is much the commoner. In the series of 42 cases of leukemia which the

[1] Centralbl. f. allg. Path. u. path. Anat., 1894, vol. v, pp. 553 and 601.
[2] *Ibid.*, 1901, vol. xii, p. 967.
[3] Virchow's Arch., 1899, vol. clvii, p. 19; *ibid.*, 1900, vol. clix, p. 40; *ibid.*, 1901, vol. clxi, p. 424.
[4] Deutsch. med. Wochenschr., 1901, vol. xxvii, p. 651.
[5] Amer. Jour. Med. Sci., 1902, vol. cxxiv, p. 653.
[6] Trans. Assoc. Amer. Phys., 1903, vol. xviii, p. 481.
[7] Deutsch. Arch. f. klin. Med., 1880, vol. xxvi, p. 368.
[8] *Ibid.*, 1896, vol. lxvii, p. 300.
[9] XV. Cong. f. inn. Med., 1897.

writer has had the opportunity of studying, 29 were of the myelogenous and 13 of the lymphatic form; while of Cabot's 66 cases,[1] 49 were myelogenous and 17 lymphatic—a proportion of almost three of the former to one of the latter, for the combined series of 108 cases.

PARASITOLOGY.

In the present state of our knowledge it is not possible to regard leukemia as a disease of infectious origin, notwithstanding the suggestiveness of the symptoms shown by many of those cases which run an acute course. Within the past few years several investigators, notably Delbert,[2] Kelsch and Vaillard,[3] Pallowski,[4] and Löwit[5] have attempted to ascribe to various micro-organisms specific etiological relationship with the condition, but these attempts have thus far been unconvincing. Löwit's researches, however, are worthy of special attention, if for no other reason than for the elaborate and painstaking study which they represent. This author believes that two distinct forms of parasites may be demonstrated in leukemia: the *Hæmamœba leukemiæ magna*, thought to be the specific cause of the myelogenous form of the disease, and the *Hæmamœba leukemiæ parva*, which he claims is the definite infective principle of the lymphatic form. These so-called "specific bodies," which are found both in the blood of the peripheral vessels and in the hematopoietic organs, have either a granular or an ameboid appearance, and bear a more or less close resemblance to the basophile granules of the leucocytes; navicular, segmenting, and vacuolated forms are also said to occur. They are either attached to or lie within the leucocytes, especially the small lymphocytes, and more rarely the other varieties of normal leucocytes and the myelocytes; in an occasional instance they are said to be found lying free in the plasma. Although it is claimed that a leucocytic infection has been produced in animals by the injection of blood presumably containing these micro-organisms, all attempts to cultivate them on artificial media have proved futile. Löwit's amebæ are demonstrated only in heat-fixed specimens, stained preferably with a steaming hot solution of Löffler's methylene-blue, after which they are washed, differentiated with a 0.3 per cent. solution of hydrochloric acid alcohol, again washed, and mounted. In specimens thus treated they stain metachromatically, and, if the acid alcohol differentiation has been properly effected, are the only elements except the basophile

[1] *Loc. cit.* [2] Bull. et mém. Soc. de chir., Paris, 1895, vol. xxi, p. 788.
[3] Annal. de l'Institut Pasteur, 1890, vol. iv, p. 276.
[4] Deutsch. med. Wochenschr., 1892, vol. xviii, p. 641.
[5] "Die Leukämie als Protozoeninfektion," Wiesbaden, 1900.

leucocyte granules which retain the color of the dye. Türk,[1] who has followed out Löwit's technic precisely, in investigating this author's claims has come to the conclusion that these "specific bodies" are in no sense of parasitic nature, but merely artefacts resulting from the action of an aqueous solution of a basic dye upon the mast cell granules, which causes the partial solution of the latter elements and deforms them. Türk claims, furthermore, that these so-called amebæ can be produced both in the normal blood of man and in the blood of rabbits.

## Myelogenous Leukemia.

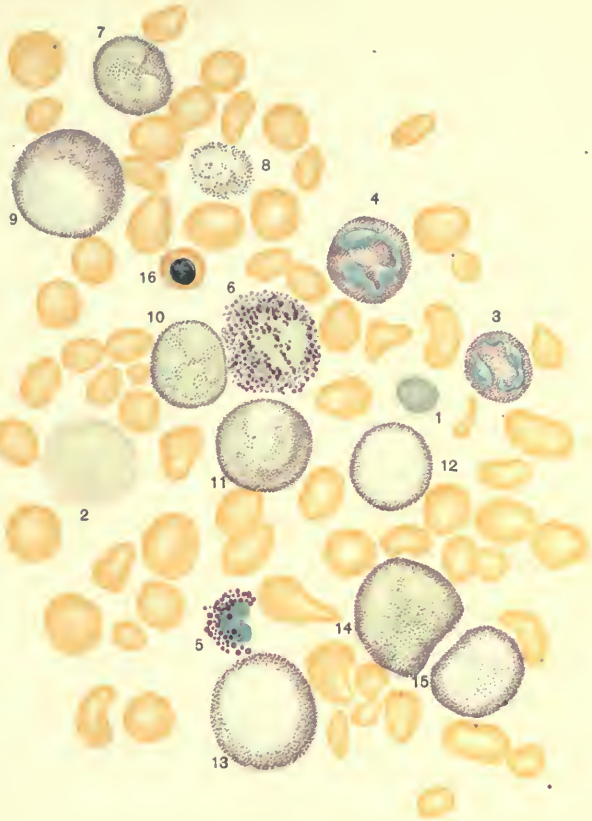
Appearance of the Fresh Blood.

The drop as it flows from the puncture is, in most instances, of a bright scarlet color, and often has a peculiar and misleading appearance of density. After brief exposure to the air, it may become resolved into a serous, scarlet fluid, in which are suspended many minute, whitish, fat-like masses; the former appears to consist of serum and erythrocytes, and the latter of adhering masses of leucocytes. It was probably this striking appearance of the leukemic blood drop that led Hughes Bennett erroneously to describe the condition as a "suppuration of the blood" before he proposed the more suitable term leucocythemia. In some cases the drop is simply much darker than normal, but it is difficult to believe that it ever resembles the chocolate-brown shade mentioned by some authors as occurring in this disease. The blood usually flows very freely from the wound, often by fine jets and spurts, especially if slight pressure is applied above the site of the puncture.

Microscopically, the field is found to contain an enormous number of leucocytes, the proportion of these cells to the erythrocytes being, by actual count, as great as 1 to 8 or 6, or even greater. Many different varieties of leucocytes may be distinguished in the fresh specimen, the most striking being the large, mononuclear, finely granular cells of round or ovoid shape. These are the myelocytes which are present in enormous numbers in this form of leukemia, of which they form a characteristic blood picture. "Fractured" leucocytes, usually eosinophiles, with a cloud of escaped granules free in the plasma in the neighborhood of the disrupted cell body, may also be observed in variable numbers, although such cells are more numerous in the dried, stained film. (See Figs. 54 and 55.)

The erythrocytes vary in number and in appearance according

[1] XVIII. Cong. f. inn. Med., Wiesbaden, 1900.




SPLENO-MEDULLARY LEUKEMIA.

(*Triacid Stain.*)

1. **Small Lymphocyte.**
2. **Large Lymphocyte.**
   Contrast this cell with the myelocytes, 10, 11, and 12, noting the presence of neutrophile granules in the latter, and their absence in the lymphocyte. The size and nuclear characteristics of all these cells are practically the same.
3. 4. **Polynuclear Neutrophiles.**
5. **Eosinophile.**
   In this "dwarf" eosinophile, ruptured during the preparation of the specimen, the granules are peculiarly arranged about the nucleus; no signs of protoplasm are distinguishable.
6. **Eosinophilic Myelocyte.**
   Note the irregularity with which the granules are stained.
7, 8, 9, 10, 11, 12, 13, 14, 15. **Myelocytes.** (*Neutrophilic.*)
   These cells vary greatly in size (compare 8 with 9), but they all have similar distinctive characteristics—a large opalescent nucleus containing a scanty chromatin network embedded in a cell body crowded with delicate neutrophile granules, precisely like those found in the polynuclear neutrophiles, 3 and 4. The nucleus of 7 is distinctly indented and somewhat denser than that of the other myelocytes. This cell probably represents a developmental phase of the myelocyte just short of its transition into a typical polynuclear neutrophile.
16. **Normoblast.**
   The erythrocytes (stained orange) show many evidences of deformity, an occasional megalocyte, many microcytes, and a few poikilocytes being present. Polychromatophilia is absent.

(E. F. FABER, *fec.*)

to the severity of the coëxisting anemia; in some instances large numbers of poikilocytes, megalocytes, and microcytes, with marked pallor of the corpuscles, may be seen, while in others the changes affecting the erythrocytes appear to be but trifling.

In fresh films which have been allowed to dry for some time Charcot-Leyden crystals may sometimes be detected. They appear as colorless, refractive crystals, shaped like octahedra, having long, pointed, sharp angles, and occurring either singly or in twos or threes, superimposed at right angles or as collections of radiating, crystalline masses. These crystals are not observed in the freshly drawn blood, being demonstrable only in films which have stood exposed to the air for at least twenty-four hours, and only occasionally even under this circumstance.

On account of the presence in the blood of such large numbers of leucocytes a very small drop should be used for making the cover-glass spreads for staining, since it is advisable to avoid overcrowding the field with these cells. No difficulty will be experienced in obtaining thin, evenly distributed spreads if this precaution is observed, especially if the cover-glasses are slightly warmed just before they are used.

COAGULATION. The coagulation of the blood and the formation of the fibrin network must be regarded as variable. In some cases, especially those with great loss of hemoglobin and erythrocytes, both processes are delayed and imperfect, as evidenced by the formation of the "raspberry-jelly" clots referred to by the German writers. But in other cases the coagulation time is unaltered, and the fibrin network is perfectly normal.

ALKALINITY. The alkalinity of the blood is usually decreased, and, as in chlorosis, it increases after the patient is given iron, in parallelism with the gain in hemoglobin and erythrocytes. In 3 cases studied by Burmin[1] an average alkalinity equivalent to 146 mgm. was found, Landois' method being employed in the investigations. Taylor[1] found an average of 380 mgm. in three tested by the von Limbeck method.

SPECIFIC GRAVITY. In cases with severe anemia the density of the blood may fall as low as 1.035 or 1.040. Grawitz[1] has reported a case in which the figure was 1.023. The fallacies in leukemia of Hammerschlag's tables of specific gravities and their hemoglobin equivalents have already been pointed out. (See p. 133.)

[1] *Loc. cit.*

HEMOGLOBIN AND ERYTHROCYTES.

Decided hemoglobin and erythrocyte loss is the invariable rule sooner or later during the course of the disease, the anemia usually being well defined at the time the patient first comes under observation, and becoming acutely marked as the termination of the illness approaches. It is generally the case that the hemoglobin loss is disproportionately greater than the decrease in the erythrocytes, thus producing a moderately low color index, but in some cases just the opposite of this is observed.[1] In the writer's 29 cases, grouped in Table VIII, the color index averaged about 0.86. The hemoglobin percentage ranged between 24 and 70, averaging 48.6, and the number of erythrocytes was as low as 572,000 and as high as 4,200,000 per c.mm., the mean average being 2,814,000; the count of these cells was diminished to one-half of the normal standard, or below this figure, in 11 of the cases examined. An analysis of Cabot's series of 42 cases of myelogenous leukemia[2] shows these average findings: hemoglobin, 43 per cent.; erythrocytes, 3,123,000 per c.mm.; and color index, about 0.68.

TABLE VIII.—HEMOGLOBIN AND ERYTHROCYTES IN 29 CASES OF MYELOGENOUS LEUKEMIA.

| Hemoglobin Percentage. | Number of Cases. | Erythrocytes per c.mm. | Number of Cases. |
|---|---|---|---|
| From 60–70 | 7 | From 4,000,000–5,000,000 | 1 |
| " 50–60 | 5 | " 3,000,000–4,000,000 | 15 |
| " 40–50 | 5 | " 2,000,000–3,000,000 | 8 |
| " 30–40 | 10 | " 1,000,000–2,000,000 | 4 |
| " 20–30 | 2 | Below 1,000,000 | 1 |
| Average, 48.6 per cent. | | Average, 2,814,000 per c.mm. | |
| Maximum 70.0 " " | | Maximum, 4,200,000 " " | |
| Minimum, 24.0 " " | | Minimum, 572,000 " " | |

Fluctuations in the hemoglobin percentage and in the number of erythrocytes may or may not accompany variations in the leucocyte count. Sometimes, as the leucocytes rise, the erythrocytes fall, but again they remain practically stationary; or, the leucocytes may progressively fall to a comparatively moderate count, coincidentally with an apparent improvement in the patient's general condition, and yet the erythrocytes do not materially gain in numbers. Taylor[3] refers to two such instances which have come

[1] It is to be remembered that hemoglobin estimates in leukemia may be unreliable (except when Dare's instrument is used), for correct readings are sometimes impossible, owing to the milkiness of the diluted blood from the presence of such immense numbers of leucocytes. About one-half of the hemoglobin figures in the accompanying table (Table VIII) were obtained by means of von Fleischl's hemometer, the remainder being based upon examinations with Oliver's and with Dare's instruments.

[2] *Loc. cit.*

[3] *Loc. cit.*

under his observation, in both of which the blood picture at certain brief intervals resembled that of pernicious anemia, for under the influence of energetic arsenical treatment the leucocytes were reduced to normal, while the oligocythemia stubbornly persisted. In a case studied for a protracted period it is possible to distinguish a general decrease in the erythrocyte count as the leucocytes increase, although the reverse may not be true. (See Chart II, p. 311.)

Examination of the stained specimen shows the presence of *nucleated erythrocytes* in practically every case of myelogenous leukemia, these cells often being many times more numerous than in grave cases of pernicious anemia. Normoblasts prevail, always being more numerous than megaloblasts; in some cases they are the only type of erythroblast to be observed; in others they are associated with a relatively moderate number of typical megaloblasts, or, more commonly, with large numbers of atypical forms, sharing the characteristics of the typical adult and embryonic nucleated erythrocytes. In the 9 cases of this variety of leukemia in which the writer has made differential erythrocyte counts the following estimates were obtained at the first examinations:

| Number. | Total Number of Erythroblasts per c.mm. | Normoblasts per c.mm. | Megaloblasts per c.mm. |
|---|---|---|---|
| 1 | 12,913 | 8376 | 4537 |
| 2 | 9,178 | 9178 | 0 |
| 3 | 8,626 | 7264 | 1362 |
| 4 | 8,064 | 6048 | 2016 |
| 5 | 5,694 | 3504 | 2190 |
| 6 | 3,234 | 1848 | 1386 |
| 7 | 2,940 | 2940 | 0 |
| 8 | 1,980 | 1980 | 0 |
| 9 | 748 | 748 | 0 |
| Average: | 5,931 | 4654 | 1277 |

Comparison of the above summary with the table giving the number and forms of erythroblasts in pernicious anemia (Table V, p. 283) illustrates two striking facts concerning these cells in myelogenous leukemia: the immense numbers in which they occur and the predominance of normoblasts over megaloblasts. Periods of temporary improvement in the patient's general health are often heralded by a notable increase in the normoblasts, but it is a noteworthy fact that during these remissions, while the leucocytes may fall decidedly, the normoblasts tend to persist in greater or less numbers.

Examples of so-called nuclear extrusion, of multinucleation,

of clover-leaf, dumb-bell, or other irregularly formed nuclei, and even, in rare instances, of karyokinesis are observed in many of the normoblasts. (See Fig. 49, p. 193.) Such alterations may be more conspicuous in highly developed cases of myelogenous leukemia than in any other disease of the blood. In an occasional normoblast the contracted, glistening, intensely basic nucleus is highly suggestive of pyknosis. No one who has done much blood work can fail to be struck with the obvious avidity displayed by the stroma of the erythroblasts for the acid fuchsin of the triple stain, a peculiarity which is exhibited in spite of good technic and the use of a reliable staining solution.

*Deformities* affecting the size and the shape of the erythrocytes may be trivial or decided, depending upon the degree of hemoglobin and erythrocyte loss present. *Polychromatophilia*, alone or associated with *basophilic stroma degeneration*, is very common in cases with great anemia, these changes affecting both the nucleated and the non-nucleated cells.

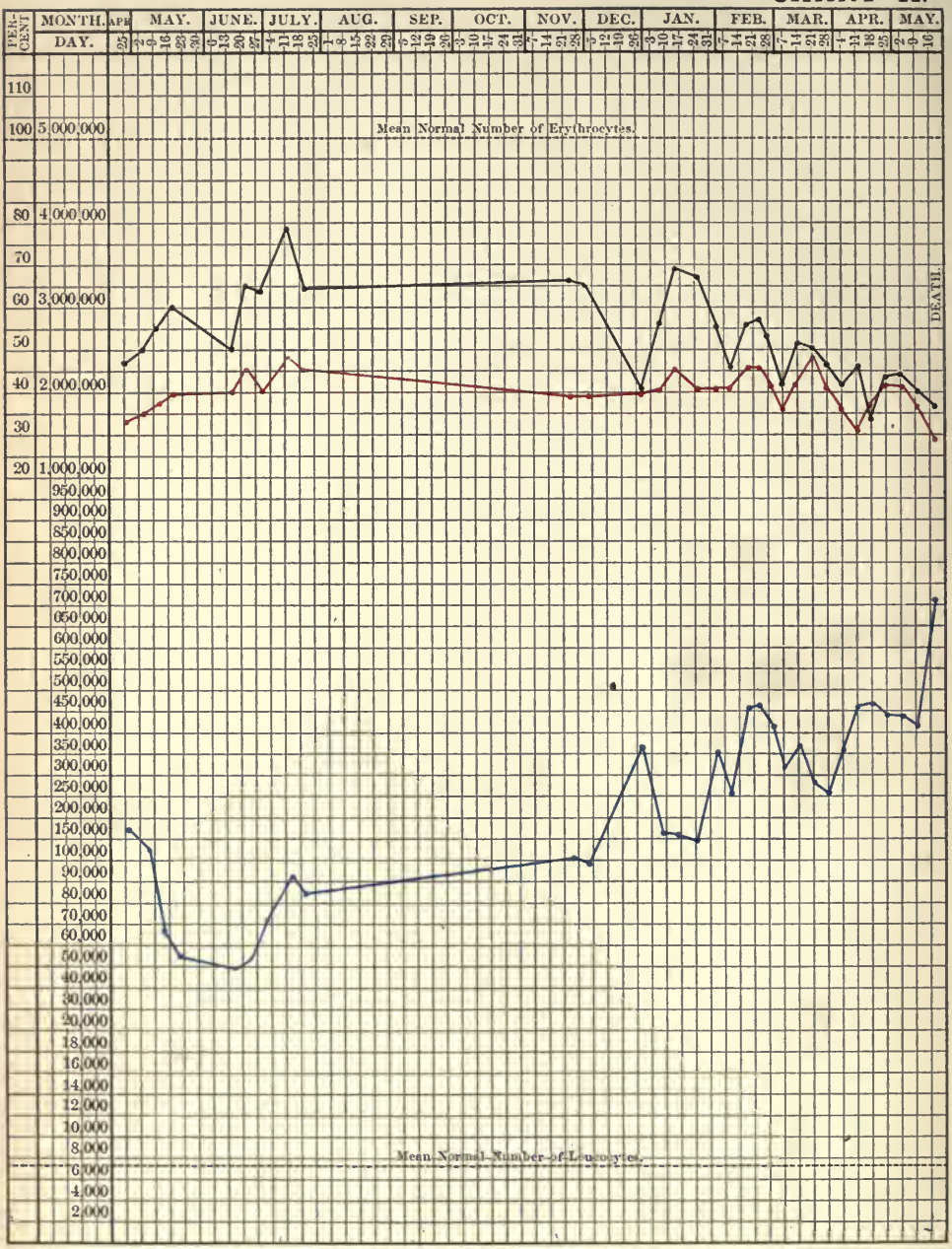
TABLE IX.—NUMBER OF LEUCOCYTES IN 29 CASES OF MYELOGENOUS LEUKEMIA.

| Leucocytes per c.mm. | Number of Cases. |
|---|---|
| Above 1,000,000 | 1 |
| From 500,000–1,000,000 | 5 |
| " 400,000– 500,000 | 4 |
| " 300,000– 400,000 | 4 |
| " 200,000– 300,000 | 6 |
| " 100,000– 200,000 | 6 |
| " 50,000– 100,000 | 2 |
| Below 50,000 | 1 |
| Average, 355,119 per c.mm. | |
| Maximum, 1,046,000 " " | |
| Minimum, 44,000 " " | |

Leucocytes.

Striking increase in the number of leucocytes is found even during the early stages of the disease. Counts in excess of 100,000 are generally expected, while in fully 70 per cent. of cases they exceed 300,000 and in about 20 per cent., 500,000 or higher. In rare instances the number of leucocytes may be as high as 1,000,000 per c.mm. One case of the writer's had 1,046,000 leucocytes. The leucocyte count may, very infrequently, equal or even exceed that of the erythrocytes, and it is easy to see that such a condition is possible, should the accompanying oligocythemia be intense. In a patient recently admitted to the Jefferson Hospital the leucocytes numbered 650,000 and the erythrocytes 572,000. In the estimates given in Table IX, showing the number of leucocytes at the time the patient first applied for treatment, the average count was 355,119 per c.mm., the highest being 1,046,000 and the lowest 44,000.

## CHART II.




SPLENO-MEDULLARY LEUKEMIA.

Red, Hemoglobin. Black, Erythrocytes. Blue, Leucocytes.

The number of leucocytes may fluctuate enormously at various times during the progress of the disease, a gain or a loss of some 200,000 cells to the c.mm. from week to week being a matter of common occurrence. Sometimes they are temporarily diminished as the result of the administration of arsenic to the point of tolerance, or of the vigorous employment of other therapeutical measures; sometimes the decrease takes place independently of the influence of remedial agencies, so far as can be determined. When arsenic is withheld, the number of leucocytes promptly increases, and in spite of its use they ultimately increase as the disease runs its fatal course. The relations of the leucocytes' fluctuations to the number of erythrocytes have already been mentioned. Hayek[1] has drawn attention to the fact that in the leukemic individual the morning leucocyte count may be greater by more than 100,000 cells per c.mm. than the estimate made during the afternoon, and vice versâ. In a case in Professor Wilson's wards at the Jefferson Hospital the writer has been able to verify this statement, the counts being as follows: 11 A. M., 144,000; 6 P. M., 256,000—a difference of 112,000 cells within a period of seven hours. The influences of digestion and other sources of error were, of course, excluded in making this observation. The occurrence of this enormous diurnal fluctuation emphasizes the importance of making the blood examination of leukemic patients at precisely the same hour each day in cases studied for a long period.

TABLE X.—QUALITATIVE CHANGES IN THE LEUCOCYTES IN 29 CASES OF MYELOGENOUS LEUKEMIA.

| | Average. | Maximum. | Minimum. |
|---|---|---|---|
| Small lymphocytes | 4.1 | 12.0 | 0.7 |
| Large lymphocytes | 9.5 | 28.4 | 0.3 |
| Polynuclear neutrophiles | 54.3 | 77.0 | 34.1 |
| Eosinophiles | 5.4 | 28.7 | 1.2 |
| Myelocytes | 20.6 | 44.0 | 7.0 |
| Mast cells[2] | 9.0 | 28.0 | 1.0 |

The possibility of encountering a case of leukemia during a period of remission, when the typical blood changes are absent, must be borne in mind, for such instances are observed from time to time, although they are very rare. For example, McCrae[3] reports a case, treated by arsenic, in which twice during a period of ten months the blood and general symptoms of the patient were typical of myelogenous leukemia, and twice were absolutely normal. When first examined, this patient's leucocytes numbered 584,000 per c.mm., three months later they had

[1] Wien. klin. Wochenschr., 1897, vol. x, p. 475.
[2] Estimates in 19 cases.
[3] Brit. Med. Jour., 1900, vol. i, p. 760.

fallen to 9250, two months after this they had risen to 178,000, and after a lapse of another five months they again fell to 5000. These fluctuations did not depend upon the influence of any intercurrent infection (see below), and the case is peculiar in that the leucocytes, as well as the erythrocytes, not only were normal in number, but also normal qualitatively, and in that the patient's splenic tumor entirely disappeared during the periods of remission. A similar case (myelogenous) is reported by Simon and Campbell,[1] in which the leucocytes, after vigorous arsenical treatment for three weeks, fell from 350,000 to 4000, with disappearance of the myelemic blood picture and of the splenic tumor. Twelve months later the blood (save for the persistence of mast cells) and spleen were still normal. Other cases have been recorded showing brief periods of temporary decline to normal in the number of leucocytes, but with the persistence of myelocytes, or of the splenic enlargement, or of both.

Here may be noted the astonishing results of Röntgen-ray therapy, alone and in conjunction with arsenic, in the treatment of myelogenous leukemia. Senn,[2] E. J. Brown,[3] C. H. Weber,[4] Grosh and Stone,[5] Bryant and Crane,[6] and Aubertin and Beaujard[7] have reported cases thus treated, in which the blood picture became normal and the splenic tumor disappeared. Time alone can determine whether such cures are symptomatic or radical.

The following summary illustrates the good effects of arsenic, and, in the patient in question, the indifferent results of *x*-ray therapy in myelogenous leukemia, observed in the Jefferson Hospital:

| | On Admission. | After Twelve Weeks' Arsenic Treatment. | After Six Weeks' Arsenic Treatment with X-ray. |
|---|---|---|---|
| Hemoglobin | 60 per cent. | 80 per cent. | 70 per cent. |
| Erythrocytes | 3,520,000 | 4,860,000 | 3,090,000 |
| Leucocytes | 341,000 | 32,000 | 147,600 |
| Small lymphocytes | 3 per cent. | 2.5 per cent. | 3.0 per cent. |
| Large lymphocytes | 1 " " | 1.5 " " | 1.4 " " |
| Polynuclear neutrophiles | 50 " " | 74.5 " " | 74.9 " " |
| Eosinophiles | 3 " " | 2.0 " " | 3.6 " " |
| Myelocytes | 15 " " | 16.0 " " | 10.0 " " |
| Mast cells | 28 " " | 3.5 " " | 7.1 " " |

The presence of *myelocytes* in large numbers is the hinge upon which the diagnosis of myelogenous leukemia must turn, for in

[1] Johns Hopkins Hosp. Bull., 1904, vol. xv, p. 181; also Med. News, 1904, vol. lxxxiv, p. 431.
[2] Med. Rec., 1903, vol. lxiv, p. 281.
[3] Jour. Amer. Med. Assoc., 1904, vol. xlii, p. 827.
[4] Amer. Med., 1904, vol. vii, p. 824.
[5] Jour. Amer. Med. Assoc., 1904, vol. xliii, p. 18.
[6] Med. Rec., 1904, vol. lxv, p. 574.
[7] Presse méd., 1904, vol. i, p. 399.

no other disease are these cells so numerous or so constantly present. A high percentage of myelocytes, irrespective of the degree of increase in the total number of leucocytes of all forms, is as essential for the diagnosis of this variety of leukemia as is a predominance of megaloblasts for the recognition of pernicious anemia. In most cases they constitute at least 20 per cent. of the different forms of leucocytes, and occasionally as high as 50 per cent. or more. In the 29 cases of the present series (Table X) the myelocytes, at the first counts, averaged 20.6 per cent., with 7 and 44 per cent. as the minimum and maximum estimates, respectively. It is the fact, not that myelocytes simply occur in this disease, but that they occur in such enormous numbers, that is of prime value in the diagnosis, since in no other condition in which this type of marrow leucocyte is found in the blood are they present in such striking abundance. For example,

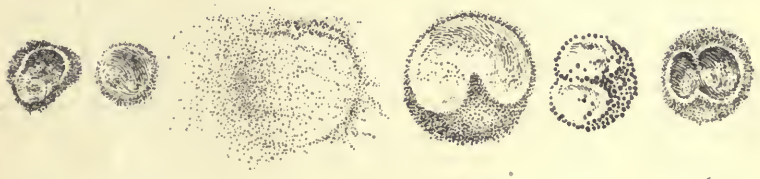



FIG. 53.—ATYPICAL FORMS OF MYELOCYTES IN MYELOGENOUS LEUKEMIA.

1, 2, Dwarf forms, with relatively large and deeply stained nuclei situated in a relatively small amount of cell body containing neutrophile granules. 3, "Fractured" myelocyte. 4, Extremely large form, with kidney-shaped nucleus. 5, Eosinophilic myelocyte with deeply constricted nucleus. 6, Myelocyte with an hour-glass constriction of the nucleus. (Ehrlich's triacid stain.)

although myelocytes are very constant in pernicious anemia, they are only about one-twentieth as numerous in this disease, on the average, as they are in myelogenous leukemia.

Many of the myelocytes are of very large size, some being quite 22 $\mu$ in diameter and occasionally of somewhat larger dimensions; others are dwarfed to no larger than the diameter of a small lymphocyte. The nuclei of these larger forms stain with relatively less intensity than those of the smaller. Indentation, apparent division, and hour-glass constriction of the myelocytes' nuclei are also frequently noted. A very common form of this cell in myelogenous leukemia is characterized by its large size and its pale, kidney-shaped nucleus, the regularly convex border of which lies in intimate contact with fully one-half the periphery of the cell body. These and other atypical forms of myelocytes are shown in the accompanying illustration (Fig. 53). The abnormalities affecting the granules of this

type of cells, as well as certain degenerative changes, do not differ from those which are found in the polynuclear neutrophiles described below.

The relative percentage of *polynuclear neutrophiles* is low, but not especially so, although, of course, the absolute number of this type of cells is greatly in excess of the normal standard, as may be demonstrated by taking into consideration the high total leucocyte count. For instance, in a case having 300,000 leucocytes per c.mm., with, say, 50 per cent. of them polynuclear neutrophiles, the actual number of the latter is 150,000 to the c.mm., or fifteen times the maximum normal number. The cases listed in Table X averaged 54.3 per cent. for this variety of leucocytes, with a range between 34.1 and 77.0 per cent. in the individual case, but other authors, with more extended series of cases as a basis for their statistics, give lower figures than these.

A feature which at once attracts attention in the examination of the stained specimen is the deviation from the normal size of a large proportion of these neutrophilic cells. Dwarfed cells, often not more than 5 or 6 $\mu$ in diameter, and large forms, some of them measuring 15 $\mu$ or even more in diameter, are common, the nuclei of the former usually staining much more sharply than those of the latter, which may exhibit a very feeble reaction toward the basic dye, and show a more diffuse and delicate chromatin structure than is the rule in normal blood. The nuclei tend to exhibit extreme polymorphism and variations in their relative size to that of the cell body, and many of the cells are deformed in shape, being drawn out into various oblong and elliptical designs or into irregular elongated masses. "Fractured" cells, from which the granules have escaped, are very commonly seen. It seems reasonable to attribute the free neutrophile granules sometimes seen in the blood in this disease to the rupture of a neutrophilic leucocyte, although the particular cell to which they belonged may be difficult to identify; the view expressed by some authors that such granules may preëxist in the plasma is scarcely to be thought of seriously. All these deformities are doubtless the result of injuries to the cells in the preparation of the cover-glass spreads, and they suggest a lowered resistance on the part of the leucocytes.

The number of granules in the polynuclear neutrophiles varies greatly in the individual cells: in some they are densely crowded throughout the protoplasm and overrun portions of the nucleus; in others they are confined to certain areas of the cell body, especially in the neighborhood of the nucleus; while in still others they are distributed singly or in twos and threes through the

protoplasm. Occasionally a cell wholly devoid of granules is observed, and, very rarely, one containing both neutrophile and a few isolated eosinophile or basophile granules. The neutrophile granules themselves vary greatly in size, being in some cells so extremely delicate and fine that they can barely be distinguished, while in others they almost equal the size of the smaller eosinophile granules.

Fine and coarse vacuolation of the nucleus and protoplasm, a fissured and cracked appearance of the nuclear chromatin, and an apparent solution of the protoplasm, with freeing of the nucleus, are the most prominent degenerative changes affecting the polynuclear neutrophiles, as well as the other varieties of leucocytes, in this disease.

The relative percentage of *lymphocytes*, small and large together, is decidedly lower than normal, although their total num-

1 2 3

FIG. 54.—ATYPICAL FORMS OF POLYNUCLEAR NEUTROPHILES IN MYELOGENOUS LEUKEMIA.

1, Cell containing both neutrophile and moderately coarse basophile granules. 2, Polynuclear cell with two ovoid nuclei and neutrophile granules, probably representing a later developmental stage than 6, Fig. 53. 3, "Fractured" polynuclear neutrophile. (No. 1 stained with Jenner's eosinate of methylene-blue, 2 and 3 with Ehrlich's triacid stain.)

ber to the c.mm. of blood is greatly increased. As shown by the cases in Table X, these cells average approximately 14 per cent. of all varieties of leucocytes, which represents a diminution to about one-half of the proportion found in normal blood. It is the small lymphocytes which suffer the greater loss, for their proportion in the differential count is sometimes not more than a fraction of one per cent., and always greatly below normal; the large lymphocytes and "transitional" forms, on the contrary, average about normal, and, indeed, may be greatly increased in the individual case. Türk's *stimulation forms* are also met with, but these cells, as a rule, are not numerous.

Atypical forms of lymphocytes are not so common in this form of leukemia as they are in the lymphatic variety. Such cells are described under the latter disease. (See p. 319.)

*Eosinophilia*, as indicated by an increase in the total number of eosinophiles, is almost invariably found, and an increase above

normal in the relative percentage of these cells sometimes, but not always, exists.[1] Thus, in the above-mentioned series the eosinophiles, which normally do not exceed 500 per c.mm., ranged from 780 to 129,150 and averaged 14,204 per c.mm., these figures corresponding to percentages of 1.2, 28.7, and 5.4, respectively.

Ehrlich's original statement regarding an increase of the eosinophiles in this form of leukemia has been contradicted by several writers, notably by von Limbeck[2] and by Müller and Rieder[3]; but these contradictions are based upon a misconception of Ehrlich's remarks, for he never claimed that an abnormally high *percentage* of eosinophiles was associated with this disease, but said simply that their *absolute number* was increased.

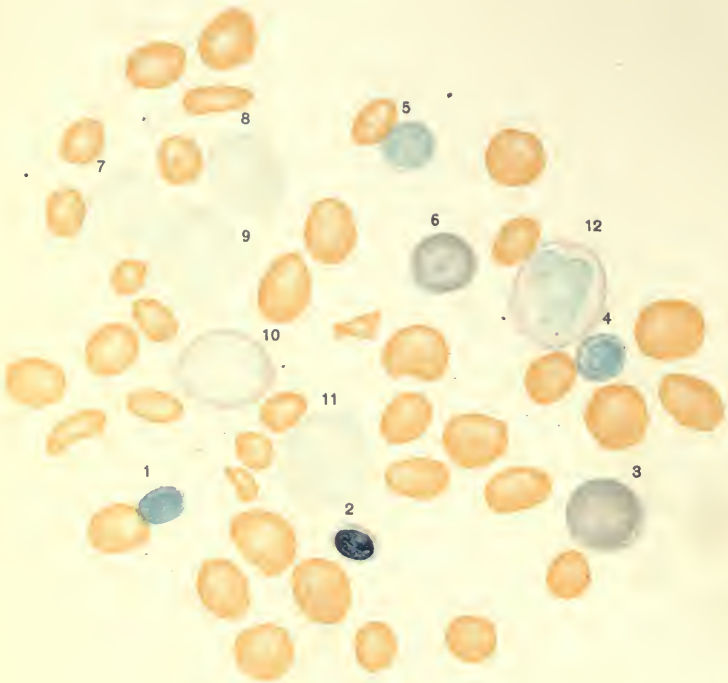
Marked variation in the size of many of the eosinophiles is commonly observed, dwarf forms, 5 or 6 $\mu$ in diameter, with densely crowded and deeply stained granules, being especially striking and apparently more numerous than the larger forms. *Eosinophilic myelocytes*, differing from ordinary neutrophilic myelocytes only in that they are studded with eosinophile granules, are very numerous, and are among the largest forms of the myelocyte found in this disease. "Fractured" eosinophiles are common, being usually more abundant than neutrophilic cells which have thus traumatically suffered. In some of the eosinophiles the granules are scanty, and in many their size varies greatly. Unusually large-sized granules are often found, especially in the dwarf cells and in the extremely large forms.

*Mast cells* with coarse, metachromatic granules are found with great constancy, being absent in but a small proportion of cases. This cell is especially suggestive of leukemia of this variety, since in no other disease does it occur in such large numbers. In 19 cases of the above series the mast cells averaged 9 per cent. in the differential leucocyte counts. In some leukemic bloods the mast cells attain an enormous size, being quite the largest cellular elements found in the specimen. They may be easily identified by their characteristic reaction toward the basic dyes, described in a previous section. (See p. 221.)

From the above remarks it may be concluded that myelocytes and mast cells are present in the circulating blood at the expense of all the normal varieties of leucocytes except the eosinophiles, and that the brunt of this decrease is sustained by the mononuclear, non-granular forms, chiefly by the small lymphocytes.

[1] Simon's case, in which 13 differential counts were made, consistently showed an absence of eosinophiles. (Amer. Jour. Med. Sci., 1903, vol. cxxv, p. 984.)

[2] *Loc. cit.* [3] Deutsch. Arch. f. klin. Med., 1891, vol. xlviii, p. 96.




LYMPHATIC LEUKEMIA.

(*Triacid Stain.*)

1, 2, 3, 4, 5, 6. **Small Lymphocytes.**
These cells show a great difference in the intensity of their reaction toward the basic dye. The smallest forms, 1, 2, 4, and 5, being richer in nuclear chromatin and staining more deeply than the larger, 3 and 6. Compare 2 with the normoblast, 16, Plate IV.

7, 8, 9, 10, 11. **Large Lymphocytes.**
Except in 10, which shows a delicate rim of fuchsin-stained protoplasm, these lymphocytes appear simply as pale chromatin-deficient nuclear structures, lacking cell bodies. Compare these cells with the myelocytes, Plate IV.

12. **Transitional Form.**
The upper edge of the nucleus is somewhat indented and the protoplasm is distinguishable; otherwise this cell resembles a large lymphocyte.

(E. F. FABER, *fec.*)

BLOOD PLAQUES.

These bodies are greatly increased in number in most cases of this form of leukemia, and may frequently be recognized in the fresh specimen and in the diluted blood in the counting chamber of the hemocytometer. They are very constantly observed in the stained film prepared by the Romanowsky method.

## LYMPHATIC LEUKEMIA.

APPEARANCE OF THE FRESH BLOOD.

In most cases the blood drop is watery-looking, pale, and thin, for in this variety of leukemia the anemia is usually very marked. The milky appearance of the drop, frequently observed in the myelogenous form of the disease, is not often noticed in the lymphatic variety.

The alterations in the coagulability, alkalinity, and specific gravity of the whole blood are similar to those met with in myelogenous leukemia.

Microscopically, the field is crowded with large numbers of leucocytes, the vast majority of which are mononuclear cells encircled by a perfectly hyaline, non-granular protoplasm. They may be quite uniformly either of small or of large size, or so many intermediate sizes may be present that it is impossible to distinguish any single predominating type. It is apparent that the leucocytes do not seem so numerous as in myelogenous leukemia, nor are their characteristics so striking, at first glance, because of the lack of granulations in their protoplasm. The difference between these hyaline cells and the granular leucocytes of the last-named disease, even although they may not happen to differ greatly in size and shape, is at once patent to the practised eye. The changes affecting the size, color, and shape of the erythrocytes vary with the degree of oligochromemia and oligocythemia present; they are generally quite decided, as in any high-grade anemia.

HEMOGLOBIN AND ERYTHROCYTES.

Marked anemia, characterized by a disproportionate diminution in hemoglobin, is the general rule in this variety of leukemia, the decrease in both hemoglobin and erythrocytes, especially in the former, being frequently greater than in the myelogenous form. In a series of 13 cases the following figures, referring to the initial examinations, were obtained:

TABLE XI.—HEMOGLOBIN AND ERYTHROCYTES IN 13 CASES OF LYMPHATIC LEUKEMIA.

| Hemoglobin Percentage. | Number of Cases. | Erythrocytes per c.mm. | Number of Cases. |
|---|---|---|---|
| From 40–50 | 1 | From 3,000,000–4,000,000 | 3 |
| " 30–40 | 4 | " 2,000,000–3,000,000 | 4 |
| " 20–30 | 4 | " 1,000,000–2,000,000 | 6 |
| " 10–20 | 4 | | |
| Average, 38.1 per cent. | | Average, 3,032,211 per c.mm. | |
| Maximum, 47.0 " " | | Maximum, 3,590,000 " " | |
| Minimum, 14.0 " " | | Minimum, 1,152,000 " " | |

In rare instances the number of erythrocytes falls below 1,000,000, and the hemoglobin so low that it is impossible to estimate the percentage at all accurately. Rapidly developing and extremely pronounced anemia is generally observed in cases which pursue an acute course.

*Nucleated erythrocytes*, chiefly of the normoblastic type, are commonly found in moderate numbers, but never, except in rare instances, usually occurring in children, are they as numerous as in the myelogenous form of the disease. As a rule, when both normoblasts and megaloblasts are present, the former vastly outnumber the latter, although occasionally one meets with a case in which this predominance of adult-type erythroblasts is less pronounced. Thus, in one of the writer's cases the total number of erythroblasts was calculated at 10,678 per c.mm., of which 8512 were normoblasts and 2166 megaloblasts; such a blood picture as this, however, is but seldom found. In general terms it may be said that the more acute the form of the disease, the more decided the oligochromemia and oligocythemia, and the more abundant the erythroblasts, the number and character of which appear to depend upon the grade of the anemia present. It should not be forgotten that in some cases of typical lymphatic leukemia nucleated erythrocytes are so scanty that they are detected only after repeated examinations.

*Deformities* of size and shape and *atypical staining* of the erythrocytes are marked in direct relation to the severity of the anemia.

Leucocytes. The number of leucocytes is largely increased, but usually much less strikingly so than in myelogenous leukemia. Counts of 500,000 or even of 1,000,000 cells have, it is true, been reported by a few observers, but only as rare examples of the extreme increase which it is possible for the leucocytes to attain in this condition. The cases in Table XII illustrate the range of the leucocytes in this variety of leukemia.

TABLE XII.—NUMBER OF LEUCOCYTES IN 13 CASES OF LYMPHATIC LEUKEMIA.

| Leucocytes per c.mm. | Number of Cases. |
|---|---|
| Above 300,000 | 2 |
| From 200,000–300,000 | 1 |
| " 150,000–200,000 | 0 |
| " 100,000–150,000 | 4 |
| " 50,000–100,000 | 3 |
| Below 50,000 | 3 |

Average, 270,822 per c.mm.
Maximum, 958,000 " "
Minimum, 38,000 " "

By examination of the stained film the identity of the leucocytes responsible for the high count is more clearly distinguishable, and it is found that the increase is due to a large absolute gain in the *lymphocytes*, the relative percentage of these cells to the other varieties of leucocytes generally being 90, 95, or even higher. In the series below summarized (Table XIII) these non-granular cells averaged 89.8 per cent. of the leucocytes, and equaled or exceeded 90 per cent. in three-fourths of the cases examined. In some instances the small lymphocytes are found to be in excess, and the field is dotted with small, deeply stained cells ranging from about 5 to 10 $\mu$ in diameter; in other instances the larger forms prevail, so that large, feebly stained cells, from about 10 to 15 $\mu$ or even larger, are in excess; while in still other cases the sizes and staining properties of the cells are so variable and atypical that it is impracticable to class them in two definite groups, large and small. It is generally believed that small lymphocytes are associated with the more chronic forms of the disease, and that the larger varieties are found in excess in the acute cases. Many of the larger forms, which possess a relatively large nucleus deficient in chromatin and a faintly basic non-granular protoplasm, are regarded as the mother-cells of the typical small lymphocytes. They are identical with the "lymphogonien" of Benda and the "leukoblasts" of Löwit, cells resident in the germinal nests of the lymphatic tissues.

TABLE XIII.—QUALITATIVE CHANGES IN THE LEUCOCYTES IN 13 CASES OF LYMPHATIC LEUKEMIA.

| | Average. | Maximum. | Minimum. |
|---|---|---|---|
| Total lymphocytes | 89.8 | 97.7 | 53.0 |
| Small lymphocytes | 51.4 | 94.0 | 21.1 |
| Large lymphocytes | 38.4 | 76.2 | 1.0 |
| Polynuclear neutrophiles | 7.6 | 45.0 | 1.6 |
| Eosinophiles | 0.6 | 5.0 | 0.1 |
| Myelocytes | 1.4 | 4.9 | 0.3 |
| Mast cells[1] | 0.1 | 1.0 | 0.0 |

[1] Estimates in 6 cases.

Various atypical forms of lymphocytes, the commonest of which are pictured below (Fig. 55), are often numerous. With basic stains, such as methylene-blue, a ragged, torn condition of the basic seam of protoplasm, with so-called "budding," is frequently demonstrable, as well as nucleolation of some cells, especially of those of large size. Nuclear indentation and division and forms characterized by a small lymphocyte's nucleus within a large lymphocyte's cell body are also common.

The relative proportion of *polynuclear neutrophiles* is markedly diminished, commonly to from about 5 or 10 per cent. of the total number of leucocytes, and sometimes even to below one per cent. These cells do not usually display the abnormal staining, the nuclear peculiarities, and the irregularities in size and shape that are so often seen in myelogenous leukemia.

*Myelocytes* are present in the great majority of cases, but always in trifling numbers, as in pernicious anemia; their proportion rarely exceeds one or two per cent. of all forms of leucocytes.


FIG. 55.—ATYPICAL FORMS OF LYMPHOCYTES IN LYMPHATIC LEUKEMIA.

1, Large lymphocyte with ragged protoplasm. Two small bits of protoplasm, the product of "budding," lie free in the plasma beside the cell. 2, Large lymphocyte showing a nucleolus. 3, Large lymphocyte containing two nuclei. 4, Small lymphocyte containing an indented nucleus. 5, Small lymphocyte containing two nuclei. 6, Cell the size of a large lymphocyte with the nucleus of a small lymphocyte. (1 and 2 are stained with eosin and methylene-blue; 3, 4, 5, and 6 with Ehrlich's triacid stain.)

The percentage of *eosinophiles* is diminished, usually to a fraction of one per cent., and in a certain proportion of cases these cells are absent from the peripheral blood. It must be remembered, however, that even with a low relative percentage figure for the eosinophiles true eosinophilia may exist, although usually not to so marked a degree as in the myelogenous form of the disease. One per cent. of eosinophiles in a leucocyte count of 100,000 means 1000 eosinophiles per c.mm. of blood, or twice the maximum number found in the normal individual. *Eosinophilic myelocytes* are rare, but they occur in small numbers in an occasional case.

Increase in the number of *basophiles* does not occur with great frequency, and both the finely granular basophilic leucocytes and the typical mast cells are generally conspicuous by their absence, in contrast to their abundance in the myelogenous variety of this disease.

From the above it is evident that in lymphatic leukemia the increase in the total number of leucocytes is dependent upon a marked absolute gain in the lymphocytes, and that in consequence of this enormous influx of mononuclear hyaline forms, the relative percentages of the other leucocytes, especially of the polynuclear neutrophiles, are correspondingly diminished.

As in the myelogenous form, the number of *blood plaques* is usually much increased.

ACUTE LEUKEMIA.

This term has been applied to a form of leukemia which pursues a rapid course suggestive of an acute infectious process, and ends fatally within a few weeks after the onset of the acute symptoms. Rapid, progressive enlargement of the lymphatic glands and a relatively small splenic tumor, associated with such clinical features as rigors and irregular pyrexia, bone pains, ulcerative stomatitis, and a decided tendency to purpura and to hemorrhages from the mucous membranes, serve to identify most cases of this rapidly fatal disease. But, as already stated, there may be no evidences of lymphatic or splenic involvement, the lesions in such cases being confined to the bone marrow or to the deep lymph nodes. Some authors limit the duration of acute leukemia to six weeks, but the time limit proposed by Fraenkel,[1] four months, is generally accepted as being more appropriate.

The disease is a rare one, for probably less than 100 authentic cases have been recorded up to the present time, although many more reputed instances have been published. Ebstein[2] collected 17 cases in 1889; Fraenkel[3] published the statistics of 10 in 1895; Bradford and Shaw[4] described, in 1898, 5 cases coming under their observation; and Fussell, Jopson and Taylor,[5] in the same year, published a collective report, embracing the statistics of 57 cases selected as representing all the true examples of acute leukemia reported during the past twenty-one years. Since this report about 30 additional cases have been described by various observers.[6]

Beyond stating that *lymphemia* is the type of blood characteristic of acute leukemia, no special description of the condition of the blood is necessary. In the majority of cases the leucocyte increase may be attributed to a marked gain in the large lympho-

[1] Deutsch. med. Wochenschr., 1895, vol. xxi, p. 639, *et seq.*
[2] Deutsch. Arch. f. klin. Med., 1889, vol. xliv, p. 343.
[3] *Loc. cit.*
[4] Medico-Chirurg. Trans., London, 1898, vol. lxxxi, p. 343.
[5] Trans. Assoc. Amer. Phys., Philadelphia, 1898, vol. xiii, p. 124.
[6] For a review of the literature of acute leukemia see (1) Hamman, Amer. Med., 1904, vol. vii, p. 138; (2) Kelly, Univ. of Penna. Bull., 1903, vol. xvi, p. 270; (3) Miller and Hess, Amer. Med., 1904, vol. vii, p. 389; (4) Mixa, Wien. klin. Rundschau, 1901, vol. xv, pp. 655 and 671; (5) Billings and Capps, Amer. Jour. Med. Sci., 1903, vol. cxxvi, p. 375.

cytes, which greatly predominate over the small forms, while the polynuclear neutrophiles, myelocytes, and eosinophiles are relatively few in number. As a rule, the more acute the case, the more decided the predominance of the large lymphocytes, which usually show well-marked evidences of nuclear and protoplasmic degenerative changes. The loss of hemoglobin and erythrocytes is generally more marked and the erythroblasts are more numerous, than in the commoner forms of chronic lymphatic leukemia. The blood may coagulate very imperfectly and the plaques are often greatly diminished in number. In a case reported by Bensaude,[1] total absence of clotting and of serum transudation was noted after the expiration of twenty-four hours.

Acute leukemia may begin as such, or either the chronic lymphatic or the myelogenous form may develop acute symptoms, with a coincident change in the condition of the blood, but this change in the myelogenous variety is extremely rare. Only nine cases of acute myelogenous leukemia are on record.[2]

Influence of Acute Intercurrent Infections.

The development of an acute infectious process in a leukemic individual commonly provokes striking changes in the behavior of the leucocytes, consisting in most instances in a decrease of their total number to the c.mm. of blood, associated sometimes with an increase in the polynuclear variety of cells and a relative diminution in the number of myelocytes. At other times there is practically no alteration in the relative proportions of the different forms as they existed in the leukemic blood prior to the onset of the complicating infection.

Among the infectious conditions acting in this manner on the leucocytes are abscess, sepsis, pneumonia, influenza, tuberculosis, and erysipelas, but it seems that rheumatic fever has no such effect. Weil,[3] who has studied the effects of colon, pneumococcus, and streptococcus infections in both forms of leukemia, comes to the conclusion that the most powerful influence upon the blood picture is exerted by streptococcus infections. Malignant disease is also capable of bringing about a leucocyte decrease characterized by a relative gain in polynuclear neutrophiles at the expense of the lymphocytes, but the loss does not appear to be so decided as that excited by a specific infectious process.

In rare instances the occurrence of an infectious disease fails to cause a decrease in the leucocytes, and thus to destroy the leukemic picture, but, on the contrary, increases them, by superim-

[1] Sem. méd., 1903, vol. xxiii, p. 57.
[2] Billings and Capps, *loc. cit.*
[3] Gaz. hebdom. de méd. et de chir., 1900, vol. v, p. 829.

posing a typical polynuclear neutrophile leucocytosis, which remains during the existence of the complicating infection the conspicuous feature of the blood. The writer has observed a typical illustration of such a change in a case of myelogenous leukemia, in which, within ten days after the onset of a complicating peritonitis, the leucocyte count rose from 245,000 to 400,000, and the proportion of polynuclear neutrophiles from 44.5 to 79 per cent., while the percentage of myelocytes fell from 20.5 to 8.

Dock[1] has collected 50 cases of leukemia complicated by various intercurrent infections, which in 27 instances were tuberculous and in 23 non-tuberculous. In leukemia plus tuberculosis the virulence of the complicating infection appears to govern the behavior of the leucocytes, which are not decidedly influenced in chronic forms of the disease, but which usually are greatly diminished in the acute miliary type. In the 23 cases of intercurrent infections other than tuberculosis Dock's analysis shows that a marked leucocyte decrease occurred in 11, a relatively slight decrease in 9, and either an increase or no change in the remaining 3. Of qualitative changes in these cases there was noted a general tendency of the leucocytic blood picture to disappear, with a decided increase, both absolutely and relatively, in the polynuclear neutrophiles. Dock's masterly monograph should be consulted for a complete account of this complicated topic, which does not lend itself to text-book discussion.

Coincidentally with the improvement in the condition of the blood there is frequently a decrease in the size of the patient's enlarged spleen and lymphatics, the period during which the leukemic condition is thus bettered, and, so to speak, held in abeyance, corresponding to the duration of the complicating infection, for the blood gradually regains its leukemic type and the glandular and splenic tumors reappear as recovery from the intercurrent disease takes place.

DIAGNOSIS. The following blood picture is characteristic of the myelogenous variety of leukemia:

*Hemoglobin.* Decided loss, averaging about 50 per cent. Color index subnormal, or high.

*Erythrocytes.* Counts average about 3,000,000 per c.mm. Erythroblasts very numerous, cells of the normoblastic type predominating.

Deformities of size and shape, polychromatophilia, and basophilic stroma degeneration marked in cases with severe anemia.

[1] Amer. Jour. Med. Sci., 1904, vol. cxxvii, p. 563.

*Leucocytes.* Increased to about 350,000 per c.mm.
Myelocytes constitute about 20 per cent. of all forms.
Relative percentage of polynuclear neutrophiles low.
Relative percentage of lymphocytes very low.
Eosinophiles absolutely, sometimes relatively, increased.
Mast cells average about 10 per cent. of all forms.
Atypical forms of neutrophiles numerous.

*Plaques.* Increased.

In lymphatic leukemia the blood changes may be briefly expressed thus:

*Hemoglobin.* Marked loss, averaging about 60 per cent. Color index low.

*Erythrocytes.* Counts average about 3,000,000 per c.mm.
Erythroblasts usually scanty, cells of the normoblastic type predominating.
Deformities of size and shape and atypical staining reaction marked in relation to the degree of anemia present.

*Leucocytes.* Increased to about 250,000 per c.mm., counts above this figure being rare.
Lymphocytes constitute about 90 per cent. of all forms.
Relative percentage of polynuclear neutrophiles strikingly low.
Relative percentage of eosinophiles diminished; rarely, an absolute increase.
Small numbers of myelocytes frequent.
Basophiles usually not increased.
Atypical forms of lymphocytes numerous.

*Plaques.* Increased.

In dealing with the differential diagnosis of leukemia it is necessary to distinguish the myelogenous from the lymphatic form, and also to differentiate both forms of the disease from a number of other conditions which may present either somewhat similar blood findings or which, apart from the condition of the blood, may have closely similar clinical manifestations. Thus, on the one hand, leucocytosis and lymphocytosis require differentiation because they produce changes in the blood which may be confused with leukemia; while, on the other hand, one must distinguish between leukemia and Hodgkin's disease, splenic anemia, and a number of conditions causing enlargement of the spleen,

neighboring organs, and lymphatic glands, because of the resemblance, even the identity in some instances, of the other clinical signs.

*Myelogenous* and *lymphatic leukemia* can be distinguished only by examination of the blood, for the distinction between these two forms of the disease cannot be based with any degree of certainty upon the gross clinical appearance of the spleen and lymphatics. Nothing can be more marked than the contrast between the two blood pictures. In the myelogenous form the leucocyte count is usually much higher, and is associated with the presence of immense numbers of myelocytes, and with an increase in the eosinophiles and mast cells; the oligocythemia is not so marked, but erythroblasts are exceedingly numerous, and, strangely, tend to persist independently of any increase in the erythrocytes which may occur from time to time. In the lymphatic form the relatively moderate leucocyte increase depends upon an excessive gain in the ungranulated cells, or lymphocytes, myelocytes being either absent or present in trifling numbers, and decided increase in the eosinophiles and mast cells being most unusual; the oligocythemia is usually decided, but erythroblasts are scanty, and stand in relationship to the degree of anemia existing. The important points of difference are, therefore, the presence of a myelocytic blood in the myelogenous form, and of a lymphocytic blood in the lymphatic variety.

*Pathological leucocytosis* may occasionally involve an increase in the total number of leucocytes equal to that found in either form of leukemia, especially in those cases in which a period of temporary improvement with a fall in the leucocyte count exists. But, aside from the more or less temporary character of the increase in leucocytes, the differential count at once shows that, unlike leukemia, the gain depends upon a large absolute and relative increase in the polynuclear neutrophiles, which constitute ordinarily 85 per cent. or more of the several forms of leucocytes.

*Lymphocytosis*, which is usually a *relative* condition, may in rare instances become *absolute*, so that, in addition to the increase in the relative percentage of lymphocytes, the total number of leucocytes in the blood is also decidedly increased. In marked instances of this sort it is obviously impossible to distinguish the blood change from that of lymphatic leukemia, and the aid of other clinical symptoms must be invoked to make the diagnosis clear. Thus, both an absolute and a relative lymphocytosis, closely simulating the lymphatic form of leukemia, have been observed in severe cases of chlorosis, in pertussis, in sarcoma of the lymphatic structures, and in acute inflammatory processes oc-

curring in young children. The author recalls an instance of marked absolute lymphocytosis in a case of pernicious anemia which seemed to justify the tentative diagnosis of lymphatic leukemia, an error which was later corrected, when the megaloblastic blood picture became apparent. In such instances, which are, fortunately, of very rare occurrence, it is true that neither the percentage of lymphocytes nor the count of leucocytes is likely to average so high as in lymphatic leukemia, but still the blood changes are sometimes very misleading, and should not be relied upon to the exclusion of other equally important symptoms.

The glandular and splenic enlargements of *Hodgkin's disease* form a clinical picture identical with either the myelogenous or the lymphatic variety of leukemia, so that these conditions are distinguishable only by the result of the blood examination. But by this means the diagnosis is made extremely simple, by finding in Hodgkin's disease either entirely normal blood or a variable degree of anemia. The number of leucocytes is usually normal, except in cases in which some complicating inflammatory or infectious process causes a moderate increase, typical of a polynuclear neutrophile leucocytosis.

In *chloroma* the blood changes may be practically those of an acute lymphatic leukemia—progressive anemia with absolute lymphocytosis. In chloroma, however, the clinical picture is made up of exophthalmos, deafness, orbital pain, elastic swellings of the orbital and temporal regions, and a tendency toward metastases of the "green tumors" in periosteal structures.

Von Jaksch's *multiple periostitis* is a symptom-complex resembling myelogenous leukemia, in that a myelocytic anemia with splenic enlargement and a tendency toward hemorrhage are symptoms common to both conditions. In the early stages of the disease described by von Jaksch, fever, drenching sweats, painful and swollen joints, and thickening of the distal extremities of the radius and ulna are observed, these distinctive symptoms being succeeded by a preagonal stage marked by a cessation of the bone pains and sweating and by an aggravation of the already marked anemia, splenomegaly, and hemorrhagic tendency.

*Still's disease*, because of the splenic and lymph-node enlargements, may superficially resemble leukemia, but in this form of infantile arthritis, besides an aleukemic blood picture, one finds multiple arthritis, especially of the periarticular structures, and usually a clear history of rickets.

The rather close resemblance which certain cases of *splenic anemia* bear to leukemia, together with the points of difference between them, have already been described. (See p. 295.)

Enlargements of the *spleen, left kidney,* and *pancreas* may lead to the belief that leukemia exists. Thus, splenic tumors due to chronic malarial infection, to amyloid disease, to cysts, and to malignant neoplasms; enlargements of the left kidney, such as can be caused by hydronephrosis, by cysts, and by malignant disease; as well as cystic tumors of the pancreas and malignant disease of the retroperitoneal glands all may, on physical examination, simulate more or less faithfully the leukemic spleen. The negative character of the blood findings will at once exclude leukemia, should one of the above-named conditions be the cause of the physical signs suggesting this disease.

*Lymphatic hyperplasia,* due to tuberculosis, to syphilis, and to malignant disease, may also be mistaken for the glandular involvement of leukemia, for such enlargements sometimes show nothing distinctive. In tuberculous adenitis the blood is either normal or anemic, if the cachectic state of the patient is marked; or, should there happen to be a secondary infection of the glands plus the tuberculous lesions, a simple polynuclear leucocytosis is found. In syphilitic adenitis there is often anemia with a moderate polynuclear leucocytosis, and sometimes with a relative lymphocytosis, especially in children. In malignant disease of the lymphatics increase in the number of leucocytes may also be noted in association with a high-grade anemia; in carcinoma the increase involves chiefly the polynuclear neutrophiles, but in sarcoma the lymphocytes may be unduly increased, though not to the extent found in lymphatic leukemia.

## VII. HODGKIN'S DISEASE.

Appearance of the Fresh Blood.

Nothing characteristic is observed either in the gross appearance of the fresh blood drop or in the unstained film, microscopically. The blood may appear normal, or it may show changes common to any secondary anemia.

The alkalinity and specific gravity of the whole blood are diminished in relation to the degree of anemia which exists. Coagulation may take place slowly, and even be as incomplete as it is in some cases of leukemia; or it may occur within the normal time limit.

Hemoglobin and Erythrocytes.

Both the hemoglobin percentage and the number of erythrocytes are normal in the early stages of the disease, and in slowly progressive cases the blood may remain unaffected for a long period. But sooner or later, as the disease pro-

gresses and a cachectic condition of the patient develops, anemia appears, gradually where the course of the disorder is slow, and rapidly in the more acute forms. Counts made when the patient first comes under observation usually average 4,000,000 or 5,000,000 cells per c.mm., but in the later stages the number frequently falls to one-half this figure or even less. The loss of hemoglobin begins earlier, and in most instances is proportionately somewhat greater, than the erythrocyte decrease, so that subnormal color indices rule—not decidedly low, but yet twenty points or so below the normal standard. In cases which develop excessive oligocythemia the index figures may be quite as high as in pernicious anemia.

In the series of 21 cases summarized in Table XIV, the hemoglobin averaged about 55 per cent., ranging between 30 and 81 per cent.; the erythrocyte count averaged 3,591,423 per c.mm., the minimum being 1,300,000 and the maximum 5,225,000, with more than one-third of the cases having 4,000,000 cells or more.

TABLE XIV.—HEMOGLOBIN AND ERYTHROCYTES IN 21 CASES OF HODGKIN'S DISEASE.

| Hemoglobin. Percentage. | Number of Cases. | Erythrocytes per c.mm. | Number of Cases. |
|---|---|---|---|
| From 80–90 | 3 | Above 5,000,000 | 2 |
| " 70–80 | 3 | From 4,000,000–5,000,000 | 6 |
| " 60–70 | 1 | " 3,000,000–4,000,000 | 10 |
| " 50–60 | 6 | " 2,000,000–3,000,000 | 1 |
| " 40–50 | 4 | " 1,000,000–2,000,000 | 2 |
| " 30–40 | 4 | | |
| Average, 55.3 per cent. | | Average, 3,591,423 per c.mm. | |
| Maximum, 81.0 " " | | Maximum, 5,225,000 " " | |
| Minimum, 30.0 " " | | Minimum, 1,300,000 " " | |

*Qualitative changes* affecting the corpuscles occur in relation to the intensity of the anemic process, deformities of shape and size and atypical staining reaction of the cells being associated with cases in which notable hemoglobin and erythrocyte losses exist, and being absent when the anemia is moderate. Nucleated erythrocytes are not common, nor are they numerous when present. Usually they are wanting, except in connection with a high-grade anemia, under which circumstance a few normoblasts may be detected, and in rare instances an occasional megaloblast.

Leucocytes.

In the average case the leucocytes are normal, both in number and in the relative percentage of different varieties. More rarely, *relative lymphocytosis* occurs, involving a decrease in the percentage of polynuclear neutrophiles, but not increasing the total number of leucocytes. An instance of this kind has been observed by the

writer, in which the relative proportion of lymphocytes to other forms of leucocytes habitually remained for some months between 70 and 85 per cent., this change affecting chiefly the large lymphocytes, while the total leucocyte count never exceeded normal.

TABLE XV.—NUMBER OF LEUCOCYTES IN 21 CASES OF HODGKIN'S DISEASE.

| Leucocytes per c.mm. | Number of Cases. |
|---|---|
| Above 20,000 | 1 |
| From 15,000–20,000 | 3 |
| " 10,000–15,000 | 4 |
| " 5,000–10,000 | 7 |
| Below 5,000 | 6 |
| Average, 8,819 per c.mm. | |
| Maximum, 21,000 " " | |
| Minimum, 1,000 " " | |

If secondary infection takes place, it soon becomes evident by an increase in the leucocytes to about 20,000 or more, principally involving the *polynuclear neutrophile cells*, at the expense of the lymphocytes—a picture of typical leucocytosis. In some instances, however, without any apparent signs of a secondary infection or of a glandular inflammation, the total count may exceed the normal standard by several thousand cells, and yet show a normal or even somewhat decreased proportion of neutrophiles. These changes in the blood picture must be distinguished from those indicating the conversion of Hodgkin's disease into true lymphatic leukemia. This transition, although exceedingly rare, probably sometimes occurs, as shown by Wende,[1] Posselt,[2] Senator,[3] Mosler,[4] Fleischer and Penzoldt,[5] and others. Should the anemia be very marked, pronounced leucopenia is commonly associated with it. In the present series (Table XV) about one-fourth of the cases showed frank leucocytosis, the highest estimate being 21,000, the lowest 1000, and the average 8819.

TABLE XVI.—QUALITATIVE CHANGES IN THE LEUCOCYTES IN 21 CASES OF HODGKIN'S DISEASE.

| | Average. | Maximum. | Minimum. |
|---|---|---|---|
| Small lymphocytes | 16.5 | 49.0 | 1.0 |
| Large lymphocytes | 10.8 | 21.0 | 0.5 |
| Polynuclear neutrophiles | 69.6 | 88.0 | 46.2 |
| Eosinophiles | 2.2 | 10.0 | 0.0 |
| Myelocytes | 0.5 | 3.0 | 0.0 |
| Mast cells[6] | 0.9 | 1.1 | 0.0 |

[1] Amer. Jour. Med. Sci., 1901, vol. cxxii, p. 836.
[2] Wien. klin. Wochenschr., 1895, vol. viii, p. 407.
[3] Berlin. klin. Wochenschr., 1882, vol. xix, p. 533.
[4] Virchow's Arch., 1888, vol. cxiv, p. 461.
[5] Deutsch. Arch. f. klin. Med., 1896, vol. lxvii, p. 300.
[6] Estimates in 11 cases.

Small numbers of *myelocytes* are not uncommon in the advanced anemia of Hodgkin's disease, but they are never more numerous than in any other condition accompanied by a similar deterioration of the blood. These cells were found in 8 of the 21 cases under consideration.

Small numbers and low percentages of *eosinophiles* are the rule, in cases both with and without leucocytosis; it is most unusual for these cells to attain the maximum normal figure, and they are sometimes wholly absent.

Neither the finely granular *basophiles* nor the typical *mast cells* are increased in this disease. The author found the latter in but a single case of 11 examined.

As in both forms of leukemia, the number of *blood plaques* in Hodgkin's disease is usually increased.

DIAGNOSIS. Although no characteristic blood changes occur in this condition, the alterations most commonly observed may be briefly summed up as follows:

*Hemoglobin.* Normal in the early stages of the disease; later, a moderate decrease, estimates averaging about 55 per cent. Color index commonly subnormal, rarely high.

*Erythrocytes.* Normal in the early stages; later, a variable degree of oligocythemia, counts averaging about 3,500,000 per c.mm.

Erythroblasts uncommon and scanty when present.

Normoblasts prevail almost exclusively, megaloblasts being very rare.

Deformities of size and shape, polychromatophilia, and basic stroma degeneration only in cases with high-grade anemia.

*Leucocytes.* Normal or moderately increased.

Either polynuclear neutrophiles or lymphocytes may be relatively increased, more commonly the former.

Small numbers of myelocytes in very anemic cases.

Eosinophiles not increased.

Basophiles not increased.

*Plaques.* Usually increased.

The absence of characteristic blood changes in Hodgkin's disease at once distinguishes the condition from its clinical counterfeit, *leukemia*, but, aside from this single disease, the blood ex-

amination is valueless in the differentiation of other conditions having somewhat similar involvement of the glandular structures. These conditions, *tuberculous* and *syphilitic adenitis*, local *lymphomatous tumors*, and *malignant neoplasms* of the lymphatics, must therefore be distinguished from Hodgkin's disease by other clinical methods, for all may provoke identical blood changes.

In reviewing the clinical history of a case of suspected Hodgkin's disease the following symptoms should be given special consideration: the gradual onset of a widespread hyperplasia of the lymphatic structures, occurring most commonly in males under middle age; the progressive character and chronicity in most cases of the disorder; the tendency in some cases toward the occurrence of unexplained febrile periods, sometimes coinciding with a rapid and marked increase in the size of the affected glands which disappears as the fever subsides; the cachexia, asthenia, and emaciation of the patient, frequently associated with gastro-intestinal and circulatory disturbances and with a tendency to hemorrhages, such as epistaxis and purpura; the presence of pressure symptoms, such as cough, dysphagia, dyspnea, edema, and pleural and peritoneal effusions; and the development of bronzing of the skin in an occasional case.

In typical cases the glandular enlargement forms a series of distinct, painless, hard tumors, each freely separable from its neighbor, and rarely caseating or suppurating. Due weight should also be given to the fact that in the majority of cases the lesion originates in the superficial lymphatic glands of the cervical region, beginning either in the occipital or in the inferior carotid triangle. The spleen is moderately enlarged in the majority of cases, and in others the liver, kidneys, suprarenals, tonsils, thymus, thyroid, and sexual organs may be involved in the lymphoid growths.

As in leukemia, remarkable improvement in the blood and other clinical features of Hodgkin's disease is reported by Senn,[1] by Steinwald,[2] by Childs,[3] and by Pusey[4] as the result of *x*-ray treatment.

*Tuberculous adenitis* usually first involves a group of glands in the submaxillary triangle, and tends to produce inflammatory adhesions between the tissues and the glandular structure, with softening, fusing; caseation, and suppuration of the glands. It is of sluggish development, often occurs in the very young, and is

[1] N. Y. Med. Jour., 1903, vol. lxxvii, p. 665.
[2] Medicine, 1904, vol. x, p. 438.
[3] N. Y. Med. Jour., 1904, vol. lxxx, p. 13.
[4] Jour. Amer. Med. Assoc., 1902, vol. xxxviii, p. 911.

almost always confined to a single group of glands. Evidences of tuberculous lesions in the lungs or in other parts of the body, especially of dental caries, cutaneous lesions of the face, and adenoid pharyngeal growths, and the discovery of tubercle bacilli in the glandular tissue are valuable evidences of the tuberculous nature of the disease, yet they do not positively exclude Hodgkin's disease, since the coëxistence of the two conditions in the same individual is possible beyond a doubt. Sternberg's belief[1] that Hodgkin's disease is essentially tuberculosis is still supported by Musser[2] and Sailer,[3] although the recent brilliant work of Dorothy Reed,[4] of Longcope,[5] and of Simmons[6] is convincing proof that the disease is a distinct clinical entity, in no way related pathologically to the tubercle bacillus.

In *syphilitic adenitis* of the neck the post-cervical groups are first affected, the glands being of cartilaginous hardness, painless, freely movable, and of small or moderate size. The glandular enlargement is often more or less general, but the affected groups do not attain a large size. A history of an initial lesion in the vicinity of the primary glandular swellings, or of the appearance of secondary symptoms, the disappearance of the glandular tumors after the administration of mercury, and the presence of Justus' test will suffice to prove the specific character of the hyperplasia.

A *local lymphoma* is limited strictly to a single group of glands, forming a painless, dense mass, free from inflammatory adhesions, caseation, and suppuration. It commonly involves the submaxillary glands, may attain a large size, and is unassociated with constitutional symptoms. Such a local lymphatic tumor cannot be distinguished from the early stage of Hodgkin's disease, for in some cases of the latter the general lymphoid hyperplasia is preceded by a period during which the only sign of the condition is a localized enlargement of a single group of glands. If, according to Osler,[7] a local tumor of this kind persists for over a year or eighteen months without involving the glands of the opposite side or of the axilla, it is almost certainly a non-malignant lymphoma.

*Sarcoma* of the lymphatic tissue forms an immovable tumor, early complicated by inflammatory processes which cause interglandular adhesions and adhesions between the glands and the

[1] Zeitschr. f. Heilk., 1898, vol. xix, p. 21. [2] Amer. Med., 1902, vol. iii, p. 13.
[3] Phila. Med. Jour., 1902, vol. x, pp. 615 and 669.
[4] Johns Hopkins Hosp. Rep., 1902, vol. x, p. 133.
[5] Bull. Ayer Clin. Lab., Penna. Hosp., 1903, vol. i, p. 4.
[6] Jour. Med. Research, 1903, vol. ix, p. 378.
[7] Cited by Bramwell, "Anemia," Philadelphia, 1899, p. 203.

surrounding tissues. The swelling is often red and inflamed, pits upon pressure, and resembles an abscess, while the skin over the site of the lesion is frequently marked by a maze of tortuous, congested, cutaneous veins, and is prone to ulcerate. If nerves are entangled in the growth, the tumor is exquisitely painful. The adjacent tissues become densely infiltrated by the sarcomatous growth, and involvement of distant organs by metastasis is likely to occur. If such be the case, microscopical search for sarcoma cells in the fresh specimen may give a definite clue. (See "Sarcoma," Section VII.) Sarcoma of the lymphatic glands may occur at any period of life.

*Carcinoma* of the lymphatic glands is secondary to an initial growth in some other part of the body, so that in the region of the neck search should be made for a primary cancerous lesion in the mouth and upper air-passages. The disease is most commonly found during the decline of life.

Finally, as Tyson so pertinently remarks,[1] it should not be forgotten that all the conditions named as possible to be mistaken for Hodgkin's disease are limited to a single group of glands, while Hodgkin's disease always extends, and the fact of such limitation is of itself sufficient to exclude the disease. This progressive involvement of the lymphatic glands, group after group, must, after all, be the mainstay in the diagnosis of doubtful cases.

## VIII. THE EFFECT ON THE BLOOD OF SPLENECTOMY.

Hemoglobin and Erythrocytes.

Excision of the spleen in man is followed by a diminution in the hemoglobin and erythrocytes, the degree of which is generally believed to be more pronounced than can be accounted for by the simple factor of hemorrhage incident to the operation. Blood regeneration is slow, especially the restoration of the hemoglobin, which is prone to increase much less rapidly than is the rule in an ordinary secondary anemia. In uncomplicated cases from one to three months' time usually elapses before the normal percentages of hemoglobin and erythrocytes are attained; in unfavorable cases persistence of the anemia for a much longer period is to be observed. Splenectomies attended by great loss of blood may excite, in addition to an extreme cellular decrease, striking qualitative changes, and in such instances the blood picture is characterized by the presence of many normo-

[1] "Practice of Medicine," Philadelphia, 1898, p. 606.

blasts, achromacytes, and corpuscles deformed in shape and size. Conspicuous post-operative anemia is especially common in patients to whom saline intravenous injections have been administered.

TABLE XVII.—THE EFFECT ON THE BLOOD OF SPLENECTOMY

| Number. | Hemoglobin. | Erythrocytes. | Leucocytes. | Small Lymphocytes. | Large Lymphocytes. | Polynuclear Neutrophiles. | Eosinophiles. | Notes. |
|---|---|---|---|---|---|---|---|---|
| 1 [1] | 65 | 5,200,000 | 2,200 | 22 | 5 | 70 | 3 | Before operation. |
| | | | | | | | | Megaloblasts and normoblasts found. |
| | 65 | 5,000,000 | 24,000 | 3.6 | 3.2 | 93 | 0.2 | 2 days after operation. |
| | | | 21,400 | | | | | 3 " " " |
| | | | 23,800 | | | | | 4 " " " |
| | | | 18,000 | | | | | 5 " " " |
| | | | 18,000 | | | | | 8 " " " |
| | 45 | 3,256,000 | 24,000 | 7.9 | 8.5 | 81 | 2.6 | 11 " " " |
| | | | | | | | | Myelocytes and mast cells found. No erythroblasts. |
| | | | 16,400 | | | | | 16 days after operation. |
| | 45 | 4,496,000 | 17,000 | 7.8 | 9 | 76.8 | 6.2 | 21 " " " |
| | | | | | | | | Myelocytes, 0.2 per cent. |
| | 40 | 3,984,000 | 20,000 | 9 | 7.4 | 82.2 | 1.4 | 27 days after operation. |
| | | | | | | | | No erythroblasts. |
| | 40 | 4,000,000 | 15,000 | 5.8 | 7.4 | 82.8 | 4 | 37 days after operation. |
| | 52.5 | 4,672,000 | 21,600 | 15.8 | 8.8 | 73.4 | 2 | 56 " " " |
| | | | | | | | | Myelocytes found; no erythroblasts. |
| | | | 16,000 | 5.6 | 10.2 | 79.8 | 3.5 | 99 days after operation. |
| | | | | | | | | Erythrocytes normal. |
| 2 [2] | 108 | 4,850,000 | 30,000 | 8 | 8 | 83 | 1 | Before operation. |
| | 100 | 4,700,000 | 39,000 | 5 | 4 | 91 | 0 | 7 days after operation. |
| | 105 | 3,630,000 | 18,000 | 15 | 6 | 78 | 1 | 60 " " " |
| | 63 | 2,750,000 | 20,000 | 5 | 10 | 84 | 1 | 3 years " " |
| 3 [3] | 45 | 1,634,000 | 12,000 | 16 | 20 | 61 | 3 | 14 days " " |
| | 87 | 2,460,000 | 20,000 | 18 | 32 | 49 | 1 | 27 " " " |
| | 110 | 4,530,000 | 27,000 | 18 | 15 | 66 | 1 | 33 " " " |
| | 100 | 3,977,000 | 8,000 | 21 | 11 | 62 | 6 | 2 years and six months after operation. |
| 4 [4] | 63 | 4,570,000 | 8,000 | | | | | Before operation. |
| | 64 | 4,970,000 | 30,000 | | | | | 3 days after operation. |
| | 77 | 5,180,000 | 65,000 | | | | | 6 " " " |
| | 66 | 4,800,000 | 17,500 | | | | | 48 " " " |
| | 85 | 4,353,000 | 11,700 | | | | | 4 m'ths " " |
| | 85 | 3,300,000 | 11,600 | | | | | 5 years " " |

[1] Warren, Annals of Surgery, 1901, vol. xxxiii, p. 513.
[2] Hartmann and Vaquez, Compt. rend Soc. biol., Paris, 1897, vol. iv, p. 126.
[3] *Ibid.* [4] Czerny, cited by Vulpius, Beiträge z. klin. Chir., 1894, vol. xi, p. 633

TABLE XVII.—THE EFFECT ON THE BLOOD OF SPLENECTOMY.—(*Continued.*)

| NUMBER. | HEMOGLOBIN. | ERYTHROCYTES. | LEUCOCYTES. | SMALL LYMPHOCYTES. | LARGE LYMPHOCYTES. | POLYNUCLEAR NEUTROPHILES. | EOSINOPHILES. | NOTES. |
|---|---|---|---|---|---|---|---|---|
| 5[1] | 60 | 4,300,000 | 22,000 | 14 | 8 | 77 | 2 | 6 days after operation. |
| | 72 | 4,408,000 | 13,000 | 13 | 19 | 66.5 | 1.5 | 19 " " " |
| | 77 | 4,420,000 | 11,200 | 12 | 18.1 | 65.2 | 2 | 26 " " " |
| | | | | | | | | Normoblasts found. |
| 6[2] | 83 | 3,280,000 | 22,000 | | | | | 4 days after operation. |
| | | 3,980,000 | 26,000 | | | | | 8 " " " |
| | 85 | 4,480,000 | 24,000 | 33 | 4 | 53 | 10 | 14 " " " |
| | 82 | 4,280,000 | 18,000 | | | | | 18 " " " |
| | 86 | 4,300,000 | 12,000 | | | | | 25 " " " |
| | 90 | 4,220,000 | 10,000 | 27 | 5 | 63 | 3 | 30 " " " |
| | 92 | 4,630,000 | 12,000 | | | | | 52 " " " |
| 7[3] | 40 | 4,100,000 | 60,000 | 6 | 12 | 81.6 | 0.3 | 7 days after operation. |
| | 50 | 4,370,000 | 46,000 | | | | | 15 " " " |
| | | 4,300,000 | 38,000 | 11.4 | 8.6 | 78.9 | 1.1 | 19 " " " |
| | | | 26,000 | | | | | 38 " " " |
| | | | 17,000 | | | | | 46 " " " |
| | | 4,500,000 | 25,000 | 7.1 | 30.1 | 60.1 | 2.3 | 2 m'ths " " |
| | 75 | 4,480,000 | 10,400 | 14.3 | 17.1 | 61.9 | 6.7 | 5 " " " |
| 8[4] | | | | 24 | 7 | 67 | 2 | Before operation. |
| | | | | 12 | 8 | 77 | 3 | 7 days after operation. |
| | | | | 15 | 5 | 80 | 1 | 30 " " " |
| | | | | 19 | 7 | 71 | 3 | 45 " " " |
| | | | | 16 | 8 | 72 | 4 | 90 " " " |
| 9[5] | 101 | 4,700,000 | 5,700 | 59.1 | 2.2 | 59.1 | 4.2 | 60 days after operation. |
| | 111 | 5,600,000 | 12,300 | 42.9 | 1.7 | 52.1 | 3.2 | 91 " " " |
| | 91 | 4,500,000 | 15,000 | 18.0 | 1.9 | 78.6 | 1.4 | 128 " " " |
| | 73 | 3,900,000 | 11,300 | 30.5 | 2.2 | 65.6 | 1.5 | 138 " " " |
| | 85 | 3,500,000 | 10,000 | 26.7 | 1.8 | 67.9 | 3.5 | 161 " " " |
| | 93 | 4,000,000 | 8,000 | 32.9 | 2.7 | 58.8 | 5.5 | 212 " " " |
| | 102 | 5,300,000 | 7,500 | 35.9 | 1.7 | 58.5 | 3.7 | 13 m'ths " " " |
| | | | | | | | | Mast cells ranged from 0.15 to 0.3 per cent. |

LEUCOCYTES.

Post-operative leucocytosis of the polynuclear neutrophile type develops promptly, and persists in most instances for from four to six weeks, according to Hartmann and Vaquez,[6] but occasionally for a longer period. Counts of between 15,000 and 30,000 represent the grade

[1] Tieken, Jour. Amer. Med. Assoc., 1903, vol. xl, p. 887.
[2] Ballance, Practitioner, 1898, vol. lx, p. 347.
[3] Heaton, Brit. Med. Jour., 1899, vol. ii, p. 476.
[4] Hartmann and Vaquez, cited by Bordet, "Des modifications du sang après la splénectomie," Thèse, Paris, 1897.
[5] Staehelin, Deutsch. Arch. f. klin. Med., 1903, vol. lxxvi, p. 364.
[6] Compt. rend. Soc. biol., Paris, 1897, vol. iv, p. 126.

of leucocytosis ordinarily found, although occasionally the increase is far greater—70,000 in a case cited by Czerny,[1] and 75,000 in one reported by Hartley.[2] After a number of months, even as late as the second or third year after the operation, there is a moderate increase in the number of eosinophiles. Small-celled lymphocytosis is a frequent but inconstant change, the development and duration of which vary greatly. It is supposed to reflect hyperactivity of the lymphatic glands. Eosinophilia commonly develops within a few months, as in a case studied by Rautenberg,[3] in which the eosinophiles increased to six times the normal percentage by the fourth week after the operation. In splenectomized guinea-pigs Kurloff[4] found during the first year after the operation a marked lymphocytosis, as high as 60 per cent. in some animals, together with a corresponding decrease in the number of granular cells, but with no alteration in the number of large mononuclear leucocytes. Eosinophilia became apparent during the second year, and coincidentally with this change a decrease in the lymphocytes to their normal percentage took place. In splenectomized dogs Nicholas and Dumoulin[5] noted post-operative leucopenia, succeeded by eosinophilia and by lymphocytosis, which, after several months, was followed by a gradual, progressive lymphocyte decrease. The polynuclear neutrophiles showed relatively high percentages, but were not decidedly increased.

The above remarks concerning the differential changes affecting the leucocytes after splenectomy must be regarded as tentative, in view of the fact that sufficient data bearing upon this question have not yet accumulated to justify more definite conclusions. Enlargement of the lymph glands, bone pains, and a marked susceptibility to various infections are frequent post-operative phenomena in splenectomized individuals.

The table on page 334 shows the condition of the blood in the few recorded cases in which thorough blood examinations have been carried out. Reports of other cases may be found in Staehelin's monograph, referred to above.

It is to be remembered that, aside from the character of the splenic lesion, these important factors also determine the degree of the post-operative anemia and leucocytosis in this procedure: the grade of the preëxisting anemia, the amount of hemorrhage during the following operation, and the patient's recuperative powers. All things being equal, the anemia is least marked and

[1] *Loc. cit.* [2] Med. News, 1898, vol. lxxii, p. 417.
[3] Deutsch. Zeitschr. f. Chir., 1902, vol. lxiv, p. 352. [4] Cited by Ehrlich, *loc. cit.*
[5] Journ. de phys. et de path. gén., 1903, vol. v, p. 1073.

## DIFFERENTIAL TABLE.

| | Leucocytosis. | Absolute Lymphocytosis. | Lymphatic Leukemia. | Myelogenous Leukemia. | Hodgkin's Disease. |
|---|---|---|---|---|---|
| *Hemoglobin.* | Unchanged. | Unchanged. | Decided decrease, averaging to about 60 per cent.<br>Relatively very low to erythrocyte loss. | Decided decrease, averaging to about 50 per cent.<br>Relatively low to erythrocyte loss. | Normal or moderate decrease, averaging to about 60 per cent.<br>Relatively high or low to erythrocyte loss. |
| *Erythrocytes.* | Unchanged. | Unchanged. | Decided decrease, counts averaging about 3,000,000.<br>Pallor usually marked.<br>Poikilocytosis variable, often excessive.<br>Erythroblasts rare, normoblasts predominating.<br>Polychromatophilia and basic stroma degeneration common.<br>Changes in diameter variable. | Decided decrease, counts averaging about 3,000,000.<br>Pallor usually marked.<br>Poikilocytosis variable, often excessive.<br>Erythroblasts numerous, normoblasts predominating.<br>Polychromatophilia and basic stroma degeneration common.<br>Changes in diameter variable. | Normal or moderate decrease, counts averaging about 3,500,000.<br>Pallor usually not notably marked.<br>Poikilocytosis usually absent or slight.<br>Erythroblasts extremely rare, normoblasts predominating.<br>Polychromatophilia and basic stroma degeneration usually absent.<br>Changes in diameter variable. |
| *Color Index.* | Unchanged. | Unchanged. | Low, averaging about 0.60. | Subnormal, averaging about 0.90. | Subnormal, averaging about 0.80. |
| *Leucocytes.* | Increased, rarely to more than 50,000.<br>Excessive polynuclear neutrophile increase with lymphocyte decrease.<br>Small numbers of myelocytes occasionally present.<br>Eosinophiles almost invariably decreased.<br>Basophiles not increased. | Increased, rarely to more than 25,000.<br>Moderate lymphocyte increase, with polynuclear neutrophile decrease.<br>Myelocytes uncommon.<br>Eosinophiles usually decreased.<br>Basophiles not increased. | Marked increase, counts averaging about 250,000.<br>Excessive lymphocyte increase with polynuclear neutrophile decrease.<br>Small numbers of myelocytes common.<br>Eosinophiles usually not increased.<br>Basophiles not greatly increased. | Striking increase, counts averaging about 350,000.<br>Excessive myelocyte increase, with polynuclear and lymphocyte decrease.<br>Eosinophiles always increased.<br>Basophiles strikingly increased. | Normal or slightly increased, counts averaging about 10,000.<br>Polynuclear neutrophiles normal or increased, with consequent lymphocyte decrease; rarely, an increase in lymphocytes.<br>Myelocytes rare.<br>Eosinophiles normal or decreased.<br>Basophiles not increased. |
| *Plaques.* | Variable. | Not increased. | Increased. | Increased. | Usually increased. |

the blood regeneration most prompt in simple *wandering spleen* and in *ague cake*, while blood deterioration is more marked and regeneration slower in *rupture* of this organ. Splenectomy for *myelogenous* leukemia is almost invariably followed by a progressive anemia and leucocytosis, and in nearly all cases by death. But five recoveries after removal of the spleen in this disease have been reported,[1] the operation having been performed in forty-two cases.

[1] Hagen, Arch. f. klin. Chir., 1900, vol. v, p. 188; also Richardson, cited by Warren, *loc. cit.*

## SECTION VI.

# THE ANEMIAS OF INFANCY AND CHILDHOOD.

## SECTION VI.

## THE ANEMIAS OF INFANCY AND CHILDHOOD.

### I. CHARACTERISTICS OF THE BLOOD IN CHILDREN.

Fetal Blood.

As a preliminary essential to the intelligent study of the various pathological conditions of the blood in children it is necessary briefly to refer to certain points of difference in the composition of this tissue in the child and in the adult. In general terms it may be stated that the younger the child, the more unformed are the different elements of the blood and the nearer its composition resembles the blood of the fetus.

In fetal blood the specific gravity, both of the whole blood and of the serum, is lower than in the adult, and coagulation is very slow and imperfect. The erythrocytes vary greatly in size and in shape, and are deficient in hemoglobin, which is loosely attached to these cells and hence becomes readily dissolved out. Zangmeister and Meissl[1] conclude that, in comparison with maternal blood, fetal blood is poorer in albumin and nitrogen and is less active in agglutinative, bactericidal, and immunizing powers.

Until about the seventh month of intra-uterine life normoblasts constitute the predominating variety of *erythrocytes*, after which period they rapidly diminish in number, until at full term few, if any, nucleated red corpuscles are found in the blood. Of the different varieties of *leucocytes*, the mononuclear forms are present in a proportion relatively excessive to the other varieties; before the seventh month this lymphocytosis is due to a high relative percentage of large lymphocytes, but after this period the proportion of small lymphocytes increases, until finally they predominate. The percentage of eosinophiles reaches its maximum at the seventh month, gradually becoming less and less as the end of the intra-uterine life approaches.

We find, therefore, that in the fetus the blood is characterized chiefly by the presence of large numbers of normoblasts, by a high relative proportion of mononuclear leucocytes, by a deficiency

[1] Münch. med. Wochenschr., 1903, vol. l, p. 673.

of hemoglobin, and by a feeble activity of the serum. The closer an infant's blood resembles this picture, the "younger" in point of development is such blood considered, and the more strongly is it said to revert to a "young" or "embryonal" type. The deficient defensive potency of the blood of the young baby may account for the proneness of infants toward infections in general.

THE BLOOD AT BIRTH.

At birth the blood of the full-term infant is of higher specific gravity and richer in hemoglobin and in corpuscular elements than that of the older child or of the adult.

The *specific gravity* of the blood of the average healthy infant at the time of birth and during the first few weeks of life is, approximately, 1.066. For normal children the average, which is reached by the beginning of the second year, varies from 1.050 to 1.058, being slightly higher in boys than in girls.

The maximum amount of *hemoglobin* is found at birth, the percentage at this time ranging from 100 to 104, according to the investigations of Hammerschlag.[1] After birth the amount of hemoglobin immediately begins to diminish, the minimum, which may be as low as 55 or 60 per cent., being attained by the end of the third week of life. It remains at or about this minimum for a variable period of time—sometimes for as long as six months—and then gradually begins to increase.

At birth the number of *erythrocytes* in the peripheral blood is decidedly higher than normal, counts of between 5,500,000 and 6,000,000 cells per c.mm. being found at this time, the highest figures being observed in those cases in which ligation of the umbilical cord has been delayed. During the first twenty-four hours of extra-uterine life this polycythemia becomes still more marked, so that the number of corpuscles per c.mm. may reach a maximum of from 7,000,000 to 8,000,000, and sometimes higher, by the end of the first day. Beginning with the second day, a gradual diminution in the number of these cells is noticed, and the normal 5,000,000 per c.mm. is reached by the end of the first week or ten days. Hayem[2] emphasizes the fact that the fluctuations in the number of erythrocytes during the early days of life stand in inverse ratio to the variations in the weight of the child, the maximum number being found at the time of the infant's minimum weight, while as the child begins to gain in weight the count decreases. Schiff[3] is inclined to attribute these fluctuations to the amount of liquids in the body, the result of feeding, showing

[1] Centralbl. f. klin. Med., 1891, vol. xii, p. 825.
[2] "Du Sang," etc., Paris, 1889.
[3] Zeitschr. f. Heilk., 1890, vol. xi, p. 17.

that in fasting children the counts are always higher than in those fed at frequent intervals.

Whatever may be the exact manner of their production, it is evident that these fluctuations are to be regarded as purely physiological in character, depending upon concentration and dilution of the blood, rather than as an expression of involvement of the blood-making organs.

The erythrocytes vary greatly in size during the first few days of post-natal life, the diameter of some cells being as small as 3.25 $\mu$ and of others as large as 10.25 $\mu$. Many observers have noticed that the small-sized cells, as a rule, predominate. Such a microcytosis in the adult would mean well-defined anemia.

*Nucleated erythrocytes* of the normoblastic type may or may not be present in the blood of new-born infants; they are commonly found in large numbers in the prematurely-born child, and also occur less numerously in many fully developed babies, notwithstanding views to the contrary expressed by some observers, notably by Hayem[1] and by Fischl.[2] In most cases normoblasts disappear from the blood after the first few days of life, and their presence after the sixth month should always be regarded as pathological.

The number of *leucocytes* at birth averages about 20,000 per c.mm., the normal average for young infants, 15,000 per c.mm., being reached by the end of the first week, after numerical fluctuations similar to those affecting the erythrocytes. From the second or third week until the sixth month a count from 10,000 to 14,000 may be regarded as normal, while for the child of one year of age the average is about 10,000. By the sixth year the number of leucocytes falls to the number normal for the adult, 7500 per c.mm. The following excellent table from Rotch[3] shows these average counts of erythrocytes and leucocytes in children from birth until the sixth year of age:

| Age. | Erythrocytes. | Leucocytes. |
|---|---|---|
| At birth | 5,900,000 | 21,000 (26,000 to 36,000 after first feeding). |
| End of 1st day | 7,000,000 to 8,000,000 | 24,000 |
| " 2d " | Generally increased. | 30,000 |
| " 4th " | 6,000,000 | 20,000 |
| " 7th " | 5,000,000 | 15,000 |
| 10th day | .... | 10,000 to 14,000 |
| 12th to 18th day | .... | 12,000 |
| 1st year | .... | 10,000 |
| 6th year and upward | .... | 7,500 |

[1] *Loc. cit.*
[2] Zeitschr. f. Heilk., 1892, vol. xiii, p. 277.
[3] "Pediatrics," Philadelphia, 1896, p. 342.

The influence of the initial feeding in infants produces a marked leucocytosis, the increase amounting to from 5000 to 15,000 per c.mm., as shown by the above table. It is probable that the habitual leucocytosis of early childhood is largely referable to a more or less continuous digestion leucocytosis. (For a further discussion of this question see "Digestion Leucocytosis," p. 231.)

The blood of infants and of young children differs greatly from that of the adult in the *relative proportions of the different forms of leucocytes*, these qualitative differences becoming less and less apparent as the child grows older, and not usually persisting beyond the tenth year. In general terms it may be said that these dissimilarities are striking in relation to the youth of the child. Compared to the adult, a differential count of the leucocytes in the child shows that the relative percentage of lymphocytes is more than twice as great, and of polynuclear neutrophiles one-half as great, while the proportion of eosinophiles is frequently much higher. In the following table, based upon data given by Gundobin,[1] these points of difference are contrasted:

| Forms of Leucocytes. | Infants. | Adults. |
|---|---|---|
| Small lymphocytes | 50 to 70 per cent. | 20 to 30 per cent. |
| Large lymphocytes and transitional forms | 6 " 14 " " | 4 " 8 " " |
| Polynuclear neutrophiles | 28 " 40 " " | 60 " 75 " " |
| Eosinophiles | 0.5 " 10 " " | 0.5 " 5 " " |

It is important to take into account these differences in making blood examinations in children, in whom one must expect to find percentages of lymphocytes which in the adult would be regarded as abnormally high.

Many of the lymphocytes differ from corresponding cells in the adult, chiefly in being of larger size, in exhibiting a greater variety of nuclear figures, and in having a more basic tendency. Karnizki[2] also professes to find an occasional myelocyte in the blood of the healthy child, and to trace a decided morphological resemblance between the lymphocytes, the transitional forms, and the neutrophiles. While it is true that myelocytes appear in the infant's blood upon slight provocation, one can scarcely regard them as normal elements; nor can a relationship between the lymphatic and the myelogenous leucocytes be established more clearly in the child than in the adult.

*Leucocytosis* in children is of extremely common occurrence,

[1] Jahrb. f. Kinderheilk., 1893, vol. xxxv, p. 187.
[2] Arch. f. Kinderheilk., 1903, vol. xxxvi, p. 42.

often arising from causes of the most trivial character, and developing to a greater degree and with much more rapidity than in the adult. It is therefore to be regarded with less significance than when it occurs in the mature. Usually the polynuclear cells are chiefly involved in the increase, but the inclination of the blood of children to revert to the embryonic type appears to cause, in many cases, a disproportionate increase of lymphocytes in relation to the other forms, this peculiarity being especially true of the various pathological leucocytoses. Physiological leucocytosis in children usually affects chiefly the polynuclear neutrophile cells.

## II. ANEMIA IN CHILDREN.

FREQUENCY.

Children, as a class, are peculiarly susceptible to anemia, for they appear to lack resisting powers against the influence of causes tending to produce pathological alterations in the blood. Thus, it is found that the same factors which in the adult have little or no effect upon the blood, are capable of producing profound alterations in its composition in the child. Severe anemias may arise in children from apparently the most trivial causes; slight hemorrhage from the navel, for instance, may light up an anemia of an intensity out of all proportion to the actual amount of the blood loss, while minor lesions of the gastro-intestinal tract are commonly associated with blood deterioration of a severe type.

GENERAL CHARACTERISTICS.

It is a notable fact that in anemic children a predominant tendency exists toward a reversion of the blood to a less mature histological type, such as that found in the blood of the fetus. Thus in children anemia of a type which in the adult is unattended by qualitative changes in the corpuscles is commonly associated with the presence of large numbers of *nucleated erythrocytes*, these cells being far more numerous than the severity of the anemia would seem to warrant. Megaloblasts are not of the same significance in infancy as in adult life. They may occur in anemias of but moderate intensity—in fact, they may predominate, as Morse has shown,[1] without necessarily constituting a sign of fatal blood disease. In Riviere's experience[2] megaloblasts, in ordinary well-marked infantile anemias, may constitute between 20 and 50 per cent. of the erythroblasts, the ratio of megaloblastic forms apparently standing in no definite parallelism

[1] Boston Med. and Surg. Jour., 1903, vol. cxlviii, p. 573.
[2] Lancet, 1903, vol. ii, p. 1419.

to the severity of the anemia of which they are symptomatic. *Poikilocytosis* and *deformities* in the size of the corpuscles also occur with far greater frequency than in the adult. Regeneration of the blood takes place slowly. The oligochromemia is relatively greater than the oligocythemia in most anemias of children, this being due probably to the fact that the hemoglobin is peculiarly prone to separate from the corpuscular stroma. Owing to this fact low color indices, as in chlorosis, are common, irrespective of the degree of corpuscular diminution.

*Myelocytes* are commonly found in the blood in all the anemias of children; they are present in larger relative percentages and in less severe pathological conditions than in the adult. The writer has noted the frequency, almost the constancy, with which typical *mast cells* and the fine *basophiles* occur in the specific infections in children, notably in enteric fever and in tuberculosis.

*Leucocytosis*, often *lymphocytosis*, and *enlargement of the spleen* are frequently associated with all forms of anemia in the young; and although these conditions are likely to coëxist, this is by no means the invariable rule. Splenic enlargement is especially common in the anemias due to syphilis, rachitis, tuberculosis, gastro-intestinal disease, malaria, and septic infection.

To epitomize, in the anemias of infancy and childhood the following prominent features of the blood are found: (*a*) The frequency of a low color index; (*b*) the common occurrence of erythroblasts and of deformities affecting the shape and size of the erythrocytes; (*c*) a tendency toward leucocytosis and splenic enlargement, and (*d*) the frequency of myelocytes and mast cells.

CLASSIFICATION.

It is owing to these peculiarities that the classification of the anemias of children is such a difficult matter. The older classifications, based upon the nature of the causal factors of the anemia and upon the presence or absence of enlargement of the spleen, have failed in many respects to prove adequate, so that it becomes necessary to adopt a simpler and more comprehensive division from which no exceptions need be made in the individual case. Such a classification has been suggested by Morse.[1] This author assuming, and rightly so, that chlorosis is a condition wholly foreign to infantile life, and that the disease described by von Jaksch as "anemia infantum pseudoleukemica" does not represent a distinct clinical condition, proposes this excellent classification, slightly modified from that of Monti:

*Primary Anemia.* Pernicious anemia.
Leukemia.

[1] Arch. Pediat., 1898, vol. xv, p. 815.

*Secondary Anemia.*

Mild anemia.
Mild anemia with leucocytosis.
Severe anemia.
Severe anemia with leucocytosis.

PRIMARY ANEMIA.

*Pernicious anemia* is rare in the young, and likely to be mistaken for other forms of severe anemia secondary to various conditions. It is probable that many of the reported cases of Biermer's anemia in infants were in reality examples of severe secondary anemia. Hutchinson,[1] in his Goulstonian lectures, gives the data of 11 authentic cases, the total number recorded up to the present time. The apparent tendency of pernicious anemia in children to become transformed into leukemia is doubtless more fanciful than real, a remark which is equally true of those few reported instances of the conversion of leukemia into pernicious anemia. In the first case the erroneous impression may arise from such evidence as marked enlargement of the spleen associated with a high leucocytosis; in the second, a temporary disappearance of the myelogenous blood-picture plus an aggravation of the existing anemia may be sufficient to convey the false impression. It must be admitted that these atypical blood changes, so common in young children, are highly confusing and difficult to interpret without the closest observation and the correlation of other clinical signs. (See "Leukanemia," p. 290.)

Infantile pernicious anemia is characterized by the same blood changes that are found in the adult, in so far as striking oligocythemia and deformities of the erythrocytes are concerned, but the blood often fails to show a high color index. A prevalence of megaloblasts and of megalocytes is not pathognomonic, as it is in the adult.

*Splenic anemia*, which is none too certain an entity in the adult, can rarely be identified in the child. The splenic enlargement in most reputed instances of this condition in early life must be regarded as symptomatic of nutritive disturbance, rather than as a distinctive factor of anemia. Hutchinson[2] uses the terms infantile splenic anemia and infantile pseudoleukemic anemia synonymously, and believes that the former is entirely distinct from splenic anemia of the adult. It must be admitted, however, that rarely the adult type of the disease is met with in children, as shown by the cases reported by Williamson,[3] Hunt,[4] Rolleston,[5]

[1] Lancet, 1904, vol. i, pp. 1253 and 1325, and 1402.
[2] *Loc. cit.*
[3] Med. Chronicle, 1893, vol. xviii, p. 103.
[4] Trans. Path. Soc. London, 1899, vol. l, p. 209.
[5] Clin. Jour., 1902, vol. xix, p. 401.

Hamill,[1] and others. Such cases show the same type of anemia, leucopenia, and lymphocytosis found in the blood of the adult suffering from this disease.

*Leukemia* in children is uncommon, but instances have been reported during all stages of infancy and childhood, even in the new-born. Acute forms of the disease are most frequently met with, the great majority of cases, according to Holt,[2] proving fatal within a year from the appearance of the first symptoms, while in many the disease runs its course in a few weeks. Lymphatic leukemia is about five times as common in children as myelogenous. Male children are more commonly leukemic than female. Conditions such as rachitis, syphilis, and malarial fever have been regarded by some authors as possessing a certain amount of importance as etiological factors, but in the vast majority of cases the cause of the disease is entirely obscure.

Of the several collected reports of leukemia in children, the two articles of Morse, giving a total of 27 cases, are by far the most valuable. In his first communication[3] 20 cases were recorded, including one of his own, tabulated below, but of this series the diagnosis, in the great majority of instances being based either upon clinical symptoms or upon inadequate examination of the blood, the reporter is led to remark that "it is highly probable that not more than half, perhaps not more than a third, of these were really cases of leukemia." In Morse's second article,[4] which deals with the acute form of the disease, seven cases, again including one of his own, also recorded below, are reported.

Although the literature of pediatrics is fairly rich in alleged examples of leukemia in children, the cases, with but a few exceptions, are reported in so unsatisfactory a manner that they cannot be regarded without reserve as typical. Those reported prior to the publication of Morse's first article—in 1894—must all be open to criticism, owing to the general disregard for differential counts shown by the various authors, and, strangely enough, this criticism must hold true for many cases recorded during the past ten years. The author has been able to collect 21 cases (Table XVIII), in all of which the differential count of leucocytes leaves no doubt as to the precise character of the disease.

In addition to the above cases several others have been reported in which the differential count of leucocytes has been either faultily made or entirely neglected. Thus, Pollman[5] believes that he has

[1] Arch. Pediat., 1902, vol. xix, p. 641.
[2] "The Diseases of Infancy and Childhood," New York, 1897, p. 806.
[3] Boston Med. and Surg. Jour., 1894, vol. cxxxi, p. 133.
[4] Arch. Pediat., 1898, vol. xv, p. 330.
[5] Münch. med. Wochenschr., 1898, vol. xlv, p. 44.

TABLE XVIII.—LEUKEMIA IN CHILDREN.

| Author. | Age. | Hemoglobin. | Erythrocytes. | Leucocytes. | Small Lymphocytes. | Large Lymphocytes. | Polynuclear Neutrophiles. | Eosinophiles. | Myelocytes. | Histological Changes in the Erythrocytes. | Type. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Morse [1] | 3 yrs. | 25 | 2,024,000 | 87,400 | 83.0 | 11.0 | 5.0 | 1.0 | 0.0 | No nucleated cells; slight variation in size, and almost none in shape. | Lymphatic. |
| Rolleston and Latham [2] | 1½ yrs. | De- | crease. | Increase. | 61.1 | | 16.5 | 1.6 | 20.8 | | Myelogenous. |
| Cassel [3] | 8 yrs. | 40 | 3,500,000 | 500,000 | [9] | | 29.7 | | 69.0 | Normoblasts in excess; poikilocytosis marked. | Myelogenous. |
| Müller [4] | 4 yrs. | 40 | 1,508,000 | 109,000 | 12.0 | 85.0 | 2.0 | 1.0 | 0.0 | A few normoblasts and megaloblasts. | Lymphatic. |
| | 4 yrs. | 25 | 2,350,000 | 209,000 | 16.0 | 82.0 | 2.0 | 0.0 | 0.0 | A few normoblasts; no megaloblasts. | Lymphatic. |
| | 4 yrs. | | 1,308,000 | 420,000 | 2.0 | 97.3 | 0.7 | 0.01 | 0.0 | A few normoblasts; no megaloblasts. | Lymphatic. |
| Morse [5] | 1 yr. | | 2,900,000 | 48,000 | 23.4 | 8.1 | 21.4 | 0.6 | 46.5 | Many normoblasts and undeveloped forms of nucleated cells; megaloblasts numerous. Poikilocytosis and polychromatophilia marked. | Myelogenous. |
| Thayer [6] | | | 1 white | to 20 red. | 97.9 | 0.7 | 1.4 | 0.08 | 0.0 | | Lymphatic. |
| Baginsky [7] | 9 yrs. | 30 | 2,000,000 | 986,000 | 5.0 | | 12.3 | 5.0 | 79.1 | Poikilocytosis and nucleation of erythrocytes. | Myelogenous. |
| Author [8] | 15 yrs. | 25 | 1,875,000 | 218,000 | 11.6 | 11.7 | 29.9 | 4.0 | 42.8 | Many normoblasts; poikilocytosis and polychromatophilia marked. | Myelogenous. |

[1] Arch. Pediat., 1898, vol. xv, p. 330.
[2] Lancet, 1898, vol. i, p. 1313.
[3] Berlin. klin. Wochenschr., 1898, vol. xxxv, p. 76.
[4] Jahrb. f. Kinderheilk., 1896, vol. xliii, p. 143.
[5] Boston Med. and Surg. Jour., 1894, vol. cxxxi, p. 133.
[6] Cited by Cabot, *loc. cit.*
[7] Jacobi's Festschr., abst. in Phila. Med. Jour., 1900, vol. v, p. 1170.
[8] Unpublished case.
[9] The differential count of leucocytes showed, in this author's language: "Of 571 leucocytes counted, there were 398 myelocytes (including eosinophiles) = 69 per cent. (!), and 170 polynuclear cells, in addition to the transitional forms = 29.7 per cent. Of 542 leucocytes counted, the number of eosinophiles was 69 = 12 per cent."

TABLE XVIII.—LEUKEMIA IN CHILDREN.—*Continued.*

| AUTHOR. | AGE. | HEMOGLOBIN. | ERYTHROCYTES. | LEUCOCYTES. | SMALL LYMPHOCYTES. | LARGE LYMPHOCYTES. | POLYNUCLEAR NEUTROPHILES. | EOSINOPHILES. | MYELOCYTES. | HISTOLOGICAL CHANGES IN THE ERYTHROCYTES. | TYPE. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bradley [1] | 8 yrs. | 18 | 1,850,000 | 85,000 | 28.0 | 69.0 | 3.0 | | | A few erythroblasts. | Lymphatic. |
| Scott [2] | 9 mos. | 20 | 3,120,000 | 105,000 | 27.0 | 30.0 | 27.5 | 7.5 | 8.0 | To 200 leucocytes, 92 normoblasts and 2 megaloblasts were found. Polychromatophilia and poikilocytosis. | Lymphatic. |
| McCrae [3] | 3 yrs. | 35 | 1,680,000 | 26,000[4] | 41.5 | 45.2 | 13.3 | 0.0 | 0.0 | No erythroblasts. Slight polychromatophilia. | Lymphatic. |
| Hutchinson [5] | 5 yrs. | 25 | 2,425,000 | 1,590,000 | 0.7 | | 30.0 | 9.0 | 60.0 | | Myelogenous |
| | 6 yrs. | 10 | 1,200,000 | 170,000 | 98.0 | | | | | | Lymphatic. |
| | 13 yrs. | 40 | 2,000,000 | 240,000 | 98.7 | | 1.2 | | | One normoblast to 105 leucocytes. | Lymphatic. |
| Carpenter [6] | 3½ yrs. | 15 | 1,568,000 | 130,000 | 99.1 | | | | | | Lymphatic. |
| Einhorn [7] | 9 yrs. | | 3,360,000 | 389,000 | 48.7 | 50.3 | 1.0 | | | | Lymphatic. |
| Guinon and Jolly [8] | 15 yrs. | 20 | 685,000 | 28,000 | 93.0 | 3.6 | 3.0 | 0.4 | | | Lymphatic. |
| McCaw [9] | 1 yr., 8 mos | 39 | 2,200,000 | 810,000 | 99.2 | | | | | | Lymphatic. |
| Geissler and Japha [10] | 5 yrs. | | 2,158,000 | 34,250 | 97.0 | | | | | | Lymphatic. |

[1] N. Y. Med. Jour., 1899, vol. lxx, p. 923.
[2] Amer. Jour. Med. Sci., 1902, vol. cxxiii, p. 20.
[3] Johns Hopkins Hosp. Bull., 1900, vol. xi, p. 102.
[4] A subsequent count showed 60,800 leucocytes, of which 99.2 per cent. were lymphocytes.
[5] Lancet, 1904, vol. i, p. 1332.
[6] Brit. Med. Jour., 1903, vol. ii, p. 463.
[7] Med. Rec., 1902, vol. lxii, p. 41.
[8] Rev. mens. des mal. de l'Enf., 1899, vol. xvii, p. 262.
[9] Rep. Soc. for Study of Dis. in Children, 1903, vol. iii, p. 251
[10] Jahrb. f. Kinderheilk., 1900, vol. lii, p. 572.

seen a case of myelogenous leukemia in a new-born infant, the count on the fourteenth day after birth being 2,500,000 erythrocytes and 312,500 leucocytes per c.mm. The latter consisted chiefly of "large mononucleated cells, with large, distinct nuclei and an abundance of protoplasm." Nucleated forms of erythrocytes were not found. Cassel,[1] in addition to his own case, tabulated above, has collected four others occurring in children under fourteen years of age.

Theodor[2] has collected from German literature six cases of acute leukemia in children between the ages of two and one-half and eight years of age, and also reports one of his own, apparently of the lymphatic form, in a boy of four years. No actual numerical estimates of the corpuscles are given, but the proportion of leucocytes to erythrocytes is stated to vary from 1 : 9 to 1 : 3. The differential count of leucocytes is also very inexact. The greater percentage apparently consisted of lymphocytes; myelocytes were fairly numerous, many of them containing mitotic figures, and normoblasts and megaloblasts were present in large numbers. Gilbert and Weil,[3] in 1899, published data of five acute lymphatic cases in children between the first and tenth years. Charon and Gratea[4] report a case of myelogenous leukemia in a child of eight years, the percentage of hemoglobin being 39, the erythrocyte count 880,000, and the leucocyte count 305,000. Instances of acute lymphatic leukemia in children have also been reported, with more or less accuracy, by Bradford and Shaw,[5] by Guinon and Jolly,[6] by Haushalter and Richon,[7] and by Bloch and Hirschfeld.[8]

SECONDARY ANEMIA.

In the above classification of the secondary anemias, under the heading of *mild anemia* are included those cases characterized by trifling reduction in the hemoglobin percentage and number of erythrocytes, and by an absence of histological alterations in these cells. The color index in these cases is usually 1.00, or slightly below, uncommonly falling to a low figure.

The term *severe anemia* includes cases having marked diminution of hemoglobin and erythrocytes, associated with deformities of shape and of size and nucleation of these cells. The hemo-

[1] Berlin. klin. Wochenschr., 1898, vol. xxxv, p. 76.
[2] Arch. f. Kinderheilk., 1897, vol. xxii, p. 47.
[3] Arch. de méd. expér., 1899, vol. ii, p. 157.
[4] Bull. Soc. roy. sc. méd. et nat., Brussels, 1896, vol. liv, p. 63.
[5] Trans. Roy. Med.-chir. Soc., 1898, vol. lxxxi, p. 343.
[6] Rev. mens. des mal. de l'Enf., 1899, vol. i, p. 262.
[7] Arch. de méd. des Enf., 1899, vol. ii, p. 356.
[8] Zeitschr. f. klin. Med., 1900, vol. xxxix, p. 32.

globin loss is especially marked, often being only one-quarter or one-third of normal, and the color index is low.

*Anemias with leucocytosis*, whether of mild or of severe type, are generally marked by a greater degree of hemoglobin and corpuscular decrease than anemias without leucocytosis. The leucocytosis is moderate in the milder forms, but in severe cases the increase in the number of leucocytes often appears to be progressive, and the relative number of leucocytes to erythrocytes occasionally attains the proportion of 1 to 100.

Histological changes in the erythrocytes are more striking in grave anemias with leucocytosis than in grave anemias pure and simple. This is especially true of the changes relating to nucleation of the cells, normoblasts and atypical forms being very numerous in the former class. As already stated, megaloblasts do not necessarily mean a fatal nor even an especially intense anemia.

Regarding the etiological factors of these secondary anemias, the following groups of causes are given by Monti[1]:

| | | |
|---|---|---|
| 1. CONGENITAL ..... | | Syphilis, tuberculosis, and other infections. |
| 2. ACQUIRED ..... | *Hemorrhagic.* | From navel, from circumcision, etc. |
| | | Purpuric diseases. |
| | | Malnutrition, improper hygiene, etc. |
| | | Syphilis, rachitis, and tuberculosis. |
| | *General.* | Gastro-intestinal diseases. |
| | | Visceral diseases. |
| | | Febrile diseases. |
| | | Septic infections. |
| | | Nephritides. |
| | | Malignant growths. |

*Syphilis*, either congenital or acquired, is responsible for a large proportion of the cases of anemia in children, especially those of a severe type, associated with enlargement of the spleen, and often also with enlargement of the lymphatic glands. The hemoglobin loss is in most instances disproportionately greater than the corpuscular decrease, so that low color indices are especially common in this disease—the misnamed "chlorosis" of syphilis. Deformities and nucleation of the erythrocytes are common in the severer types, and in such forms polychromatophilic changes and excessive decrease in the number of erythrocytes are usually present. A leucocyte increase is present in the secondary stage, and is usually associated with the grave anemia of this disease; the relative percentage of lymphocytes is increased, and of polymorphous forms decreased; and small numbers of myelocytes are common. From the writer's experience, in the average case of moderate severity the percentage of hemoglobin varies from

[1] Wien. med. Wochenschr., 1894, vol. xliv, pp. 401, 464, 516, 560, and 613.

about 40 to 50, the erythrocytes are reduced to about 3,000,000 to 3,500,000 per c.mm., and the leucocyte count is in the neighborhood of 20,000; but in severe cases the erythrocytes may be reduced to 1,000,000, and the leucocytes increased to 50,000 or more. As in the adult, Justus' test proves of value in the diagnosis of many anomalous cases. Labbé and Armand Delille[1] insist that in congenital syphilis the blood picture may precisely resemble that of von Jaksch's disease (*q. v.*), but that the blood promptly returns to normal after energetic mercurialization.

In *rachitis* there is usually well-marked anemia, accompanied by decided enlargement of the spleen, such cases having most decided blood changes. The hemoglobin percentage tends to range lower than the percentage of corpuscles, so that low color indices prevail; but in the individual case neither the oligochromemia nor the oligocythemia is generally as marked as in syphilis. In severe cases, deformed and nucleated erythrocytes and a small percentage of myelocytes are commonly found. The number of leucocytes is, as a rule, moderately increased, and relatively high percentages of lymphocytes are common.

Uncomplicated *tuberculosis*, due to pure infections with the tubercle bacillus, produces an anemia which varies in degree with the severity of the constitutional effects of the disease. The hemoglobin and erythrocytes are usually but slightly decreased, the former suffering a relatively greater loss, and the number of leucocytes does not rise above normal. If to the tuberculous process a septic infection is superadded, the anemia becomes severer, and leucocytosis, involving the polymorphous forms of cells, occurs. Splenic enlargement is common and sometimes marked. The anemia of tuberculosis is in no way referable to the infection itself, but depends upon the drain on the albumins of the blood due to the presence of a long-continued cachexia and upon secondary infections.

*Gastro-intestinal diseases*, especially those of chronic character, cause most marked anemia. Chronic inflammations of the intestines strikingly affect the blood, the percentage of hemoglobin frequently falling to one-quarter of normal or even lower, and the number of erythrocytes being decreased to one-half of normal or lower. Deformities affecting the shape and size of the erythrocytes and nucleation of these cells are of frequent occurrence. A leucocyte increase, involving in many instances the lymphocytes, is usually present, and small percentages of myelocytes have been observed. Splenic enlargement is frequently a conspicuous clinical sign. It should be remembered that in acute forms

[1] Sem. méd., 1903, vol. xxiii, p. 50.

of gastro-intestinal disorders, in which profuse diarrhea and vomiting occur, concentration of the blood takes place, causing temporary polycythemia which may for a time hide the real degree of the blood deterioration. The anemias found in this class of diseases are apparently to a large extent autointoxicative in character, depending to a less degree upon insufficient nutrition.

In *enteric fever* the blood picture does not differ essentially from that seen in the adult suffering from this affection, absence of leucocytosis or leucopenia with progressive anemia being found with great constancy. The difference is simply one of degree, the changes developing more rapidly but persisting for a shorter period in the child than in the adult. Churchill's studies [1] show that the leucopenia is most decided during the second week of the fever; and that the anemia is especially apparent during the first three weeks, after which the hemoglobin and erythrocytes begin steadily to increase, reaching normal by about the fifth week. In 12 cases studied by Stengel and White [2] the hemoglobin ranged from 68 to 83 per cent., the erythrocytes from 3,320,000 to 5,200,000, and the leucocytes (in uncomplicated cases) from 3800 to 12,320. Polynuclear leucocytosis was observed in 3 cases as the result of inflammatory complications. Two uncomplicated cases showed fractional percentages of myelocytes, but there were no other differential changes of any consequence. Head [3] believes that in uncomplicated cases the leucocytes never number more than 10,000 per c.mm. The writer has found that the alkalinity of the blood varies within wide limits in infantile typhoid, tests by Engel's method in 6 consecutive cases showing a range of from 373 to 692 mgm. NaOH. The rapidity of coagulation also was found to vary greatly, clotting taking place in as short a time as thirty-seven seconds in one instance, and not occurring for four minutes and thirty-five seconds in another. Morse [4] concludes that the serum test appears earlier, is less marked, and persists for a shorter period in children than in adults. In a nursling it should be remembered that a positive reaction may be of uncertain value, for the reason that the agglutinating power may be transmitted from mother to child through the milk, both during the active stages of the disease and also during and after convalescence.

Under the title "*anemia infantum pseudoleukemica*" von Jaksch [5] has described a condition which he regards as a form of

[1] Boston Med. and Surg. Jour., 1903, vol. clviii, p. 692.
[2] Arch. Pediat., 1901, vol. xviii, pp. 241 and 321.
[3] *Ibid.*, 1902, vol. xix, p. 253.
[4] *Ibid.*, 1901, vol. xviii, p. 338.
[5] Wien. klin. Wochenschr., 1889, vol. ii, p. 435.

primary anemia peculiar to the young child. The blood changes, which are not characteristic of the disease in question, as this author admits, consist of (*a*) marked oligocythemia and oligochromemia; (*b*) extensive and persistent leucocyte increase; and (*c*) striking structural alterations in the erythrocytes. Associated with these changes in the blood, and of equal importance in diagnosing the disease, constant enlargement of the spleen and, less commonly, enlargement of the liver are found.

The number of erythrocytes is greatly decreased, usually to from 2,000,000 to 3,000,000 per c.mm., but sometimes falling to 1,000,000 or even to a lower figure, as in one of von Jaksch's cases, in which the count was only 820,000. The hemoglobin loss is also great—relatively more so than the corpuscular decrease.

The leucocyte gain is decided, averaging, in the majority of cases, from 30,000 to 50,000 corpuscles per c.mm., and in some instances exceeding 100,000. In some cases the increase involves principally the polynuclear neutrophiles, while in others the lymphocytes are the cells chiefly affected. The cells show a most striking dissimilarity of form and of size, and a highly confusing variety of forms atypical in size, shape, and nuclear morphology is encountered. This "polymorphous" state of the leucocytes is a point insisted upon by von Jaksch in his description of the condition. The number of eosinophiles varies within wide limits, the percentage of these cells being normal, decreased, or increased. Small percentages of myelocytes have been observed.

The histological changes affecting the erythrocytes consist in marked poikilocytosis, deformities of size, loss of color, and nucleation. Poikilocytes, megalocytes, and microcytes occur in large numbers, and the pallor of most of the corpuscles is extreme. Normoblasts are the most common form of erythroblasts observed, but occasionally the occurrence of atypical forms, small and large, and of megaloblasts has been noted. Karyokinetic changes in these cells are not uncommonly seen, and polychromatophilia is of frequent occurrence.

The spleen is enlarged in all cases, sometimes moderately, but often very greatly, so that the organ extends far below the costal margin, and occupies the entire upper left part of the abdominal cavity. The spleen is extremely indurated, and may show capsular thickening from perisplenitis. The increase in the size of the organ is due to a hyperplasia. Enlargement of the liver is not constant in all cases, and when present does not reach a size corresponding to that of the spleen, as is the case in leukemia; the lower border of the liver is not rounded, but distinctly sharp. In a certain proportion of cases the lymphatic glands are slightly

enlarged, but never to any notable extent. Changes in the bone marrow, common to any severe anemia, have been observed in some cases.

The disease occurs most frequently in infants between the ages of seven and twelve months, and is rarely met with in children over four years old. By some writers it is supposed to be slightly more common in children of the male sex.

In many cases a previous history of rachitis, syphilis, or long-standing gastro-intestinal disease is obtained, although von Jaksch denies the existence of these etiological factors in his cases.

The onset of the symptoms is slow and insidious, and the pallor of the skin, blanching of the mucous membranes, and other signs of anemia slowly develop, with the gradual enlargement of the spleen, until these clinical manifestations become marked. In all cases there is excessive loss of strength, and in a great many a high degree of emaciation.

Von Jaksch's disease, if untreated, tends to pursue a progressively grave course, ending fatally; but, under suitable treatment, the splenic tumor decreases in size, the leucocytosis disappears and the hemoglobin and erythrocytes return to normal.

Pseudoleukemic anemia of infants is not generally considered as a separate clinical entity, but is regarded rather as a form of severe secondary anemia associated with marked leucocytosis and splenic enlargement. It may be due to a number of different causes, the most prominent among which are syphilis, rachitis, and chronic gastro-intestinal disease. The conflicting reports of different authors concerning this disease, and the incompleteness with which the leucocytes have been studied in many instances, render it probable that in some of the reported cases pernicious anemia and leukemia have masqueraded as typical examples of the condition described by von Jaksch.

*Bacteriemia*, generally referable to preagonal infections, appears to occur with great frequency in the young child during the course of many acute diseases. Delestre's careful studies[1] of general blood infections in children tend to show that infants born before full term are peculiarly susceptible to this condition. Using careful technic, this author examined 40 children, ranging in age from a few days to four years, all of whom were believed to be suffering from infections which bade fair to end fatally within a few days, at the latest. Of the 32 fatal cases of this series, bacteria were found in the blood during life in 14, while of the 8 who recovered, but one gave a positive result. The bacterium found with greatest frequency was the streptococcus, while staphylococci,

[1] Annal. de gynécol. et d'obstet., 1901, vol. lv, p. 51.

pneumococci, colon bacilli, and influenza bacilli were isolated more rarely. It was furthermore shown that premature babies seemed especially susceptible to streptococcus and colon infections, and that nursing infants several months old were more prone to suffer from the effects of the staphylococcus.

The blood changes occurring in *pertussis*, *pneumonia*, *diphtheria*, *scarlet fever*, *measles*, *varicella*, and other infectious diseases of childhood are considered in Section VII.

# SECTION VII.

# GENERAL HEMATOLOGY.

# SECTION VII.

## GENERAL HEMATOLOGY.

### I. ABSCESS.

General Features.

The rate of *coagulation* is, as a rule, somewhat slower than normal. Hyperinosis is conspicuous, and under the microscope the *fibrin* network appears abnormally dense and thick. The *iodin reaction* may be detected in the dried blood film by the method described in a previous section. (See p. 226.) These remarks, as well as those which follow, do not apply to purely tuberculous or "cold" abscesses, the effects of which are referred to elsewhere.

Hemoglobin and Erythrocytes.

If the absorption of toxic material from an abscess is great enough to produce a systemic effect upon the patient, anemia of an intensity parallel to the severity of the poisoning sooner or later develops. This fact is sufficient to explain why the grades of anemia in purulent conditions vary within such wide limits. The size and the site of the abscess do not appear primarily to determine the degree of the associated blood changes, although, other circumstances being equal, a large, deep-seated collection of pus is likely to have a more harmful effect than one of small size and superficial situation. Chronicity of the lesion seems to go hand in hand with an increase in the blood deterioration—few persons harboring pus for a protracted period fail to show decided signs of anemia.

In many cases, especially the acute, the only noticeable change is a moderate oligochromemia, but in chronic cases different degrees of ordinary secondary anemia are commonly encountered, amounting in an exceptional instance to a reduction of hemoglobin to as low as 20 or 30 per cent. of the normal standard, and to an erythrocyte decrease to between 2,000,000 and 3,000,000 cells to the c.mm. Such profound losses are, of course, unusual, for in the majority of patients with anemia the hemoglobin is above 50, and the corpuscles above 60, per cent. of normal. The average color index for 134 German Hospital cases, listed below,

was 0.77. The condition of the hemoglobin and erythrocytes in these patients is shown by the following summary:

| Hemoglobin Percentage. | Number of Cases. | Erythrocytes Per c.mm. | Number of Cases. |
|---|---|---|---|
| From 90–100 in | 4 | Above 5,000,000 | in 10 |
| " 80–90 " | 15 | From 4,000,000–5,000,000 " | 41 |
| " 70–80 " | 27 | " 3,000,000–4,000,000 " | 68 |
| " 60–70 " | 23 | " 2,000,000–3,000,000 " | 9 |
| " 50–60 " | 23 | " 1,000,000–2,000,000 " | 6 |
| " 40–50 " | 22 | | |
| " 30–40 " | 13 | | |
| " 20–30 " | 7 | | |
| Average, 59 per cent. | | Average, 3,797,470 per c.mm. | |
| Maximum, 95 " | | Maximum, 5,970,000 " " | |
| Minimum, 20 " | | Minimum, 1,320,000 " " | |

If marked anemia exists, a variable grade of cell deformity, atypical staining, and nucleation is also to be observed. If the latter change is evident, it will be found that the great majority, if not all, of the nucleated corpuscles belong to the normoblastic class.

Leucocytes.

Practically the same influences governing the behavior of the leucocytes in most other infections also determine their increase and decrease in abscess. Thus, in both trivial and in extensive pus foci the number of leucocytes may be normal or even subnormal; in the former instance because systemic reaction is not provoked, and in the latter because it is overpowered. Leucocytosis may also be absent in case toxic absorption is impossible, owing to the complete walling-off of the abscess. In all other instances save these a definite and usually well-marked leucocytosis occurs, amounting on the average to a count of about twice the mean normal standard, but often greatly exceeding this figure in the individual case. The size of the primary abscess cannot be estimated by the height of the leucocytosis, but a tendency of the pus to extend is almost always accompanied by a distinct increase in the number of cells in excess of the figure originally estimated. Complete evacuation of the abscess is soon followed by a disappearance of the leucocytosis and iodophilia, but so long as the pus remains ineffectually drained, these signs persist.

Analysis of the "first counts" in 258 cases of various forms of abscess (excluding appendicitis) shows that in 184, or 71.3 per cent., the leucocyte count was in excess of 10,000. The general range of the leucocytes in abscess is illustrated by the following table:

| Leucocytes per c.mm. | Number of Cases. |
|---|---|
| 40,000–50,000 | 2 |
| 30,000–40,000 | 5 |
| 20,000–30,000 | 28 |
| 15,000–20,000 | 59 |
| 10,000–15,000 | 90 |
| 5,000–10,000 | 70 |
| Below 5000 | 4 |

Average, 13,931 per c.mm.
Highest, 42,000 " "
Lowest, 550 " "

A more detailed study of these cases, directed toward the number of the leucocytes in relation to the site of the abscess, may be expressed thus in tabular form:

| Site of Abscess. | Cases. | Average. | Highest. | Lowest. | Cases with Leucocytosis. |
|---|---|---|---|---|---|
| Pelvic | 164 | 13,837 | 42,000 | 550 | 107 or 66.9 per cent. |
| Kidney | 30 | 13,924 | 33,600 | 6,000 | 26 " 86.6 " |
| Superficial | 26 | 13,168 | 24,000 | 4,800 | 19 " 73.0 " |
| Empyema | 10 | 17,180 | 31,800 | 11,200 | 10 " 100.0 " |
| Lung | 8 | 12,128 | 17,650 | 8,200 | 6 " 75.0 " |
| Liver | 8 | 14,922 | 23,400 | 9,300 | 6 " 75.0 " |
| Gall-bladder | 6 | 15,650 | 21,200 | 9,500 | 5 " 83.3 " |
| Brain | 6 | 13,406 | 18,560 | 6,800 | 5 " 83.3 " |

A polynuclear neutrophile gain generally accounts for the increase when leucocytosis is present, and, rarely, this differential change may be found without any increase in the total number of cells. In some instances, and these are not so uncommon as is generally believed, the increase affects all forms of cells proportionately. A few myelocytes are not uncommon in cases having a decided anemia or a high leucocytosis.

Diagnosis. The presence of leucocytosis, especially if associated with hyperinosis and a positive iodin reaction, is suggestive of abscess rather than of other lesions, such as *aneurisms*, *gummata*, *hematomata*, and *benign neoplasms*. An absence of one or all of these signs, on the other hand, is not sufficient to exclude pus. The distinctions, as

shown by the blood, between pyogenic and tuberculous abscesses and malignant disease are considered under the last-named conditions.

## II. ACROMEGALY.

The following counts illustrate the blood changes found in two cases of this disease, the first showing practically normal blood, except for a moderate relative lymphocytosis and an absence of eosinophiles, and the second simply a well-marked secondary anemia with a high color index.

| | Case I. | Case II. |
|---|---|---|
| Hemoglobin | 86 per cent. | 60 per cent. |
| Color index | 0.93 | 1.04 |
| Erythrocytes | 4,620,000 per c.mm. | 2,880,000 per c.mm. |
| Leucocytes | 8,000 per c.mm. | 4,890 per c.mm. |
| Small lymphocytes | 31.7 per cent. | 21.0 per cent. |
| Large lymphocytes | 2.1 " | 7.0 " |
| Polynuclear neutrophiles | 66.2 " | 71.0 " |
| Eosinophiles | 0.0 " | 1.0 " |
| Basophiles | 0.0 " | 0.0 " |
| Myelocytes | 0.0 " | 0.0 " |

The erythrocytes showed moderate deformities of size and shape in the anemic case, but no signs of nucleation nor of basophilic stroma degeneration were observed. Coagulation, fibrin formation, and the number of plaques were apparently normal.

## III. ACTINOMYCOSIS.

*Anemia*, marked by a disproportionately great hemoglobin decrease, is sometimes found, and *leucocytosis* appears to be an almost constant change, judging from the small number of reports available. In a case of actinomycosis of the arm the writer found 55 per cent. of hemoglobin, with 4,985,000 erythrocytes and 12,000 leucocytes per c.mm. The number of blood plaques was greatly increased and the erythrocytes were pale, but not deformed, nucleated, nor basophilic. The percentages of the leucocytes were: small lymphocytes, 25.5; large lymphocytes, 7.3; polynuclear neutrophiles, 60.0; eosinophiles, 2.4; myelocytes, 3.2; basophiles (finely granular), 1.0; and mast cells, 0.6.

Erving[1] reports 4 cases with leucocyte counts of 10,000, 12,000, 21,000, and 36,200; Ewing's case[2] gave 21,500; and Cabot's two[2] showed leucocytoses of from 20,000 to 31,700. As a rule, actinomycosis excites a higher leucocytosis when deep organs (liver, lungs) are involved than when the lesion is situated in superficial parts of the body (jaw, elbow, abdominal wall) where free drainage is favored. It is probable that the grade of both the anemia and the leucocytosis depends largely upon the amount of septic absorption originating from the lesion.

## IV. ACUTE YELLOW ATROPHY OF THE LIVER.

Malignant jaundice appears to be associated with a moderate *polycythemia*, so far as can be determined by the limited number of blood counts made in this disease up to the present time. The *leucocytes* are moderately increased in number, but show no peculiar differential changes. In two cases, reported by Grawitz[3] and by Cabot,[4] respectively, the counts of erythrocytes were 5,150,000 and 5,520,000, and the number of leucocytes 12,000 and 16,000 per c.mm. *Bacteriological examination* of the blood has thrown no definite light upon the nature of this apparently infectious process. In many cases *hemoglobinemia* and *lipacidemia* have been detected.

## V. ADDISON'S DISEASE.

HEMOGLOBIN AND ERYTHROCYTES.

Moderate anemia is commonly, and decided anemia occasionally, associated with this condition, although the prime importance of this symptom insisted upon by Addison himself appears to be somewhat exaggerated, in the light of our more accurate methods of blood study. The "anemiated eye" of Addison does not always mean anemia. In advanced cases the blood picture may be characterized by marked hemoglobin and erythrocyte losses, by the presence of numerous poikilocytes and microcytes, and by small numbers of normoblasts; the hemoglobin readings in such instances range between 20 and 40 per cent., and the erythrocyte counts between 2,000,000 and 3,000,000 per c.mm., or even less. Tschirkoff[5] reports cases in which, notwithstanding the coëxistence of a notable oligocythe-

[1] Johns Hopkins Hosp. Bull., 1902, vol. xiii, p. 261.
[2] *Loc. cit.*
[3] *Loc. cit.*
[4] *Loc. cit.*
[5] Zeitschr. f. klin. Med., 1890, vol. xix, p. 87.

mia, the hemoglobin percentage remained normal or above normal, and this peculiar condition he referred to an increase in the amount of corpuscular reduced hemoglobin. This author also detected the presence of methemoglobin and melanin in the blood of patients suffering from Addison's disease. The polycythemia which is sometimes met with in this condition is doubtless to be attributed to such factors as vasomotor changes and blood inspissation from emesis. Treatment with suprarenal extract tends to improve the anemia, but to what extent and how permanently is undetermined.

Leucocytes.

The number of leucocytes is usually normal or below normal, and extreme leucopenia has been repeatedly noted. Relative lymphocytosis and sometimes a moderate increase in the number of eosinophiles are the most familiar differential changes, together, in some instances, with the presence of a few myelocytes and basophiles.

## VI. ANTHRAX.

Nothing definite is known of the behavior of the *hemoglobin* and *corpuscles* in this infection. Only occasionally can the anthrax bacillus be isolated from the peripheral blood, since general invasion of the circulation by this organism is rare. Blumer and Young[1] succeeded in finding the organism in the blood of a single case of anthrax septicemia, both in ordinary cover-glass specimens as well as by culturing.

## VII. APPENDICITIS.

Hemoglobin and Erythrocytes.

Fully three-fourths of all cases of appendicitis, whatever their character, show a loss of at least 30 per cent. of hemoglobin, while in about one case in five the erythrocytes are diminished 1,000,000 or more to the c.mm. From an analysis of the cases tabulated below it appears that the average hemoglobin loss amounts to about 25 per cent., and the average decrease in erythrocytes to about 15 per cent. of the normal standard. The anemia, which may usually be attributed to the effects of septicemia, is most frequent and most marked in long-standing cases of appendicular abscess, in which type of the disease the hemoglobin may fall to between 30 and 40 per cent., and the corpuscles to between 2,000,000 and 3,000,000 per c.mm.

[1] Johns Hopkins Hosp. Bull. 1895, vol. vi, p. 127.

In such instances the risk, actual or reputed, of operating upon a patient having so low a percentage of hemoglobin must be recalled by the surgeon. (See p. 164.) Anemia, usually of a more moderate grade, is also frequently found in catarrhal cases, and in the individual instance it may reach as high a grade as in the purulent form of the disease. The blood impoverishment in such instances depends probably upon the debilitated state of the patient, apart from the appendix inflammation.

The following table illustrates the range of the hemoglobin and erythrocytes, as shown by the initial examinations of 139 cases[1] in Dr. J. B. Deaver's wards at the German Hospital:

| | Number of Cases. | |
|---|---|---|
| Hemoglobin Percentage. | Non-purulent. | Purulent, Perforative, and Gangrenous. |
| Above 100 | 1 | 0 |
| From 90–100 | 1 | 4 |
| " 80–90 | 9 | 20 |
| " 70–80 | 13 | 31 |
| " 60–70 | 13 | 23 |
| " 50–60 | 6 | 7 |
| " 40–50 | 2 | 6 |
| " 30–40 | 0 | 3 |
| Highest | 102 per cent. | 100 per cent. |
| Lowest | 45 " | 38 " |
| Average | 69.5 " | 72.5 " |
| Erythrocytes per c.mm. | | |
| Above 5,000,000 | 6 | 14 |
| From 4,000,000–5,000,000 | 27 | 60 |
| " 3,000,000–4,000,000 | 11 | 15 |
| " 2,000,000–3,000,000 | 1 | 5 |
| Highest | 5,660,000 per c.mm. | 5,710,000 per c.mm. |
| Lowest | 2,050,000 per c.mm. | 2,100,000 per c.mm. |
| Average | 4,295,955 per c.mm. | 4,381,234 per c.mm. |

Qualitative changes in the erythrocytes are neither common nor important, occurring only in cases with decided anemia, and consisting simply in deformities of shape and of size. Erythro-

[1] Similar figures were found in the examination of more than 700 additional cases not here recorded.

blasts apparently do not occur, although there is no reason why they should not, if the anemia happens to be of a type of sufficient severity to provoke marrow changes.

Leucocytes.

In simple appendicular inflammation uncomplicated by pus, gangrene, or peritonitis there is, as a rule, little or no increase in the number of leucocytes, although in an exceptional case the leucocytosis is fairly well defined. Thus, of the 45 cases of this form of the disease below referred to, less than 9 per cent. were accompanied by a count in excess of 15,000, the maximum estimate being 17,100, and the average 8987 per c.mm. A relatively high count in this variety of appendicitis may usually be attributed to a limited periappendicular peritonitis. In some instances it is possible that the increase may be due to blood inspissation from vomiting and purging, or that it may simply represent a blood finding of the associated anemia.

In cases with abscess, gangrene, or general peritonitis a well-marked leucocytosis is the general rule. Few cases of appendicular abscess fail to increase the leucocyte count to at least 15,000 or 20,000 to the c.mm., although it is to be remembered that should the purulent focus happen to be so effectually walled off that absorption of toxic material is practically prevented, so decided an increase does not develop. A trivial increase, or, indeed, an absence of leucocytosis, is also met with in an occasional grave case (such, for instance, as one complicated by a general purulent peritonitis), owing to the prostration of the patient from the systemic poison of the infection. As shown below, the *average* count in purulent and gangrenous appendicitis is higher than the *maximum* count in the catarrhal form of the affection.

A high leucocytosis suggests either a localized abscess or a general peritonitis, for the differentiation of which other clinical data are absolutely essential. The belief is current that if a marked leucocytosis occurs early in the attack, peritonitis is probable, and if it occurs after the first week, a local accumulation of pus is suggested. While this is undoubtedly true in many instances, in many others the condition of the appendicular lesion may be wrongly interpreted if too great reliance is placed on the behavior of the leucocytes in connection with the period of the attack.

Increase in the purulent focus and extension of peritonitis are betrayed by an increase in the leucocyte count, provided that the patient's powers of reaction are not too greatly crippled. In operative cases thorough evacuation of the abscess is followed within a few days by a decline to normal in the number of leuco-

cytes. Persistence of the leucocytosis after the third or fourth day following the operation may usually be attributed to undrained pus pockets or to a general peritonitis.

In non-operative cases with abscess the leucocytosis, which becomes well developed by the fourth or fifth day of the attack, persists but does not tend to increase if the lesion remains localized; it gradually decreases as the pus collection disappears; and it suddenly increases if the process extends.

To sum up, absence of or slight leucocytosis suggests (*a*) simple catarrhal appendicitis; (*b*) fulminant appendicitis; or (*c*) a localized pus focus from which no absorption occurs. Well-marked leucocytosis indicates (*a*) a local abscess from which absorption of toxins occurs; (*b*) general peritonitis; or (*c*) gangrene.

The following table shows the range of the leucocytes in the German Hospital cases to which reference has been made:

| Leucocytes per c.mm. | Number of Cases. | |
|---|---|---|
| | Non-purulent. | Purulent, Perforative, and Gangrenous. |
| Above 50,000 | 0 | 1 |
| From 40,000–50,000 | 0 | 0 |
| " 35,000–40,000 | 0 | 2 |
| " 30,000–35,000 | 0 | 0 |
| " 25,000–30,000 | 0 | 6 |
| " 20,000–25,000 | 0 | 16 |
| " 15,000–20,000 | 4 | 38 |
| " 10,000–15,000 | 10 | 24 |
| " 5,000–10,000 | 25 | 7 |
| Below 5000 | 6 | 0 |
| Highest | 17,100 per c.mm. | 58,500 per c.mm. |
| Lowest | 1,600 " " | 6,000 " " |
| Average | 8,987 " " | 17,955 " " |

The qualitative changes found in high leucocyte counts are those typical of an ordinary polynuclear neutrophile leucocytosis —a large absolute and relative gain in polynuclear forms at the expense of the hyaline mononuclear cells.

Findings essentially like the above have been reported by Cabot,[1] Richardson,[2] Bloodgood,[3] and Joy and Wright,[4] in America; by Longridge,[5] Gulland,[6] and French,[7] in Great Britain; by

[1] *Loc. cit.* [2] Amer. Jour. Med. Sci., 1899, vol. cxviii, p. 635.
[3] Prog. Med., 1901, vol. iv, p. 216. [4] Med. News, 1902, vol. lxxx, p. 628.
[5] Brit. Med. Jour., 1902, vol. ii, p. 1511; also Lancet, 1902, vol. ii, p. 74.
[6] Scottish Med. and Surg. Jour., 1903, vol. xii, p. 157.
[7] Brit. Med. Jour., 1904, vol. i, p. 1136.

Curschmann,[1] Wassermann,[2] Dützmann,[3] Federmann,[4] Gerngross,[5] Coste,[6] Kühn,[7] and Stadler,[8] in Germany; and by Cazin and Gros,[9] in France.

The detection of *iodophilia* is a great diagnostic aid. Like leucocytosis, it measures the intensity of the toxemia, but it also develops in greatly anemic patients, irrespective of their systemic reaction to an appendicular lesion. Iodophilia is most marked in cases with abscess, gangrene, and local or general peritonitis; in pus cases the reaction persists so long as the pus remains pent up, but it rapidly disappears after adequate drainage is established—usually, in the author's cases, within thirty-six hours or so. Thus it appears that the iodin reaction has much the same meaning as leucocytosis, but it is even a more sensitive sign than the latter, since it frequently betrays a sepsis too slight to excite, or so grave as to stifle, a leucocyte increase. Locke,[10] from a study of 61 cases of appendicitis, lays stress on iodophilia as a negative sign; rarely, if ever, does it fail to develop when sepsis is present.

DIAGNOSIS.

The conditions which may more or less closely simulate an acute attack of appendicitis are numerous, and unfortunately it happens that just those lesions in which the resemblance is closest often produce blood changes identical with those of appendicitis. Thus, leucocytosis is the rule in *pyosalpinx*, *ovarian abscess*, *ectopic pregnancy*, *pyonephrosis*, *perinephritic abscess*, *hepatic abscess*, *empyema of the gall-bladder*, *mesenteric thrombosis*, and *malignant disease of the cecum*, all of which may be confused with an appendicular abscess. Iodophilia also occurs in these conditions, save perhaps in ectopic gestation.

Such a large proportion of cases of *hepatic* and *renal colic* are accompanied with acute inflammatory complications, giving rise to leucocytosis, that these conditions cannot be differentiated with any degree of confidence from appendicitis simply by an examination of the blood. The same is true of *dysmenorrhea*, in which inflammatory changes in the uterus may constitute the factor of a leucocyte increase. *Acute gastritis* is sometimes accompanied by a well-marked leucocytosis, and sometimes by

[1] Münch. med. Wochenschr., 1901, vol. xlviii, pp. 1907 and 1962.
[2] Arch. f. klin. Chir., 1903, vol. lxix, p. 392.
[3] Sem. méd., 1903, vol. xxiii, p. 324.
[4] XXII. Cong. f. Chir., Berlin, 1903; also Sem. méd., 1904, vol. xxiv, p. 206.
[5] Münch. med. Wochenschr., 1903, vol. l, p. 1586.
[6] *Ibid.*, 1902, vol. xlix, p. 2038.
[7] *Ibid.*, 1902, vol. xlix, pp. 2033 and 2085.
[8] Jour. Amer. Med. Assoc., 1903, vol. xli, p. 216.
[9] Sem. méd., 1903, vol. xxiii, p. 141.
[10] Boston Med. and Surg. Jour., 1902, vol. cxlvii, p. 289.

none at all, so that the blood count cannot be relied upon as a clue in distinguishing this disease from appendicitis.

Simple *enteralgia*, *gastralgia*, and *ovarian neuralgia* may be ruled out if a leucocyte increase is present, as also may be *intestinal obstruction*, provided that the latter is not complicated by inflammatory changes, by gangrene, or by malignant disease. In *lead colic* there is often a pronounced leucocytosis, especially in patients with acutely toxic symptoms; but granular basophilia of the erythrocytes can be detected even in the earliest stages of plumbism, while in appendicitis this change is found only in highly anemic subjects.

The presence of a leucocytosis is sufficient to exclude a non-inflammatory *ovarian cyst* and *a movable kidney*, and the same sign is of no little value in ruling out *enteric fever* if no leucocyte-raising complications are apparent.

The simple fact of the presence or absence of a leucocytosis is more often misleading than useful in the diagnosis of appendicitis, for this sign, to be of any real value, must invariably be correlated with other more clinical manifestations. Appendicitis should never be ruled out because leucocytosis is absent, nor should a moderate leucocyte count be considered an indication of the benignancy of the lesion. A count in excess of 20,000, particularly if it persists or increases, may be relied upon as a certain sign of pus or its consequences, and is sufficient to warrant operative interference if the symptoms point to the appendix as the seat of the trouble. Counts of less than 20,000 cannot be depended upon to reflect the character of the local lesion, since an increase to practically this figure may be found occasionally in mild catarrhal cases, as well as in those with purulent foci. In the writer's experience the behavior of the leucocytes throws a much clearer light upon the progress of the disease in both operative and non-operative cases than it does upon the initial diagnosis, which should be determined chiefly by other clinical methods.

The absence of iodophilia is a dependable sign that no active inflammation of the appendix exists, although its presence does not necessarily mean appendicitis, as remarked above.

## VIII. ARTHRITIS DEFORMANS.

In spite of their pallor, sufferers from arthritis deformans seldom have decided anemia. Recent hematological work in this disease shows that most cases have normal *hemoglobin* and *erythro-*

*cyte* values, and that anemia, when it does develop, is generally moderate and traceable to causes other than the joint lesions. McCrae's report[1] shows an average hemoglobin percentage of 70.6 in 33 cases, and an average erythrocyte count of 4,468,000 per c.mm. in 29. Erving's counts[2] in 40 cases show an average hemoglobin percentage of 94, with a range between 80 and 100, and an erythrocyte count averaging 5,112,000, with a range between 4,148,000 and 5,980,000. These figures evidence a much less marked anemia than that detailed by earlier writers, for example by Bannatyne,[3] who found that the hemoglobin commonly ranged between 40 and 80 per cent., and the erythrocytes between 3,000,000 and 4,000,000. Histological changes in the erythrocytes are conspicuous by their absence.

*Leucocytosis* rarely accompanies arthritis deformans, and when present, can generally be referred to some complicating lesion. Of McCrae's 33 cases, but 9 showed definite leucocytosis, the average count being 7600; in Erving's 40 cases the leucocytes exceeded 10,000 per c.mm. in but 5 instances, and averaged 8885. Differential changes are trifling and do not occur in all cases. They consist in nothing more than a moderate diminution in the polynuclear neutrophiles with a proportionate lymphocyte increase.

## IX. ASIATIC CHOLERA.

GENERAL FEATURES.

In many cases there is great difficulty in obtaining a sufficient quantity of blood for clinical examination, even from a deep puncture. This peculiarity, which has been attributed to excessive dryness of the tissues from drains upon the body fluids, is most pronounced in the algid stage of the disease.

The great decrease in the alkalinity of the blood in Asiatic cholera, sometimes spoken of as an *acid* reaction, was first determined by C. A. Schmidt[4] by a series of elaborate analyses made in 1850, since which time similar findings have been noted by Cantani,[5] Straus,[6] and others.

The density of the blood mass is found to be increased, especially in those cases in which the blood is highly inspissated; in such instances the specific gravity may rise to as high as 1.073.

The agglutination of cholera vibrios by the blood serum of

[1] Jour. Amer. Med. Assoc., 1904, vol. xlii, p. 1.
[2] Amer. Med., 1903, vol. vi, p. 440.
[3] Lancet, 1896, vol. ii, p. 1510.
[4] "Charakteristik der epidemischen Cholera gegenüber verwandten Transsudationsanomilien," Leipsic, 1850.
[5] Centralbl. f. d. med. Wissenschaft, 1894, vol. xxii, p. 785.
[6] Compt. rend. Soc. biol., Paris, 1883, vol. iv, p. 569.

cholera patients was first applied as a clinical test by Achard and Bensaude,[1] these investigators finding that the reaction may occur as early as the reputed first day of the illness and as late as the fourth week after recovery. Clinically, the test may be made either with dried blood or with serum.

HEMOGLOBIN AND ERYTHROCYTES.

The studies of Biernacki[2] and of Okladnych,[3] which together include the investigation of 62 cases, furnish the most complete data concerning the changes affecting these elements. Both of these observers found a more or less marked polycythemia with a proportionate increase in the hemoglobin percentage, the erythrocyte count in many cases being between 6,500,000 and 7,500,000, and in one case reaching a maximum of 8,000,000. The increase may often be observed within a few hours after the onset of the infection. Concentration of the blood is to be considered as the cause of these high counts, which are, as a rule, highest in cases characterized by pronounced emesis and purging. No constant relation between the degree of polycythemia and the gravity of the infection can be distinguished.

LEUCOCYTES.

The above-quoted authors found high-grade leucocytosis to be the almost invariable rule, the increase in leucocytes being not parallel with, but rather relatively greater than, the accompanying increase in erythrocytes. It occurs both in mild and in severe cases, as early as within twelve hours after the onset of the disease, and as late as the third, fourth, or sixth day. It may be present both in the algid stage and in the stage of reaction, but is likely to be more decided in the former. The degree of leucocytosis may range from a minimum count of 14,000 to a maximum of 60,000 cells per c.mm., the case of average severity showing an increase to about 25,000 or 30,000. Preagonal leucocytosis may be pronounced, counts of 50,000 being not uncommon. Biernacki states that "all cases which in the algid stage show a leucocytosis of 40,000 to 60,000 soon prove fatal." On the contrary, an absence of leucocytosis cannot be regarded as a surety that the patient will recover. In a trivial infection distinct leucopenia has been observed, but this is rare. Rogers,[4] who invariably found leucocytosis in 23 cases, also believes that the higher the count, the worse the prognosis. Of 9 of his patients with counts of less than 20,000, 4 died; of 14 with counts ranging

[1] Presse méd., 1896, vol. xvi, p. 504.
[2] Deutsch. med. Wochenschr., 1895, vol. xxi, p. 795.
[3] Cited by Biernacki, *loc. cit.*
[4] Lancet, 1902 vol. ii, p. 659.

between 20,000 and 46,000, 11 died. Concentration of the blood does not altogether account for the leucocytosis of cholera, for the influence of the specific infection as a factor is thought to be most active.

The leucocytosis involves chiefly the polynuclear neutrophiles, but not so conspicuously as in most other infections, since the percentage of these cells in cholera seldom exceeds 80. Rogers first described this peculiarity, and also drew attention to the behavior of the lymphocytes. The small lymphocytes, he found, are usually outnumbered to the extent of two to one by the large mononuclear forms, the latter's increase becoming more marked as the disease progresses, and being especially so in fatal cases. He finds that an excessive increase in this form of cell is of bad prognosis: of 18 cases with counts exceeding 2000 large lymphocytes per c.mm., 14 died; while of 5 in which these cells numbered less than 2000 but a single patient died. The eosinophiles rarely numbered more than a fraction of one per cent. Sherrington [1] has found the mast cells notably increased in some instances.

DIAGNOSIS. Leucocytosis is more constant and tends to reach a higher degree in Asiatic cholera than in any other non-choleraic disease with similar symptoms. In *acute dysentery* and in *ptomain poisoning*, however, the counts may be identical with those of cholera, but the characteristic lymphocyte formula of the latter is wanting. From the viewpoint of prognosis this differential change and the height of the total leucocyte count are obviously helpful signs.

## X. ASTHMA AND EMPHYSEMA.

In long-standing cases moderate *secondary anemia* involving chiefly a hemoglobin loss may be found, for in many instances the general debility of the patient or the presence of lesions of other organs is quite adequate to give rise to such a change. In cyanotic patients the anemia may be hidden by the polycythemia arising from circulatory disturbances, this deceptive blood concentration being most conspicuous during an asthmatic paroxysm.

Little or no increase above the normal standard in the number of *leucocytes* is the usual condition, although these cells may show a considerable increase in cases associated with acute bronchitis, and also during an asthmatic attack. Gabritschewsky,[2] Fink,[3] von

[1] Proc. of the Roy. Soc., London, 1894, vol. lv, p. 189.
[2] Arch. f. exp. Path. u. Pharm., 1890, vol. xxviii, p. 83
[3] Inaug. Diss., Bonn, 1890.

Noorden,[1] Billings,[2] and others have called attention to the presence of an eosinophile increase in both asthma and emphysema. From 10 to 20 per cent. of this type of cells is not an unusual proportion, both in cases with and in those without leucocytosis, while in one case Billings has reported three consecutive counts of 33.9, 38.2, and 53.6 per cent. respectively, with corresponding total leucocyte estimates of 7600, 7500, and 8300 per c.mm. In true bronchial asthma the eosinophile increase develops shortly before the paroxysm, and persists during and for a short time after it, disappearing in the interval between the seizures. This sign is regarded of value in differentiating true bronchial asthma from the dyspnea due to renal and cardiac lesions, since in the latter the eosinophiles are never increased, and it is also considered of some clinical utility in heralding an impending asthmatic paroxysm.

## XI. BRONCHITIS.

With the exception of a slight *oligochromemia*, which is frequently present in severe cases with high temperatures, the erythrocytes and their hemoglobin content remain practically normal in all forms of bronchial inflammation.

*Acute catarrhal bronchitis* of the larger tubes is ordinarily unattended by leucocytosis, but, unfortunately for diagnostic purposes, an occasional case shows a marked increase. Thus, in four of Cabot's seventeen cases[3] the counts were 17,600, 23,500, 26,000, and 41,000 respectively, while in eleven the leucocytes numbered more than 10,000 per c.mm. In *chronic bronchitis* leucocytosis rarely if ever occurs. Extension of the inflammation to the finer tubules and vesicular structure causes a leucocytosis identical with that of croupous pneumonia (*q. v.*).

Inflammation of the tracheobronchial glands, according to Carrière,[4] sets up a decided mononucleosis. Lichtwitz and Sabrazes[5] found this change in the blood of children with nasopharyngeal adenoids, the mononuclear increase in such cases disappearing after removal of the growths.

[1] Zeitschr. f. klin. Med., 1892, vol. xx, p. 98.
[2] N. Y. Med. Jour., 1897, vol. lxv, p. 691.
[3] *Loc. cit.*
[4] Sem. méd., 1902, vol. xxii, p. 44.
[5] Arch. de méd. des Enf., 1901, vol. iv, p. 120.

## XII. BUBONIC PLAGUE.

General Features.

Slow, imperfect *coagulation* of the blood has been found, and, in virulent cases, absolute non-coagulability. This was noted by Alice M. Corthorn [1] in 10 of 12 fatal cases of pest, whose blood, kept sealed in Wright's tubes for three weeks and longer, showed no sign of clot formation, but resembled simply a dark-red, treacle-like fluid. Jennings [2] found that rouleaux formation is but feebly exhibited.

Since 1894, when Kitasato and Yersin, working independently, simultaneously discovered the *Bacillus pestis bubonicæ* in the circulating blood of patients infected with plague, this organism has been repeatedly isolated from the blood by many different observers. In a careful bacteriological study of 27 cases Ogata [3] also frequently found in the blood, especially in severe infections, a micro-organism morphologically similar to Fränkel's pneumococcus, the significance of this unidentified organism being undetermined. The same observer calls attention to the fact that blood from patients convalescent from nineteen to sixty-five days, although giving negative results by cultural methods, when injected into mice proves rapidly fatal to these animals, in whose tissues the plague bacillus may be recovered in pure culture. The relatively large number of positive results to be obtained from bacteriological blood examinations in this disease, especially in its septicemic form, attaches to the procedure no small diagnostic value. The German Plague Commission's results [4]—43 positive findings in a series of 124 cases examined—are probably representative of the value of blood culturing in this infection. Powell's studies [5] show but 15 positive cultures in 117 cases. Cultural methods with blood drawn directly from a vein give, of course, the most favorable results, but the bacilli may be often detected in the stained cover-glass specimen of finger blood, in which they appear as short rods, tending to group together in chains or in pairs, exhibiting bipolar staining and decolorizing by Gram's method. In view of the fact that the peripheral blood contains but small numbers of the bacilli, Rees [6] advises making large films on slides rather than cover-glass specimens, should direct examination of the stained film be attempted.

[1] Brit. Med. Jour., 1902, vol. i, p. 1143. [2] "Manual of Plague," London, 1903.
[3] Centralbl. f. Bakt. u. Parasit., 1897, vol. xxi, p. 769.
[4] Cited by Novy, Amer. Jour. Med. Sci., 1901, vol. cxxii, p. 416.
[5] Indian Med. Gaz., 1904, vol. xxxix, p. 41.
[6] Brit. Med Jour., 1900, vol. ii, p. 1236.

The agglutination of the plague bacillus by the blood serum from plague subjects has been noted by a number of different investigators, but thus far no clinical application of the reaction has been made. The inconstancy with which the reaction occurs—for it may frequently be absent in both the mildest and the most severe cases—and the variable degrees of serum dilution necessary for its production appear to bar the acceptance of the test as a reliable diagnostic sign. The British Indian Plague Commission [1] concludes that "no practical value attaches to the method of serum diagnosis in the case of plague."

The striking bactericidal action of plague serum upon the *Bacillus pestis* has been taken by Row [2] as the basis of a clinical test. A drop of blood serum from a plague patient is mixed with a loopful of a saline emulsion of the *Bacillus pestis*, and from this mixture a hanging-drop culture is made and placed in the dark for twenty-four hours at laboratory temperature. The cover-glass is then fixed, stained with thionin, and examined microscopically. If the serum used in the test is from a person infected with plague, the twenty-four-hour-old culture shows either no growth of the bacillus or, at the most, a very few distorted and atypical organisms. In control cases, with normal serum, the growth is abundant. Row considers this test of real value in estimating the protective effects of Haffkinization. Its usefulness as a clinical means of diagnosing plague is restricted by the time required (twenty-four hours) for the completion of the reaction, though in suspected cases with poorly developed symptoms the test may prove of value.

HEMOGLOBIN AND ERYTHROCYTES.

According to Aoyoma's studies,[3] the hemoglobin and the erythrocytes are both decidedly increased above normal in the majority of cases. Of the six cases examined by this writer, five showed marked polycythemia, the highest count being 8,190,000, and the average 6,976,666. Septic cases may develop secondary anemia of variable intensity. Qualitative changes in the erythrocytes, it is to be presumed, are not conspicuous, since no mention of such alterations is made.

LEUCOCYTES.

In two-thirds of the cases just quoted marked increase in the number of leucocytes was found, the gain being greater than is ordinarily met with in any condition except leukemia; the count exceeded 100,000 in four instances, and averaged for the six 96,666. In fulminant

[1] Brit. Med. Jour., 1903, vol. i, pp. 1093, 1155, 1220, 1279.
[2] *Ibid.*, 1902, vol. ii, p. 1895.
[3] Mittheilungen aus d. Med. Fac. d. Kaiserlich-Japanischen Universität, Tokio, 1895, vol. iii, p. 115.

types of plague extreme leucopenia, due to the overpowering toxemia, is not unusual, and in very mild cases the number of leucocytes may be normal. The increase was due usually to a disproportionately large percentage of polynuclear neutrophiles, but in some cases "large and small mononuclear white cells" were observed. The eosinophiles were, as a rule, conspicuous by their absence. Rogers,[1] whose leucocyte counts varied from 20,000 to 60,000, also observed a lymphocytosis, and emphasizes the fact that in most cases the polynuclear neutrophiles show little or no relative increase. Contrary to Aoyoma's findings, Zinno[2] has noted, in an occasional case, a great abundance of eosinophiles of the myelocytic type, of the ordinary polynuclear variety, and of a form transitional between the two; in one of his cases the eosinophilic myelocytes numbered 13 per cent. of the total leucocyte count.

The *blood plaques* are in most cases notably increased in number.

## XIII. BURNS.

Rapidly developing and marked polycythemia, equally striking leucocytosis, and a plaque increase are the features of clinical interest in the blood picture of the severely burned. To the naked eye the blood has a deep purplish color and flows very sluggishly.

Locke's studies[3] of ten cases of severe burns show that within a few hours after the accident a marked increase in the number of *erythrocytes* takes place, amounting in favorable cases to between 1,000,000 and 2,000,000 cells to the c.mm., and in fatal cases to between 2,000,000 and 4,000,000. This polycythemia may be explained chiefly by venous stasis and partly by loss of the blood plasma. Structural changes in the erythrocytes are not conspicuous. The *leucocytes* rapidly increase, reaching a count of from 30,000 to 40,000 per c.mm. in non-fatal burns, and of 50,000 or more in patients who die. The percentage of polynuclear neutrophiles increases, although not to so high a figure as is commonly found in ordinary inflammatory leucocytosis. Degenerative changes, especially of their protoplasm, involve many of these cells, as well as the other granular leucocytes, and are marked in parallelism to the severity of the lesion. Myelocytes occur in small numbers, particularly in cases having a high leucocytosis. The *blood plaques* are greatly increased in practically every instance.

[1] Lancet, 1902, vol. ii, p. 660.
[2] Centralbl. f. allg. Path. u. path. Anat., 1902, vol. xiii, p. 410.
[3] Boston Med. and Surg. Jour., 1902, vol. cxlvii, p. 480.

## XIV. CHLOROMA.

The blood changes in this rare disease closely resemble those of lymphatic leukemia, namely, marked anemia with absolute lymphocytosis. The *hemoglobin* and *erythrocytes* progressively diminish as the disease runs its course, and eventually may sink quite as low as in pernicious anemia. In a case reported by Dunlop[1] the hemoglobin fell from 32 to 12 per cent., and the erythrocytes from 1,800,000 to 850,000 per c.mm. during a period of but four weeks. Estimates not dissimilar from these have been recorded by a number of other writers, notably by Rosenblath and Risel,[2] by Gümbel,[3] and by Weinberger.[4] Misshapen and otherwise degenerate erythrocytes, together with a variable number of normoblasts, make their appearance as the anemia increases in degree.

High *leucocyte* counts, involving absolute lymphocytosis (small celled), are the rule in the cases thus far reported. Exceptionally there is simply lymphocytosis of the relative form, as in a case reported by Bramwell[5] with 95 per cent. of lymphocytes and a leucocyte count of 8000. Early in the disease the total count is not excessive,—20,000 or 30,000,—but after a few weeks it may attain much higher figures—75,000 to 100,000 or more. Dunlop's case at one time showed a leucocyte count of 245,000 per c.mm. Differentially, the count in chloroma differs but slightly from that of lymphatic leukemia. The percentage of lymphocytes is rarely so high as in the latter, and mast cells are not so numerous, but myelocytes are more abundant. Eosinophiles, both polynuclear and myelocytic, were numerous in a case studied by Dock.[6] These, however, are minor differences, and do not serve as reliable criteria of differentiation.

Chloroma has a superficial resemblance to *exophthalmic goiter*, and very closely counterfeits *acute lymphatic leukemia*. Indeed, one is strongly tempted to agree with Dock in considering it a malignant form of leukemia, from which it differs chiefly in being more violently neoplastic and in forming greenish infiltrations and metastases. Graves' disease is readily differentiated by the blood picture. The clinical differences between chloroma and leukemia are dealt with under the latter disease. (See p. 326.)

[1] Brit. Med. Jour., 1902, vol. i, p. 1072.
[2] Deutsch. Arch. f. klin. Med., 1902, vol. lxxii, p. 1.
[3] Virchow's Arch., 1903, vol. clxxi, p. 504.
[4] Zeitschr. f. klin. Med., 1903, vol. l, p. 383.
[5] Lancet, 1902, vol. i, pp. 451 and 520. [6] Med. News, 1904, vol. lxxxiv, p. 955.

## XV. CHOLELITHIASIS.

General Features.

In impacted calculi with jaundice, *coagulation* is frequently but not invariably delayed, but in gall-stone complicated by phlegmonous cholangitis or other purulent sequelæ, hyperinosis is observed, and coagulation is generally more rapid than normal. In 28 cases of cholelithiasis the writer found that clotting occurred in less than five minutes in 7, in from five to ten minutes in 16, and in longer than ten minutes in 5. In 60 per cent. clotting was delayed, the coagulation time for these cases averaging eight and one-half minutes. The general effects of bile upon the blood, elsewhere noted, may also be detected when marked jaundice develops. (See "Cholemia" and "Icterus.")

Positive results from *bacteriological examination* of the blood have frequently been obtained in cholelithiasis, streptococci having been isolated by Netter,[1] staphylococci and colon bacilli by Sittmann,[2] streptococci and pneumococci by Canon,[3] and various bacteria of unknown identity by other investigators.

| Hemoglobin Percentage. | Number of Cases. | Erythrocytes per c.mm. | Number of Cases. |
|---|---|---|---|
| From 90–100 | 12 | Above 5,000,000 | 13 |
| " 80–90 | 23 | From 4,000,000–5,000,000 | 58 |
| " 70–80 | 43 | " 3,000,000–4,000,000 | 34 |
| " 60–70 | 20 | " 2,000,000–3,000,000 | 10 |
| " 50–60 | 8 | " 1,000,000–2,000,000 | 1 |
| " 40–50 | 5 | | |
| " 30–40 | 1 | | |
| " 20–30 | 3 | | |
| Below 20 | 1 | | |
| Average, 73.5 per cent. | | Average, 4,080,117 per c.mm. | |
| Maximum, 98.0 " | | Maximum, 5,390,000 " " | |
| Minimum, 15.0 " | | Minimum, 1,040,000 " " | |

Hemoglobin and Erythrocytes.

Moderate oligochromemia is found in the greater proportion of cases, but a decided loss either of hemoglobin or of erythrocytes is comparatively rare. In general terms it may be conservatively stated that the hemoglobin loss on the average amounts to about 30 per cent., and that the cel-

[1] Progrès méd., 1886, vol. xiv, p. 992.
[2] Deutsch. Arch. f. klin. Med., 1894, vol. liii, p. 323.
[3] Deutsch. med. Wochenschr., 1893, vol. xix, p. 1038.

lular decrease approximates 15 per cent. of the normal standard. In occasional instances, notably those in which suppuration or sepsis coëxists, the anemia is of a more intense grade, and may be associated with various changes indicative of cellular degeneration. In 116 cases of cholelithiasis the foregoing estimates of the hemoglobin and erythrocytes were obtained at the initial examinations.

LEUCOCYTES.

Simple gall-stone does not of itself excite the slightest increase in the number of leucocytes, but nevertheless leucocytosis, typically polynuclear in type, is a rather common feature of the blood picture in this disease, owing to the fact that such a large percentage of cases is complicated by acute inflammatory changes. Thirty-three of the 116 cases just mentioned had a count of more than 10,000 cells to the c.mm. The following résumé of the examinations illustrates the range of leucocytes in the series:

| Leucocytes Per c.mm. | Number of Cases. |
|---|---|
| From 20,000–30,000 | 6 |
| " 15,000–20,000 | 8 |
| " 10,000–15,000 | 19 |
| " 5,000–10,000 | 74 |
| Below 5,000 | 9 |

Average, 9,623 per c.mm.
Maximum, 26,000 " "
Minimum, 4,500 " "

DIAGNOSIS.

The presence of a leucocytosis excludes simple biliary colic, and indicates as the cause of the increase some other lesion, such, for example, as *phlegmonous cholangitis* or *cholecystitis*, *hepatic abscess*, *peritonitis*, or *malignant disease*. *Hepatic* and *renal* colics cannot be differentiated by the blood count.

The surgeon should remember that cases with delayed coagulation may bleed freely, even fatally, when the knife is used, and that to such patients remedies which promote clotting should be given before operating.

## XVI. CYANOTIC POLYCYTHEMIA.

In this new clinical entity, first described by Saunby and Russell,[1] the *hemoglobin* and *erythrocyte* values attain extraordinarily

[1] Lancet, 1902, vol. i, p. 515.

high figures. Osler,[1] who has studied the condition in detail, reports one case with a hemoglobin percentage of 165 and an erythrocyte count of 10,200,000 per c.mm., while in a case examined by J. N. Hall[2] the hemoglobin was 200 per cent. (making it necessary to dilute the blood doubly in order to make the test with the von Fleischl hemometer), and the erythrocyte count reached 9,949,600. Estimates not differing greatly from these have also been reported by Cabot,[3] Saunby and Russell,[4] C. G. Stockton,[5] Türk,[6] Vaquez and Quiserne,[7] and others. The blood is thick and tarry, flows sluggishly from the puncture, and coagulates with great rapidity. The cause of the enormous polycythemia is a moot point. Osler[8] proposes as a factor hyperviscosity of the blood, in consequence of which the intracapillary flow is impeded. Gibson[9] attributes it to peripheral stasis dependent upon myocardial weakness.

Osler's disease, as it may be fittingly termed, occurs in middle age, and, aside from the blood findings, is characterized by idiopathic and permanent cyanosis, by splenic and sometimes hepatic enlargement, and almost invariably by albuminuria. In the cases thus far studied no clinically demonstrable lesion of the heart or lungs has accounted for the cyanosis, nor has autopsy revealed tuberculosis of the spleen, which in its primary form is associated with cyanosis and polycythemia of a moderate degree.

The *leucocytes* are usually not increased, although in an occasional instance they have been found to number about double the maximum normal standard. Differentially they are also normal.

## XVII. DENGUE.

Graham,[10] of Beyrouth, recently announced that dengue is due to a protozoon resembling in some respects the malarial parasite. He believes that such an organism is constantly present in the disease, and that it undergoes an evolution within the erythrocytes at their expense. Furthermore, it is held that the parasite is harbored and conveyed by a species of mosquito, the *Culex fatigans*. Graham's work still lacks confirmation, and hence cannot be

[1] Amer. Jour. Med. Sci., 1903, vol. cxxvi, p. 187.
[2] Amer. Med., 1903, vol. v, p. 1026.
[3] Boston Med. and Surg. Jour., 1899, vol. cxli, p. 574; *ibid.*, 1900, vol. cxlii, p. 275.
[4] *Loc. cit.*
[5] Medical News, 1903, vol. lxxxii, p. 948.
[6] Cited by Osler, *loc. cit.*
[7] Sem. méd., 1902, vol. xxii, p. 235.
[8] Brit. Med. Jour., 1904, vol. i, p. 121.
[9] Lancet, 1903, vol. ii, p. 1560.
[10] Med. Rec., 1902, vol. lxi, p. 204.

considered conclusive. Study of the corpuscles in this disease seems to have been generally neglected.

## XVIII. DIABETES MELLITUS.

General Features.

The *alkalinity* of the blood, according to the investigations of Minkowski,[1] is appreciably diminished, especially in cases in which coma either impends or exists. The change, however, cannot be regarded as constant, since in none of the five cases lately studied by Golla[2] did the alkalinity figures differ materially from normal.

Orlowsky[3] in two cases of diabetes mellitus appreciably increased the blood alkalinity by giving warm alkaline enemata, the change thus effected being more decided and persisting longer than when caused by the administration of similar drugs by the mouth. He believes in treating diabetic coma in this manner, the alkali being given for some time after urgent symptoms have disappeared. *Lipemia* is not uncommon, the amount of fat in some instances being so large as to produce a milky appearance of the blood drop, evident to the naked eye, although in most instances the condition is recognizable only by the detection of fat globules under the microscope. Not infrequently diabetic blood has a peculiar salmon color. Neisser and Derlin[4] report a case of diabetes which showed 20 per cent. (!) of fat in the inspissated blood obtained by venesection. *Lipacidemia* may be detected in diabetic coma. *Glycemia* is present, and can be demonstrated by the detection of grape-sugar in relatively large amounts, even as great as 5.7 parts per thousand, according to Grawitz,[5] or 9 per thousand, according to Hoppe-Seyler.[6] (See p. 141.)

*Williamson's Test.*—This reaction, devised by Williamson[7] in 1896, depends upon the fact that a warm alkaline solution of methylene-blue is decolorized when mixed with a minute quantity of glucose. Twenty c.mm. of the suspected blood, obtained by puncturing the finger, are measured by means of Gower's hemocytometer pipette, and blown out into 40 c.mm. of distilled water contained in a small test-tube. To this mixture are then

[1] Mitth. a. der med. Klinik. z. Königsberg, 1888.
[2] Lancet, 1903, vol. i, p. 1230.
[3] Russkiy Vrach, 1901, vol. xxii, pp. 1190 and 1222.
[4] Zeitschr. f. klin. Med., 1904, vol. li, p. 428.
[5] *Loc. cit.*
[6] Virchow's Arch., 1858, vol. xiii, p. 104.
[7] Brit. Med. Jour., 1896, vol. ii, p. 730; also Lancet, 1900, vol. ii, p. 320.

added, in the order given, 1 c.c. of a 1:6000 aqueous solution of methylene-blue and 40 c.mm. of a six per cent. aqueous solution of potassium hydrate. In a second test-tube the same proportions of normal blood and reagents are mixed, to be used as a control. The color of both mixtures is precisely the same—moderately deep bluish-green. Both tubes are placed in a beaker filled with boiling water, in which they are allowed to remain for four minutes, at the end of which time the test fluid containing the diabetic blood will have turned a dingy yellow color, while the color of the control mixture remains unchanged. Care must be taken to use not more than 20 c.mm. of blood, since a positive reaction may be more or less closely counterfeited with non-diabetic blood should three or four times this quantity be employed. It is essential, therefore, to measure the blood accurately, and not to trust to the approximate method used by some, of simply taking two drops of blood as the equivalent of the required 20 c.mm.

Williamson's reaction is presumably due solely to the action of the grape-sugar contained in diabetic blood, and if this proves true, it is not unreasonable to predict that the principle of the test may be elaborated into a method for estimating the percentage of sugar in the blood. Positive reactions occur constantly in diabetes, sometimes even after the disappearance of every trace of sugar from the urine, and, so far as investigations up to the present time have shown, negative results are invariably met with in other diseases.

*Bremer's Test.*—Bremer,[1] having noticed in diabetes mellitus peculiar affinities of the erythrocytes for various anilin dyes, has devised upon this basis an ingenious test for the recognition of diabetic blood. Several thick films from a suspected case, controlled by the same number of preparations made from normal blood, are prepared, preferably on slides, and heated in an oven to a temperature of 135° C., after which they are set aside to cool. Both sets of films are then stained for about two minutes with a one per cent. aqueous solution of Congo-red (mixed freshly just before using), thoroughly washed in running water, and dried between bits of filter-paper. Thus treated, diabetic blood is either colored pale greenish-yellow or is entirely unstained, while normal blood stains typically the red color of the dye. Using the same method of heat fixation, other anilin dyes may be employed to demonstrate this peculiar behavior of diabetic blood. For example, with a one per cent. aqueous solution of methylene-blue the diabetic specimen stains yellowish-green and

[1] N. Y. Med. Jour., 1896, vol. lxiii, p. 501 (*Lit.*); also Med. Record, 1897, vol. xii, p. 495.

the normal film blue. Diabetic blood, on the contrary, treated with a one per cent. aqueous solution of biebrich-scarlet, takes the color of the dye in a typical manner, while normal blood remains practically uncolored. Ehrlich's triacid stain, as well as mixtures of methylene-blue and eosin and methyl-green and eosin, have also been used to demonstrate the reaction. The cause of Bremer's reaction is unknown, but apparently it is not due to the effect of glucose; many authors are inclined to attribute it to excessive acidity of the blood. Positive results with this test cannot be regarded as pathognomonic of diabetes mellitus, since they have been reported with the blood of persons suffering from exophthalmic goiter, multiple neuritis, leukemia, and Hodgkin's disease.

HEMOGLOBIN AND ERYTHROCYTES.

There are no constant changes to be found in these elements. Normal hemoglobin percentages and erythrocyte counts are observed in most cases, while in others in which the cachexia is pronounced a well-marked secondary anemia may exist. James,[1] in a study of 13 cases, found the number of erythrocytes over 6,000,000 in 5; 5,000,000 plus in 5; 4,000,000 plus in 2; and 3,000,000 plus in 1. The hemoglobin percentage was over 100 in 3 cases; 60–70 in 8; and 50–60 in 2. The alterations in the hemoglobin and erythrocytes in diabetes have been attributed by the older writers[2] chiefly to the effects of blood concentration and dilution. Thus, it was believed that in cases with excessive polyuria the blood became inspissated and the count thus increased, while in cases with pronounced glycemia the blood became diluted and the count lowered as a consequence of the fluid transfer from the tissues into the capillaries provoked by the presence in the blood of a large percentage of sugar. It is obvious that these influences, if active, are sufficient to render the blood count in diabetes of little or no practical value, since, on the one hand, perfectly normal blood, if diluted, may appear anemic, while, on the other hand, anemic blood, if concentrated, may seem normal. James[3] contends, however, that the polycythemia is real, and is not dependent upon inspissation, for were the increase merely relative, it would naturally be accompanied by an increase in the density of the blood, and this in his experience never occurred, the specific gravity figure for his series ranging between 1.054 and 1.060.

[1] Edinburgh Med. Jour., 1896, vol. xlii, p. 193.

[2] Lit. cited by Leichtenstern, "Unters. über d. Hg-Gehalt d. Blutes," Leipsic, 1878.

[3] *Loc. cit.*

LEUCOCYTES.

High digestion leucocytosis is the most constant change affecting these cells, but this is not found in every case. Isolated examples of leucocytosis, apparently independent of this influence, have been reported, but in the great majority of diabetics the leucocyte count is normal. The presence of small numbers of myelocytes in cachectic patients is the only qualitative change to which attention has been directed. Mahogany-colored granules, either within the leucocytes or extracellular, may usually be demonstrated by the iodin method. No numerical change in the *blood plaques* has been reported.

DIAGNOSIS.

Williamson's reaction is of real value, especially in the recognition of cases with temporary disappearance of sugar from the urine and in diabetic coma. Bremer's test and the iodin reaction are to be regarded as symptomatic, not necessarily of diabetes. The other blood findings are without diagnostic value.

## XIX. DIPHTHERIA.

HEMOGLOBIN AND ERYTHROCYTES.

Usually the changes in the hemoglobin and erythrocytes are, at the most, trifling, for in about two-thirds of all cases these elements are practically normal, while in the other one-third moderate anemia, more marked in severe than in mild cases, is found. The anemia does not develop until about the middle of the first week of the disease, and is, as a rule, characterized by a diminution of hemoglobin roughly proportionate to the corpuscular loss. Degenerative changes are rare, consisting usually of nothing more than occasional polychromatophilia; nucleation and deformities of size and of shape are absent. Regeneration of the blood takes place slowly, and, as the loss of hemoglobin is made up less rapidly than that of the corpuscles, the color index, which is approximately normal early in the disease, later falls considerably.

The loss of hemoglobin does not often exceed 15 per cent., nor is the decrease of erythrocytes usually greater than from 500,000 to 750,000 cells to the c.mm. Concentration of the blood, which frequently occurs during the febrile period, may cause striking temporary polycythemia.

Morse,[1] in single examinations of 30 cases treated without antitoxin, found the count of erythrocytes above 5,000,000 in

[1] Boston Med. and Surg. Jour., 1895, vol. cxxxii, pp. 228 and 252.

21, and below 4,000,000 in but a single instance, a woman with chronic anemia; several of his counts were in the neighborhood of 6,000,000. From this author's monograph[1] the following counts reported by other investigators are taken: Bouchut and Dubroisay,[2] 4,305,000 as the mean average of 93 counts in 84 cases; Gilbert,[3] an average of 4,500,000 in 58 counts in 22 cases; Carter,[4] an average of 4,253,000 in 13 cases; and Filè,[5] an average of 4,588,000 in 18 counts in 10 cases, some of which had received antitoxin. Billings,[6] in a painstaking study of 7 cases untreated with antitoxin, in which 36 counts were made, found a moderate but distinct decrease in hemoglobin and erythrocytes in 5 cases, the loss first becoming apparent by the third or fourth day, and being proportionate to the severity of the infection. This author's first counts, all made during the first week of the disease, ranged from 5,200,000 to 6,122,000, the average being 5,611,285, the hemoglobin for the same period ranging from 70 to 98 per cent. and averaging 90 per cent. Subsequent counts in this series showed that the greatest loss of hemoglobin averaged 12 per cent., ranging from 1 to 30 per cent., and that the maximum corpuscular loss averaged 878,500, varying from 227,000 to 2,040,000.

It is generally observed that in cases treated with antitoxin the anemia is decidedly less than in those treated by other methods, and, in fact, a majority of cases thus treated suffer no decrease at all.

LEUCOCYTES.

Well-marked leucocytosis, beginning probably within a few hours after the infection first occurs, characterizes the average case of diphtheria of moderate severity. An analysis of the statistics derived from 276 counts made by reliable investigators[7] shows that over 90 per cent. of all cases are accompanied by a more or less marked increase in the number of leucocytes.

In the majority of cases the number of leucocytes is not increased above 30,000 per c.mm., but a much greater leucocytosis is sometimes encountered. Thus, the maximum counts of several authors are as follows: Felsenthal, 148,229[8]; Ewing, 72,000[9];

[1] Med. and Surg. Reports of the Boston City Hospital, 1899, tenth series, p. 138.
[2] Compt. rend. Soc. biol., Paris, 1877, vol. lxxxv, p. 158.
[3] Traité de Méd. Charcot-Bouchard, vol. ii, p. 485.
[4] Univ. Med. Mag., 1894–95, vol. vii, pp. 17, 81, and 158.
[5] Lo Sperimentale, 1896, vol. l, p. 284. [6] Med. Record, 1896, vol. xlix, p. 577.
[7] Ewing, Morse, Billings, Carter, Schlesinger, Filè, Gabritschewsky, Bouchut and Dubroisay, Rieder, Felsenthal, and Gilbert.
[8] Arch. f. Kinderheilk., 1893, vol. xv, p. 178.
[9] N. Y. Med. Jour., 1893, vol. lviii, p. 713.

Gabritschewsky, 51,000[1]; Morse, 48,000[2]; Carter, 48,280[3]; Billings, 38,600[4]; and Gilbert, 31,000.[5]

This increase is to be regarded as a rough gage of the reaction of the individual against the effects of the toxic products of the disease; it is, therefore, absent in very mild cases, where little or no reaction is excited, and in severe cases in which the patient's resisting powers are overwhelmed by the intoxication.

In favorable cases the maximum leucocytosis is reached coincidentally with the height of the disease, and the increase gradually fades away during convalescence, having in most cases entirely ceased by the time the membrane has disappeared, but occasionally persisting after the subsidence of all local and systemic manifestations of the illness. In unfavorable cases high leucocyte counts persist until death, or "in somewhat prolonged cases, with much septic absorption, there may be an uninterrupted decrease of leucocytes continuing up to the fatal termination" (Ewing). No constant relation has been determined between the leucocytosis and the extent of the local lesion, the degree of tonsillar and glandular swellings, or the height of the fever, although in individual cases some authors have suggested that such relationship may be distinguished.

The effects of antitoxin upon the leucocytes are well illustrated by the conclusions of Ewing,[6] based upon 228 counts made in 53 cases before and after the injection of the serum. As the result of these investigations this author concludes that antitoxin, within thirty minutes after its injection, causes a hypoleucocytosis. In favorable cases, after the injection, the original height of the leucocytosis is not again attained, but in severe and less favorable cases the dose of antitoxin is followed in a few hours by a hyperleucocytosis exceeding that found in the primary count. In malignant cases the administration of antitoxin may be followed immediately either by rapid hyperleucocytosis or by extreme hypoleucocytosis and death. Bize[7] finds that in some cases the initial serum injection may not affect the leucocytes because of the insufficiency of the dose, and that repeated injections are sometimes required to modify the count. This investigator has also called attention to the pronounced leucocytoses which accompany eruptions due to antitoxin.

The leucocytosis of diphtheria, as a rule, involves the polynuclear neutrophile cells, most cases showing about 80 per cent. of this variety, but in an occasional instance there may be a

[1] Annal. de l'Institut Pasteur, 1894, vol. viii, p. 673. [2] *Loc. cit.*
[3] *Loc. cit.* [4] *Loc. cit.* [5] *Loc. cit.*
[6] *Loc. cit.* [7] Arch. de méd. des Enf., 1901, vol. iv, p. 102.

well-marked increase in the mononuclear forms, considerably in excess of the percentage found in health. Relative lymphocytosis has been observed both during convalescence and in fatal cases with leucopenia, and absolute lymphocytosis may occur at the height of the disease in cases with high total leucocyte counts. In two of Ewing's cases[1] the estimates showed 60 per cent. of lymphocytes in a count of 72,000 leucocytes, and 62 per cent. of these cells in a count of 22,500.

Besredka[2] believes that marked polynuclear leucocytosis is a good prognostic sign, especially if this form of cells shows a strong tendency to increase after injection of antitoxin. On the contrary, cases which fail to show such a change he regards as grave, usually as fatal. This characteristic of high percentages of polynuclear neutrophiles is also regarded by many other authors as a favorable clinical sign, and a low percentage as unfavorable.

Ewing,[3] by staining the leucocytes with gentian-violet (50 c.c. of normal salt solution to which one drop of a saturated alcoholic solution of gentian-violet is added), has deduced certain conclusions from the reaction of the leucocytes to this dye, to which he is inclined to attribute great prognostic value. He believes that the numbers and percentages of poorly stained leucocytes, and usually of ameboid figures, invariably increase in unfavorable cases, without relation to the total number of cells found in the blood. In his experience any considerable increase of poorly stained leucocytes, especially if associated with a decrease of the well-stained cells, was invariably the forerunner of a grave or fatal change in the patient's condition. In favorable cases, after treatment with antitoxin, he noted that the polymorphous forms show a decidedly increased affinity for gentian-violet, this characteristic often being observed within twelve hours after the injection of the serum. Failure of this peculiarity to develop he regards as a very unfavorable prognostic sign.

The proportion of eosinophiles is exceedingly variable, these cells sometimes being absent, and at other times found in large numbers—four or five per cent. Kucharzewski[4] concludes, from experimental work with animals, that a high percentage of eosinophiles is of favorable prognosis and that a low percentage is unfavorable.

Engel[5] found variable percentages of myelocytes in both favor-

[1] "Clinical Pathology of the Blood," 2d ed., New York and Philadelphia, 1904.
[2] Annal. de l'Institut Pasteur, 1898, vol. xii, p. 305.
[3] N. Y. Med. Jour., 1893, vol. lviii, p. 713.
[4] XIV. Internat. Med. Congress, Madrid, Apr. 23–30, 1903; abst., Jour. Amer. Med. Assoc., 1903, vol. xl, p. 1673.
[5] Deutsch. med. Wochenschr., 1897, vol. xxiii, pp. 118 and 137.

able and unfavorable cases, especially in the latter, and he considers their presence in relatively high percentages (2 per cent. or higher) as an unfavorable prognostic indication. In 7 of Engel's fatal cases the percentage of myelocytes ranged from 3.6 to 14.6, but they never exceeded 1.5 per cent. in patients who recovered. An absence of myelemia, however, is no guarantee of recovery, for this sign is absent in about one out of every four fatal cases.

DIAGNOSIS.

Examination of the blood in diphtheria gives no information which is not clearly shown by other clinical signs, and it must be regarded as of no value as an aid to diagnosis. The leucocytosis in this disease, if the very benign and the very severe cases are excluded, is, as a rule, proportionate to the intensity of the infection.

From a prognostic point of view it appears that, as in pneumonia, an absence of leucocytosis occurring in obviously severe infections is an unfavorable indication. The presence of a large percentage of myelocytes has a similar meaning. Pronounced lymphocytosis is also regarded as an unfavorable prognostic sign.

## XX. ENTERITIS.

ENTERITIS AND DIARRHEA.

In *acute catarrhal enteritis* the same changes occur that are found in acute gastritis, namely, little or no alteration in the hemoglobin and erythrocytes, and an inconstant, moderate leucocytosis. Profuse watery dejecta lead, of course, to more or less blood concentration, by depletion of the body-fluids, and hence polycythemia may be a transient sign. In the *summer diarrheas of infants* Knox and Warfield[1] found that the leucocyte count, although usually increased, varies so widely that the mere presence of a high or a low count is of practically no definite diagnostic importance. An increase in the relative percentage of polynuclear neutrophiles, even with a normal number of leucocytes, suggests the onset of inflammatory intestinal complications. These findings Zahorsky has fully corroborated.[2] In *chronic enteritis* and in *gastro-enteritis* the interference with the patient's nutrition plus a drain upon the albuminoids may in course of time give rise to a decided anemia. Leucocytosis is not a characteristic of such cases.

[1] Johns Hopkins Hosp. Bull., 1902, vol. xiii, p. 167.
[2] N. Y. Med. Jour., 1903, vol. lxxviii, p. 505.

Dysentery.

In *dysentery* and in *ulcerative* and *phlegmonous enteritis* acute forms of secondary anemia are frequently met with, especially in patients who pass much blood by the bowel. Leucocytosis, often of high degree, is also common in these lesions.

In 38 cases of uncomplicated *amebic dysentery* Futcher[1] found that the hemoglobin averaged 63 per cent. and the erythrocytes 4,802,000 per c.mm., while in 43 cases the leucocytes averaged 10,600. In 15 cases complicated by amebic abscess of the liver the hemoglobin averaged 66 per cent., the erythrocytes 4,250,000, and the leucocytes 18,350. Doubtless in both series the high erythrocyte values may be referred to blood concentration. The value of the leucocyte count in the diagnosis of amebic hepatic abscess is doubtful. In most abscess cases, such as in the 11 reported by Schlayer,[2] the leucocytosis is high (averaging in this series 25,000 and ranging between 18,000 and 62,000); but in other cases low counts (6000, 9000, and 11,000 in Osler's series[3]) are not incompatible with pus. In Rogers' experience[4] the count is higher in small, deeply seated abscesses than in those superficially situated. In differentiating malarial fever from the intermittent pyrexia of hepatic abscess the presence of leucocytosis practically excludes the former.

As a means of differentiating amebic from bacillary dysentery the serum test is of value, for the *Bacillus dysenteriæ* is clumped by the blood of persons suffering from bacillary (Shiga[5]) dysentery, but not by the blood of those infected with the amebic form of the disease. Rogers[6] has used the test extensively in India with great success.

On similar grounds it may be presumed, until proof to the contrary is shown, that Shiga's organism is unaffected by the blood of those infected with so-called dysentery due to the bacilli of Ogata, of Lessage, and of Roger, and to the *Balantidium coli.*

Shiga's claim,[7] that the *Bacillus dysenteriæ* is never clumped by the blood of non-dysenteric diseases, needs revision, in the light of the researches of Park,[8] Pilsbury,[9] and Strong.[10] These observers have found that dysentery bacilli of the Shiga, the Flexner, and the Kruse strains occasionally clumped with the blood of persons suffering from such diseases as alcoholic

[1] Jour. Amer. Med. Assoc., 1903, vol. xli, p. 480.
[2] Münch. med. Wochenschr., 1903, vol. l, p. 1372.
[3] Med. News, 1902, vol. lxxx, p. 673. [4] Brit. Med. Jour., 1902, vol. ii, p. 850.
[5] Centralbl. f. Bakt. u. Parasit., 1898, vol. xxiii, p. 599.
[6] Brit. Med. Jour., 1903, vol. i, p. 1315.
[7] *Loc. cit.* [8] Jour. Med. Research, 1903, vol. ix, p. 180.
[9] Med. News, 1903, vol. lxxxiii, p. 1078. [10] Rep. Surg.-Gen., U. S. A., 1900.

enteritis, tuberculosis, enteric fever, appendicitis, pernicious anemia, and pleurisy—even in dilutions as high as 1:30, 1:50, and 1:100. Still, it is safe to regard a positive reaction with the *Bacillus dysenteriæ* as the most valuable single clinical sign of acute bacillary dysentery in adults in whom a recent chronic inflammation of the intestine can be ruled out. The test, to be dependable, should be made with at least a 1:20 dilution, the time-limit being not more than two hours. Pilsbury found that false positive reactions do not occur with the blood of non-dysenteric children under one year of age.

SPRUE.

Attempts to isolate organisms by *bacteriological examination* of the blood were unsuccessful in Goadby's hands,[1] although the most careful methods were employed.

The *hemoglobin* and *erythrocytes* are markedly reduced, and in a remarkably short time after the onset of the acute symptoms the cellular loss becomes so severe as to simulate that of true pernicious anemia. Counts of between 1,000,000 and 2,000,000 were found to be the rule by Bassett-Smith,[2] with correspondingly low hemoglobin values. There are also decided structural changes in the erythrocytes, many of which are deformed in shape and size (especially microcytes) and show unnatural pallor. A few normoblasts, but no megaloblasts, may be encountered. The blood plaques are scanty.

The *leucocytes*, according to the last-named writer, are not increased, and in some instances fall to between 1000 and 2000 per c.mm. Relative lymphocytosis, small-celled in type, relative eosinophilia (in the exceptional case), and fractional percentages of myelocytes are the other leucocyte findings in this grave form of enteric disease.

EFFECTS OF PURGES.

The effects upon the blood of *saline purges* were first determined by Brouardel,[3] and later studied by Grawitz[4] and by Hay.[5] The investigations of these authors have shown that the administration of a purgative dose of Epsom or Glauber salts is followed within about thirty minutes by an appreciable increase in the number of erythrocytes, and that within an hour the count of these cells is fully 1,500,000 more than before the purge was given; three hours after this maximum is reached the count is again normal. When a certain degree of concentration is obtained by these means, continued administration of the salt produces neither additional con-

[1] Brit. Med. Jour., 1903, vol. ii, p. 644.
[2] *Ibid.*, 1903, vol. ii, p. 641.
[3] Union méd., 1897, vol. xxii, p. 405.
[4] *Loc. cit.*
[5] Jour. Anat. and Physiol., 1882, vol. xvi, p. 435.

centration nor further purgation. Common table salt is also a most energetic factor of blood density, even more so than either Epsom or Glauber salts. Purgative doses of jalap, croton oil, and other drugs of this class are also followed by more or less polycythemia.

## XXI. ENTERIC FEVER.

General Features.

The *alkalinity* of the blood is generally decreased, a change which may be due partly to the effect of the pyrexia and toxemia and partly to the anemia. Dare and Funke[1] found subnormal alkalinity figures in 20 of 23 cases of enteric fever, but were unable to determine the relationship of this change to the other clinical manifestations. Drouin[2] also found similar alkalinity values in this infection.

The *coagulation time* of the blood is appreciably diminished in the early stages of typhoid, sometimes to such a degree as to favor intestinal hemorrhage. During convalescence the rapidity of clotting is decidedly increased, and this tendency may be regarded as a possible factor of thrombosis. Wright and Knapp[3] suggest that the increased coagulability is due to the excessive amount of calcium salts present in the blood at the time of convalescence, and that this excess depends chiefly upon the large quantity of lime salts ingested by a patient kept on a milk diet. These authors advise, as a preventive measure against thrombosis, partial decalcification of the milk by the addition of sodium citrate (20 to 40 gr. to the pint) as soon as the danger of intestinal hemorrhage is over.

Bacteriology.

Enteric fever is practically always a specific bacteriemia, and the *Bacillus typhosus* can be cultured from the circulating blood in more than 75 per cent. of all cases. From 200 to 300 c.c. of nutrient broth sown with 2 or 3 c.c. of blood gives satisfactory results, although some investigators, notably Schottmüller,[4] prefer to mix the blood directly with melted agar, at a temperature of 45° C., and to plate the inoculation, so as to get some idea of the number of colonies grown.

The typhoid bacteriemia usually develops early in the disease, and therefore frequently may be demonstrated some days before the appearance of the serum reaction. Cultures made during the

[1] Johns Hopkins Hosp. Bull., 1903, vol. xiv, p. 175.
[2] "Hémo-alcalimétrie et Hémo-acidimétrie," Thèse de Paris, 1892, No. 83
[3] Brit. Med. Jour., 1902, vol. ii, p. 1706; also Lancet, 1902, vol. ii, p. 1531.
[4] Münch. med. Wochenschr., 1902, vol. xlix, p. 1562.

first week of the fever yield a higher percentage of positive results than those made during the second and third weeks. With defervescence the bacilli disappear from the blood, but they reappear with a relapse, although not with a simple recrudescence of the fever—a significant fact when the question arises of determining the presence of a true reinfection. It is the general experience that the severer the type of the infection, the more abundant the bacteria in the blood. The following tabulation illustrates the frequency of positive results in cases examined by modern methods:

| Authority. | Number of Cases. | Positive Results. | Percentage of Positive Results. |
|---|---|---|---|
| Schottmüller [1] | 212 | 182 | 86 per cent. |
| Hewlett [2] | 125 | 90 | 72 " " |
| Courmont; Lesieur [3] | 57 | 54 | 95 " " |
| Harris; Kerr [4] | 56 | 31 | 55 " " |
| Busquet [5] | 43 | 43 | 100 " " |
| Warfield [6] | 43 | 33 | 76.5 " " |
| Kühnau [7] | 41 | 11 | 27 " " |
| Lesieur [8] | 36 | 36 | 100 " " |
| Reudiger [9] | 27 | 20 | 74 " " |
| Cole [10] | 15 | 11 | 73 " " |
| Castellani [11] | 14 | 12 | 86 " " |
| Orlovsky [12] | 12 | 10 | 83 " " |
| Auerbach; Unger [13] | 10 | 7 | 70 " " |
| Courmont [14] | 9 | 9 | 100 " " |
| Total: | 700 | 549 | Average: 78.4 per cent. |

Coleman and Buxton's analysis [15] of 453 collected cases shows the following results of blood culturing during the first four weeks of the fever:

| Week of Fever. | Number of Cases. | Cases Positive. | Per Cent. Positive. |
|---|---|---|---|
| First | 85 | 79 | 93 per cent. |
| Second | 198 | 151 | 76 " " |
| Third | 115 | 65 | 56 " " |
| Fourth | 55 | 18 | 33 " " |

During recent years numerous instances of paracolon (paratyphoid) infection, clinically counterfeiting typical enteric fever,

[1] Münch. med. Wochenschr., 1902, vol. xlix, p. 1562.
[2] Med. Rec., 1901, vol. lx, p. 849.
[3] Sem. méd., 1902, vol. xx, p. 408.
[4] Jour. Amer. Med. Assoc., 1902, vol. xxxix, p. 1000.
[5] Presse méd., 1902, vol. l, p. 593.
[6] Bull. Ayer Clin. Lab., Penna. Hosp., 1903, vol. i, p. 77.
[7] Zeitschr. f. Hyg. u. Infectionskr., 1897, vol. xxv, p. 492.
[8] Gaz. hebd. de méd. et chir., 1902, vol. xlix, p. 1128.
[9] Medicine, 1903, vol. ix, p. 258.
[10] Johns Hopkins Hosp. Bull., 1901, vol. xii, p. 203.
[11] Centralbl. f. Bakt. u. Parasit., 1902, vol. xxxi, p. 477.
[12] Phila. Med. Jour., 1903, vol. xi, p. 959.
[13] Deutsch. med. Wochenschr., 1900, vol. xxvi, p. 796.
[14] Jour. de physiol. et de path. gen., 1902, vol. iv, p. 154.
[15] Med. News, 1904, vol. lxxxiv, p. 1046.

have been reported, in which a paracolon bacillus has been isolated from the blood during life. Among those reporting such cases may be mentioned Gwyn,[1] Longcope,[2] H. W. Allen,[3] Hewlett,[4] Johnston,[5] and Libman.[6] Of 60 cases clinically typhoid studied by Coleman and Buxton,[7] 2 yielded blood cultures of the *Bacillus coli communis*. In the late stages of the disease pyogenic bacteria have been found in the blood.

*Examination of the Rose Spots.*—Formerly the cultivation of typhoid bacilli from the rose spots was attended by indifferent success, but the favorable results with this procedure recently announced by a number of authors must stamp it as a distinct aid to the diagnosis of typhoid. Neufeld,[8] basing his experiments upon the belief that the bacteria lodge and multiply in the spots protected from the bactericidal action of the blood, examined these lesions in 14 cases and obtained positive results in 13. His findings have been corroborated by the work of Curschmann,[9] who found the bacilli in 14 of 20 cases; of Richardson,[10] whose results were positive in 5 of 6 cases; of Kozarinoff,[11] who found positive results in 12 of 17 cases; of Seemann,[12] who reports positive findings in 32 of 34 cases; and of Scholz and Krause,[13] who found bacilli in the spots in 14 of 16 cases examined. The latter investigators emphasize the statement that the bacilli are prone to disappear from the spots after from three to five days, and that, to insure the best results, the examinations must be made as soon as possible after the appearance of the roseola. The most favorable results from spot culturing yet reported are those of Pollaco and Gemelli[14]—invariably positive findings in 50 consecutive cases. All investigators agree that, in the great majority of instances, spot cultures give positive results several days before the appearance of the serum test. The chief disadvantages to this method of diagnosis appear to be the absence of the roseola in some cases, its late development in others, and the possibility of not always being able to distinguish typhoid spots from other eruptions.

The technic used by Richardson[15] is simple, and, judging

[1] Johns Hopkins Hosp. Bull., 1898, vol. ix, p. 54.
[2] Amer. Jour. Med. Sci., 1902, vol. cxxiv, p. 209.
[3] *Ibid.*, 1903, vol. cxxv, p. 96. [4] *Ibid.*, 1902, vol. cxxiv, p. 200.
[5] *Ibid.*, 1902, vol. cxxiv, p. 187. [6] Jour. Med. Research, 1902, vol. viii, p. 1.
[7] *Loc. cit.* [8] Zeitschr. f. Hyg. u. Infectionskr., 1899, vol. xxx, p. 498.
[9] Münch. med. Wochenschr., 1899, vol. xlvi, p. 1597
[10] Phila. Med. Jour., 1900, vol. v, p. 514.
[11] N. Y. Med. Jour., 1903, vol. lxxviii, p. 196.
[12] Wien. klin. Wochenschr., 1902, vol. xv, p. 580.
[13] Zeitschr. f. klin.Med., 1900, vol. xli, p. 405.
[14] Centralbl. f. inn. Med., 1902, vol. xxiii, p. 121. [15] *Loc. cit.*

from his results, trustworthy. After having washed the skin of the part with alcohol and ether, the spot is frozen with an ethyl-chlorid spray, after which it is crucially incised. Its substance is then removed by scraping with a small skin-curette, and transferred to a tube of nutrient bouillon. A second tube is inoculated with the blood which collects as soon as the effects of freezing have worn off, both cultures being then incubated and examined in the usual manner. At least five or six spots should be thus treated in each case, and two tubes, one for the scrapings, the other for the blood inoculation, used for each spot.

SERUM TEST.

If blood serum from a case of enteric fever is mixed with a broth culture of the *Bacillus typhosus* and a small drop of this mixture placed upon a slide and examined under the microscope, it will be observed

FIG. 56.—A POSITIVE REACTION.

Large clumps of motionless bacilli separated by open spaces. The few bacteria outside of the clumps are devoid of motility.

FIG. 57.—A PSEUDO-REACTION.

A few small clumps of bacilli having impaired motility. Persistent motility of the bacteria in other parts of the field.

that the bacilli, instead of continuing to dart actively to and fro across the field, as they do in the pure culture, are attracted to each other, lose their power of propulsion, and become grouped together in large agglutinated clumps of irregular outline, which, after the lapse of a variable length of time, become more and more compact and homogeneous. In the typical positive reaction the field of the microscope shows islands of clumped bacilli, separated from each other by large open spaces, containing perhaps a few isolated organisms the motility of which is decidedly inhibited at first, and finally entirely lost. If the clumps are of very large size, they produce a peculiar grayish mottling of the specimen visible to the naked eye, a point to which attention

was first directed by Greene.[1] In a small proportion of cases the clumps undergo a granular change, and then become entirely destroyed; in some instances they remain unaltered for several days; and in still others they may break up after a few hours, the field then becoming refilled with isolated, actively motile bacteria.

In a certain percentage of instances the agglutinated, motionless masses of bacilli may be observed as soon as the specimen is brought into focus, so that the reaction may be said to have taken place immediately. In other instances some little time elapses before the character of the test can be determined, and in such reactions the formation of the clumps, from their inception out of two or three bacteria to their completion, when they consist of several hundred organisms tightly glued together into a densely crowded mass, may be studied advantageously. In these slower reactions, while early clumping may progress to some extent, many isolated bacteria are seen, the motility of which persists for a variable period, gradually growing less and less, until finally, with more or less crippled powers of propulsion, the organisms are attracted to the clump centers with which they are ultimately incorporated, either becoming adherent at first approach, or, as is usually the case with the more active bacilli, circling around the edges of the clump for some little time before becoming attached to it. Still other reactions are characterized by an almost immediate cessation of motility, followed by tardy agglutination, and usually by the formation of clumps of smaller size than those noted in a prompt and immediate reaction.

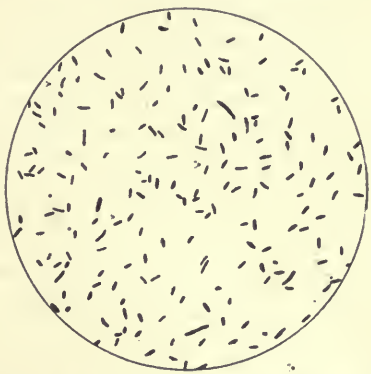


FIG. 58.—BACILLUS TYPHI ABDOMINALIS. The bacilli are actively motile throughout the field.

If the reaction is negative, the motility of the bacilli persists and the formation of clumps is not observed, regardless of the time during which the specimen is watched. Not unless agglutination is marked and entire loss of motility occurs may a reaction be considered positive; and pseudo-reactions resulting in the formation of small masses of more or less motile organisms, together

[1] Med. Record, 1896, vol. l, pp. 697 and 805.

with persistent motility of many unclumped bacteria in other parts of the field, cannot be regarded as typical in any sense. Clumping of small numbers of bacteria sometimes occurs in the pure culture during its growth, and this source of error must be eliminated by habitually examining the culture before each test or series of tests.

*Technic.*—In order to exclude all sources of error, such as may arise from the clumping of the typhoid bacillus by non-typhoid serum, provided that the latter is sufficiently concentrated and is allowed enough time to exert its agglutinative powers, the reaction can be considered of diagnostic value only under the following two conditions: first, that the blood to be tested must always be diluted with at least twenty volumes of the culture; and, second, that loss of motility and clump formation must occur within an arbitrary time limit of ten minutes. Under these conditions it has been shown that agglutination of the typhoid bacillus is produced only by the blood from a patient who is or who has been infected with enteric fever, save in exceptional cases. (See p. 403.) In some cases of typhoid the reaction occurs in much higher dilutions, frequently with dilutions of 1: 50, or 1 : 100, or even higher. Some habitually work with higher dilutions than 1 : 20, but they extend the time limit of the reaction proportionately to the degree of the dilution used.

Cultures from twelve to twenty-four hours old, grown in neutral peptone bouillon from a stock agar-agar culture, are best adapted for the test. It is advisable to keep all the cultures at room temperature, and to transplant the stock agar growths not oftener than once a month, since cultures "forced" by incubation and by frequent transplanting may give rise to false reactions with non-typhoid blood. The cultures should, of course, be absolutely uncontaminated, and must respond typically to the recognized tests for their identification.

The test may be conducted either *microscopically*, by the dried blood method, or by the use of fluid blood or fluid serum; or *macroscopically*, the method preferred by Widal.[1]

The *dried blood method*, perfected and popularized by Wyatt Johnston,[2] is to be chosen whenever it is necessary to send the blood sample any distance for examination, and where the examiner finds it convenient to carry with him to the patient's bedside the test-tubes required for the methods next to be described. Johnston's method is especially adapted for use by health boards, by which bodies it is now extensively employed in nearly all the large cities in this country.

[1] Bull. méd., 1896, vol. x, pp. 618 and 766.
[2] N. Y. Med. Jour., 1896, vol. lxiv, p. 573.

The technic of collecting the blood specimens is exceedingly simple. After having punctured the finger or ear in the usual manner, several separate drops of blood are collected upon the surface of some non-absorbent material, preferably glass, then dried, placed in an envelop or other protective covering, and tested at the examiner's convenience. Glass slides or slips of non-absorbent Bristol board or paper are most commonly used for collecting the blood samples, and specimens thus obtained may be kept for several months without aseptic precautions and still retain their agglutinative powers.

If the specimen has been collected on glass, one of the crusts is moistened with a drop of sterile water and worked up into a thin paste with a platinum loop, after which complete solution and proper dilution of the blood are effected by adding twenty drops of typhoid bouillon and mixing thoroughly. If the sample has been dried on a paper or cardboard surface, the blood crust may be cut out with a pair of scissors and placed to soak, face downward, in a watch-glass containing twenty drops of the culture.

From one of these mixtures of blood and typhoid culture a minute portion is transferred to the center of a clean cover-glass, which is at once inverted over a "concave slide," sealed with cedar oil, and examined as a hanging-drop with a $\frac{1}{6}$-inch dry objective, using dim illumination. A plain glass slip is used by many workers instead of a "hollow slide," but either will prove satisfactory.

If the *fluid blood method* is used, the dilution is made at the patient's bedside, by adding one drop of the whole blood as it flows from the puncture to twenty drops of typhoid bouillon contained in a small test-tube. The mouth of the tube is then closed by a cotton plug, and its contents are thoroughly mixed by vigorous shaking. At the time of the test, which must not be delayed more than a few hours after the dilution is made, a small drop of the mixture is removed from the tube and examined microscopically in the usual manner. In order to insure accurate dilutions, a graduated pipette is needed for measuring the blood and the culture, for the drops of both liquids must be of exactly the same size. Either the special pipettes devised for serum testing or the Thoma-Zeiss leucocytometer will prove satisfactory for this purpose. In lieu of either of these instruments a graduated pipette may readily be made from a bit of glass tubing or an ordinary medicine dropper.

If the *liquid serum method* is chosen, fifteen or twenty drops of blood, drawn by making a rather deep puncture, are allowed to flow into a narrow-calibered test-tube, and set aside for a few

minutes until coagulation has taken place. As soon as the clot has formed the nose of the graduated pipette is thrust into the test-tube, and one drop of the fluid serum sucked up and diluted with twenty drops of typhoid bouillon contained in a second test-tube. The preparation for microscopical examination is then made from this dilution. If the requisite apparatus is at hand, the serum may be obtained by centrifugalization.

If the *macroscopical method* is employed, the whole procedure must be carried out under the strictest aseptic precautions, for otherwise the growth of contaminating bacteria may interfere with the reaction, owing to the length of time required for the completion of the experiment.

The blood from which the serum is obtained is aspirated from one of the superficial veins of the arm, according to the technic employed in bacteriological examinations, and then immediately expelled into a sterile test-tube, which is plugged with cotton and set aside until clotting occurs. If it is desired to send the specimen any distance, the blood may be drawn up into a glass bulb, previously sterilized by heat, and then sealed at both ends. Blood collected in this manner will preserve its agglutinative properties and remain sterile indefinitely.

Having thus obtained the serum from the whole blood, the test is carried out by adding the serum in definite dilutions to either a twenty-four-hour-old bouillon culture of the typhoid bacillus or to sterile bouillon inoculated with a typhoid culture at the time of the test.

In the first instance, a 1 : 20 dilution of serum and twenty-four-hour typhoid bouillon is made in a sterile test-tube, which is then plugged and placed in an incubator, where it remains for about twelve hours at a temperature of 37° C. At the expiration of this time a positive reaction may be recognized by the presence of dense, whitish, flaky masses (composed of large clumps of agglutinated bacteria), forming in the typical positive reaction a thick precipitate at the bottom of the test-tube, which contrasts with the perfectly clear appearance of the supernatant bouillon. If the reaction is negative, the tube shows simply the uniform cloudiness of an ordinary typhoid bouillon culture.

In the second instance a 1 : 20 dilution of serum and sterile neutral peptone bouillon is made, the mixture then being inoculated with a small loopful of typhoid bacilli derived from either a bouillon or an agar culture. The contents of the test-tube are then thoroughly mixed, and the preparation incubated at 37° C. for twenty-four hours. A positive reaction is characterized after this length of time by the formation of a similar grayish-white

precipitate at the bottom of the tube, underlying an unclouded layer of fluid. In negative reactions the typical cloudiness of the typhoid growth is diffused throughout the bouillon.

In both of these macroscopical methods control tubes of normal serum and typhoid bouillon should invariably be prepared, and incubated side by side with the specimen of serum to be tested.

Both tests may be carried out at ordinary room temperature, but more certain and more typical results are obtained when the tubes are incubated at a temperature of 37° C.

*The Test with Dead Cultures.*—Reudiger,[1] modifying Ficker's technic,[2] has devised a very satisfactory serum test with a solution of dead Eberth bacilli, made by adding 1 c.c. of formalin to 100 c.c. of typhoid bouillon. Four drops of the suspected blood are mixed with 2 c.c. of a 1 : 500 aqueous solution of formalin, and, after laking has occurred, 1 c.c. of this blood solution is mixed in a small test-tube with 4 c.c. of the dead culture. This gives approximately a 30 : 1 dilution of the blood. The test-tube is then set aside in a vertical position, with the result that, if the reaction is positive, a flocculent precipitate will appear within an hour or two, falling to the bottom of the tube as a granular sediment, with clearing of the supernatant bouillon within from twelve to twenty-four hours. By microscopical examination this sediment is found to consist of tightly clumped masses of bacilli.

In his clinic at the Jefferson Hospital the writer uses this test, in connection with the more rapid hanging-drop method with live bacilli, in all suspected enterics, and regards it as quite as accurate as the older method, although, of course, much slower. Dried blood as well as fresh may be employed, if the precaution is taken to powder it finely before adding it to the formalin solution. Reudiger obtained positive results with test cultures killed a year previously.

*The Choice of a Method.*—The choice between the methods of serum testing described above must depend largely upon the circumstances under which the test is to be made.

The dried blood method, as already remarked, is best adapted to health board necessities, where samples of blood are collected by the general practitioner and sent by mail to the laboratory. The chief objection to this method is the impossibility in many cases of making accurate dilutions, since usually it can only be *assumed* that a given crust of blood represents the same volume in its liquid state as the drop of culture with which it is diluted. If the examiner collects the specimens himself, quite accurate dilu-

[1] Jour. Infect. Dis., 1904, vol. i, p. 236.
[2] Berlin. klin. Wochenschr., 1903, vol. xl, p. 1021.

tions may be obtained if a graduated pipette or a platinum loop of fixed size is used to measure both the blood and the culture. Another drawback is the fact that more or less typical agglutination occasionally occurs with non-typhoid blood, although Johnston believes that this source of error may always be eliminated by using cultures of sufficient attenuation.

In hospital work either the fluid blood or fluid serum should be chosen, for exact dilutions may be made by these methods, and if the test is made within a reasonable length of time after the dilution, bacterial contamination need not be feared. The writer, a warm advocate of Johnston's method in the early days of serum testing, has now discarded it wherever possible in favor of the more accurate and equally simple test performed with the fluid whole blood or serum.

The macroscopical method with fluid serum is too slow, and requires too elaborate bacteriological apparatus ever to be adopted for general clinical use. Reudiger's method is obviously of great value to the practitioner who has not access to a laboratory.[1]

*Value of the Serum Test.*—Fully 95 per cent. of all typhoids give a positive reaction at some period of the disease, usually as early as the eighth day, as nearly as it is possible to compute this period. An error of about three per cent. must be allowed for, on account of the occurrence of positive or misleading results with non-typhoid blood. The statistics of the Philadelphia Bureau of Health[2] are of interest in demonstrating the usefulness of the test in routine public health work. These data, covering a period of six years (1897–1902), show that, of 22,521 tests by the dried blood method, in 19,080 cases diagnosed as enteric fever, the discrepancy between the laboratory and the clinical diagnosis ranged between 3.9 and 8.3 per cent. Rosenberger's collection[3] of 17,280 cases from other sources shows 16,352 positive results, or 94.6 per cent.

In some instances repeated negative results are found until convalescence is well established, and, rarely, cases are encountered

[1] A. J. Wolff (Amer. Jour. Med. Sci., 1903, vol. cxxv, p. 661) has devised a clever method of serum testing with the patient's blood and feces. Bouillon cultures of the patient's feces are incubated for twelve hours, mixed, in definite dilution, with his blood, and examined microscopically. If Eberth bacilli exist in the culture thus made, they become clumped and immobilized, while the motility of the colon organisms also present is unimpaired. If no reaction occurs, the patient's blood should be tested in the usual manner as a control. Wolff reports uniformly positive results in 35 cases of enteric fever with this modification of the Widal test, and claims that reactions occur from the fourth to the seventh day—in spite of the general impression that the stools in typhoid do not contain the Eberth bacillus before the tenth or eleventh day of the disease.

[2] A. C. Abbott, Annual Report of the Division of Bacteriology, Pathology, and Disinfection of the Philadelphia Bureau of Health, 1903, p. 112.

[3] Proc. Path. Soc. Phila., 1904, vol. vii, p. 97.

in which the reaction never occurs at any time during the entire course of the illness. The blood may lose its clumping powers at about the time of defervescence, or, on the other hand, this peculiarity may persist for months or even years after the attack. This last source of error in the test may be due to the presence of unsuspected foci containing typhoid bacilli, but sometimes it is apparently independent of such factors. Before pronouncing upon the value of a positive reaction in the individual case the occurrence of a previous attack of typhoid and the presence of lesions which may be due to the Eberth bacillus (osteomyelitis, cystitis, arthritis, cholecystitis, etc.) must be excluded.

The reaction may be positive on one day of the disease or for a series of days, and negative on the next day or succeeding days. Its character is apparently uninfluenced by the intensity of the infection, for although, as a rule, the reaction is usually prompt and marked in severe infections, it may be just as marked in mild cases. A certain relationship seems to exist between the height of the fever and the intensity of the reaction, for the latter is usually most decided at the period of maximum pyrexia.

*Positive Reactions in Non-typhoid Conditions.*—In a number of conditions other than enteric fever the blood may acquire a more or less decided agglutinative action toward the Eberth bacillus, but, with rare exceptions, such reactions are attributable to low dilutions, to a prolonged time limit, and, perhaps, to a previous attack of typhoid. Typhus fever, malarial fever, sepsis, pneumonia, tuberculosis, acute osteomyelitis, and influenza are among the most important diseases thus simulating a true typhoid reaction, and a positive test in any of these conditions must, to be conclusive, occur in a high dilution—1 : 50, 1 : 60, or higher. In Weil's disease positive reactions are common, even in high dilutions, and this fact tends to corroborate Weil's original suggestion, that this disease in reality is nothing more than typhoid aborted by the supervention of jaundice. Or, as Lubowski and Steinberg suggest,[1] the reaction may sometimes be due to a *Bacillus proteus* infection, since immune proteus serum may agglutinate the Eberth bacillus, in very high dilutions. The blood of patients suffering from other forms of jaundice also has a moderately strong agglutinative effect upon the typhoid bacillus, although bile itself has no such action. With proper technic, however, the blood of jaundice cases need not be a source of error, for Libman,[2] in a study of 35 such instances, failed to find in any one of them greater agglutinative effect than is often

[1] Deutsch. Arch. f. klin. Med., 1904, vol. lxxix, p 396.
[2] Med. News, 1904, vol. lxxxiv, p. 204.

seen in normal blood. Errors of technic and latent or previous typhoid infection are the probable explanation of most of the positive results with bilious blood and typhoid cultures, reported by Koenigstein, Kochler, Greenbaum, and other earlier students of this question.

In paratyphoid fever the blood serum usually does not clump the Eberth organism, although in an occasional instance it does so, even in high dilutions. In doubtful cases, therefore, the agglutination reaction with the typhoid bacillus should always be supplemented by similar tests with paratyphoid cultures, as well as by bacteriological examination of the blood for the isolation of a specific bacterium.

In the light of our present knowledge of the serum test its value appears to be less than its first enthusiastic advocates were inclined to urge. A positive reaction, obtained by a skilled worker whose technic as to dilution, time limit, and culture has been exact, is the most valuable single sign of enteric fever, although it cannot be regarded as absolutely pathognomonic. On the other hand, a negative result with the test is no proof of the absence of the disease.

HEMOGLOBIN AND ERYTHROCYTES.

Anemia develops shortly after the beginning of the fever, slowly and progressively increasing in intensity throughout the course of the disease, and persisting during the early weeks of defervescence. During the first week there is little or no decrease in the number of erythrocytes, although the hemoglobin loss appears to begin coincidentally with the first manifestations of the infection. Normal erythrocyte counts are the rule during the first seven days, but there are few cases of typhoid which fail to show a hemoglobin loss amounting to at least 15 or 20 per cent. during this period. Whether this early oligochromemia is due to the influence of the fever, or whether it represents the actual prefebrile state of the patient's blood, is difficult to decide. By the second week the corpuscular decrease becomes evident and steadily grows more and more marked as the disease progresses, reaching its maximum at about the end of defervescence. Thayer [1] distinguishes a slight accentuation of the oligocythemia between the third and fourth weeks, the decrease continuing until the seventh, when a still more decided fall occurs, followed in the eighth week by a considerable rise. A slow rise in the erythrocyte curve is observed after defervescence, and, indeed, sometimes before the end of the febrile stage, until by the end of the fourth or fifth week of convalescence the count is again

[1] Johns Hopkins Hosp. Reports, 1900, vol. viii, p. 487.

normal. The hemoglobin after the first week follows the same general course as the erythrocytes, but its decrease during the early weeks is relatively greater and its regeneration slower during the post-febrile period. These data, as well as those relating to the leucocytes, have been confirmed by W. A. Winter.[1]

Thayer's analysis [2] of the blood examinations in enteric fever, made in the Johns Hopkins Hospital during a period of eleven years, furnishes a striking illustration of the development of the anemia during the progress of the disease. Arranged according to the week of the fever, the following hemoglobin and erythrocyte averages in uncomplicated cases are shown:

HEMOGLOBIN.
(165 estimates.)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1st week, | 21 | estimates | 76.1 | per cent. | 6th week, | 6 | estimates | 62.1 | per cent. |
| 2d " | 51 | " | 72.8 | " " | 7th " | 4 | " | 50.5 | " " |
| 3d " | 34 | " | 66.2 | " " | 8th " | 3 | " | 56.9 | " " |
| 4th " | 20 | " | 60.5 | " " | 9th " | 4 | " | 47.7 | " " |
| 5th " | 20 | " | 57.8 | " " | 10th " | 2 | " | 66.5 | " " |

ERYTHROCYTES.
(265 counts.)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1st week, | 32 | counts | 4,913,312 | | 7th week, | 8 | counts | 3,309,125 | |
| 2d " | 86 | " | 4,692,428 | | 8th " | 7 | " | 3,652,285 | |
| 3d " | 59 | " | 4,429,208 | | 9th " | 6 | " | 3,509,966 | |
| 4th " | 36 | " | 4,222,236 | | 10th " | 1 | " | 3,920,000 | |
| 5th " | 22 | " | 4,118,590 | | 11th " | 1 | " | 2,109,333 | |
| 6th " | 7 | " | 4,028,428 | | | | | | |

Seventy-four typhoid patients, both with and without complications, examined in the German and the Jefferson Hospitals showed the following averages, the first estimates being taken in all cases in which multiple examinations were made:

| WEEK. | | | | HEMOGLOBIN. | ERYTHROCYTES. |
|---|---|---|---|---|---|
| 1st week, | 14 | cases | | 77.4 per cent. | 4,789,285 per c.mm. |
| 2d " | 30 | " | | 66.5 " " | 4,161,233 " " |
| 3d " | 13 | " | | 58.8 " " | 3,555,000 " " |
| 4th " | 6 | " | | 49.6 " " | 3,490,833 " " |
| 5th " | 6 | " | | 53.1 " " | 2,445,000 " " |
| 6th " | 2 | " | | 47.5 " " | 3,165,000 " " |
| 7th " | 2 | " | | 47.5 " " | 3,335,000 " " |
| 8th " | 1 | " | | 40.0 " " | 2,790,000 " " |

As a rule, the degree of a typhoid anemia is parallel to the severity of the attack, but this is not invariably true, since a mild case may be associated with a most intense anemia. In the series

[1] Dublin Jour. Med. Sci., 1901, vol. cxii p. 249.

[2] *Loc. cit.*

included in the last tabulation the most marked instances of anemia showed hemoglobin and erythrocyte estimates of 40 per cent. and 1,720,000; 40 per cent. and 1,850,000; and 50 per cent. and 1,800,000, respectively. The most striking example of oligochromemia showed a hemoglobin percentage of 20, with a corresponding erythrocyte count of 2,470,000. Considerably lower estimates than these have been reported by a number of other observers, but they are uncommon.

The effects of the cold tub and of excessive diarrhea and sweating may cause a temporary polycythemia from concentration of the blood, and these sources of high counts must be excluded in making examinations during the early weeks of the disease. In four cases examined by the writer to determine the effects of the cold plunge it was found that the average erythrocyte increase after the bath amounted to 813,000 corpuscles per c.mm., and the hemoglobin gain to 8 per cent. Hemorrhages, if severe, may cause an abrupt fall in the erythrocyte count, often succeeded by a more or less successful attempt at regeneration, in an effort to compensate for the blood loss.

*Qualitatively*, the cells show no peculiar changes, poikilocytosis, irregular staining affinities, and deformities of size occurring in relation to the intensity of the anemia. Erythroblasts are comparatively rare, being absent or few in number in the average case. Normoblasts may be found in cases with high-grade anemia and as a sequel to hemorrhage. Megaloblasts are very rare, an occasional cell of this type being observed now and then only in severe cases.

Leucocytes.

A steady, slow decrease in the number of leucocytes becomes evident after the first week of the fever, the lowest counts being found during the fifth or sixth week, after which an increase, which may be either permanent or transient and followed by a still more decided leucopenia, is observed. It appears that the latter change accompanies cases with severe post-febrile anemia, although sufficient data are lacking to justify absolutely positive conclusions on this point. In uncomplicated cases the normal count becomes reestablished by about the fourth week of convalescence. The leucopenia of typhoid corresponds in a general way to the severity of the attack, and although not marked in the average, in the individual case it may be striking, counts of from 2000 to 3000 being not at all uncommon.

Kast and Gutig,[1] in 105 cases, found the leucocytes below 7000 in 97, between 7000 and 9000 in 6, and 9000 or higher in 2

[1] Deutsch. Arch. f. klin. Med., 1904, vol. lxxx, p. 104.

cases. Contrary to the experience of others (see below), these authors found little or no leucocytosis as the effect of non-typhoid complications, which in their cases generally caused simply a polynuclear increase with no disturbance of the leucopenia.

Thayer's report of 832 counts in uncomplicated cases shows the following range of the leucocytes, according to the week of the disease:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1st week, | 119 | counts | 6442 | 8th week, | 14 | counts | 6614 |
| 2d " | 258 | " | 6251 | 9th " | 7 | " | 5057 |
| 3d " | 200 | " | 5528 | 10th " | 2 | " | 5000 |
| 4th " | 117 | " | 5431 | 11th " | 3 | " | 5333 |
| 5th " | 70 | " | 5510 | 12th " | 2 | " | 5000 |
| 6th " | 25 | " | 5690 | 13th " | 1 | " | 8000 |
| 7th " | 14 | " | 6132 | | | | |

The leucocyte estimates of the 74 hospital typhoids referred to above averaged:

| | | | | |
|---|---|---|---|---|
| 1st week, | 14 | cases | 8026 | leucocytes per c.mm. |
| 2d " | 30 | " | 6713 | " " " |
| 3d " | 13 | " | 7076 | " " " |
| 4th " | 6 | " | 4400 | " " " |
| 5th " | 6 | " | 5766 | " " " |
| 6th " | 2 | " | 6250 | " " " |
| 7th " | 2 | " | 4500 | " " " |
| 8th " | 1 | " | 8000 | " " " |

Disregarding the week of the fever, the number of leucocytes in these cases ranged as follows:

| | | | |
|---|---|---|---|
| Above | 10,000 | in | 7 cases. |
| From | 9,000–10,000 | in | 3 " |
| " | 8,000– 9,000 | " | 8 " |
| " | 7,000– 8,000 | " | 14 " |
| " | 6,000– 7,000 | " | 8 " |
| " | 5,000– 6,000 | " | 10 " |
| " | 4,000– 5,000 | " | 11 " |
| " | 3,000– 4,000 | " | 8 " |
| " | 2,000– 3,000 | " | 4 " |
| " | 1,000– 2,000 | " | 1 " |

Highest, 16,000 per c.mm.
Lowest, 1,333 " "
Average, 6,706 " "

It appears from these figures that counts in excess of 10,000 per c.mm. may be looked for in more than ten per cent of all cases, such an increase being due either to the effects of blood concentration from diarrhea, sweating, vomiting, or cold tubbing, or to some hidden or frank complication. In four of the seven relatively high counts above noted the cause was plain—croupous pneumonia in two, cholecystitis in one, and furunculosis in one.

In the other three, all of which were made in patients whose fever had not yet run seven days, the factors of the increase were undetermined; possibly it was due to physiological blood inspissation.

Inflammatory complications, such as otitis, abscess, pneumonia, severe bronchitis, peritonitis, cystitis, periostitis, and phlebitis, give rise to a prompt leucocytosis in patients whose vital powers are sufficiently strong to react against the process. Intestinal hemorrhage is usually followed by an increase reaching its maximum within twenty-four hours after the blood loss, and disappearing within a week. Intestinal perforation may promptly be followed by a leucocytosis, the increase developing within a few hours. Thayer has observed that in some instances the increase in the number of leucocytes succeeding the perforation may tend to diminish and disappear with the aggravation of the symptoms, and that not infrequently there is a complete absence of leucocytosis and sometimes a diminution in the number of leucocytes after this accident. He also considers that the prospect of relief by surgical interference is best in those cases with a leucocytosis, the absence or disappearance of this sign following a perforation being an indication of the malignancy of the infection or the prostration of the patient.

*Qualitative changes* are absent or inconspicuous during the first two weeks of the fever, but during the third week a slow, progressive decrease in the relative percentage of polynuclear neutrophiles with a consequent increase in the mononuclear ungranulated forms, begins, this change becoming most marked at about the end of defervescence. In 23 of the writer's cases the percentage of polynuclears averaged, according to the week of the disease, 75.0 per cent. for 8 cases in the first week; 70.9 per cent. for 7 in the second week; 50.2 for 4 in the third week; 60.0 per cent. for 2 in the fourth week; and 64.0 and 68.0 per cent. for a single case in the fifth and sixth weeks, respectively. Higley[1] finds that the polynuclear neutrophiles decrease much earlier in the disease, 9 of his cases examined during the first week showing an average of 59.4 per cent. for these cells.

Thayer has found that the mononuclear cells which are most markedly increased are "elements containing nuclei not much larger than those of lymphocytes, and often presenting the general appearance of a lymphocyte nucleus, with the exception of the slight affinity for coloring matters. The size of these cells is usually about that, or but little larger than that, of the ordinary polymorphonuclear neutrophile." The typical small lymphocyte and the transitional forms undergo little or no increase.

[1] Med. News, 1903, vol. lxxxiii, p. 1140.

The eosinophiles are almost invariably decreased, both absolutely and relatively, and are often absent during the active febrile stages. The relative percentages of these cells in the above cases averaged 0.87, rising as high as 5 per cent. in only 2 cases, and being entirely absent in 11.

Myelocytes in small numbers may be found in severe forms of post-typhoid anemia, but they are absent during the active period of the infection. Türk's "stimulation forms" are met with under the same conditions.

Thayer's elaborate report of the Johns Hopkins Hospital cases includes the following averages of the differential leucocyte counts:

| Week. | Small Mononuclear. | Large Mononuclear. | Polynuclear Neutrophile. | Eosinophile. |
|---|---|---|---|---|
| 1st week, 12 counts | 12.9 per cent. | 12.4 per cent. | 74.0 per cent. | 0.5 per cent. |
| 2d " 39 " | 14.6 " " | 13.4 " " | 70.9 " " | 0.8 " " |
| 3d " 34 " | 21.5 " " | 11.6 " " | 66.3 " " | 0.3 " " |
| 4th " 19 " | 20.1 " " | 14.4 " " | 65.0 " " | 0.4 " " |
| 5th " 8 " | 18.2 " " | 19.7 " " | 61.7 " " | 0.3 " " |
| 6th " 4 " | 22.6 " " | 13.5 " " | 57.7 " " | 6.0 " " |
| 7th " 1 " | 23.7 " " | 34.4 " " | 37.3 " " | 4.6 " " |
| 8th " 1 " | 24.2 " " | 16.8 " " | 56.9 " " | 2.1 " " |

According to Hayem,[1] the number of *blood plaques* is markedly decreased during the febrile period of the fever, as in any other condition characterized by pyrexia.

Diagnosis. The blood examination furnishes four clinical signs of positive value in the diagnosis of enteric fever: the serum reaction; a subnormal leucocyte count or at least an absence of leucocytosis; bacteriemia; and in cases with roseola the detection of the Eberth bacillus by spot culturing. The influence of complications upon the behavior of the leucocytes must, however, always be borne in mind.

*Acute miliary tuberculosis, cerebrospinal meningitis, malarial fever*, certain atypical cases of *pneumonia* and *influenza*, and *septicemic and pyemic processes*, such as ulcerative endocarditis, are the diseases most frequently confounded with typhoid, and in their differentiation the blood report often gives just the essential clue.

Acute miliary tuberculosis, if a pure infection, shows a similar absence of a leucocyte increase, and in excluding this disease reliance must be placed on the Widal test and upon blood culturing. Influenza is not characterized by leucocytosis, and must be differentiated from typhoid by the aid of the same methods of

[1] "Du Sang," etc. Paris, 1889.

examination. Cerebrospinal meningitis, pneumonia, and septic and pyemic conditions may be differentiated by their association with a more or less well-marked leucocytosis. In the last-named processes bacteriological examination of the blood not infrequently gives conclusive results. *Paratyphoid fever*, often clinically identical with genuine enteric fever, shows also a similar anemia and leucocyte range. As a rule, paratyphoid blood does not agglutinate cultures of the Eberth bacillus; if it does so, the reaction is less marked than with cultures of the paratyphoid organism. Blood culturing is the court of final appeal in distinguishing these two closely related infections. The differentiation of *malarial fever* is referred to under this disease. (See p. 472.)

## XXII. ERYSIPELAS.

General Features.

In severe infections Türk[1] has noted a decided increase in the quantity of *fibrin* and in the number of *blood plaques*, but in the case of average severity these changes are not to be observed. Drouin[2] has found that the *alkalinity* of the blood is greatly decreased. Negative results from *bacteriological examination* of the blood are the rule, although streptococci (Fehleisen), diplococci (Pfähler), and other pyogenic bacteria invade the blood at and near the erysipelatous lesions.

Hemoglobin and Erythrocytes.

Moderate anemia, characterized by a somewhat disproportionate hemoglobin loss, is common in the severer forms of the disease, but not in mild cases. The decreases are not notable, amounting on the average to a loss of not more than 10 or 20 per cent. of corpuscles and of about 30 per cent. of hemoglobin. Maragliano's degenerative changes of corpuscular structure have occasionally been found.

Leucocytes.

Leucocytosis of the polynuclear neutrophile type is the usual finding, but mild cases frequently run their course without provoking the slightest increase. Except in isolated instances, the leucocytosis is not high, the counts usually being about 15,000, and rarely more than 20,000, cells to the c.mm. Von Limbeck[3] and Chantemesse and Rey[4] have shown that the leucocyte and temperature curves maintain a definite parallelism in the majority of cases, and that the diminution in the leucocytosis, as a rule, anticipates the fall in temperature. There is, however, no apparent rela-

[1] *Loc. cit.*
[2] Thèse de Paris, 1892, No. 83, p. 108.
[3] *Loc. cit.*
[4] Presse méd., 1899, vol. vi, p. 316.

tionship between the height of the count and the degree of pyrexia, for moderate leucocytoses are not incompatible with strikingly high temperatures. It is generally agreed that the highest counts are found in the severest cases, provided that the patient's resisting powers are acting normally. An extension of the lesion is generally accompanied by an increase in the leucocytosis.

With the onset of convalescence, as the leucocytosis disappears, the normal percentages of the different forms of corpuscles, disturbed during the febrile period, are reëstablished by a rapid increase in the small lymphocytes and eosinophiles, and a decrease in the polynuclear neutrophiles; the percentage of large lymphocytes remains stationary, and the eosinophiles are absent during the height of the attack, according to Chantemesse and Rey. Small percentages of myelocytes and an occasional "stimulation form" are commonly found during the active stages of the leucocytosis.

## XXIII. EXOPHTHALMIC GOITER.

There are no characteristic changes in the *hemoglobin* and *erythrocytes*, although an anemia indistinguishable from typical chlorosis is not an infrequent feature. Such cases must be distinguished from so-called "thyroid chlorosis," or chlorosis with thyroid hypertrophy, by means of other clinical symptoms. In nine cases of which the writer has notes the hemoglobin averaged 78.6 per cent., and the erythrocytes 4,715,571 per c.mm. It seems likely that in cases of Graves' disease characterized by excessive diaphoresis, emesis, and diarrhea, the blood concentration thus produced may be sufficient more or less effectually to obscure the real grade of anemia existing.

The *leucocytes* are not increased in number, and leucopenia of a decided degree is frequently observed. Of the above cases, the leucocyte average was 4698 per c.mm., none of the counts exceeding 10,000. Relative lymphocytosis is a common change, and moderate increase in the percentage of eosinophiles an occasional finding.

## XXIV. FEVER.

It is a well-recognized fact that more or less *hemoglobin* and *erythrocyte* losses follow pyrexia maintained for any length of time, but an attempt to demonstrate the exact cause or group of causes of this anemia involves the analysis of a most complex problem in physiology, about which the most skilled investigators

express diametrically opposite opinions. Some maintain that sufficient actual destruction of the corpuscles occurs as the result of fever to account for their decrease in number, while others attribute the loss largely, if not wholly, to the influence of vasomotor changes. Maragliano[1] has shown that capillary contraction accompanies the period of active pyrexia, while Reinert[2] suggests that the blood is diminished in volume by the excessive drain upon the body fluids occurring at this time. In septic fevers, furthermore, additional inspissation of the blood is produced by the influence of bacteria and their products. These factors, tending to inspissate the blood, favor the production of polycythemia, which change is to be observed during the stage of active fever. But as defervescence sets in the conditions are reversed, for the capillaries then dilate, the draining away of the fluid elements of the blood ceases, and, consequently, dilution of the blood now occurs. Anemia, therefore, develops coincidentally with the disappearance of the fever. It is undetermined whether this postfebrile anemia is the result purely of these physical causes or of these causes plus a certain amount of real hematocytolysis due to high temperature. It seems reasonable to regard both factors as active.

*Coagulation* and *fibrin* behave so erratically that no definite statements regarding them are justified. In the incipient stage of septic fevers coagulation is much delayed, according to Schmidt,[3] but during the later stage it occurs more rapidly than normal. The *leucocytes* in this class of fevers are generally increased in number.

The *alkalinity* of the blood undergoes wide variations in different febrile states, but it cannot be said that these changes, which are probably due to complex chemical processes rather than to the primary effect of the fever, are constantly parallel, either to the degree of pyrexia or to the behavior of the leucocytes. Löwy and Richter[4] state that increased alkalinity occurs coincidentally with the stage of hypoleucocytosis—a statement which Strauss,[5] Löwit,[6] and others have verified. Fodor and Rigler's experiments[7] have proved that the pyrexia following infection with pathogenic bacteria ultimately effects a diminution in the alkalinity of the blood, and that this change is sometimes preceded by a dis-

[1] Berlin. klin. Wochenschr., 1887, vol. xxiv, p. 797.
[2] "Die Zählung der rothen Blutkörperchen," Leipsic, 1891.
[3] Pflüger's Arch., 1875, vol. xi, pp. 291 and 515.
[4] Deutsch. med. Wochenschr., 1895, vol. xxi, p. 526.
[5] Zeitschr. f. klin. Med., 1896, vol. xxx, p. 315.
[6] "Die Lehre v. Fieber," Jena, 1897.
[7] Centralbl. f. Bakt. u. Parasit., 1897, vol. xxi, p. 134.

tinct primary increase. The conclusions voiced by von Jaksch,[1] Krause,[2] and other earlier writers, that decreased alkalinity is a constant accompaniment of febrile processes, cannot be unreservedly accepted, if von Limbeck's[3] later statements to the contrary are to be believed.

## XXV. FILARIASIS.

Occurrence.

Filariasis, the pathological condition depending upon the presence in the body of the parental and embryonic forms of the *Filaria sanguinis hominis*, is of wide-spread distribution throughout the tropics and subtropics, being prevalent in various districts of Africa, India, Australia, China, Japan, South America, and the islands of the South Pacific and the West Indies, and, as mentioned below, having been found to a limited extent in North America.

Parasitology.

Six distinct species of embryo blood worms, the parental forms of which do not enter the circulation, have been demonstrated in the peripheral blood of man, these parasites being known by the general term *Filaria sanguinis hominis*. These different filariæ, according to the nomenclature suggested by Manson,[4] are distinguished by the names *Filaria nocturna*,[5] *Filaria diurna*, *Filaria perstans*, *Filaria demarquaii*, *Filaria ozzardi*, and *Filaria magalhāesi*. To but a single member of this group, the *Filaria nocturna*, has an undisputed pathological rôle been assigned, this parasite being regarded as the cause of various forms of ulcer, lymphangitis, lymph varices, lymph scrotum, tropical elephantiasis Arabum, endemic chyluria, chylous ascites, and other tropical diseases of more or less obscure nature. The *Filaria perstans*, Manson conjectures, may possibly be the cause of that peculiar tropical disease known as African kra-kra, or "craw-craw." Christy[6] couples this worm etiologically with the "tick fever" of Uganda, and has shown that a variety of tick (known locally as the *Bibo*) acts as its intermediary host. Bastian[7] suggests that the embryonic forms of the *Filaria perstans* are in reality embryos of a species of *Tylenchus*, which infests the roots of the banana. By eating the fruit thus contaminated it is

[1] Zeitschr. f. klin. Med., 1887, vol. xiii, p. 380.
[2] Zeitschr. f. Heilk., 1889, vol. x, p. 106.
[3] Centralbl. f. inn. Med., 1895, vol. xvi, p. 649.
[4] "Tropical Diseases," 3d ed., New York, 1903.
[5] The author is greatly indebted to Dr. F. P. Henry and to Dr. J. H. Gibbon for the opportunity of making repeated blood examinations in two cases of *Filaria nocturna* infection occurring in their respective hospital services.
[6] Thompson Yates and Johnston Lab. Rep., 1903, vol. v, p. 187.
[7] Lancet, 1904, vol. i, p. 286.

possible that some of the worms ingested may bore through the gut and reach the mesentery, where they rest and develop, and whence they later pass into the general circulation via the lymph stream. Low,[1] on the contrary, has pointed out that the distribution of the *Filaria perstans* does not always correspond to that of banana cultivation, and contends that this organism is in no way related to the genus *Tylenchus*. This investigator[2] has disproved the belief, once held, that the *Filaria perstans* is the specific cause of "sleeping sickness." The other filariæ (*diurna*, *demarquaii*, *ozzardi*, and *magalhãesi*) possess no interest from a diagnostic standpoint, since their life history and pathological significance are still obscure.[3]

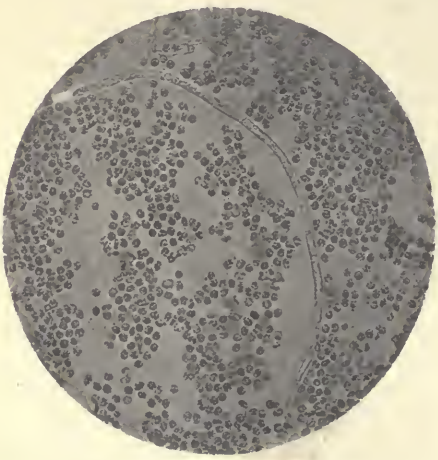


FIG. 59.—THE FILARIA NOCTURNA.
From a photomicrograph of the parasite in a fresh blood film.

THE FILARIA NOCTURNA. This is by far the most important member of the above-named class of blood worms, being the one most familiarly known of all, as well as the one of greatest clinical interest, because of the interesting pathological lesions which it is capable of exciting. In this country cases of filariasis due to the *Filaria nocturna* have been reported by a number of different observers, Guiteras,[4] de Saussure,[5] Mastin,[6] Slaughter,[7] F. P. Henry,[8] Dunn,[9] and Lothrop and Pratt[10] having met with the disease. A few of these cases have been regarded by their reporters as indigenous, but the great majority of them, it is safe to state, were directly imported from the tropics. To the writer's knowledge, at least five cases have been diagnosed in Philadelphia during the last six years.

[1] Lancet, 1904, vol. i, p. 420. [2] Brit. Med. Jour., 1903, vol. i, p. 722.
[3] For a complete description of filariasis and of the various forms of the filariæ the reader should consult Manson's text-book, above mentioned. Davidson's "Hygiene and Diseases of Warm Climates" (Edinburgh, 1893) contains an excellent account of the histological structure of filariæ.
[4] Med. News, 1886, vol. xlvii, p. 399. [5] *Ibid.*, 1890, vol. lvi, p. 704.
[6] Annals of Surg., 1888, vol. viii, p. 321. [7] Med. News, 1891, vol. ii, p. 649.
[8] *Ibid.*, 1896, vol. xviii, p. 477. [9] Trans. Coll. Phys. Phila., 1898, p. 80.
[10] Amer. Jour. Med. Sci., 1900, vol. cxx, p. 525.

As may be inferred from the name, the embryos of the *Filaria nocturna* are found in the peripheral blood most abundantly at night, the vast majority of the parasites retiring into the deeper circulation during the daytime. From late in the afternoon until about midnight they make their way into the peripheral vessels in progressively increasing numbers, with more or less fluctuation, the maximum number being found at the latter time, after which they begin to grow less and less numerous, until, by about eight o'clock in the morning, they have practically all disappeared from the superficial circulation and reëntered the deeper vessels, in which they remain until the close of the day. This peculiar periodicity is well illustrated by a recent series of investigations made by Lothrop and Pratt,[1] who have charted the phenomenon in one case, showing the approximate number of parasites to the c.mm. of blood as follows: 4 P. M., 100; 6 P. M., 275; 8 P. M., 1300; 10 P. M., 900; 12 M., 1500; 2 A. M., 700; 4 A. M., 900; 6 A. M., 125; 8 A. M., 125; and 10 A. M., 100. The highest number ever observed by these authors was 2100 embryos per c.mm., the specimen in which this count was made having been taken at midnight. This characteristic periodicity, it should also be remarked, is completely reversed if the individual harboring the parasite reverses his habits of life, sleeping during the day and moving about at night. If such should be the case, the worms will appear in the peripheral blood during the daytime, the patient's period of rest, and seek the deeper circulation at night, the patient's period of activity.

The painstaking studies of Manson[2] and Low[3] have shown that mosquitos (*Culex fatigans* and several of the genus *Anopheles*) are the intermediate hosts of this parasite, which may be found alive in the stomachs of these insects after they have fed upon a filarious individual. Ecdysis takes place in this organ, and the embryos, after having cast their sheaths, manage eventually to penetrate the thoracic muscles of their host, in which situation they undergo a developmental phase. This lasts about eighteen days, after which the larvæ thus evolved escape from the thorax and ultimately reach the insect's labium and proboscis, whence they are directly introduced in the blood of man by the bite of the infected mosquito. The parasites having been inoculated into a human being in this manner, make their way to some part of the lymphatic system, in which they lodge, sexually mature, fecundate, and beget the innumerable embryo forms which, via the lymph stream, find their way into the circulating blood.

*Appearance in Fresh Blood.*—In the unstained blood film the

[1] *Loc. cit.* [2] *Loc. cit.* [3] Brit. Med. Jour., 1902, vol. i, p. 1472.

parasite appears under the microscope as a long, slender, graceful worm possessing a most remarkable degree of activity. It measures about $\frac{1}{80}$ of an inch in length and $\frac{1}{3000}$ of an inch in diameter, and is of a pearly-gray color, with perhaps the faintest suggestion of a yellowish tone in certain lights. Its general appearance conveys to one, at first glance, the impression of a thin, transparent tube, through which a rapidly flowing stream of liquid is constantly circulating. The head (cephalic end) is gracefully rounded, while the tail (caudal end) gradually tapers for about one-sixth the entire length of the animal, and ends in a fine-pointed extremity. The worm is cylindrical in shape, of regular outline, and consists of a central body enveloped in a distinct, loosely fitting, hyaline, structureless sheath, which is about as much too large for the body as the thumb of an adult's glove would be for the little finger of a child. Thus, that part of the sheath temporarily unoccupied by the body is prone to collapse, folding upon itself and trailing after the worm at either or both extremities as a twisted, whip-like ribbon. The greater part of the body appears to be of a homogeneous structure when examined in the freshly prepared slide, but after the specimen has been kept for several hours, coarse granulations begin to stipple its surface, first developing in the center and gradually spreading toward the periphery. (See Fig. 60.) A series of fine striations, like the milling on a coin, may be observed running along both edges of the body at right angles to its long axis. A viscus, appearing as a mass of granular material, occupies a part of the central third of the worm's body, running parallel to its long axis. Upon careful examination with an oil-immersion objective rhythmical dimpling or puckering movements may generally be observed at the tip of the cephalic end of the embryo; these movements, which occur with more or less regularity at the rate of from twenty-five to forty times a minute, have been attributed to the act of respiration. As the

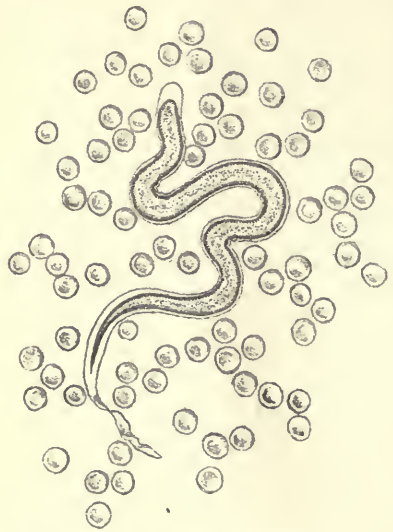


FIG. 60.—FILARIA NOCTURNA.
Showing beginning granular degeneration of the body of the parasite in a fresh blood film.

wriggling of the worm becomes less active close observation will show that these pouting movements are caused by the alternate covering and uncovering of the cephalic end by a delicate, six-lipped prepuce. The sudden projection and the equally rapid retraction of a filamentous fang or tongue-like organ from the worm's uncovered head may also be noted in some instances, but this characteristic is so difficult to make out that it may usually be looked for in vain. At a point about one-fifth of the entire length of the worm posterior to the head it is possible to make out a triangular, slightly luminous patch, shaped like the letter V, this spot being known as the V-shaped patch, regarded by Manson as a rudimentary generative organ. A second spot, somewhat similar to it in appearance but smaller in size, may occasionally be seen at a point just above the tail of the parasite; this spot Manson is inclined to regard as the rudimentary anus.

The movements of the worm are rapid and violent in the extreme; so much that they are followed with difficulty with any but a low-power dry objective. The parasite is never at rest: one moment it may be curled up into a tight bunch, like a coil of rope; the next moment it may suddenly straighten out and become rigid for an instant, only to resume its incessant contortions and twistings, which throw it into every conceivable shape. If particular attention is paid to the point, it will be noticed that, however rapid and complicated may be its movements, the parasite is never seen to turn completely over laterally. The accompanying series of sketches of the *Filaria nocturna* in a fresh blood slide illustrate a few of the different forms which this parasite may assume (Fig. 61). The worm seems to move about among the blood corpuscles with graceful and quick undulations of its body and abrupt whip-like strokes of its tail, butting its head against the more resisting masses of cells or else seeking a less difficult passage around them, always in motion, but never, it appears, with any definite aim to its exertions. Contrary to the views expressed by most observers, that the movements of the *Filaria nocturna* are not truly propulsive in character, the writer has repeatedly noticed that this worm sometimes travels several times the distance of the diameter of the microscope field ($\frac{1}{6}$-inch objective, one-inch ocular, and 160 mm. tube-length), although in most cases its excursions were limited to a measured area not exceeding half a dozen square microns. It cannot be denied that these apparently progressive movements of the worm may possibly be due to the currents in the blood plasma, but they certainly seem to have every characteristic of a true locomotive force. After the slide has been kept for a few hours, the movements

of the worm, at first so confusingly rapid, gradually become slower and slower, and these torpid, more deliberate turnings and twistings may be accurately followed under an immersion-lens. If the parasite happens to become confined in a little pool of plasma surrounded by rouleaux of half-dried erythrocytes, an accident which often happens when the drying of the film has spread inward some little distance from the edges of the cover-glass, its finer structure and characteristics may be studied with great ease and accuracy.

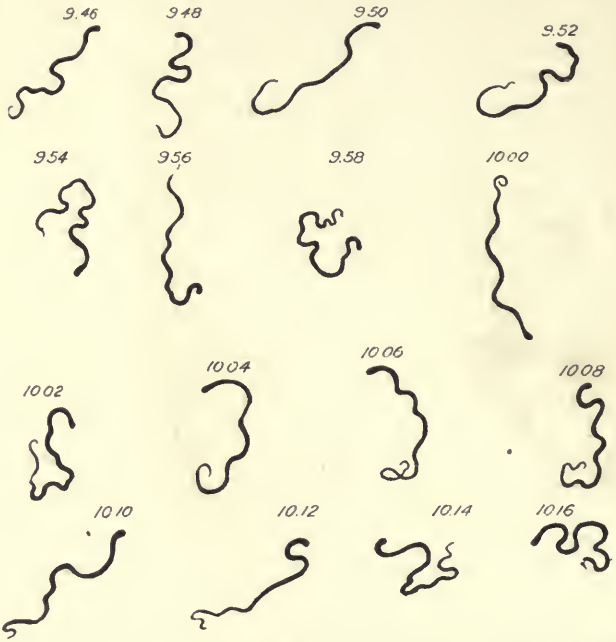



FIG. 61.—SHOWING THE CHANGES IN THE SHAPE OF THE FILARIA NOCTURNA DURING THE PERIOD OF HALF AN HOUR.
The sketches, made at two-minute intervals, all represent the same parasite.

The phenomenon of ecdysis, or shedding of the worm's sheath, with the consequent escape of its naked body into the plasma, occurs when slides containing the live filariæ are kept for some hours in a cold (not freezing) place. It commonly happens that just before the death of the parasite an occasional erythrocyte or leucocyte becomes tightly adherent to the sheath, swinging to and fro with the now lazy, torpid movements of the animal.

*Technic of Examination.*—A rather large drop of finger blood, taken from the patient late in the evening, preferably

toward midnight, is placed between a slightly warmed slide and cover-glass, the edges of which are immediately sealed with cedar oil or with vaselin. The parasite should be searched for with a low-power dry objective, a ⅔-inch lens being most useful for this purpose, and the attention of the examiner directed especially to portions of the field which may show any unnatural agitation of the blood cells. In specimens prepared in this manner the filariæ will usually remain active for several days, generally for at least forty-eight hours, and sometimes for a longer period, as in Henry's[1] experience, this author having kept them alive for ten days in a cold room.

*Staining the Filariæ.*—Films fixed for fifteen minutes in equal parts of absolute alcohol and ether and stained with thionin give the clearest-cut pictures, the multitude of small nuclei which crowd the body of the filariæ being sharply differentiated by the use of this dye. Fixation by heat or by formalin cannot be employed without risk of injuring the finer structure of the embryo. Fair results may also be obtained by staining with methylene-blue or with Jenner's or Wright's stain, but the definition is not nearly so satisfactory with these solutions as it is with thionin. The technic suggested by Manson[2] (washing out the hemoglobin of the erythrocytes with water, drying, fixing in alcohol, and staining with methylene-blue or with hematoxylin) has proved unreliable in the writer's hands. The same comment may be made regarding attempts to demonstrate the structure of the worm by staining with fuchsin, as has also been recommended.

HEMOGLOBIN AND ERYTHROCYTES.

The presence in the circulation of the *Filaria nocturna* does not appear of itself to be a factor of any conspicuous changes in the erythrocytes and their hemoglobin content. The high-grade anemia sometimes associated with filariasis, mentioned by the Bancrofts[3] and by Ehrlich and Lazarus,[4] is due, no doubt, to such complications as hematuria, severe chyluria, and chronic diarrhea. In two cases the writer found hemoglobin percentages of 85 and 88, and erythrocyte counts of 4,200,000 and 4,876,000 per c.mm., respectively. These figures may be taken as representative for the average case, judging from the limited data available.

Neither structural changes, nor irregular staining affinities of the cells and the occurrence of nucleated erythrocytes have been reported in connection with the disease.

[1] *Loc. cit.*
[2] *Loc. cit.*
[3] Australasian Med. Gaz., 1894, vol. xiii, p. 6.
[4] *Loc. cit*

LEUCOCYTES.

In the early stages of filariasis a distinct leucocytosis is generally found, but as the disease progresses the number of leucocytes gradually diminishes, and in cases of long standing the count does not exceed the physiological limits of health, except as the result of some complication. Thus, in the first case quoted above the leucocyte count was found to be 41,000 per c.mm., but this increase was regarded purely as a post-operative rise, the patient having been operated upon for a supposed varicocele less than twenty-four hours before the blood examination was made. The count in the second case, one of several years' duration, was 8000.

The relative percentage of mononuclear non-granular leucocytes is somewhat higher than normal, with a consequent decrease in the proportion of polynuclear neutrophiles. The eosinophiles either remain at a maximum normal percentage or may be distinctly in excess of this figure. This statement, made by the writer in 1901, has since been verified by several observers, notably by Calvert,[1] Gulland,[2] Coles,[3] Vaquez,[4] Sicard,[5] and Clerc.[6] Calvert has shown that the eosinophilia, like the leucocytosis, diminishes as the infection becomes chronic, and that its development is cyclical, in that it follows by a few hours the periodicity of the embryo worms in the peripheral blood. In the author's two cases the percentage of eosinophiles ranged from 3.4 to 9.5; in Gulland's case, from 3.0 to 12.0; in Calvert's three cases, from 6.0 to 22.0; and in Coles' two cases, from 15.0 to 17.0. The three French authors mentioned above report eosinophilia varying from 7.5 to 12 per cent. It may be noted here that the *Filaria loa*, found in the subconjunctival tissues, may also excite eosinophilia—53 per cent. in a case reported by Wuntz and Clerc[7]; and that in patients harboring the *guinea-worm* a similar eosinophile increase develops—as high as 36.6 per cent. in cases studied by Balfour.[8] In six cases of guinea-worm infection Powell[9] found eosinophile percentages of 4.7, 5.5, 7.5, 7.5, 8, and 12.2, respectively. The lymphocytes frequently appear as cells having a deeply stained eccentric nucleus surrounded by an abnormally large area of protoplasm, the general appearance of these cells being similar to those in the illustration shown on page 320, Fig. 55. Typical coarsely granular mast cells may be found in small numbers or they may be entirely absent, as may also be the finely gran-

[1] Johns Hopkins Hosp. Bull., 1902, vol. xiii, pp. 23 and 133; also Jour. Amer. Med. Assoc., 1902, vol. xxxix, p. 1523.
[2] Brit. Med. Jour., 1902, vol. i, p. 831.
[3] *Ibid.*, 1902, vol. i, p. 1137.
[4] Sem. méd., 1902, vol. xxii, p. 418.
[5] *Loc. cit.*
[6] *Loc. cit.*
[7] Sem. méd., 1903, vol. xxiii, p. 420.
[8] Lancet, 1903, vol. ii, p. 1649.
[9] Brit. Med. Jour., 1904, vol. i, p. 73.

ular forms of basophiles. The presence of myelocytes has not been noted.

DIAGNOSIS.

The detection of the *Filaria nocturna* in the blood serves at once to differentiate *idiopathic* from *parasitic chyluria*, *hydrocele* from *lymph scrotum*, *hernia* and other *tumors of the groin* from *parasitic inguinal varicosities* (Bancroft's "helminthoma elastica"), and *filarial orchitis* from other *inflammatory conditions of the testes*. *Non-parasitic lymphedema*, affecting, for example, the legs, can but rarely be distinguished by the blood findings from true *elephantiasis Arabum*, since in the latter disease it is exceptional to find filariæ in the general circulation.

## XXVI. FRACTURES.

Blake, Hubbard, and Cabot[1] conclude, from a study of 38 cases, that in simple uncomplicated fractures the number of *leucocytes* is seldom increased to any extent, a statement which applies also to complicated fractures in the great majority of instances. Of 23 simple fractures examined by these authors, in but 10 was the count higher than 10,500 per c.mm., and of these, only 6 exceeded 12,000. The highest estimate was 15,400, in a fracture of the pelvis, and the next highest, 14,800, in a broken leg. Of 15 complicated fractures, but 2 showed any decided increase in the number of leucocytes, namely, a fracture of the tibia and fibula, with symptoms suggestive of fat embolism, in which the count was 15,600; and a case of fractured ribs with injury of the lung, in which the leucocytes numbered 14,900 two days after the accident. An estimate of 5400 cells was made in a compound fracture of the leg two hours after the accident.

*Lipemia* is occasionally met with in fractures of the long bones involving injury of the fatty marrow.

## XXVII. GASTRITIS.

HEMOGLOBIN AND ERYTHROCYTES.

In the *acute form* there is no deviation from normal in the number and hemoglobin value of the erythrocytes, except in the event of hyperemesis, which, through concentration of the blood, may cause a transient polycythemia. In the *chronic form* secondary anemia frequently develops, and occasionally reaches an extreme grade, should the gastric lesion be suffi-

[1] Annals of Surg., 1901, vol. xxxiv, p. 361.

cient to interfere radically with the digestion and absorption of food. In instances of this sort the quantitative changes may simulate those of true pernicious anemia, but the qualitative changes typical of this disease are invariably wanting. In passing, it seems pertinent to recall the reputed etiological relationship, distinguished by some authorities, between gastric tubule atrophy and pernicious anemia. Well-defined secondary anemia (without erythroblasts) was found by Einhorn[1] in but 4 of 15 cases of gastric achylia, but in none were the qualitative blood changes of pernicious anemia detected. In cases associated with gastrectasis and hyperacidity, blood inspissation from emesis is a common change.

A synopsis of J. A. Lichty's studies[2] of the hemoglobin and erythrocytes in 98 cases of various gastric disorders shows the following average values:

| Condition. | Number of Cases. | Hemoglobin Percentage. | Erythrocytes per c.mm. |
|---|---|---|---|
| Hyperchlorhydria .. | 39 | 90.9 | 5,556,000 |
| Hypochlorhydria ... | 13 | 83.5 | 5,431,000 |
| Gastric achylia .... | 6 | 92.1 | 5,680,000 |
| Gastric dilatation .. | 11 | 85.6 | 5,623,000 |
| Gastric neurasthenia | 13 | 87.2 | 5,274,000 |
| Chronic gastritis.... | 14 | 91.0 | 5,498,000 |

From other investigations, Lichty also determined that in the above-named diseases there is no definite relationship between the condition of the blood, the urine, and the gastric contents.

In *gastroptosis* the blood remains normal in the great majority of patients; in small proportion either a mild secondary anemia or a chlorotic blood picture develops. Francine[3] found the latter change in 3 of 100 cases. Of 55 patients studied by Steele and Francine,[4] the color index averaged 0.95 in 24, 0.9 in 20, and fell as low as 0.50 in but a single instance. The theory of Meinert,[5] that chlorosis is a factor of gastroptosis, has no foundation in fact.

Leucocytes.

In *acute gastritis* leucocytosis of the polynuclear neutrophile type is common, although not constant; the increase is most notable in the severest cases, but even in these the count seldom exceeds 15,000 or 20,000. Hyperinosis also usually exists. In *chronic cases*

[1] Med. Rec., 1903, vol. lxiii, p. 321. [2] Phila. Med. Jour., 1899, vol. iii, p. 326.
[3] Proc. Phila. Co. Med. Soc., 1902, vol. xxiii, p. 447.
[4] Jour. Amer. Med. Assoc., 1902, vol. xxxix, p. 1173.
[5] "Zur Aetiologie der Chlorose," Wiesbaden, 1894.

an absence of leucocytosis is the rule, while leucopenia, resulting from defective absorption, is an occasional finding. Relative lymphocytosis is commonly associated with leucopenia and sometimes with normal leucocyte counts. In a small proportion of cases digestion leucocytosis is either delayed or absent.

DIAGNOSIS.

The presence of a leucocytosis is a valuable sign in ruling out *enteric fever*, should the diagnosis lie between this disease and acute febrile gastritis. This sign, however, cannot be employed to differentiate other acute infections, such, for instance, as *appendicitis*.

The blood furnishes no sure means of differentiating chronic gastritis from *gastric cancer*, although a persistent leucocytosis is very suggestive of the latter; unfortunately, digestion leucocytosis is neither constantly absent in cancer nor invariably present in gastritis.

Certain cases of chronic gastric catarrh, with atrophy of the stomach tubules, in course of time develop a clinical picture very like that of true *pernicious anemia*, since they present not only a similar cachexia, but also a very striking diminution in hemoglobin and erythrocytes. But pernicious anemia is characterized by the presence of nucleated erythrocytes the majority of which are megaloblasts, while in the secondary anemia of gastric catarrh erythroblasts are uncommon, and if present, show a predominance of cells of the normoblastic type.

## XXVIII. GASTRIC ULCER.

HEMOGLOBIN AND ERYTHROCYTES.

The average case shows a loss of approximately 40 per cent. of hemoglobin and of 1,250,000 erythrocytes to the c.mm., and, owing to this prevalence of a disproportionately large oligochromemia, low color indices are the rule. The individual case may show a much greater degree of anemia, but no matter how marked the cellular decrease, it is always far outstripped by the diminution in the percentage of hemoglobin. In fact, in some instances the latter alone is subnormal, the blood condition of chlorosis being thus faithfully counterfeited. The average index for the cases tabulated below was 0.75.

Profuse hemorrhage may provoke a very marked anemia, while protracted emesis tends to concentrate the blood, thus masking its real condition.

The several degenerative changes affecting the erythrocytes common to any severe anemia may be present if the blood de-

terioration is sufficiently profound. After a severe hemorrhage a few normoblasts not infrequently appear in the blood temporarily, and an occasional cell of this type may be found at other times in cases with marked cachexia.

The following summary illustrates the hemoglobin and erythrocyte ranges in 33 cases:

| Hemoglobin Percentage. | Number of Cases. | Erythrocytes per c.mm. | Number of Cases. |
|---|---|---|---|
| From 80–90 | 5 | Above 5,000,0000 | 2 |
| " 70–80 | 5 | From 4,000,000–5,000,000 | 16 |
| " 60–70 | 7 | " 3,000,000–4,000,000 | 7 |
| " 50–60 | 1 | " 2,000,000–3,000,000 | 7 |
| " 40–50 | 6 | " 1,000,000–2,000,000 | 1 |
| " 30–40 | 5 | | |
| " 20–30 | 4 | | |
| Average, 57 per cent. | | Average, 3,798,000 per c.mm. | |
| Maximum, 89 " " | | Maximum, 5,200,000 " " | |
| Minimum, 20 " " | | Minimum, 1,090,000 " " | |

Futcher[1] gives the following data of 82 cases: the hemoglobin percentage in 42 cases averaged 58, ranging from 12 to 105; the erythrocyte count in 44 cases averaged 4,071,000, or from 1,012,000 to 4,071,000; and the leucocyte count in 45 cases averaged 7500, varying from 1100 to 40,000.

Greenough and Joslin[2] report hemoglobin estimates in 73 cases, of which 34 were below 50 per cent. and 64 below 80 per cent. Of their 43 erythrocyte counts, 24 were below 4,000,000 per c.mm., the color index for this series averaging 0.67, and ranging from 0.35 to 1.41.

Leucocytes. Absence of leucocytosis is the rule, for an increase occurs only after taking food or in the event of some complication, such as hemorrhage or perforation. But the fact must be recalled that hemorrhage by no means invariably raises the count; for example, hematemesis is a symptom in fully 50 per cent. of patients suffering from ulcer of the stomach, yet in not more than 30 per cent. of all cases, both those with and those without this symptom, does the number of leucocytes exceed 10,000 to the c.mm. Perforation always excites leucocytosis, except when the patient is overwhelmingly toxic.

[1] Amer. Med., 1904, vol. viii, p. 53.
[2] Amer. Jour. Med. Sci., 1899, vol. cxviii, p. 167.

The behavior of the leucocytes in the above-mentioned series of cases may be expressed thus:

| Leucocytes per c.mm. | Number of Cases. |
|---|---|
| Above 20,000 | 2 |
| From 15,000–20,000 | 2 |
| " 10,000–15,000 | 6 |
| " 5,000–10,000 | 18 |
| Below 5,000 | 5 |

Average, 8,778 per c.mm.
Maximum, 29,400 " "
Minimum, 2,400 " "

In cases with leucocytosis the increase affects chiefly the polynuclear neutrophiles; in those without leucocytosis minimum normal or distinctly subnormal percentages of these cells are not uncommon, and a total absence of eosinophiles is the general rule, these changes being counterbalanced by a proportionate increase in the small lymphocytes.

Diagnosis. Hematology gives no aid in distinguishing gastric ulcer from *gastralgia*, *duodenal ulcer*, and simple *gall-stone colic*, in all of which leucocytosis is absent. A well-defined leucocytosis points to *acute gastritis* rather than to ulcer. The differences in the blood pictures of gastric ulcer and *cancer* are referred to under the latter disease. (See "Malignant Disease.")

## XXIX. GLANDERS.

Data are wanting regarding the condition of the *hemoglobin* and *erythrocytes* in human glanders, but it is known that *leucocytosis* is the rule. The *Bacillus mallei* has been obtained by antemortem blood culturing and by Duval[1] and by von Jaksch.[2] Heanley[3] claims that, with a dilution of 1 : 2500 and a time limit of twelve hours, a specific *serum reaction* occurs with the bacillus of glanders and glanders blood serum. In lower dilutions similar results may be obtained with the serum of patients suffering from variola and scarlatina.

[1] Arch. de méd. exper., 1896, vol. viii, p. 361.
[2] "Clinical Diagnosis," 4th ed., London, 1899.
[3] Lancet, 1904, vol. i, p. 364.

## XXX. GONORRHEA.

The *hemoglobin* and *erythrocytes* are unaltered, but the acute febrile stage of specific urethritis is usually accompanied by a moderate *polynuclear leucocytosis*, which, in the event of any of the inflammatory complications of clap, may be much aggravated. Sabrazés[1] found that the increase usually does not exceed double the mean average normal count. Giorgi,[2] contrary to general opinion, found an absence of leucocytosis, sometimes leucopenia, the rule, together with a relative mononucleosis, at the expense of the polynuclear neutrophiles. Some authors formerly claimed that circulatory *eosinophilia* was a feature of this disease, but the investigations of Vorbach[3] have shown that such a change, while occurring sometimes, is by no means constant; he found in 20 cases that the percentage of eosinophiles ranged from as low as 0.05 to as high as 11.5. Bettmann[4] believes that eosinophilia is especially frequent in posterior urethritis, an observation which thus far is unique.

In gonorrheal endocarditis and in gonorrheal arthritis the gonococcus has been repeatedly cultured from the blood during life. In distinguishing gonorrheal arthritis from rheumatic fever a positive iodin reaction is suggestive of the former.

## XXXI. GOUT.

Garrod's[5] earlier teachings regarding the lowered *alkalinity* of the blood in acute gout have been contradicted, apparently with ample proof, by the later researches of Levy,[6] who failed to find a diminution in any of the 17 cases which he investigated by the most approved methods. Still more recently Levy's conclusions have been corroborated by Watson.[7] During an acute gouty seizure *hyperinosis* is an almost invariable finding.

It is questionable whether or not the amount of *uric acid* in the blood is greater during the acute stages than in the interval between them, but it is nevertheless a fact that in many gouty persons uric acid crystals can be demonstrated in the blood by the "thread-test"—a reaction by no means peculiar to this disease, as already pointed out. (See p. 144.)

[1] Sem. méd., 1902, vol. xxii, p. 435.
[2] *Ibid.*, 1904, vol. xxiv, p. 39.
[3] Inaug. Dissert., Wurzburg, 1895.
[4] Arch. f. Dermat. u. Syph., 1899, vol. xxxix, p. 227.
[5] "Gout and Rheumatic Gout," London, 1876, p. 80.
[6] Zeitschr. f. klin. Med., 1898, vol. xxxvi, p. 336.
[7] Brit. Med. Jour., 1900, vol. i, p. 10.

The *cellular elements* show no characteristic alterations, and are normal, except in long-standing cases, in which an ordinary secondary anemia may develop in the course of time. During the acute attack a moderate increase in the number of leucocytes, affecting chiefly the polynuclear neutrophiles, may or may not be found. A relative increase in the eosinophiles is also sometimes encountered, in cases both with and without an increase in the leucocyte count. In one case of the writer's the blood examined during the height of a severe paroxysm showed 100 per cent. of hemoglobin, 7,125,000 erythrocytes, and 14,000 leucocytes per c.mm., the only peculiar differential change being the presence of myelocytes in the proportion of 0.4 per cent. The occurrence of these cells in gout has also been mentioned by Watson,[1] who found them in small numbers both during and between the acute seizures.

The same observer also states that he found (apparently in increased numbers) cells resembling blood plaques as large as 4 $\mu$ in diameter, often forming "very irregular, torn-looking masses."

The worthlessness of Neusser's so-called perinuclear basophilic granules as a diagnostic sign of gout has been alluded to in a previous section (p. 228).

## XXXII. HEMORRHAGIC DISEASES.

GENERAL FEATURES.

From a hematological standpoint *scurvy*, *hemophilia*, and the various forms of *purpura* may conveniently be considered together, since the blood changes in all of these conditions are similar, and in none are characteristic.

The *specific gravity* of the blood varies with the degree of anemia present, but only in exceptional instances does it fall to an excessively low figure. Aiello[2] estimated it as low as 1.043 in a case of purpura hæmorrhagica in which the erythrocyte loss ranged between 50 and 60 per cent. The same investigator also detected, by spectroscopical examination, methemoglobin in the blood in this form of purpura, which he attributes directly to autointoxication from the absorption of the products of decomposition occurring within the intestinal canal. Immerman[3] believed that in the late stages of hemophilia an increase in the total quantity of the blood, or a true plethora, exists, but this view is not entertained at the present time.

Various *bacteria*, especially streptococci, staphylococci, and

[1] *Loc. cit.* [2] Rif. med., 1894, vol. ii, p. 103.
[3] Ziemssen's Handb. spec. Pathol. u. Ther., 1879, vol. xiii, p. 2.

bacilli, have been found in the circulating blood by a number of observers, both in scurvy and in those forms of purpura due to infectious diseases. No special clinical significance, however, can be attached to these findings. The specific properties claimed by Letzerich[1] for his *Bacillus purpuræ* are not generally credited.

The *alkalinity* of the blood, according to the studies of Cantani[2] and others, is generally decreased in the hemorrhagic diatheses, although more recent investigators have disputed this fact, having found it higher than normal. Wright,[3] judging from his studies of the blood alkalinity in 7 cases of scurvy, believes the disease to be a condition of acid intoxication. He found in 3 of these cases that the alkalinity corresponded to the figure N. 100, and to N. 200, N. 150, N. 110, and N. 80, respectively, in the remaining 4. As determined by this author's method, the alkalinity of normal blood is expressed by the formula N. 35, which means, in other words, that the degree of alkalinity is such that a mixture of one volume of a thirty-five-fold diluted normal acid with an equal volume of blood serum is just sufficient to prevent the latter from reacting with sensitive blue litmus-paper. Opposed to Wright's views are the results obtained by Lamb,[4] who, in a study of 11 cases of scurvy, found no diminution in the alkalinity of the blood. His cases were investigated by Wright's method, and showed alkalinity values ranging between N. 30 and N. 35.

The *coagulation* of the fresh blood drop is, as a rule, slow, and sometimes incomplete, these characteristics being observed with especial frequency in hemophilics. In such subjects Wright[5] determined that clotting may fail to occur until after the lapse of over an hour after the withdrawal of the blood from the vessels, while in other instances the coagulation time ranged from nine to fourteen minutes. In 8 cases of scurvy Lamb[6] found that the coagulation time ranged from one and one-quarter to four minutes, and averaged about three and one-half. An average clotting time of four and one-third minutes was found by Hutchinson in 5 cases of infantile scurvy.[7] Of 5 cases of purpura studied by Sicard,[8] but one showed clot retraction with exudation of serum. Grawitz[9] has called attention to the fact that in cases with long-continued hemorrhage the clotting may be abnormally rapid, as is the case with normal blood after this accident. This, however, must be an exception to the general rule, for, unlike

[1] Zeitschr. f. klin. Med., 1890, vol. xviii, p. 517.
[2] "Spec. Pathol. u. Ther. der Stoffwechselkrankh.," Leipsic, 1884.
[3] Lancet, 1900, vol. ii, p. 1556.
[4] *Ibid.*, 1902, vol. i, p. 10.
[5] Brit. Med. Jour., 1893, vol. i, p. 223.
[6] *Loc. cit.*
[7] Lancet, 1904, vol. i, p. 1261.
[8] Amer. Jour. Med. Sci., 1899, vol. cxviii, p. 466.
[9] *Loc. cit.*

normal blood, that of hemophilics generally clots more and more imperfectly as the amount of the hemorrhage increases. This is also true of Werlhoff's purpura, according to Roncagliolo.[1]

HEMOGLOBIN AND ERYTHROCYTES.

There are no characteristic changes affecting the erythrocytes and hemoglobin, the blood picture being that of secondary anemia of variable intensity. In the majority of well-marked cases the erythrocytes do not suffer a loss of more than 1,000,000 or 2,000,000 to the c.mm., but the hemoglobin tends toward a proportionately greater decrease, making a low color index the rule. This is particularly noticeable in scurvy, in which condition the hemoglobin loss is often twice as great as that of the cells; in fact, some cases show simply oligochromemia, with a normal number of erythrocytes. In seven cases of infantile scurvy examined by the writer the hemoglobin percentage ranged between 35 and 65, averaging 43.8, and the erythrocyte count between 2,950,000 and 5,100,000 per c.mm., the average being 3,527,071. In three of these cases, with counts of 5,100,000, 4,900,000, and 4,814,000, respectively, the corresponding hemoglobin estimates were 52, 50, and 65 per cent. In severe cases, for example, of scurvy and purpura hæmorrhagica the count may fall to less than 1,000,000 and the hemoglobin to 20 per cent. or lower, these changes being accompanied by all the qualitative alterations typical of a profound secondary anemia which sooner or later may prove fatal. Muir[2] reports a case of purpura in which the hemoglobin was only 11 per cent., and the erythrocytes 800,000 per c.mm. Still more remarkable is the anemia reported by Talley[3] in a case of scurvy—17 per cent. of hemoglobin and 370,000 erythrocytes per c.mm. In mild cases the blood may be absolutely normal in every respect. Regeneration is rapid in cases which pursue a favorable course. It is well known that hemophilics appear to be less susceptible to the ill effects of hemorrhage than other individuals, and that in this condition recovery from blood losses is usually rapid and uneventful, in spite of their number, extent, and chronicity.

LEUCOCYTES.

The leucocytes are usually increased both in purpura and in scurvy, but in hemophilia a decided leucopenia may develop in spite of the existing hemorrhages. The increase is typically polynuclear in most instances, although a relative excess of lymphocytes may occur. Stengel[4] found this change most striking in two cases of

[1] Sem. méd., 1903, vol. xxiii, p. 368. [2] Brit. Med. Jour., 1900, vol. ii, p. 909.
[3] Jour. Amer. Med. Assoc., 1902, vol. xxxix, p. 1086.
[4] "Twentieth Century Practice of Medicine," New York 1896, vol. vii, p. 485.

purpura hæmorrhagica, and the writer has noticed an exaggeration of the lymphocytic tendency of children's blood in a number of cases of infantile scurvy. In 4 of the 7 cases of this condition referred to in the preceding paragraph, the total percentage of lymphocytes was between 60 and 66; in 3 the percentage of polynuclear neutrophiles was from 27 to 35; the eosinophiles averaged a low normal figure, and in all but a single case myelocytes were found, ranging in percentage from a minimum of 1 to a maximum of 6, and averaging 2.5 per cent. The actual number of leucocytes varied between 8000 and 25,000, and averaged 15,557 per c.mm., all but a single case having a decided increase. Denys[1] has called attention to the presence of large numbers of leucocytes in the different stages of degeneration, both in scurvy and in the infectious form of purpura.

Blood Plaques.

In all the hemorrhagic conditions above mentioned the blood plaques are usually much diminished in number and sometimes are absent. Especially is this the case in grave forms of scurvy and of purpura hæmorrhagica. Hayem[2] believes that a marked diminution in the number of plaques plus a deficiency in clotting is a pathognomonic sign of the latter disease. Lenoble[3] considers that in all cases of true purpura the plaques are increased in size but diminished in number, and that (as in malarial fever) they lose their characteristic viscidity and consequently their racemose grouping. These peculiarities, plus the presence of normoblasts and the failure of the blood clot to retract, this author believes to be the specific hematological formula of this disease, whatever may be its clinical variety.

## XXXIII. HEPATIC CIRRHOSIS.

Hemoglobin and Erythrocytes.

In the early stages of *atrophic cirrhosis*, so long as the patient's general health is maintained, the blood remains practically normal, or shows, perhaps, only a moderate diminution in hemoglobin. But as the disease progresses and the patient suffers from gastro-intestinal catarrh, hemorrhages, and circulatory embarrassment, an ordinary secondary anemia sooner or later becomes apparent, the intensity of this change depending upon the severity of the primary disease and its associated lesions. Most advanced cases show a loss of from 2,000,000 to 3,000,000

[1] Centralbl. f. allg. Pathol. u. path. Anat., 1893, vol. iv, p. 174.
[2] Compt. rend. l'Acad. sc., Paris, 1896, vol. cxxiii, p. 894.
[3] Sem. méd., 1902, vol. xxii, p. 355.

cells to the c.mm., and a few, an even greater oligocythemia. Hemorrhages, either repeated and small or single and profuse, constitute the factor of a profound anemia in many instances. The average case of Lænnec's cirrhosis loses about 40 per cent. of hemoglobin and 30 per cent. of erythrocytes, while in the individual case the count may fall to between 1,500,000 and 2,000,000. The color index, as a rule, is moderately reduced; it averaged 0.86 for a series of 40 well-advanced cases examined at the German Hospital, a synopsis of which shows these hemoglobin and erythrocyte values:

| Hemoglobin Percentage. | Number of Cases. | Erythrocytes per c.mm. | Number of Cases. |
|---|---|---|---|
| From 90–100 | 1 | From 4,000,000–5,000,000 | 10 |
| " 80–90 | 6 | " 3,000,000–4,000,000 | 18 |
| " 70–80 | 8 | " 2,000,000–3,000,000 | 10 |
| " 60–70 | 5 | " 1,000,000–2,000,000 | 2 |
| " 50–60 | 8 | | |
| " 40–50 | 7 | | |
| " 30–40 | 5 | | |
| Average, 60.4 per cent. | | Average, 3,526,825 per c.mm. | |
| Maximum, 98.0 " " | | Maximum, 4,970,000 " " | |
| Minimum, 30.0 " " | | Minimum, 1,800,000 " " | |

The effects of *ascites* upon the blood are probably twofold and diametrically opposed. Primarily it is thought to cause more or less anemia by reason of the steady drain exerted upon the albumin of the blood, but this deterioration may be effectually masked by a polycythemia due either to peripheral stasis or to inspissation of the blood caused by the rapid transudation of liquids from the vessels. This last factor is no doubt the cause of the polycythemia noted by von Limbeck[1] in cases after paracentesis. On the other hand, Grawitz[2] has demonstrated that a decrease in the hemoglobin and erythrocyte values may follow this operation, in cases in which the presence of a large ascitic exudate interferes with the circulation sufficiently to produce capillary stagnation and a consequent polycythemia, which the tapping dispels.

Anemia is apparently more striking and more common in *hypertrophic cirrhosis* than in ordinary gin-liver. Judging from a rather limited experience in 16 cases, the writer finds a greater tendency toward corpuscular than toward hemoglobin loss, and consequently toward higher color indices. The index for

[1] *Loc. cit.* [2] *Loc. cit.*

these cases averaged 0.91, and in two it reached the figures 1.02 and 1.00, respectively. For the series the hemoglobin averaged 52.9 per cent., the minimum being 20 and the maximum 85 per cent. The average erythrocyte count was 2,895,324, and ranged from as low as 1,100,000 to as high as 4,290,000 per c.mm.

The usual degenerative and other qualitative changes accompanying any severe secondary anemia may be found in the anemias of liver cirrhoses; and, in addition, Hayem[1] has observed that in the hypertrophic variety there seems to be a marked tendency toward megalocytosis. It may here be noted that in Hanot's disease Netter[2] has detected staphylococci and Kirikow[3] diplococci in the peripheral blood during life. The effect of bile upon the blood is also apparent in many cases of Hanot's cirrhosis. (See "Icterus," p. 434.)

LEUCOCYTES.

In the great majority of *atrophic cirrhoses* the number of leucocytes either remains normal or is distinctly decreased, while a few show a moderate degree of intermittent leucocytosis, to be regarded in all probability as a post-hemorrhagic change. It is questionable whether or not the jaundice present in some cases accounts for a leucocyte increase, although some authorities profess this belief. The leucocytes in the 40 cases tabulated above ranged thus:

| LEUCOCYTES PER C.MM. | NUMBER OF CASES. |
|---|---|
| From 10,000–15,000 in | 4 |
| " 5,000–10,000 " | 26 |
| Below 5,000 " | 10 |
| Average, 6,921 per c.mm. | |
| Maximum, 12,000 " " | |
| Minimum, 3,000 " " | |

In the 16 cases of *hypertrophic cirrhosis* the leucocytes averaged 9385 per c.mm., the lowest count being 4100, and the highest 21,600. Seven of the estimates were above, and nine below, 10,000 cells to the c.mm. Much higher counts than these, however, have been reported by others. While it must be admitted that leucocytosis is more frequent in this than in the atrophic variety, Hanot and Meunier's[4] claim that it is a constant symptom of hypertrophic cirrhosis is by no means justified.

The leucocytoses of both these forms of the disease depend

[1] "Du Sang," Paris, 1889. [2] Progrés méd., 1886 vol. xiv, p. 992.
[3] St. Petersburg med. Wochenschr., 1900, vol. xvii, p. 353.
[4] Compt. rend. Soc. biol., Paris, 1895, vol. ii, p. 49.

upon an absolute and relative increase in the polynuclear neutrophiles, at the expense of the other forms of cells.

DIAGNOSIS.

The blood examination fails to provide any dependable signs by which cirrhosis is distinguishable from other lesions of the liver, but a good idea of the inroads made by the disease upon the patient's health may be gained by determining from time to time the grade of the anemia present.

## XXXIV. HYDATID DISEASE.

HEMOGLOBIN AND ERYTHROCYTES.

In the reported blood studies of this condition the hemoglobin and erythrocytes have varied so greatly that it seems fair to regard the presence of an anemia as due to factors other than the echinococcus infection. Some cases show perfectly normal values, or, at the most, trifling hemoglobin losses, while in others a rather severe type of secondary anemia develops. Low color indices rule.

LEUCOCYTES.

Leucocytosis with eosinophilia is the important feature of the blood picture. In Seligmann and Dudgeon's case,[1] the first to be reported, the leucocytes numbered as high as 17,000 per c.mm., and the eosinophiles reached 57 per cent., the actual count of these cells being, therefore, 9690 to the c.mm., or nearly twenty times the maximum normal number. Longridge's case[2] had 9 per cent. of eosinophiles in a leucocyte count of 18,400, and the reports of others, notably Launois and Weil,[3] Tuffier and Milian,[4] Archard and Clerc,[5] Darguin and Tribondeau,[6] attest that this form of leucocyte increase is constant in hydatid disease, no matter in what region of the body the cysts are situated. As in other forms of helminthiasis, the eosinophilia of hydatid disease may disappear in cases of great chronicity. The polynuclear neutrophiles are greatly diminished, the lymphocytes remain about normal, and the basophiles are distinctly increased. After evacuation of the cysts the leucocytosis and eosinophilia promptly disappear, and the other forms of cells again attain their normal proportions. Memmi[7] has produced eosinophilia experimentally by the injection of hydatid fluid.

[1] Lancet, 1902, vol. i, p. 1764.
[2] *Ibid.*, 1902, vol. ii, p. 44.
[3] Sem. méd., 1902, vol. xxii, p. 378.
[4] *Ibid.*, 1902, vol. xxii, p. 75.
[5] Arch. gén. de méd., 1902, vol. clxxxix, p. 743.
[6] Presse méd., 1902, vol. viii, p. 142.
[7] Riv. crit. di clin. med., 1901, vol. ii, p. 233

DIAGNOSIS. In hydatids versus abscess or solid tumor the presence of eosinophilia is strongly in favor of the former, other eosinophile-increasing causes being, of course, eliminated.

## XXXV. HERPES ZOSTER.

The blood changes in shingles have been studied by Sabrazes and Mathias,[1] who found no appreciable diminution in the *hemoglobin* and *erythrocytes* and no structural changes affecting the latter. *Leucocytosis* develops as early as the first day of the eruption, and progressively increases until about the third day, after which it gradually diminishes until, by the fifth day, the count again reaches the normal figure. A secondary leucocytosis accompanies the period of desiccation and desquamation. A gain in the polynuclear neutrophiles and eosinophiles is accountable for the leucocytosis, which in some instances is associated with a few myelocytes.

## XXXVI. ICTERUS.

GENERAL FEATURES. Simple catarrhal jaundice *per se* produces little or no effect upon the blood, except in the most pronounced cases. The most conspicuous change consists in a greenish-yellow discoloration of the serum, due to the presence of *bile*. In patients suffering from obstructive jaundice—due, for instance, to gall-stones—a surgical operation may be complicated by dangerous, even fatal, hemorrhage, owing to the slow and imperfect *coagulation* of the blood. The coagulation time of the blood in this form of obstructive jaundice is referred to elsewhere. (See "Cholelithiasis," p. 380.) In the Jefferson Hospital within three years four patients with jaundice due to malignant disease of the biliary apparatus have bled to death after operation. The quantity of *fibrin* is not increased. The *specific gravity* of the whole blood increases in relation to the intensity of the icterus, but the density of the serum is unaffected. In severe cases de Rienzi[2] found that the *alkalinity* of the blood was decidedly reduced. The blood serum of patients affected with icterus, especially Weil's disease, may exhibit a moderate *agglutinative action* upon the Eberth bacillus, the colon bacillus, the cholera spirillum, and other bacteria. Koehler[3]

[1] Rev. de sc. méd., 1901, vol. xxi, p. 251.
[2] Virchow's Arch., 1885, vol. cii, p. 218.
[3] Cited by Libman, Med. News, 1904, vol. lxxxiv, p. 204.

attributes this clumping power to the taurocholic acid constituent of the bile. (See "Enteric Fever," p. 403.)

HEMOGLOBIN AND ERYTHROCYTES.

In mild cases the hemoglobin and erythrocytes remain unaltered, but in severe jaundice a moderate anemia is not uncommon, characterized by an absence of rouleaux formation and by evidences of endoglobular degeneration marked out of all proportion to the grade of the cellular decrease. This association of a moderate oligocythemia with striking degenerative changes in the corpuscles appears to be peculiar to this affection. In cases with symptoms of cholemia these degenerative changes are even more notable, but here the hemoglobin and erythrocyte losses also are more pronounced.

The effects produced upon the hemoglobin and erythrocytes by catarrhal jaundice are illustrated by this table of examinations in 40 cases:

| Hemoglobin Percentage. | Number of Cases. | Erythrocytes per c.mm. | Number of Cases. |
|---|---|---|---|
| From 90–100 | 4 | Above 5,000,000 | 4 |
| " 80–90 | 10 | From 4,000,000–5,000,000 | 19 |
| " 70–80 | 13 | " 3,000,000–4,000,000 | 8 |
| " 60–70 | 4 | " 2,000,000–3,000,000 | 7 |
| " 50–60 | 2 | " 1,000,000–2,000,000 | 2 |
| " 40–50 | 3 | | |
| " 30–40 | 2 | | |
| " 20–30 | 2 | | |
| Average, 72.0 per cent. | | Average, 3,956,000 per c.mm. | |
| Maximum, 97.0 " | | Maximum, 5,344,000 " " | |
| Minimum, 28.0 " | | Minimum, 1,500,000 " " | |

Von Limbeck[1] has observed that the volume of the individual erythrocyte is markedly increased. The cells' diameters, according to Vaquez,[2] average 8 or 9 $\mu$, and not infrequently are as large as 12 $\mu$. Originally von Limbeck,[3] and later Lang[4] and Vaquez and Ribierré,[5] also noted that in jaundice the erythrocytes are highly resistant to the action of hypotonic sodium chlorid solutions and of distilled water. This change probably is preceded by a period of susceptibility on the part of the erythrocytes to the action of circulating biliary poisons, and its development suggests that in time the cells acquire a tolerance against the very toxins which primarily acted deleteriously upon them. It is possible

[1] *Loc. cit.*
[2] Sem. méd., 1902, vol. xxiii, p. 245.
[3] *Loc. cit.*
[4] Zeitschr. f. klin. Med., 1902, vol. xlvii, p. 153.
[5] Sem. méd., 1902, vol. xxiii, p. 246.

that in some instances the anemia is actually greater than the blood count indicates, for polycythemia, according to Becquerel and Rodier,[1] may develop by reason of inspissation of the blood from the action of bile.

LEUCOCYTES.

Most observers report that no leucocytosis occurs in simple catarrhal jaundice, but Grawitz,[2] on the contrary, states that he finds a constant increase in "uncomplicated cases of icterus," the count ranging in some instances as high as from 30,000 to 40,000 to the c.mm. This author's report, however, does not represent the general consensus of opinion. In the writer's experience, about one-fifth of all cases of catarrhal jaundice show a leucocyte count higher than 10,000 to the c.mm. The following estimates in 40 cases are the basis for this statement:

| LEUCOCYTES PER C.MM. | NUMBER OF CASES. |
|---|---|
| From 20,000–30,000 | 4 |
| " 15,000–20,000 | 0 |
| " 10,000–15,000 | 4 |
| " 5,000–10,000 | 29 |
| Below 5,000 | 3 |

Average, 9,361 per c.mm.
Maximum, 26,000 " "
Minimum, 3,600 " "

Severe cases with *cholemia* may and usually do give rise to a well-developed leucocytosis. In *experimental cholemia* in animals, caused by the injection of bile and of biliary salts and pigments, Gilbert and Herscher[3] invariably noted a decided leucocytosis, the extent and persistence of which indexed the animals' defensive powers against the toxin.

DIAGNOSIS.

The association of icterus with leucocytosis, except in obviously cholemic patients, suggests some purulent lesion or malignant disease as the factor of jaundice, rather than uncomplicated angiocholitis.

## XXXVII. INFLUENZA.

General invasion of the circulation by the influenza bacillus occurs very rarely, and the positive results from *bacteriological examination* of the blood claimed by Canon,[4] Klein,[5] and their

[1] Arch. de Physiol. norm. et path., 1874, vol. i, p. 509.
[2] *Loc. cit.*
[3] Sem. méd., 1902, vol. xxii, p. 197.
[4] Virchow's Arch., 1893, vol. cxxxi, p. 401.
[5] Baumgarten's Jahresb., 1893, vol. ix, p. 206.

contemporaries must be regarded as unsubstantiated, in the light of the large number of negative findings by Pfeiffer[1] and by Kühnau.[2] Slawyk[3] has recently succeeded in cultivating this organism from the blood of a patient whose predominant symptoms suggested epidemic meningitis. Castellani[4] was able to detect pneumococci, but not Pfeiffer's bacilli, in the blood of patients having influenza complicated by catarrhal and croupous pneumonia. Jehle,[5] although he admits the rare occurrence of the influenza bacillus in the blood of uncomplicated influenza, claims to have obtained many positive blood cultures of this organism in diphtheria, in pertussis, and in several of the exanthemata—measles, scarlet fever, and varicella. He attributes these findings to the fact that these diseases predispose to a secondary infection, especially to an influenzal bacteriemia.

The *hemoglobin* and *erythrocytes* are normal in the great majority of cases, a moderate diminution in these elements having been found only occasionally.

Uncomplicated influenza is one of the few examples of an acute infection unaccompanied by a *leucocytosis*, although in some instances *hyperinosis* may be observed in the early stages of the attack. Rieder[6] states that a complicating catarrhal pneumonia causes either a moderate increase in the number of leucocytes or none at all, but that in a post-influenzal croupous pneumonia the leucocytosis of this condition develops typically.

It is unfortunate that an absence of leucocytosis is common to both *enteric fever* and influenza, for these two diseases are not infrequently confused. The serum test, however, generally is conclusive if typhoid exists, and blood cultures are even more definite. Should a frank leucocytosis be present, *croupous pneumonia*, rather than influenza, is suggested.

## XXXVIII. INSOLATION.

In the acute stages of thermic fever the *hemoglobin* and *erythrocyte* values are unduly high, owing to the concentration of the blood from the excessive loss of body fluids by the lungs and the skin. Lambert,[7] for example, has observed a hemoglobin percentage of 125 in a sunstroke patient, while Vincent[8] states

[1] Deutsch. med. Wochenschr., 1893, vol. xix, p. 816. [2] *Loc. cit.*
[3] Zeitschr. f. Hyg. u. Infectionskr., 1899, vol. xxxii, p. 443.
[4] Fortsch. d. Med., 1901, vol. xix, p. 781.
[5] Zeitschr. f. Heilk., 1901, vol. xxii, p. 190.
[6] Münch. med. Wochenschr., 1892, vol. xxxix, p. 511.
[7] Loomis-Thompson, "A System of Practical Medicine," New York, 1898, vol. iii, p. 877. [8] Thèse d. Bordeaux, 1887–88, No. 8, p. 7.

that the erythrocytes may number as high as 300,000 per c.mm. in excess of the normal average count. A more or less pronounced destruction of the erythrocytes also occurs both during and after the acute stages of insolation, and this factor is responsible for the anemia, sometimes decided, which subsequently develops. Owing to the coëxistence of these two conflicting factors the real extent of the hemolysis cannot be determined until after the disappearance of the symptoms leading to blood concentration. This hemolysis is thought to depend upon the presence in the blood of some toxic element, since the hyperpyrexia itself is insufficient to cause disorganization of the cells. Schultze and Ranvier's[1] experiments have proved that such changes begin only when an animal is subjected to a temperature of from 54° to 56° C. (129.2° to 132.8° F.). Levene and Van Gieson[2] have shown that the blood serum of sunstruck patients is a highly active blood poison to animals when injected intravenously.

Some investigators have found an increased number of *leucocytes*, but others have been unable to detect any such change, so that leucocytosis must be regarded as an inconstant sign, depending, perhaps, more upon the degree of blood condensation than upon any specific influence of the heat-stroke. Pigmented leucocytes have been observed in cases in which there existed marked signs of blood destruction.

Wood[3] found that a decreased alkalinity or even an acidity of the blood was a conspicuous postmortem change, but evidence is lacking to show that the reaction of the blood is altered during life.

## XXXIX. INTESTINAL HELMINTHIASIS.

General Features.

The presence in the intestinal canal of certain parasites, notably the *Bothriocephalus latus* and the *Ankylostomum duodenale*, is capable of provoking anemia of marked intensity in the individual harboring them. The *Ascaris lumbricoides* also may be held responsible for anemia in some instances, but the blood changes attributable to this parasite are, as a rule, much less marked than those commonly met with in the two preceding forms of helminthiasis. Ostrovosky[4] has called attention to a case, unique of its kind, of fatal progressive anemia attributable to the presence in the intestine of the long threadworm, *Trichocephalus dispar*. Severe secondary anemia,

[1] Cited by Vincent, *loc. cit.*
[2] Cited by Lambert, *loc. cit.*
[3] "Thermic Fever or Sun-stroke" (Boylston Prize Essay), Philadelphia, 1872.
[4] Russkiy Vrach Sept. 30, 1900; abst., N. Y. Med. Jour., 1900, vol. lxxii, p. 826.

not approaching the true pernicious type, has been found in this infection by E. Becker.[1] The cause of these anemias is generally attributed to two factors: interference with absorption of food from the intestinal canal due to the catarrhal inflammation therein existing, and the systemic effects on the host of certain soluble and absorbable toxic products eliminated by the parasites. That such poisons are produced, and that they undoubtedly can act in this deleterious manner, has been abundantly proved by many different investigators, among whom Hubner,[2] Reyner,[3] Schaumann,[4] Askanazy,[5] and Lussana[6] may be named as authorities whom the student should consult for more detailed information on this topic. It is probable that in ankylostomiasis the anemia is also kept up partly by the constant drain on the system caused by the direct abstraction of blood by the parasite, and partly by the so-called "plasmotropic" action of the blood derivatives absorbed from the gut. Grawitz[7] explains this form of blood destruction by assuming that the presence of blood in the intestine is attended by the elaboration of certain toxic substances which, when absorbed, influence the bone marrow, liver, and spleen so as to provoke increased hemocytolysis. The details of this type of blood destruction have been described by the writer elsewhere.[8]

HEMOGLOBIN AND ERYTHROCYTES.

The blood changes due to *Bothriocephalus latus* infection are by far the most interesting, from the clinician's standpoint, since the anemia caused by this worm may in some individuals exactly simulate primary pernicious anemia. The blood pictures of the two conditions may be identical, both being characterized by marked and disproportionate oligocythemia, and consequently by a high color index and by the presence of nucleated erythrocytes, the majority of which conform to the megaloblastic type. Megalocytes and erythrocytes stippled with basophilic areas also are usually numerous. This so-called bothriocephalus anemia has been aptly described by Ehrlich[9] as "a pernicious anemia, with a known and removable cause." It is distinguishable from true pernicious anemia solely by the fact that after the expulsion of the worm by the administration of appropriate vermifuges the megaloblastic type of blood and the anemia rapidly disappear, and the patient makes an uneventful recovery.

[1] Deutsch. med. Wochenschr., 1902, vol. xxviii, p. 468.
[2] Deutsch. Arch. f. klin. Med., 1870, vol. vii, p. 7.
[3] *Ibid.*, 1886, vol. xxxix, p. 31.
[4] "Bothriocephalus-Anämie," Berlin, 1894.
[5] Zeitschr. f. klin. Med., 1893, vol. xxiii, p. 80; *ibid.*, 1895, vol. xxvii, p. 492.
[6] Rivista clin., 1889, vol. iv, p. 750.
[7] Deutsch. med. Wochenschr., 1901, vol. xxvii, p. 908.
[8] Amer. Med., 1902, vol. v, p. 571.
[9] *Loc. cit.*

The anemia of ankylostomiasis (uncinariasis), while it may reach a very high grade of development, still does not counterfeit pernicious anemia. Griesinger's Egyptian chlorosis, the brick-makers' anemia of the Germans and the miners' anemia of the Italians, as well as many forms of tropical anemia, are all due to the effects of this nematode. The hemoglobin and erythrocyte loss may individually be as great as is seen in Biermer's disease, but low color indices rule. The erythrocytes are commonly found in a state of marked deformity, as to both shape and size, poly-chromatophilia may be noted, and erythroblasts are often seen, but never, so far as our present knowledge indicates, is there a prevalence of megaloblasts, as there is both in bothriocephalus and in pernicious anemias.

LEUCOCYTES.

Save in ankylostomiasis, leucocytosis does not accompany any of the above-named forms of helminthiasis, except as the effect of some complication. In ankylostomiasis it is more frequent the earlier the stage of the disease, and usually disappears by the time the anemia is well defined. Boycott and Haldane's 34 cases[1] ranged between 3800 and 56,000 per c.mm., the 4 highest (averaging 36,250) being in patients ill not longer than six months, and the 4 lowest (averaging 5875) occurring in cases of from two to four years' standing. Rogers[2] found an average count of 5338 in 12 cases, and Ashford[3] an average of 7000 in 19 cases. These cases were probably of considerable chronicity. Sandwith[4] noted, contrary to the general rule, that the number of leucocytes increases as the patient convalesces.

As already pointed out (see p. 256), a conspicuous feature of the blood is the frequent, but not constant, occurrence of both a relative and an absolute increase in the percentage of eosinophiles. This change is especially well marked soon after the infection occurs, but may disappear in time, even though the worm is not expelled—a fact first proved by Boycott and Haldane,[5] and one to which may be attributed the inconstancy of eosinophilia in helminthiasis of various types. The individual's age and resisting powers are, of course, contributing factors. The eosinophilia may be moderate or it may be enormous—in one case of ankylostomiasis reported by Ashford,[6] 40 per cent., and in another, 53·5 per cent.;[7] 72 per cent. in a case of the same disease and 34 per cent. in a patient harboring the *Tænia mediocanellata*,

[1] Jour. Hyg., 1903, vol. iii, p. 121.
[2] Brit. Med. Jour., 1900, vol. ii, p. 544.
[3] N. Y. Med. Jour., 1900, vol. lxxi, p. 552.
[4] Lancet, 1894, vol. i, p. 1365.
[5] *Loc. cit.*
[6] *Loc. cit.*
[7] Amer. Med., 1903, vol. vi, p. 301.

these instances having been reported by Leichtenstern.[1] In *bilharziasis* high eosinophile figures have also been reported, for example, 47.6 per cent. (Boycott[2]); 33.6 per cent. (Russell[3]); 33 per cent. in one case, and an average of 16.4 per cent. for 50 cases (Douglas and Hardy[4]); 20 per cent. (Coles[5]); and 18.4 per cent. (Balfour[6]). Even the *Oxyuris vermicularis*, although it is not considered to be a factor of anemia, may cause a well-marked increase in the percentage of eosinophiles, these cells sometimes constituting from 10 to 15 per cent. of all forms of leucocytes. In three cases of *Strongyloides intestinalis* infection, reported by Price,[7] eosinophilia was absent.

Relatively high percentages of mononuclear forms, especially the large, and of basophiles are other differential changes occasionally observed.

## XL. INTESTINAL OBSTRUCTION.

The *hemoglobin* and *erythrocytes* are unaffected, except in obstruction due to malignant disease or associated with hemorrhage, in which there may be a moderate secondary anemia.

*Leucocytosis* is a frequent, though not a constant, accompaniment of all forms of ileus, even those with comparatively slight symptoms. The increase is most constant in obstruction depending upon malignant disease or complicated by gangrene and peritonitis, and in this class of cases it tends to reach the highest figures, except in the event of grave intoxication. Bloodgood[8] regards the presence of a high leucocytosis (20,000 to 30,000) on the third or fourth day after the onset of symptoms as a favorable indication for operative interference, but he considers that low counts (below 10,000) under the same circumstances indicate extensive gangrene-peritonitis, or that the patient will be so depressed that reaction cannot follow relief of the obstruction.

## XLI. KALA-AZAR.

PARASITOLOGY.

In many cases malarial parasites are detected in the blood of individuals suffering from kala-azar, a finding which points to a coincident malarial infection. Bentley,[9] by splenic puncture, demonstrated in

[1] Cited by Ehrlich, *loc. cit.*
[2] Brit. Med. Jour., 1903, vol. ii, p. 1267.
[3] Lancet, 1902, vol. ii, p. 1540.
[4] *Ibid.*, 1903, vol. ii, p. 1009.
[5] Brit. Med. Jour., 1902, vol. i, p. 1137.
[6] Lancet, 1903, vol. ii, p. 1649.
[7] Jour. Amer. Med., Assoc., 1903, vol. xli, p. 651.
[8] Amer. Med., 1901, vol. i, p. 306.
[9] Cited by Ross, Brit. Med. Jour., 1904, vol. i, p. 160.

kala-azar the newly described *Leishmania donovani*, an observation subsequently confirmed by Rogers,[1] who found these bodies in 6 of 7 cases of this disease, as well as in 15 of 30 other febrile conditions associated with splenomegaly and striking cachexia. Leishman-Donovan bodies were first found *intra vitam* by Leishman[2] in blood aspirated from the enlarged spleen of a patient affected with so-called "dum-dum fever," and they were first demonstrated *post-mortem* by Donovan[3] in smears from the spleens in cases of obscure Indian fevers. Marchand and Ledingham[4] have similarly detected these organisms in a patient dead of an irregular pyrexia, with anemia and splenomegaly, and Manson and Low,[5] Neave,[6] and Swan[7] found them in cases of tropical splenomegaly. Manson, Low, and Christophers[8] detected Leishman-Donovan bodies in intestinal ulcers.

The parasites are found but rarely in the peripheral blood, and only when the patient's fever is high, 103° or 104° F.

The organisms in question appear, singly and in clusters, as oval or spherical bodies, 2 or 3 $\mu$ in diameter, and consisting of a limiting membrane inclosing two distinct masses of chromatin: one relatively large and of circular or ring-shaped contour, the other much smaller, rod-shaped, and, as a rule, situated perpendicularly to the circumference of the larger mass (Fig. 62). The precise significance of these organisms is unsettled. Leishman[9] suggests that they may be simply evolution forms of trypanosomata or of a closely related flagellate organism, and the statements of Marchand and Ledingham[4] carry a similar inference. More recently Leishman[10] pointed out the resemblance of his bodies to the *Helcosoma tropicum*, found by J. H. Wright[11] in the pus of that form of tropical ulcer known as Delhi sore. It is possible that they are identical with the so-called plasmodia found in Delhi sore by Cunningham[12] in 1885. Laveran[13] and

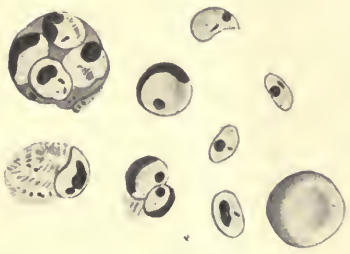


FIG. 62.—LEISHMAN-DONOVAN BODIES.

[1] Cited by Ross, Brit. Med. Journ., 1904, vol. i, p. 160.
[2] *Ibid.*, 1903, vol. i, p. 1252.
[3] *Ibid.*, 1903, vol. ii, p. 79.
[4] Lancet, 1904, vol. i, p. 149.
[5] Brit. Med. Jour., 1904, vol. i, p. 183.
[6] *Ibid.*, 1904, vol. i, p. 1252.
[7] *Ibid.*, 1904, vol. i, p. 1487.
[8] Brit. Med. Jour., 1904, vol. ii, p. 11
[9] *Loc. cit.*
[10] Brit. Med. Jour., 1904, vol. i, p. 303.
[11] Jour. Med. Research, 1903, vol. v, p. 472.
[12] "Scientific Memoirs by Medical Officers of the Army of India," Calcutta, 1885.
[13] Bull. de l'Acad. de méd., Paris, 1903, vol. l, p. 238.

Donovan[1] believe that the organisms belong to the genus piroplasma, while Ross[2] is strongly inclined to the view that they represent a novel organism, possibly of the genus cercomonas.

Rogers'[3] success in developing trypanosomata from cultures of Leishman-Donovan bodies is of the highest importance, if confirmed.

HEMOGLOBIN AND ERYTHROCYTES.

Well-defined secondary anemia sooner or later develops, due in part to the extreme malnutrition of the subject and in part to the effect of a coincident ankylostomiasis, for, as Bentley[4] has shown, this type of helminthiasis is almost universally prevalent in natives stricken with kala-azar. Rogers[5] found about 3,500,000 per c.mm. as the average number of erythrocytes in his series of cases. With the persistence of such an anemia structural changes are to be expected—poikilocytosis, deformities of size, polychromatophilia, and sometimes a few normoblasts.

LEUCOCYTES.

The leucocyte count is subnormal in the majority of cases, and extreme leucopenia may supervene. Relative lymphocytosis, involving especially the large cells, with a consequent decrease in the proportion of polynuclear neutrophiles, is the general rule. Neave[6] found 11 per cent. of small, and 67 per cent. of large, lymphocytes in one case. In 10 cases Donovan[1] found that the mononuclear forms averaged 32.8 per cent., of which 23.6 per cent. were large lymphocytes; and a still more decided mononucleosis was found by Rogers[7] in 22 cases. Because of the concomitant ankylostomiasis, an eosinophile increase is common, the percentage of these cells frequently rising to 10 or 12. Five per cent. of mast cells were found in a case studied by Swan.[8]

The *blood plaques*, according to Bentley,[9] are generally very abundant, even in the pyrexial stage of the disease, and appear to exhibit multiple areas, staining more deeply than the surrounding body of the cell.

DIAGNOSIS.

Hematologically, kala-azar closely simulates *malarial fever*, save in two particulars—the absence, in uncomplicated cases, of the malarial parasite and of pigment and the great increase in the number of plaques. The presence or absence of eosinophilia is naturally

[1] Lancet, 1904, vol. ii, p. 613.
[2] Brit. Med. Jour., 1903, vol. ii, p. 1261; also *ibid.*, 1904, vol. ii, p. 98.
[3] *Ibid.*, 1904, vol. ii, p. 29; also Lancet, 1904, vol. ii, p. 215.
[4] Brit. Med. Jour., 1902, vol. ii, p. 872.
[5] *Ibid.*, 1904, vol. ii, p. 647.
[6] *Ibid.*, 1904, vol. i, p. 1252.
[7] *Ibid.*, 1904, vol. ii, p. 647.
[8] *Loc. cit.*
[9] *Loc. cit.*

of no diagnostic significance. The blood pictures of kala-azar and of *Malta fever* are very similar, aside from the parasitology of the blood. Although the blood of kala-azar patients clumps Bruce's micrococcus in low dilutions, no such reaction occurs in high dilutions—1 in 80 or higher.

## XLII. LEPROSY.

The studies of Winiarski[1] and of P. K. Brown[2] show that in the early stages of this disease neither the *hemoglobin* nor the *erythrocytes* suffer any deterioration, but that in advanced leprosy, especially in cases with extensive ulcerative lesions, the anemia may be striking—quite as marked, in fact, as in a moderately severe case of true Biermer's anemia. In such instances there is a conspicuous oligocythemia in comparison to the oligochromemia, and the counts may fall to below 2,000,000 to the c.mm. A tendency toward megalocytosis and high color indices has been observed, the index in some counts being as high as 1.7. Normoblasts may occur alone or in association with a few megaloblasts, but the latter never predominate. Polycythemia, resulting from peripheral stagnation, may be a feature of some cases. The number of *leucocytes* is not increased, but a relative lymphocytosis is a commonly observed differential change affecting these cells. An eosinophilia of 8.7 per cent. was present in a leper examined by Boston.[3]

Brown,[4] Streker,[5] and Boston[6] have succeeded in demonstrating the *Bacillus lepræ* in the circulating blood during life. These organisms, as a rule, were found to be inclosed in the leucocytes, and, more rarely, lay free in the plasma. On the other hand, Bibb[7] failed in 30 cases to find the bacillus by blood culturing, although he obtained positive results constantly with blood aspirated from the leprous tubercles.

The *serum diagnosis* of leprosy has not yet come into general clinical use, although positive clump reactions with cultures of the leprosy bacillus and the serum of lepers have been observed.

[1] St. Petersburg med. Wochenschr., 1892, vol. ix, p. 365.
[2] Trans. California State Med. Soc., 1897, vol. xxvii, p. 168.
[3] Proc. Phila. Co. Med. Soc., 1903, vol. xxiv, p. 6.
[4] *Loc. cit.*
[5] Münch. med. Wochenschr., 1897, vol. xliv, p. 1103.
[6] *Loc. cit.*
[7] Amer. Jour. Med. Sci., 1894, vol. cviii, p. 539.

## XLIII. MALARIAL FEVER.

PARASITOLOGY.

The specific cause of malarial fever is a form of blood parasite generally known as the *Plasmodium malariæ* or the *Hæmamœba malariæ*, an organism classified among the Sporozoa, according to Metschnikoff.[1] First accurately described in 1880 by Laveran,[2] a medical officer of the French army stationed in Algeria, our knowledge of the parasite and its relation to the malarial fevers have been furthered chiefly by the researches of Richard,[3] also a French army surgeon; of Grassi and Filetti,[4] in Sicily; of Mannaberg,[5] in Austria; of Marchiafava and Celli,[6] Bastianelli and Bignami,[7] and Golgi,[8] of the Italian school; of Manson[9] and Ross,[10] in England; and of Councilman and Abbott,[11] Sternberg,[12] Osler,[13] Dock,[14] Thayer and Hewetson,[15] and Craig,[16] in America. In addition to these principal investigators, numerous workers in other parts of the world have materially advanced our knowledge of the subject.[17]

*Developmental Cycle in Man.*—The malarial organism gains entrance to the erythrocyte of man, in which it pursues a definite cycle of development at the expense of its corpuscular host. Existing in its earliest stages as a hyaline, ameboid body within the substance of the corpuscle, the parasite increases in size, and

[1] Centralbl. f. Bakt. u. Parasit., 1887, vol. i, p. 624.

[2] "Nature parasitaire des accidents de l'impaludisme," Paris, 1881.

[3] Gaz. méd. d. Paris, 1882, vol. iv, p. 252.

[4] Centralbl. f. Bakt. u. Parasit., 1890, vol. vii, pp. 396 and 430; *ibid.*, 1891 vol. ix, pp. 403, 429, and 461; *ibid.*, 1891, vol. x, p. 449.

[5] "The Malarial Parasites," New Sydenham Soc. Trans., London, 1894, vol. cl, p. 241.

[6] Fortsch. d. Med., 1885, vol. iii, p. 787; *ibid.*, 1888, vol. vi, p. 450; also Festschr. z. Virchow's 70. Geburtstag, 1891, vol. iii, p. 187.

[7] Riforma med., 1890, vol. vi, pp. 860, 866, and 872; also Lancet, 1898, vol. ii, p. 1461.

[8] Arch. per le sc. méd., 1886, vol. x, p. 109.

[9] Lancet, 1896, vol. i, pp. 695, 751, and 831; also Brit. Med. Jour., 1894, vol. ii, p. 1306.

[10] "Report on the Cultivation of Proteosoma (Labbé) in Gray Mosquitoes," Calcutta, 1898.

[11] Amer. Jour. Med. Sci., 1885, vol. lxxxix, p. 416; also Med. News, 1887, vol. i, p. 59.

[12] Med. Record, 1886, vol. xxix, pp. 489 and 517.

[13] Phila. Med. Times, 1886, vol. xvii, p. 126.

[14] Med. News, 1890, vol. lvii, p. 59; *ibid.*, 1891, vol. lviii, pp. 602 and 628.

[15] Johns Hopkins Hosp. Reports, 1895, vol. v, p. 3.

[16] "Estivo-autumnal Malarial Fevers," New York, 1901.

[17] For an exhaustive bibliography the reader should consult Thayer's admirable monograph, "Lectures on the Malarial Fevers," New York, 1897. An authoritative account of the malarial fevers in all their phases is given in Celli's book, "Malaria According to the New Researches," English translation by J. J. Eyre, London and New York, 1900. The estivo-autumnal fevers are well dealt with in Craig's book above noted.

derives fine pigment granules from the hemoglobin of its host, which it ultimately destroys at the time its full maturity is attained. Full development of the parasite having been reached, it divides into a number of segments, which, by the rupture of the blood cell, are set free to enter fresh, uninvaded erythrocytes and there to initiate a new developmental cycle of similar characteristics. Segmentation or sporulation of a group of parasites is accompanied by a paroxysm, which in all probability is due to the influence of certain toxic material liberated at this time.

The favorable effect of quinin in malaria King[1] attributes to the fluorescence of the drug, whereby violet rays flood the blood and thus deprive the parasites of the light requisite for their sporulation. This ingenious theory is based upon the fact that light is essential for fluorescence, and upon the hypothesis that the malarial parasite must have light in order to segment. It explains why quinin cures the paroxysms of tertian and quartan fevers, and also why it fails materially to influence those due to estivo-autumnal crescents. In the first two infections the organisms circulate freely in the peripheral blood, where there is enough light to develop fluorescence, while in the latter the organisms are largely confined to the deep circulation, where, because of darkness, no fluorescence occurs. In addition to quinin, fraxin and æsculin possess fluorescent properties and are also therapeutically active in paludism.

In order to complete its full life cycle, the malarial parasite must also pass through a developmental phase within the bodies of certain mosquitos, for it has been shown that these insects not only act as the intermediate hosts of the parasite, but also carry the infection by means of their bite. These important discoveries were first made by Ross,[2] whose conclusions were shortly confirmed by Grassi, Bignami, Bastianelli,[3] and by others of the Italian school.

*Developmental Cycle in the Mosquito.*—While in the human body the malarial parasite pursues an asexual cycle, terminating in segmentation, in the body of mosquitos of the genus *Anopheles* it follows out a true sexual cycle. In the blood of man certain of the parasites which do not undergo segmentation constitute sexual forms of the organism, known as *gametes*, which, after having been imbibed by the mosquito while biting a malarious

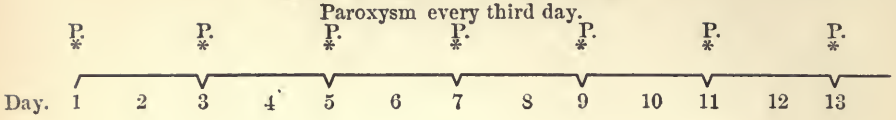
[1] Amer. Jour. Med. Sci., 1902, vol. cxxiii, p. 1025.

[2] *Loc. cit.*

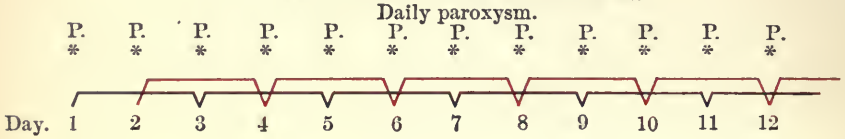
[3] Reale Accademia dei Lincei. Estratto dal, vol. vii, 2° sem., ser. 5a., fasc. 11°. Seduta del 4 dicembre, 1898; abst. in Progressive Med., Philadelphia, 1899, vol. i, p. 287; also Bignami, Lancet, 1898, vol. ii, pp. 1461 and 1541; also Grassi, Il Policlinico. 1898, vol. v, p. 469.

CHART III.

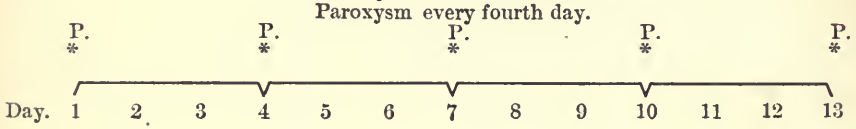
SINGLE TERTIAN INFECTION.
Paroxysm every third day.



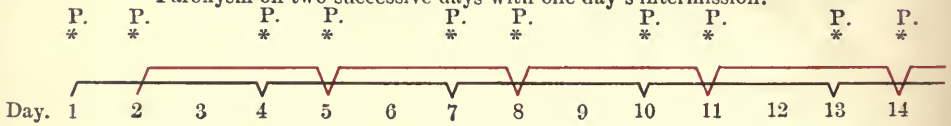

DOUBLE TERTIAN INFECTION.
Daily paroxysm.




SINGLE QUARTAN INFECTION.
Paroxysm every fourth day.




DOUBLE QUARTAN INFECTION.
Paroxysm on two successive days with one day's intermission.




TRIPLE QUARTAN INFECTION.
Daily paroxysm.

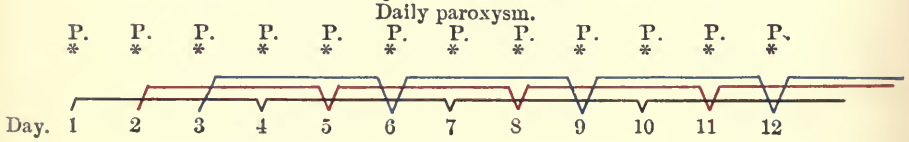



**CHART ILLUSTRATING THE DIFFERENT TYPES OF FEVER RESULTING FROM INFECTION WITH SINGLE AND WITH MULTIPLE GROUPS OF MALARIAL PARASITES.**

The duration of the parasites' cycle of development is expressed by colored lines, thus:

Black: First group of parasites.
Red: Second " " " "
Blue: Third " " " "
P: Paroxysm.

individual, develop into impregnated bodies by reason of the fecundation of the female sexual elements, or *macrogametes*, by the free flagella, or *microgametes*, which have become detached from the male sexual elements, or *microgametocytes*. The resultant fertilized bodies develop into motile *pseudo-vermicules*, which, having penetrated the muscular wall of the mosquito's stomach, lodge and become encysted in this situation and are now known as *zygotes*. From the latter are derived large numbers of delicate spindle-shaped cells, or *sporoblasts*, which, by the rupture of the *zygote's* capsule, are set free, and, as *sporozoids*, make their way into the salivary gland of their host, whence they pass by way of the salivary duct into the proboscis of the insect, and consequently into the circulating blood of the individual stung by the infected mosquito. The *sporozoids* thus inoculated into the blood of man penetrate his erythrocytes, in which, as the young hyaline forms of the malarial parasite, they pursue the typical developmental cycle to be described below.

Thus, it has been definitely shown that mosquitos of the genus *Anopheles* are capable of transmitting malarial infection from man to man, and it is now generally believed that this, the only proved method of malaria transmission, is probably the sole means by which the disease is spread. The ordinary house-mosquito, of the genus *Culex*, is not concerned in the transmission of malaria, since it has been shown that the parasites do not follow out a developmental cycle within the body of this insect.

For a complete review of the "mosquito theory" of malaria, embracing the work of Ross, Manson, MacCallum, and the Italian school, the reader should consult Thayer's "Recent Advances in our Knowledge Concerning the Ætiology of Malarial Fever,"[1] Futcher's "A Critical Summary of Recent Literature Concerning the Mosquito as an Agent in the Transmission of Malaria,"[2] and Howard's "Mosquitoes."

*Varieties of the Malarial Parasite.*—Three distinct varieties of the parasite are recognized, each of which has been found constantly associated with a specific type of malarial infection. These three varieties are:

1. *The parasite of tertian fever*, associated with a regularly intermittent type of fever, with paroxysms every third day.

2. *The parasite of quartan fever*, associated with a regularly intermittent type of fever, with paroxysms every fourth day.

3. *The parasite of estivo-autumnal fever*, associated with the more irregular types of fever.

[1] Proc. Phila. Co. Med. Soc., 1900, vol. xxi, p. 211.
[2] Amer. Jour. Med. Sci., 1899, vol. cxviii, p. 318.

The parasites of tertian and of quartan fever exist in the blood of the infected individual in great groups, consisting of immense numbers of organisms, *all of which are approximately at the same stage of development*, and therefore undergo sporulation at about the same period. This fact serves to explain the regularity of the tertian and quartan paroxysms. On the other hand, in estivo-autumnal infections this regular grouping of the parasites is often wanting, and large numbers of this organism commonly exist in the blood in *different stages of development*. Sporulation thus taking place at irregular intervals, irregularity in the occurrence of the estivo-autumnal paroxysms is extremely common.

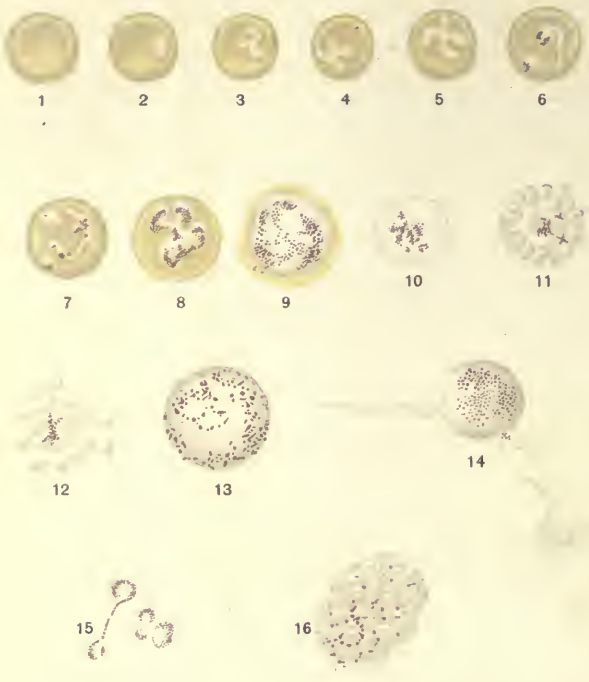
As the development of these three types of the malarial parasite progresses, certain forms are evolved which possess more or less common characteristics, so that it is convenient to speak of these forms, which represent the maturing phases of the organism, as follows:

(*a*) The intracellular hyaline forms.
(*b*) The intracellular pigmented forms.
(*c*) The extracellular pigmented forms.
(*d*) The segmenting forms.
(*e*) The flagellate forms.

Furthermore, in parasites of the estivo-autumnal type additional forms—those of the crescent group—are met with, these varieties being peculiar to this type of infection and never occurring in tertian and quartan fevers.

Tertian infections constitute the prevailing type of malarial fever in almost all countries in which the disease exists. Quartan fevers are relatively uncommon, except in certain limited districts —parts of Sicily, for example?—in which a large proportion of the cases conform to this type. Estivo-autumnal fevers are especially common in the tropics, but this type of the disease is by no means incompatible with temperate regions. In Philadelphia and its environs tertian infections are about five times as common as those of the estivo-autumnal type, while quartan malaria is practically unknown. The writer has seen but a single instance of quartan infection in this vicinity, and this case was without doubt imported.

*The Parasite of Tertian Fever (Plate VI)*.—The tertian parasite attains its full development in about forty-eight hours, segmentation of a single group of organisms at this interval producing the characteristic paroxysms every third day. Infection with two distinct groups of parasites, each maturing on successive days, gives rise to a quotidian type of fever, characterized by the occurrence of daily paroxysms. (See Chart III, p. 447.) Infection with more




THE TERTIAN PARASITE.

1. *Normal erythrocyte.*
2, 3, 4, 5. *Intracellular hyaline forms.*
6, 7. *Young pigmented intracellular forms.* In 6 two distinct parasites inhabit the erythrocyte, the larger one being actively ameboid, as evidenced by the long tentacular process trailing from the main body of the organism. This ameboid tendency is still better illustrated in 7, by the ribbon-like design formed by the parasite. Note the delicacy of the pigment granules, and their tendency toward peripheral arrangement in 6, 7, and 8.
8. *Later developmental stage of 7.* In 7, 8, and 9 enlargement and pallor of the infected erythrocyte become conspicuous.
9. *Mature intracellular pigmented parasite.*
10, 11, 12. *Segmenting forms.* In 10 is shown the early stage of sporulation—the development of radial striations and peripheral indentations coincidentally with the swarming of the pigment toward the center of the parasite. The completion of this process is illustrated by 11 and 12.
13. *Large swollen extracellular form.* Note the coarse fused blocks of pigment. (Compare size with that of normal erythrocyte, 1.)
14. *Flagellate form.*
15. *Shrunken and fragmenting extracellular forms.*
16. *Vacuolation of an extracellular form.*

NOTE.—The original water-color drawings were made from fresh blood specimens, a Leitz $\frac{1}{12}$-inch oil-immersion objective and 4 ocular, with a Zeiss camera-lucida, being used.

(E. F. FABER, *fec.*)

than two groups is extremely rare, and produces an atypical and irregular type of fever.

Anticipation of the paroxysm, which is especially frequent in tertian fever, may be explained by a precocity displayed by a group of parasites, by virtue of which their development is so rapid that the stage of sporulation is reached before the expiration of forty-eight hours. On the contrary, should the development of a group happen to be slower, requiring more than forty-eight hours for its full maturity and sporulation, then the paroxysm is retarded.

If a specimen of fresh, unstained blood from a case of tertian fever is examined during the period immediately or shortly following the malarial paroxysm, it will be observed that many of the erythrocytes contain small, pale, transparent foreign bodies, dim of outline, more or less markedly ameboid in character, and of a peculiar dirty, grayish-pearl tint. These bodies, known as the *intracellular hyaline forms*, or *amebulæ*, represent the youngest forms of this organism, being derived from the sporulation of the immediately preceding group of parasites. They may occasionally be found in the peripheral blood toward the latter part of the paroxysm, and for a short time after the occurrence of this phenomenon.

The ameboid movements of these hyaline bodies form one of their most striking features, and in consequence of this trait their shape is constantly altered. At one moment the parasite appears as a flattened spherical or oval disc, measuring 2 or 3 $\mu$ in diameter; the next instant it may change to the shape of a jack-stone, or become a stellate design or take the form of an anvil. The succession of figures which the organism may resemble is limitless. As the parasite increases in size long pseudopodia, like the delicate tendrils of a vine, are alternately thrown out and retracted, reaching here and there through the corpuscular substance with uncertain but sudden motility. In the active parasite these pseudopodia appear as long, delicate, gracefully curved branchings of the protoplasm, usually terminating in a spherical, knob-like extremity, and measuring 4 or 5 $\mu$ in length in many instances. Occasionally the parasite seems to have formed a perfect ring, either because of the thinning out of its central portion, or, rarely, by reason of the fusion of two short pseudopodia between which a small portion of the corpuscle becomes imprisoned. The outline and color of the hyaline body are quite characteristic, at least to the eye of the practised observer. Usually described as quite colorless, the parasite rather possesses a distinctive pearly tint, overlaid in patches by strata of corpuscular substance of varying depth,

so that in certain lights the yellowish-green color of the erythrocyte predominates and obscures the true color of the organism to some extent. Usually but a single hyaline body, situated somewhat eccentrically, is found in the corpuscle; less commonly, two or more are harbored.

The next stage in the development of the organism, the collection of pigment granules derived from the hemoglobin of the erythrocyte, is reached toward the latter part of the first twenty-four hours following the paroxysm. By this time the size of the parasite has increased to about half that of its corpuscular host, and it is now known as an *intracellular pigmented form.*

The pigment appears as a collection of exceedingly fine, yellowish-brown granules, which are usually most densely distributed near the peripheral rather than the central portion of the parasite. In the large spherical forms of the latter most of the pigment is arranged in a series of irregular clumps, loosely strung together by delicate, wavy connecting lines consisting of individual granules; or the rim of the parasite may be paralleled for the greater part of its extent by a pigment design not unlike a wreath or a hoop. The individual granules are observed to be in active, incessant motion, their violent oscillations hither and thither forming a picture that at once arrests the attention of the observer.

In many of the ameboid figures a polar distribution of the pigment is noticeable, the greater part of the granules being situated, in fine clumps, in the knob-like extremities of the several pseudopodia; and even in these situations the typical tendency of the pigment to arrange itself eccentrically is striking.

As the parasite matures it becomes of still larger size, more and more pigmented and less and less ameboid, the latter characteristic becoming quite or almost entirely lost by the time it attains its full growth. The pigment, fine, of yellowish-brown color, and eccentrically distributed in the earlier forms, is at this period of the organism's growth much coarser, darker in color, and more scattered throughout the protoplasm. Some of the granules are fused into minute, dark-colored spikes and rods, in contrast to the discrete, dot-like granules of the younger parasites.

Coincidentally with these changes striking alterations are apparent in the invaded erythrocytes. These cells become progressively paler and more swollen as the development of the parasite goes on, until at the time of the latter's full maturity (attained after a growth of about forty hours' duration) the corpuscles have become almost entirely decolorized, and appear now as hyaline or pale yellowish rims encircling the parasite, the size of which is now approximately equal to that of a normal erythrocyte.

Just before and during the next paroxysm, or from about forty to forty-eight hours after the preceding chill, the parasite attains its full maturity and the stage of sporulation occurs. Coincidentally with this, *segmenting forms* of the parasite, also known as *sporocytes*, begin to appear in the blood. In tertian infections segmentation occurs to a greater extent in the deep than in the peripheral circulation, but if finger blood is obtained two or three hours before the chill, a few "segmenters" will almost always be found if the search for them is careful and thorough. In cases in which the number of parasites has been scanty during the preceding days of the attack it may be impossible to detect these forms in spite of careful, skilled observation.

Segmentation is heralded by a tendency of the pigment granules to collect in or near the center of the parasite in one large or in several smaller compact clumps or fused masses. This having taken place, a number of minute, somewhat refractive points may be seen with more or less distinctness, the majority of these spots being confined to the peripheral portion of the organism, which by this time has lost a great deal of its earlier clear, hyaline appearance, and has become dully opaque and somewhat granular. Following the development of these refractive points, indistinct parallel linear shadings, usually fifteen or twenty in number, extending from the periphery of the parasite toward the central collection of pigment, may be discerned; and coincidentally with this change the rim of the parasite becomes wrinkled, then distinctly corrugated, each corrugation capping a pair of these radiating shadings. The latter finally become the dividing lines of fifteen or twenty spores or segments, of somewhat round or ovoid shape, radiating in an irregular figure toward the central pigment mass. By careful focusing each segment is found to contain a central refractive spot, the whole collection being surrounded and held together by the shell of the erythrocyte, now so decolorized that it is scarcely visible.

Finally, when segmentation is completed, the spores—for as such these segmenting bodies must now be considered—are freed from the body of the corpuscle which has served until this time as their limiting capsule. The latter having apparently ruptured, the spores escape, either by gradually emerging several at a time, or by the simultaneous and extremely abrupt exit of their whole number. The spores, which now lie free in the blood plasma, surround the remains of the central pigment mass in an irregular group which has been likened in appearance to a bunch of grapes. Sooner or later they wander off through the plasma and disappear from view, the inference being that they invade fresh erythrocytes

and thus initiate a new cycle of development of another forty-eight hours' duration. Although visual proof of this invasion is lacking, the fact that hyaline bodies, biologically similar to these free spores, are found in the erythrocytes at or shortly after the time of segmentation, must be regarded as sufficiently strong evidence of the truth of this inference.[1] Most of the liberated pigment is carried off through the blood, to be deposited in various organs, while some of it is taken up by phagocytes. These cells probably also engulf any free spores which fail to penetrate the erythrocytes.

The preceding remarks refer to the typical circle of the parasite's development, from the smallest hyaline intracellular body to the full-grown pigmented segmenting variety, from which the former is derived. But all the parasites of one group do not pursue this routine, some escaping prematurely from the erythrocyte at an early period of their life history, others continuing to develop further, and losing their corpuscular capsule just prior to the time segmentation begins in the other parasites of the same group. In consequence of the latter change another distinct class of tertian parasites, the *extracellular pigmented forms*, or *gametes*, is produced, and it is the varieties of this class that we now have to consider.

In the first instance, the young, slightly pigmented parasite escapes from its corpuscular host through an apparent breach in the surface of the latter. The immediate effect of its contact with the blood plasma is to convert it into a deformed, dwarfed body of protoplasm, which sooner or later becomes wholly devoid of ameboid motion. It is often fragmented and divided into two or more small rounded masses, each containing an amount of pigment seemingly disproportionate to its size, compared to the quantity found in the intracellular forms. Sometimes two of these pigmented spheres are joined to each other by a filmy connecting thread of protoplasm, from 3 to 5 $\mu$ in length, forming a design which may be compared to a miniature chain-shot. After the lapse of a short length of time the outlines of these bastard forms of the parasite become almost indistinguishable. The erythrocytes from which they have escaped become completely decolorized and invisible shortly after this accident has occurred.

In the second instance, in which the parasite loses its corpuscular envelop just before the time of segmentation, the resulting spherical extracellular body is usually of large size, often 9 to

[1] Christy's drawings (Brit. Med. Jour., 1903, vol. ii, p. 645), showing free hyaline organisms adhering to and apparently entering the erythrocytes, are of interest in connection with this question.

12 $\mu$ in its greatest diameter, or, in the smaller forms, about the size of the normal erythrocyte. It is filled with actively moving pigment granules, wreathed in the center of the parasite, arranged peripherally, or scattered throughout its body, and standing out in bold relief against the background formed by the pale surface of the parasite. The granules in this form of the organism are usually quite dark in color, some of them being welded and fused into minute spiculate figures, while others remain free and distinct. As a rule, male gametes contain pigment in the form of a more or less compact central mass, while in female gametes the pigment is arranged in the form of a loose loop or wreath in the center of the organism.

These extracellular pigmented bodies are of especial interest, for the reason that from them develop those most striking varieties of the malarial parasite, the *flagellate forms*. The earliest evidence of the process of flagellation is seen in the strikingly increased activity of the pigment, the oscillations of the granules growing more and more violent with the approach of the phenomenon. Then, one or more long, almost transparent tentacular processes are observed suddenly to burst from the periphery of the parasite, their violent and incessant whipping about in the plasma causing more or less disturbance of the blood corpuscles in their vicinity. The pigment granules, meanwhile, have swarmed together into a loose mass at or near the center of the main body. The length of the flagella varies from 4 to 5 to 20 $\mu$ or longer, their average breadth being somewhat less than 0.5 $\mu$. They frequently possess one or more bulbous swellings, usually at their distal extremity, occasionally at their proximal end, and also at other points along their course intermediate to these situations. They may or may not contain a few fine and active dotlets of pigment situated in the swollen extremity, or sprinkled as fine stipplings along their course.

The ultimate disposition of the flagella occurs in one of two ways: they either become detached from the large spherical parasite, and, as free flagella, wander off through the plasma, propelled by their own ameboid movements, which finally cease, after which they soon disappear from view; or, remaining attached to the large body, they are observed to disappear by apparently reëntering the large parasite and becoming reincorporated with its protoplasm. Flagellate forms do not occur in the circulating blood, and are not found in the fresh specimen until some little time, usually from ten to twenty minutes, has elapsed after the withdrawal of the blood from the body They are most easily found in blood which has been taken from the patient just before

the onset of a paroxysm. The nature and functions of these flagellate bodies were first clearly determined by MacCallum,[1] who proved that the flagella are true male sexual organs, actively concerned in the process of fertilization, to which reference has already been made. (See p. 446.) The parasites from which they develop are obviously male gamete forms, or microgametocytes.

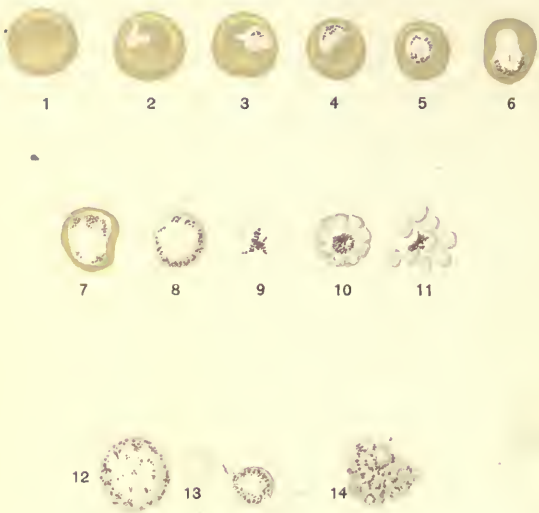
Some of the gamete forms, failing to develop flagella, undergo vacuolization, often become exceedingly misshapen, and sometimes fragmented, these changes being regarded as degenerative in character. A parasite thus affected loses its regularly spherical outline, and may so alter in appearance that it resembles a gourd or a partly inflated balloon. Constrictions at one or more points may appear, and in the little knobs thus cut off from the main body of the organism a few actively motile pigment granules are usually imprisoned. Small portions of the original body, containing active pigment, may become extruded and float off through the plasma, but sooner or later the pigment in these fragmented bits loses its motility and the bodies themselves become deformed and so indistinct of outline that they are lost to view. These degenerative forms closely resemble those derived from prematurely escaped intracellular parasites, except that the latter, as a rule, contain finer and less abundant pigment.

2. *The Parasite of Quartan Fever (Plate VII).*—The quartan parasite completes its cycle of development in about seventy-two hours, thus producing a paroxysm every fourth day. Infection with two separate groups of parasites is marked clinically by a paroxysm occurring on each of two successive days, separated by one day of intermission. Infection with three groups of parasites produces daily paroxysms, the resulting quotidian type of fever being similar to that due to double tertian infections. (See Chart III, p. 447.)

Ordinarily, the quartan parasite's cycle of development is extremely regular, the period required for its maturation seldom deviating from seventy-two hours. It is owing to this that anticipation and retardation of the paroxysm, so common in tertian infections, are rare in the quartan types of fever.

The *young hyaline forms* of the quartan parasite closely resemble those of the tertian organism: they have the same hyaline appearance, the same indistinct outline, and the same sort of ameboid movement. While the quartan hyaline body is usually described as being of smaller size and less ameboid than the

[1] Jour. Exper. Med., 1898, vol. iii, p. 117; also Johns Hopkins Hosp. Bull., 1897 vol. viii, p. 236.




THE QUARTAN PARASITE.

1. *Normal erythrocyte.*
2. *Intracellular hyaline form.*
3. *Young pigmented intracellular form.* Note the coarseness, dark color, and scantiness of the pigment granules.
4, 5, 6, 7. *Later developmental stages of 3.* Note the peripheral distribution of the pigment in all the parasites from 3 to 8. (Compare size and color of the erythrocytes in 5, 6, and 7 with 7, 8, and 9, Plate VI.)
8. *Mature intracellular form.* Note that the stroma of the erythrocyte is no longer demonstrable.
9, 10, 11, *Segmenting forms.* In 9 are shown the characteristic radiating lines of pigment. (Compare with 10, 11, and 12, Plate VI, and with 10, 11, and 12, Plate VIII.)
12. *Large swollen extracellular form.* (Compare with 13, Plate VI.)
13. *Flagellate form.* (Compare with 14, Plate VI.)
14. *Vacuolation of an extracellular form.*

(E. F. FABER, *fec.*)

similar tertian form, these differences are not well enough marked to be of practical application. At this stage of its life history the organism of quartan fever possesses no distinctive characteristics by which it may be differentiated from the tertian variety of a similar period of growth. It is not until it has matured to the stage of pigmentation that it is possible to discern points of distinction by which its identity may be fixed—characteristics which become more and more striking as development of the parasite progresses, and which relate to its color, outline, pigment, and ameboid powers, as well as to changes affecting its corpuscular host.

The outline of the *intracellular pigmented form* is much more distinct than that of the tertian, its margins contrasting rather than blending with the color of the surrounding erythrocyte. The appearance of its protoplasm is also quite different, being apparently denser in consistence, more highly refractive, and unobscured by the color of the overlying corpuscular substance. Thayer[1] has happily compared this difference in refraction and distinctness of outline between the tertian and quartan parasites to the difference between a pale hyaline and a waxy cast in the urine—a comparison which precisely expresses these points of dissimilarity.

The pigment granules, fine, yellowish-brown, and violently motile in the tertian variety, are coarse, dark brown or almost black, and sluggishly motile in the quartan form. They early tend to form little spicula and rods, intensely dark in color, and compactly arranged, being frequently grouped together in masses like coffee-grounds in one corner of the parasite.

By the time the organism reaches about one-half or two-thirds the size of the corpuscle in which it is contained, it may be observed that its ameboid movements, which in the earlier stages of its existence were quite active, have now become sluggish, slow, and inconspicuous. In consequence of this limited motility the long tentacular shoots of protoplasm, so familiar in the tertian form, are not seen, the quartan parasite inclining to form resting figures, oval, round, or somewhat elongated in outline. The pigment does not oscillate violently, but moves about a more limited area with a sort of deliberate, tugging motion. It is distributed about the periphery, which it parallels for only a short distance, not tending to produce the wreathed designs commonly observed in the tertian organism at a corresponding stage of its maturity.

As the parasite matures its ameboid powers progressively di-

[1] "Lectures on the Malarial Fevers," New York, 1897.

minish, until at a period usually after the forty-eighth hour following the last paroxysm little or no motility either of protoplasm or of pigment is distinguishable.

The corpuscular host meanwhile undergoes striking changes in comparison to the erythrocyte invaded by the tertian organism. Instead of becoming swollen and pale, as in the latter instance, it becomes, on the contrary, shrunken, darker colored, and sometimes "brassy." It is not until segmentation is imminent, or from about ten to twelve hours before the impending paroxysm, that decolorization of the blood corpuscle becomes marked. At this period of its cycle the parasite measures about 7 or 8 $\mu$ in diameter, and is apparently, although not actually, unconfined by a corpuscular envelop, the latter now having become rapidly decolorized and finally quite invisible.

As *segmentation* approaches the pigment collects in the center of the sporocyte, which now becomes more opaque and develops a number of refractive dots, which later become the nuclei of from six to twelve segments, developed by a progressive deepening of parallel radial striations extending from the periphery to the center of the parasite. The segmenting quartan parasite forms a perfect rosette, the individual spores being of equal size and of the same shape, and the collected mass of spores being very symmetrically arranged. Coincidentally with segmentation a new group of young hyaline parasites may be found in the hitherto uninvaded erythrocytes, indicating the beginning of another cycle of the parasite's development, which, if unchecked, persists for seventy-two hours.

Thayer[1] mentions a star-like arrangement of the pigment in the early stages of the segmenting quartan organism, as if the granules had flowed inward in distinct streams during the process of collection, and this picture he is inclined to consider characteristic. The author is able to verify Thayer's observation, having, in a limited experience with the quartan parasite, never failed to find this peculiarity, its absence having been equally conspicuous in malarial organisms of other types.

The quartan parasite completes every phase of its development in the circulating blood, so that all stages of its cycle, from the earliest hyaline forms to the segmenting and flagellate bodies, may be studied in the peripheral blood.

*Extracellular pigmented forms*, which have parted with all traces of their corpuscular capsule without having undergone segmentation, may also be observed. These gametes average less in diameter than similar forms of the tertian parasite, the largest

[1] *Loc. cit.*

of the quartan forms being about equal in size to the smallest of the tertian. Their pigment granules are coarse, very dark colored, and situated chiefly toward the periphery, with a greater or less drifting inward of individual pigment clumps apparently composed of two or three agglutinated coarse granules. The difference in the distribution of the pigment in the male and female quartan gametes is more difficult to appreciate than in corresponding tertian bodies.

*Flagellate bodies*, smaller in size and containing coarser granules than corresponding tertian forms, develop from these swollen extracellular parasites, the onset of flagellation being portended by increased activity and centralization of the pigment in direct anticipation of the appearance of the flagellate appendages.

Degenerate forms of the parasite, vacuolized, fragmented, and otherwise deformed, may also be observed, but with less frequency than in tertian fever, probably for the reason that extracellular forms of the quartan parasite are not so numerous as those of the tertian organism. The writer has especially noticed the infrequency of fragmentation and other deformity of those organisms which have prematurely emerged from their corpuscular host, atypical varieties of the more mature free bodies being comparatively much commoner.

3. *The Parasite of Estivo-autumnal Fever (Plate VIII).*—The developmental cycle of the estivo-autumnal parasite exhibits marked irregularity as to the length of time required for its completion, in contrast to the routine forty-eight- and seventy-two-hour cycles in which the tertian and quartan organisms round out their life histories. In some instances the cycle of the estivo-autumnal parasite is of only twenty-four hours' duration, while in others it is quite forty-eight hours, or perhaps longer. This inconstancy of type is thought to depend upon some peculiarity of the organism, by virtue of which the time required for its maturation may widely fluctuate under different conditions of quite obscure character. It is not generally believed that the common types of fever, quotidian and tertian, respectively, depend upon infection with two special forms of the estivo-autumnal parasite, although this view is held by some authors, notably by Mannaberg [1] and by Marchiafava and Bignami,[2] all of whom recognize both a quotidian and a tertian variety of the organism; the former, furthermore, describes a pigmented and an unpigmented form of the quotidian variety.

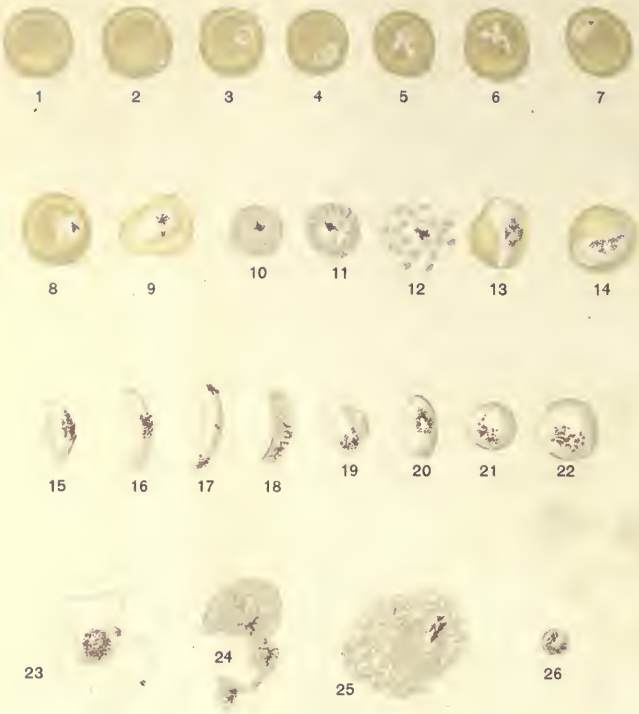
Certain phases of the *young hyaline forms* of the estivo-

[1] Nothnagel's "Spec. Path. u. Ther.," Vienna, 1899, vol. ii, p. 68.
[2] New Sydenham Soc. Transl., London, 1894, vol. cl, p. 1.

autumnal parasite bear a striking resemblance to similar forms of the tertian and quartan organisms, but other phases are, on the contrary, just as strikingly dissimilar. As a rule, the estivo-autumnal amebula is much smaller than those just described, its margins are more sharply defined from the corpuscular substance, and it appears to possess a greater degree of refraction. But these are minor points of difference, the chief distinction relating to the peculiar morphological changes to be observed in these immature parasites. At one moment they may appear as pale, rounded or somewhat oval bodies, situated rather toward the periphery of the corpuscle than in its center, and usually possessing active ameboid movements which produce various stellate and forked designs. On closer observation certain other striking changes may be noted in these round forms. These changes consist in the formation of the so-called ring-shaped bodies, due to the development of a more or less marked biconcavity of the hitherto flattened hyaline body, either in its center, in event of which the parasite appears as a true ring or hoop, or more toward its periphery, in which instance a figure resembling a signet-ring is produced. These figures remain visible for a variable length of time, the parasite meanwhile being apparently in a resting stage, but sooner or later its ameboid powers are reasserted, with the result that the biconcavity abruptly disappears, converting the ring-shaped body into its original form of an ameboid, flattened disc. This successive alteration in shape, from disc to ring to disc, regardless of the other changes in shape, is highly characteristic of the estivo-autumnal organism, and is fully as valuable a diagnostic sign as the more striking pictures of the maturer forms, to be considered later. The size of the ring-shaped parasites varies from less than 2 $\mu$ in diameter to about 3 $\mu$. They are rarely situated in the exact center of the corpuscles, more commonly being found lying midway between the center and the periphery, or, indeed, quite upon the latter.

As the parasite matures, pigment, in the form of a few exceedingly fine, scattered granules, begins to appear. The granules are very few in number, dark brown in color, and are usually situated toward the edge of the organism. They may or may not be motile, usually not. Strikingly *pigmented forms* of the estivo-autumnal parasite are never observed, in marked contrast to the abundant fine pigment of the tertian forms and to the coarse granules typical of the quartan varieties.

The development of the parasite up to this stage can be studied in the peripheral blood, but the older forms of the pigmented bodies, and their final division into spores, by segmentation, occur




THE ESTIVO-AUTUMNAL PARASITE.

1. *Normal erythrocyte.*
2, 3. *Young hyaline ring-forms.*
4, 5, 6. *Intracellular hyaline forms.* In 4 the parasite appears as an irregularly shaped disc with a thinned-out central area. In 5 and 6 its ameboid properties are obvious.
7. *Young pigmented intracellular form.* Note the extreme delicacy and small number of the pigment granules. (Compare with 6, Plate VI, and with 3, Plate VII.)
8, 9. *Later developmental stages of 7.*
10, 11, 12. *Segmenting forms.*
13, 14. *Crescentic forms at early stages of their development.*
15, 16, 17, 18, 19. *Crescentic forms.* In 15 and 19 a distinct "bib" of the erythrocyte is visible. Vacuolation of a crescent is shown in 18, and polar arrangement of the pigment in 17.
20. *Oval form.*
21, 22. *Spherical forms.*
23. *Flagellate form.*
24. *Vacuolation and deformity of a spherical form.*
25. *Vacuolated leucocyte apparently enclosing a dwarfed and shrunken crescent.*
26. *Remains of a shrunken spherical form.*

(E. F. FABER, *fec.*)

almost exclusively in the deeper circulation, and must be followed out in blood obtained from one of the internal organs, such as the spleen, which may be aspirated for this purpose, although the procedure is not without risk to the patient. In the finger blood the writer has never seen presegmenting forms more mature than those represented by the young, slightly pigmented parasite, and has never had the good fortune to meet with segmenting bodies except in specimens derived from the spleen. The general rule is to find in the peripheral blood nothing more than hyaline, ameboid, and ring-shaped bodies, or, perhaps, a few organisms containing two or three minute granules of pigment.

If, now, a drop of blood, aspirated from the spleen, is examined, the remainder of the parasite's cycle may be traced with fair accuracy. As it approaches the *stage of segmentation*, the parasite develops into a spherical body, measuring from about 2 to 6 $\mu$ in diameter, and having a distinct outline which limits it from the surrounding substance of the erythrocyte, which it only partly fills. The pigment granules, which by this time are moderately but never strikingly increased in number, show a marked tendency to become concentrated near the center of the organism. They here exist as a tightly clumped, compact mass, in which the identity of the individual granules is completely lost, as they have now become fused into a single distinct, dark-colored, round or somewhat elongated mass.

As segmentation commences the parasite becomes opaque, minute refractive areas paralleling the periphery develop, and radial shadings, which later divide the body usually into from eighteen to twenty spores, become apparent. The segmenting body is smaller than that of the tertian and quartan parasites, but it usually resembles the former as to the arrangement and number of the individual segments.

A marked characteristic of the estivo-autumnal infections is the early occurrence of degenerative changes in the invaded erythrocytes. These changes, the "erythropyknosis" of the Italian school, consist in the development of a pronounced "brassy" appearance of the blood cell, together, in many instances, with distinct crenation along its periphery and in various portions of its flat surface. Occasionally there appears to be a distinct concentration of the hemoglobin about the parasite, leaving portions of the corpuscle quite colorless. This corpuscular degeneration occurs early, even in those cells occupied by the youngest hyaline bodies, and grows more and more marked, as a rule, as the parasite matures. Simple decolorization of the erythrocyte appears to follow no fixed rule, for segmenting bodies have been observed

both in perfectly hyaline and in apparently unchanged corpuscles. In Thayer's experience the rim of the blood cell surrounding the parasite has usually been entirely devoid of color.

After the infection has existed for a week or more examination of the peripheral blood, which until now has contained perhaps only ring-shaped organisms, reveals the presence of other highly characteristic forms of the estivo-autumnal parasite, the *round*, *ovoid*, and *crescentic* bodies, all belonging to the crescent group, representing the gamete forms of the organism. These forms, which are never present in the circulation during the first days of the fever, are prone to persist in the blood for a long period after the disappearance of the earlier forms of the parasite, and even after all the clinical manifestations of the attack have vanished. Unlike other forms of the malarial parasite, those of the crescent group are peculiarly resistant to the effects of the administration of quinin, large doses of this drug having in many instances no appreciable effect in causing their disappearance from the peripheral circulation.

Crescents are of intracellular origin, being transformed stages of the full-grown, pigmented, intracellular spherical bodies which have not been involved in the process of segmentation. These gamete forms continue their development within the corpuscle, from which they derive more and more pigment, thus causing progressive decolorization of their host, until finally all that remains of the corpuscle is a thin shell surrounding the crescent. As its growth progresses the parasite first loses its regular spherical contour, and then becomes drawn out into a long, narrow, spindle-shaped body, which finally becomes bent in the shape of a crescent, the convexity of which lies next to, and for some distance parallels, one margin of the now almost colorless erythrocyte.

Owing to the fact that the early development of the crescents occurs almost exclusively in the deeper circulation, only the later phases of their evolution are ordinarily observed in the peripheral blood. In fresh blood they appear as highly refractive, crescent-shaped bodies, measuring about 6 or 8 $\mu$ from pole to pole, and possessing a distinct double outline, as if they consisted of a central darker body inclosed in a lighter colored membranous envelop. Adhering to the concave surface of the crescent a more or less distinct "bib," the remnant of the corpuscular host, may usually be observed. It varies in color from pale yellow to an almost indistinguishable shade of light lemon, yet it always, on close observation, retains sufficient of the corpuscular color to distinguish it from the parasite to which it is attached. The "bib"

completely bounds the concavity of the crescent in some instances, extending from pole to pole; in other instances—and this is of commoner occurrence—it is of smaller size, extending over only the central portion of the concavity. Occasionally crescentic bodies totally devoid of all traces of their corpuscular host are found, but these forms are rare. The pigment is usually arranged in a moderately compact clump or wreath-like design, in the center of the crescent; less commonly the granules are scattered along the long axis; and very rarely a distinct polar grouping of the pigment at both ends of the crescent is seen. The pigment granules may or may not show active motility. In the fresh specimen it will be noted that in the male crescents the pigment tends to collect centrally in an irregular mass, while in the female crescents it is generally arranged in the form of a wreath.

The ovoid bodies, which are simply transitional forms of the crescents, are of symmetrically oval shape, and show the same refractive protoplasm and apparently double outline observed in the latter. The pigment, which is generally motionless, is arranged in an elongated clump in the center of the ovoid, and a partly decolorized, bib-like corpuscular attachment apparently clings to one side of the body. The long diameter of the ovoid body measures approximately 5 or 6 $\mu$ and its short axis is about 2 or 3 $\mu$ across.

The round forms, derived from the crescentic and ovoid bodies, are the direct antecedents of the flagellate organisms. They appear as perfect spheres, 4 or 5 $\mu$ in diameter, either attached to a more or less yellowish remnant of the erythrocyte or lying entirely free. Their pigment is prone to form a central wreathed or ringed design, or to be massed centrally.

The approach of flagellation is preceded by unusual activity of the central pigment mass, coincidentally with which indications of motility about the periphery of the parasite become apparent. The flagella, which are finally seen to reach out from different points on the periphery of the body, are similar in appearance to those of the tertian and quartan organisms. Their size, however, is about midway between that of these forms. Rarely, a free flagellum may be seen to penetrate and fertilize a female gamete (represented by one of the round non-flagellate forms), which in consequence becomes actively motile, loses its spherical contour, and exhibits violent agitation of its contained pigment.

Degenerative changes of the crescentic, ovoid, and round bodies occur, being evidenced by the development of vacuoles and occasionally by apparent fragmentation.

PIGMENTED LEUCOCYTES AND PHAGOCYTOSIS.

Pigmented leucocytes are found in the blood of all types of malarial infection, and this fact alone, irrespective of the presence of the parasites themselves, is an extremely valuable diagnostic clue to the condition.

In tertian and quartan infections the large mononuclears and polynuclear neutrophiles are the pigment-bearing cells, the granules being found scattered either in fine masses or in fused angular blocks throughout the bodies of the leucocytes. Although both of these forms of leucocytes show this evidence of having acted the rôle of phagocytes, actual visual proof of the performance of this function by the mononuclear forms is wanting. The phenomenon of the phagocytosis by the polynuclear leucocytes may, however, be watched in the fresh specimen, and these cells may be seen to engulf bits of free pigment, flagellate bodies, bastard forms of extracellular parasites, and even, rarely, true segmenting bodies. Distinct periodicity characterizes the performance of phagocytosis in tertian and quartan infections, this process being most conspicuous at the time of segmentation, during and shortly after the paroxysm, when the extracellular forms of the organism are present in the blood in greatest number. Phagocytosis is sometimes seen during the interparoxysmal interval, when only the extracellular forms of parasites which have prematurely escaped from their corpuscular host are attacked.

In estivo-autumnal infections macrophages, derived from the spleen, bone marrow, liver, and blood vessel endothelium, act as phagocytes, as well as the mononuclear and polynuclear cells, which alone exercise this function in the regularly intermittent fevers. Phagocytosis is much less periodical than in tertian and quartan infections, for while it is true that pigmented leucocytes are most numerous in the blood at the time of segmentation, it is also true that they may be observed in great numbers during the interval—a fact which is explained chiefly by the practically continuous segmentation which goes on in these infections because of the presence in the blood of multiple groups of the parasite. Phagocytosis in estivo-autumnal fever differs from that of tertian and quartan infections in that in the former inclusion of both parasite and corpuscular host may occasionally be observed—a phenomenon which does not occur in the latter. Thus, in addition to free pigment and extracellular, segmenting, and flagellate forms, the phagocytic leucocytes are found also to contain whole or portions of necrobiotic erythrocytes, some of the latter, perhaps, inclosing parasites. Osler[1] has observed the phagocytosis of

[1] Brit. Med. Jour., 1887, vol. i, p. 556.

crescentic forms, and the writer believes that he has seen the result of this phenomenon in a single instance. (See Plate VIII, Fig. 25.)

DIFFERENTIAL TABLE OF THE MALARIAL PARASITES.

| Tertian Parasite. | Quartan Parasite. | Estivo-autumnal Parasite. |
|---|---|---|
| Cycle, Forty-eight Hours. | Cycle, Seventy-two Hours. | Cycle, Twenty-four to Forty-eight Hours or Longer. |
| Hyaline body larger than that of quartan and estivo-autumnal organisms; outline indistinct; ameboid movements exceedingly active; long pseudopodia common. | Hyaline body smaller than that of tertian, but usually larger than that of estivo-autumnal organism; outline distinct; ameboid movements slow, except in early forms; marked pseudopodial branching uncommon. | Hyaline body smaller than that of tertian and quartan organisms; outline very sharp and distinct; ameboid movements active in early stages; ring- and disc-shaped forms. |
| Pigment granules fine, very active, and of yellowish brown color; more or less peripherally arranged. | Pigment granules coarse, sluggish, and of dark-brown color; peripheral arrangement striking. | Pigment granules exceedingly fine and scanty; may be either motionless or motile; peripheral arrangement often marked. |
| Mature parasite about 7 $\mu$ in diameter. | Mature parasite about 5 $\mu$ in diameter. | Mature parasite from 1.5 to 7 $\mu$ in diameter. |
| Segmenting body consists of from 15 to 30 segments, arranged in an irregular racemose figure about one or more central pigment clumps. | Segmenting body consists of from 6 to 12 segments, arranged in regular rosette form about a single, compact, central pigment mass, the latter often being radially grouped in the early stages of sporulation. | Segmenting body consists of from 18 to 20 or more segments, arranged either as a regular rosette or irregularly about a single compactly fused central pigment clump. |
| Preflagellate form consists of swollen, spherical pigmented body as large as 10 to 12 $\mu$ in diameter. | Preflagellate form consists of swollen, spherical pigmented body as large as 6 to 8 $\mu$ in diameter. | Preflagellate form consists of spherical pigmented body, 5 to 6 $\mu$ in diameter, and derived from crescentic and ovoid forms, with which they are associated. |
| Flagellate form larger than that of quartan and estivo-autumnal parasite. | Flagellate form smaller than that of tertian and estivo-autumnal parasite. | Flagellate form smaller than that of tertian, but larger than that of quartan, parasite. |
| Erythrocyte becomes very pale and swollen. | Erythrocyte becomes dark and contracted. | Erythrocyte becomes brassy and crenated. |

*Technic of the Blood Examination.*—For diagnostic purposes the fresh, unstained blood film should be invariably preferred to the dried, stained specimen, for in the latter not only are the ameboid movements of the parasite and the dancing of the pigment necessarily lost, but much of the morphology and the finer structure of the organism is also greatly altered. The blood is obtained in the usual manner, and a drop used which is small enough to insure an exceedingly thin film, consisting of a single layer of corpuscles, each lying edge to edge, so that every portion of their flat surfaces may be readily searched for foreign bodies. Thick, dehemoglobinized films are useful for the diagnostic examination of blood in which the parasites are very scanty, but they are unsuitable for accurate histological study. (See p. 77.) If the examination is likely to be prolonged, it is advisable to ring the cover-glass with cedar oil or with vaselin, to prevent crenation of the corpuscles.

Dried blood films, prepared in the usual way, may be used in case the specimens must be sent some distance for examination. Such specimens must be stained with various anilin dyes, as already directed. Polychrome methylene-blue, in the form of Wright's or Goldhorn's solutions, gives the sharpest differentiation of the parasite's histological structure, but solutions of thionin and of eosin and methylene-blue also will prove useful. (See pp. 82 and 88.)

No magnification can be too great in studying the finer points of the malarial parasite, so that a $\frac{1}{12}$-inch oil-immersion objective, with at least a $1\frac{1}{2}$-inch ocular, should be habitually employed for the microscopical examination. While it is frequently convenient to search for individual parasites with a $\frac{1}{5}$- or a $\frac{1}{6}$-inch lens, one cannot well dispense with an immersion objective in distinguishing their finer characteristics. The substage condenser and iris diaphragm should be so adjusted that the field is dimly illuminated, and not drowned in a flood of white light. When the ameboid movements of the parasite are to be studied at length, a warm stage is useful, but not essential, if the temperature of the room is not too low.

The best time for the examination is a few hours before the onset of the expected paroxysm, at which period it is common to find full-grown pigmented organisms and often an occasional segmenting form, if the specimen is from a tertian or quartan infection. In estivo-autumnal fever relatively large ring- and disc-shaped bodies, containing exceedingly delicate pigment granules, are usually abundant at this time. Intracellular hyaline forms are most numerous in the blood a few hours subsequent to the paroxysm in all three forms of the infection.

The writer would urge the beginner systematically to study the development of a group of parasites by examining the blood of a single case of malarial fever at frequent intervals between the paroxysms. For example, the life history of the tertian parasite, from the youngest hyaline amebula to the segmenter and the flagellate body, may be traced in most cases of tertian fever if the blood is examined every three or four hours, day and night, for a period of forty-eight hours. Such a collated series of observations, although they entail close and tiresome application for the time, will prove more profitable to the student in his comprehension of the organism's developmental cycle than dozens of haphazard examinations made in many different cases at odd times.

To the unpractised eye a number of artefacts occurring in fresh blood specimens may for a time be confused with the malarial parasite, but careful observation linked to an increased familiarity with the appearance of the organism and of its counterfeits will eliminate such sources of error. The following are the principal objects which require to be differentiated from the malarial parasite: (1) The central biconcavity of the normal erythrocytes; (2) morphological changes in the erythrocytes, and (3) hemokoniæ.

1. At first glance the pale *central biconcavity* of the erythrocyte somewhat resembles the young hyaline tertian parasite, for each has an indistinct outline which merges with the surrounding corpuscular substance. But the parasite is rarely in the center of the blood cell, it is actively ameboid, and it possesses a characteristic pearly-gray appearance. On the other hand, the pale area of the corpuscle is in the center of the normally shaped blood cell, it never exhibits ameboid powers, and its appearance is clean white or yellowish-white. It is, of course, uncolored in the stained specimen.

2. The morphological changes in the erythrocyte, which may be mistaken for malarial organisms, are those produced by *vacuolization*, *crenation*, and *fragmentation* of these cells. *Vacuoles* appear as highly refractive, clean-cut, spherical bodies which possess more or less oscillating, rotary motility, in contrast to the dimmer, more vaguely outlined, truly ameboid forms of the hyaline malarial parasite. The spicula of crenated red cells may in a very dim illumination of the object appear at first glance somewhat like the coarse granules of the mature pigmented parasite, but a change of focus and a wider-open diaphragm immediately dispels the illusion. *Fragmentation* of the erythrocytes, as the result of thermic influences, may produce a most *bizarre* and peculiar variety of designs, the most confusing of which is a

sort of flagellate appendage which appears to originate in a fragmented sphere of corpuscular substance, to which it is attached. The size of this body, however, is far too small to be mistaken seriously for a true malarial flagellate body, for its spherical portion, which is unpigmented and tinged with hemoglobin, measures only about 2 $\mu$ in diameter; while the flagellate appendage, usually single, is represented by a colorless, thin line not often longer than 3 or 4 $\mu$, and tremulously motile, not ameboid like the flagellum of the malarial body. This sort of a flagellate figure is very commonly seen in blood slides which have become chilled.

3. *Hemokoniæ* are readily distinguished by their very small size, spherical contour, and glistening, fat-like appearance. It sometimes happens that one of these granules of "blood dust," in its Brownian excursion across the field of the microscope, lies over the flat surface of an erythrocyte, simulating for the moment a small, hyaline, intracellular parasite. It seems probable, also, that one of these granules, observed just at the instant it crosses the rim of the blood cell, has been mistaken for a hyaline spore in the act of invading an erythrocyte, by those who believe that they have witnessed this remarkable phenomenon. (See p. 452.)

HEMOGLOBIN AND ERYTHROCYTES.

Well-marked anemia, developing early during the course of the disease, and proportionate in degree to the severity of the attack, is a conspicuous clinical sign in the malarial fevers.

Dionisi,[1] Thayer,[2] and other authors have observed that a loss of hemoglobin and a diminution in the number of erythrocytes occur after every paroxysm, this being due largely to the destruction of immense numbers of parasite-containing corpuscles by the maturation of the organisms, and in part to the presence in the blood of other substances destructive to the uninvaded red cells. The loss is especially marked after the early paroxysms, being of slighter degree after those occurring later in the course of the disease. On the other hand, *during* the paroxysm a tendency on the part of the erythrocytes to increase in number has been noted.

The loss is more moderate in the regularly intermittent tertian and quartan types of malaria than in the estivo-autumnal form. In the former types, the regenerative powers of the blood are usually prompt and vigorous, so that the normal number of cells is almost restored by the onset of the succeeding paroxysm. It is owing to this fact that repeated paroxysms must occur before the anemia becomes striking.

[1] Lo Sperimentale, 1891, f. iii and iv, p. 284. [2] *Loc. cit.*

In the estivo-autumnal form the loss is far greater, a decrease of 500,000 or more corpuscles per c.mm. sometimes occurring after a single paroxysm, so marked a loss as this being associated especially with cases in which excessive numbers of parasites are present in the blood. Even in non-febrile cases of larval malarial fever Marchiafava and Bignami [1] have observed more or less anemia. Organisms of the crescent group appear to exert no influence in causing diminution in the number of erythrocytes. Regeneration of the blood is slow in the estivo-autumnal fever, so that the loss of hemoglobin and of corpuscles is not made up during an interparoxysmal interval, in consequence of which more marked and graver anemias are commoner than in the tertian and quartan fevers. If the anemia is markedly developed during the early stages of the infection, the corpuscular decrease is aggravated slightly, if at all, by the following paroxysms.

In malarial hemoglobinuria an enormous destruction of corpuscles occurs, "a destruction," in the words of Thayer,[2] "too great, probably, to be dependent wholly on the disintegration of parasitiferous elements. We are compelled . . . to suppose the existence of some condition which renders the *uninfected* red blood corpuscles unusually vulnerable, possibly some change in the blood serum by which its isotonicity is markedly disturbed."

Usually the hemoglobin loss is relatively less than the corpuscular decrease, fairly high color indices being the general rule, but in some cases both are parallel. In estimates made by the author in 45 cases of malarial fever, nearly all of the tertian type, the hemoglobin averaged 67 per cent. of normal, ranging, in the individual case, from 19 to 97 per cent.

The variations in hemoglobin were as follows:

| Hemoglobin Percentage. | Number of Cases. |
|---|---|
| From 90–100 | 2 |
| " 80–90 | 12 |
| " 70–80 | 11 |
| " 60–70 | 7 |
| " 50–60 | 4 |
| " 40–50 | 5 |
| " 30–40 | 2 |
| " 20–30 | 1 |
| " 10–20 | 1 |

Average, 67 per cent.
Maximum, 97 "
Minimum, 19 "

[1] *Loc. cit.*

[2] *Loc. cit.*

The loss of corpuscles varies within wide limits, being most marked in severe and in long-standing cases. Counts as low as 500,000 per c.mm. have been reported,[1] and the number falls to from 1,000,000 to 2,000,000 in a considerable proportion of cases. In the above series the average of the 45 counts showed 2,585,688 erythrocytes per c.mm., individual cases varying from 1,410,000 to 5,250,000. The range of the counts is shown thus, in tabular arrangement:

| Erythrocytes per c.mm. | Number of Cases. |
|---|---|
| Above 5,000,000 | 2 |
| From 4,000,000–5,000,000 | 22 |
| " 3,000,000–4,000,000 | 8 |
| " 2,000,000–3,000,000 | 8 |
| " 1,000,000–2,000,000 | 5 |

Average, 2,585,688 per c.mm.
Maximum, 5,250,000 " "
Minimum, 1,410,000 " "

In 7 cases clinically designated as "malarial cachexia," in which parasites were not found in the circulating blood, the following results were obtained: hemoglobin ranged from 40 to 52 per cent., the average being 45.5; color index, from 0.41 to 1.13, averaging 0.66; and erythrocytes, from 2,300,000 to 4,861,000 per c.mm., with an average of 3,406,250.

As regeneration of the blood, which is generally slow, takes place, the normal percentage of hemoglobin is reached more slowly that that of the corpuscles—in fact, in some instances of post-malarial anemia subnormal hemoglobin percentages persist for indefinite periods after convalescence has been established.

Histological changes in the erythrocytes are marked in relation to the severity of the anemia. Pallor of the corpuscles is often conspicuous, and poikilocytosis and deformities of size are present in severe cases. In such instances small percentages of normoblasts and of atypical nucleated forms are not infrequently found, sometimes in association with an occasional megaloblast. In severe cases both polychromatophilia and basic granular degeneration of the erythrocytes are familiar findings.

The peculiar "brassy" appearance of the erythrocytes ("*globuli rossi attonati*" of the Italians) invaded by the estivo-autumnal parasite has already been noted (p. 459).

In tertian fever, and only in this type of malaria, the infected erythrocytes, when stained by Romanowsky's method, show Schüffner's granules, recognized as a reddish mottling of portions of the cells not occupied by the parasites.

[1] Kelsch, Arch. Physiol., 1875, vol. ii, p. 690; *ibid.*, 1876, vol. iii, p. 490.

In estivo-autumnal fever the infected cells, when similarly stained, show minute clefts and cracks and coarse, irregular areas reacting basically. Stephens and Christophers[1] demonstrate these signs of necrobiosis by staining chloroform-fixed films by the Romanowsky method for one hour, and differentiate them from the familiar basic stippling and the reddish Schüffner's granules of erythrocytes harboring the tertian parasite.

The following four types of post-malarial anemia are distinguished by Bignami and Dionisi.[2]

1. Anemias in which examination of the blood shows alterations similar to those observed in secondary anemias, from which they differ only in that the leucocytes are diminished in number. The greater part of these cases go on to recovery; a few, without any further change in the hematological condition, pursue a fatal course.

2. Anemias in which the examination of the blood shows alterations similar to those seen in pernicious anemia—prevalence of megaloblasts. These cases end fatally.

3. Anemias which are progressive as a result of the lack of compensation by the marrow for losses brought about by the infection. At autopsy the marrow of the long bones is found to be wholly yellow, while the marrow of the flat bones is also poor in nucleated erythrocytes.

4. Chronic anemias of the cachectic, which differ from the above-mentioned types by clinical and anatomical characteristics in that the special symptoms of malarial cachexia prevail, while one observes, postmortem, a sort of sclerosis of the bone marrow. The marrow of the long bones is red and of an increased consistency; the giant cells are very numerous and many are necrotic; the nucleated erythrocytes are very rare, and the colorless polynuclear corpuscles are present in small numbers.

LEUCOCYTES.

Distinct leucopenia, or at least an absence of leucocytosis, is almost invariably found in the uncomplicated cases of malarial fever, the exceptions to this general rule occurring during the grave paroxysms of the pernicious type of fever.

The subnormal range of the leucocytes in malarial fever was early noted by Kelsch,[3] and has been repeatedly confirmed by other investigators since the former's statement of the fact. Billings,[4] in particular, has carefully studied this question, and his

[1] Brit. Med. Jour., 1903, vol. i, p. 730.

[2] Centralbl. f. allg. Path. u. path. Anat., 1894, vol. v, p. 422. (Cited by Thayer and Hewetson, "The Malarial Fevers of Baltimore," Baltimore, 1895, p. 58.)

[3] *Loc. cit.*

[4] Johns Hopkins Hosp. Bull., 1894, vol. v, p. 89.

examinations, 100 in number, show that the number of leucocytes averaged 4323 per c.mm., or a decrease of about 38 per cent. below normal. In 71 counts made by this reporter in 16 cases, to determine the effects of the malarial paroxysms on these cells, it was found that during the early part of the paroxysm their number gradually increased, the maximum being reached, as a rule, two or three hours after the chill. Following this maximum increase the number steadily and progressively decreased, hour by hour, until the minimum was reached during the period of subnormal temperature, at the end of the paroxysm. During the afebrile interval the number of leucocytes is distinctly subnormal, but it rises slightly again just before the onset of the following chill, so that the average count is slightly higher immediately before the chill than during the rest of the interval.

In the author's series, above referred to, the average of 45 counts showed 5622 leucocytes per c.mm., the lowest count being 2000, and the highest 12,800. All these counts were made during the interval between the paroxysms, in uncomplicated cases, so far as it was possible to determine.

The counts ranged as follows:

| Leucocytes per c.mm. | Number of Cases. |
|---|---|
| Above 10,000 | 3 |
| Between 8,000–10,000 | 9 |
| " 6,000– 8,000 | 9 |
| " 4,000– 6,000 | 10 |
| " 2,000– 4,000 | 14 |
| Average, 5,622 per c.mm. | |
| Maximum, 12,800 " " | |
| Minimum, 2,000 " " | |

In the 7 cases of "malarial cachexia" the number of leucocytes to the c.mm. ranged from 4500 to 44,000, the average being 16,971. Five of these cases had distinct leucocytosis, a condition believed by Thayer to occur in some of the post-malarial anemias, usually those following short-lived infections.

Relative lymphocytosis, sufficiently decided to become a striking characteristic of the condition, is practically a constant qualitative change. As a rule, the increase affects chiefly the large lymphocytes. Christophers and Stephens,[1] in a study of "blackwater fever," found that this type of cells often constituted 20 per cent., 30 per cent., or even 50 per cent. of the total number of leucocytes; furthermore, they state that this relative increase bears an inverse relation to the temperature curve, being least marked during the

[1] Lancet, 1901, vol. i, p. 848.

pyrexia and greatest during the periods of apyrexia. This feature of the blood picture, which is of considerable importance, has also been noted by Delafly,[1] by Rogers,[2] and by Daniels.[3] The percentage of eosinophiles is, as a rule, subnormal, and this variety of cells is frequently absent; more rarely they are slightly increased, especially in some of the post-malarial anemias. Myelocytes in small numbers are very commonly found, in the writer's experience, especially in estivo-autumnal infections and in cases with pronounced anemia. In 9 differential counts, made in cases of the series above referred to, the relative percentages of the different forms of leucocytes averaged as follows:

| | |
|---|---|
| Small lymphocytes | 15.33 per cent. |
| Large lymphocytes and transitional forms | 15.94 " |
| Polynuclear neutrophiles | 67.00 " |
| Eosinophiles | 0.83 " |
| Myelocytes | 0.51 " |

Practically the same figures were obtained from the similar examination of 5 cases of anemia associated with malarial cachectic conditions.

The *blood plaques* are greatly decreased in number, as in other febrile conditions. They fail to agglutinate in both tertain and quartan fevers, according to Zeri and Almazia,[4] unless cinchonization is pushed sufficiently to antidote the infection. In normal blood and in the blood of various non-malarial fevers the plaques clump together in masses just before and during the process of clotting. Ducchesi's method may be used to show this phenomenon macroscopically. (See p. 199.)

Diagnosis. The detection of the specific parasite in the circulating blood is proof positive of malarial fever, the exact type of which may be determined by close study of the organism's peculiarities. Even if nothing more definite than pigmented leucocytes is found, the evidence is strongly in favor of some form of paludism. The progressive anemia and the leucopenia involving a relative decrease in the polynuclear neutrophiles are also valuable side-lights on the diagnosis. An obscure intermittent fever which shows leucocytosis is almost certainly not malarious.

The chills and pyrexia of *sepsis* and of *tuberculosis* are not in-

[1] Brit. Med. Jour., 1903, vol. i, p. 725.
[2] *Ibid.*, 1902, vol. i, p. 827; also Lancet, 1903, vol. i, p. 1500.
[3] Cited by Manson, Lancet, 1902, vol. i, p. 1377.
[4] Il Policlin., 1903, vol. ix, p. 485.

frequently misinterpreted as symptoms of malarial fever. In septicemia leucocytosis is usually found, but even should the leucocytes not be increased in number, they fail to show the relative lymphocytosis of malarial blood. In pure tuberculosis the blood picture of malaria may be counterfeited, in so far as the quantitative and qualitative leucocyte changes are concerned, and in such instances the parasite must be demonstrated to settle the diagnosis.

*Enteric fever*, like malaria, shows anemia, an absence of leucocytosis, and relative mononucleosis. But in typhoid the small lymphocytes are increased, while in malaria the large mononuclear forms are in excess. Minor points of difference are the more rapid onset of the anemia, the greater frequency of decided leucopenia, and the tendency toward higher percentages of myelocytes in malaria. The pertinence of positive examinations for the specific parasite and for the serum reaction is obvious. It may be added that in those rare instances of coincident typhoid and malaria the blood of the same individual may contain malarial parasites and give a positive serum reaction with the Eberth bacillus. In such cases blood cultures prove the presence of the typhoid infection.

## XLIV. MALIGNANT DISEASE.

### Carcinoma.

General Features.

There is no deviation from normal in the *coagulability* and the amount of *fibrin*, except in the event of ulcerative and inflammatory changes affecting the tumor, but, should these conditions be present, coagulation may occur with abnormal rapidity, and the density of the fibrin network almost invariably increases. The *specific gravity* may or may not be subnormal, according to whether or not the percentage of hemoglobin, which it parallels, is reduced. The *alkalinity* of the blood is almost always decreased in gastric cancer, according to Krokiewicz.[1]

Relatively large amounts of *sugar* (as high as 3 parts per 1000) have been found in the blood of patients suffering from various forms of carcinoma, especially visceral cancer, in contradistinction to more superficial growths, involving, for example, the skin and mucous membranes. In no other disease except diabetes has more than one-third of the above-named quantity of sugar been detected in the blood, according to the analyses made by Trinkler.[2]

[1] Arch. f. Verdauungskr., 1900, vol. vi, p. 25.
[2] Centralbl. f. d. med. Wissensch., 1890, vol. xxviii, p. 498.

Examination of the blood for the detection of a *specific parasite* of cancer has thus far proved unconvincing, although much careful work has been done with this purpose in view. None of the many bacteria exploited as the cause of cancer has fulfilled Koch's law, and many of them have been shown to be artefacts. The protozoan theory of cancer, defended chiefly by Ruffner,[1] Gaylord,[2] and Feinberg,[3] has been criticized on the grounds that the so-called protozoa found in the neoplasms are nothing more than cell inclusions and degenerations. The same stricture has been urged against the theory advocated by Russell,[4] Sanfelice,[5] and Plimmer,[6] who interpret the factor of cancer as a yeast fungus. The careful studies of 33 cases of cancer by Maragliano[7] have apparently disproved the statements of a number of authors, who claimed to have cultivated blastomycetes from the circulating blood in this disease.

HEMOGLOBIN AND ERYTHROCYTES.

During its incipiency carcinoma gives rise to practically no changes in the erythrocytes or their hemoglobin content, or, at the most, causes simply a moderate diminution in the latter. As the disease progresses and extends, and as the cachexia of the patient becomes more pronounced, a secondary anemia develops, attaining but a moderate degree in some instances, but in others becoming so extreme as to simulate in some particulars true pernicious anemia. The anemia of cancer differs from other forms of secondary anemia in stubbornly persisting, or at the most improving but slightly, under treatment. Since the hemoglobin loss usually anticipates the cellular decrease, the blood picture of early cancer not infrequently resembles that of chlorosis. Later, however, these conditions may be reversed, so that the index rises. In the author's experience, the average hemoglobin loss has amounted to about 33 per cent., and the erythrocyte decrease to about 22 per cent., of normal. The color index tends to range moderately below normal, usually from 20 to 30 points below the standard of health. It averaged 0.86 for the 145 cases grouped below. As just intimated, it is generally lower in the early than in the late stages of the disease. In operative cases of carcinoma it has been observed that the regeneration time of the hemoglobin averages at least two-thirds longer than in

[1] "Sur les Parasites des Tumeurs Epithéliales Malignes," 1896.
[2] Amer. Jour. Med. Sci., 1901, vol. cxxi, p. 503.
[3] "Das Gewebe und die Ursache der Krebsgeschwülste," 1903.
[4] Brit. Med. Jour., 1900, vol. ii, p. 1356.
[5] Zeitschr. f. Hyg. u. Infektionskr., 1898, vol. xxix, p. 463.
[6] Practitioner, 1899, vol. lxvii, p. 430.
[7] Gaz. degli Ospedali e. d. Clin., 1900, vol. xxi, p. 1538; also Sem. méd., 1901, vol. xxi, p. 63.

other diseases treated surgically, and that the loss of hemoglobin after operation is usually not less than 15 per cent. Bierfreund[1] finds that the percentage of hemoglobin after the removal of the tumor never equals that found before the operation.

The oligocythemia is occasionally most striking, for in some cases the counts may range as low as between 1,000,000 and 2,000,000, such a degree of decrease apparently being most commonly found in septic cases and in gastric cancer. F. P. Henry's statement[2] that he has never seen a case of the latter disease in which the erythrocytes fell below 1,500,000 to the c.mm. has been generally corroborated, although counts below this figure have been occasionally reported. In one of the cases of cancer of the stomach included in the table given below the count was 1,001,000 per c.mm., and the hemoglobin percentage 50; in another case the count was 1,240,000 and the hemoglobin 56 per cent.

Polycythemia may occur as a temporary condition in gastric and esophageal cancer, as the result of blood concentration due to vomiting, to diarrhea, or to lack of ingested fluids. In such instances the number of erythrocytes not uncommonly exceeds 6,000,000 or 7,000,000 per c.mm., and, exceptionally, even a higher figure.

The following table illustrates the alterations in the amount of hemoglobin and number of erythrocytes, as determined by the examination of 145 cases of various forms of carcinoma:

| Hemoglobin Percentage. | Number of Cases. | Erythrocytes per c.mm. | Number of Cases. |
|---|---|---|---|
| From 90–100 | 5 | Above 5,000,000 | 13 |
| " 80– 90 | 30 | From 4,000,000–5,000,000 | 66 |
| " 70– 80 | 32 | " 3,000,000–4,000,000 | 39 |
| " 60– 70 | 29 | " 2,000,000–3,000,000 | 18 |
| " 50– 60 | 21 | " 1,000,000–2,000,000 | 9 |
| " 40– 50 | 12 | | |
| " 30– 40 | 10 | | |
| " 20– 30 | 4 | | |
| " 10– 20 | 2 | | |
| Average, 67.0 per cent. | | Average, 3,897,923 per c.mm. | |
| Maximum, 94.0 " " | | Maximum, 5,900,000 " " | |
| Minimum, 12.0 " " | | Minimum, 1,001,000 " " | |

In gastric cancer Osler and McCrae[3] report an average of 49.9 per cent. of hemoglobin in 52 cases, and an average erythrocyte

[1] Langenbeck's Arch., 1890–91, vol. xli, p. 1.
[2] Arch. f. Verdauungskr., 1898, vol. iv, p. 1.
[3] "Cancer of the Stomach," London and Philadelphia, 1900, p. 115.

count of 3,712,186 in 59 cases. In two cases the count was less than 1,500,000. An average color index of 0.63 was found in this series. The author, in a series of 46 cases, found the anemia less decided, as shown by these averages: hemoglobin, 67.5 per cent; erythrocytes, 4,020,978; color index, 0.84. Lang[1] finds that the isotonicity of the erythrocytes is increased in this disease, and this change he attributes to the organism's attempt to combat some obscure hemolytic agent peculiar to the cancerous process.

Deformities of shape and of size are marked in relation to the grade of the anemia which exists. Poikilocytes may be quite as numerous and as striking as in true pernicious anemia, while the alterations affecting simply the size of the cells tend toward microcytosis rather than megalocytosis. Polychromatophilia and basophilic degenerative changes are frequently to be seen in grave cases with marked cachexia.

Erythroblasts are very common, especially in cancer with decided cachexia and high-grade anemia, but their occurrence is by no means limited to such cases, as they may also be found in blood which shows but trifling quantitative deterioration. It may be stated as an accepted fact that nucleated erythrocytes occur in cancer more frequently than in any other variety of secondary anemia, except that accompanying sarcoma.

Normoblasts are generally found to the exclusion of other forms, although in an exceptionally grave case an occasional megaloblast and atypical "mesoblast" may be encountered. The important point to be remembered is that cells of the adult, normoblastic type invariably predominate, since megaloblasts, when present, are never so numerous as normoblasts.

LEUCOCYTES.

Leucocytosis is a frequent but not a constant feature of the blood picture in carcinosis, for more cases are encountered in which the number of leucocytes is normal than those in which an increase prevails. Judging from the statistics of patients treated in the German Hospital, leucocytosis is present in less than one-third of all forms of cancer, or in 31 per cent. In general terms, it may be said that tumors characterized by active inflammatory changes, by hemorrhage, by rapid growth, or by extensive metastases are accompanied by a well-marked leucocyte increase, while non-inflammatory, slowly developing, localized tumors do not raise the count. Thus, a large carcinoma of the liver or kidney, for instance, may cause a leucocytosis of 30,000 or 40,000 to the c.mm., while a small, limited skin cancer may exist without provoking

[1] Zeitschr. f. klin. Med., 1902, vol. xlvii, p. 153.

the slightest increase. Thorough extirpation of the growth is followed by a decline in the leucocytosis, the normal count being reached by the time the wound has entirely healed. Hayem[1] is the authority for the statement that in mammary cancer recurrence of the growth after its removal may be detected by a reappearance of the leucocytosis, which antedates all other physical signs. The constancy of this change, as well as the question of its occurrence in cancer involving other structures, still remains to be investigated.

It seems reasonable to attribute the origin of cancer leucocytosis chiefly to the presence of inflammatory changes and to hemorrhage in the tissues in the neighborhood of the growth, although in some instances it seems possible that positive chemotaxis may be excited by the absorption of toxins derived from the breaking down of the neoplasm itself. The strength of the patient's powers of resistance as a determining factor of the increase must also be taken into account in this as in other diseases.

In the writer's experience, leucocytosis is most constant and most striking in cancer of the liver, least frequent in cancer of the uterus, and least conspicuous in cancer of the stomach. In cancer of the esophagus absence of leucocytosis is the rule, while in many cases a decided leucopenia may exist. Skin cancers, unless ulcerated and inflamed, do not raise the count.

The 145 cases on the study of which the above observations are based may be summarized as follows:

| Seat of Growth. | Number of Cases. | Number and Percentage of Cases with Leucocytosis. | Average Count. | Maximum Count. | Minimum Count. |
|---|---|---|---|---|---|
| Stomach . | 69 | 17 or 24.6 per cent. | 7,858 | 23,400 | 1,000 |
| Uterus .. | 22 | 3 " 13.6 " | 11,225 | 24,000 | 3,200 |
| Rectum . | 15 | 4 " 26.6 " | 9,650 | 16,000 | 6,000 |
| Breast .. | 18 | 6 " 33.3 " | 10,163 | 31,500 | 5,200 |
| Liver ... | 10 | 8 " 80.0 " | 17,549 | 40,800 | 8,000 |
| Bowel ... | 7 | 5 " 71.4 " | 11,185 | 16,300 | 7,000 |
| Pancreas | 4 | 2 " 50.0 " | 10,850 | 18,200 | 6,660 |

As compared with the above, this summary of Cunliffe's 71 cases[2] shows a decidedly higher leucocyte average and range:

[1] *Loc. cit.*

[2] Med. Chronicle, 1903, vol. xxxviii, p. 333.

| Seat of Growth. | Number of Cases. | Average Count. | Maximum Count. | Minimum Count. |
|---|---|---|---|---|
| Stomach | 10 | 17,280 | 36 800 | 5 200 |
| Uterus | 8 | 22,800 | 59,200 | 7 000 |
| Rectum | 10 | 12,780 | 20,200 | 6,800 |
| Breast | 18 | 11,400 | 24,800 | 7,800 |
| Esophagus | 13 | 13,700 | 39,800 | 10,000 |
| Tongue | 12 | 13,400 | 24,800 | 7,800 |

In cancer of the stomach *digestion leucocytosis* is usually, though by no means invariably, absent. The frequency with which this phenomenon is absent is shown by the following compilation of the data of various authorities who have studied this question:

| Author. | Number of Cases. | Absent. | Present. |
|---|---|---|---|
| Hoffman | 24 | 21 | 3 |
| Osler and McCrae | 22 | 12 | 10 |
| Cabot | 20 | 19 | 1 |
| Schneyer | 18 | 18 | 0 |
| Capps | 17 | 15 | 2 |
| Krokiewicz | 17 | 13 | 4 |
| Douglas | 11 | 6 | 5 |
| Hartung | 10 | 10 | 0 |
| Müller | 5 | 5 | 0 |
| | 144 | 119 | 25 |

These figures, referring to 144 cases, show that digestion leucocytosis is absent in 82.6 per cent. of gastric carcinomata. But the presence of the phenomenon in practically one case out of five is sufficient to weaken materially the former belief that absence of digestion leucocytosis is a diagnostic sign of this disease. Furthermore, it has also been shown by Hoffman [1] and others that the sign may be absent in a number of other diseases of the stomach, as well as in some apparently healthy individuals.

Rencki,[2] from an investigation of 15 cases, concludes that the digestion leucocytosis of gastric cancer, when present, averages an increase of 3500 cells, and that this acme is reached the third or fourth hour after taking food.

Differential counts usually show percentages of polynuclear neutrophiles ranging between 80 and 90, with a corresponding decrease in the large and small lymphocytes, in cases with leucocytosis, and not infrequently also in those without. This

[1] Zeitschr. f. klin. Med., 1898, vol. xxxiii, p. 460.
[2] Arch. f. Verdauungskr., 1901, vol. vii, pp. 234 and 392.

change is not to be considered constant, since relatively high percentages of lymphocytes, especially of the large variety, have occasionally been observed. The eosinophiles are usually decreased, or, indeed, they may be absent in cases with pronounced leucocytosis; in a certain proportion of cases, in spite of the abnormally high leucocyte count, their relative percentage remains within the limits of health. Myelocytes are extremely common, small numbers of these cells (usually not higher than a fraction of one per cent.) occurring in at least a majority of all cases of cancer. In cancer with bone metastases these cells are much more abundant. (See p. 259.) The presence of a few basophiles is sometimes to be noted, particularly often in association with conspicuously high leucocytoses.

## Sarcoma.

General Features.

The changes affecting the *fibrin*, the rate of *coagulation*, and the *specific gravity* of the blood are similar to those prevailing in cancer, and therefore require no further mention.

In contrast to the hyperglycemia of carcinoma, the researches of Trinkler, previously referred to, tend to show that in sarcoma no increase above normal in the amount of *sugar* in the blood can be detected. *Bacteriological examinations* of the blood have thus far given no definite results.

Loeper and Louste [1] report having found sarcoma cells in the blood of three cases of sarcoma, one affecting the neck, one the shoulder, and one being a general sarcomatosis. These observers centrifugalized a mixture of 20 drops of finger blood and 15 c.c. of a one per cent. aqueous solution of acetic acid, and detected in the resulting sediment cells precisely similar in morphology and other biological characteristics to those of the neoplasms in question. In carcinoma, on the contrary, specific cytological findings in the blood were absent. These experiments, if substantiated, should be of signal value in the diagnosis of deep-seated sarcomata. They tend also to corroborate the belief that sarcoma, but not cancer, spreads by the blood stream.

Hemoglobin and Erythrocytes.

The changes in the hemoglobin and erythrocytes are not materially different from those found in cancer, for the genesis of the blood deterioration is doubtless similar in all forms of malignant disease. Some authors believe that the anemia tends to reach a higher degree in sarcoma than in carcinoma, but the truth of this contention certainly does not appear to be

[1] Sem. méd., 1904, vol. xxiv, p. 36.

indisputably established. In the writer's experience, the intensity of the anemia is practically similar in both these forms of neoplasms, or, if anything, somewhat more striking in cancer, both individually and on the average. In a series of 34 cases of sarcoma the following data were obtained:

| Hemoglobin Percentage. | Number of Cases. | Erythrocytes per c.mm. | Number of Cases. |
|---|---|---|---|
| Above 100 | 1 | Above 5,000,000 | 5 |
| From 90–100 | 1 | From 4,000,000–5,000,000 | 13 |
| " 80–90 | 8 | " 3,000,000–4,000,000 | 12 |
| " 70–80 | 6 | " 2,000,000–3,000,000 | 3 |
| " 60–70 | 6 | " 1,000,000–2,000,000 | 1 |
| " 50–60 | 6 | | |
| " 40–50 | 4 | | |
| " 30–40 | 1 | | |
| " 20–30 | 1 | | |
| Average, 65.5 per cent. | | Average, 3,962,705 per c.mm. | |
| Maximum, 101 " | | Maximum, 5,400,000 " " | |
| Minimum, 25 " | | Minimum, 1,400,000 " " | |

Poikilocytes, microcytes, megalocytes and atypically stained cells are common in cases with pronounced anemia, and in such instances erythroblasts, the majority of which are always normoblasts, are also to be looked for.

Leucocytosis, while inconstant in sarcoma, is without doubt more frequently associated with this lesion than with carcinoma. Statistics have also been advanced to demonstrate that the counts range higher than in cancer, but the cases on record are still far too few to warrant this conclusion. The behavior of the leucocytes in both forms of malignant disease is probably influenced by the same group of factors. In the cases summarized in this series, leucocytosis was found in 45 of the 145 carcinomata, or in 31 per cent., and in 20 of the 34 sarcomata, or in 58.8 per cent. In the latter the counts varied as follows:

Leucocytes.

| Leucocytes per c.mm. | Number of Cases. |
|---|---|
| From 30,000–40,000 | 1 |
| " 20,000–30,000 | 2 |
| " 15,000–20,000 | 5 |
| " 10,000–15,000 | 13 |
| " 5,000–10,000 | 13 |
| Average, 12,282 per c.mm. | |
| Maximum, 40,000 " " | |
| Minimum, 5,000 " " | |

The increase usually involves a large absolute and relative gain in the polynuclear neutrophiles at the expense of the lymphocytes, although in an occasional instance the latter reach a disproportionately high percentage, while the former decline to a subnormal figure. It may be added that either of these differential changes may also occur in the absence of an increase in the total number of leucocytes. The percentage of eosinophiles is usually subnormal, and not infrequently these cells may be searched for in vain. Rarely, marked eosinophilia has been reported in sarcomata with bone metastases, but such findings are by no means constant. Decided myelemia (as high as 10 or 15 per cent.), according to Kurpjuweit,[1] may develop as the result of the altered hemogenesis excited by invasion of the bone marrow by malignant tumors. In all forms of sarcoma small numbers of myelocytes are to be observed as frequently as not, these cells being about as common and as numerous as they are in cancer—a remark which is also true of basophiles.

DIAGNOSIS. The clinical resemblance between certain forms of malignant disease (especially those in which the lesion remains obscure or undemonstrable) and *pernicious anemia* is often very close, on account of the striking degree of cachexia apparent in both. But the blood changes found in these two conditions, although similar in some respects, are sufficiently characteristic to afford the necessary diagnostic clue. These differences, already referred to in a preceding section, may, for the sake of emphasis, be expressed as follows:

| *Malignant Disease.* | *Pernicious Anemia.* |
|---|---|
| Color index usually moderately low. | Color index almost always high. |
| Oligocythemia usually marked. | Oligocythemia invariably extreme. |
| Tendency toward microcytosis. | Tendency toward megalocytosis. |
| Erythroblasts common, normoblasts always predominating. | Erythroblasts constant, megaloblasts always predominating. |
| Leucocytosis common. | Leucocytosis rare. |
| Lymphocytosis rare. | Lymphocytosis common. |

It is to be noted that of the above changes, but one is characteristic—the invariable predominance of megaloblastic cells in

[1] Deutsch. Arch. f. klin. Med., 1903, vol. lxxvii, p. 553.

pernicious anemia and their minority or absence in those cases of malignant disease in which nucleated erythrocytes are found.

Should a doubt arise as to whether a tumor is *benign* or malignant in character, the fact is to be remembered that the presence of a persistent leucocytosis, especially if accompanied by anemia, is decidedly in favor of its malignancy. Should it be necessary to distinguish between a malignant growth and an obscure *pus focus* with sepsis, the blood examination, aside from culturing, is useless, since both leucocytosis and hyperinosis may or may not exist in either condition; if, however, bacteriological findings are positive, the existence of a septicemia is obvious.

As a means of differentiating *carcinomata* and *sarcomata*, the chemical examination of the blood for sugar should prove of the greatest clinical value, if further research substantiates the claims made that hyperglycemia is constant in the first, and absent in the second, type of neoplasms.

As a means of distinguishing gastric cancer from *gastric ulcer* the blood count is, unfortunately, of doubtful utility. The presence of a persistent, well-marked leucocytosis is a very significant sign of cancer, since in ulcer the count is not increased except as the result of hemorrhage, perforation, or digestion. On the other hand, an absence of leucocytosis is of no value in determining which condition is present, owing to the fact that no increase occurs in a large proportion of stomach cancers. Tuffier,[1] who found that tumors of epithelial origin cause mononucleosis, contends that it is possible by this sign to differentiate gastric cancer and ulcer. This observation was not confirmed by Monisset and Tolot,[2] whose studies show that it is impossible to take the differential count as a criterion of diagnosis. Recent investigations have fully corroborated Löwit's view that the absence of digestion leucocytosis in gastric cancer has about the same diagnostic value as the absence of hydrochloric acid and the presence of lactic acid.

The chief points of distinction in the blood pictures associated with the two diseases in question are illustrated by this table:

| *Gastric Cancer.* | *Gastric Ulcer.* |
|---|---|
| Anemia usually marked. | Anemia usually moderate, except after hemorrhage. |
| Erythroblasts common. | Erythroblasts rare. |
| Leucocytosis common. | Leucocytosis rare. |
| Absence of digestion leucocytosis the rule. | Absence of digestion leucocytosis the exception. |

[1] Presse méd., 1901, vol. viii, p. 246.

[2] Rev. de méd., 1902, vol. xxii, p. 844.

If the diagnosis lies between carcinoma, *amyloid disease*, and *gumma* of the liver, the presence of a leucocytosis suggests the first; should it lie between cancer and *hypertrophic cirrhosis* of the liver, high leucocytosis (30,000 or more) is strongly in favor of the first, since although the leucocytes may be increased moderately in this variety of cirrhosis, they do not reach a strikingly high figure. In an instance of cancer versus *echinococcus cyst* of the liver a high eosinophilia strongly argues the latter condition. Hyperinosis, if present, is also a sign suggestive of cancer, rather than of these other liver diseases.

## XLV. MALIGNANT ENDOCARDITIS.

GENERAL FEATURES.

The blood changes in malignant or ulcerative endocarditis are essentially those of a grave septicemia, described elsewhere, and do not, therefore, require extended consideration in this place.

According to the studies of Grawitz,[1] Kraus,[2] Sittman,[3] Kühnau,[4] James and Tuttle,[5] Thayer and Lazear,[6] and others, the chances of securing definite results from *bacteriological examination* of the blood are good in this disease. An analysis of these authors' work shows that various micro-organisms, notably pneumococci, gonococci, streptococci, and staphylococci, are demonstrable by culture of the peripheral blood with great frequency.

HEMOGLOBIN AND ERYTHROCYTES.

The loss of hemoglobin and erythrocytes is likely to be marked, and, in acute cases, extremely rapid and often most excessive—sometimes as great as in typical pernicious anemia. Structural degenerative changes are common, as in any severe anemia, and in many acute cases hemoglobinemia may be observed. As a rule, the loss of hemoglobin and erythrocytes is not markedly disproportionate, so that moderately subnormal color indices are commonest.

The following counts, by Dr. Uhle, of a profoundly septic patient at the German Hospital, illustrate the striking degree of anemia, as well as the intermittent and moderate leucocytosis which may develop in a grave case:

[1] Charité-Annal., 1894, vol. xix, p. 154.
[2] Zeitschr. f. Heilk., 1896, vol. xvii, p. 117.
[3] Deutsch. Arch. f. klin. Med., 1894, vol. liii, p. 323.
[4] Zeitschr. f. Hyg. u. Infektionskr., 1897, vol. xxv, p. 492.
[5] Med. and Surg. Report of the Presbyterian Hosp., New York, 1898, vol. iii, p. 44.
[6] Jour. Exper. Med., 1899, vol. iv, p. 81.

| Date. | Hemoglobin Percentage. | Erythrocytes. per c.mm. | Leucocytes per c.mm. |
|---|---|---|---|
| 11- 4-99 | 30 | 1,590,000 | 8,000 |
| 11- 8-99 | 26 | 1 243,000 | 8,400 |
| 11-11-99 | 30 | 2,010,000 | 12,800 |
| 11-16-99 | 28 | 1,810,000 | 8,000 |
| 11-20-99 | 19 | 2,130,000 | 14,000 |
| 11-25-99 | 24 | 2,170,000 | 12,800 |
| 12- 1-99 | 25 | 1,710,000 | 9,600 |
| 12- 5-99 | 35 | 2,750,000 | 16,000 |
| 12-16-99 | 41 | 3,530,000 | 7,200 |
| 12-27-99 | 36 | 2,330,000 | 4,800 |
| 1- 5-00 | 50 | 3,350,000 | 8,000 |
| 1-11-00 | 38 | 2,760,000 | 4,000 |

Leucocytes.

An increase in the number of leucocytes, more commonly moderate than marked, and characterized by a high percentage of polynuclear neutrophiles, is the usual finding, except in profoundly septic patients, in whom the count may be normal or subnormal during the greater part of the illness, as shown by the above table. Absence of leucocytosis is not infrequent in this disease, doubtless because in a large proportion of cases the depressant effects of the poison predominate. In no other infection is a better illustration offered of the relationship between the behavior of the leucocytes, the intensity of the disease, and the patient's powers of reaction. Occasionally, a striking preagonal increase develops, or, on the contrary, death may be ushered in by a decided leucopenia.

Diagnosis.

In many instances the diagnosis of malignant endocarditis is materially facilitated by the blood examination, and in some it can be made only by this means. A positive result from blood culturing at once gives a definite clue to the real character of the disease, and this procedure should be undertaken in every doubtful case. Malignant endocarditis with marked constitutional symptoms is perhaps most frequently confused with *enteric fever* and occasionally with *malarial fever*. Both of these infections may be excluded if a leucocytosis exists, unless, of course, this sign is obviously due to some complication. It should also be remembered that in malignant endocarditis the anemia develops early and tends to attain a marked degree with great rapidity, while in the other two fevers it does not become striking until the post-febrile stage of the disease is reached. No comment is necessary on the value of obtaining a positive serum reaction or of detecting the malarial parasite as a means of distinguishing this trinity of infections.

## XLVI. MALTA FEVER.

Most cases are accompanied by a moderate, progressive *secondary anemia*, becoming most marked at about the end of the febrile period, and involving, according to Bruce,[1] an average loss of about 1,500,000 erythrocytes to the c.mm. The most severe anemia is found in cases complicated by profuse epistaxis and by hemorrhage from the bowel, but those in which these symptoms are absent may show simply a slight oligochromemia, as demonstrated by a case studied by Musser and Sailer.[2] Bassett-Smith[3] found that the average erythrocyte loss ranges between 20 and 40 per cent. below the normal standard, and that the hemoglobin deficiency is relatively greater. In severely cachectic patients he noted a decided increase in the number of plaques, together with poikilocytosis, a tendency toward microcytosis, but never nucleated erythrocytes. This observer also has determined that the phagocytic powers of the leucocytes are diminished and the bactericidal properties of the blood lowered in this infection. Frank *leucocytosis* does not develop, except as the result of hemorrhage, but occasionally the number of leucocytes is slightly increased—to about 12,000 or 13,000 per c.mm. Charles[4] states that during the acute stages of the infection he has found a notable relative increase in the large lymphocytes. In Bassett-Smith's cases the percentage of mononuclear leucocytes ranged from 26 to 76, the polynuclear neutrophiles being relatively diminished. Counts higher than 6600 were not found.

Bassett-Smith[5] cultured the *Micrococcus melitensis* from the peripheral blood in all cases during the early stages and in severe pyrexial relapses, thus confirming the earlier findings of Gilmour, Shaw, and Zammit.[6] In the finger blood of a case clinically identical with Malta fever Manson[7] found spirilla similar to, yet differing somewhat from, the *Spirillum obermeieri*. Wright and Smith[8] found that the blood serum of patients suffering from Malta fever clumps Bruce's micrococcus but produces no agglutination of the *Bacillus typhosus*. The diagnostic value of this *serum test* in differentiating Malta and enteric fevers has since been corroborated by the reports of Aldridge,[9] Musser and

[1] Brit. Med. Jour., 1889, vol. i, p. 1101.
[2] Phila. Med. Jour., 1898, vol. ii, p. 1408.
[3] Brit. Med. Jour., 1902, vol. ii, p. 861.
[4] *Ibid.*, 1898, vol. ii, p. 607.
[5] *Ibid.*, 1904, vol. ii, p. 325.
[6] Cited by Bruce, *ibid.*, 1904, vol. ii, p. 323.
[7] *Ibid.*, 1904, vol. i, p. 538.
[8] Lancet, 1897, vol. i, p. 656.
[9] *Ibid.*, 1898, vol. i, p. 1394.

Sailer,[1] Kretz,[2] Cox,[3] Bassett-Smith,[4] Craig,[5] and others. A 1:50 dilution with a thirty-minute time limit appears to give the most satisfactory results, but in many instances prompt reactions occur with dilutions of from 1:100 to 1:250. (See p. 444.)

## XLVII. MEASLES.

GENERAL FEATURES.

The amount of *fibrin* is either normal or decreased, except in the event of a marked inflammatory complication, which may produce hyperinosis. The *blood plaques* are decreased in number during the febrile period.

A peculiar bacillus has been isolated from the blood of 6 cases of measles by Arsamaskoff,[6] but specificity is not unreservedly claimed for it. Zlatogoroff[7] has also cultured a novel bacillus from the blood in 17 of 24 cases, the organism in question closely resembling that isolated from the secretions of the eye and nose. Protozoa of undetermined character have been detected in the blood by Weber.[8]

HEMOGLOBIN AND ERYTHROCYTES.

The hemoglobin and erythrocytes are practically unchanged in typical cases. When a decrease does occur, it is trifling, amounting at the most to a loss of from 250,000 to 500,000 corpuscles, and of about 15 or 20 per cent. of hemoglobin. The great majority of cases have counts of 5,000,000 cells to the c.mm. Qualitative changes in the erythrocytes are absent.

LEUCOCYTES.

In the uncomplicated case of measles the number of leucocytes is either normal or subnormal. The latter change is very common, the decrease of leucocytes being most marked at the height of the fever during the stage of eruption, and their number again reaching normal coincidentally with the fading of the eruption and the beginning of desquamation. The count may fall to 3000 or 4000 per c.mm. during the period of maximum temperature. Combe[9] believes that leucopenia is constant in all uncomplicated cases, and that the diminution in the number of cells amounts to at

[1] *Loc. cit.*
[2] Lancet, 1898, vol. i, p. 221.
[3] Phila. Med. Jour., 1899, vol. iv, p. 491.
[4] *Loc. cit.*
[5] Amer. Jour. Med. Sci., 1903, vol. cxxv, p. 105.
[6] Amer. Year-book of Med. and Surg., 1900, p. 317.
[7] N. Y. Med. Jour., 1904, vol. lxxx, p. 419.
[8] Centralbl. f. Bakt. u. Parasit., 1897, vol. xxi, p. 286.
[9] Arch. de méd. des Enf., 1899, vol. ii, p. 345.

least one-half the normal number; he finds that the decrease begins during the last two days of the invasion period, and persists through the stage of exanthem. The first two days of the invasion period, however, are characterized by a moderate leucocytosis, chiefly involving, according to Renaud,[1] the polynuclear neutrophiles. This author, as well as Combe, also found a striking degree of relative lymphocytosis, first developing during the early days of the eruption. All cases, however, do not show this increase in mononuclear forms, for in some the relative percentages of the different varieties of leucocytes remain as in health. Cases with decided adenitis and those with persistent diarrhea most frequently show this lymphocyte increase. The eosinophiles are usually either diminished or else entirely absent during the febrile period of the disease; occasionally they reach a high normal standard, but are not increased, as in scarlet fever. Reckzeh[2] found that, as a rule, the eosinophiles do not reach their normal value until the end of the second week after invasion.

Should leucocytosis develop, it should be attributed to some acute inflammatory complication, such as bronchopneumonia, croupous pneumonia, or severe bronchitis.

DIAGNOSIS.

In cases with anomalous symptoms the existence of *scarlet fever* may often be excluded by the absence of leucocytosis. Absence of increase in fibrin and eosinophiles is also suggestive in ruling out this infection. If the diagnosis lies between measles and *syphilitic roseola*, the absence of leucocytosis points to the former. The initial stage of *variola* has been mistaken for measles, but the blood examination is of no aid in differentiating these two conditions, as leucocytosis is not found in small-pox at this stage of its development. *Rötheln* does not give rise to blood changes distinguishable from those of true measles. This was true of nine cases examined by Plantenga.[3] Tchistovitch[4] found, in four cases, either normal blood or a very slight neutrophile increase.

## XLVIII. MENINGITIS.

HEMOGLOBIN AND ERYTHROCYTES.

The condition of the hemoglobin and erythrocytes has not been extensively studied in this disease, but so far as the data at present available show, the only notable change to be observed consists of a moderate oligochromemia. This

[1] Arch. de méd. des Enf., 1901, vol. iv, p. 22.
[2] Zeitschr. f. klin. Med., 1902, vol. xlv, p. 107.
[3] Arch. de méd. des Enf., 1903, vol. vi, p. 129.
[4] Russkiy Vrach, 1904; abst., Jour. Amer. Med. Assoc., 1904, vol. xlii, p. 809.

change, however, is inconstant, for, as a rule, both the number of corpuscles and their hemoglobin value are normal, or, perhaps, somewhat above normal.

These statements, as well as those relating to the leucocytes, apply to the various non-tuberculous inflammations of the cerebral and spinal pia-arachnoid and dura mater, acute leptomeningitis and pachymeningitis, and epidemic cerebrospinal meningitis. The blood changes associated with tuberculous meningitis are described elsewhere. (See pp. 546 and 549.)

LEUCOCYTES.

Well-defined leucocytosis is found in the great majority of instances, the counts usually ranging in excess of 20,000 to the c.mm., and tending to attain highest figures in purulent meningitides.

Forty-seven cases of various non-tuberculous meningeal inflammations have been observed by Williams and by Cabot,[1] in all but two of which the leucocytes at the first examinations numbered more than 10,000 to the c.mm., and in the individual case as high as 40,000 and 50,000. The two instances in which the first counts failed to show leucocytosis were cases of epidemic cerebrospinal meningitis, 36 of which were included in the entire series. In 37 cases of cerebrospinal fever Koplik[2] found leucocytosis a constant sign, the counts ranging from 12,000 to 55,000, and exceeding 25,000 in 55 per cent. of the cases. The highest leucocytoses were found in fatal cases, in which lumbar puncture showed a thick, turbid, pus-like fluid.

The myth, still entertained to some extent, that tuberculous and non-tuberculous meningitis differ in that the former does not cause leucocytosis, should have been dispelled long ago. Thus, while Türk[3] found this sign in 32 out of 35 (or 91.4 per cent.) counts in non-tuberculous cases, he also noted it in 4 out of 8 counts in the tuberculous form, the maximum estimate in the latter being 20,800 cells per c.mm. Rieder[4] has reported a count of 14,400 in one case of tuberculous meningitis, and in another, 7800 and 5900 cells; leucocytosis was constant in this author's 10 counts in non-tuberculous cases, the maximum being 29,300. Examples of this sort could be still further multiplied to demonstrate that leucocytosis occurs with great frequency in tuberculous meningitis.

The most common differential change consists in an absolute and relative increase in the polynuclear neutrophiles, this alteration tending to become most striking when the total leucocyte count is excessively high. In cases with a normal count, or with only

[1] *Loc. cit.*
[2] Med. News, 1904, vol. lxxxiv, p. 1065.
[3] *Loc. cit.*
[4] *Loc. cit.*

a moderate increase, Türk observed a relatively high percentage of large lymphocytes and transitional forms, and he has further called attention to the fact that the eosinophiles are either absent or decreased to a small fraction of one per cent. in practically every count, irrespective of the presence or absence of an increase in the total number of leucocytes.

DIAGNOSIS. Between *tuberculous* and *non-tuberculous* meningitis an absence of leucocytosis strongly suggests the former, although the presence of a leucocytosis does not of necessity exclude it.

*Epidemic cerebrospinal meningitis* sometimes resembles such infections as *enteric fever*, *typhus fever*, *pneumonia*, and malignant forms of *variola*. In attempting these diagnoses, the presence of a leucocytosis almost invariably excludes typhoid, but the behavior of the leucocytes is of no avail as a means of differentiating pneumonia. In variola the early development of a large-celled mononucleosis, often with myelemia, proves a helpful sign. Most cases of typhus show a normal or subnormal number of leucocytes, but some with moderate leucocytosis have been reported.

*Acute meningitis* cannot be distinguished by the blood examination from *cerebral hemorrhage* and *abscess*, since in all these conditions high counts are the rule. Cabot[1] believes that *hysteria*, *lead encephalopathy*, *diabetic coma*, *sunstroke*, and *narcotic* or *alcoholic intoxication* can be excluded by the presence of a leucocytosis, and that, should the diagnosis lie between meningitis, on the one hand, and *uremia* and *post-epileptic* coma, on the other, an absence of leucocytosis is sufficient to exclude meningitis, although its presence is of no diagnostic value. It is possible that bacteriological examination of the blood may furnish definite information, for Gwyn[2] has succeeded in repeatedly cultivating the *Diplococcus meningitidis intracellularis* from the blood of a case of epidemic cerebrospinal fever. Several investigators have found pneumococci in the blood in cases of acute meningitis.

## XLIX. MYXEDEMA.

*Anemia*, involving chiefly the hemoglobin, is a finding in perhaps four-fifths of all cases, judging from Murray's[3] and Bramwell's[4] records of 56 patients. More rarely, high grade anemia is found in this condition, as in a case examined by Le Breton,[5] in

[1] *Loc. cit.* [2] Johns Hopkins Hosp. Bull., 1899, vol. x, p. 112.
[3] "Twentieth Century Practice of Medicine," New York, 1895, vol. iv, p. 710.
[4] "Anemia," London, 1899, p. 309.
[5] Bull. soc. méd. des hôp. de Paris, 1895, vol. xii, p. 22.

which the loss of hemoglobin amounted to 45, and the loss of erythrocytes to 66, per cent. of the normal standard, with a color index of 1.91. This author, as well as Kraepelin,[1] in several instances has observed a general increase in the diameter of the erythrocytes and the presence of erythroblasts, but such changes are not ordinarily encountered.

The *leucocytes* are moderately increased in a small proportion of patients, but never reach notably high figures; in fully three-fourths of cases their number does not exceed the maximum normal limit. In a case published by Putnam,[2] a small number of myelocytes was found, but no other differential changes of special interest have been reported.

A prompt increase in the hemoglobin and erythrocytes follows the administration of thyroid extract in appropriate doses, but, on the other hand, excessive thyroidization rapidly aggravates the anemia, according to Bramwell.[3]

## L. NEPHRITIS.

GENERAL FEATURES.

Important contributing factors of the blood changes in this condition are albuminuria, hemorrhage, circulatory disturbances, and the character of the disease with which the renal lesion may be associated. The fact that so many other circumstances are capable of playing active etiological rôles serves to explain the great dissimilarity of the blood pictures in different nephritides and at different stages of the same nephritis.

Marked albuminuria produces in course of time a notable drain upon the serum proteids and a less conspicuous deterioration of the corpuscles, especially affecting their volume. By this agency, therefore, the *specific gravity* of the whole blood is diminished, in close relationship with the extent of the drain produced. It is still a disputed question whether or not edema may also be held responsible for this change. The investigations by Houston,[4] of the edema of anemia, tend to prove that in renal disease practically no direct relationship exists between the condition of the blood and the extent of the dropsy. At least there is no demonstrable relationship in cases with gradually developing edema, owing doubtless to the promptness with which the blood mass corrects any tendency to dilution. In cases with hematuria as a prominent symptom the familiar picture of a post-hemorrhagic anemia

[1] Deutsch. Arch. f. klin. Med., 1892, vol. xlix, p. 587.
[2] Amer. Jour. Med. Sci., 1893, vol. cvi, p. 125.
[3] *Loc. cit.*
[4] Brit. Med. Jour., 1902, vol. i, p. 1464.

may be encountered, and in kidney inflammations which accompany an acute infectious process the effects of the latter upon the blood are to be remembered.

The amount of *fibrin* may be found to be increased, especially in contracted kidney; the rate of *coagulation* is, so far as has been determined, exceedingly inconstant.

Von Jaksch,[1] von Limbeck,[2] and others have drawn attention to diminished *alkalinity* of the blood as a sign anticipating and accompanying uremic attacks.

*Bacteriological examination* of the blood proves negative, except in the terminal stages of nephritis, when evidences of a general circulatory invasion by micro-organisms may sometimes be detected. Thus, excluding this factor, James and Tuttle[3] failed to demonstrate pathogenic bacteria in the blood of six successive chronic cases; while, on the other hand, White[4] obtained growths of streptococci in three consecutive cases of chronic parenchymatous nephritis, on the second, third, and fourth days before death, respectively, these positive findings being attributed to terminal septicemia.

HEMOGLOBIN AND ERYTHROCYTES.

In *acute parenchymatous nephritis* the hemoglobin and erythrocyte values may remain perfectly normal, or, as is more usual, a moderate secondary anemia develops, of which a greatly disproportionate oligochromemia is a notable feature. The grade of the anemia is highest in cases with marked albuminuria and hematuria, but only exceptionally is a loss of more than 2,000,000 per c. mm. cells noted. Laache[5] estimates the average loss in hemoglobin at 26 per cent. and in erythrocytes at 19 per cent., and considers that the decrease is much greater in acute than in chronic cases. Hayem[6] is authority for the statement that striking anemia develops only in cases with hematuria.

In *chronic parenchymatous nephritis* most observers state that moderate hemoglobin and erythrocyte decreases are the most notable findings, but some report severe anemia the grade of which is likely to be most intense in cases with marked, persistent albuminuria and with associated lesions of other organs. Sörensen[7] found that the count of erythrocytes in this form of renal disease averaged 4,700,000 to the c.mm., but in the writer's experience a much more pronounced loss has been observed—an average hemoglobin percentage of 57.1 and an average ery-

[1] Zeitschr. f. klin. Med., 1887, vol. xiii, p. 350. [2] *Loc. cit.* [3] *Loc. cit.*
[4] *Loc. cit.* [5] "Die Anämie," Christiania, 1883.
[6] *Loc. cit.* [7] Cited by Grawitz, *loc. cit.*

throcyte count of 3,971,206 per c.mm., in a series of 15 cases. A synopsis of the examinations in these cases shows the following data: Hemoglobin percentage: 80–90 in 1; 70–80 in 2; 60–70 in 4; 50–60 in 3; 40–50 in 2; and 30–40 in 3. Erythrocyte counts: above 5,000,000 in 2; 4,000,000–5,000,000 in 4; 3,000,000–4,000,000 in 8; 2,000,000–3,000,000 in 1. The maximum hemoglobin estimate in this series was 82, and the minimum 30, per cent.; the maximum number of erythrocytes per c.mm. was 5,520,000, and the minimum 2,270,000. The average color index was 0.71.

Polycythemia, masking the real condition of the blood, is not at all uncommon; it may arise from some such cause as cyanosis or the sudden development of an extensive edema. Every clinician must have been repeatedly struck by the evident discrepancy between the blood report and the pinched, waxy, nephritic facies.

In *chronic interstitial nephritis*, so long as circulatory disturbances do not exist, the condition of the blood remains practically normal, but as soon as the compensatory hypertrophy of the left ventricle becomes inadequate, the blood changes identified with uncompensated valvular heart disease develop, and various degrees of apparent anemia and polycythemia become evident from time to time. These factors, the importance of which is insisted upon by Grawitz,[1] no doubt serve to explain most of the blood changes found in sclerotic kidney, but it seems obvious that neither the malnutrition of the patient nor the considerable hemorrhages from which he often suffers should be disregarded as possible causes of blood deterioration.

All the structural changes affecting the erythrocytes in secondary anemia may occur in association with any of the preceding varieties of nephritis, should the accompanying anemia be sufficiently striking.

Leucocytes.

In *acute parenchymatous nephritis* leucocytosis may develop in the early stages of the disease, and persist for some time after convalescence is established. Cabot,[2] who attributes the increase to the effects of hemorrhage and of uremia, found it present in about 80 per cent. of his 50 cases, the maximum count being 50,000 per c.mm. Of 12 cases in which these two factors were excluded the writer found that the number of leucocytes was above 10,000 per c.mm. in 9.

In the 15 cases of *chronic parenchymatous nephritis* above mentioned, the number of leucocytes averaged 8626 per c.mm., the maximum being 16,000 and the minimum 4000. Four of

[1] *Loc. cit.* [2] *Loc. cit.*

the counts were in excess of 10,000; 9 from 5000–10,000; and 2 below 5000.

*Chronic interstitial nephritis* does not of itself influence the number of leucocytes.

*Uremia* may or may not be associated with leucocytosis; the change is to be noted in the majority of nephritides in which this complication supervenes, but it is by no means constant. Dopter and Gourand[1] have shown that in rabbits the removal of one kidney is followed by a transient, doubtless post-operative, leucocytosis, but that after a double nephrectomy the consequent uremic intoxication provokes a leucocytosis which persists until the animal's death.

In all the above forms of kidney inflammation the leucocytosis, if present, is of the polynuclear neutrophile type.

DIAGNOSIS.

The blood count is of no diagnostic value in nephritis, nor can it always be relied upon to indicate accurately the richness of the blood in cellular elements, owing to the frequent prevalence of factors which cause dilution and inspissation.

## LI. NERVOUS AND MENTAL DISEASES.

NEURITIS, BERI-BERI, NEURALGIA, BRAIN TUMOR.

In a single case of febrile *multiple neuritis* Cabot[2] found a moderate degree of secondary anemia, with leucocytosis, the counts, 8 in number, ranging from 16,000 to 28,700 per c.mm., and the latter figure being reached during the post-febrile period of the attack. This author also noted a moderate anemia and leucocytosis in 4 of 6 cases of *alcoholic neuritis*, but found the number of leucocytes normal in 25 cases of *plumbic neuritis*.

*Beri-beri*, according to Spencer,[3] is usually associated with a well-defined secondary anemia, in some instances characterized by striking qualitative changes affecting the size and shape of the erythrocytes. The leucocytes, both in number and in the relative percentages of their different varieties, remain normal, except in the acute stages of the infection, when an increase in the eosinophiles may develop. Fajardo[4] has detected a spore-forming, pigment-producing hematozoön, and Rost[5] a diplobacillus, in the blood of beri-beri patients, each of which organisms has

[1] Sem. méd., 1903, vol. xxiii, p. 14.
[2] *Loc. cit.*
[3] Lancet, 1897, vol. i, p. 32.
[4] Centralbl. f. Bakt. u. Parasit., 1900, vol. xxvii, p. 249.
[5] Lancet, 1901, vol. i, p. 66.

been regarded by their respective discoverer as the specific cause of the disease. Other investigators, notably Affleck,[1] have obtained negative results from bacteriological blood examinations. In the blood of negroes suffering from *akatama*, a form of peripheral neuritis endemic in Central Africa, Wellman[2] has found the parasites of both tertian malarial fever and of filariasis; he concludes, however, that neither of these parasites has any etiological bearing on akatama, for they are frequently harbored by natives exempt from this disease.

*Neuralgia*, whatever its seat, is capable of exciting neither anemia nor leucocytosis. Excessive polycythemia (9,000,000 erythrocytes per c.mm., with 125 per cent. of hemoglobin) was found by F. P. Weber[3] in a case of *erythromelalgia*.

The blood in *brain tumor* usually deviates in no manner from the normal, although rarely a moderate leucocytosis has been observed. In five cases the writer found that the hemoglobin averaged 72.2 per cent. (ranging from 70 to 79), and the erythrocyte count 3,800,000, the maximum being 4,270,000 and the minimum 2,860,000 per c.mm. None of the cases showed leucocytosis, the average count of leucocytes being 7320. This is a distinct contrast to *cerebral abscess* and *hemorrhage*, in both of which conditions leucocytosis is the general rule. The condition of the blood in *meningitis* has already been described. (See p. 486.)

FUNCTIONAL NEUROSES.

*Neurasthenia*, *hypochondriasis*, and *hysteria*, while they do not primarily serve as factors of blood deterioration, are in some instances associated with other conditions which lead to moderate secondary anemia, usually involving chiefly the hemoglobin, and but rarely causing any appreciable diminution in the number of erythrocytes. But, as a rule, functional neurotics have normal blood in spite of their anemic appearance. Luxemberg,[4] in a study of 40 cases of hysteria and neurasthenia, found that polycythemia was common, having repeatedly noted erythrocyte counts as high as 6,000,000, and even in one instance 7,300,000, per c.mm.; he attributes this to vasomotor changes, possibly due in large part to the effect of the examination itself. Reinert,[5] examining 74 cases of these two forms of neurosis, found a moderate hemoglobin diminution in many cases of hysteria, but normal blood in neurasthenia. In sexual neurasthenia, however, anemia is not at all uncommon, in the writer's experience. MacPhail[6]

[1] Edinburgh Med. Jour., 1900, vol. viii, p. 33.
[2] Jour. Trop. Med., 1903, vol. vi, p. 267.
[3] Brit. Med. Jour., 1904, vol. i, p. 1017.
[4] Centralbl. f. inn. Med., 1899, vol. xx, p. 533.
[5] Münch. med. Wochenschr., 1895, vol. xlii, p. 305.
[6] Jour. Mental Sci., 1884, vol. xxx, pp. 378 and 488.

speaks of the marked anemia usually found in insane masturbators, and every clinician who has made many routine blood counts must have been struck with the fact that the pallid, pasty face of the confirmed masturbator but seldom falsely reflects the state of the sufferer's blood.

The functional neuroses are not accompanied by leucocytosis, but, on the other hand, in many cases a decided leucopenia is present. In all a relatively increased proportion of lymphocytes may frequently be observed, while in hysteria the number of eosinophiles may be relatively in excess of the normal standard.

General Paresis, Dementia, Melancholia, Mania.

MacPhail,[1] in a prize essay submitted to the Medico-Psychological Association of Great Britain in 1884, concludes that these mental diseases are in many instances closely associated with a more or less decided anemia, although in no sense can blood deterioration be regarded as a factor of insanity. In *general paresis* this observer found subnormal hemoglobin values, averaging about 67 per cent., on the patient's first admission to the hospital, but later, as the patient profited by the improved hygienic environment, this value rose, only again to fall to an average of 52 per cent. in the terminal stages of the affection. The oligocythemia steadily increased as the disease progressed, and occasionally reached in the individual case a minimum count of between 3,000,000 and 4,000,000 erythrocytes per c.mm.; it was more striking during the active and completely paretic stages than during the intervening periods of quiescence. A well-defined leucocytosis was constant, and many of the counts made shortly before death reached high figures. Diefendorf,[2] in 11 paretics, also noted a progressive anemia, generally of the so-called "chlorotic" type, together with a steady gain in the polynuclear neutrophile leucocytes, which attained its acme during the terminal stage. At this time there developed a distinct leucocytosis, as well as an increase in the hemoglobin and erythrocyte figures. Paralytic seizures were accompanied by moderate leucocytosis, usually not exceeding a count of 20,000. Capps,[3] in a study of 19 cases, found that the hemoglobin averaged 85 per cent. and the erythrocytes 4,789,900 per c.mm.—figures which may be compared with Smyth's average estimates[4] in 40 cases: hemoglobin, 68.7 per cent., and erythrocytes, 4,700,000. Capps states that the majority of cases show a moderate leucocytosis, averaging an increase of 22 per cent. in

[1] *Loc. cit.*
[2] Amer. Jour. Med. Sci., 1903, vol. cxxvi, p. 1047.
[3] *Ibid.*, 1896, vol. cxi, p. 650.
[4] Jour. Mental Sci., 1890, vol. xxxvi, p. 504.

excess of the normal standard, but that in the incipient stages of the disease the number of leucocytes usually is not increased. An average count of 8800 was noted by Somers[1] in 5 cases. Relatively high percentages of polynuclear neutrophiles, with a diminution in the small lymphocytes, are common differential changes, while the relative numbers of large lymphocytes and eosinophiles may be higher than normal. The eosinophiles were found, by Roncoroni,[2] to be regularly increased in paretic excitement—sometimes as high as 20 or 25 per cent. Kippel and Lefas,[3] in 22 cases, invariably found from 1 to 4 per cent. of basophiles; early in the disease a polynuclear neutrophile increase was the rule, but in the later stages it declined until finally lymphocytosis, usually but relative, supervened.

*Convulsions* and *apoplectiform attacks* tend to produce blood concentration, and therefore temporarily increase the hemoglobin and erythrocyte values. During and following such seizures an abrupt rise in the leucocyte curve, characterized by a striking absolute and relative gain in the large lymphocytes, and, rarely, by the appearance of myelocytes, was observed by Capps, who has also described a small mononuclear neutrophilic leucocyte, resembling a dwarf myelocyte, as peculiar to the condition in question. (See p. 222.) Burrows[4] believes that the leucocytosis associated with convulsions, not only in general paralysis, but in other conditions, bears a definite relation to the severity of the fit, and that the increase is in part the result of the muscular contractions attending the convulsion, and in part represents an actual pathological leucocytosis. In 23 cases of general paresis a lowered blood alkalinity was constantly found by Pugh,[5] the diminution being most notable in the acuter forms of the disease and in connection with convulsive seizures. *Acute delirium* from any cause also provokes leucocytosis.

In *dementia*, according to Smyth,[6] both the hemoglobin and the erythrocytes are decidedly lower than in the preceding condition, his averages for this disease being 53.7 per cent. of hemoglobin and a count of 4,070,000 erythrocytes in a series of 12 cases. In 10 cases of *melancholia* he found that the hemoglobin averaged 69.7 per cent. and the erythrocytes 4,684,000, while Steel,[7] in 35 cases of this disease, estimated the average hemoglobin value at 75 per cent. and the average erythrocyte count at 3,000,000.

[1] Bull. N. Y. State Hosp., 1896.
[2] Arch. d. Psychiat. Sc., 1894, vol. xv, p. 293.
[3] Sem. méd., 1902, vol. xxii, p. 393.
[4] Amer. Jour. Med. Sci., 1899, vol. cxvii, p. 503.
[5] Jour. Mental Sci., 1903, vol. xlix, p. 71.
[6] *Loc. cit.*
[7] Amer. Jour. Insanity, 1892, vol. xlix, p. 604.

In *acute mania* anemia of the so-called "chlorotic" type usually may be observed; this blood change becomes aggravated by each acute maniacal outbreak, but after recovery from these attacks the deficiency is rapidly restored. In acute continuous mania Bruce[1] believes that the number of leucocytes stands in direct relation to the improvement of the patient, convalescents showing a progressive leucocytosis with a high percentage of polynuclear neutrophiles, which persists after recovery, but which rapidly diminishes should a relapse occur. This investigator[2] also cultured from the blood a diplobacillus, indifferently agglutinable by the serum of patients suffering from mania, and non-pathogenic for laboratory animals. Bruce interprets his findings as evidence that some cases of mania, at least, are true infections. Opposed to the above leucocyte formula of acute mania are the observations of Johnson and Goodall,[3] who found the count highest during the acute stages and lowest during states of remission and recovery. These investigators have also shown that in 60 per cent. of all cases of mania, paresis, and other forms of insanity the patient's blood clumps the colon bacillus. This suggests that in these diseases the normal inhibition of this organism's growth in the gut is interfered with, and in consequence its proliferation is excessive enough to excite toxemia. Somers'[4] leucocyte counts in 19 dements averaged 10,743, in 19 melancholics 7947, and in 19 maniacs 8315. The alkalinity of the blood remains normal, except in patients with great motor restlessness, in whom subnormal figures are the rule. The following table of averages by Wherry[5] relates to the blood changes in 95 cases of dementia, melancholia, and mania:

| Form of Insanity. | Hemoglobin. | Erythrocytes. | Leucocytes. |
|---|---|---|---|
| Mania, acute, men | 84 per cent. | 3,780,000 | 7,800 |
| Mania, acute, women | 68 " | 3,554,400 | 7,600 |
| Melancholia, acute, men | 78 " | 4,051,200 | 6,400 |
| Melancholia, acute, women | 77 " | 3,793,600 | 7,400 |
| Mania, chronic, men | 82 " | 3,798,400 | 7,100 |
| Mania, chronic, women | 71 " | 3,708,000 | 8,200 |
| Melancholia, chronic, men | 77 " | 3,624,000 | 6,700 |
| Melancholia, chronic, women | 77 " | 3,764,800 | 7,200 |
| Dementia, men | 71 " | 3,518,400 | 7,600 |
| Dementia, women | 73 " | 2,976,000 | 9,200 |

[1] Jour. Mental Sci., 1903, vol. xlix, p. 441.
[2] *Ibid.*, 1903, vol. xlix, p. 219.
[3] Lancet, 1903, vol. ii, p. 470.
[4] *Loc. cit.*
[5] Amer. Med., 1901, vol. ii, p. 70.

In *family periodic paralysis* J. K. Mitchell [1] finds that the hemoglobin and corpuscular figures and the differential leucocyte counts do not deviate from normal. High blood alkalinity was detected, both during the paralytic attacks and in the intervals between them.

EPILEPSY. CHOREA. TETANY.

In *epilepsy* a moderate anemia appears to be the general rule. Smyth's studies [2] of 50 cases show an average of 62.8 per cent. of hemoglobin, and 4,520,000 erythrocytes per c.mm. MacPhail [3] asserts that prolonged attacks of excitement notably increase the anemia, but that the habitual administration of bromids seems in no manner to produce deleterious effects upon the blood. Furthermore, this author observed that a close relationship can be distinguished between the patient's gain in weight, the decrease in the anemia, and the mental improvement, and that in patients who recovered, the regeneration of the blood became practically complete. Distinct leucocytosis seldom occurs in epilepsy, except as the result of a convulsion. Kuhlmann,[4] for example, found the leucocytes in excess of normal but once in a study of 16 cases. In a series of 7 cases, Pearce and Boston[5] found well-marked chloro-anemia and usually a moderate leucocyte increase. Differentially, the most conspicuous changes were a reduction in the polynuclear neutrophiles and the inconstant presence of small numbers of myelocytes.

Pugh's studies[6] of 40 epileptics tend to show that the alkalinity of the blood is subnormal during the period between epileptic attacks, that it decidedly falls immediately before a fit, and that a further fall occurs soon after a fit is over. Pugh attributes the lowered alkalinity partly to an accumulation of acid toxins in the blood and partly to an output of sarcolactic and carbonic acids as the result of the violent convulsive attacks. The administration of strontium bromid and potassium bicarbonate for a time restores the normal blood alkalinity and apparently diminishes the number of fits—but only for a brief period, since it is impossible permanently to influence the reaction of the blood by drug giving. Bra and Chaussé [7] describe a "neurococcus" in the blood during epileptic fits, and claim to have cultured the alleged germ and to have produced convulsions in animals by its injection. Bra's claim for his neurococcus as the cause of idiopathic epilepsy has been con-

[1] Brain, 1902, vol. xxv, p. 109; also Trans. Assoc. Amer. Phys., 1899, vol. xiv, p. 345.
[2] *Loc. cit.*
[3] *Loc. cit.*
[4] Bull. N. Y. State Hosp., 1897.
[5] Medicine, 1904, vol. x, p. 123.
[6] *Loc. cit.*
[7] Rev. Neuroli. and Psychiat., 1903, vol. i, p. 689

troverted by Tirelli and Brossa,[1] who obtained uniformly negative bacteriological blood findings in this condition.

In *chorea* slight anemia, usually of the "chlorotic" type, occurs with frequency, but not with constancy, for many cases habitually show normal hemoglobin and erythrocyte values. It seems scarcely necessary to remark that the belief once entertained, that blood deterioration was a causal factor of this disease, is obviously erroneous. Burr,[2] in a study of the hemoglobin and erythrocytes in 36 cases, concludes that a moderate diminution in both of these elements is the general finding, and that a high grade of anemia occurs only as the result of some complication. The oligocythemia usually does not exceed a loss of more than 1,000,000 cells per c.mm. in uncomplicated cases. The leucocytes are not increased, but differential counts may detect a relatively large percentage of eosinophiles, according to the reports of Zappert[3] and others. *Tetany* is not of itself a cause of blood impoverishment.

## LII. OBESITY.

From Kisch's studies[4] it is evident that the *hemoglobin* values are notably high in most corpulent individuals, and in some excessively increased. In 79 of 100 cases of obesity examined by this author the hemoglobin percentage exceeded 100, while in the remaining 21 moderate oligochromemia was found. The maximum reading in this series was 120 and the minimum 55 per cent. Actual *anemia*, however, is not incompatible with this class of patients, as demonstrated by Leichtenstern[5] and by Oertel.[6] The latter also maintains that in some instances true *plethora* exists, and furthermore professes to recognize two distinct forms of obesity, an anemic and a plethoric. Data regarding the *leucocytes* in this condition are wanting.

## LIII. OSTEOMALACIA.

The *hemoglobin* and *erythrocytes* do not exhibit any marked deviations, being in most instances normal, or but moderately diminished. The anemia, when present, is characterized by a hemoglobin loss relatively exceeding that of the corpuscles.

[1] Rif. Med., 1903, vol. xix, p. 934.
[2] Univ. Med. Mag., 1896, vol. ix, p. 188.
[3] Zeitschr. f. klin. Med., 1893, vol. xxiii, p. 227.
[4] *Ibid.*, 1887, vol. xii, p. 357.
[5] "Untersuch. u. d. Hg-Gehalt d. Blutes," Leipsic, 1878.
[6] "Allgem. Ther. d. Kreislaufsstör.," Leipsic, 1884; also Deutsch. Arch. f. klin. Med., 1892, vol. i, p. 293.

The *leucocytes* also remain approximately normal in number, slight fluctuations above and below this standard being the only numerical change thus far noted. Relative lymphocytosis has been found by Ritchie [1] and by Tschistowitch,[2] while Neusser [3] and others have observed in many cases a moderate increase in the eosinophiles, and the presence of small numbers of myelocytes. None of these differential changes, however, is to be considered constant in this condition. According to von Limbeck,[4] the *alkalinity* of the blood remains practically unaltered, although von Jaksch[5] formerly maintained that it was considerably diminished.

## LIV. PANCREATITIS.

In interpreting the blood picture of pancreatitis due attention should be paid to the influence of possible attendant lesions, especially those such as cholangitis, peritonitis, icterus, and diabetes. *Acute hemorrhagic pancreatitis* is so frequently part and parcel of abscess, gangrene, and sepsis that more or less anemia is to be expected. As a rule, the hemoglobin and erythrocyte losses are moderate, although exceptionally the values approximate but 25 or 30 per cent. of normal. The dual factors, hemorrhage and acute inflammation, not to mention infection and necrosis, are ideal theoretical reasons for a leucocytosis, and, practically, it is found that these factors are active. Seven examinations in four cases of acute pancreatic inflammation in the German Hospital gave these averages: hemoglobin, 57.1 per cent., ranging between 26 and 88; erythrocytes, 3,205,714 per c.mm., ranging between 1,550,000 and 4,460,000; and leucocytes, 19,292 per c.mm., ranging between 11,600 and 32,000. The histological changes are those attending secondary anemia and a polynuclear neutrophile leucocytosis.

*Chronic pancreatitis*, being a slow sclerosis, has little or no effect upon the blood. Nine estimates in three German Hospital cases averaged 80.2 per cent. of hemoglobin, with extremes of 54 and 99; 4,700,000 erythrocytes per c.mm., or a range of from 3,600,000 to 5,900,000; and 7188 leucocytes per c.mm., ranging between 4000 and 11,300. In non-inflammatory *pancreatic cyst* a similar blood picture is found, while if the cyst is actively inflamed, a varying degree of leucocytosis generally develops. In *pancreatic lithiasis* the blood changes may be those of acute

[1] Edinburgh Med. Jour., 1896, vol. xlii, p. 208.
[2] Berlin. klin. Wochenschr., 1893, vol. xxx, p. 919.
[3] Wien. klin. Wochenschr., 1892, vol. v, p. 41.
[4] *Loc. cit.*
[5] Zeitschr. f. klin. Med., 1887, vol. xiii, p. 350.

or chronic pancreatitis or of malignant disease, depending upon the effects of the stones. The blood in *pancreatic malignant neoplasm* has already been considered. (See "Malignant Disease," p. 472.)

Aside from the facts that anemia and leucocytosis attend acute rather than latent inflammations of the pancreas, and that a leucocytosis argues the malignancy of a tumor of this organ, the blood examination is of no definite value in recognizing pancreatic lesions.

## LV. PERICARDIAL EFFUSION.

The *hemoglobin* and *erythrocytes* remain normal, or, if anemia is found, it may be referred to other coëxisting conditions.

*Leucocytosis* of the polynuclear neutrophile type is practically a constant change in the non-tuberculous forms, but in tuberculous pericarditis the leucocytes apparently do not increase. From a diagnostic viewpoint the presence of a leucocytosis is of real value in excluding the latter condition, as well as *cardiac dilatation;* this sign is also strong evidence against the existence of a serous *pleural effusion*, which, if left-sided, may simulate pericarditis.

## LVI. PERITONITIS.

HEMOGLOBIN AND ERYTHROCYTES.

Anemia is frequently found, the degree of which largely depends upon the character and the chronicity of the inflammation. In general *purulent peritonitis*, especially in cases of comparatively long standing, the hemoglobin and erythrocyte diminution may be excessive—to between 20 and 30 per cent. for the former, and to between 2,000,000 and 3,000,000 per c.mm. for the latter. With such an anemia as this the erythrocyte loss is commonly very disproportionate to that of the hemoglobin, so that high color indices rule; for example, in three of the cases summarized below, the indices were 1.12, 1.01, and 1.00 respectively. The several qualitative changes accompanying any severe secondary anemia are also commonly to be observed. *Serous peritonitis* has but little effect in provoking a cellular decrease, although it usually causes a slight but definite oligochromemia, so that in such cases the color indices are moderately subnormal. On the average, it may be stated that peritonitis involves a loss of about 30 per cent. of hemoglobin and of 25 per cent. of erythrocytes.

The following summary of 54 cases, none of which was appendicular, shows the grade of anemia prevailing in this disease:

| Hemoglobin Percentage. | Number of Cases. |
|---|---|
| From 90–100 | 2 |
| " 80– 90 | 15 |
| " 70– 80 | 10 |
| " 60– 70 | 9 |
| " 50– 60 | 6 |
| " 40– 50 | 7 |
| " 30– 40 | 3 |
| " 20– 30 | 2 |

Average, 68.4 per cent.
Maximum, 93.0 "
Minimum, 20.0 "

| Erythrocytes per c.mm. | Number of Cases. |
|---|---|
| Above 5,000,000 | 5 |
| From 4,000,000–5,000,000 | 23 |
| " 3,000,000–4,000,000 | 14 |
| " 2,000,000–3,000,000 | 7 |
| " 1,000,000–2,000,000 | 5 |

Average, 3,756,035 per c.mm.
Maximum, 5,670,000 " "
Minimum, 1,290,000 " "

Leucocytes. Provided that the patient's resisting powers react normally, septic peritonitis constantly causes a typical leucocytosis of the polynuclear neutrophile variety. Generally speaking, 80 per cent. of cases show a leucocyte count of 10,000 or higher. It cannot be stated with certainty that the increase is greater in purulent than in serous inflammations, for any variety of peritonitis, except the tuberculous, may provoke a striking leucocytosis. As already remarked in the discussion of appendicitis, extension of the process is heralded by an abrupt rise in the leucocyte curve. As in other infections, leucocytosis may be absent, or leucopenia may exist, in cases of a profound, crippling character. The number of leucocytes in the preceding 54 cases ranged as follows:

| Leucocytes per c.mm. | Number of Cases. |
|---|---|
| Above 45,000 | 1 |
| From 35,000–45,000 | 2 |
| " 25,000–35,000 | 2 |
| " 20,000–25,000 | 5 |
| " 15,000–20,000 | 12 |
| " 10,000–15,000 | 19 |
| " 5,000–10,000 | 12 |
| Below 5,000 | 1 |

Average, 15,526 per c.mm.
Maximum, 46,000 " "
Minimum, 4,400 " "

Diagnosis. The presence of leucocytosis is sufficient evidence for the exclusion of *tuberculous peritonitis*, so-called *hysterical peritonitis*, and *rheumatism of the abdominal muscles*. This sign, however, cannot safely be

employed to differentiate between peritonitis and *acute enteritis*, certain forms of *intestinal obstruction*, and rupture of a *tubal pregnancy* or of an *abdominal aneurism*, all of which may cause more or less leucocyte increase. (See "Appendicitis," p. 370.)

Cabot[1] regards the association of marked leucocytosis with hyperinosis as strongly in favor of a peritoneal inflammation rather than of such conditions as *non-malignant bowel obstruction*, *malignant disease*, *hysteria*, and *phantom tumors*.

## LVII. PERTUSSIS.

*Iodophilia* was detected by Crisafi[2] in 16 of 20 cases examined, but no relationship could be traced between this sign and the presence of glycosuria, which developed in 4 of the patients. In so far as can be learned from the scanty literature at present available, the *hemoglobin* and *erythrocyte* values remain normal in this disease. *Lymphocytosis*, generally relative, but sometimes absolute, is a characteristic finding in whooping-cough. As a consequence of this change there is a coincident diminution in the polynuclear neutrophiles and eosinophiles. Fröhlich and Meunier,[3] who originally determined this fact, found in 30 cases an average of 27,800 leucocytes per c.mm., the individual counts ranging from a minimum of 15,500 to a maximum of 51,150. De Amicis and Pacchioni[4] have corroborated this observation, although they consider that the increase is somewhat less, having found an average count of 17,943 for their cases. Wanstall,[5] in 15 cases, and Stengel and White,[6] in 4, obtained even lower total leucocyte values (no increase being noted in many instances), but found a lymphocyte increase the rule. The lymphocytosis develops during the early stages of the disease, before the cough begins, and usually persists for some time after, convalescence is established. As a general rule, it may be stated that the younger the child, the more notable the increase. Such complications as bronchitis, catarrhal pneumonia, and otitis do not appear appreciably to exaggerate it. According to Ehrlich,[7] it is to be attributed to the stimulation and swelling of the tracheo-bronchial lymphatic glands.

The fact that a marked lymphocyte increase occurs in the early catarrhal stages of the disease, antedating the development of the

[1] *Loc. cit.*
[2] Brit. Med. Jour. Epit., 1904, vol. i, p. 49.
[3] Compt. rend. Soc. biol., Paris, 1898, vol. v, p. 103.
[4] Clinica Medica, 1899, vol. iv, p. 103.
[5] Amer. Med., 1903, vol. v, p. 62.
[6] Arch. Pediat., 1901, vol. xviii, pp. 241 and 321.
[7] *Loc. cit.*

typical cough, is of diagnostic value, as is also the presence of iodophilia.

## LVIII. PLEURISY.

### Serous Pleurisy.

Hemoglobin and Erythrocytes.

In acute cases it is customary to find normal hemoglobin and erythrocyte values, or, at the most, simply a moderate oligochromemia; in those of longer standing, with decided debility of the patient, anemia, sometimes of a considerable degree, is not an uncommon finding. Thus, in an instance of this sort the writer found but 38 per cent. of hemoglobin and 3,300,000 erythrocytes per c.mm., together with the corpuscular degenerative changes to be expected in an anemia of this intensity. It is to be remembered that a rapidly developing pleural effusion may so concentrate the blood as to cause a temporary polycythemia, disguising the actual quantitative changes.

Leucocytes.

Absence of leucocytosis is the general rule, probably for the reason that almost all serous pleurisies are of tuberculous origin. Exceptionally a moderate, intermittent increase is found, chiefly affecting the polynuclear neutrophiles, and due possibly to the influence of some intercurrent process, such as a secondary pneumococcus infection. A notable increase in the eosinophiles may often be found in hemorrhagic pleural effusions. In children a leucocytosis sometimes occurs, apparently independent of secondary infections. It is quite evident that the behavior of the leucocytes cannot be used as a means of differentiating tuberculous from non-tuberculous effusions.

Morse,[1] in a study of 224 examinations made in 20 cases, comes to the conclusion that there is no definite relation between the leucocyte count and the duration of the disease, the degree of pyrexia, the amount of the effusion, and its increase and diminution. Neither could he determine that the contamination of the fluid by blood and by microscopical pus produced the slightest effect upon the number of cells. In Morse's counts the number of leucocytes exceeded 10,000 to the c.mm. in 5.8 per cent., while in Cabot's 99 cases[2] this figure was exceeded in 14.1 per cent., the average count for the latter being 6130.

[1] Amer. Jour. Med. Sci., 1900, vol. cxx, p. 658.

[2] *Loc. cit.*

## Purulent Pleurisy.

Hemoglobin and Erythrocytes.

The changes in the hemoglobin and erythrocytes do not differ conspicuously from those prevailing in primary serous pleurisy, although evidences of a decided anemia are to be observed somewhat more frequently.

In 8 of the writer's 10 cases of empyema the hemoglobin loss exceeded 50 per cent. of the normal, 38 per cent. being the minimum, 73 per cent. the maximum, and 46 per cent. the average, estimate. The erythrocytes were below 2,000,000 to the c.mm. in 2 instances, averaging 3,500,000, with 1,540,000 as the minimum and 4,600,000 as the maximum, counts.

Leucocytes.

Leucocytosis, ordinarily of a high grade, accompanies the great majority of cases, the increase involving mainly the polynuclear neutrophile cells at the expense of the lymphocytes. It is more usual to find the count above than below 20,000 to the c.mm., and in an exceptional instance it may even exceed 50,000. Aspiration of the pus is followed by a decline, and its reaccumulation by a rise, in the leucocyte curve. The extent of the primary purulent accumulation cannot be gaged with any accuracy by the degree of the leucocyte increase.

The following counts in a case of empyema examined at the German Hospital will serve to illustrate the high leucocytosis sometimes seen in this condition:

| Date. | Hemoglobin Percentage. | Erythrocytes per c.mm. | Leucocytes per c.mm. |
|---|---|---|---|
| Jan. 16, 1900 .. | 84 | 4,460,000 | 23,200 |
| " 17, 1900 .. | 88 | 5,380,000 | 42,400 |
| " 18, 1900 .. | 82 | 4,320,000 | 45,000 |
| " 19, 1900 .. | 82 | 4,430,000 | 40,800 |
| " 20, 1900 .. | 83 | 4,383,000 | 23,320 |
| " 21, 1900 .. | 82 | 4,410,000 | 44,300 |
| " 22, 1900 .. | 81 | 4,330,000 | 40,600 |
| " 23, 1900 .. | 71 | 3,985,000 | 37,300 |
| " 24, 1900 .. | 67 | 4,360,000 | 53,500 |
| " 26, 1900 .. | 83 | 4,240,000 | 47,100 |
| " 27, 1900 .. | 71 | 3,480,000 | 48,100 |

In 10 other cases above noted a leucocyte increase was in-

variably found, the counts averaging 17,180 and ranging from 11,200 to 31,800 per c.mm.

DIAGNOSIS. The presence of a well-developed leucocytosis points to *pneumonia* or *empyema*, rather than to simple *serous pleurisy*, but it does not differentiate between these first two conditions. On the other hand, an absence of leucocytosis does not surely exclude pneumonia and empyema, although it is extremely suggestive that neither exists. *Malignant neoplasms* of the lungs and pleura also cause a decided leucocyte increase, as does *actinomycosis*.

## LIX. PNEUMONIA.

GENERAL FEATURES. In the case of average severity, *coagulation* is exceedingly rapid, and the amount of fibrin greatly increased, the network being dense, coarse, and formed with great rapidity. The hyperinosis tends to persist for some time after the disappearance of the pyrexia and the signs of lung involvement. In severe infections, occurring in individuals of good resisting powers, the change is especially striking, but in fatal cases, overwhelmed by the disease, a fibrin increase is not observed. High temperature and extensive infiltration of the lungs are associated with marked hyperinosis. In children the *specific gravity* of the blood is usually high during the febrile period, falling to normal as resolution takes place; in cases with marked cyanosis the concentration of the blood also raises its density. Attempts to apply the *serum test* in this disease have generally been disappointing, most reports having shown that pneumococci are either unaffected by the serum of pneumonia patients or, at the most, agglutinate slowly and atypically. (See p. 526.) Rosenow,[1] however, reports positive findings in 77 of 83 cases examined, and Huber [2] has applied the test in 10 cases, claiming uniformly positive results, the reaction appearing as early as the fifth day of the disease, increasing in intensity toward crisis, and slowly disappearing by about the tenth day.

BACTERIOLOGY. Pneumonia, like enteric fever, is an example of an acute infection which in the great majority of instances must be classed as a true *bacteriemia*, even in its early stages. With modern technic it is now possible to culture the pneumococcus from the circulating blood in about 70 per cent. of all cases of acute pneumonia, although until within recent years positive bacteriological findings were the exception

[1] Medicine, 1903, vol. ix, p. 435.
[2] Centralbl. f. inn. Med., 1902, vol. xxiii, p. 417.

rather than the rule. Since 1900 the following statistics have been recorded: Prochaska,[1] 50 cases, all positive, with 12 deaths; Rosenow,[2] 83 cases, 74 positive, mortality, 45 per cent.; Silvestrini and Sertoli,[3] 16 cases, 15 positive; Pieracinni,[4] 28 cases, 11 positive; Landi and Cionini,[5] 27 cases, 25 positive; Kinsey,[6] 25 cases, 19 positive,[7] of which 31 per cent. died; and Cole,[8] 30 cases, 9 positive, all of which were fatal. In contrast with these figures the results of the earlier investigators should be compared. Of 49 cases studied by Beco,[9] 7 were positive, with 5 deaths. Sello,[10] in 48 cases, found 12 positive results, of which 10 ended fatally. Kraus[11] examined 21 cases, with but 2 positive findings, both in fatal cases.

Franklin W. White,[12] in 19 carefully studied cases of pneumonia, obtained positive results in 3 patients, all of whom died; of the 16 negative cases, 7 proved fatal. Sittmann[13] found the pneumococcus in 6 of 16 cases examined by him, in 4 cases by cultural methods, and in 2 in stained cover-slip preparations of the blood; of these 6 positive cases, 4 died, and of the 10 negative cases but a single one ended fatally. Kohn[14] examined 32 cases, obtaining positive results in 9, of which number 7 cases were fatal, while the other 2 finally recovered after a grave infection; of this author's 23 negative cases recovery took place in 8. James and Tuttle,[15] in their studies of 12 cases, 2 of which were fatal, failed in every instance to obtain positive findings.

Analysis of the above data affords a total of 159 positive findings in which the outcome of the disease is definitely stated by the observer, and of these 159, 96 ended fatally—a mortality of 60.7 per cent. It is obvious, from these figures, that pneumococcemia, although it means a well-marked infection, is by no means a hopeless sign, as was once believed.

[1] Centralbl. f. inn. Med., 1900, vol. xxi, p. 1145; also Deutsch. Arch. f. klin. Med., 1901, vol. lxx, p. 559.

[2] *Loc. cit.*

[3] Centralbl. f. allg. Path. u. pathol. Anat., 1900, vol. xi, p. 447.

[4] *Ibid.*, 1900, vol. xi, p. 460.

[5] Deutsch. med. Wochenschr., 1901, vol. xxxvii, p. 296.

[6] Jour. Amer. Med. Assoc., 1904, vol. xlii, p. 759.

[7] The proportion of bouillon to blood in this series was 15 or 20 to 1. In another series of 25 cases, with a 6 : 1 dilution, only 3, or 12 per cent., of the cultures were positive.

[8] Johns Hopkins Hosp. Bull., 1902, vol. xiii, p. 136.

[9] Rev. de méd., 1899, vol. xix, pp. 385 and 461.

[10] Zeitschr. f klin. Med , 1898, vol. xxxvi, p. 112.

[11] Zeitschr. f. Heilk., 1896, vol. xvii, pp. 117 and 138.

[12] Jour. Exper. Med., 1899, vol. iv, p. 425.

[13] Deutsch. Arch. f. klin. Med., 1894, vol. liii, p. 323.

[14] Deutsch. med. Wochenschr., 1897, vol. xxiii, p. 136.

[15] Med. and Surg. Rep. of the Presbyterian Hosp., New York, 1898, vol. iii, p. 46.

HEMOGLOBIN AND ERYTHROCYTES.

During the active stages of the fever the hemoglobin and erythrocytes are either normal or very slightly diminished. But polycythemia also may occur, as the result of the fever's influence in causing contraction of the peripheral vessels, or from cyanosis. During the post-febrile period moderately low counts are usually found, being due possibly to the hemocytolytic effects of the fever, and to a dilution of the blood caused by the decreased arterial tension which occurs at this stage of the illness. The loss, in the writer's experience, amounts in the average case to about 20 per cent. of the normal number of cells, with a slightly greater hemoglobin decrease—approximately 25 per cent. Poikilocytosis and other structural changes in the cells are to be noted only in severe cases. In 109 hospital cases of croupous pneumonia the hemoglobin and erythrocyte values were within the following limits:

| HEMOGLOBIN PERCENTAGE | NUMBER OF CASES. | ERYTHROCYTES PER C.MM. | NUMBER OF CASES. |
|---|---|---|---|
| From 90–100 | 8 | Above 5,000,000 | 3 |
| " 80– 90 | 38 | From 4,000,000–5,000,000 | 60 |
| " 70– 80 | 29 | " 3,000,000–4,000,000 | 41 |
| " 60– 70 | 14 | " 2,000,000–3,000,000 | 4 |
| " 50– 60 | 12 | " 1,000,000–2,000,000 | 1 |
| " 40– 50 | 7 | | |
| " 30– 40 | 1 | | |
| Average, | 75.0 per cent. | Average, | 4,048,833 per c.mm. |
| Maximum, | 98.0 " | Maximum, | 5,070,000 " " |
| Minimum, | 26.0 " | Minimum, | 1,880,000 " " |

LEUCOCYTES.

In pneumonia, as in other acute infections, the severity of the infective process and the intensity of the reaction on the part of the organism are the factors which determine the behavior of the leucocytes. In the great majority of cases a well-marked leucocytosis develops at or soon after the time of the initial chill, and persists until shortly after the temperature has fallen to normal.

A high leucocytosis indicates a severe infection in an individual of strong resisting powers. A moderate increase indicates either a slight infection coupled with good resistance, or an intense infection with an inadequate reaction. Little or no leucocyte increase also suggests one of two diametrically opposite conditions: either an infection too trivial to excite reaction, or one so severe as to overpower the organism, stifling reaction. Ewing[1]

[1] N. Y. Med. Jour., 1893, vol. lviii p. 715.

has found that, as a rule, the increase is greater in cases with extensive lung involvement than in those with limited lesions, but this parallelism between the degree of leucocytosis and the extent of the pneumonic process is approximate, and does not always hold good. In a general sense it applies only to cases which react well toward the disease. There is no relationship between the degree of increase and the degree of fever during the active stages of pneumonia.

In the average well-marked case the number of leucocytes usually ranges between 20,000 and 30,000 per c.mm., the latter figure being only rarely exceeded, as, for example, in severe sthenic cases, in which the count may rise to 40,000 or 50,000, or even higher. To illustrate the constancy of leucocytosis in pneumonia the table given below shows that this sign developed in about two-thirds of the 153 cases examined. Summing up a total of 470 cases reported by various observers, it is found that the average "first count" of the leucocytes, during the febrile stage of the disease, was 22,693, this figure applying to all cases, both with and without leucocytosis. In the writer's experience the average has been distinctly lower—16,066 per c.mm. Absence of leucocytosis is of unfavorable prognosis, except in patients in whom the clinical type of the infection is obviously mild. The occurrence of a high leucocytosis is of no definite prognostic value, since it indicates simply a marked infection and good resisting powers.

The following table shows the range of the leucocytes in 153 hospital cases of pneumonia:

| Leucocytes per c.mm. | Number of Cases. |
|---|---|
| Above 50,000 | 1 |
| From 40,000–50,000 | 4 |
| " 30,000–40,000 | 2 |
| " 20,000–30,000 | 24 |
| " 15,000–20,000 | 35 |
| " 10,000–15,000 | 34 |
| " 5,000–10,000 | 49 |
| Below 5,000 | 4 |

Average, 16,066 per c.mm.
Maximum, 83,600 " "
Minimum, 3,200 " "

To these figures may be added the counts made in 10 cases of *catarrhal pneumonia*, which ranged between 8000 and 40,000, and averaged 15,210 per c.mm. Seven of the cases showed definite leucocytosis—namely, a count exceeding 10,000.

In cases terminating by crisis the leucocytes begin to diminish

either a short time before or after the temperature commences to decline, the normal number being reached, in most cases, within twenty-four or forty-eight hours after crisis occurs, although in a small proportion of cases the decrease is much slower, the count sometimes not reaching normal until a week after the temperature has dropped. False crises, although they may cause a striking drop in the temperature, do not cause a decline of the leucocyte curve.

In cases ending by lysis the decrease in the number of leucocytes and the decline in the temperature begin simultaneously, but the latter reaches normal much more rapidly than the former; the leucocyte decrease progresses more gradually than in the cases ending by crisis, and the normal count is often not reached until a week or ten days after the temperature has fallen to the normal figure. At the beginning of lysis a correspondence may be distinguished between the diurnal fluctuations of the temperature and leucocyte curves, although no such relation is apparent during the febrile period of the disease.

It is an interesting fact that in about half of all cases, whether ending by crisis or by lysis, the maximum count of leucocytes is attained *during* the period of temperature decline.

Von Jaksch's [1] idea of injecting substances to cause leucocytosis in pneumonia where this phenomenon was absent, hoping thereby to benefit the patient, has not been attended by the favorable results which he anticipated. Leucocytosis is as promptly induced in the pneumonic as in the healthy individual, by the injection of nuclein, for example, but without beneficial effect upon the patient's condition, a fact which must be regarded as evidently signifying that an absence of leucocytosis in fatal cases is not the cause of death, as Billings [2] remarks. Borini [3] injected digitalis and aleuron into rabbits inoculated with virulent pneumococci, and found that the animals treated with digitalis survived the infection longer than those to which aleuron was given. Both substances caused leucocytosis, but that excited by aleuron was transient, while that caused by digitalis persisted for some time—a fact to which Borini believes the beneficial effects of digitalis in the treatment of pneumonia are largely due. In a series of German Hospital cases reported by J. C. Wilson [4] the injection of antipneumococcus serum was followed by a marked increase in the number of leucocytes, though his method of treatment did not perceptibly lower the mortality.

[1] Cited by Cabot, *loc. cit.* [2] Johns Hopkins Hosp. Bull., 1894, vol. v, p. 112.
[3] Centralbl. f. Bakt. u. Parasit., 1902, vol. xxxii, p. 207.
[4] Jour. Amer. Med. Assoc., 1900, vol. xxxv, p. 595.

Hare[1] has drawn attention to the fact that while leucocytosis is checked by antipyretics, it is not arrested by cold sponging, an observation which prompts Cabot[2] to declare in favor of the latter method of reducing temperature in pneumonia.

The leucocytosis of pneumonia is of the typical variety—that is, it is due to a large absolute and relative increase in the polynuclear neutrophiles, with a consequent relative decrease in lymphocytes. The proportion of eosinophiles is much reduced, and frequently these cells are entirely wanting. This is regarded as an unfavorable sign by Becker,[3] who states that he has never found eosinophiles in fatal cases. It is of interest to contrast with this circulatory poverty in eosinophiles the great abundance of these cells in serous blister fluid of pneumonia patients, as determined by Audibert.[4] In 27 cases of various diseases this observer found the highest percentages of eosinophiles in pneumonia—much higher, for example, than in pleurisy, phthisis, influenza, rheumatic fever, or erysipelas. With the decline of the temperature and the fading away of the leucocytosis, the percentage of polymorphous cells rapidly falls to normal or subnormal, and the lymphocytes and eosinophiles increase until they regain their normal percentages. The latter cells usually begin to reappear in the circulation a day or two before defervescence, and in some instances a striking post-febrile eosinophilia develops. In 20 cases showing marked leucocytosis, Billings found the following averages: lymphocytes, 9.6 per cent.; polynuclear neutrophiles, 91.2 per cent.; eosinophiles, 0.2 per cent. Heim[5] found a similar degree of polynuclear neutrophile increase in 19 cases. In 3 of Billings' counts in fatal cases showing no leucocytosis it was found that the various forms of leucocytes remained in their normal relative proportions. The leucocytes usually respond to the *iodin reaction*, most strikingly in cases with high leucocytosis. In such instances myelocytes are generally numerous.

In the pneumonias of children the possibility of *lymphocytosis* should be remembered, for although a true lymphocytosis is rare, it sometimes occurs, giving rise to false impressions if clinical signs other than the examination of the blood are neglected. (See p. 253.)

During the period of fever the *blood plaques* are markedly decreased in number, and often, indeed, altogether disappear from the blood, but after the crisis they reappear in great abundance,

[1] Therapeutic Gaz., 1898, vol. xii, p. 153.
[2] *Loc. cit.*
[3] Deutsch. med. Wochenschr., 1900, vol. xxvi, p. 558.
[4] Presse méd., 1902, vol. xv, p. 1256.
[5] Arch. de méd. des Enf., 1901, vol. iv, p. 21.

the fresh specimen taken at this time often being flooded with these bodies.

DIAGNOSIS. In atypical cases the presence of a well-marked leucocytosis is a helpful sign in excluding such conditions as *serous pleurisy*, *enteric fever*, *typhus fever*, *malarial fever*, and *influenza*. In the differentiation of croupous from *catarrhal pneumonia*, *empyema*, and *acute meningitis* the leucocyte count furnishes no tangible clue, since it is high in all these conditions; the same is true of some cases of *acute bronchitis*. An acute *apical pneumonia*, if associated with leucocytosis, is almost invariably to be considered non-tuberculous.

As previously stated, absence of leucocytosis in a case with well-defined chest signs is of a grave prognosis, but the presence of a leucocytosis is by no means always of good augury. Persistence of a high leucocyte count is suggestive of delayed resolution, empyema, or gangrene, and a sudden reëstablishment of the leucocytosis, after its disappearance at the time of crisis, points to a recurrent attack of the disease. Reappearance of the eosinophiles and disappearance of the iodin reaction indicate the termination of the acute phase of the illness. Post-critical iodophilia is most suggestive of delayed resolution or of a more serious pulmonary sequela.

Detection of the pneumococcus in the peripheral circulation indicates a severe infection, but it is not *per se* a grave prognostic sign.

## LX. POISONING.

A synopsis of the blood changes produced by various toxic substances is given in the following table, these changes consisting chiefly in hemocytolysis, methemoglobinemia, anemia, polycythemia, and leucocytosis.

| NAME OF POISON. | EFFECTS UPON THE BLOOD. |
|---|---|
| Alcohol | Anemia; often leucocytosis.[1] |
| Amyl nitrite | Methemoglobinemia.[2] |
| Acetanilid | Marked anemia, with many erythroblasts and stroma degeneration; leucocytosis; increase of plaques;[3] eosinophilia;[4] methemoglobinemia.[5] |

[1] Cabot, *loc. cit.* [2] Grawitz, *loc. cit.*
[3] Stengel and White, Univ. of Penna. Med. Bull., 1903, vol. xv, p. 462.
[4] Leredde and Pautrier, Sem. méd., 1903, vol. xxx, p. 224.
[5] Müller, Deutsch. med. Wochenschr., 1887, vol. xiii, p. 27.

| Name of Poison. | Effects upon the Blood. |
|---|---|
| Ammonia | Leucocytosis.[1] |
| Antipyrin | Methemoglobinemia.[2] |
| Arseniuretted hydrogen | Hemoglobinemia.[3] |
| Aspidium | Hemocytolysis.[4] |
| Bromin | Methemoglobinemia.[5] |
| Carbon monoxid | Methemoglobinemia; diminution of oxygen content;[6] polycythemia; leucocytosis; carbonyl hemoglobin.[7] |
| Chloral | Leucocytosis.[1] |
| Chloroform | Oligochromemia; leucocytosis;[8] oligocythemia;[9] diminished alkalinity.[10] |
| Chromic acid | Methemoglobinemia.[3] |
| Corrosive metallic salts | Anemia; leucocytosis.[1] |
| Ether | Oligochromemia; leucocytosis.[11] |
| Fusel oil | Hemocytolysis; methemoglobinemia.[12] |
| Guaiacol | Hemocytolysis; leucocytosis; relative lymphocytosis.[13] |
| Hydrocyanic acid | Methemoglobinemia.[14] |
| Iodin | Methemoglobinemia.[5] |
| Lead | Anemia; granular basophilia; often leucocytosis.[15] |
| Nitrobenzene | Methemoglobinemia; megaloblastic anemia;[16] eosinophile leucocytosis.[17] |
| Nitroglycerin | Methemoglobinemia.[3] |
| Opium | Occasionally leucocytosis.[1] |
| Phenacetin | Methemoglobinemia.[18] |
| Phosphorus | Polycythemia; occasionally leucocytosis;[19] slow coagulability.[20] |
| Potassium chlorate | Methemoglobinemia; anemia; leucocytosis.[21] |

[1] Cabot, *loc. cit.* [2] Müller, *loc. cit.* [3] Grawitz, *loc. cit.*
[4] Georgiewsky, Beitr. z. path. Anat. u. z. allg. Path., 1898, vol. xxiv, p. 1.
[5] Hayem, Compt. rend. Soc. biol., Paris, 1886, vol. cii, p. 698.
[6] Lacassagne, Martin, and Nicloux, Sem. méd., 1903, vol. xxiii, p. 25.
[7] Yarrow, Amer. Med., 1904, vol. iv, p. 338.
[8] Holman, Jour. Amer. Med. Assoc., 1902, vol. xxxix, p. 939.
[9] Loewy and Paris, Compt. rend. Soc. biol., Paris, 1902, vol. liv, p. 188.
[10] Baccarani, Gaz. degli Osped. e. d. Clin., 1900, vol. xlii, p. 445.
[11] DaCosta and Kalteyer, Annals of Surg., 1901, vol. xxxiv, p. 329.
[12] Futcher, Amer. Med., 1901, vol. ii, p. 210.
[13] Wyss, Deutsch. med. Wochenschr., 1894, vol. xx, p. 296.
[14] Kobert, "Lehrb. d. Intoxicationen," Stuttgart, 1893.
[15] Grawitz and Hamel Deutsch. Arch. f. klin. Med., 1900, vol. lxvii, p. 357
[16] Ehrlich and Lindenthal, Zeitschr. f. klin. Med., 1896, vol. xxx, p. 427.
[17] Personal observation.
[18] Krönig, Berlin. klin. Wochenschr., 1898, vol. xxxii, p. 998.
[19] Von Jaksch, Deutsch. med. Wochenschr., 1893, vol. xix, p. 10.
[20] Cevidalli, Phila. Med. Jour., 1903, vol. xi, p. 248.
[21] Bradenburg, Berlin. klin. Wochenschr., 1895, vol. xxxii, p. 583.

| Name of Poison. | Effects upon the Blood |
|---|---|
| Potassium permanganate | Methemoglobinemia.[1] |
| Ptomains | Leucocytosis.[2] |
| Pyrodin | Hemocytolysis.[3] |
| Pyrogallol | Methemoglobinemia.[4] |
| Snake and scorpion venom | Hemoglobinemia;[5] agglutination of erythrocytes;[6] diminished coagulation;[7] leucocytosis.[8] |
| Sodium nitrite | Methemoglobinemia.[4] |
| Tansy | Leucocytosis.[2] |
| Toadstools | Hemoglobinemia.[9] |
| Toluene | Hemoglobinemia.[10] |
| Toluylendiamin | Marked anemia; hemocytolysis; eosinophile leucocytosis; increase of plaques.[11] |
| Turpentine | Methemoglobinemia.[12] |

## LXI. RABIES.

Courmont and Lesieur[13] have determined that an excessive increase in the number of polynuclear neutrophiles is a constant change in the blood of patients suffering from hydrophobia, and that analogous findings are met with experimentally in rabid dogs, guinea-pigs, and rabbits. The polynuclear gain is frequently, but not invariably, associated with an increase in the total number of leucocytes. It may amount to as much as 98 per cent., and first develops during the period of incubation, becoming emphasized with the appearance of the clinical symptoms of the affection, and reaching a maximum just before death. The authors referred to believe that an absence of polynucleosis is sufficient to rule out rabies in a suspected case, although its presence cannot be regarded as pathognomonic of the disease.

[1] Hayem, *loc. cit.* [2] Cabot, *loc. cit.*
[3] Tallquist, "Exper. Blut-gift Anämie," Berlin, 1900. [4] Grawitz, *loc. cit.*
[5] Mitchell and Stewart, "A Contribution to the Study of the Effect of the Venom of Crotalus adamanteus upon the Blood," Washington, 1898; also Rogers, Lancet, 1904, vol. i, p. 349. (As a rule, colubrine venoms causes slighter blood destruction than those of the viperine type. According to Elliot [Lancet, 1904, vol. ii, p. 143], the venom of the common krait causes neither hemolysis nor abnormal clotting.)
[6] Flexner and Noguchi, Proc. Phila. Path. Soc., 1903, vol. vi, p. 88.
[7] Lamb, Glasgow Med. Jour., 1903, vol. lix, p. 80.
[8] Auché and Vaillant, Jour. de Méd. de Bordeaux, 1901, vol. xxxi, p. 29.
[9] Kobert, *loc. cit.* [10] Vast, Thèse de Paris, 1889.
[11] Schwalbe and Solley, Virchow's Arch., 1902, vol. clxviii, p. 399.
[12] Hayem, *loc. cit.* [13] Sem. méd., 1901, vol. xxi, p. 61.

## LXII. RELAPSING FEVER.

PARASITOLOGY.

The specific cause of relapsing fever, a spirillum discovered by Obermeier in 1868,[1] may be found in the peripheral blood of patients suffering from this disease, only during and shortly before the febrile paroxysm, the organism disappearing from the general circulation during the interparoxysmal afebrile period. The number of parasites found in a blood film varies within wide limits, and does not generally stand in any definite parallelism to the severity of the infection or to the degree of pyrexia.

Microscopically, the spirilla of Obermeier appear in the fresh

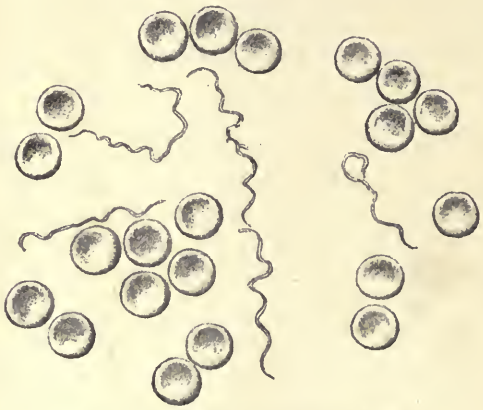


FIG. 63.—SPIRILLA OF RELAPSING FEVER.

blood as delicate, homogeneous, thread-like bodies twisted into the form of spirals, occurring singly or in groups of several organisms, radiating from a common center (Fig. 63). The length of the parasites varies from 16 to 40 $\mu$, or approximately from two to six times the size of the normal erythrocyte. They possess an active vibratile motility, exerted in the direction of their long axes, by virtue of which they are propelled and constantly altered in shape. Owing to this characteristic motility the presence of the parasites is usually first betrayed to the examiner by the whipping about of the blood corpuscles in their immediate proximity. The spirilla remain alive for only a short time after the withdrawal of the blood, and are so extremely sensitive to external influences that the addition even of distilled water causes them rapidly to

[1] Centralbl. f. d. med. Wissensch., 1873, vol. xi, p. 145.

disappear. Since nothing is known of their life history, the cause of their disappearance from the peripheral circulation during the intermissions of the disease is not known.

Both Sarnow[1] and von Jaksch[2] have called attention to the presence of certain refractive bodies, similar to diplococci, which may be found in the blood during the intermission, provided that another paroxysm is impending. The last-named authority believes that he has observed the metamorphosis of these bodies into short thick rods from which the typical spirilla eventually are evolved, and he tentatively regards them as spores of the latter. The views of this investigator have not, however, been generally accepted up to the present time.

Afanassiew[3] has described, in addition to the specific spirilla, peculiar bacteria which he found in the blood during the paroxysm. The organisms in question resemble bacilli with rounded poles, and appear to be invested by non-staining, hyaline sheaths. Some of them measure not more than 5 or 6 $\mu$ in length, while others appear as filamentous threads fully 10, 12, or 14 $\mu$ long, this increase in size being demonstrable in the fresh specimen watched for some time under the microscope. Afanassiew asserts that, unlike Obermeier's spirilla, the bodies may be cultivated on bouillon, gelatin, agar, and blood serum; he further claims that, in three patients inoculated with a twenty-four-hour-old bouillon culture of the organism, periods of pyrexia, recurring at ten-day intervals, were produced, and that in the blood of one of the patients thus treated numerous bacillary and filamentous forms were discovered. These investigations, as yet unconfirmed by other workers, are to be regarded only in the light of an interesting observation.

Melanin granules, either free or within the protoplasm of the leucocytes, are frequently seen in the blood, especially just after a paroxysm, and phagocytes containing engulfed spirilla may also be found at this time.

*Technic of Examination.*—Fresh specimens of blood, taken during the paroxysm, from the patient's finger or ear, are most suitable for microscopical examination. The motility and finer structure of the spirilla are seen most clearly with a $\frac{1}{12}$-inch oil-immersion objective, but for making the preliminary search a lower power, dry lens is more convenient, a $\frac{1}{6}$- or $\frac{1}{8}$-inch objective being useful for this purpose.

Dried films, fixed by one of the chemical methods of fixation

[1] Inaug. Dissert., Leipsic, 1882.
[2] "Clinical Diagnosis," 3d ed., London and Philadelphia, 1897, p. 50.
[3] Centralbl. f. Bakt. u. Parasit., 1899, vol. xxv, p. 273.

already described (p. 80), may be stained preferably by fuchsin, or the method of Günther (p. 112) may be used. For diagnostic purposes stained specimens are never to be preferred to the fresh blood film.

*Löwenthal's Reaction.*—The ingenious blood test devised by Löwenthal[1] furnishes a means of recognizing relapsing fever during the afebrile period, when the spirilla cannot be detected in the blood. It is conducted in the following manner: A drop of blood from a suspected case is mixed with a drop containing motile spirilla, the latter being taken from a patient during the paroxysmal stage of the disease. The mixture thus made is sealed between a slide and cover-glass, and incubated at body temperature for half an hour, at the end of which time it is examined under the microscope. If the suspected case is one of relapsing fever, the spirilla will have become quite motionless and collected together in regular masses, while if the case is one of some other disease, the motility of the parasites remains unimpaired. A control specimen, prepared from normal and spirilla-containing blood, must always be similarly incubated and examined with each test. If no such reaction as that described occurs within a time limit of two hours and one-half at the most, it is safe to regard the suspicious case as one not of relapsing fever. In 35 cases of this disease Löwenthal obtained about 85 per cent. of positive results, while in 14 cases of fever due to other causes the reaction was uniformly absent.

The reaction, which takes place in abortive and mild cases as well as in the severer forms of the disease, is thought to be dependent upon the presence in the blood of specific bactericidal products.

HEMOGLOBIN AND ERYTHROCYTES.

Von Limbeck,[2] quoting von Böckmann, states that there is a decrease in the number of erythrocytes and in the hemoglobin value in cases of relapsing fever, but neither the mode of production of such an anemia nor the exact morphological changes by which it is characterized has been carefully investigated, so far as can be ascertained. The losses are observed to occur during and for a few days after each paroxysm, but they are partly compensated during the interparoxysmal period.

LEUCOCYTES.

A variable degree of increase in the number of leucocytes, often of high grade, has been described by Laptschinski[3] as associated with the paroxysmal stage of the disease, this observer having noted that the poly-

[1] Deutsch. med. Wochenschr., 1897, vol. xxiii, p. 560.
[2] *Loc. cit.* [3] Centralbl. f. d. med. Wissensch., 1875 vol. xiii, p. 36.

nuclear neutrophiles were especially involved, and that the relative number of leucocytes to erythrocytes was in some instances as high as 1 to 37. During the period of intermission this leucocytosis disappears. This author, as well as von Böckmann[1] and Heidenreich,[2] also noted that the period of maximum leucocytosis was reached just after the crisis. Melkich,[3] in 14 cases of spirillum fever, noted that the highest counts were attained twenty-four hours before the crisis, after which the leucocytosis persisted for a day or two and then rapidly declined, the effect of each succeeding relapse being to accentuate this post-critical hypoleucocytosis.

The same worker, in collaboration with Kalyapin,[4] also determined that in this infection no direct relation exists between the leucocyte count and the richness of the blood in alexins. These substances, which are found most abundantly during the initial paroxysm, begin to diminish just before the crises, still further decrease during the afebrile periods, and again increase with the febrile recurrences. With convalescence the alexins rise to high figures and remain so until recovery takes place.

Phagocytosis occurs with great constancy in the peripheral blood. Ivanoff,[5] who first demonstrated this fact, has shown that phagocytic leucocytes containing fragments of spirilla are almost invariably present in the finger-blood of this infection. Especially is this the case in immunized monkeys, in whose blood intracellular spirilla are the rule and extracellular spirilla the exception.

DIAGNOSIS. The detection of the spirillum in the blood immediately differentiates relapsing fever from *typhus fever*, the onset and initial symptoms of which not infrequently prove confusing, and it may also be added that in such instances the absence of this organism during the stage of pyrexia is strong evidence for excluding the first-named disease. During the afebrile period, when the symptoms may suggest, for example, *malarial fever*, Löwenthal's reaction should be attempted, and the malarial parasite searched for. Melanemia, it must be recalled, may be encountered in each of these infections.

[1] *Loc. cit.* [2] "Untersuch. ü d. Par. d. Rückfallstyphus," Berlin, 1877.
[3] Russkiy Vrach, 1903; abst., Med. News, 1903, vol. lxxxiii, p. 1031.
[4] *Ibid.* [5] Centralbl. f. Bakt. u. Parasit., 1897, vol. xxii, p. 117.

## LXIII. RHEUMATIC FEVER.

GENERAL FEATURES.

*Coagulation* of the blood takes place within the normal time limit, or it may be delayed considerably. The amount of *fibrin* is markedly increased, especially during the most acute stages of the illness. Contradictory reports have been made by different authors concerning the *alkalinity*, some having found it diminished, and others having been unable to detect any such alteration. According to Hutchinson,[1] the general consensus of opinion is against any notable disturbance of the normal figure. In chronic articular rheumatism with coëxisting anemia a slight diminution of the alkalinity is occasionally observed.

The *bacteriology* of the blood in this disease has for many years been the object of much careful study, but thus far specific properties have not been generally conceded to any definite organism, although many different bacilli, streptococci, staphylococci, and diplococci have been cultivated from the circulating blood during life. Most suggestive are the investigations of Paine and Poynton,[2] who discovered a micrococcus, growing in streptococcal chains, in the blood of 18 cases of rheumatic fever. This organism is closely allied to, if not identical with, that previously described by Wasserman,[3] Triboulet,[4] and others. The same micrococcus has been cultured by Beaton and Walker[5] in 15 cases—8 of acute rheumatic fever, 4 of rheumatic endocarditis, and 3 of chorea. Rabbits inoculated with this organism die after developing fever, arthritides, endocarditis, pericarditis, and sepsis, and from their cadavers the micrococcus can be recovered in pure culture. Shaw,[6] who recently confirmed the above findings, succeeded in carrying out two successful inoculation experiments with monkeys. These investigators claim to have differentiated this "*Micrococcus rheumaticus*" from the ordinary *Streptococcus pyogenes* (which Singer[7] believes to be the germ described) by Marmorek's method of culturing it upon filtered streptococcus-bouillon in which streptococci of human origin fail to develop.

[1] Lancet, 1896, vol. i, p. 615.

[2] Brit. Med. Jour., 1900, vol. ii, pp. 861 and 932; also *ibid.*, 1901, vol. ii, p. 779.

[3] Berlin. klin. Wochenschr., 1899, vol. xxxvi, p. 638.

[4] Rev. de méd., Paris, 1888, vol. xviii, pp. 189 and 329; also Triboulet, Coyon, and Zadoc, Bull. Soc. méd. des hôp. de Paris, 1897, vol. xiv, p. 1343; also "Le Rheumatisme articulaire aigu en bacteriologie," Triboulet et Coyon, Paris, 1900.

[5] Brit. Med. Jour., 1903, vol. i, p. 237; also Walker, Practitioner, 1903, vol. lxx, p. 185.

[6] Jour. Path. and Bact., 1903, vol. ix, p. 158.

[7] *Ibid.*, 1901, vol. ii, p. 780.

Additional proof of the specificity of this organism, together with a study of the toxic specificity of rheumatic fever and of the mode of infection, is necessary before the organism in question can be universally credited as the exciting factor of the disease.

HEMOGLOBIN AND ERYTHROCYTES.

Few cases of acute rheumatic fever are unaccompanied by anemia, the intensity of which generally bears a fairly close relation to the severity and the duration of the illness. In acute attacks of short duration the hemoglobin falls to about 70 or 80 per cent. and the erythrocytes to 4,000,000, but in cases of longer standing the losses are likely to be more pronounced, the count often being not more than 3,000,000 or thereabouts. The color index usually is moderately subnormal, and may tend to remain so after the attack, even though the rise normalward of the erythrocyte count may have become well established. In the exceptional case, however, it may be quite as high as in pernicious anemia; in 4 of 32 cases noted below the index was above 1.00. In chronic rheumatism a moderate oligochromemia is usually the only evidence of anemia that can be detected, unless the patient happens to be decidedly cachectic. In the writer's experience, the erythrocytes fall below 3,000,000 in about one case in every five; in an occasional case the anemia may be intense, as in two of those tabulated below, with hemoglobin figures of 26 and 30 per cent. and counts of 1,242,000 and 1,590,000, respectively. In the following table of 32 cases it will be noted that the anemia averages greater than most authors report:

| Hemoglobin Percentage. | Number of Cases. | Erythrocytes per c.mm. | Number of Cases. |
|---|---|---|---|
| From 90–100 | 3 | From 4,000,000–5,000,000 | 16 |
| " 80–90 | 5 | " 3,000,000–4,000,000 | 9 |
| " 70–80 | 7 | " 2,000,000–3,000,000 | 5 |
| " 60–70 | 3 | " 1,000,000–2,000,000 | 2 |
| " 50–60 | 8 | | |
| " 40–50 | 2 | | |
| " 30–40 | 0 | | |
| " 20–30 | 4 | | |
| Average, 63 per cent. | | Average, 3,686,648 per c.mm. | |
| Maximum, 92 " | | Maximum, 4,980,000 " " | |
| Minimum, 26 " | | Minimum, 1,242,000 " " | |

The average hemoglobin percentage in 33 cases studied by McCrae [1] was 73.4, and the average erythrocyte count, 4,636,000.

[1] Amer. Med., 1903, vol. vi, p. 22.

Cabot's 43 cases [1] averaged 67 per cent. of hemoglobin and 4,400,000 erythrocytes per c.mm.

Should the cellular loss reach a high grade, deformities of shape and size, polychromatophilia, and, rarely, nucleated erythrocytes of the normoblastic type, may be observed.

LEUCOCYTES. Leucocytosis of the typical polynuclear neutrophile type is almost always present during the acute stages, but it is found only exceptionally in the subacute form of rheumatism, and practically never exists in the chronic variety. The count does not often exceed twice the maximum number of cells found normally, but occasionally it reaches a figure as high as 30,000 or 40,000 per c.mm. It is in cases with intense pyrexia, with endocarditis or pericarditis, and with pulmonary complications that high leucocytoses are most commonly found.

In 32 cases the leucocyte counts ranged as follows:

| LEUCOCYTES PER C.MM. | NUMBER OF CASES. |
|---|---|
| Above 20,000 | 2 |
| From 15,000–20,000 | 9 |
| " 10,000–15,000 | 8 |
| " 5,000–10,000 | 11 |
| Below 5,000 | 2 |

Average, 12,218 per c.mm.
Maximum, 31,200 " "
Minimum, 4,800 " "

McCrae's leucocyte figures in 36 cases averaged 12,370, and in but 9 was the count below 10,000 at the first examination.

In Cabot's cases, above referred to, the number of leucocytes averaged 16,800, and ranged from 4700 to 39,000.

Türk [2] has noticed that in many instances well-marked postfebrile eosinophilia develops, and that in favorable cases a relatively high percentage of eosinophiles persists during the acute stage of pyrexia. Both Korowicki [3] and Patella [4] describe a mononucleosis in cases with endocarditis, but the writer has been unable to verify this finding.

DIAGNOSIS. The blood changes are uncharacteristic, and do not serve as a means of differentiating this condition from other lesions in which the joint involvement and the constitutional manifestations are more or less

[1] *Loc. cit.* [2] *Loc. cit.*
[3] Deutsch. Aerzte-Zeitung, 1903, vol. i, p. 241.
[4] Sem. méd., 1903, vol. xxiii, p. 368.

similar. Thus, in *acute gout*, in *multiple secondary arthritis*, and in *septic arthritis* due to pyemia the same grade of anemia, leucocytosis, and hyperinosis may be observed. In the latter condition blood culturing may be helpful.[1] Iodophilia means *gonorrheal arthritis* rather than rheumatic fever. (See p. 228.)

## LXIV. SCARLET FEVER.

General Features.

In cases associated with pronounced anginal symptoms and with marked leucocytosis *coagulation* of the fresh blood drop is rapid and the amount of *fibrin* decidedly in excess of normal. In many cases a slight increase of fibrin is observed at the period of beginning desquamation.

The *specific gravity* is unchanged in the average case, but in those complicated by acute parenchymatous nephritis, in consequence of the drain on the albumins of the blood thus produced, it may fall to a very low figure—to 1.030, according to Peiper and Hammerschlag.[2] In 12 cases studied by van den Berg,[3] the specific gravity ranged from 1.031 in complicated cases to 1.060 in uncomplicated cases of the average severity.

The *specific micro-organism* of scarlet fever has not yet been isolated, either from the blood or other tissues, although in recent years many different bacteria have been described as causative factors. Class[4] claims to have discovered in the blood and throats of scarlet fever patients a diplococcus, named by him the *Diplococcus scarlatinæ*, which he considers specific, and this claim has received the support of a number of other investigators, Gradwohl,[5] Jaques,[6] and Page[7] being among those who found the bacterium in question. Baginsky and Sommerfeld[8] conclude, as have some earlier writers, that the clinical features of scarlet fever are due to a general streptococcus infection, having found this organism in the blood of 42 fatal cases. Class,[9] in a later communication, hints that his diplococcus and Baginsky and Sommerfeld's streptococcus are identical, since the former often develops strep-

[1] See articles on Pneumococcus Arthritis by Herrick (Amer. Jour. Med. Sci. 1902, vol. cxxiv, p. 12) and by Cole (Amer. Med., 1902, vol. iii, p. 905).
[2] Centralbl. f. klin. Med., 1891, vol. xii, pp. 217 and 825.
[3] Arch. f. Kinderheilk., 1898, vol. xxv, p. 321.
[4] Med. Rec., 1899, vol. lvi, p. 330.
[5] Phila. Med. Jour., 1900, vol. iv, p. 688.
[6] *Ibid.*, p. 552. [7] Jour. Bost. Soc. Med. Sci., 1899, vol. iii, p. 344.
[8] Berlin. klin. Wochenschr., vol. xxxvii, p. 588.
[9] Jour. Amer. Med. Assoc., 1900, vol. xxxv, p. 799.

tococcus forms in young cultures made from the blood. Any one who has read Class' description of his organism must be struck with its resemblance to the diplococcus found in scarlet fever blood by Crajkowski,[1] in 1895. Both Hektoen[2] and Jochmann[3] have isolated the streptococcus during life in a large series of scarlet fever patients, the former in 12 per cent. and the latter in 15.5 per cent. of cases examined. Hektoen's findings suggest no definite relationship between the severity of the disease and the presence of a bacteriemia, for the latter may develop in mild, uncomplicated cases, and may not occur in fatal ones; as a rule, however, more colonies grow in cultures made from severe types of the disease. Jochmann decides that the presence of a streptococcemia does not modify the symptomatology of scarlet fever; excluding those dying of nephritis, one-half of his fatal cases gave cultures of the streptococcus. Mackie[4] found streptococci and staphylococci in 3 of 6 cases by blood culturing during life. From the findings just stated it is clear that streptococci in scarlet fever play the rôle not of a specific factor, but rather of a secondary infection, the relationship of which to the primary infective agent remains obscure. The streptococci found in scarlet fever cannot be differentiated from ordinary forms of this organism; and the immunity conferred by an attack of scarlet fever protects only against this exanthema, and not against ordinary streptococcus infections.

Jehle[5] states that he has repeatedly isolated the influenza bacillus from the blood of young children ill with scarlet fever. It may be added that Mallory's protozoa,[6] found in the skin of scarlet fever patients, have not been demonstrated in the circulating blood.

HEMOGLOBIN AND ERYTHROCYTES.

Most observers agree that the scarlatinal infection, unless complicated, produces but trifling changes in the hemoglobin and erythrocytes, moderate anemia characterized by a disproportionate diminution of hemoglobin being the general rule in the cases in which any changes are noted.

Widowitz[7] found that the percentage of hemoglobin, normal at the beginning of the illness, slowly diminished during the febrile period, in a degree commensurate to the intensity of infec-

[1] Centralbl. f. Bakt. u. Parasit., 1895, vol. xviii, p. 116.
[2] Jour. Amer. Med. Assoc., 1903, vol. xl, p. 685.
[3] Deutsch. Arch. f. klin. Med., 1903, vol. lxxviii, p. 209.
[4] Lancet, 1904, vol. i, p. 494. [5] Zeitschr. f. Heilk., 1901, vol. xxii, p. 190.
[6] Boston Soc. Med. Sci., Dec. 15, 1903; also Jour. Amer. Med. Assoc., 1904, vol. xlii, pp. 31 *et seq.*
[7] Jahrb. f. Kinderheilk., 1888, vol. xxvii, p. 380; vol. xxviii, p. 25.

tion, and gradually returned to normal during convalescence. Pée[1] noticed in severe cases a pronounced "chloro-anemia," characterized by notable pallor and variations in the size of the corpuscles. Hemoglobinemia has also been occasionally observed.

The number of erythrocytes is generally between 4,000,000 and 5,000,000 per c.mm. in the case of average severity, the minimum count being reached at about the time of the decline of the temperature; but in complicated cases the anemia is more marked, and histological degenerative changes of the corpuscles have been noted during the period of desquamation. Van den Berg's examinations of 12 cases[2] show that the count is usually above 4,000,000 per c.mm., except in severe cases complicated by acute nephritis or endocarditis, in the event of which a rapid and striking anemia is produced, the hemoglobin sometimes being as low as 25 per cent., and the corpuscles diminishing to as low as 2,000,000 per c.mm. In addition to these complications severe streptococcus septicemia may account for a high grade of scarlatinal anemia. From an analysis of the cases reported by Zappert,[3] Felsenthal,[4] Widowitz,[5] Hayem,[6] and others the average loss of erythrocytes in all cases amounts to about 1,000,000 cells to the c.mm., but Kotschetkoff[7] notes a more decided average reduction, this author stating that they progressively decrease to about 3,000,000, and that regeneration is slow and gradual, not being completed for a period of six weeks.

LEUCOCYTES.

A well-marked leucocytosis, the count usually ranging between 20,000 and 30,000 per c.mm., occurs in the majority of cases, often first appearing several days in advance of the cutaneous eruption, and persisting in some cases long after convalescence has been established. Its duration varies widely in different instances: in some cases, not necessarily of a severe type, the leucocytosis persists for ten, or even thirty, days; while in others, usually of a mild type, it disappears before the temperature has fallen to normal. The maximum degree of increase is reached from four to six days after the onset of the illness.

In asthenic cases the number of leucocytes is increased but slightly, or not at all; but in the well-nourished child the degree of leucocytosis may be regarded as a rough gage of the intensity of the infection, being usually greater in severe than in mild cases. The increase appears to bear no fixed relationship either to the

[1] Inaug. Dissert., Berlin, 1890.
[2] *Loc. cit.*
[3] Zeitschr. f. klin. Med., 1893, vol. xiii, p. 292.
[4] Arch. f. Kinderheilk., 1892, vol. xv, p. 82.
[5] *Loc. cit.*
[6] St. Petersburg. med. Wochenschr., 1892, vol. i, p. 914.
[7] Russkiy Vrach, 1891, vol. xii, p. 919.

anginal infection or to the glandular involvement, for marked leucocytosis has been observed in cases with mild angina unaccompanied by swelling of the glands. Neither can any clear relation be established between the leucocytosis and the character of the temperature, the period of desquamation, and the inflammatory complications of the ear and kidneys.

In all of van den Berg's cases the number of leucocytes was in excess of normal, the "first counts" averaging slightly more than 17,000 per c.mm., and the leucocytosis being higher than 30,000 in only 2 cases. The investigations of the other authors above referred to give practically the same results, although somewhat higher counts have been made in some instances. Mackie[1] found leucocytosis constant in 25 cases, and in one patient with severe anginal symptoms the count rose to 93,300. He failed to observe any signs of a leucocyte increase until twenty-four hours after the appearance of the rash.

The leucocytosis is generally due to an increase in the polynuclear neutrophiles, these cells ranging from 85 to 90 per cent.; but in some instances the increase is more evenly divided between the polymorphous and mononuclear forms, so that from 70 to 80 per cent. of the former and from 15 to 30 per cent. of the latter may be found. The writer has noticed the presence of large numbers of the so-called transitional mononuclear leucocytes and of an occasional myelocyte. Van den Berg has noted the presence of small numbers of myelocytes in grave cases. Contrary to the rule which holds good in most febrile conditions, the number of eosinophiles in favorable cases of scarlet fever remains normal, or, indeed, may be decidedly increased. In the majority of favorable cases the eosinophile increase begins after the patient has been ill two or three days, and attains a maximum during the second or third week, after which it progressively diminishes, the percentage reaching normal by about the sixth week. In very grave cases a decrease or absence of these cells is usually found. In cases with nephritic complications their increase is thought to be favorable. The proportion of eosinophiles is usually from 4 to 5 per cent. of the other forms, sometimes even 10 or 15 per cent., especially during the post-febrile period of the disease. Bowie,[2] from a study of 167 cases, concludes that normal or subnormal eosinophile values after the first forty-eight hours mean a severe infection, and that the graver the case, the longer the persistence of these low figures.

The *blood plaques* are normal at the beginning of the attack,

[1] Lancet, 1901, vol. ii, p. 525.
[2] Jour. Path. and Bacteriol., 1902, vol. viii, p. 82.

but a large increase in their number is said to occur during the period of desquamation.

DIAGNOSIS. The presence of leucocytosis and persistence of the eosinophiles are suggestive signs in distinguishing scarlet fever from *measles*, since in uncomplicated cases of the latter disease these changes are absent. Disappearance of the eosinophiles is regarded as a bad prognostic sign.

## LXV. SEPTICEMIA AND PYEMIA.

GENERAL FEATURES. The blood changes found in those conditions due to the presence in the circulating blood of septic bacteria or their toxins, *general septicemia*, *sapremia*, and *pyemia*, are similar, and therefore may be considered together under the above heading. An apparently trivial infected wound may give rise to just as severe blood changes as an intense pyemia with wide-spread metastatic abscesses, since these alterations depend upon the virulence of the infection and the reaction which it provokes, rather than upon the character of the exciting lesion and the specific nature of the offending organisms. Clinically, these blood changes may be associated with such conditions as infected wounds, osteomyelitis, malignant endocarditis, puerperal fever, septic joints, and many other lesions for which various septic micro-organisms are held responsible.

The amount of *fibrin* is often appreciably increased in cases in which the reaction against the infection is well marked, especially in the early stages of the illness. A decrease in fibrin is common in patients with pronounced anemia and in those who readily succumb without reaction against the infection.

Thus far the *serum test* has given no reliable clinical information in this class of diseases, although several clinicians of the French school claim occasionally to have observed typical clumping of streptococcus bouillon cultures with the serum of patients suffering from streptococcus infections, such as streptococcus infected wounds, sepsis, puerperal fever, and erysipelas; but negative results were obtained in testing bouillon cultures of the staphylococcus with the serum of staphylococcus septicemia. The evidence brought forward to show that the serum of patients suffering from colon infections clumps cultures of the colon bacillus is by no means conclusive; for many races of the colon bacillus, it may be recalled, clump spontaneously and are agglutinated by normal serum.

If a test-tube containing blood serum of a patient suffering from pneumococcus septicemia is inoculated with a pure culture of the pneumococcus, it will be found that, after twenty-four hours' incubation, the serum still remains free from turbidity, and shows simply a slight sediment composed of pneumococci, capsuleless and glued together in tenacious clumps or in serpentine, trailing designs. Pneumococci grown in normal serum cloud the liquid, and develop a new growth, consisting of encapsulated, isolated organisms. Favorable results have been reported by several Continental writers who have used this test clinically, but its diagnostic value must still be regarded as questionable.

For a review of the literature of serum diagnosis in sepsis and in other conditions the reader should consult Rosenberger's comprehensive article.[1]

BACTERIOLOGY.

Blood cultures in sepsis are more frequently sterile than productive, but negative results neither exclude the existence of a septic process nor necessarily indicate a favorable prognosis. On the other hand, positive results are often of the greatest value in the diagnosis of obscure cases of sepsis, in which the clinical manifestations are more or less vague. As pointed out by Welch,[2] blood cultures in which the *Staphylococcus pyogenes albus* is demonstrated have little significance in the prognosis of the case, whereas the presence in the blood of the other pyogenic cocci is a sign of intense infection.

The results obtained by different investigators in the bacteriological examination of the blood in septicemia vary within wide limits, these variations being explained partly by the differences in the technical methods used by each reporter and partly perhaps by the nature of the infection. Petruschky[3] obtained 17 positive results in the examination of 59 cases of sepsis, streptococci being found in 15 and staphylococci in 2 instances. Sittman[4] examined 53 cases of septicemia, and succeeded in isolating streptococci in 4, staphylococci in 11, and pneumococci in 6. Czerniéwski[5] in 37 cases of puerperal sepsis obtained positive results in 10, pure cultures of streptococci being found in all the grave infections. Symes[6] obtained positive cultures in 9 of 31 cases of sepsis, the staphylococcus, the streptococcus, the pneumococcus, and the *Micrococcus tetragenus* having been the

[1] Proc. Path. Soc. of Phila., 1904, vol. vii, p. 97.
[2] Dennis' "System of Surgery," Philadelphia, 1895, vol. i, p. 251.
[3] Zeitschr. f. Hyg. u. Infektionskr., 1894, vol. xvii, p. 59.
[4] Deutsch. Arch. f. klin. Med., 1894, vol. liii, p. 323.
[5] Arch. f. Gynäkol., 1888, vol. xxxiii, p. 73.
[6] Brit. Med. Jour., 1901, vol. ii, p. 709.

organisms identified. Kühnau's investigations[1] show a much lower percentage of positive findings than are commonly reported, for this author, in 23 cases of septicopyemia, obtained growths in only 3 instances, while but a single positive finding resulted from the examinations of 12 cases of ulcerative endocarditis. Krauss,[2] who has had a very large experience in the bacteriology of the blood in various infectious diseases, reports 7 positive results in a series of 22 cases of septicemia, ulcerative endocarditis, and erysipelas. White,[3] in 18 severe cases of sepsis, all of which were fatal, obtained positive findings in 4: the *Streptococcus pyogenes* 3 times, and the *Staphylococcus pyogenes aureus* once. Canon[4] obtained 11 positive results in the examination of 17 cases of septicemia, pyemia, and osteomyelitis. Hirschlaff[5] obtained the streptococcus or staphylococcus 7 times in cultures made from 8 cases of sepsis. James and Tuttle,[6] in 6 severe septic infections, succeeded in finding the streptococcus in 2 instances. Brieger[7] obtained uniformly negative findings in the examination of 6 cases of puerperal sepsis. Similar results have also been reported by Neumann,[8] who obtained negative findings in blood cultures from 5 cases of pyemia. Grawitz[9] cultured pyogenic cocci only once in his examination of 7 cases of malignant endocarditis.

Consideration of these figures, together with the statistics of a number of other reporters of smaller series of cases, furnishes a total of 316 cases of sepsis in which it is reasonable to presume that the bacteriological examination of the blood has been made by dependable methods. Of these 316 cases, positive results were obtained in 107, while the remaining 209 proved negative—a percentage of 33.8 for the former. This analysis, however, is not to be regarded as equivalent to the statement that bacteriological examination of the blood gives positive diagnostic information in one-third of all cases, for the results of a single reliable observer, rather than the aggregate figures of several, are to be considered in order to arrive at a true estimate of the value of this procedure.

HEMOGLOBIN AND ERYTHROCYTES.

Anemia, of a grade proportionate to the intensity of the infection, is the rule in septic cases, regardless of the specific nature of the infective process. In very acute cases the diminution of hemoglobin and erythrocytes may be so excessive

[1] Zeitschr. f. Hyg. u. Infektionskr., 1897, vol. xxv, p. 492.
[2] Zeitschr. f. Heilk., 1896, vol. xvii, p. 117.
[3] Jour. Exper. Med., 1899, vol. iv, p. 425.
[4] Deutsch. Zeitschr. f. Chirurg., 1893, vol. xxxvii, p. 571.
[5] Deutsch. med. Wochenschr., 1897, vol. xxiii, p. 766.
[6] *Loc. cit.* [7] Charité-Annal., 1888, vol. xiii, p. 198.
[8] Berlin. klin. Wochenschr., 1888, vol. xxv, p. 143.
[9] Charité-Annal., 1894, vol. xix, p. 154.

and so rapid that an abrupt downward curve in the erythrocyte line of the blood chart may be detected from day to day, even from morning until night, in some instances. This rapidly developing type of anemia is associated especially with fulminant cases of puerperal septicemia, in which counts of less than 1,000,000 cells per c.mm. have been frequently reported. In a case of this sort the writer found the hemoglobin reduced to 20 per cent. and the erythrocytes to 730,000 per c.mm.

In less severe cases the development of the anemia is slower and of a more moderate grade, the hemoglobin being reduced to 40 or 50 per cent., and the erythrocytes to about 2,500,000 or 3,500,000 per c.mm.

The following estimates show the blood changes found in a case of puerperal sepsis during a period of four months:

| Date. | Hemoglobin Percentage. | Erythrocytes per c.mm. | Leucocytes per c.mm. |
|---|---|---|---|
| April 29, 1901 | 57 | 3,380,000 | 17,200 |
| May 16, 1901 | 52 | 3,390,000 | 14,200 |
| June 2, 1901 | 38 | 2,640,000 | 29,400 |
| " 9, 1901 | 22 | 2,000,000 | 33,100 |
| " 12, 1901 | 25 | 1,600,000 | 19,000 |
| " 16, 1901 | 30 | 1,850,000 | 16,800 |
| " 21, 1901 | 25 | 1,902,250 | 27,200 |
| " 27, 1901 | 35 | 2,339,000 | 19,200 |
| July 5, 1901 | 30 | 2,050,000 | 8,600 |
| " 22, 1901 | 30 | 2,300,000 | 6,000 |
| Aug. 6, 1901 | 35 | 3,150,000 | 15,000 |
| " 15, 1901 | 39 | 3,787,000 | 10,500 |
| " 22, 1901 | 48 | 3,637,000 | 9,200 |
| " 25, 1901 | 52 | 3,899,000 | 9,000 |

The color index is diminished moderately, but not excessively, save in an occasional instance; it averaged 0.85 for the series on page 529. Hemoglobinemia is found in occasional instances of grave character. Most writers lay stress on the excessively watery condition of the serum, particularly in those cases in which the development of anemia is early, marked, and rapid.

Deformities of shape and size and atypical staining phenomena are marked in relation to the degree of the anemia; they are rarely conspicuous, except in long-standing cases. The same remarks apply to the presence of nucleated erythrocytes. Granular basophilia is found with more or less constancy in severe cases.

In 79 hospital cases of septicemia and pyemia the hemoglobin and erythrocyte values were as follows:

| Hemoglobin Percentage. | Number of Cases. | Erythrocytes per c.mm. | Number of Cases. |
|---|---|---|---|
| From 90–100 | 1 | Above 5,000,000 | 4 |
| " 80–90 | 14 | From 4,000,000–5,000,000 | 21 |
| " 70–80 | 9 | " 3,000,000–4,000,000 | 29 |
| " 60–70 | 14 | " 2,000,000–3,000,000 | 17 |
| " 50–60 | 13 | " 1,000,000–2,000,000 | 7 |
| " 40–50 | 15 | Below 1,000,000 | 1 |
| " 30–40 | 6 | | |
| " 20–30 | 6 | | |
| Below 20 | 1 | | |
| Average, 58.9 per cent. | | Average, 3,430,687 per c.mm. | |
| Maximum, 92.0 " | | Maximum, 5,970,000 " " | |
| Minimum, 19.0 " | | Minimum, 730,000 " " | |

Leucocytes.

Leucocytosis is always present in those cases in which the infection, either moderate or marked, occurs in a patient whose powers of resistance are sufficiently strong to react against the poison. The increase in the number of leucocytes is usually moderate, counts of from 15,000 to 25,000 being commonest. In trifling infections, not sufficiently marked to produce activity of the leucocyte-forming organs, and in lethal cases, in which the system is overwhelmed by the toxins, not only does leucocytosis fail to develop, but sometimes distinct leucopenia may be observed. These facts render the occurrence of leucocytosis in septicemia an inconstant sign, for it is no uncommon experience to examine case after case of undoubted sepsis without encountering any increase in the leucocytes above normal. In the series tabulated below frank leucocytosis was found in 92 instances, or in approximately 70 per cent., while in 11 cases, or about 8 per cent., there was distinct leucopenia, the count in one being only 2000 per c.mm. All the cases not showing leucocytosis were either very mild or very severe infections.

The increase affects chiefly the polynuclear neutrophiles, which are both relatively and absolutely increased at the expense of the mononuclear forms. Mast cells and myelocytes in small numbers, are common. In a profoundly anemic case of sepsis Kline[1] found striking eosinophilia—40 per cent; a decided diminution of these cells is, however, the common finding. In all forms of sepsis, and especially in puerperal fever, the *iodin reaction* occurs

[1] Centralbl. f. inn. Med., 1899, vol. xx, p. 97.

with great constancy. Iodophilia is a more dependable sign of sepsis than the behavior of the leucocytes, since it is present in many cases so toxic as to stifle leucocytosis.

One hundred and thirty-five cases in which leucocyte counts were made showed the following averages:

| Leucocytes per c.mm | Number of Cases. |
|---|---|
| Above 40,000 | 1 |
| From 30,000–40,000 | 6 |
| " 20,000–30,000 | 14 |
| " 15,000–20,000 | 27 |
| " 10,000–15,000 | 44 |
| " 5,000–10,000 | 32 |
| Below 5,000 | 11 |

Average, 15,040 per c.mm.
Highest, 41,600 " "
Lowest, 2,000 " "

Diagnosis. The value of the blood examination as an aid to the diagnosis of septic conditions must be regarded as more or less uncertain. In cases with clinical manifestations suggesting at once *enteric fever*, *malarial fever*, and septicemia the presence of leucocytosis is highly suggestive of the latter condition, for in typhoid and in malaria leucocytosis rarely exists, except in the event of some complication. The early development of a rapidly increasing anemia would also point to sepsis rather than to typhoid or malaria, for in the latter fevers the anemia, although it begins early, does not reach a high grade until comparatively late in the course of the illness. The presence of a positive serum reaction, or the discovery of malarial parasites in the blood, will, of course, at once determine the diagnosis. If the diagnosis lies between sepsis and *miliary tuberculosis*, increase in the number of leucocytes points to the former. The iodin reaction may be present in all the conditions noted above.

In cases without leucocytosis but with marked iodophilia a bad prognosis is justified, for the reason stated above.

## LXVI. SPOTTED FEVER OF MONTANA.

PARASITOLOGY.

Ovoid bodies, presumably hematozoa, have been found first by Wilson and Chowning[1] and later by Anderson[2] and by Cobb[3] in the circulating blood of those ill with the disease known as spotted fever, or tick fever, prevailing in the Bitter Root Valley in Montana. The former investigators have labeled their discovery the *Piroplasma hominis.*

The ovoid bodies in question remind one of both the Texas fever and the malarial parasite, though they differ from the former in being larger and in possessing ameboid motility, and from the common forms of the latter in being unpigmented. In the fresh blood three forms of the piroplasma, each ovoid in shape, were found within the erythrocytes: a small non-motile form, 1 to 2 $\mu$ in length by 1 $\mu$ in width; a larger, actively ameboid form, 3 to 5 $\mu$ in length by 1 to 1.5 $\mu$ in width, and showing a dark granular spot at one end; and a twin form, consisting of two pear-shaped bodies lying with their tapered ends approaching, and bearing a granular spot at each end. Extracellular diplococcic-like forms, 0.5 to 1 $\mu$ in size, and not endowed with motion, were also identified. In the dry film the bodies are stained best by one of the basic dyes, such as methylene-blue or thionin. The theory is tempting that the infection of spotted fever is conveyed by a variety of tick known as the *Dermacentor reticulatus.*

HEMOGLOBIN AND ERYTHROCYTES.

Moderate anemia, usually with a low color index, is the rule. The hemoglobin percentage may fall as low as 50 or 60, but the erythrocyte count remains at about 4,000,000 cells per c.mm. The effect of high altitude in masking an anemia must, however, be taken into consideration, for the counts in all the reported cases of spotted fever were made at an elevation of 3500 feet above the sea-level. No structural changes in the erythrocytes have been noted.

LEUCOCYTES.

The leucocytes are slightly increased—to 12,000 or 13,000 per c.mm.—and show, differentially, nothing abnormal save, perhaps, a moderate increase in the large lymphocytes at the expense of the small hyaline cells.

DIAGNOSIS.

The specificity of the ameboid bodies found in this disease seems to be well established, and their detection in the blood should prove a

[1] Jour. Amer. Med. Assoc., 1902, vol. xxxix, p. 131.
[2] Amer. Med., 1903, vol. vi, p. 506.
[3] Public Health Rep., 1902, vol. xvii, p. 1868.

means of excluding *malarial* and *enteric fevers*. From the latter the absence of a serum reaction and the presence of a moderate leucocyte increase are additional points of differentiation.

## LXVII. SYPHILIS.

BACTERIOLOGY.

Micrococci (Hallin; Martineau), pleomorphous bacilli (van Niessen), cocci-bacilli (Klebs), bacteria resembling the Klebs-Löffler organism (Joseph and Piorkowski), and spore-like bodies (Klotzsch; Lostorfer) are some of the prominent "organisms" which, during the last three decades, have been found in the circulating blood of syphilitics and have been exploited as the exciting cause of the disease. The vogue of these findings, as well as that of those relating to the presence of a syphilitic organism in various tissues, has been ephemeral. Lustgarten's bacillus, regarded by many as specific, has thus far resisted artificial cultivation. De Lisle and Jullien, in 1901, claimed to have cultured a bacillus from the blood of syphilitics, and to this microbe they ascribe specific properties. The bacillus in question is said to occur constantly in the blood during the secondary manifestations of the infection, and to excite indurated ulcer, adenitis, and other luetic signs when inoculated into animals. It possesses peculiar cultural traits, for an account of which the reader is referred to De Lisle's original article.[1]

HEMOGLOBIN AND ERYTHROCYTES.

During the early stages of the infection, in the interval between the appearance of the initial lesion and the development of secondary symptoms, the blood changes closely counterfeit those of typical chlorosis, a fact which has led to the use of the term "syphilitic chlorosis" to describe the blood picture of early lues. The hemoglobin progressively falls until the loss approximates 20 or 30 per cent., while the number of erythrocytes remains normal or is but slightly diminished, in consequence of which the color index is low. As secondary symptoms appear oligocythemia usually develops, and in some instances reaches a high grade. There is a close relationship between the intensity of the infection and the intensity of the anemia. In the tertiary and hereditary forms of the disease if treatment is neglected, the count may fall to approximately 1,000,000 cells, and the hemoglobin to 20 per cent. or even less, while extreme poikilocytosis, megalocytosis, and microcytosis may be present, together with

[1] Amer. Med., 1903, vol. vi, p. 474.

numerous normoblasts and, perhaps, a few megaloblasts—the so-called "syphilitic pernicious anemia." But the anemia seldom reaches this grade, since most syphilitics receive adequate treatment early in the course of the disease. Löwenbach and Oppenheim,[1] from a recent study of 36 cases, have shown that a diminution of the hemoglobin and iron content, with trifling oligocythemia, is the usual finding in the tertiary stage.

After the administration of mercury both the hemoglobin and the erythrocytes begin to increase, the former more slowly than the latter, until treatment has been continued for about two or three weeks, but should this drug be given for longer than this period, just the opposite effect is produced—first a diminution in the hemoglobin percentage, followed later by oligocythemia. Ossendowski[2] found that the initial increase is more rapid after intramuscular injections of mercury than after its administration by inunction or by the mouth. He also determined that the effects of potassium iodid as a regenerator of the hemoglobin content are more decided than those of mercurials.

Buffa[3] concludes, from a study of 21 cases, that the hemogenesis excited by mercury is temporary, owing chiefly to the feeble resistance of the newly bred cells, many of which perish prematurely. The ultimate effect of this drug, therefore, is hemolytic, although it may antidote the poison of the disease. Extreme hemoglobin loss in patients undergoing mercurialization is regarded as prognostic of severe tertiary manifestations as the infection matures. The intravenous injection of mercuric chlorid rapidly causes hemoglobinemia in syphilitics. It is a well-recognized clinical fact that the blood changes provoked by syphilis are likely to be more marked in women than in men, other things being equal.

*Justus' Test.*—This reaction, described by Justus,[4] depends upon the presumption that in untreated cases of congenital, secondary, and tertiary syphilis, a single dose of mercury, administered either by inunction or by subcutaneous or intravenous injection, causes a hemoglobin loss of from 10 to 20 per cent. within about twenty-four hours, this abrupt decline being followed within a few days by a rise in the hemoglobin value to a somewhat higher figure than that first observed before the drug was given. In Justus' last communication,[5] relating to over 500 cases, data are given which show that the test is positive in from 70 to 80

[1] Deutsch. Arch. f. klin. Med., 1903, vol. lxxv, p. 22.
[2] Inaug. Dissert., Dorpat, 1903. [3] Sem. méd., 1903, vol. xxiii, p. 75.
[4] Virchow's Arch., 1894, vol. cxl, pp. 91 and 533.
[5] Deutsch. Arch. f. klin. Med., 1903, vol. lxxv, p. 1.

per cent. of all cases of florid syphilis, and that it disappears with the involution of the specific lesions and reappears with their recurrence. It is further claimed that negative findings are constant in healthy persons and in those suffering from non-syphilitic diseases. Failures to obtain good results Justus attributes to faulty diagnosis and to wrong technic—at least 3 gm. of blue ointment for an adult or 1 gm. for a child must be used for the inunction, the final hemoglobin estimate being made the following morning. Cabot and Mertins[1] obtained positive results in 7 syphilitics, and also in one case of chlorosis and in one of tertian malarial fever, but in their hands the test proved negative in 32 control cases of other diseases. Regarding the exceptional non-syphilitic diseases in which the reaction may prove positive, Brown and Dale[2] state that such cases are characterized by striking oligochromemia. A thorough study of the test has been made by Jones,[3] who examined 53 cases, of which number 35 were syphilis, and 18 cases of other diseases. Of the former, 17 were active syphilis untreated, and of these the test was positive in 13 and negative in 4; 15 cases of chancre yielded but 7 positive results, these occurring most frequently in chancre with adenitis; in two cases of latent syphilis and in one of active syphilis under treatment the test failed. Tucker,[4] Huger,[5] Christian and Foerster,[6] and Oppenheimer and Löwenbach[7] have also reported similar inaccuracies in the test. From the statistics of these investigators (121 cases) Ewing's analysis[8] credits the test with positive findings in 62 per cent. of cases of active syphilis, in 30 per cent. of chancre, and in 67 per cent. of chancroid. In the writer's experience, limited to 9 cases, the success of the test has been uniform.

The diagnostic value of Justus' test is greatly restricted by its frequent failure in early initial lesions and in latent syphilis, and its occasional failure in the early part of the secondary stage, periods when a pathognomonic test would prove of the greatest aid. The fact that positive reactions may occur in non-syphilitic diseases, in which hypersensitiveness to the action of mercury is to be presumed, obviously is against the test's specificity.

**Leucocytes.** The number of leucocytes, which remains approximately normal during the preëruptive stage of the disease, usually increases moderately with

[1] Boston Med. and Surg. Jour., 1899, vol. cxl, p. 323.
[2] Cincinnati Lancet-Clinic, 1900, vol. xliv, p. 261.
[3] N. Y. Med. Jour., 1900, vol. lxxi, p. 513.
[4] Phila. Med. Jour., 1902, vol. ix, p. 846.
[5] *Ibid.*, p. 849. [6] Univ. Med. Mag., 1900, vol. xiii, p. 634.
[7] Deutsch. Arch. f. klin. Med., 1901, vol. lxxi, p. 425.
[8] "Clinical Pathology of the Blood," 2d ed., New York and Philadelphia, 1903, p. 342.

the appearance of the secondary symptoms. Their total number rarely equals twice the maximum normal standard, and the gain is due, in the great majority of instances, to an increase in the non-granular hyaline forms, the percentage of polynuclear neutrophiles being relatively low. Many authors maintain that the eosinophiles are increased, but Peter,[1] who has especially investigated this question, emphatically states that in no form and at no stage of syphilis has he observed eosinophilia. In the leucocyte increase frequently found in the high-grade anemia of tertiary syphilis the lymphocytosis is especially striking, and the presence of small numbers of myelocytes is common. Under the influence of mercurial or iodid treatment the leucocyte count diminishes, the lymphocytes decrease, and the polynuclear neutrophiles grow more numerous.

The writer has found *iodophilia* in severe syphilitic anemia.

In patients whose blood is approximately normal no numerical changes in the *blood plaques* occur. In severe syphilitic anemia the plaques are increased—a change for which Vörner[2] blames the anemia, not the syphilis.

DIAGNOSIS. But slight diagnostic value can be attached to the changes in the blood in this disease. The association of a low color index and a leucocyte increase chiefly of the lymphocytes is suggestive, but nothing more. Justus' test, if positive, strengthens the pertinence of the preceding signs, provided that all sources of fallacy can be excluded; absence of the reaction by no means excludes syphilis. The distinctions between tertiary syphilitic anemia and true pernicious anemia have already been discussed. (See p. 289.)

## LXVIII. TETANUS.

In a fatal case treated with antitoxin Cabot[3] found 70 per cent. of hemoglobin and 11,900 leucocytes per c.mm., with no decrease in the number of eosinophiles, as is usual in most febrile states. In two other cases, also fatal and treated with antitoxin, he found leucocytoses of 19,600 and 18,200, respectively.

## LXIX. TONSILLITIS.

As a general rule, no appreciable changes are found in the *hemoglobin* and *erythrocytes*, although in severe cases the former is sometimes diminished. *Leucocytosis* of a moderate grade may or may not develop, depending largely upon the character of the

[1] Dermatolog. Zeitschr., 1897, vol. iv, p. 669.
[2] Deutsch. med. Wochenschr., 1902, vol. xxviii, p. 897.
[3] *Loc. cit.*

tonsillar inflammation. When present, the increase involves principally the polynuclear neutrophiles, and the total leucocyte count rarely exceeds 15,000 cells to the c.mm. Leucocytosis is less common and less decided in follicular tonsillitis than in quinsy. In the latter Pée,[1] Rieder,[2] and others have observed counts in excess of 20,000.

The leucocyte count is of no aid in differentiating tonsillitis, *diphtheria*, and *streptococcus inflammations* of the throat.

## LXX. TRICHINIASIS.

HEMOGLOBIN AND ERYTHROCYTES.

It is generally agreed that there are no changes in the hemoglobin and erythrocytes attributable to the influence of this infection, high counts and hemoglobin estimates, often polycythemia, being the rule. Rarely, well-marked anemia may be found, due to some other cause, as in a case reported by Kerr,[3] in which the erythrocytes numbered between 3,300,000 and 3,340,000 per c.mm.

LEUCOCYTES.

T. R. Brown[4] first made the important announcement that acute cases of trichiniasis are accompanied by a well-marked increase in the number of leucocytes, characterized by an absolute and relative gain in the eosinophiles. This observation has since been corroborated by a number of other workers whose results are tabulated below.

Unfortunately, eosinophilia cannot be regarded as constant in this condition, as shown by the following count made by the writer in a typical case of trichiniasis occurring in J. Chalmers Da Costa's surgical service at St. Joseph's Hospital:

| | |
|---|---|
| Hemoglobin | 80 per cent. |
| Erythrocytes | 4,400,000 per c.mm |
| Leucocytes | 12,000 " " |
| Small lymphocytes | 36.7 per cent. |
| Large lymphocytes and transitional forms | 6.5 " |
| Polynuclear neutrophiles | 56.1 " |
| Eosinophiles | 0.5 " |
| Myelocytes | 0.2 " |
| Basophiles | 0.0 " |

Repeated examinations by others showed practically these figures, the eosinophiles at no time being increased. The lesions

[1] Inaug. Dissert., Berlin, 1890. [2] *Loc. cit.*

[3] Phila. Med. Jour., 1900, vol. vi, p. 346.

[4] Johns Hopkins Hosp. Bull., 1897, vol. iii, p. 79; also Jour. Exper. Med., 1898, vol. iii, p. 315.

in this patient were most striking, as they involved the greater part of the right lower extremity, from calf to thigh. Excised bits of muscles from the affected parts were found to be swarming with trichinæ and rich in eosinophile cells. It is possible that in such instances as this the absence of eosinophilia may be attributed to the overwhelming nature of the toxins, which, by their repellant action, stifle eosinophile proliferation in the marrow. This, indeed, has been proved by Opie,[1] who found that in dogs mortally infected with trichinæ the circulatory eosinophiles rapidly diminished, and at the same time those of the marrow, mesenteric glands, and intestinal mucosa showed marked degenerative changes. In other (milder) cases, as the disease becomes chronic, the eosinophilia of the early stages tends to disappear, as is the rule in other forms of helminthiasis. Howard[2] failed to find an eosinophile increase in a single case, although large numbers of these cells were detected in the muscle lesions, and Drake[3] and Schleip[4] also report trichiniasis without eosinophilia.

LEUCOCYTE COUNT AND PERCENTAGE OF EOSINOPHILES IN 84 CASES OF TRICHINIASIS.

| Name of Reporter. | Number of Cases. | Total Number of Leucocytes per c.mm. | Relative Percentage of Eosinophiles to Other Forms of Leucocytes. |
|---|---|---|---|
| T. R. Brown[5] | 3 | 8,000–35,000 | 8–68.2 |
| Gwyn[6] | 1 | 17,000 | 33–65.9 |
| Kerr[7] | 2 | 10,000–25,000 | 18.1–86.6 |
| Blumer and Neuman[8] | 9 | 6,000–24,000 | 8–50.4 |
| Stump[9] | 1 | ..... | 52 |
| Cabot[10] | 4 | 1,410–25,000 | 7.8–37 |
| Atkinson[11] | 1 | 28,000 | 35–58.5 |
| Gordinier[12] | 2 | ..... | 31.9–77 |
| H. Brooks[13] | 1 | 18,000 | 15–83 |
| Patek[14] | 1 | ..... | 30 |
| Gould[15] | 1 | 9,800 | 23 7–30.6 |
| Cheney[16] | 1 | 12,000–15,000 | 10–17 |
| Schleip[17] | 57 | 5,300–22,600 | 1.2–62.2. |

[1] Amer. Jour. Med. Sci., 1904, vol. cxxvi, p. 477.
[2] Phila. Med. Jour., 1899, vol. iv, p. 1085.
[3] Jour. Med. Research, 1902, vol. iii, p. 255.
[4] "Die Homberger Trichinosisepidemie und die für Trichinosis pathognomonische Eosinophilie," Leipsic, 1904.
[5] *Loc. cit.* [6] Centralbl. f. Bakt. u. Parasit., 1899, vol. xxv, p. 746. [7] *Loc. cit.*
[8] Amer. Jour. Med. Sci., 1900, vol. cxix, p. 14.
[9] Phila. Med. Jour., 1899, vol. iii, p. 1318.
[10] Boston Med. and Surg. Jour., 1897, vol. cxxxvii, p. 676; also "Clinical Examination of the Blood," 5th ed., New York, 1904.
[11] Phila. Med. Jour., 1899, vol. iii, p. 1243.
[12] Med. News, 1900, vol. lxxvii, p. 965. [13] Med. Rec., 1900, vol. lviii, p. 885.
[14] Amer. Med., 1901, vol. i, p. 513. [15] *Ibid.*, 1903, vol. vi, p. 515.
[16] Amer. Med., 1903, vol. vi, p. 985. [17] *Loc. cit.*

The other differential changes, which are unimportant, consist in a corresponding relative decrease in the polynuclear neutrophiles, and occasionally, in the early stages of some cases, in a similar diminution in the lymphocytes. Mast cells and myelocytes, in small numbers, have also been observed, although not constantly.

Blumer and Neuman's studies of 9 cases of epidemic trichiniasis[1] lead them to conclude that the degree of leucocyte increase corresponds in a general way to the severity of the attack, relatively severe cases being attended with a higher and more persistent increase than the milder attacks; on the other hand, the intensity of the infection does not necessarily correspond to the degree of cosinophilia. The latter may persist for months after the disappearance of the leucocytosis and the apparent convalescence of the patient, but just how long it does last is as yet undetermined.

DIAGNOSIS. The presence of an eosinophile leucocytosis, usually of a high grade, may be the only indication of trichiniasis in obscure cases in which the characteristic symptoms of the infection are wanting, and in such instances the change is to be regarded as a most valuable aid to diagnosis. Absence of this sign, however, does not definitely exclude the disease.

## LXXI. TRYPANOSOMIASIS.

PARASITOLOGY. Nepveu,[2] in 1898, published his observations on finding, eight years previously, trypanosomata in the circulating blood of man, but not until the discoveries of Forde,[3] Dutton,[4] Manson,[5] and Daniels[6] were published did human trypanosomiasis become recognized as a distinct entity in tropical medicine. To Forde and to Dutton is due the credit of first adequately describing the organism. Since the pioneer labors of these investigators our knowledge of the condition has been extended by the studies of Castellani,[7] Bruce,[8] Baker,[9] Todd,[10] Leishman,[11] Nabarro,[12] Sambon,[13] Lave-

[1] *Loc. cit.* [2] Compt. rend. Soc. biol., Paris, 1898, vol. v, p. 1172.
[3] Jour. Trop. Med., 1902, vol. v, p. 261.
[4] Brit. Med. Jour., 1902, vol. ii, p. 881; 1904, vol. ii, p. 369; also Dutton and Todd, "First Report of the Trypanosomiasis Expedition to the Senegambia (1902) of the Liverpool School of Tropical Medicine and Medical Parasitology," Lancet, 1903, vol. ii, p. 1727.
[5] Brit. Med. Jour., 1903, vol. i, pp. 720 and 1249; vol. ii, p. 645.
[6] *Ibid.*, 1903, vol. i, p. 1249. [7] *Ibid.*, 1903, vol. i, p. 1431.
[8] *Ibid.*, 1903, vol. ii, p. 1343. [9] *Ibid.*, 1903, vol. i, p. 1254.
[10] *Ibid.*, 1903, vol. ii, p. 645. [11] *Ibid.*, 1903, vol. i, p. 1252.
[12] Lancet, 1904, vol. i, p. 229. [13] *Ibid.*, 1904, vol. i, p. 228.

ran,[1] and others. Aside from the blood findings, human trypanosomiasis is characterized by progressive asthenia, wasting, edema, splenic tumor, accelerated pulse and respirations, and irregular fever of a relapsing type. The disease runs an exceedingly chronic course, and has received the name "trypanosoma fever." Castellani[2] was the first to demonstrate the presence of trypanosomata in the blood and cerebrospinal fluid of persons suffering from African "sleeping sickness," and later Bruce,[3] having confirmed these results, repeated the original suggestion of Maxwell Adams that many, if not all, of the cases of so-called trypanosoma fever in reality represent early stages of sleeping sickness. The presence of Castellani's streptococcus, and of Bettencourt's diplostreptococcus (probably the same organism) in the cerebrospinal fluid of patients infected with this disease is thought to mean a concomitant infection. These conclusions have recently been voiced by the Royal Society's Sleeping Sickness Commission.[4] Singularly enough, the Portuguese Commission,[5] headed by Bettencourt, did not find trypanosomata in sleeping sickness, which they attribute to a variety of streptococcus.

There is possibly a relationship (although such is still unproved) between the parasite of trypanosomiasis and certain obscure tropical maladies, such as kala-azar, dum-dum fever, tropical splenomegaly, and the undetermined hyperpyrexias of West Africa.

The organism found in man, tentatively named *Trypanosoma gambiense*, is regarded as a form of trypanosoma distinct from those responsible for tsetse-fly disease, surra, dourain, and mal de caderas, in the lower animals. The parasites multiply by fission, but evidences of this process are rarely, if ever, found in the circulating blood of the periphery. They are conveyed by a species of tsetse-fly, *Glossina palpalis*, which acts simply as a mechanical carrier of the parasite, and not as a host for the evolution of a sexual cycle. Laveran[6] has shown that the administration of arsenic to animals infected with the trypanosoma causes a rapid disappearance of the parasites from the general circulation, and it has long been recognized that this drug has a favorable action in the treatment of trypanosoma disease in the lower animals. Ehrlich

[1] Compt. rend. Acad. d. sc., Paris, 1904, vol. cxxxviii, p. 841.
[2] Brit. Med. Jour., 1903, vol. i, pp. 1218 and 1431.
[3] *Ibid.*, 1903, vol. ii, pp. 1008 and 1291; 1904, vol. ii, p. 367.
[4] Bruce, Nabarro, and Greig, "Report of the Sleeping Sickness Commission of the Royal Society of London," Brit. Med. Jour., 1903, vol. ii, p. 1343; also Dutton, Todd, and Christy, "First Progress Report of the Expedition of the Liverpool School of Tropical Medicine and Medical Parasitology to the Congo, 1903," *ibid.*, 1904, vol. i, p. 186; *ibid.*, 1904, vol. ii, p. 369.
[5] "Doenga do Somno," Lisbon, 1902. [6] Sem. méd., 1904, vol. xxiv, p. 68.

and Shiga[1] effected permanent cures in trypanosomatous mice by the use of trypan red (a dye belonging to the benzopurpurin series), given hypodermically and by the mouth. They also succeeded in establishing, by the use of this drug, a transient immunity against the infection.

In the fresh specimen of blood the parasite appears as a minute, worm-like organism which glides about, with undulatory motility, between the collections of blood corpuscles (Fig. 64). It is readily recognized as a flagellated protozoön, one end of which is bluntly conical, and the other drawn out into a whip-like flagellum (rarely two flagella occur), while a transparent, flange-like process or undulatory membrane is attached along one side

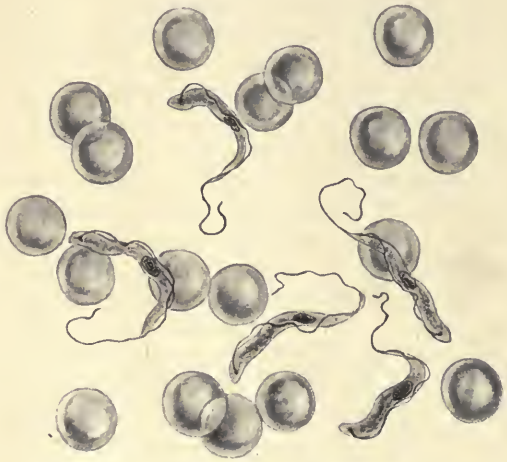


FIG. 64.—TRYPANOSOMA GAMBIENSE.
From a stained specimen furnished by Dr. J. E. Dutton.

of the body. The latter is a rather short, granular structure, provided with a highly refractile spot, or nucleus, near the blunt end. The movements of the worm, both forward and backward, are effected by a sort of screw-like motility, beginning in the flagellated process and shared by the undulatory membrane and by the body protoplasm. In the fresh specimen the trypanosomata perish within from one to three hours after the blood is drawn. In the stained film Dutton[2] found that the parasites measured from 18 to 25 $\mu$ in length and from 2 to 2.8 $\mu$ in width, and that they bear, just posterior to the nucleus, a centrosome or micronucleus. With Wright's or Leishman's polychrome methylene-blue stains

[1] Berlin. klin. Wochenschr., 1904, vol. xli, pp. 329 and 362. [2] *Loc. cit.*

the protoplasm is colored pale blue, and the nucleus, centrosome, and flagellum red. The organisms stain better with carbolfuchsin than with the weaker basic dyes, such as hematoxylin.

HEMOGLOBIN AND ERYTHROCYTES.

In the cases of trypanosomiasis thus far studied there has been found well-defined, although not excessive, anemia. In no instance has the hemoglobin fallen below 36 per cent., nor the erythrocytes below 2,825,000 per c.mm., and in most cases the loss amounted to about 20 or 25 per cent., affecting both elements proportionately. The erythrocytes deviate but little from their normal size and shape, and normoblasts (found only occasionally, in small numbers) are the only form of nucleated erythrocytes encountered.

LEUCOCYTES.

The leucocyte counts, absolute and differential, are not unlike those of the malarial fevers, showing either a normal or a subnormal total estimate and a decided relative lymphocytosis affecting the large lymphocytes. These cells are increased to twice or thrice their normal percentage, at the expense of the polynuclear neutrophiles, without appreciable alteration in the proportion of small lymphocytes and eosinophiles. Mast cells ($\frac{1}{4}$ to $\frac{1}{2}$ per cent.), as well as indeterminate hyaline cells, resembling those found in myelogenous leukemia, were also noted in the cases examined by Duncan, Daniels, and Low.[1] Daniels[2] pictures these cells as having a relatively large nucleus, staining, by the Romanowsky method, a delicate pink splotched with purple, encircled by a narrow zone of blue non-granular protoplasm.

DIAGNOSIS.

The detection of trypanosomata in the blood is obviously the key to the diagnosis, but in cases in which no organisms can be found, a well-defined anemia with a low leucocyte count, characterized by an excess of large lymphoid cells and by moderate basophilia, gives a very suggestive blood picture.

## LXXII. TUBERCULOSIS.

GENERAL FEATURES.

A pure infection with Koch's bacillus of tuberculosis is capable of producing comparatively slight alteration in the composition of the blood, such changes as may be associated with tuberculous processes, whatever organs they involve, being due chiefly to secondary infection with other bacteria, usually of pyogenic

[1] Cited by Manson and Daniels, Brit. Med. Jour., 1903, vol. i, p. 1249.
[2] "Studies in Laboratory Work," London, 1903, p. 68.

type, and not to the disease *per se*. The prolonged ill effects of tuberculosis upon body nutrition must also in time cause more or less blood impoverishment, but it is a well-recognized clinical fact that the changes are, as a rule, trivial in comparison with the gravity of the disease and the apparent degree of cachexia. The above facts are sufficient to explain the reason for the varied blood pictures found in tuberculosis—pictures ranging from those of practically normal blood to those of most intense anemia, and from leucopenia to frank leucocytosis.

BACTERIOLOGY.

In a limited number of cases of acute miliary tuberculosis the specific bacillus has been isolated from the blood during life by culturing and by intraperitoneal inoculation in animals; but in this as well as in the other forms of the disease this procedure generally results negatively so far as the detection of the tubercle bacillus is concerned. Nattan-Larrier's method[1] of examining fluids for tubercle bacilli is worthy of trial. An injection of 2 or 3 c.c. of blood is made into a guinea-pig's mammary gland, in which the organisms rapidly multiply. A sample of milk expressed from the inoculated gland is examined, after the lapse of a few days, by the technic used in sputum staining, with the result that in positive cases tubercle bacilli are found, generally within from five to ten days after the injection. In advanced septic cases of pulmonary tuberculosis, streptococci, staphylococci, blastomycetes, and other micro-organisms have been found in the blood, but only rarely, for the septic process tends to remain localized in the lungs, rather than to invade the general circulation.

*Serum Test.*—Arloing and Courmont[2] have succeeded in preparing cultures with which they claim that the serum diagnosis of tuberculosis can be carried out. Glycerin peptone bouillon, inoculated with an old, attenuated culture of the tubercle bacillus and thoroughly agitated each day to insure homogeneity of the culture, finally develops a growth in which the bacilli are uniformly disseminated and actively motile. Blood serum from the suspected case is mixed in small test-tubes with the culture thus prepared, in proportions of 1 to 5, 1 to 10, and 1 to 20, and the tubes inclined at an angle of 45 degrees, being examined at intervals of two, ten, and twenty-four hours. A positive reaction is indicated by a clarification of the mixture and the deposition of small flakes or granules in the bottom of the tube, while microscopically it may be seen that the bacilli are clumped and motionless. With this technic, reactions occurring after the lapse of twenty-

[1] Presse méd., 1903, vol. ii, p. 838.
[2] Congrès pour l'Etude de la Tuberculose, 1898.

four hours are without clinical significance. Or Koch's method[1] of using a solution of sterile "tubercle powder" may be employed. A test solution is made by dissolving pulverized sterile tubercle cultures in normal salt solution, and then centrifugalizing the fluid, so as to obtain a clear, opalescent liquid free from bacilli. The test fluid thus prepared is mixed with the suspected blood serum, sedimentation occurring after twenty-four hours in positive reactions. With normal serum in a dilution of 1 to 5 positive reactions do not occur, and they occur but rarely with tuberculous serum in a dilution higher than 1 to 20. A peculiarity about this test is that it takes place in an inverse ratio to the intensity of the infection, and hence it fails in advanced and virulent cases in which presumably there is already an excessive auto-intoxication with tuberculin. Excluding such cases, Arloing and Courmont found that positive reactions were constant in all tuberculous patients, but, unfortunately, they also found similar results in some normal individuals and in various non-tuberculous diseases.

Bendix[2] found the test successful in 34 of 36 cases of tuberculosis, the two failures being in instances of overwhelming infections; he also claims that normal blood and the blood from other diseases give negative results. Nine cases of pulmonary tuberculosis, 4 of pleurisy, and 17 of various non-tuberculous affections were examined by Mongour and Buard.[3] All the phthisis cases and 3 of the 4 pleurisies, which were tuberculous, were positive, the case not reacting proving to be non-tuberculous. In 15 of 17 other diseases the results of the test corresponded with the clinical diagnosis and the autopsy findings. Similar results in tuberculous pleurisy were obtained by P. Courmont,[4] who found positive reactions in 10 of 11 cases clinically tuberculous, while of 9 cases clinically non-tuberculous 4 were positive and 5 negative. In 12 cases of ascites, 7 due to hepatic cirrhosis failed to react, but the other 5, all clinically tuberculous, gave positive results. Results distinctly less favorable than those reported by other investigators are published by Beck and Rabinowitch,[5] but it is not at all improbable that these discrepancies may be attributed, at least in part, to the use of unsuitable cultures. According to these authors' experiments, only 6 of 17 cases of incipient lung tuberculosis were positive, and but 4 of 16 advanced cases. Of 5 suspected cases that reacted to tuberculin injections, but one gave a positive serum reaction. They furthermore found

[1] Deutsch. med. Wochenschr., 1901, vol. xxvii, p. 829.
[2] *Ibid.*, 1900, vol. xxvi, p. 224.
[3] Compt. rend. Soc. biol., Paris, 1898, vol. v, p. 1142; also Buard, Jour. de phys. et path. gén., 1900, vol. ii, p. 797.
[4] Congrès pour l'Etude de la Tuberculose, 1898.
[5] Deutsch. med. Wochenschr., 1900, vol. xxvi, p. 400.

that positive reactions may occur in healthy persons, and in rheumatic fever, bronchitis, hepatic cirrhosis, and croupous pneumonia. Von Gebhardt and Torday,[1] using Arloing's homogeneous cultures, found that in 56 of 75 tuberculous patients and 35 of 96 non-tuberculous diseases the test was positive, and that a similar result occurred in 3 of 5 healthy persons examined. Rumpf and Guinard[2] obtained positive results in 90 of 107 cases. Kazarinoff,[3] who examined 73 tuberculous and 10 normal persons, found the test uniformly positive in the former and negative in all the latter except one. Ivanov[4] obtained the reaction in 14 of 21 cases tested. Romberg[5] has determined that the serum of more than 50 per cent. of persons who fail to show clinical evidences of tuberculosis possesses a more or less agglutinative property.

The serum test in tuberculosis, as at present elaborated, must be considered of questionable diagnostic value, since it has been shown that it may occur in normal individuals and in non-tuberculous diseases, and that it may often be negative in affections undoubtedly tuberculous. The reaction appears to become less and less marked as the disease advances, and to be more striking in mild than in severe lesions. Marchetti and Stefanelli,[6] for example, in a study of 73 cases of pulmonary and abdominal tuberculosis, found that 88 per cent. of early, mild cases and but 42 per cent. of late, grave cases reacted positively to the test. Romberg,[7] in 105 cases, found that the reaction occurred in 80 per cent. of cases in the first stage of phthisis, in 66 per cent. in the second stage, and in 57 per cent. in the third stage. Wright[8] values the test chiefly as an index to the organism's ability to elaborate antibactericidal substances, as shown by the development of the reaction in the blood of patients subjected to therapeutic inoculations of tubercle vaccine. As compared with Widal's typhoid reaction, the test of Arloing and Courmont is crude and untrustworthy.

HEMOGLOBIN AND ERYTHROCYTES.

The change most frequently observed is a moderate loss of hemoglobin, with little or no decrease in the number of erythrocytes, and a low color index, resembling somewhat the blood picture of chlorosis. In such instances poorly colored, small-sized corpuscles may be numerous, but poikilocytes and other structural alterations in the cells are absent. In cases in

[1] Münch. med. Wochenschr., 1902, vol. xlix, p. 1171.
[2] Deutsch. med. Wochenschr., 1902, vol. xxviii, p. 131.
[3] Jour. Amer. Med. Assoc., 1902, vol. xxxviii, p. 362.
[4] Sem. méd., 1902, vol. xxii, p. 207.
[5] Deutsch. med. Wochenschr., 1901, vol. xxvii, p. 292.
[6] Riv. Crit. d. Clin. Med. Fir., 1903, vol. iv, pp. 657, 673, and 689.
[7] Münch. med. Wochenschr., 1902, vol. xlix, p. 89.
[8] Lancet, 1903, vol. i, p. 1299.

which the effects of a complicating septicemic process are active the above changes may be aggravated, and a secondary anemia of variable intensity is thus developed. The oligocythemia becomes marked and more proportionate to the oligochromemia, the color index consequently rising; deformities of shape and size and degenerative stroma changes become evident; and in severe cases an occasional normoblast may stray into the circulation, especially after the occurrence of a hemorrhage. But these qualitative changes, even in advanced cases with marked cachexia, are comparatively uncommon, and, when present, are usually not striking in spite of the gravity of the disease.

Appelbaum[1] points out that in a group of phthisis cases characterized by the alar chest, by underweight, and often by a scrofulous history, a decided anemia is likely to develop long before physical and bacteriological signs become manifest. Both Baumholtz[2] and Gozdzicki[3] have determined, in a large series of cases, that the resistance of the erythrocytes is lowered proportionately to the intensity of the systemic infection.

Finally, in a large proportion of tuberculous patients, neither the hemoglobin nor the erythrocytes fall below the normal standard, this being the rule both in incipient cases and in those which, although of greater chronicity, have escaped mixed infection or have successfully withstood the ill effects of the constitutional drain. Incipient cases may even show polycythemia with excessive hemoglobin figures.

In 25 hospital cases of *pulmonary tuberculosis* in various stages the writer found the hemoglobin percentage from 20 to 30 in 1; from 30 to 40 in 4; from 40 to 50 in 4; from 50 to 60 in 5; from 60 to 70 in 4; from 70 to 80 in 6; and from 80 to 90 in 1. The lowest estimate was 20, and the highest 83, per cent. The erythrocytes were in excess of 5,000,000 in 3 cases; from 4,000,000 to 5,000,000 in 10; from 3,000,000 to 4,000,000 in 11, and from 2,000,000 to 3,000,000 in 1. The minimum count was 2,660,000, and the maximum 5,500,000, cells per c.mm. M. L. Stevens[4] found these averages in 100 cases of phthisis: Males—hemoglobin, 76.7; erythrocytes, 5,039,000; color index, 0.76; leucocytes, 14,060; specific gravity, 1.056. Females—hemoglobin, 72; erythrocytes, 4,373,000; color index, 0.73; leucocytes, 12,666; specific gravity, 1.054. These estimates were made in Asheville, at an altitude of 2300 feet above sea-level, and therefore are doubtless too high for the average patient.

[1] Berlin. klin. Wochenschr., 1901, vol. xxxix, p. 7.
[2] Sem. méd., 1900, vol. xx, p. 319.
[3] *Ibid.*, 1903, vol. xxiii, p. 131.
[4] Med. Rec., 1902, vol. lxii, p. 133.

From a study of 43 cases of *coxalgia*, *vertebral tuberculosis*, and *tuberculous osteomyelitis*, Dane[1] concludes that most cases of tuberculous disease of the bones and joints do not cause a decrease in the number of erythrocytes, although they do, however, affect the percentage of hemoglobin, giving rise to a mild degree of "chloro-anemia," so-called. An analysis of his series shows that the hemoglobin percentage ranged from 80 to 90 in 2 cases; from 70 to 80 in 11; from 60 to 70 in 24; from 50 to 60 in 4; and from 40 to 50 in 2. The erythrocytes numbered 5,000,000 or more in 24 cases, ranging between 6,000,000 and 7,000,000 plus in 6; from 4,000,000 to 5,000,000 in 15; from 3,000,000 to 4,000,000 in 3; and from 2,000,000 to 3,000,000 in 1. According to P. K. Brown's investigations of 73 cases of bone tuberculosis,[2] the erythrocytes decrease only in long-continued and extensive lesions occurring in young children, and in secondary septic infections, while the hemoglobin is diminished practically in all cases, the loss depending upon the same factors which influence the erythrocytes. He also observed that the patient's return to health is indicated by a tendency of the blood to return to the normal standard. In about 15 per cent. of this author's cases there was an erythrocyte loss of 1,000,000 or more cells per c.mm., and in all but some half dozen the hemoglobin was diminished, in one case to as low as 15 per cent.

In cases with secondary septic infection the anemia disappears as the patient's recuperative powers become active, but should the system be overwhelmed by the intensity of the pyogenic process, the anemia either remains stationary or grows more marked.

In other forms of the disease—*tuberculous adenitis*, *meningitis*, *pericarditis*, *pleurisy*, *peritonitis*, and lesions of the *genito-urinary system*—the changes affecting the erythrocytes and their hemoglobin content do not differ from those already described. Well-developed secondary anemia is not uncommon in the two last-named forms of tuberculosis, while in the glandular variety disproportionately low hemoglobin values are frequently found. It is to be recalled that apparent polycythemia may be encountered in both tuberculous peritonitis and pleurisy, due in the former instance to the inspissating effect of the purging and in the latter to the same effect produced by the sudden accumulation of an extensive exudate.

LEUCOCYTES.

Much the same factors which influence the erythrocytes also determine the behavior of the leucocytes in the different forms of tuberculo-

[1] Boston Med. and Surg. Jour., 1896, vol. cxxxiv, pp. 529, 559, and 589.
[2] Trans. Med. Soc. of State of California, 1897, vol. xxvii, p. 168.

sis. In cases of unmixed infection these cells do not rise above the normal limits of health, but the moment the tuberculous lesion becomes complicated by a secondary infectious process, such as a septicemia, the accident is heralded by a prompt increase in their number. For example, in a simple tuberculous adenitis the count is normal, but should the glands ulcerate, fistulate, and become septic, a leucocytosis at once develops. As a rule, the qualitative changes are inconspicuous, although in some forms of the disease, as will be shown below, there is a tendency toward lymphocytosis. Increase in the number of leucocytes, characterized by a relative gain in the lymphocytes and eosinophiles, usually develops during the reactionary fever following the injection of tuberculin.

The theory that Neusser's "perinuclear basophilic granules" are a favorable prognostic sign in tuberculosis has been effectually exploded, since later research has proved that these so-called granules are simply artefacts. (See p. 228.) *Iodinophile* cells are generally found in septic cases, but not in pure tuberculosis.

In *pulmonary tuberculosis* leucocytosis may be symptomatic of cavities, of rapidly spreading bronchopneumonia, and of acute pleurisy. It also usually follows hemorrhage of any considerable extent, and may develop as the effect of a tuberculous diarrhea. No definite relationship apparently exists between the degree of pyrexia and the leucocyte count. Incipient cases of simple tuberculous infiltration and pure lung cirrhosis are not accompanied by an increase. Of the 25 cases above referred to, about one-half showed a moderate leucocytosis, in 12 the count being 10,000 or higher; in 6 between 9000 and 10,000; in 2 between 8000 and 9000; and in 2 between 3000 and 8000 per c.mm. The highest estimate was 22,000, and the lowest, 3152. Differential counts in 11 of the cases having an increase of 10,000 or more revealed no qualitative changes other than those typical of an ordinary polynuclear neutrophile leucocytosis. It may be added that in 6 of these 11 counts the eosinophiles were entirely absent. Myelocytes, in fractions of one per cent., were found in cases with high-grade anemia.

Swan,[1] from a careful study of 25 cases of phthisis, concludes that an absence of eosinophiles is an unfavorable prognostic sign, but that an increase of these cells while the patient is under treatment indicates that the progress of the disease has a tendency to become arrested. Pesel[2] regards basophilia as an index to the progress of phthisis, having found a basophile increase in cases improving under bettered hygiene and a diet rich in nitrogen.

[1] Jour. Amer. Med. Assoc., 1904, vol. xlii, p. 696.
[2] Med. Press and Circular, 1903, vol. lxxvi, p. 474

A. M. Holmes[1] believes that it is possible to estimate not only the degree of the tuberculous process, but the degree of the individual's recuperative powers, by a careful study of the leucocytes, using a special technic of staining with acid and basic dyes. Briefly, he considers that the pretuberculous stage is characterized by an absence of leucocytosis, a slight decrease in the lymphocytes, little or no increase in the polynuclear neutrophiles, more or less abundant débris from cell disintegration, and feeble differentiating powers of the cells. In the stage of early incipiency he finds that there may or may not be leucocytosis, accompanied by a gain in the polynuclear neutrophiles at the expense of the lymphocytes as the disease advances, together with well-marked signs of cell disintegration and impaired differentiation. In the advanced stage, with cavity formation and extensive distribution of the lesions through the lungs, the preceding signs are thought to be still more strongly emphasized, especially those relating to the quantity of débris derived from cells undergoing dissolution. While it is true that the above changes in the leucocytes may be found in many cases of pulmonary tuberculosis, they by no means occur in all, nor can they be regarded as characteristic of this disease. Any septic or purulent process may cause a similar polynuclear neutrophile increase, while the presence of degenerating forms of cells is not at all uncommon in such conditions.

The numerical variations in the leucocytes in *coxalgia*, *Pott's disease*, and other forms of *joint* and *bone tuberculosis* are well illustrated by the following analysis of the large number of counts made by Brown[2] and by Dane[3] in these conditions.

| Leucocytes per c.mm. | Brown's 122 Counts. | Dane's 51 Counts. |
|---|---|---|
| Above 30,000 in | 1 | 4 |
| From 20,000 to 30,000 in | 8 | 12 |
| " 18,000 " 20 000 " | 4 | 1 |
| " 16,000 " 18,000 " | 5 | 2 |
| " 14,000 " 16,000 " | 16 | 4 |
| " 12,000 " 14,000 " | 22 | 12 |
| " 10,000 " 12,000 " | 18 | 7 |
| " 9,000 " 10,000 " | 19 | 3 |
| " 8,000 " 9,000 " | 9 | 1 |
| " 7,000 " 8,000 " | 8 | 1 |
| " 6,000 " 7,000 " | 8 | 4 |
| " 5,000 " 6,000 " | 4 | 0 |
| Maximum | 31,250 | 41,369 |
| Minimum | 5,100 | 6,063 |

[1] Jour. Amer. Med. Assoc. 1897, vol. xxix, p. 828.
[2] *Loc. cit.* [3] *Loc. cit.*

In the great majority of instances the high counts picture a polynuclear neutrophile leucocytosis, but this is not invariably the rule, since in an occasional case the gain depends chiefly upon an increase in the lymphocytes. Low counts may also be characterized by a relative lymphocytosis, this change being most common and most marked in young children and in the profoundly cachectic.

From the results of the studies made by the above-mentioned writers it may be concluded that in these forms of bone tuberculosis high leucocyte counts generally signify that an abscess either exists or impends, although, on the contrary, low counts do not necessarily preclude the presence of an abscess. High counts, especially those of rapid development, point to a secondary pyogenic infection, while slowly developing, moderate leucocytoses appear to be compatible with simply a sudden increase in the activity of the tuberculous process. In the presence of an abscess low counts usually indicate a pure tuberculous pus collection. Cases in which, at the first operation, the pus was proved sterile show an increased leucocyte count when the wound becomes infected with pyogenic bacteria. In these post-operative leucocytoses due to secondary infection the count persists very high for a few days, and then gradually falls unless the sepsis is so acute as to threaten life, in the event of which it may still remain high until a crisis is reached. Should the pyogenic infection be so severe as to overcome the patient's resisting powers, the leucocytosis either fails to develop or else disappears, if it is already present. As in pulmonary tuberculosis, the leucocyte count and the degree of pyrexia apparently stand in no parallelism.

Absence of a leucocyte increase is the rule in uncomplicated *acute miliary tuberculosis*, *tuberculous adenitis*, *pleurisy*, and *pericarditis*, whereas in *tuberculosis of the genito-urinary apparatus* high counts are not uncommon, owing to the frequency of secondary infections in such lesions. In *tuberculous peritonitis*, especially in early life, the count may also be high, probably always as the result of coëxisting inflammatory processes. In Rotch's[1] 23 cases in young children the leucocytes averaged 16,435, and ranged between 5400 and 44,000 per c.mm. Such high counts as these rarely occur in the adult. In *tuberculous meningitis*, unlike other forms of tuberculosis, leucocytosis is the rule. It occurred in 75 per cent. of Cabot's[2] 43 cases, the counts in some cases ranging as high as 40,000 to 50,000. Of 26 cases of tubercu-

[1] Jour. Amer. Med. Assoc., 1903, vol. xl, p. 69. [2] *Loc. cit.*

lous meningitis in children studied by Koplik,[1] 40 per cent. showed a count of 20,000 to 25,000, results corresponding to those found by Osler[2] in the adult.

DIAGNOSIS.

The presence of a leucocytosis in a lesion obviously tuberculous, whatever its seat, is usually to be interpreted as a sign of some complicating secondary infection, the chief exceptions to this general rule being those infrequent cases in which the sudden extension of a purely tuberculous bone disease may cause a moderate, progressive rise in the count. A positive iodin reaction also points to a mixed infection. In *pulmonary tuberculosis*, if the influences of bronchopneumonia and hemorrhage can be ruled out, leucocytosis almost invariably indicates the presence of cavity formation, and in *bone tuberculosis* the superposition of a pyogenic process. In *peritoneal, pleural,* and *pericardial effusions* low counts suggest an unmixed tuberculous affection unless the leucopenic influences of a virulent infection are to be found. The diagnosis between *acute miliary tuberculosis* and *enteric fever* has been referred to under the latter disease. (See p. 409.) Blood cultures should be made in every case of doubtful miliary tuberculosis, for positive results, although rare, are conclusive when present. The leucocyte count may be quite as high in tuberculous as it is in non-tuberculous *meningitis*.

## LXXIII. TYPHUS FEVER.

PARASITOLOGY.

Lewaschew[3] claims to have found, in the finger blood of a large number of typhus patients, a micrococcus, occurring both singly and in pairs, which he characterizes as the *Micrococcus exanthematicus*, and regards as the pathological agent of infection. A diplococcus has been isolated by Balfour and Porter,[4] from blood obtained by puncture of the thumb, in 36 of 43 cases of typhus examined by these authors. In a large number of control cases, including measles, scarlet fever, and enteric fever, the organism in question was uniformly absent, except in the last-named disease, in which it was discovered in 40 of the 46 cases studied. Cultures of this parasite when injected intravenously into rabbits produced a rapidly fatal septicemia in these

[1] Med. News, 1904, vol. lxxxiv, p. 1065.
[2] "Principles and Practice of Medicine," 4th ed., New York, 1901.
[3] Russkiy Vrach, 1894, Nos. 2 and 3; abst. in Sajous' Annual, 1895, sec. H, p. 45.
[4] Edinburgh Med. Jour., 1899, vol. vi, p. 522.

animals. In 6 cases of typhus Gotschlich[1] reports having found protozoan bodies resembling Smith's *Piroplasma bigeminum* of Texas fever. The organisms in question were observed in three different developmental stages—intracellular forms, extracellular ovoids and spheres, and free flagellated bodies. These investigations, while interesting as pathological studies, throw no definite light upon the etiology of typhus fever.

HEMOGLOBIN AND ERYTHROCYTES.

From the limited data at present available it appears that at the beginning of the attack the amount of hemoglobin and the number of erythrocytes remain unchanged, but that later a moderate degree of anemia appears, being most marked during the period of apyrexia. Tumas' careful studies[2] of two cases, in which altogether 25 examinations were made, showed a hemoglobin range of from 50 to 94 per cent., with from 3,450,000 to 5,360,000 erythrocytes per c.mm., the minimum figures for both being observed during the second week of the infection. The presence of structural degenerative changes and of erythroblasts has not been recorded. In the acutest forms of the disease hemoglobinemia has been noted.

LEUCOCYTES.

Absence of leucocytosis, with occasional counts showing a decided leucopenia, is the rule, as in enteric fever, according to conclusions of the most careful investigators of this question. Even the coëxistence of another infection, alone sufficient to give rise to leucocytosis, seems to have no effect in provoking an increase, as evidenced by one of Tumas' cases, complicated by diphtheria, in which the number of leucocytes never exceeded 9600 per c.mm.; in his other case they once rose to 17,000 after a profuse sweat, but with this exception the counts all ranged between 1600 and 9600. Ewing[3] found a maximum count of 9000 in a study of 4 cases, 2 of which were fatal. It has not yet been determined whether or not specific qualitative changes affect the leucocytes in this disease.

DIAGNOSIS.

In differentiating typhus fever from *epidemic cerebrospinal meningitis* the presence of a frank leucocytosis should be regarded as highly symptomatic of the latter. The behavior of the leucocytes fails to be of service in distinguishing typhus from *typhoid*, since in neither of these infections are these cells increased in number; here, however, the serum test and blood culturing prove of signal utility.

[1] Sem. méd., 1903, vol. xxiii, p. 298.
[2] Deutsch. Arch. f. klin. Med., 1887, vol. xli, p. 323.
[3] "Clinical Pathology of the Blood," 2d ed., Philadelphia and New York, 1904.

Absence of leucocytosis is also associated with *malignant measles*, the early stages of which may remind one of typhus fever.

## LXXIV. VACCINATION.

Billings[1] who has carefully studied the effects of vaccination on the blood, finds that no changes are produced in the *hemoglobin* and *erythrocytes* by this procedure. Pallor, with other signs of anemia, developing in young children after vaccination has been described as "post-vaccinal anemia" by Bellotti,[2] who attributes the change, if such it be, to the hemolytic effect of the vaccine virus. Bellotti's conclusions, since they are not fortified by blood counts, must be accepted with doubt.

Moderate but definite *leucocytosis*, the counts averaging about 15,000 per c.mm., is characteristic. The leucocytosis is of the inflammatory type, and reaches its maximum coincidentally with the height of maturation of the vaccine pustule, fading away as the latter desiccates. Sobotka[3] has observed a secondary leucocytosis, beginning about the tenth or twelfth day, and often persisting for as long as six days, the height of the count corresponding in a general way to the severity of the local lesion and to the activity of the virus.

## LXXV. VALVULAR HEART DISEASE.

STAGE OF COMPENSATION.

In well-compensated valvular lesions of the heart, irrespective of their character, the blood shows no deviation from its normal composition, for such lesions of themselves are incapable of giving rise to blood changes. If the latter are observed in cases of this kind, they should be attributed to other factors rather than to the heart disease.

ACUTE RUPTURE OF COMPENSATION.

In cases associated with acute failure of compensation changes in the blood picture, the intensity of which runs parallel to the severity of the circulatory disturbances, sooner or later become manifest. These changes, consisting in the production of a so-called serous plethora, depend chiefly upon a reduction in blood pressure, in consequence of which the blood mass becomes diluted by transudation into the vessels of fluids from the

[1] Med. News, 1898, vol. lxxiii, p. 301.
[2] Gaz. degli Ospedali e. d. Clin., 1903, vol. xxiv, p. 587.
[3] Zeitschr. f. Heilk., 1893, vol. xiv, p. 349.

surrounding lymph spaces. It is also highly probable that this surcharging of the blood mass with liquids is aggravated by the disturbances in the functions of the heart and kidneys whereby the elimination of the superfluous watery constituents of the blood is hindered. Oertel[1] remarks that it seems not unlikely that another factor in the production of this hydremia may be found in the increased consumption of liquids, which he has noted in many patients suffering from valvular disease. Examination of the blood at this stage of the disease shows that there is a diminution in the albuminoid constituents and in the *specific gravity* of the blood, that the percentage of *hemoglobin* falls, and that *oligocythemia* proportionate to the latter develops; the *leucocytes*, unlike the erythrocytes, do not decrease, but their number remains within normal limits. The observer must be careful not to mistake the blood picture of hydremia for that of a true anemia, from which it is distinguishable only by taking into consideration other clinical signs and symptoms.

In chronic valvular lesions, myocarditis, and dilatation, Schott[2] found, as the result of his treatment by baths and gymnastics, a decided hemoglobin increase. In the average case it amounted to a gain of about 20 per cent., and usually was associated with a blood pressure rise ranging from 20 to 30 mm. of mercury.

Effects of Stasis.

In cases of chronic valvular disease with stasis, dyspnea, and cyanosis, a very different picture from that just described presents itself. The hydremia gives way to a concentration of the blood mass, this change being due mainly to the increased outflow of plasma from the vessels into the neighboring tissues, and perhaps to the excessive loss of water, especially through the lungs, as Grawitz[3] has suggested. Stengel[4] offers as an explanation of this inspissation of the blood two other factors: the lagging of the erythrocytes in the peripheral arterioles and venules, and the increase in the viscosity of the blood. Calabresse[5] argues that in some instances the polycythemia may be absolute, being a sign of hematopoietic hyperactivity excited by an excess of carbonic acid in the blood. Other signs of active hemogenesis, such as the presence of normoblasts, are required, however, to make this view reasonable.

At this period of valvular disease the *specific gravity* and the proportion of albuminoid principles of the blood rise,

[1] Deutsch. Arch. f. klin. Med., 1892, vol. xxxi, p. 293.
[2] Brit. Med. Jour., 1904, vol. i, p. 536.
[3] *Loc. cit.*
[4] Proc. Path. Soc. of Phila., 1898, vol. i, p. 137.
[5] Sem. méd., 1903, vol. xxiii, p. 388.

and high *hemoglobin* values with more or less decided *polycythemia* are found, the erythrocyte count commonly being in the neighborhood of 6,000,000 per c.mm., or, in some instances, notably those of congenital heart disease, as high as from 7,000,000 to 8,000,000. It is common to find many megalocytes, and, as Labbé[1] has shown, a great increase in the proportion of reduced hemoglobin in the blood. The polycythemia, it should be remembered, may be sufficient completely to mask a coëxisting anemia; in fact, it must be admitted that no reliable data concerning the true condition of the blood are obtainable in valvular disease of the heart, except during the stage of perfect compensation.

The behavior of the *leucocytes* is variable: their number may be normal, or, on the other hand, a decided, but not an excessive, leucocytosis may be present. Should this be the case, the increase will be found to involve principally the polynuclear neutrophile cells at the expense of the other forms.

Grawitz[2] has drawn attention to the fact that a form of stroma degeneration is frequently met with in valvular disease, being evidenced by the unnatural readiness with which the hemoglobin tends to become diffused in the plasma within a short time after the removal of the blood from the body. This, while it cannot be termed a true hemoglobinemia, at least appears to demonstrate that the stroma and its hemoglobin are less firmly combined than they are in perfectly normal blood.

The efforts made by some authors to associate certain blood conditions with definite valvular lesions seem to the writer farfetched. The changes just described are thought by some to be especially prone to occur in affections of the mitral segments, and other authors even go so far as to state that disease of these valves is more often associated with transient apparent anemia or with chronic polycythemia than lesions of the aortic valves, the blood in the latter conditions being usually normal or but slightly impoverished. After all, the general disturbances dependent upon the lesion, and not the lesion *per se*, account for the alterations of the normal blood picture which have been observed in heart disease of this type.

## LXXVI. VARICELLA.

Thomson and Brownlee[3] report having found small spherical hyaline bodies in the blood of persons suffering from chicken-pox, both in the prodromal stage and during the first week of the disease.

[1] Sem. méd., 1903, vol. xxiii, p. 33. [2] *Loc. cit.*
[3] Brit. Med. Jour., 1903, vol. i, p. 241.

Uncomplicated chicken-pox produces no alteration in the *hemoglobin* and *erythrocytes*, but in cases complicated by extensive necrotic processes ("varicella escharotica") or by hemorrhage ("varicella hæmorrhagica") a variable degree of symptomatic anemia may be encountered.

The *leucocytes* behave erratically both as to number and as to kind. In about one-third of the reported cases the stage of active pustulation was associated with a moderate neutrophile increase, ranging from 1000 to 4500 cells above normal. In Engel's case,[1] the first on record, this was accompanied by a disappearance of the eosinophiles, which, after the pustules healed, rose to 16 per cent. Practically similar quantitative changes were found by Nobecourt and Merklen[2] in their series of 15 cases, but in one-half the large lymphocytes were in excess and myelocytes were noted in one-third. In contrast to these findings, Weil and Descos,[3] while meeting with polynuclear neutrophile leucocytosis in about one case in three, deny that either lymphocytosis or myelocytosis is found in varicella, and emphasize the value of this negative finding as a sign for differentiating this condition from small-pox. Stengel and White[4] report normal leucocyte counts in uncomplicated cases.

It is obvious that the contradictory reports of various observers must be reconciled by further investigation before the blood examination in varicella can have any dependable clinical bearing.

## LXXVII. VARIOLA.

General Features.

During the first few days of the attack the fibrin network is normal, but as the stage of pustular eruption is reached, a decided *hyperinosis* develops. Streptococci have been found in the blood repeatedly by Widal and Benzacon,[5] and also by Waele and Sugg.[6] The streptococcus found by the last-named authors is clumped by variolous blood serum and by that of successfully vaccinated subjects, but not by the serum of unvaccinated persons nor by ordinary antistreptococcus serum. Pfeiffer[7] has attached specific properties to apparently ameboid bodies which he discovered in small-pox patients' blood, and to this view a number of other

[1] XV. Cong. f. inn. Med., 1897.
[2] Jour. de physiol. et de path. gén., 1901, vol. iii, p. 439.
[3] *Ibid.*, 1902, vol. iv, p. 504.
[4] Univ. of Penna. Med. Bull., 1901, vol. xiv, p. 310.
[5] Centralbl. f. allg. Path., 1896. vol. vii, p. 569.
[6] Brit. Med. Jour. Epit., 1904, vol. i, p. 8.
[7] "Handb. d. spec. Therap.," 1894, vol. i, p. 229.

workers, notably Guarnieri[1] and Wasielewski,[2] also subscribe. Other amebæ have been found under similar circumstances by Reed,[3] Weber,[4] Ishigami,[5] Thomson and Brownlee,[6] and others. None of these discoveries has elucidated the etiology of variola. A protozoan body (*Cytoryctes variolæ*) has been found in the skin epithelium and once in the blood of small-pox patients by Councilman, Magrath, and Brinckerhoff,[7] who consider this organism the specific cause of the disease. This claim, although based upon convincing experimental work, has still to endure the test of corroborative research.

HEMOGLOBIN AND ERYTHROCYTES.

Post-febrile anemia, first becoming apparent when defervescence is established, is the rule, the decrease in hemoglobin and corpuscles being usually decided, and not infrequently excessive. This is especially true in hemorrhagic and confluent variola, in which conditions a loss of 2,000,000 or 3,000,000 cells per c.mm. may occur with great rapidity. The loss of hemoglobin begins slightly earlier than that of the corpuscles, but later both elements are usually diminished proportionately.

During the febrile period of the disease the number of erythrocytes is approximately normal, or even increased, in case the blood becomes concentrated by the influence of the temperature.

Qualitative changes in the erythrocytes are not marked, except in cases with severe anemia, in which nucleation, poikilocytosis, and deformities of size may be noted. In such instances hemoglobinemia may also occasionally be detected. Regeneration of the blood is said to be exceedingly slow.

LEUCOCYTES.

In the majority of cases a moderate but distinct leucocyte increase develops, first becoming apparent in the early stages of the disease, reaching its maximum during pustulation, and gradually declining during desiccation of the pocks, unless prolonged by some complication. In the uncomplicated case the normal count is regained by the end of the second or the early part of the third week. The count usually ranges between 10,000 and 20,000, although such factors as hemorrhage, confluence, and secondary infection tend to exaggerate these figures. In a series

[1] Arch. per le sci. med., 1897, vol. xvi, p. 403.
[2] Zeitschr. f. Hyg. u. Infectionskr., 1901, vol. xxxviii, p. 212.
[3] Jour. Exper. Med., 1897, vol. ii, p. 515.
[4] Centralbl. f Bakt. u. Parasit., 1897, vol. xxi, p. 286.
[5] *Ibid.*, 1902, vol. xxxi, p. 794.
[6] Brit. Med. Jour., 1903, vol. i, p. 241
[7] Jour. Med. Research, 1903, vol. iv, p. 372; *ibid.*, 1904, vol xi, p. 12; also Calkins, *ibid.*, p. 136.

of 36 cases studied by Roger[1] the count ranged from 6000 to 15,000 in 19; from 15,000 to 20,000 in 12; from 20,000 to 30,000 in 3; and from 30,000 to 35,000 in 2. The count is likely to be higher in the unvaccinated than in the vaccinated, and may rapidly diminish under the influence of serum therapy, and, in some fatal cases, just before death.

The behavior of the leucocytes in small-pox is probably due to the action of the specific variolous toxin, which, if sufficiently active, provokes an increase of these cells. This change develops too early in the disease to be dependent solely upon the effects of secondary infection of the pocks, which accident the older writers—Pick,[2] Halla,[2] Brouardel,[2] and others—maintained was the real determining factor. Furthermore, the increase involves chiefly the large lymphocytes, which cells, according to Courmont and Montgard,[3] are in excess even when there is extensive streptococcic pustulation of the lesions; when, however, genuine secondary furunculosis and abscesses complicate the variolous process, a neutrophile leucocytosis promptly supervenes. These findings, which have been corroborated by Weil,[4] by Ewing,[5] and by Roger,[6] strongly suggest that pustulation is a specific change, and not merely the expression of a secondary contamination of the pocks with pyogenic bacteria, as was the former belief. Myelocytes, sometimes in considerable numbers, are also found with great constancy, and are especially abundant in the severer types of the infection. The eosinophiles may be greatly increased in hemorrhagic small-pox, and small percentages of mast cells are to be noted in cases with high leucocyte counts.

The *blood plaques* are decreased in number during the period of fever, being sometimes absent from the blood at this stage of the disease.

*Varioloid*, unless associated with suppuration, does not give rise to anemia nor to leucocytosis.

Diagnosis. The presence of a definite mononucleosis, involving principally the large lymphocytes and the myelocytes, is highly suggestive. Such a blood picture is sufficient evidence to exclude *measles*, for which the prepustular stage of variola has been mistaken, and to suggest true, rather than *modified, small-pox*. In *pustular syphilide* and in the purpuric type of *cerebrospinal meningitis*, which may counterfeit variola, the blood shows a typical polynuclear neutrophile

[1] "Infectious Diseases," Eng. trans. by Gabriel, New York and Philadelphia, 1903, p. 260.
[2] Cited by von Limbeck, *loc. cit*
[3] Province méd., 1900, vol. xv, p. 481.
[4] Sem. méd., 1900, vol. xx, p. 222.
[5] "Clinical Pathology of the Blood," New York and Philadelphia, 2d ed. 1904, p. 296.
[6] *Loc. cit.*

leucocytosis. Owing to the current conflicting views (see p. 555) it is impossible at present to base the differentiation of variola and *varicella* upon the state of the blood.

## LXXVIII. YELLOW FEVER.

GENERAL FEATURES.

Slow *coagulation* and deficiency or even complete absence of the *fibrin network* are common, these peculiarities being observable often in the earliest stages of the disease, apparently beginning coincidentally with the introduction of the infecting principle.

The identity of the specific cause of this disease is still unknown. Sanarelli's claim,[1] that his *Bacillus icteroides* is to be found in the circulating blood of yellow fever patients during life, has been very generally disproved. Reed and Carroll[2] have shown that this bacterium is identical, morphologically and biologically, with the hog-cholera bacillus, and Agramonte[3] and the members of the United States Yellow Fever Commission[4] (Reed, Carroll, Agramonte, and Lazear) report uniform failures to isolate Sanarelli's bacillus either by antemortem blood cultures or by postmortem examinations of the blood and organs of persons dead of yellow fever. In passing, it may be of interest to add that Finlay's theory,[5] that yellow fever is transmitted by means of the mosquito's bite, has been confirmed beyond question by the experiments of this Commission, which has identified the *Stegomyia fasciata* as the offending insect. The French Yellow Fever Commission[6] has fully corroborated these findings. The character of the infective principle thus harbored by the mosquito is unknown—possibly it belongs to that class of invisible (ultramicroscopic) organisms now believed to excite certain specific infections. Finlay[7] suggests that it may be a protozoön similar to the malarial plasmodium, which undergoes one developmental cycle in the human body and another in the mosquito, the former being an asexual reproduction accompanied by the elaboration of powerful toxins to which the fever is to be attributed. J. C.

[1] Annal. de l'Institut Pasteur, 1897, vol. xi, p. 433; also Brit. Med. Jour., 1897, vol. ii, p. 7; also Med. Record, 1897, vol. lxii, p. 117.

[2] Jour. Exper. Med., 1900, vol. v, p. 216; also Carroll, N. Y. Med. Jour., 1904, vol. lxxix, p. 241.

[3] Med. News, 1900, vol. lxxvi, p. 249.

[4] Phila. Med. Jour., 1900, vol. vi, p. 790; also Jour. Amer. Med. Assoc., 1901, vol. xxxvi, p. 431.

[5] Jour. Amer. Med. Assoc., 1901, vol. xxxvi, p. 1040.

[6] Jour. Amer. Med. Assoc., 1904, vol. xlii, p. 1369.

[7] Lancet, 1903, vol. i, p. 1711.

Smith[1] and Parker, Beyer, and Pothier[2] have discovered what they regard as a protozoan parasite, called by them the *Myxococcidium stegomyiæ*, in the bodies of *Stegomyia fasciata* fed upon the blood of yellow fever patients, but in no others. The inference from their announcement, that these bodies represent the specific cause of yellow fever undergoing its mosquito phase, has been weakened considerably by Carroll's later and apparently convincing demonstration[3] that the so-called protozoön is merely a yeast fungus such as is commonly found in the mosquito fed upon overripe bananas to which yeast has been added.

In a series of 23 cases Tombleson[4] claims to have found in finger blood peculiar polymorphous organisms, appearing as short oval bacilli, as bacilli with rounded ends, and as long beaded rods.

HEMOGLOBIN AND ERYTHROCYTES.

Pothier's studies of 154 cases at the New Orleans Isolation Hospital, in 1897,[5] show that a more or less decided loss of hemoglobin commonly occurs during the active stages of the infection, and that the normal percentage is slowly regained during and after convalescence; during the febrile period the hemoglobin ranged from 50 to 90 per cent., and during convalescence from 64 to 80 per cent. He found that the erythrocyte count never fell below 4,280,000 per c.mm., and that even in a fatal case it might be normal. Lack of parallelism between the hemoglobin percentage and the specific gravity of the blood is a peculiarity to which Albertini[6] has drawn attention, this investigator having repeatedly noted a considerable fall in the blood density without a corresponding loss of hemoglobin. Sternberg[7] has noted the absence of quantitative changes affecting the erythrocytes in this disease, stating that, "although there is no general destruction of the red corpuscles, it is probable that a considerable number of these elements perish, for the serum contains free hemoglobin, which gives it a yellow color even as early as the third or fourth day." This hemoglobinemia is common in all cases, but especially so in fatal cases just before death. The results of these investigations by Pothier and by Sternberg are contradictory to the views expressed by earlier writers, who have been

[1] Science, 1903, vol. xviii, p. 530.
[2] Report of Working Party No. 1, Yellow Fever Institute, P. H. and M. H. S., Washington, 1903.
[3] Jour. Amer. Med. Assoc., 1903, vol. xli, p. 1341.
[4] Lancet, 1903, vol. ii, pp. 594 and 1781.
[5] Jour. Amer. Med. Assoc., 1898, vol. xxx, p. 885.
[6] Rev. de Méd. Trop., 1903, vol. iv, p. 73
[7] U. S. M. H. Service Report on the Etiology and Prevention of Yellow Fever, Washington, 1890.

accustomed to describe the cellular elements of the blood in yellow fever as profoundly altered.

Decided degenerative changes in the erythrocytes have not been observed, although it has been asserted by Jones[1] that these cells "present under the microscope certain peculiar appearances which are referable to the action of certain extraneous excretory matters in the blood." A few nucleated cells of the normoblastic type are reported to have been found occasionally.

LEUCOCYTES.

The behavior of the leucocytes in yellow fever is extremely variable, their number being subnormal in some cases, and decidedly, but not strikingly, increased in others. In the series of Pothier, just quoted, the counts ranged between 4660 and 20,000 per c.mm. In five counts by John Guiteras[2] the leucocytes ranged from 3200 to 11,400 per c.mm., averaging 5400. The increase, when present, involves chiefly the polynuclear neutrophiles, the relative proportion of these cells usually being in excess of 85 or 90 per cent. Small numbers of myelocytes were found occasionally by Cabot[3] in differential counts of 12 films of yellow fever blood.

Sternberg[4] has described certain relatively large, highly refractive, spherical granules in the protoplasm of the leucocytes, which he is inclined to regard as an evidence of fatty degeneration of these cells; these granules were especially abundant in severe cases, nearly every leucocyte containing some of them. They are not, however, peculiar to yellow fever, since they have been found in the blood of patients suffering from beri-beri, and even in the blood of normal individuals, residents of the tropics.

DIAGNOSIS.

Until the cause of yellow fever is discovered, the blood examination can afford little or no aid in the recognition of this infection. The frequency of hemoglobinemia in yellow fever, and its absence, so far as is known, in *dengue*, may serve as a hint of some importance in differentiating these two fevers. In differentiating *malarial fever*, the examination of the blood for the malarial parasite will usually give definite information, and the occasional presence of a well-developed polynuclear leucocytosis in yellow fever should not be forgotten. The lack of relationship between the specific gravity and the hemoglobin value of the blood in the latter is also significant.

[1] Jour. Amer. Med. Assoc., 1895, vol. xxiv, p. 403.
[2] Brit. Med. Jour., 1902, vol. i, p. 366. [3] *Loc. cit.* [4] *Loc. cit.*

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