ON

THE PHOTOCHEMISTRY OF THE RETINA

AND ON

VISUAL PURPLE,

TRANSLATED FROM THE GERMAN

OF

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EDITED, WITH NOTES,

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PREFACE.

It is now more than twelvemonths since I undertook to publish an English translation of Prof. Kühne's treatises on *The Photochemistry of the Retina and on Visual Purple.* Circumstances 'over which I have had no control' have, until now, prevented my intention from being accomplished. Meanwhile further investigations have largely changed the aspects and the prospects of the subject. When Boll first announced his discovery of the sensitive purple colour of the retina he expressed the very natural conviction that it was directly connected with the act of vision; and, as will be seen from the following pages, the earlier results obtained by Kühne promised at first to afford a facile solution of many visual problems. As, however, thanks to the energetic labours of this latter observer[1] and his talented assistant Dr A. Ewald, rapid pro-

[1] As the reader is possibly aware, Kühne has in some quarters been reproached for having made use of Boll's discovery. To Boll must undoubtedly be given the credit of the discovery that the retinas of many animals possess a sensitive purple colour (for the mention of it by previous observers (see p. 15) does not amount to a discovery), and he therefore will rightly share in all the fame belonging to the subsequent developement of that discovery. At the same time the study of Boll's writings can leave no doubt on the mind of the candid reader that at first Boll did not realize that the visual purple undergoes

gress was made in our knowledge of the matter, it became step by step more and more evident that the mountain top was far more distant than had at first been imagined. From the first Kühne seems to have felt, and he very early (see p. 18) stated his conviction that the visual purple was not 'the visual substance,' a knowledge of the changes of which would at once enable us to bridge over the gap between the waves of the luminous ether and the waves of the visual nervous impulses, but rather a type, shewing us, in a dim way, the manner in which the problems of the existence and behaviour of such a visual substance or such visual substances might be attacked; and, in the minds of the thoughtless, he may seem, in demonstrating (see Appendix, Note E) the possibility of vision and even colour vision without visual purple, to have destroyed, with his own hands, the value of his own handiwork.

More thoughtful consideration, however, will lead us to see reason to hope, rather than to despair, in such an apparent failure. The discovery of the visual purple, in the form in which it was first laid hold of by the general public, seemed, as people say, 'too good to be true;' and it is no little consolation to know that the first premature delusions have been corrected with the least possible delay. On the other hand there remains the important fact that we can now point to positive photochemical

changes through the action of light after the death of the animal; he attributed the bleaching after death to post mortem decomposition. It was Kühne who was the first to observe that light bleaches the visual purple after death. This discovery made quite independently of Boll and in contradiction to Boll's statements is ample justification for Kühne's continuing his investigations with a view to determining the bearings of what he 'himself had found out.' When the following pages have been read, it will be at once seen that the bleaching of the retina by light after death is the key to the whole matter. In the second edition of my *Text-Book of Physiology* (p. 414, l. 19 from bottom small print, "which after a few seconds exposure to light," &c.) the desire to condense the greatest amount of information into as few lines as possible, has led me to use a form of expression which makes me seem to attribute to Boll what really belongs to Kühne: I gladly take this opportunity of correcting this inadvertence.

actions taking place in the retina: the first stone of a true theory of vision (*i. e.* of the origin of peripheral visual nervous impulses) has been laid. For some time past the minds of physiologists have been drawing near to the conception of a visual substance or of visual substances as the basis of vision. The speculations of Hering (*Zur Lehre vom Lichtsinne. Wien. Sitzungsberichte*, LXVI.—LXX.), though in their present form they may be spoken of as crude, and as perhaps raising more difficulties than they remove, have this great merit, that by the hypothesis of a visual substance (or visual substances) they bring visual (and by analogy other specific) sensations into the same category as ordinary nervous impulses and even muscular contractions (we might perhaps go so far as to say as protoplasmic molecular movements in general), and thus open up the way by which the phenomena of a visual sensation, a nervous impulse, the act of secretion, and a muscular spasm may be made to illustrate each other.

Awaiting patiently the future discovery of the exact nature and relations of what may be called the true visual substances, we shall still be justified in maintaining that the visual purple is, if not directly at least indirectly, connected with vision; and, in this sense, I have not hesitated to retain the name 'visual purple,' in spite of the fact that frogs appear to see perfectly well in spite of the absence of any store of it in their retinas, and that many animals which never at any time seem to possess such a store, have nevertheless very respectable vision; and I trust that an account in the English language of its behaviour and properties will prove not unacceptable to the physiologists and psychologists of this country.

The translation has been made by Mrs Foster, who has found the task of converting Prof. Kühne's somewhat idiomatic German into readable English not free from difficulty; we trust, however, that his meaning has been in all cases correctly rendered, even if the diction should at times seem capable of improvement. I have gone carefully over the whole of the translation myself, and have added some notes in the form of an Appendix; and Prof. Kühne has himself kindly revised the proofs.

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July, 1878.

PART I.

PHOTOCHEMISTRY OF THE RETINA.

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In a recent communication to the Berlin Academy, Herr Fr. Boll announced the beautiful and, beyond doubt, important discovery, that the bacillary layer of the retina of all animals is in the living condition not colourless, as has been hitherto supposed, but of a purple red colour. During life, says Boll, the proper colour of the retina is continually being destroyed by the light which falls into the eye; it is restored in the dark, and after death only remains a few moments. Animals which have been kept in the light are therefore very unsuitable for demonstrating the living colour of the retina, and the eyes of animals which have been exposed to the sun for a long time before death remain absolutely colourless. These facts illustrate the relation of the retinal colour to light on the one hand, and to vital conditions on the other.

Whoever has busied himself with the retina will be reminded by Boll's discovery (and thereby receive a wholesome admonition of the limits of his own ability), that he has already seen something of the kind before. He will perhaps remember that puzzling blood-clot—which at one moment he saw, or thought he saw, under the retina, and which the next moment disappeared. What he then passed over so lightly was nothing less than the key of the secret, how a nerve can be excited by light. In other words, it was the first fact disclosing the existence of photo-chemical processes in the retina.

During my first attempts to test the truth of Boll's statements, I was under the impression, strengthened by what Boll had said, that the greatest haste was necessary in removing the eye, and taking out the retina; but I very soon convinced myself that a considerable time may be allowed for the operation, since the visual purple, as I call the proper colour of the retina, may continue to exist for some time after the retina has ceased to be in a physiologically fresh condition, and even after death is only destroyed by light. Under the light of a good gas-lamp the retina may be quite leisurely spread out, and is then seen to lose its colour very gradually, for what takes place under bright daylight in half a minute, lasts in this case from 20 to 30 minutes, that is to say, much longer than Boll gives as the duration of tissue-life. And in the dark, or in the light of a sodium flame, the purple does not pass away at all, either in the frog or rabbit, at least not for 24 or even 48 hours, in spite of decomposition evidently setting in.

By the discovery of these facts, the path of experimentation with the visual purple is freed from many difficulties. The necessary preparations can be conducted leisurely in a dark chamber lighted by a sodium flame, and the object then brought out for examination into diffuse daylight. A room such as is used by photographers, into which the light passes through yellow glass or paper, answers the same purpose, but less perfectly.

As we do not exactly know how long the rods or their parts remain alive after death, I treated the retinas of frogs in the sodium chamber with the most various reagents, many of which were without doubt capable of producing great structural alterations, to see whether the colouring and sensibility to light were thereby affected. The colour was destroyed by heating to 100° C., by alcohol, by glacial acetic acid, by soda solution, both concentrated, and 10 per cent. It was not affected by a solution of sodium chloride 0·5 per cent., by strong ammonia, by sodium carbonate solution, by saturated sodium chloride solution, by alum, lead acetate, acetic acid 2 per cent., or tannic acid 2 per cent., by lying in glycerine for 24 hours, by ether, or by being dried upon a glass plate. In all these latter instances, the retina, when brought into daylight, was found to be still red, and bleached more or less rapidly, the purple passing after 1 or 10 minutes into chamois colour[1], which gradually disappeared, until there was scarcely anything of it visible. The depth of the colour naturally depended upon the other conditions of the retina, upon its being transparent, or cloudy. When it was opaque it gave an opportunity of proving the correctness of Boll's statement, that it is the outer layer, therefore really the layer belonging to the rods, which is coloured; for an opaque retina is seen to be white in front, the red appearing at the back only. The colour comes out most beautifully after the action of ammonia, which makes the retina very transparent; and the red of a retina treated with ammonia withstands the action of light from 10 to 20 times longer than an unchanged retina exposed to light of equal intensity. The dried membrane also retains its colour for a long time, but it also yields gradually to the light.

From what has already been said concerning the methods of obtaining coloured retinas, it is obvious that all kinds of light are not equally active in bleaching the visual purple. This is untouched by the photo-chemically inactive (or only slightly active) rays of the line *D*[2]. It is only the very strongly coloured retinas (such as those of Rana temporaria) which shew any colour in sodium light, and then only a trace of it; and sodium light is not quite monochromatic. The retina of a living rabbit examined with an ophthalmoscope in the approximately monochromatic sodium light, appears bluish white, with something of a mother-of-pearl sheen, the vessels coming out with astonishing distinctness, like black lines drawn with ink. So also the pupil of an albino rabbit, when the eye is illuminated obliquely, looks quite black. Indeed the sodium light, which is so easily rendered sufficiently intense, may be expressly recommended as suitable for delicate ophthalmoscopic investigation.

In order to ascertain which are the particular rays which bleach the visual purple, I placed retinas spread out on glass

[1] See Appendix, note A. [2] See however p. 57.

plates, in blackened moist chambers, covered them with glass slips, on which I had fastened strips of tin foil about one mm. broad, and then placed over them coloured plates of glass, or glass beakers filled with coloured solutions. For red I used blood solutions of such a strength that neither yellow nor orange were any longer visible in their absorption spectra; also red glass plates, which, however, allowed some amount of purple as well to pass through. For blue I used ammoniacal cupric oxide, and for green, coloured glass plates, the spectrum of which consisted of only a narrow green band. Under the blood there was really no bleaching at all; under the red glass the first trace of it appeared after 6 hours; in the blue light the bleaching began after 2 hours, and in the green after from 4 to 5 hours. Of course, owing to the intensity of the light under which these experiments were conducted not being very great, and also not really comparable, one cannot from them arrive at accurate conclusions concerning the problem; but the apparently more powerful action of the more refractive rays, particularly of the blue light, was brought out clearly[1]. On removing the cover-glass from the bleached preparations, there was seen, where the retina was protected by the strips of foil, a beautiful band of unchanged purple, in other words a positive photogram. I found the purple was as little changed by lithium light as by the blood-red, while, as might be expected, magnesium light very rapidly destroyed it. Once bleached, by whatever means, the purple never returned, either in the dark, or in different coloured light, or by being exposed to heat, or by the ultra red rays coming through a smoked glass illuminated by the sun[2].

After I had followed out the experiments I have described, with animals which had, according to Boll's directions, been kept in the dark, I was anxious to see how the retina of a frog would appear, which, immediately after exposure of the eye to light while the animal was still alive, had been prepared as rapidly as possible in a chamber of yellow light. According to Boll's opinion, I expected to see it bleached to a perceptible degree; I found it on the contrary as red as others. It is

[1] See Appendix, note B. [2] See Appendix, note C.

therefore unnecessary to keep the animal in the dark just previous to the experiment. As the daylight was not very bright, the sky being cloudy, though quite sufficient for microscope work, I tried illumination with magnesium light, but that also was followed by the same result. I therefore came to the conclusion that Boll was mistaken in ascribing the failure that he once had in demonstrating the presence of the purple, to the fact that the frog had been kept in the light; if he found his preparations equally bleached, this must have been due to exposure to light during the time they were being manipulated.

To discover why it was that the visual purple remained thus apparently unchanged in the physiological act of vision, I isolated the retina of a frog and laid it on a glass plate, leaving the other in the bulb, which I had however extirpated and had opened wide by an incision along the equator. Both preparations were then brought into daylight which was still not very bright, and were left there until the first was perfectly bleached; the second one was then brought back into a chamber lighted by sodium light, the retina taken out, laid upon glass, and brought out again into ordinary light; it was at first of a deep red, but quickly became colourless. The sky not being clear, I conducted the same experiments with a magnesium lamp, and always found that the visual purple was restored so long as the retina remained in the eye lying upon the choroid, though it was protected from light and air by nothing more than thin capillary layers of the vitreous humour. On the following day, when the mid-day sun was almost clear, and shining so brightly that I was unable to look up at it, I repeated the experiment, by bisecting a frog's eye, removing the vitreous humour, and exposing the posterior half containing the retina to the sun for four minutes. I found even then red specks in the chamois-coloured retina, only the edges being completely bleached. In an entire bulb which I had exposed properly arranged to the same sunshine for 25 minutes, I still found very faint red spots in the midst of a great deal of chamois colour, but this was probably owing to the pupil becoming very contracted during the illumination. Since I always prepared the retina in sodium light in these experiments, it might be thought that the period of photo-chemical rest thus occasioned, although short, in some way or other caused the return of the purple. This however is not the case, for if a divided eye be held up against the daylight for a period quite sufficient to bleach an isolated retina, and then, while the light is still falling on it, the retina be rapidly drawn out of the bulb, this will always be found to be at the first moment beautifully red. It is seen that my observations support those of Boll in this limited sense, that the retina may be bleached in living animals, after long continued exposure to direct sunlight; I may moreover add that frogs which had been left to themselves for several days, in a glass case, in a very sunny place, at the end of that time had colourless retinas. While I do not agree with Boll's experience of the effects of "a moderately lighted chamber," we are at one as to the results of more intense illumination.

If the photo-chemical processes which take place in the retina separated from the eye, are taken as representing those which are going on in the living eye, the visual purple may be conceived of as continually being destroyed by the act of seeing, and by some process or other being as continually renewed; and indeed Boll has expressed some such view as this. The oculist led by experience would immediately seek for the process of regeneration in the nourishment brought by the circulating blood; for this is a favourite way of accounting for most of these kinds of events. In the case in point, however, the matter is far less complicated.

That which restores the visual purple is something nearer to hand, and cannot, in a frog's eye at all events, depend on the constant renewal of the blood, since an eye, when taken out and opened, exhibits the same apparent indifference to light as when connected with the whole body and the nutritive currents. If therefore we are correct in supposing the sensitive purple to be continually restored, the regeneration must proceed from something lying behind or on the rods, that is, from the retinal epithelium or from the choroid. Something must be there which either prevents the purple becoming lost, or re-creates it. The idea at once suggested itself that the mere pigment as pigment had something to do with the matter, because a more intense action of light is to be expected if the retina, which usually only receives light from the front, is in addition lighted from behind, as is the case when it is spread out upon a white surface, instead of lying in its natural condition upon a velvety black ground. It could not however be supposed that this would protect it so long and so completely as is found to be the case. Moreover, I could not discover that spreading out the retina with the rods turned downwards upon a dead black surface had much effect upon the time of bleaching; and the following experiments will perhaps shew that the evident continual renewal of the sensitive colour is due to something else than the pigment, which, as is well known, is not present in albinos, and in many animals lies behind a tapetum.

As a proof that it is the choroid, including the retinal epithelium, which alone protects the purple from bleaching in light, it is sufficient to remove the retina in such a way, that a number of black shreds of the choroid still adhere to it, and then to spread it out on a thin glass slip, and expose it on every side. The manipulation for this purpose is not difficult, if the bulb is so cut out that a hole is left at the entrance of the optic nerve, and consequently the spot got rid of which offers resistance to the removal of the retinal membrane. From a bulb so manipulated, it is very easy to get a retina spread out without a crease, especially if a few meridian incisions are made in it. These trivial matters are important, because in badly prepared and creased retinas the pigment actually prevents the access of light to certain parts of the retina. If now the dark strips are taken off the fully bleached preparation, the parts which lie under them will be found to be very deeply coloured.

Another experiment, which demonstrates the same thing, consists in tearing the bisected bulb slightly until as usual some folds of the retina bulge out, then letting the light shine in, and quickly pulling out the whole retina. Where the folds were, white stripes will be found, all the rest remaining red.

I also made the following experiment. Having made an equatorial incision, I seized the retina along a considerable portion of its edge, and very carefully lifted it up from the pigment layer over a good half of its area. I then slipped a thin morsel of porcelain under the raised portion, and exposed the whole to daylight, until it was completely bleached. Of course the bleaching can only be ascertained with certainty in the flap which is raised, as the visual purple cannot be recognized in the dark reflecting hollow fundus of the eye. I next allowed the bleached retina to sink slowly back in sodium light on to its natural support, and to lie for some minutes upon it, making sure the meanwhile that my manipulation had been successful in avoiding the formation of creases; and then I drew the entire retina away. It was uniformly red all over, and one could not even discover a line to mark the limit of the half which had been lifted up. A retina therefore bleached by light may regain its purple colour by simple contact with its natural back-ground. Still it remained to make the whole experiment in active light. This also succeeded, but the half which was restored was somewhat paler than the other. I have no doubt of the success of this operation undertaken by anyone, and I even go a step farther and recommend the cutting out of a flap, the bleaching of it upon a plate and the laying it back again upon the exposed pigment, by which it will be seen that any piece replaced in a normal manner always recovers its purple again. The regeneration has so often and so well succeeded with me by this method, that I was seriously led to try with a piece of tissue paper whether the cup of the eye did not contain a small quantity of red secretion; the morsel of tissue paper however came out moist it is true, but quite colourless.

In a frog's eye such experiments carried out with necessary care need not be hurried; nevertheless the regeneration of the purple, unlike the mere continuance of the colour or the maintenance of its sensitiveness to light, is dependent on the action of living tissues, and when the tissues cease to be alive the regeneration can no longer take place. I have placed the eyes of frogs in 0·5 per cent. Na Cl at 43° C. for 10 minutes, then bisected them, exposed them to the light, and always then found the retinas colourless. Since the retinas of the eyes so heated were still red before exposure to light, they must have been bleached by the light. The same thing occurred in eyes which had died after being kept a day at about 20° C. It may be here mentioned that *these failures* in dead eyes prove that the pigment, considered as mere pigment and not as a living tissue, is ineffectual as regards the maintenance of the visual purple.

If the regeneration of the purple depends on a continuance of vital activity in the pigmentary support of the rods, it is obvious that the rapidly decomposing organs of mammals are ill suited for these experiments. Here rapidity of manipulation is above all things necessary, and yet I have often succeeded in drawing off from the hinder half of the eye of a rabbit, beautifully red pieces of the retina, even after it had been exposed to the light for two minutes, that is for a time quite sufficient to bleach an isolated piece until the only colour left is that belonging to the blood-vessels. Still even in an albino rabbit, where the circumstances must be allowed to be peculiarly favourable, I think I have been able to distinguish between the colouring of a piece of retina which has remained in its natural position, from that of one which had been removed, especially when the former, after the bleaching of the latter, had been spread in the same way on porcelain. Nevertheless I cannot speak with certainty on this point, since the retinas of the specimens of albinos at my command for this demonstration were taken from a variety which just now is very difficult to get here, and in spite of being kept long in the dark failed to shew a really good intense purple, and after being exposed to light exhibited only a slight variable pale orange colour, which indeed is not an altogether unknown feature in mammalian retinas.

It would be especially interesting to investigate this orange colour[1], which perhaps co-exists in company with the purple, since Boll has made the very important observation, that blue rods are very frequently present in the retina of the frog. That there are many albino eyes possessing a well-developed

[1] See Appendix, note B.

purple I saw in later experiments, of which I will give an account on some future occasion.

The last-named series of experiments lead me back to the conclusion that the maintenance after death of the vital conditions in the outer layer of the visual apparatus is to be recognized, not by the existence of the visual purple, and its destruction by light, but by its relative indifference towards light, and I think that the being able to detect this reaction in the frog's eye for so long a time after death shews a happy agreement with Holmgren's and McKendrick and Dewar's beautiful experiments upon the galvanic currents of the retina, and their changes during stimulation by light.

What part of the choroid serves to regenerate the purple can for the present only be conjectured[1]; probably it is not to be sought so much in the dermic layer of the choroid as in the epithelium in which the rods are embedded, and which has been rightly considered as part of the retina. The retina, so long as it is maintained in its natural connections with this epithelium, resembles not so much a photographic plate as a whole photographic workshop, in which the operator, by bringing new sensitive material, is always renewing the plates, and at the same time washing out the old image.

[1] See Part II. and Appendix, notes D and F.

PART II.

VISUAL PURPLE.

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The conjecture which was expressed in the introduction to the former treatise, that the red colour of the retina had been seen by many observers long before it had obtained so great an interest through Boll's communications, may be readily confirmed by studying the rich literature on the structure of the retina. Apart from A. Krohn's discovery of the red rods in the Cephalopoda quoted by Boll, their occurrence in vertebrates was first suspected by H. Müller in 1851. The importance of the subject will justify a verbatim quotation of the essential statements.

A. Krohn says in his paper delivered on Sept. 4th, 1839[1], "Close by the dark stripes the fibres (visual rods) are of a reddish colour, but at their ends turned towards the vitreous membrane quite colourless; the transparency and the rose-red sheen of the inner surface of the retina are thus accounnted for."

This is confirmed by Hensen in p. 39 of his paper on the eyes of Cephalopoda[2] in the following words: "In the fresh retina they (the rods) possess red, shining, homogeneous contents."

M. Schultze remarks in speaking of the eyes of Cephalopoda in 1869[3]: "The rose-red rolour depends upon a diffuse colouration of the entire thickness of the bacillary layer (Plate I,, Fig.

[1] Printed in the *Verhandlungen der Leop. Carol. Akad.* Bd. XIX. 11, 1842, "Supplemental Observations on the Structure of the Eyes of Cephalopods."

[2] Leipzig, 1865.

[3] *Archiv f. Mikrosc. Anatomie*, Bd. II. S. 3.

1, coloured drawing), but is however only visible in fresh specimens, as already mentioned by Krohn. Under the microscope it is recognizable only in thick masses of separated rods."

Schultze further remarks that he has seen the most beautiful rose-red in a large specimen of Loligo almost entirely free from pigment. Well-known, moreover, are the descriptions and beautiful figures which the same great authority on the retina has given in his memoir on the compound eyes of crustacea and insects [1], in which he treats of the gigantic purple coloured rods of these animals.

As far as vertebrates are concerned the first accounts are found in the epoch-making memoirs of Heinrich Müller. In 1851 [2] Müller writes: "The rods of frogs when lying thickly together appear to be in themselves of a somewhat red colour, and an isolated rod may be seen to be alternately colourless and coloured according as it is seen sideways or on end. In 1856 [3] Müller returns to this: "The substance of rods is often seen to be reddish, as I have remarked in my first notice, if it is of sufficient thickness; as for instance when a rod is viewed on end, or several are seen lying together, one over another. This colouring is not uniform all over, but is sometimes stronger, sometimes weaker, often unobservable; and although it appears in eyes which are quite fresh, it may perhaps depend on an imbibition of the colouring matter of blood. Also the colouring which appears in the cones of birds is diffused by imbibition over the surrounding parts."

Six years later Fr. Leydig [4] writes: "The rods of Amphibia (Rana, Pelobates for example), if they lie in great numbers together, possess a rose-red colour; in the case of many fishes, for example Cobitis fossilis, they have a yellowish sheen. The fresh retina of the frog appears to the naked eye to be of a lively, satiny red."

After this it is not till 1866 that the red rods of the vertebrata are spoken of. This time again by M. Schultze (in Vol.

[1] Bonn, 1868.

[2] *Zeitschrift f. Wiss. Zoologie*, III. S. 234—237.

[3] *Ibid.* VIII. S. 1—122.

[4] *Lehrb. d. Histologie*, 1857, S. 238, 239.

II. of his *Archiv*, p. 199), who says: "Remarkably long rods are seen in the rat, the freshly removed retina of which, spread out with the choroidal surface uppermost, shews a strikingly marked satiny appearance, with a red sheen similar to the retina of the owl and the frog." In the same work, p. 208, he further says: "This (the retina of the owl) exhibits in a very remarkable degree the red satiny sheen, which is present in those mammals in which the rods are of an unusual length."

It will be seen from these extracts that the investigators lay most weight upon the fresh condition of the retina, without thinking of its relations to light, and that the colouring is in their view connected in part with the length of the rods; while the repeated mention of the satiny appearance and the glittering colours gives rise to the supposition in the minds of most investigators that the appearance depends rather upon interference than upon actual pigment.

A similar view is directly enunciated by Boll, who, while considering colouring as a direct indication of tissue life, proposes to investigate whether it is caused by interference or by a pigment.

Without wishing to say that the literature on the retina offers us no further account of the red colour of the rods, I will conclude this brief notice by referring to the memoir of E. Rose upon the action of santonin, in which he speaks of a red and greyish-red colouring of the retina of the rabbit, which I take as referring to the visual purple[1].

In continuing my investigation on the visual purple, I do not specially aim in this work at giving a systematic review of its distribution in the animal kingdom; still less so, since the facts which have been given leave no doubt of its frequent occurrence; a large number of invertebrates possess red rods[2], and among vertebrates the colour has been recognized in osseous and cartilaginous fishes, in amphibia, in birds[3], and in mammals.

With the discovery of the sensitiveness to light of the visual

[1] Virchow's *Archiv*, Bd. XVIII. 15, 16.

[2] It would appear, however, that the pigment of the invertebrate retina, being stable and not sensitive to light, must be something quite different from visual purple. Kühne, *Untersuch.* Bd. I. Hft. 4, p. 456.

[3] Boll says it is present in the pigeon, but see p. 26.

purple, the idea has very commonly arisen, as I gather from numerous letters and other utterances, that we are now in a position to understand tolerably well how the excitation of the optic nerve is brought about by the action of light. I can to a very limited extent only agree to this opinion, although for the purpose of investigation I feel obliged to put before myself the hypothesis, which indeed forces itself on every one—that the various decomposition products of the visual purple, namely, the orange, the yellow, and in particular the colourless substances, serve as chemical stimuli for the ends of the optic nerve, while the original visual purple serves as an inert medium having no effect upon them. I would not however venture to make this hypothesis without joining to it the caution that the visual purple must not be looked upon as the only substance in the retina which is sensitive to light. The idea that the movement of the luminous æther is transformed in the retina into chemical processes, is one which has been floating about for many years, even when there was no suspicion of the existence of the visual purple, and there was nothing to justify the assumption that the substances necessary for such an action might be recognised by their colour. It must be looked upon as peculiarly fortunate that one of these is recognizable by this quality. It must first be proved that there is blindness where the visual purple is blanched, and it still remains to be proved that all visual organs are provided with the purple. A second supposition which has met with much acceptance concerns the significance of the products of bleaching, in connection with the question as to what are the structural elements of the retina which are first laid hold of by the light; in other words, what are the real terminations of the optic fibres. It is easy to imagine that the finest radiating processes of the simple conducting fibres embrace the refractive bodies in the inner limbs of the rods, and spread out in the red outer limb. In that case the final terminations, which might continue to be considered similar to the fibres of the optic trunk and indeed to all genuine nerves in respect to structure, composition, and irritability, would be in the most simple manner stimulated, through the medium in which they lie becoming loaded by photo-chemical action with some caustic agent. If this view is correct, one would imagine that the posterior surface of the retina would in the moment of illumination serve as a stimulus to the fresh transverse section of the motor nerve of a frog. Since the first day that I began to be occupied with the visual purple I have often made such an experiment with the most sensitive preparations, and under circumstances varying as much as possible in respect to the duration and intensity of the illumination; but I have never seen any contraction of the frog's limb take place, not even when a dazzling ray of sunlight was suddenly allowed to fall into the dark chamber upon the retina which was laid over the nerve. As it may be doubted if a transverse section of a nerve, in which the medullary sheath might easily cover up the axis cylinder, is as sensitive to the action of a chemical stimulus as the very delicate fibres which possibly exist in the rods may be, I have tried the bleaching of the retina upon the outer as well as the inner surface of the skin of a frog prepared for exhibiting reflex action, but always without any result; that is to say, without any irritation of the cutaneous nerves being manifested by reflex acts. I do not consider this hypothesis disproved by such experiments, against which many and obvious objections, which need not be at present discussed, may be raised. I am, however, of opinion that one ought to give the preference to another view.

When we examine nerves which are sensitive to chemical stimulation, it is always found in every case that the conducting fibres terminate in peculiar epithelial organs; and that these latter differ from ordinary nerve-fibres in respect to structure, composition and irritability can be doubted by no one. Such is the structure, *par excellence*, of the nerves of smell and taste; and such a sensory epithelium is not to be regarded as a mere envelope for the nerve-fibrillæ which pass through it and project over the surface like hairs or bristles. If this were the case we might predicate similar sensitiveness where these terminal fibrillæ were found, and we should expect accordingly to feel a burning in the eye by means of Cohnheim's nerves in the epithelium of the cornea, at the same time that we perceive musk or oil of roses by the nose. Shall we then make an exception in the case of the terminations of the optic nerve and its sensory epithelium, in the day when we find in it substances which are chemically changed by the action of light, and recognize in the excitation by light a chemical stimulation? Certainly not. The rods appear to us now physiologically also as sensory epithelium, like olfactory and gustatory cells; their cuticula, that is the outer limbs, being the part which is decomposable by light, while the nucleated protoplasmic inner limbs appear as that part which bleaching products throw into excitation. By this theory there is room left for the possibility of a thread of the body of the cell, or a process of semi-fluid matter containing such a thread, stretching far into the termination of the outer limb, as a soft Ritter's fibre, or as the core of a Hensen's canal. As the olfactory cells are changed and stimulated by the most minute quantities of odorous substance, so minute as to defy calculation, so we may suppose can the visual cells be acted upon by the least trace of the bleaching substances which reach them. This conception of the origin of visual sensations affords at the same time a desirable link in the doctrine of the development of the higher sensory organs, which, in the case of the retina, has been clearly and logically carried out in Schwalbe's[1] exposition of the subject. Whoever is acquainted with the minute structure of the retina will here ask the question, what signification under such a view can be attached to those structures which were investigated with such care by M. Schultze and described by him as fibres in the furrows of the surface of the rods, and as the thread apparatus and the fibre-baskets around the inner limbs? In reference to this it must not be forgotten that the retinal epithelium of the choroid has been proved to be a very important physiological or chemical constituent of the retina, or, stated shortly, as a purpurogenous gland, the cells of which can scarcely fail to possess a very peculiar complicated innervation. Now I do not see that irritable fibres can arise from other source than from the nervous mass of the retina. And since one knows that the projections of the epithelium cells, which Czerny[2] ten years ago

1 *Handbuch der Ophthalm. v. Gräfe u. Saemisch.*

2 *Wien. Akad. Ber.* LVI.

suspected to be pseudopodic processes, are structures in the highest degree variable (in which, for example, the pigment during life changes its place in a most striking manner, and arranges itself in layers), it may easily be believed that not only the thread apparatus, the thread baskets, and upper fibres, but even those needles of M. Schultze's passing through the external limiting membrane are in part something more than mere cuticular thickenings, or cement material—are indeed very fine nerve-fibrillæ. In the last place, I must not allow still another hypothesis to be passed over, according to which the changes in the visual purple may be the result of, and not, as we have been supposing, the cause of an irritation by light of specific nervous elements. The sensitiveness to light of all dead retinas clearly enough contradicts this; but before I was aware of that, I had not omitted treating fresh retinas of frogs in the dark with every kind of electric stimulus; the consequence was, as every one could now predict, entirely negative : the visual purple was never bleached. Not to pass over anything, it may here be remarked that one might conceive of the terminal organ of the optic nerve exhibiting sensitiveness to light by means of substances sensitive perhaps to light and yet colourless, which on their part first destroy the purple,—we might suppose this if the unchangeableness of this latter towards the most varied chemical influences was not already known. This does not exclude the importance of experiments which would prove alongside of the bleaching other chemical decompositions in the retina by light; only I myself have not been fortunate with these, in so far at least as the simplest means, namely, the litmus reaction, has never shewn any change of the alkalescence. A fresh retina of a frog is, after the most thorough washing away of the vitreous humour in ·5 per cent. sodium chloride, clearly alkaline, and when pressed on litmus paper or Liebreich's tablets leaves a distinct blue mark. If the contrary appearance comes out, this is caused by the red rods being drawn into the pores and roughnesses of the paper ; when the reaction is confined to a circle surrounded by the fluid, the clearest blue is never missing, and it never will be seen passing into red in the light, while when the red masses of rods obscure the reaction the blue will be recognized as coming afterwards in the light. If I mashed up a retina in an agate mortar and then let the mass bleach in the light, this still gave an alkaline reaction.

The foregoing considerations caused me in the next place to examine whether all those elements of the retina which up to this time had been considered as sensitive to light contained the visual purple. It immediately struck me that I had never recognized a trace of purple in the cones of the frog In the retina of a frog which has been smoothly spread out, with the choroidal surface in contact with the cover-glass of a flat moist chamber, the cones, as is well known, may by proper focussing be readily seen between the rods, in the deeper layers where they are specially recognizable by the highly refractive bodies of their inner limbs. I have never been able to discover here any red colouration, and with a higher focus the space between the rods is never found otherwise than complementary to the visual purple, that is to say blue-green, just as it is in those spaces between the rods in which the cones are absent. This blue-green colour is visible also, as Boll has already mentioned, in a certain number of the rods, namely, in such as are somewhat turbid; and which generally, unlike their clear red neighbours, do not, when an object is slipped in between the mirror and the diaphragm of a microscope, shew the Leeuwenhoek images, which Boll and M. Schultze found reflected from the rods of the frog. This green colouration toning down into grey which, arising first from simultaneous, is increased by successive contrast, will invariably be seen even in the rents and holes of the preparation, provided these are not too large, and particularly if they are filled with some cloudy substance which obstructs the perfect passage of the light. And in cases where the rods are lying down and scattered about like the ears of corn in a cornfield which has been beaten down by wind and rain, (a state of things which by the naked eye is recognized in the fresh red retina by the occurrence of shiny satiny streaks) this is seen in the microscopic view as large streaks and tracts of a blue-green colour. The origin of these striking appearances is that the rods are not sufficiently red to allow of the purple being recognized except in the direction of their long axis. One must be very quick not to lose the time of the most intense colouration, if one wishes to recognize the colour in the isolated rods lying on their sides. Perhaps moreover besides the want of depth in the transverse diameter, the reflection of light from the outer surface of the rod is unfavourable to the perception of the colour. It will be understood from this why the turned-up edge of a preparation of the retina only appears red when the rods lie one over another in sufficient numbers; and why the circles and lines of the turned-up rods, which without the red surroundings appear to be grey, must appear a bluish-green by contrast when seen between those which are recognizable as being red. That the rods which remain standing, if they appear to possess the blue-green colour, are in reality not coloured, may be proved by inserting a disk of black cardboard with fine holes pierced in it in the place of the ocular micrometer. The red ground is then covered, and by turning round the object on the slide, one can bring into view at one time red and at another colourless grey, less refractive transverse optical sections of the rods. If all the rods are bleached, still those which are the complementary ones may be always clearly recognized, although their colour has of course disappeared, by their inferior transparency. It is quite different with the really green, that is, strictly grass green rods—which are present in the retina of the frog. These are mostly transparent, they give the Leeuwenhoek images, they bleach somewhat slower than the red, they stand the isolation test of the ocular diaphragm; and when I have occasionally met with them isolated, and standing on end, their colour has been fully developed. I do not doubt that Boll had these rods particularly in mind in his second communication to the *Acad. d. Lincei* of 7 Jan. 1877 S. 3, 4. Notwithstanding this, I am unable to bring their occurrence into relationship with the colour of the light to which the eyes had been exposed: a relationship which Boll suspected after making experiments upon frogs which had lived under green glass. In frogs which had been kept in the dark I found this kind of rod also inconstant.

As the outer limbs of the cones in the retina of the frog are very short and come to a fine point, it is possible that a purple colouration, even if present, might not be recognized. I have sought evidence of this in the retinas of birds (pigeons and fowls), which possess a large number of cones; although here I had from the outset to expect difficulties of observation on account of the well-known pigment-globules, and other colourations of the inner limb of the cones. The result of my numerous observations was negative: generally there was no visual purple at all to be recognized in the retinas of these birds. It was impossible for my sense of colour, as well as that of all other persons before whom I laid the fresh retina, to perceive any change of colour in diffuse day or direct sunlight—either in the central portion which in daylight appears to be chiefly coloured by red pigment-globules or in the peripheral greenish-yellow coloured portions. Since both regions contain rods as well as cones, and since at the periphery most elements are free from pigment-globules, I suppose one ought to see the visual purple of birds if it exists. Where in fresh preparations heaps of separated outer limbs lay in thick layers together, I have never seen any indication of purple. Therefore pigeons and fowls have no coloured substance in the outer limb which can be changed by light; and this proves that sight does not entirely depend upon the presence of such a substance. From M. Schultze's discoveries upon the eyes of nocturnal birds, we know that intensely coloured pigment-globules are not present in the retinas of all birds; and if doubts are found cast here and there upon this important statement of the morphology of the retina, this is most probably due to the modest statement of the great histologist, who does not pronounce decidedly on this point. The doubts of many observers may be due to the fact that they are unable to reconcile the observation of the absence of red globules in the owl's eyes, the globules in this animal being at the most pale yellow, with the mention of a strongly marked red colour in the rods, an appearance which previous to the discovery of visual purple had not been understood. At the present moment this latter circumstance, which is quite casually related by Schultze, is of peculiar importance; and I have not doubted for a moment that it is correct, and that it indicates a further fundamental difference in the nature of the retinas of day and night birds.

Having obtained a living owl (*Strix passerina, s. Athene noctua*, Retzius), I am in a position to confirm Schultze's[1] discovery. The bird was brought to me in a very lively state early in the morning, and kept for four hours in the dark, before being decapitated. On opening the eye the retina remained attached to the vitreous humour free from pigment — so that it was cut away from the entrance of the optic nerve without any injury. It shewed strong absorption in sodium light and appeared quite grey in it; this arose from the unwontedly intense purple (almost passing into blue) which spread uniformly over the entire posterior surface. I found, as M. Schultze said, that the rods were longer than in all other vertebrates, and not very narrow. Between the rods there were in many places cones visible with delicate less shiny outer limbs, which could not be called short, though they certainly only reached to one quarter of the length of the rods. The refractive bodies at the junctions of the inner and outer limbs were colourless. The purple did not bleach in light more rapidly than in other animals, during which it passed into a somewhat persistent orange, and here, as in all cases, was present only in the rods. When M. Schultze stated that in the owl the yellow cone-globules are only less intensely coloured than in other animals and not entirely absent, it being only the red ones which are lacking, I cannot help thinking that in making this statement (and we must remember that he was treating the subject in a very general manner only) he was influenced by having seen the orange of the entire retina—a phenomenon which could hardly have escaped his notice. His accounts and figures of *Strix aluco* and *Strix noctua* are too positive for me not to recognize that they distinctly differ from mine; for, as I have said, I have never been able in any species to discover any yellow colour in the globules in question; as soon as the orange was

[1] *Archiv f. Mikroscop. Anat.* II.

bleached, which took place in about 45 minutes in imperfect afternoon light, not only the whole retina appeared quite colourless to the naked eye but also the globules transmitted no yellowish tinge even when their surroundings were no longer coloured. In the black pigment epithelium whose cells are provided at the side of the rod with a tuft of remarkably long processes, I found no transparent pigment-globules.

In another owl, *Aluco stridua* (*Syrnium aluco*, Linn.), I found the retina, which tore close by the pecten and came away with the vitreous humour free from pigment, provided with fine rods which were somewhat less long and correspondingly appeared weaker, and less uniform with regard to the purple colour. The purple in which a violet tone was very prominent, passed when exposed to light into a pale chamois before it became entirely bleached, never becoming orange or pure yellow. Between the rods lay a fair number of cones, of which the greater number were provided with colourless, or at the utmost very extremely faint yellow globules, while a smaller number were distinctly yellow or orange coloured, and a very few isolated ones were of a red colour.

In the retina of a tower falcon (*Tinnunculus alandarius*, Brisson) I found, contrary to my expectations after the experience of the absence of the purple in the pigeon and the fowl, richly purple coloured rods again present; but these together with their quite intense violet colour were arranged in stripes and spots in a still more striking manner than in the Aluco, and confined to those places where there were few cones or only such as had colourless or very feebly coloured globules. Where cones with red yellow or greenish yellow globules were found in any number, the surrounding rods were colourless. Unfortunately I could not sufficiently early examine the foveæ of the retina of the falcon, so that I can give no opinion upon the presence or absence of the purple in these important spots. As Boll decides upon the presence of the purple in the eyes of pigeons, I have striven by increasing the material to arrive at greater certainty. My hopes that the albino pigeon, with the deep ruby red pupil, perhaps might be without the disturbing pigment-globules were not fulfilled;

for I found this retina did not differ from that of other pigeons. The cones contained yellow, greenish yellow and red pigment-globules, the rods had no perceptible trace of purple. As in the retina of all pigeons there was often the appearance of a pale flesh-coloured colouring in the mosaic of the rods which lay between remarkably ruby red cones; yet this was seen only in those places where the preparation was not quite smooth and the persistency of the coloured sheen even in direct sunlight excluded the supposition that the tint was due to visual purple. According to all appearance, the visual purple in the bird's eye is deficient in proportion as the retina is provided with other more stable means of absorbing coloured light, I mean the coloured globules of the cones. This is at least the case in the nocturnal and predaceous birds, and is entirely true as regards the pigeon and the fowl.

Besides the owl, I have been able to make investigations concerning the purple upon another species of nocturnal animal. In the bat (*Rhinolophus hipposideros*, Bechst), which is plentiful here, I tried to make sure of the colour of the retina. Unfortunately the eye of this species is so small that one must be content to cut it in pieces and to separate it out upon the microscope slide; this I naturally carried out with sodium light—a treatment which the reader must presuppose in all the experiments which are described in this treatise, where the contrary is not expressed—using animals which had been kept in the dark. I have gone further, and killed those animals in which I found no visual purple, after they had been kept a long time in the dark with a bandage over their heads, and I took in hand the preparation of their eyes with the greatest haste, either in the minimum of light which sufficed for the operation or with a blue light from an ammoniacal solution of oxyde of copper whose freedom from red, yellow and green rays had been proved by the spectrum, and with which one worked scarcely more conveniently than in darkness. I took these precautions because one could not tell whether there might not be present visual pigments far more sensitive or more affected by light of lower refrangibility, than is the visual purple. In spite of all these precautions, I have never been able to perceive even slight indications of red colouring in the rods of the bat, which are certainly very minute, and which, as is well known, do not surround any cones. I must therefore maintain that the rods in this species which I have examined are colourless, even if colouration should really be proved to exist in other species of this great order where the rods are longer.

Without wishing to say that genuine rods which are not red are altogether useless for vision, it appears to me doubtful on account of their mere existence in the bat to infer a power of sight, at all events one at all comparable to our own sense; since we know from Spallanzani's famous experiment, which Brücke in his "Vorlesungen über Physiologie" justly interprets from the stand-point of the knowledge of the present day, that these animals shew no awkwardness in their movements after their eyes have been destroyed. With the same right that we conclude from the behaviour of blinded bats that they possess an astonishingly fine sense of touch and sensitiveness to rays of heat and the like, we may also conclude, that they are certainly not accustomed to sight in any manner of which we have experience, and indeed have no need of it. The difference between their vision and ours may be almost as great as that between the sense of smell in sporting dogs and in a human creature. But it is intelligible that in cases where other peculiarly finely developed senses render a more acute vision unnecessary, that there may yet exist a morphological visual apparatus which is fairly well constructed, but yet is deficient in one of the chemical constituents which are sensitive to light. It seems preferable to ascribe to the bat four senses only, rather than six, as our predecessors wished to do.

Another animal that especially lives in the dark, namely the badger, whose eye in relation to the size of its head and body must be called small, exhibits a well-developed purple in the retina. I received the animal alive, placed it for three hours in the dark, and immediately prepared the eye in sodium light. The retina bleached quickly in light, passing

[1] Wien, 1875.

through orange into yellow. I found the rods very small, considerably shorter and narrower than in the rabbit, for example. The background of the eye shewed a large tapetum, not of a triangular but of a half-moon form, in which, somewhat corresponding to the centre, near the edge, but in the bright part, the entrance of the optic nerve was situated. There was no coloured interference to be remarked in this tapetum, only a bright satiny sheen.

Among fishes the eel and the loach, as they are chiefly inhabitants of dark mud, may be reckoned as nocturnal animals. M. Schultze makes a similar remark in reference to the eel, of whose retina he observes that it only contains rods, no cones. I have found the retina of both fishes purple: in the loach only faintly so, but in the eel more intensely purple-coloured than in any other animal, with the exception of the owl, which in this respect slightly exceeds it. When exposed to light the retinas were often very intensely yellow; to this fact may probably be referred Leydig's statements in reference to the loach; there are however differences to be noticed. I saw, for instance, in an eel which had died in darkness, the purple pass when exposed to the light into a deep orange-yellow which did not bleach for two days[1], and then did not bleach fully, whereas in another specimen the yellow came out very faintly and disappeared after being exposed for an hour to a dull light. As the retina of an eel, being free from cones, contains no pigment-globules, and the rods being of a considerable length are uncommonly rich in purple, a certain agreement with the arrangements in the owl's eye is evident. A specimen of *Petromyzon fluviatilis* which I examined shewed quite evidently a faint purple colouring of the retina, which disappeared in the light. The animal came into my possession in a condition upon which I could not depend, so that I could not be certain that it had not while already dead been exposed for some time to the light[2].

It is of the greatest importance to know for certain whether

[1] See Appendix, note B.

[2] In another specimen obtained in a perfectly lively state and examined with all proper precautions the visual purple though present was decidedly faint.

the purple is always absent from the cones. The sensory epithelium in man consists in the yellow spot chiefly, and in the fovea centralis, in the place of the clearest vision, where there certainly is also sensitiveness to colour, entirely of cones. Up to this time I have unfortunately only been able to examine one pair of human eyes which were in any way in a condition in which they could be of any use. Dr Fischer, Assistant Physician at the hospital at Pforzheim, to whom I am greatly indebted for it, took the precaution to shut out the light for about half a minute before death (March 19th), and to have a dark covering laid over the head and the eyes of the corpse. This last came early on the 21st of March into the Anatomical School of this place, when the eyes were immediately extirpated by Dr Ewald under a cloth. The cornea was already very cloudy, the bulbs tolerably soft and surrounded with a great deal of fat. I opened the first eye by means of a circular incision, somewhat in front of the equator. As the vitreous humour slid out the greater part of the posterior half of the retina came out with it—torn away over a wide extent round the papilla. When brought out of the sodium-lit chamber into the daylight, the posterior surface shewed a very clear, uniformly distributed, purple colour, which quickly passed into chamois and yellow and finally disappeared. The rest of the retina still remaining in the eye was, after an incision round the optic nerve, removed under sodium chloride ·5 per cent.; it exhibited the same colour and sensitiveness to light. The yellow spot was very clearly recognizable, there was no red perceptibly mixed with its yellow. The fovea centralis was not clearly visible, but there was certainly no red spot in the place where it was. In the immediate neighbourhood of the yellow spot the retina shewed an extremely faint tinge of red, so that the macula was surrounded by an almost colourless broad zone, which passed with indistinct gradations into the redder parts. The state of the other eye was similar; in this the retina after being halved behind the equator was exposed to light quite uninjured, with the exception of the small portion cut off round the papilla. There was no pigment epithelium clinging to either of the retinas. As the eyes were forty-eight hours old, one could not conclude with certainty from what was found in them, that the portions of the human retina which are rich in cones possess very little purple—and that the portions which consist entirely of cones, the macula lutea and the fovea, have none, however probable that may be, since the outer limbs of the cones are the most perishable, and I cannot be sure that they did not remain partly attached to the epithelium, while the corresponding portion of the rods remained with the retina. Microscopical investigation could not decide this point, for I found the rods and the cones entirely changed in the usual way by death. With reference to the colour-blindness of the peripheral portion of the human retina, it was interesting to ascertain the limit of the purple towards the front. In this I was successful in the second eye. I drew the whole contents of the eye out, under salt solution, from the sclerotic and the cornea, after which the retina was separated with the greatest facility from the uvea and the pigment epithelium as far as the ora serrata without being torn; from that point to the lens the task was more difficult, but I succeeded after careful traction. The last separated portion remained covered with brown pigment, which allowed no other colour to be visible; but it certainly did not cover up any, for the purple ceased at at least two mm. behind the periphery of the brown zone, with a boundary line by no means diffused. The eyes belonged to the body of an old and corpulent woman, whose lenses were yellow, and tolerably soft. My observations on the visual purple of the human eye confirm, as one sees, those of Fuchs and Welponer[1], Schenk and Zuckerkandl[2], but with respect to the extent of the purple, touch upon a circumstance of the greatest importance, which has not yet been considered, and upon which I am anxious to arrive at greater certainty. As I had no opportunity of investigating the quite fresh eyes of an executed criminal to overcome the doubts into which the observation of human eyes two days old had led me[3], there only remained for me to investigate the eyes of apes. By the kind assistance of the Director of the Zoological Gardens in

[1] *Wiener Med. Wochenshft. d. Js.* S. 221.
[2] *Allgem. Wien. Med. Ztg.* 13. März, 1877.
[3] See Appendix, note C.

Hamburg, Dr Bolau, to whom I am peculiarly indebted for procuring for me many of the animals used in this work, I obtained a good living specimen of *Macacus cynomolgus*. The ape after being kept in the dark for 24 hours was stupefied with chloroform, the head cut off and the eyes immediately extracted in sodium light, and further treated as the human eyes were under salt solution. I was not successful in emptying the vitreous humour well in either eye, or in separating the retina from the choroid coat after the papilla had been cut round. For that reason I placed the anterior as well as the posterior section in alum of 4 per cent. and first drew the retina out after 24 hours, an operation which was easily accomplished. Both retinas shewed a pale purple colour, with a striking decrease in the part surrounding the yellow spot, in which as well as in the fovea there was not the slightest trace of red visible. Examined under the microscope there was found in the fovea of one of the retinas a very small three-cornered slit, towards which the cones crowded with their long narrow outer limb, and which did not give the impression as if a number of cones had remained attached to the epithelium and had fallen out, but seemed rather like a rent. In the other eye the posterior surface of the corresponding part of the retina was continuous, and in all sections which were made from this and other parts might be recognized an unbroken border composed of the outer limbs of the rods and cones. The alum, it is true, caused these structures to shrink, but the rods could be well distinguished from the cones, and among the latter could be recognized the elongated ones belonging to the fovea. Lastly, to make quite certain, the surface of the epithelium of the fundus of the eye was scraped off little by little, and examined for outer limbs. In the second eye, in which the fovea was uninjured, none of these were found; in the first, isolated ones appeared, and here and there several sticking together. I maintain, after the results obtained from investigating the first retina, that it is proved that in the eyes of apes the fovea centralis and its nearest surroundings contain no visual purple, while I must leave it undecided, for the periphery of the yellow spot, whether the rods which are placed there, and in a wider circle between the cones, possess any purple. In the apes the purple extends faintly, as in man, as far as the ora serrata. Yet I found the limit of the red somewhat closer to it and more diffused than in human eyes.

When the rods are scattered, or lie between a considerably larger number of cones, each of these may be intensely purple-coloured, but not recognizable in the general appearance of the surface, if the cones are colourless. Should these latter, in spite of our not being able to perceive the purple, still contain a trace of this substance, still it must be considered how slight this must be if it disappears from observation in the fovea in the thick border formed by the extremely long outer limbs. Shades of the purple which might correspond to the different degrees of sensitiveness to colour in the human retina were, as has been already said, not recognizable in this latter, and just as slightly in the retina of the ape. Any one who has the opportunity of examining a fresh human eye will probably be able to decide definitely as to the absence of the purple from the spot of the most distinct vision, and to confirm that to which my observation on the human eye and the conclusion from analogy with the eyes of apes could only give a high degree of probability[1].

I was led by the statements of Leydig, M. Schultze and others, that in the retinas of snakes no rods are present but only cones and these free from coloured globules, to examine the eye of *Tropidonotus natrix;* and as far as this species is concerned I can thoroughly confirm the fact. I have carefully searched through a good number of such retinas but I could discover no trace of red. Nothing was visible except a yellow tinge, so slight as to be scarcely worth mentioning, and which moreover withstood exposure to light. Up to this time I have found no retina which shews the appearance of the images of Leeuwenhoek more perfectly than does this; I mention this fact as proving by the way that in spite of the smallness of this snake's eye one may very well succeed in obtaining a normally spread-out retina, and that one can bring the cones into view in the direction of their long axes. As they have

[1] See Appendix, note C.

even in that position no perceptible red colouring, the purple must be entirely absent from them. Here again is the retina of an animal, which without any doubt can see very well, from which the visual purple and indeed all visual colour substances are entirely absent. I found the retina of *Coronella laevis* exhibiting exactly the same features.

I can shew, not as strikingly as in the snake, but nevertheless quite undoubtedly, the absence of visual purple in another reptile. *Anguis fragilis* has yellow-coloured globules (so well known in the lizards), situated at the point of junction of the inner and outer limbs of the cones, as well as other colourless and similarly refractive structures of this sort. As the yellow globules are in many places a considerable distance apart, there can be no doubt that there is no visual purple between them. I have never been able to perceive in the retinas of slow-worms, which have been kept in the dark for a long time, anything but a faint yellow colour which is unchangeable by light. Therefore I must concur in Boll's doubts as regards the occurrence of the purple colour in the lizards.

I found the difference between the purple rods and the uncoloured cones very marked in the retina of the carp. The retina is of a bluish purple colour, recalling that of the eel, but one can see with an unassisted eye that the colour is interrupted, and therefore very imperfectly saturated—a condition of things which is occasioned by the peculiarly large number of colourless cones which are scattered between the red rods. Boll's statement concerning the purple of the osseous fishes is hereby confirmed, and 1 can add the same as to the retina of a cartilaginous fish. It is true that the retina from a shark, which had been kept in the dark and was brought to me 48 hours after death, was only a purple-coloured jelly, which immediately escaped from the back part of the eye and exposed a magnificent tapetum which shone like polished silver. I could, however, in this mass determine the persistency of the colour in the dark, the change to yellow and lastly the complete bleaching in light. In one of these eyes, which had been laid open in the dark for an hour, I saw to my great surprise the whole of the retinal mass flooded by a clear purple solution, which when poured upon a plate exhibited the same behaviour to light as the mass itself. Except the beautifully iridescent crystals of the epithelium of the tapetum, which I maintain are guanin combined with lime, there were no coloured solid particles to be seen in the solution.

It is well known that the newts have rods with slightly conical outer limbs, in which M. Schultze suspected a transition, both in form and function, to cones. In a marked manner these large structures are always very faintly coloured red. It is therefore all the more astonishing to be able to recognize very well the faint colouration in these outer limbs, even when they are broken off, floating about, and lying on their sides. Perhaps this apparent contradiction—viz. that the colour, when looked at in the direction of the long axis appears so faint, while when seen in the transverse diameter, of course at the lower thicker part only, it is so strongly marked—depends on the purple being deposited at the surface only of the conical outer limbs, and not, as in other cases, distributed throughout their whole substance.

Incomparably splendid is the appearance of the *Salamandra maculosa*, with its true fairly cylindrical outer limbs of rods, the large masses of which render intelligible the more intense purple colour of the retina as compared even with that of the frog.

Finally it may be interesting to know that the visual purple may appear in intra-uterine life, in rods which have never been exposed to the light. I found the retina of a fœtal calf 65 centimetres in length, which had hair on the snout, head, tail and feet, quite clearly coloured purple; the colour when exposed to light, first passed into yellow and then entirely disappeared; in this case the rods were clearly recognizable under the microscope as delicate short palisades. This agrees with Schultze's statement that in sheep's embryos the first rudiments of the outer limbs of the rods are already manifest by the time the hair appears, and also as far as the purple is concerned with the observations of Fuchs and Welponer (*l. c.*) on human fœtus of 7—9 months.

In an embryo calf 44 cm. in length, I failed to find any rods. The same was also the case in new-born rabbits, in which I can confirm Schultze's often misunderstood discovery that the outer limbs are scarcely developed.

In speaking as I have done hitherto of the red colouration of the retina in general terms as visual purple, I thereby intend to express that it depends on a particular material peculiar to the outer limb of the rods, on a substance, that is, composed of one or several coloured chemical bodies. This can scarcely be doubted, since I have demonstrated how entirely independent is the colour of the rods from the numerous structural changes which take place in the other retinal structures below the layer of the outer limbs. Whatever change obliterates the colour of the retina must attack or destroy the colouring substance, that is the visual purple itself. In the first part of this treatise I have narrated a series of cases in which the retina was handled in different ways, rather with a view to shewing that the colour is independent of the structure, and that the changes induced by light are not the result of changes in the structure of the rods, than with a desire of fixing with certainty the reactions of the visual purple. The numerous experiments which have still to be related will afford many illustrations of this truth, so that I need not enter into it more fully here.

Although the facts already known give no cause to suspect that the light can give rise to visible changes in the structure of the rods[1] and of other portions of the retina, I have not left unused the opportunity which the observation of the colour of so many dark preparations offered to me, in order at the same time to study the changes in form of the rods which have been so often described with a view of determining what share light had in the matter. I can nevertheless only report that the well-known opacities and striations, as well as a more or less distinct appearance of a satiny sheen, are changes which take place in the extirpated retina as rapidly in the dark as in the light, and possibly arise from osmotic processes, dif-

[1] See Appendix, note G.

ferences of tension, coagulations and the like. These changes affect the colour only so far as opaque retinas shew a less saturated appearance than transparent ones; and on this point the chief importance must be attached to the fact that this change already shews itself at the time when the sheen and the refraction in the outer limbs have begun to diminish, which events are the first token of that delamination of the outer limbs of the rods so carefully investigated by M. Schultze. In the perfectly fresh retina it is quite impossible to see any striation of the rods corresponding to the laminæ, a fact which is worth remembering in reference to Zenker's theory of the production of standing waves, by means of the rays which fall upon and are reflected by the laminæ. I do not mean that the laminæ do not pre-exist, for their appearance as the result of so many and various, and easily controlled chemical actions forbids the idea that they are merely artificial products; but the column of laminæ does not behave during life like a set of separate laminæ as Zenker's theory demands, but like one which is composed of glass plates cemented together with balsam, while after death it may be compared to a similar series delaminated by a softening of the cement. The thing which after postmortem decomposition becomes a set of laminæ seems accordingly to behave during life more like a glass rod. The living rod is composed not of relatively thick plates with a minimum of intermediate substance, but of alternate layers of equal thickness, which, though having the same refractive index, differ entirely in their chemical composition. M. Schultze's beautiful investigations teach us that the visual rods of the crustaceæ are composed of conspicuous layers of a purple substance alternating with a colourless one, the two possessing very different imbibition equivalents, that of the coloured ones being distinctly the greater.

Very different from the above indifference of the structures of the rods, which only holds good in isolated retinas free from epithelium, are the very striking changes of the rods, as well as other events not concerned with the purple which manifest themselves as the result of the action of light on the retina in situ and during life. Since here, however, there is at least one important factor at work in the process of regeneration, I will at present abstain from entering more fully into the matter.

The reactions which I studied on the retina, and will now describe, had for their object the discovery of a solvent for the rods or for parts of them, in order to obtain the visual purple as an isolated substance. Whatever good reason had formerly been given for the existence of this substance, I had from the beginning said to myself that such an idea only floated in air until the purple had been obtained either in solution or in a solid form free from all the remains of its structural substratum. For this purpose I could of course only use such things as I knew from experience did not destroy the colour in the dark; many were thereby at once excluded, such as caustic alkalis, concentrated acids, and even the most dilute mineral acids, alcohol, &c.

Former digestion experiments with the retina had shewn to myself and A. Ewald[1] (what has since been fully confirmed by Dr Kühne, through study of digested retina sections in the Institute here) that all outer limbs of rods leave something behind, possibly an envelope, which on account of its complete insolubility in trypsin as well as in peptic acid, and on account of its resistance to caustic alkalis, may be considered neurokeratin, as well as a substance which indeed shrinks very much during digestion but remains recognizable by its fatty aspect, and which is soluble in boiling alcohol and benzol, though not in ether or cold alcohol. This last substance agrees with the characters (dependent on the presence of cerebrin) of the medullary substance of nerves, of which indeed M. Schultze supposed the rods to be composed. As is known from M. Schultze and Rudneff's researches, the outer limbs are rapidly darkened by osmic acid just like the medulla of a nerve, yet it must here be remarked that they never assume the peculiar steel coloured, almost blue-black tint of the latter.

If the visual purple is connected with cerebrin I expected to be able to free it from that with the help of benzol; but dried or moist retinas of the frog and of the ox thoroughly

[1] *Verhandl. d. Naturhist. Med. Ver. in Heidelberg.* I. Hft. 5.

treated, first with ether and afterwards with benzol, never gave a coloured filtrate, although the purple was not bleached. So also with acetic or salicylic acid or with ether holding ammonia, none of the purple passed into solution; and the same negative facts were obtained with etherial fat solutions and with fat containing benzol. Warming to 50° C. (which did not destroy the purple) in pure olive or almond oil, after the retina had been previously treated with ether to free it from fat, was also without any effect; so also digestion with ammonia, glycerine, oil of cloves, turpentine, and extraction with chloroform, bi-sulphide of carbon, &c.

Rollett's important observations on the freezing of blood-corpuscles have made known a method of separating hæmoglobin, that is of a colouring matter, from a substratum, which on account of it containing lecithin and cerebrin may well be compared with the medulla of nerves or with the ground substance of the rods; and this seemed to point the way to a method of isolating the visual purple. I froze a dozen fresh frogs' retinas at – 13° C., thawed them, froze them and thawed again four times, and then examined them under the microscope. The rods were much altered, somewhat thickened, doubled in length, and split up into laminæ in as beautiful a manner as I have ever seen. When I rapidly froze another retina directly under the cover-glass of the moist chamber, and let it thaw under the microscope, immersing the objective in a drop of glycerine, I saw that the change took place moderately quickly, and if I slid and pressed the preparation the rods stuck together in beautiful transparent red lumps. When I, meanwhile, froze my eleven retinas (which in thawing had collected a few drops of moisture) together with a few drops of ½ per cent. saline solution, and let them thaw once more, I obtained from the whole through a miniature filter a colourless filtrate. It may here be remarked in passing, that the fluid as well as the lumps of the retina on the filter had plainly an alkaline reaction, and that the latter after bleaching in light shewed no change in their reaction towards sensitive litmus-paper. Since the rods must in this case have given up all the fluid they contained, this observation affords a conclusive extension of the former statement (p. 21) as to the unchangeableness of the alkalinity of the retina through stimulation by light.

In spite of this first unfavourable result, I still continued to make experiments based on the analogy of the chemical constitution and relations of the red blood-corpuscles, and of the medulla of nerves, with those of the red rods; and I have no reason to repent of so doing. Just as bile is a means for the solution of the blood-corpuscles, so is it also for the medulla of nerves[1], and even for the fresh axis cylinder. Bile, as is known, dissolves lecithin, and indeed it usually contains this body. It also, especially on slight warming, as I have satisfied myself, dissolves the much less soluble cerebrin. As to the way in which it works on the rods of the retina, that once seen will never be forgotten. A fresh frog's retina placed in a drop of bile under a cover-slip, immediately breaks out into most wonderful movements; at the edge the rods shoot out like rockets, and where the bile comes in contact with the separated freely moveable outer limbs, these may be seen to curl up like worms, and then suddenly, with a jerk, to stretch out straight again and shoot forward lengthwise; it is just at this moment that the longitudinal striation becomes first visible, then the whole column of the superimposed row of the laminæ may be seen, and finally the whole vanishes. It often is as if a roll of coins was shot out of a tube, or like a cartridge of grape-shot. If the drop of bile employed be too small, many rods remain unchanged for some time, and one has the opportunity of watching the process take place gradually in individual rods. This may take place at one end only, or at both ends at the same time, or may begin in the middle; and the breaking up at the spot acted upon is preceded by a change in the refractive power, difficult to describe. At times, a somewhat thick canal, often possessing swellings, is visible in the axis, around which not laminæ but rings are disposed, and as these split up in a radial direction and thus give rise to a longitudinal striation, one can well imagine such a structure of the rods as Hensen[2]

[1] Compare A. Ewald and W. Kühne, *l. c.*

[2] *Virchow's Archiv*, Bd. 39. Taf. XII. Fig. 8.

represents. It is of course understood that for these observations, purified bile, that is to say the watery solution of crystalline colourless ox gall, must be used. I recommend keeping the solution under ether, and not to employ cholate preparations which have been preserved dry, for it has repeatedly happened to me, in this way, to obtain fluids that in spite of a suitable alkaline reaction would not properly dissolve either blood-corpuscles or the medulla of nerves.

The most important action of the bile upon the retina consists in the solution of the visual purple, and the goal of our wishes would soon be reached by help of it, if it were not that the rods of dead mammalian eyes resist the means. The retinas of the still warm eyes of rabbits and oxen, it is true, very readily yield up their purple to the bile; and when I placed in the solvent about thirty ox retinas, only a few hours old, removed cleanly under chloride of sodium ·5 per cent., I obtained a mixture in which, according to all appearance, the purple was thoroughly dissolved. On filtration, however, the filtrate came through hardly coloured at all, and contained, as indicated by the spectrum, a little hæmoglobin which was unchanged by light. Corresponding to this one finds in the case of frogs' retinas which have been kept moist for 24 hours, that the bile does not exhibit the explosive action just described, but only a slow breaking up, leading it is true to the same final result. During this action it brings about no true solution of the rods, but reduces them to a much swollen mass, which, however, does not usually stop the filter. I must also remark that even the fresh rods are never completely dissolved by the bile, however much they may appear to be so; if the preparation is gradually diluted with water, considerable remains, probably consisting, in part, of neurokeratin, are always to be met with. The cells of the retinal epithelium are very perfectly and very suddenly dissolved by bile, both in the fresh and in the dead condition; their dark pigment granules stream away from them in all directions, leaving behind them in the case of frogs the well-known yellow refractive drops.

More exact studies on visual purple will only be possible when a new method of obtaining it from dead retinas is found, or when one will be able to work with a large number of still warm retinas in a yellow chamber in the immediate neighbourhood of a slaughterhouse. The eyes of oxen will certainly always be subject to this inconvenience, that some hæmoglobin will be dissolved with the visual purple, because the retinal vessels invariably contain blood. This it is true has not prevented my making an experiment with the few ox eyes which have been brought to me still warm, and thus obtaining some cub. centim. of good clear filtered visual purple solution. The experiment, however, was so far a failure, inasmuch as the solution did not completely bleach in the light. I could not, for example, determine whether the substance giving to the retina a distinctly pure red tint contrasting strongly with the true purple-red, sometimes even bluish-red, of the rabbit and the frog, passed also into solution together with the proper visual purple. In all absorption and bleaching experiments in coloured light, the presence of hæmoglobin would prove an insurmountable difficulty. I have therefore for the present to content myself with experiments carried out with materials at once scanty and difficult to obtain, which I prepared for myself from the eyes of rabbits and frogs.

Before I say anything more about these it will be desirable to give an account of some experiments which I undertook in the meantime with dead ox eyes, to discover other means of preparing the visual purple, not because they have already led to that end, but because they give further insight into the relations of the remarkable substance with which we are occupied.

It is for example possible to obtain from such retinas an insoluble residuum which consists only of neurokeratin and visual purple. For this purpose the dead retinas of the ox eyes are first extracted with purified bile and then with water, after this with acetic acid $\frac{1}{2}$ per cent., which must be removed as much as possible on the filter by washing with water. Further what remains after the filtration is digested for 24 hours at 40° C. with an active solution of trypsin, containing

1. p. c. salicylic acid[1], the resulting residue again washed on a filter, spread out upon a glass plate, dried in 40° C., and extracted with ether and benzol. The benzol solution is then left to evaporate, the residue moistened with water and washed out with concentrated ammonia, all remains of the latter being got rid of by evaporation and washing. All these operations must of course be conducted in the dark, using sodium light when anything has to be seen. The residue of the retina thus obtained is free from fat, lecithin and cerebrin, contains no albumin, or nuclein, no mucin, finally no gelatin, since the latter is readily dissolved by trypsin after previous treatment in a salicylic solution with dilute acetic acid; it consists entirely of the neurokeratin of the retinal nervous epithelial apparatus with the purple attached to it. The colour of this mass is a deep orange-red, and changes in light into a colourless grey within a very short time, direct sunlight bringing about the result in less than a minute. Properly dried over sulphuric acid it is scarcely changeable in light, but quickly bleaches if again moistened. It is seen by this how capable of resistance the purple is. In fact this body, whose sensitiveness to light apparently exceeds all the hitherto known substances which are photo-chemically decomposable, resists attacks which overcome most of the constituents of living bodies as well as many other substances.

But more, it is well known that many things which resist trypsin digestion are decomposed by putrefaction. During the experiments instituted to determine whether the visual purple was dissolved by alkaline trypsin solutions (which gave a negative result), the various mixtures after five or six hours became, as usual, putrid; nevertheless the purple remained unchanged. I have kept the stinking mass of bacteria for more than a week, during which a blackish material was deposited in addition to the purple; the latter, however, remained distinctly recognizable, and when some of the disgusting mass was poured out on a plate, the orange-red was bleached by the light.

[1] Since salicylic acid by itself or in the form of one of its salts bleaches the purple when mixed with it, care must be taken to treat the retina with the above mixture carefully prepared beforehand.

With respect to procuring the materials in these experiments, for which I was obliged to use about thirty eyes, it may here be mentioned for the advantage of future investigators, that we found no difficulty in the slaughterhouses here in being allowed to place black bandages on the eyes of the oxen before other preparations for slaughter were made. The eyes were removed with as little exposure to light as possible, under an opaque cloth, whereupon they were carried in a deep covered vessel into the laboratory. With the assistance of a trustworthy servant, up to this time no ox eyes have come under my hand already bleached. In order to obtain the retina with the least loss, I recommend the following procedure. The eye is divided along the equator and the vitreous humour allowed to fall out with a rush from the posterior half; while the eye rests on the table the optic papilla is pressed from within with a suitable cork-borer, whereby a circular incision is made just round the entrance of the optic nerve, and the retina itself can then be easily drawn away under chloride of sodium of 0·5 per cent. with a pair of very delicate hooked forceps. In doing this, one must begin at the edge of a portion lying in the tapetum, and must guard against tearing it, for if the membrane once gets into disorder it slips about over the forceps, and the rods, rubbed off, flow down as a thick mass. An attempt to obtain the rods by shaking the retina in a thin salt solution failed on account of the impossibility of filtering the mass, which moreover did not settle down clear when allowed to stand.

I have already said that the retinas of frogs and rabbits are to be preferred to those of oxen, when pure visual purple is required. In the rabbit, the blood-vessels are restricted, as is known, to streaks of medullated nerve-fibres, which curve away on both sides of the papilla; when the retina is being drawn away, after the separation of the optic nerve by means of a small cork-borer, both those streaks must be cut away. The retina, however, tears so easily that it must always remain a most critical business to remove it with tolerable success. In the case of the frog, after much loss of time and unnecessary trouble with so small an object, which must be the excuse for the minuteness of the following account, I have learnt to remove the eye and retina in the following manner. The animal is decapitated low enough down, the skin of the neck taken hold of with a cloth and drawn forwards over the nose, the skull divided transversely just behind the eyes, the upper jaw cut away on either side and the small remains of the head rather removed from the eyes than the eyes from it. I then take the eye between my fingers, remove the muscles, and with a flat curved thoroughly sharp pair of scissors cut away the optic nerve while I compress the eye in such a manner that the entrance of nerve bulges out somewhat backwards. In doing so one must take care to make the cut as cleanly as possible, the resistance of the sclerotic envelope being overcome at a single stroke, otherwise the eye will not possess that absolutely necessary very small hole without which the retina never can be drawn out quite smooth with one pull. Lastly, the bulb must be divided and the retina taken out. Retinas to which the pigment is attached may be thrown away, as it is seldom possible to separate by filtration the finely-divided black granules from the solution of the visual purple. It would appear that the delicate vascular network which lies on the anterior surface of the retina of the frog is removed in the manipulations just described, for I have never been able to detect any hæmoglobin in the solution of the purple thus obtained. In order to gain this latter I have been accustomed for every twenty frogs' retinas to use ½ or at most 1 c. c. of cholate solution of about 5 per cent. strength. Each retina is placed in this before a fresh one is prepared, and remains in it for 24 hours. A very small test-tube containing scarcely 1·5 c. c. served as a vessel, in which the retina is allowed to sink slowly down without shaking. According to all appearance the purple dissolved in the bile possesses very slight diffusibility, for even after 24 hours there is seen immediately above the retina a very intensely coloured zone, above which in turn the fluid is quite colourless. By slowly inclining the glass to and fro one tries to disperse the colour through the fluid, and this on the second day may be done repeatedly. Finally the fluid is filtered, the clear upper layers being first brought on the filter by means of a fine pipette, the retina being left till last, when all the clear part has gone through. It will take about 24 hours until the last drop is filtered; if the mass has been shaken, it very often does not filter at all. It need hardly be stated that a miniature funnel with a very small filter must be used. I have myself taken the trouble to prove that all colour may be taken away entirely from the residue on the filtering by washing with colourless bile; but I do not recommend this plan of increasing the purple by washing, because the slightly coloured wash-water is hardly worth collecting, while, on the other hand, the red saturated filter paper and the varnish-like homogeneous agglutinated retinas when removed from it can be employed for many important experiments.

The filtrated solution of the visual purple is perfectly clear and of a beautiful carmine red colour. At first I thought I perceived a somewhat blue fluorescence, but I have reason to suppose that in the first experiment in which I and others fancied we saw this, it was traces of the black epithelium pigment, whose appearance deceived us. In the light the solution became first orange, then yellow, finally colourless like water. The peculiar chamois colour which appears in the retina as it bleaches, often occurs in the purple solution but not so clearly. In direct sunlight the dissolved purple bleaches in a moment; in diffuse daylight with very varying rapidity, apparently dependent on the intensity of the light, being considerably longer during the afternoon, although our eyes at that time seemed to be quite as much, or even more affected by the light than in the morning or mid-day. This circumstance, which is well known to the photographer and not inexplicable, is also very perceptible in the bleaching of the purple while still in situ in the retina. Time and fitting material have up to this time failed me for going more fully into the chemical reactions of the visual purple[1]. Whoever considers the trouble of preparing the purple will not be surprised that I first of all employed it in another direction, and any one who remembers the disadvantages which the presence of bile acid in

[1] See Appendix, note H.

the solution offers to chemical experiments will not wonder that I remained content with the results which have been already given until I had succeeded in obtaining the purple dissolved in a more convenient medium. Let it at present suffice to say that the purple solution, concentrated on ground-glass plates at 40° C. or in the desiccator, dries to a beautiful purple-coloured varnish which in a perfectly dried condition passes even in direct sunlight extremely slowly into orange, and then remains unchanged by light until it has been again remoistened.

It was most important first of all to determine on the solution of the visual purple, the absorption of light of different wave-lengths, which up to this time had never been accomplished in the retinas themselves by means of the usual methods of spectral analysis either with transmitted or reflected light. I have repeatedly attempted to become acquainted with the absorption spectrum of the retina, by holding the glass plate on which it had been spread out before the slit of the spectrum apparatus; either the absorption was too weak or there were so many horizontal shadows and stripes in the image that nothing could be made out clearly. One can never fold or lay simple retinas over one another without getting such disturbances of the spectrum, and I have met with the same difficulty when I have brought a pile of frogs' retinas between the slit and the source of light. An experiment of leaving the mass to swell up in ammonia or trying to make it more homogeneous by allowing it to lie in glycerine produced no improvement.

The solution of the purple brought before the slit of the apparatus shewed in all the degrees of concentration prepared by me diffuse spectra only; the absorption begins in the yellow, being very weak in the yellow of the D line, increases to E, the rise being very rapid at the beginning of the green, and a further increase at the junction of the green-blue and blue, and decreases again towards the violet. The beginning, transition, and end are however so extraordinarily diffuse that it would not be possible to determine the curve more accurately without a special photometric method. From reasons hereafter to be discussed, peculiar importance was laid on the points, whether any absorption was already present at D, and whether the diminution in the violet was considerable. I think I may decide in the affirmative as regards both points :—for the first, because the retina held up before sodium light appears grey when it is purple-red, and when more faintly coloured shews grey stripes on a clear ground, if the rods have been brushed away in streaks; as a further reason may be adduced the indubitable weakening of the intensity of the bright D line in the sodium spectrum, which is perceived when the slit is for one half covered with a glass holding the visual purple, and on the other with one holding water. The beginning of the absorption in the yellow is also unmistakable when the continuous spectrum of a gas flame is used either with a weak light or with a narrow slit, and the same is seen by daylight suitably toned down. With respect to the violet, experiments in gaslight at first did not leave me in doubt. I here saw the absorption compared with that in the whole extent of the blue considerably less, but when I employed the violet end of the more convenient spectrum of daylight I was doubtful and thought I saw the contrary. The contradiction disappears when one reflects that the purple in daylight quickly fades into orange or yellow, and that was what I saw in the spectrum. If one makes haste and employs daylight of just sufficient intensity, one can no longer be in doubt that the visual purple solution allows at first much of the violet rays to pass through. When this is not the case, the eye without any further assistance can recognize the beginning of the change into yellow, and which corresponding to the spectroscopic image now shews, besides a shading of the violet, a marked clearing up from the yellow to the green-blue. This behaviour appeared to me not unimportant, because it renders difficult the easy and in many respects seductive assumption that the purple represents a mixture of two pre-existing colours, a red yellow and a blue violet. If this were so it would be intelligible why in the first moments of the illumination so much violet passes through. On this point the chemistry of the visual purple must decide.

Towards the end of the bleaching, it is only the blue and violet rays which are held back from the solution, which is continually becoming more and more brightly yellow; at the conclusion of the decomposition however they again become visible. The decolouration is then complete; the solution looks like water. As to what takes place in the ultra-violet rays, for reasons to be mentioned below, I wish to defer making any statement until I have made further investigations.

Although the absorption spectrum of the visual purple cannot be compared with the well-known spectra, rich in characteristic lines and bands, afforded by the many pigments and coloured solutions which have been so carefully studied, still a knowledge of it may furnish many points with reference to the relation of the purple to the different coloured bodies which are present in the eye or in the retina. Besides nothing justifies the propriety of its name more than the fact that it principally reflects or transmits red, orange and violet, for it is a mixture of violet and red which is called purple, and from which we produce purple. Lastly, the following will shew that the absorption stands in close relation to the chemical decomposition of the absorbents in coloured light.

From the beginning of these investigations I have placed before me the aim of making quite clear the behaviour of the visual purple in monochromatic light; and if after three months I have not been so successful as I could have wished, the sky must bear a portion of the blame, for an unprecedented continued clouding of the sun from the first days of January onwards led me to seize upon all imaginable artificial methods of compensating for the loss of the solar spectrum, although only imperfect results were to be expected from them. However, as the living eye seldom is exposed to monochromatic illumination, I shall also give these results below as useful for our vision.

Obviously the objective spectrum was the means of determining the absorptive power of visual purple in its natural position in the retina compared with that of its solution—a matter which appeared to me indispensable in order to demonstrate the identity of the extracted and the pre-existing substance. It was indeed chiefly by this means first shewn that hæmoglobin, for instance, is the natural colouring matter of the red corpuscles. I hoped that the dispersion in the retinal membrane which interfered with all experiments in which the retina was placed between the source of light and the prism, would not prove an obstacle in the case of the objective spectrum, a hope which was realized as soon as I placed the frog's retina laid on a slip of milky glass in the spectrum of a Drummond light. The little moist membrane spread out on its front surface and somewhat curled up, presented a convex surface to the light, and resembled a glistening drop when illuminated by a weak sodium flame, took the colour of the surface on which it rested when exposed to the red, appeared cloudy in the yellow and when moved from green to violet looked like a glittering black pin's head, while in the violet it seemed distinctly brighter and even as if itself of a bright violet sheen; in no part of this spectrum was any appreciable bleaching obtained within 25 minutes.

For experiments with the solar spectrum the following arrangements were used. The sunlight, which was reflected from the mirror through the slit, fell upon an achromatic Steinheil lens of sufficient aperture, which was placed at twice its focal length behind the slit. At half the focal length behind the lens an equilateral Steinheil flint-glass prism was placed in a position of minimum deviation. For the purpose of receiving the spectrum I used slips of milky glass, on to which I fastened the retinas in the plane of formation of the images of the slit. The retinas readily adhere to the glass, notwithstanding the vertical position of the support, if one carefully removes with blotting paper the fluids of the eye which have come out with the retina. Beneath the row of ten or twelve retinas placed close together side by side I fastened a strip of paper in order to indicate the position of Frauenhofer's lines, and the limits of the visible portion of the spectrum. With my arrangement the spectrum was 6 cm. long, so that the first retina lay half in the ultra-red, the last almost entirely in the ultra-violet. The same space could be well covered with two rabbit's retinas cut into strips and placed in continuation. When the height of the spectrum was 3 cm. one could combine with each experiment a second one, by allowing a small section of some chosen colour to pass through another slit above the strip of milky glass, and after its transmission through a suitably arranged lens refracting it by means of a second prism. I thus obtained, as was done by Helmholtz[1], a second short spectrum of one colour, whose ends yielded on each side a monochromatic light of great purity, which could be used for acting on the retina. The intensity is of course in this case considerably less than with the first spectrum.

With a width of the first slit ranging from 0·3 to 0·1 mm., which must be altered according to the varying intensity of the sunlight as recognized in the spectrum, the action of the monochromatic light sufficed for the experiments; the second slit scarcely admits of being taken less than 1·5 to 1 mm. wide. I was always careful with the first spectrum to place the strip which carried the retinas in such a position that the Frauenhofer lines could be seen distinctly and in large numbers on the slip of paper; they cannot readily be distinguished on the milky glass. The D line had before commencing the experiment been made conspicuous by the use of a sodium flame behind the slit, and its position carefully marked.

Since the retinas of frogs kept in the dark shew strong individual differences, one must not omit to satisfy oneself of the uniform colour of the whole series of retinas by a hasty glance in daylight before they are exposed to the action of the spectrum, in order that one may separate out those which are too red, or too pale, or too bluish-red, and replace them by others; it is also necessary in every experiment to place a retina on the strip entirely outside the spectrum, for subsequent comparison. In addition to this, one must take retinas retaining the least remains of epithelium and pigment, for these make the purple more persistent; since I must here confirm what was expressed as a conjecture in the preceding publication, namely, that it is essentially and entirely the retinal epithelium which regenerates the visual purple or protects that colour from destruction by light. Retinas which, as shewn by the condition of the emptied fundus and intact uvea, retain no trace of the choroid proper, but only the pig-

[1] Compare Poggendorff's *Ann.* Bd. 94 and *Physiol. Optik*, S. 264.

ment epithelium or its processes, behave in respect to their colour as resistant against light as those previously described, to which the choroid or pieces of it had been left attached. It therefore seems to me probable that one reason why the purple of cold-blooded animals is generally longer in bleaching than that of warm-blooded animals, is on account of the retinas of the former so often retaining functional products of the purpurogenous epithelium. The agents which are concerned in the purpurogenous function appear to be soluble or at least diffusable[1], since unmistakable red zones and lines are formed during the bleaching in the parts round the shreds of the epithelium, especially when such a retina is placed in the dark after bleaching. Perhaps one may also refer to this the curious fact, that in vertically arranged preparations the under surface is always the last to bleach, and that when a series of retinas are placed one above the other with their edges overlapping, the lowest one often bleaches many minutes later than the uppermost one, provided that the illumination does not produce a too rapid total bleaching. This does not arise from the breaking off and tearing away of entire rods, since it may be observed in cases where the surfaces of the rods which are turned towards the light are beginning to dry up. As a last precaution in starting conclusive experiments with the spectrum, it remains to be mentioned that the retinas must be kept in a uniform state of moisture. Drying has such a great influence on the time requisite for bleaching, especially in that stage when the passage from orange to yellow and white occurs, and this influence increases so much more quickly towards the red end of the spectrum, that this fact alone may lead to the greatest deception if it remains unnoticed. In order to avoid this, I frequently sprinkle over the preparation with a small spray machine, such as is used for freshening up flowers.

Of the experiments which were made on frog's retinas, the details of one of the best, carried out with all the necessary precautions by means of the narrowest slit and the purest sunlight, may now be advantageously recorded.

On the 11th of March, at 11 a.m., the sky being cloudless

[1] See Appendix, note D.

and of a deep blue colour (there was a frost), 10 uniformly purple retinas of Rana temporaria were spread out in a row touching each other, and placed in the spectrum in the following manner :

No. I. *a* (1st half), in the ultra-red.
„ I. *b* (2nd half), „ red.
„ II. *a* (1st half), „ orange.
„ II. *b* (2nd half), over D to the beginning of the green-yellow.
„ III. .... in the green-yellow and pure green into the middle between D and E.
„ IV. *a* (1st half), in the blue-green.
„ IV. *b* (2nd half), „ green-blue to F.
„ V. .... „ cyan } between F and G.
„ VI. .... „ indigo }
„ VII. .... „ violet.
„ VIII. *a* (1st half), „ end of the violet to H.
„ VIII. *b* (2nd half), „ beginning of the ultra-violet.
„ IX. X. .... „ ultra-violet.

The absorption begins in II. *b*, ends before VIII. *a*.

At 11.20 a.m., the slit was covered up and the row of retinas was examined by the light of a match. III. was already bright chamois colour ; II. *b*, IV., V., VI., VII., were bleached in decreasing series, that is VII. was still most strongly coloured ; I., VIII., IX., and X., when compared with the test retina, were not in the least bleached.

11.45 a.m., second examination as before, III., IV., V. were almost entirely bleached ; VI., VII. very pale, but somewhat more coloured than the preceding ones ; II. *b* was distinctly paler ; I., VIII., IX. and X., unchanged.

12.28 p.m., I., VIII. *b*, IX. and X. were still red ; I. *a* most intensely so ; I. *b* shewed perceptible diminution of the red colour, also VIII. *a*.

12.43 p.m., VIII. *a* was entirely bleached, the outer fourth of I. *b* toward II. was quite pale, its other portion being chamois coloured.

The experiment was concluded by drying the strip in the dark, in order to be able to examine it more accurately by daylight, with the least alteration arising from the bleaching being continued. The row of retinas then shewed the following appearance: I. was reddish-orange, only the edge turned towards II. being yellow; II., III., IV. and V. were very pale yellow; VI., VII. strikingly colourless, and quite white with a bluish tinge in the spots where traces of a diffuse black pigment existed; VIII. *a* was a rosy chamois colour; VIII. *b*, reddish chamois; IX. and X. similarly coloured, only more intensely. Even IX. and X. and I. can be readily distinguished in colour from that of the dried test retina, which still possessed the proper purple tint.

From the upper portion of the spectrum which stretched over the strip of milky glass, a portion of the blue-green a millimeter wide was conducted through a second slit on to a second combination lens-prism, and two frog's retinas were so placed at the second short purely blue-green spectrum in such a way that the half of each extended beyond its edge on the blue side. The exposure began at 11.5 a.m.; at 11.25 a.m. the halves within the spectrum had become decidedly of a reddish-orange colour, at 11.45 a.m. were distinctly paler but still orange, and at 12.30 p.m. were chamois colour.

After these and other experiments I feel able to affirm that the action of monochromatic light on visual purple takes place in the following manner:

(1) Monochromatic light decolourizes and bleaches visual purple as white light does, only considerably more slowly, corresponding to its lesser intensity.

(2) Of all kinds of monochromatic lights the following act with decreasing rapidity: greenish-yellow, yellowish-green, green, bluish-green, greenish-blue, cyanic blue, indigo blue, violet, later pure yellow and orange, still later ultra-violet and red. The extreme red and ultra-violet rays are not entirely without action, but the commencement of the ultra-violet is more active than that of the visible red.

(3) The transitional stages of visual purple as it passes to white, namely the products of bleaching,

orange, chamois, and pale yellow, are least resistant to monochromatic light in indigo and violet, are more so at the beginning of ultra-violet than in the range from cyanic blue to orange, and are most resistant in pure red.

The effect of spectral illumination might perhaps be more striking on the retina of the rabbit than on that of the frog. I placed accordingly in the spectrum immediately after the above-recorded experiments the two retinas of a coloured rabbit which had been removed in salt solution, and cut into strips, taking care to place the more intensely-coloured narrow purple band which they contain as uniformly as possible in one line. The width of the aperture remained 0·1 mm. After six minutes (at 12.56 p.m.) the part from D to E was already bleached, at 1 p.m. it was bleached up to G, at 1.30 p.m. I could still recognize a purple colouration in the violet and pure red portions, somewhat more intense in the violet, here however the membrane happened to be thicker than elsewhere. At 1.57 p.m., bleaching was perceptible in the red and violet and in the beginning of the ultra-violet. When brought now into daylight the latter portion appeared chamois-coloured, the former reddish; even in this case one could recognize as far as the commencement of the indigo a certainly palish-yellow colouration which contrasted distinctly with the colourless portion corresponding to the indigo and beginning of the violet.

The most striking result of these spectrum observations (and the one most discordant with my first observations, see p. 6, carried out by means of absorption colours, and not by monochromatic solar rays) is the most welcome fact that precisely those rays which most affect our eyes and therefore appear to be the most intense, namely, the greenish-yellow, are those by which the visual purple itself is the most changed. Having regard to the significance of this fact, I have not omitted to obtain in the manner above described a second purified partial spectrum of this part of the spectrum; and here undoubtedly the most intense action was visible at that portion of a frog's retina which was exposed to the end the least green.

The action was here so much greater than in the previously described blue-green spectrum, that it could be recognized within 16 minutes, and in 54 minutes the purple had become changed into the palest yellow. The experiment was conducted on the 10th of March, between 12.30 and 1.30 p.m., the sky, although cloudless, not being entirely free from haze, somewhat whitish-blue in fact, and the less intensity of the sunlight on this day, compared with that of the following day (see above), manifested itself even in the first spectrum in the much greater length of time required for the total bleaching.

Slight as appears to be the influence especially of the red, but also of the violet and ultra-violet rays, it is nevertheless certainly not entirely absent, and is probably sufficient to explain the fact that our sense of light can be excited by these colours. That portions of the blue and violet, although they are a long time in bleaching, in the end do so all the more completely, deserves special notice. It would, I think, be unprofitable if I were to attempt to carry out any further deductions from the facts which have just been established; these indeed will readily suggest themselves to every one. I rather turn to the less thankful but more necessary task of attempting to fill up the gaps in our knowledge of the facts in the almost boundless region thus opened out to us. Want of sunlight and of the necessary refractive quartz apparatus, has hitherto prevented my taking properly in hand any decisive experiments upon the ultra-violet rays, desirable as these would be in reference to the observations of Helmholtz and Setschenow on the fluorescence of the retina, and especially to the fact proved by Helmholtz, that the eye can directly perceive the most refrangible solar rays. In company with Mr A. Ewald, who has assisted me in the foregoing investigations in the most active and generous manner, I hope to be able soon to communicate further results, on this as well as on other points in the doctrine of visual purple[1]. Meanwhile the foregoing experiments may suffice to shew that in the visual purple there exist qualities which one would expect to find in a body serving as the means of producing vision by chemical stimulation.

[1] See Appendix, note B.

Concerning our subsequent observations I will only here make the preliminary statement, that as the year drew on and the sunlight became more intense, we found the time of monochromatic action reduced to about a third of that previously given, and that the bleaching scale previously determined holds good also for spectra in which the relative width of the individual colours was quite different from that which was described above.

I come now to the experiments on impure coloured light. As soon as the spectral yellow on both sides of the line D proved to be active although slow in its operation, I said to myself that the sodium flame could not be altogether without effect, although throughout my long use of it I never had occasion to complain of it, and the photographers most distinctly prefer it to candle-light. Hour after hour and day after day have I worked with visual purple and retinas in the sodium chamber without experiencing any drawback; and yet it is possible to bleach purple by means of this light alone. One has only to bring the retina as near to the flame as the radiation of heat will permit (this is best done by means of a suitably inclined mirror or sheet of paper) in order to find it bleached to a pale yellow colour. Care must be taken by means of a chimney having a lateral aperture to prevent the rays from the red-hot platinum wire and sodium nodule falling directly on the retina, for it makes generally more than thirty minutes difference when the rays which proceed from these objects are joined to the pure sodium rays. It is impossible to avoid other rays of various refrangibility, which, as is well known, are not wanting in the sodium flame. Hence these positive results, though they are of use inasmuch as they remove the contradiction arising out of my previous negative ones, will not enable us to state as certain that it is precisely the yellow rays in the sodium flame which are the cause of its being active, probable as this may seem on account of their great luminosity. I need hardly mention that retinas exposed for two hours to the blue flame of a Bunsen burner, in the same way as to the sodium flame, shewed no change of colour.

Thallium and indium when vaporised in a Bunsen burner, afford the only means of obtaining real artificial monochromatic light; but I have tried in vain to produce any obvious change in retinas by means of these beautiful green and blue flames. The light is not intense enough for the purpose, and for very obvious reasons cannot be kept up for hours at a time. I made an attempt to burn thallium in oxygen, with the result that this remarkable metal completely fused with the iron spoon in which it was placed. It can be no more burnt in oxygen than can zinc.

My first experiments upon the influence of absorption colours upon visual purple gave rise to the suspicion that mixed light bleaches the retina the more quickly, the larger the number of the more refrangible rays which it contains. This held true for a long series of coloured but not absolutely monochromatic lights; thus the frog's retina bleaches extraordinarily rapidly in the bright blue flame which runs down a cylinder filled with nitric oxide containing a few drops of bi-sulphide of carbon; one has only to make the retina follow the flame down each cylinder through three or four cylinders in succession in order to make the purple completely disappear. The same effect is produced by burning bi-sulphide of carbon in oxygen. Both flames are well known for their extraordinarily rapid action on a mixture of chlorine and hydrogen and on most photographic preparations. When nitrous oxide is burnt after being conducted over cotton wool impregnated with bi-sulphide of carbon, the flame thus obtained, which has a blue core surrounded by a yellow shell, is far less active.

A retina placed before the narrowest (1 mm. diameter) part of a Geissler nitrogen tube, through which I sent the sparks of a small induction machine worked by six Bunsen chromic acid elements, bleached in twenty minutes to a bright chamois, that is to say, in a time which is short compared with the slight intensity of the visual sensation produced by this light, and which gives rise to the idea that the effect is largely due to the intense ultra-violet rays which are present in it in considerable quantity. With a fluoride of silicon tube, giving a beautiful blue light in which the red rays were very weak, and the blue by far the most intense, and which contained very little violet and so little ultra-violet that it gave rise to no distinct fluorescence in quinine, I could not succeed in bleaching either retinas or solutions of visual purple. Before the light of a fluoride of boron tube, on the other hand, the retina bleaches in a very short time, namely in ten minutes, to a pale violet, and in twenty minutes becomes quite colourless. In this light, it is true, all rays with the exception of those of the blue are present in considerable quantity, nevertheless the violet and ultra-violet rays seem to be the most intense, and to give rise to the whitish lavender colour which it possesses. When the light was allowed to pass through a layer of ammoniacal cupric oxide increased by means of a Hermann's Hæmoscope, until all the red end of the spectrum, including the green-blue, was absorbed, the retina exposed to it shewed no sign of bleaching although a piece of quinine paper held in it shewed most distinct fluorescence. The experiment was also negative when the copper solution was held before the nitrogen tube, but this was probably due to the unavoidable loss of intensity undergone by the rays as they passed through it. Other experiments on coloured light, obtained by means of absorptive solutions, I have made, but these probably will not be very instructive to those who live under a more favourable sun than we do. So far my only means of continuing my researches was to expose eyes and retinas in coloured light for a longer time than I had hitherto done, and I therefore spared no trouble to procure thoroughly transparent colours. Coloured glasses could not be depended on, owing to the small choice they offered and to the difficulty of obtaining intermediate tints. It was absolutely impossible to obtain in this way a violet free from blue, and I found that the so-called violet glass might much more justly be called purple, for it let through a considerable quantity of red. Red, green and blue-violet could however be obtained in sufficient purity in the following manner: red by a mixture of carminic acid[1] and picric acid (not the so-called picro-carmine), blue-violet by a solution of ammoniacal cupric oxide,

[1] Compare Rollett (*Untersuch. a. d. Inst. zu Graz.* Hft. 2, p. 158).

green by cupric sulphate and picric acid. As a source of light I used at first not daylight, which varied to a most extraordinary degree, but the gas flame of an Argand burner, which could be kept at a fairly constant brightness. This was enclosed in a lantern of blackened metal constructed with radially disposed chambers, in which were placed the vessels containing the respective solutions. Very useful for this purpose were the angular vessels with the square bases of 10 cm. sq. which are used in houses in the ordinary batteries for electric bells. The concentration of the solution was graduated by directing the spectroscope against the brightest part of the flame and then adding material until the spectrum as seen through the solution was narrowed to the desired limits. From red to orange there was no difficulty; in the case of green and blue, at the edges of which sources of error make their appearance, such a concentration was chosen that in the case of green no yellow was visible on the one side of the spectrum, the edge appearing reddish, and no blue on the other side, the edge appearing blue-green; in the case of the blue the concentration was such that no green was seen on one side of the spectrum (the edge here having often the false appearance of being red), while on the other side the somewhat weakened violet could just be discerned. In the chamber which was intended to give green light, the lantern of course contained two vessels placed one behind the other, since it would not do to mix the cupric sulphate with the picric acid; on the outer surface all light was cut off from the vessels except for a slit of about 6 cm. broad and 10 cm. high, before which were placed the retinas or small holders to fix a living animal in position. Isolated moist retinas behaved with this method very much in the same way as was related in my first communication; except that I obtained a more rapid action, since the new arrangement with its more constant source of light permitted one to work with a less powerful absorption than was the case when I had to guard against the enormous variations in the intensity of daylight by always employing solutions of great concentration. I strove moreover to quicken the effects by washing the retinas in weak saline solution, by most carefully removing all portions of epithelium, and by throwing the light on the object with a properly inclined mirror in order that it might act as powerfully as possible. I thus found that in a blue, which seemed to my eye so deep that I was unable to manipulate in it much better than in the dark, the visual purple bleached in two or three hours, while in the green, which to my eye appeared very luminous, it took from three to four hours. In the red, which produced a most brilliant light, the action first became distinct after the lapse of 16 hours, and it was not till 24 hours that all traces of colour vanished from the retina. An owl's retina with highly-developed purple colour required 72 hours before it became perfectly colourless, and shewed, even after 48 hours, spots not only of yellowish-red and brick-red, but even of real bluish-purple. It will of course be understood that this last preparation, as well as all the others, was carefully protected from drying during the whole time of the long experiment. Several frogs' retinas were, during the experiment, cursorily examined in daylight, and some which happened to be placed under cover-slips were examined with the microscope; and these enabled me to state that in the blue light the stage of orange and yellow colouration was that which was most rapidly passed through. Bearing in mind Boll's already mentioned interesting discovery of the green rods and his remarkable statements about their increase in the eyes of living frogs, after these had been kept exposed to coloured, that is to green light, attention was especially directed to these as well as to the colourless grey opaque rods, which have been already described by me. As far as I could see, the matter was altogether inconstant; grass-green rods were present even in considerable numbers in the preparation which had been exposed to the three kinds of light. The same was the case also with the grey rods; and where the retina before all exposure to party-coloured light was rapidly examined in darkened daylight, and brought before the lantern still thoroughly well coloured, I have not been able to notice any change produced by the coloured light in this direction, certainly no increase of the green rods at the expense of the red or grey ones. I have however observed that the true grass-green rods keep their colour longest in green light, are less preserved in the blue and least of all in the red. A retina taken direct from a dark frog, and therefore already provided with grass-green rods, is on this account in the most suitable condition for demonstrating this wonderful variety, when it has been kept so long in the green light that all the red rods have become bleached. Under what circumstances the green rods arise I shall discuss later on.

In passing on now to the behaviour of the purple in the living eye, I must first of all recall the regeneration of the photographic bacillary layer by means of the retinal epithelium which I have previously described, since without this it will be impossible to understand what I have to say. Boll[1] says, "The proper colour of the retina is being continually destroyed during life by the light which falls into the eye. Diffuse daylight makes the purple colour of the retina pale. Longer action of direct sunlight (blinding) completely decolourizes the retina. In the dark the intense purple colour is speedily re-established." All this refers to the behaviour of the purple during life, and not to the sensitiveness to light (which it may be added Boll did not notice), nor to the behaviour of the retina removed from the eye together with the epithelium, which, as we have seen, is modified by the processes of regeneration taking place. Considering the brevity of the above-cited statement which deals with the most important processes taking place during life, we ought to give it the widest and most favourable interpretation; we may therefore suppose that the author, in dwelling upon the regeneration in the dark, having treated both eyes of the same frog with an equal and sufficient amount of illumination until the bleaching might be supposed to have taken place, extirpated one eye, taking care that during the preparation of the retina no subsequent bleaching could occur, then brought the frog into the dark and after some time determined the return of the red colour in the other eye. As far as I can see, there is still another but less trustworthy method, namely, to carry out the experiment on several frogs, and to examine the one set immediately, the other not until they have been kept some time in the dark. If these are the experiments on which Boll's statements rest, they may be confirmed by any one who

1 *Bericht. d. Berl. Acad.*, 26 Nov. 1877.

cares to repeat them, paying attention meanwhile to the following facts.

Even the extirpated uninjured frog's eye needs a large amount of light in order to manifest even traces of change in its visual purple, much more so is the eye still in connection with its owner, that is, *intra vitam.* Without direct sunlight, I have never succeeded in a room in obtaining frogs' retinas free from visual purple, not even when I allowed the animals to leap about before the window the whole day long, whether under a glass or uncovered and only secured by a long thread. Only when I treated the animal in the same way in the open air (in January and February) was I successful; and even then not always, as is intelligible when it is remembered that many frogs know how to protect themselves against an injurious light by drawing in their eyes and closing their nictitating membrane. Against direct sunlight they are accustomed, strange to say, to make very little use of this protection. When one considers the tremendous difference of intensity between the light of the open air and that of a so-called fully lighted room, one would expect no other change in living eyes, provided that they possess no internal means of protection against bleaching, than that which is found actually to occur: in a room nothing but direct sunlight will produce a complete bleaching. I recommend therefore that such experiments as these should be conducted in the open air, placing the frogs under glass, on a white support, early in the morning, and examining them about noon. The retina will then in many cases be found perfectly colourless, without even a trace of yellow, in other cases it will be not merely reddish, but a bright purple. It will be remembered that in p. 9 it was stated that a removed and bleached retina regained its purple after being allowed to remain in contact for a few minutes with the choroidal epithelium which in the living eye forms its natural support. From this it might be supposed that frogs deprived of their visual purple would regain it after even a very short stay in the dark. It must be remembered however that visual purple is, during life, so exceedingly durable over the whole retina, that the long and intense exposure which is necessary to cause its disappearance may possibly also interfere with the functions of the regenerative epithelium, and indeed a regeneration experiment never produces quite the full tone of redness as before, p. 9, and this the less so, the more intense the light employed. This certainly is due to changes which the epithelium substance has undergone, as may be shewn by the following experiment. The retina is removed carefully from the eye and exposed until it has become bleached. The fundus of the eye is now exposed to intense light for 20 to 30 minutes, the retina meanwhile being put back into the dark. If the retina is now carefully replaced upon its old support, the red colour will scarcely, or not at all, return, even though the rods be allowed to remain for hours in contact with the epithelium. This might be referred to decomposition (death) of the epithelium; as a matter of fact, however, it is not due to this, but to the action of the light, since an equally long exposure of the uncovered epithelium in the dark, scarcely at all affects its power of regeneration, as may be shewn by the positive result of an experiment the reverse of the above in which the retina only is exposed to daylight. The epithelial regenerator is therefore as well as the retina itself sensitive to light; from which it follows, that there is no need to hurry with the preparation in the dark, when it is desired to prove the colourlessness of the retina of a frog whose visual purple has been destroyed by sunlight. I know no experiment demonstrating the regeneration of the purple of the rods by means of the epithelial nest in which they lie so simple, certain, and independent of manual dexterity, as the following. A frog is placed for several hours in the sun, both eyes are then taken out in the dark, and one is examined in order to ascertain that the retina is perfectly colourless; this being the case, the other eye is kept for an hour or an hour and a half in the dark before it is examined; the retina of this will be found to be of a splendid purple colour. The regeneration begins in most cases in about 30 minutes, and is generally complete in about twice that time.

Having thus learnt the extraordinary intensity or correspondingly long duration necessary to bleach the frog's eye during life, I could hardly hope to produce changes by means of weak coloured lights. I made, however, several experiments in the open air with my coloured solutions, by placing these in the so-called crystallization dishes, the sides of which were blackened but the bottom left transparent. I chose, of course, those which were sufficiently deep for the purpose. Each of these I put on the top of a porcelain capsule in which the frog was placed, a ring of black velvet being placed round the lip of the capsule, between it and the dish, in order that no light should come in at the sides. The general results were as follows. The frogs which during the day had been placed beneath the blue glasses, the sky being very cloudy, shewed the palest purple. In the case of those placed beneath the green glasses (the colour being obtained by a mixture of soluble Berlin blue and picric acid), the purple was of a redder tinge; it was darkest of all under the red. This agrees very well both with my earlier results and especially with the later statements of Boll, made before the Accademia d. Lincei. In other cases when the sun happened for a while to have broken through the clouds, a very sensible decrease of the purple was seen even under the red glasses; sometimes indeed this amounted to a complete bleaching, but I cannot be sure that in these cases all the light which passed through the solutions, and so was able to act upon the retina, really consisted of red rays only. As to any distinct relation between the colour of the illumination and the prominence of grass-green transparent rods, no more was shewn by these experiments than by those previously mentioned as conducted on isolated retinas; that is to say, nothing definite came out. All that could be noticed was that the grey rods shewed all the more striking contrast colours the more intense the colouration of their neighbours; hence they inclined to a blue-green tint in proportion as the retina was brick-red, or assumed a pure grey-green tint in proportion as the colour of the rest of the rods maintained its true purple hue.

Since these experiments under the open sky gave such little result, I could hardly hope to be more successful with artificial light, which for other reasons was preferable; still it was possible with these to make up in time what was lacking in intensity. One might well suppose that the short, and, for the most part, very dull days of January and February might be replaced by a gas-lamp burning day and night, which, since frogs for the most part never go to sleep when it is warm, would give their eyes no rest. Many frogs, it is true, took a dislike to the lantern and turned their backs to it, and hid their heads, or when they were prevented from doing this, shut their eyes. In the case of some, however, which were not so obstinate, I succeeded very well in finding the purple changed after 20 or 30 hours. In the blue light this was carried on until there were exceedingly small remains of the purple, which, when exposed to daylight, changed into a very pale yellow before all colour finally disappeared. After 48 hours indeed, the retinas of some of the frogs were found to be totally bleached. In green light the loss of colour was very much less, and in the red none was seen at all.

That one cannot by the above-described method expect to produce any very considerable change in the retina, will appear evident when we consider that the frogs when they simply stare, so to speak, through the solutions fail to do exactly that thing which is most likely to affect their retinas. In order that the greatest effect may be produced, they ought to be continually looking about, so that they may expose all parts of their retinas to the more powerful rays which come from the image of the flame itself, and not simply to the surrounding diffused light; and the movements of their eyes ought to be so rapid and carried out in such a way, that the parts of the retina exposed to the more feeble light should have no time to regain their purple before they are again exposed to the stronger and more direct rays. After some positive results in the midst of a very large number of negative ones, I was about to give up the whole arrangement had I not accidentally made the following observation. One of the frogs placed in the blue light had kept his eye steadily fixed on the flame, and I found in the retina, although it had been exposed to the light for only 14 hours, a most beautiful image of the gaslight standing out perfectly colourless on the deep red ground of the bacillary mosaic. The retina came out of the eye wonderfully smooth, without any merit due to me, and lay spread out completely flat on the cover-glass, every rod standing upright with its pigment-free end uppermost. These circumstances serve to render unusually distinct a clear sharply outlined centrally disposed colourless spot, such as I found in it; and when I examined this with a low power of the microscope, it was impossible not to recognize the image of the flame with its two tongue-shaped points. This suggested a simple means of obtaining at pleasure photographs on the retina. It is only necessary to render the frog motionless with urari, and having cut away the nictitating membrane, and having caused the eyes to project somewhat, by stuffing a ball of paper into the mouth, to expose the animal for about two hours before a flame. The best distance is about 35 or 40 cm. I can only recommend the experiment, however, to those who have plenty of time to devote to it, for even the most practised hand will not be able to say beforehand whether he will succeed in placing the removed retina in such a way on the cover-slip, as to render distinctly visible any image of the flame which may be present. In the various experiments I made with a view of finding out the most favourable focal distance, I was however repeatedly successful.

Without any doubt there can be no better proof given for the hypothesis that light acts upon the retina in the eye as in the *camera* it does upon the photographic plate, than the image of the flame which I have just mentioned. I became aware of this in the frog's eye, where it was accidentally discovered. I had for a long time endeavoured to obtain it in the eyes of the larger mammals; with what results my two last communications in the *Centralblatt f. d. Med. W.* (1877) Nr. 3 and 4, will shew, and when I made the definite statement which forms the concluding paragraph of the first part of this work (see p. 12), I had already succeeded in obtaining the first optogram, which with all its imperfection sufficed to make optography credible, and to make one think that the fixation of the image in the eye was practicable.

Perhaps I may here be permitted to disclaim any share on my part in the popular interpretation which has been given to my discovery. Far be it from me to rob of the prize of a first discovery those imaginative persons, on this or the other side of the ocean, who have seen in the eye of a murdered person the image of the murderer. Indeed it is no pleasant thing to find a serious study considered as a fit companion for such ideas. There are many things which I should like to say on this point, which I will rather leave unsaid, and simply express the wish, that my readers will expect from me no corroboration of the various popular accounts to which my name has been in a most unusual manner attached.

After I had discovered that the changeability of the purple of living and dead retinas was dependent only on light, I said to myself that it must be possible in the extirpated eye to discover after the removal of the object the well-known images which the dioptric apparatus throws upon the fundus. The method which I adopted for that purpose may not have been the best and the shortest, so that the detailed account of my experiments, perhaps, will rather shew how one should not proceed with work of this kind, than indicate the proper steps for arriving at the desired end. I imagine, however, it will not be well to omit them in the following account, since in similar instances many things have come to light, which even an experienced investigator perhaps may not have noticed, because they lay in a direction which, though offering great interest, was not in his immediate line of work.

In face of the wonderfully complete knowledge concerning the dioptrics of the eye, which, thanks to the conspicuous ability of the inquirers of present and past times, we now possess, I have little more to say concerning the small inverted retinal image, than that I have always attempted to bring it into view as sharply as possible before I attempted to fix it on the retina. The eyes of albino rabbits answer best for this, as in them the light shines beautifully through the sclerotic, and it is well known these are frequently used for obtaining the image of distant objects. Coloured or even black rabbits are almost equally useful, although this might scarcely be believed, from the appearance of the dark back-ground of the eye. Nevertheless, the pigment of the retina may be very dark, and yet the whole eye may be fairly transparent, provided the choroid is only moderately coloured, and the sclerotic is thin enough, (as is the case with rabbits), since the rods reach through the pigment right to the colourless bases of the epithelial cells. In rabbits' eyes the transparency may be explained, when the uvea is only slightly coloured, by the retinal pigment existing in a very thin layer; but there are eyes, as, for example, among birds, which, in spite of a much greater development of the pigment, allow a very good image to be seen through the thin sclerotic. In such cases the reason of the transparency is to be sought for in the fact, that on the one hand the choroid is only slightly pigmented, while on the other the retina, after the fashion of a brush made of threads of glass, is really pressed through the deep black ground of pigment until it reaches the transparent layer immediately below. Retinas which come out covered with the pigment-layer, and when looked at sideways appear like black satin, will, when the light is allowed to fall perpendicularly through them, often create great surprise in the minds of those who attempt in this way to recognize that projection of the rods through the posterior surface of the epithelium, to which, as far as I know, attention was first called by M. Schultze. The matter seems to me here worth attending to, on account of its bearings on the reflection of those rays of light whose course is parallel to the long axis of the rods, and in reference to the function of the refractive lens-like bodies, each of which, lying as it does in front of an outer limb, probably forms its partial image at the posterior end of the rod. It is also of interest at the present moment, when a great deal of discussion is going on, as to whether the visual purple can be seen by the ophthalmoscope, from which question the visibility of the other pigments must not be excluded.

I have given up distinct examination of the image in other eyes than those of rabbits and birds, since even with the most careful avoidance of extraneous light, no image whatever is seen from behind; in dogs, calves, sheep, swine, and oxen, this is entirely due to the thickness of the sclerotic, not to the retina or choroid pigment, for the image may be seen shining extremely well through the latter in the pig's eye when the sclerotic has been taken away.

It is well known that in the living eye of man, in the case of blonde individuals, the image can often be seen on the outside of the sclerotic. I do not think however that this is due to the slighter development of pigment; on the contrary, I suspect that in the majority of cases, the reason lies in the tenuity of the sclerotic, through which indeed the pigment may be seen shining with a bluish tinge. Lastly, in the frog it is only at the optic entrance that the light shines through, and in the eyes of these animals it is not the sclerotic, which as is well known is very transparent, or the retinal epithelium which stops the light, but exclusively the strongly pigmented choroid.

Distant objects are seen so distinctly on the back of the rabbit's eye, that if one could get equally sharp optograms one would have every reason to be content. With objects near at hand, such as rows of windows on house-sides, there is this disadvantage—that they appear too small; more satisfactory results are gained with the images of bright window surfaces crossed with dark sashes, such as are formed in an eye placed some few feet off them. Images obtained by means of the small figured discs, described a little way back, remain quite distinct when these are brought as near to the eye as 30 or even 19 cm. I have attempted to determine this last focal distance in many ways, by means of the sodium flame for instance, when I was working in the dark. The individual features of this came out very nicely, but I have not succeeded in obtaining as great perfection as I could wish. I strove to avoid all traction and changes of external pressure, by leaving the eye in its place in the bisected head, and getting at it by taking away the brain, portions of skull and contents of the orbit. I failed, however, with every linear object which I used, to succeed in what I particularly desired to do, namely, to determine the focal distance of the eye by means of Scheiner's experiment. The use of a photograph gave me the most useful results, the glass negative of a head larger than life-size being held before the sky. The outlines and the points of correspondence in the retinal image may, as a rule, be very well seen when the eye is placed 25 cm. behind the object. It seemed useless to attempt anything more exact, since the eye is subject after death to progressive internal changes, very difficult to follow, which interfere with the distinctness of the image. This depends chiefly on the well-known opacity which makes its appearance in the refractive media, which is all the more inconvenient, inasmuch as it diminishes the intensity of the incident rays, particularly those which are optographically most active; and, as Helmholtz states, prevents the investigation of the dead organ by means of the ophthalmoscope, and so renders this instrument useless for determining the refractive index of various parts of the eye. Other hindrances are caused by changes in the iris, in the accommodation mechanism, and in the intra-ocular pressure. The iris immediately after death is so narrow that the pupil is dilated to the utmost. Very soon, however, this becomes strongly constricted again, and then later returns to a medium condition. Accompanying changes in the accommodation muscles also probably occur, quite apart from variations in the internal pressure. These, of course, from their anatomical relations, affect the form and position of the lens. Lastly, there are changes taking place in the shape of the whole bulb including the cornea. In fact, there occur at the same time a large number of processes, all of which must so much influence the image, that each eye must be tried by itself. As to which is the best method of proceeding, about that I have no doubt. One must work with urarised and atropinised rabbits, the refraction of whose eye must be determined before the optographical experiment by means of the ophthalmoscope. I have not, however, been able to spare time to master the necessary manipulation, which, as is well known, requires much practice.

A glance into the opened eye of an animal which is not an albino teaches one that after the vitreous humour has been removed, or the concave fundus inverted by drawing the sclerotic over a convex surface, the visual purple cannot be seen with sufficient distinctness to permit a colourless marking on it to be recognized even if it existed. By long experience and practice I have indeed learnt to tell whether the retina as it lies on the pigment will appear red or colourless when it is removed, and I can always recognize the visual purple in a frog's eye by a reddish-brown shading of the black ground; but I could not undertake to distinguish the fainter from the deeper portions. The visual purple is most easily recognized *in situ*, when it lies over the slightly-coloured opalescent, or rather silver-glistening, tapetum of the dog's eye. It is not quite so well seen on the greenish-blue and satiny tapetum of herbivora. In neither of these cases, however, is it so distinct that one would be able to recognize an optogram on it. In the case of the rabbit's eye, which possesses no tapetum, the retina must be wholly removed before the image can be seen. This is a critical operation, and not unfrequently the tearing of the delicate membrane brings a laborious experiment to an unhappy end. Unable to overcome these difficulties, I turned to albino rabbits, in which, as I already knew, the purple-red colour of the retina could be recognized in its natural position.

The rabbit's retina, as far as its colour is concerned, is divided in a peculiar manner into two portions, into a pale upper and a dark-red lower portion. The optic nerve in these animals ascends after its entrance into the orbit high up on the eye, being closely applied for a considerable distance to the outer hinder surface of the bulb; after piercing the bulb it takes a direction at right angles to its former course, and diverging on both sides gives rise to the well-known white streak of medullated nerve-fibres, which appears to form the only tract of the retina bearing blood-vessels. The retina is thus bisected by this white streak into an upper smaller and a lower larger division. Under the white streak is seen a second, of a dark purple-red, of about the same breadth. This is sharply defined from the upper lighter-coloured division of the retina, but shaded off gradually into the lower redder division, at about the level of the retinal horizon, and stretches on either side a long way towards the front. The medullated fibres accordingly lie entirely in that portion of the retina which is poor in purple;

and hence the "blind spot" of the rabbit, which must be thought of as a dark broad band, lies in the lower part of the field of vision, which we may suppose is but seldom used. The redness of this red streak is not occasioned by the blood-vessels which lie underneath it, and may always be demonstrated in removed rabbits' retinas. Along it the purple is far more intense than elsewhere, and it disappears, passing meanwhile into yellow, so very much later than in other parts of the retina, that it remains for a long time still visible in preparations which have been otherwise largely bleached. Finally, of course, it also becomes indistinguishable. I have not investigated the point as to what arrangement of the retinal elements gives rise to this streak, and can only throw out the suggestion, that the slight increase of thickening which is observable in it depends on the unusual length of the rods. Traces of something similar may also be seen in the eyes of many other animals. In the redder lower division of the retina of a leucotic rabbit, the purple is seen to be uniformly spread over its whole surface, and when the animal is bled to death, the remains of blood in the choroidal vessels do not usually disturb the beautiful appearance. When the posterior half of the bulb is turned over the front half—the lens, which serves as an excellent support, being retained in position—the whole retina looks exactly like a beautiful rose-leaf. If it be then brought from the sodium light into daylight, the disappearance of the colour may be watched until nothing remains but that of the blood, which, as far as the veins are visible, is always arranged in streaks. With regard to the regeneration and relative resistance to light of the purple in such preparations, I must repeat, with greater distinctness, what I have already said in pages 10, 11. A flap of the retina removed from the colourless choroid, and placed by the side of the rest of the retina, with its anterior surface towards the light, undoubtedly bleaches more quickly than those parts which have been left *in situ*. The difference appears more striking when after about half a minute a piece of the latter is rapidly removed and looked at side by side with the former isolated flap on the same support of white porcelain. Much more striking and visible, even after four times the length of exposure, is the result when coloured rabbits' eyes are used; this I imagine arises from the fact, that not only the purple, but also the purpurogenous factors of the epithelium are protected by the pigment against the action of light. Perhaps I might here be allowed to suggest the hypothesis, that the black pigment serves as a protection, not so much for the rods as for the purpurogenous epithelium; for I do not see what harm can be done by the light passing through on to the sclerotic, which indeed is an unavoidable result of the arrangement of almost all eyes, and I understand still less of what use to the rods the laterally disposed sheaths of pigment can be, for, as Brücke proved, the inner cylindrical surfaces of the rods must reflect all the rays which do not fall parallel to their axes. If it be supposed that the pigment serves as a means of protecting the outer cylindrical envelopes of the rods from the light which falls between them, it is in this case unintelligible why the epithelial processes sometimes contain no pigment, and sometimes have it arranged in layers, separated by long intervals. One sees what a host of questions further investigations on the epithelium will have to deal with. Though it succeeds so easily in the frog, I have not yet in the case of the rabbit succeeded in restoring the colour to a bleached piece of retina by replacing it on the exposed epithelium kept in the dark. Without the frogs I therefore should not have been able to bring forward so satisfactory a proof. I believe, however, that one may with reason suppose that this activity of the epithelium is present in the opened eye of coloured rabbits for at least two minutes after death, though the energy is continually decreasing.

My first optographical experiment I conducted in the following manner: In the completely opaque wooden wall of my thoroughly blackened windowless dark chamber, which was placed behind another room, serving for the heliostat and for optical operations, I bored a hole, which I covered with a circular diaphragm of 5 mm. diameter. The optical chamber was closed by window-shutters, with the exception of a ground-glass pane, upon which the bright noonday light fell. In order to see how this bright pane, which was about 5·77 metres from the wooden wall just mentioned, came out as an image in the rabbit's eye, I first of all hung over it an intensely coloured chrome-yellow tissue paper, and arranged an eye in the following way. An albino rabbit, after being kept 15 min. in the dark, was decapitated; one eye was removed from the head under sodium light, somewhat cleared at its posterior surface, and fastened on to the edge of a cork by means of needles run through the remains of the conjunctiva. Thus prepared, the eye was placed in position in the dark chamber with the cornea pressing softly against the diaphragm. The image was visible on the sclerotic, on one side of the optic nerve, some length of which had been left attached to the eye, and so far beneath the point of entrance of the nerve into the bulb, that I was sure that it fell on the more deeply coloured division of the retina, and could readily mark its place in the appropriate quadrant. Thereupon the yellow curtain was removed from the pane, and the eye after five minutes' exposure was taken away, divided along the equator and examined in feeble gaslight. Since I could as yet recognize no image on it, I brought the preparation out into darkened daylight, and shewed it to several witnesses. There was evident on the retina a most distinct brighter diffused spot, the small dimension of which corresponded to those of the image previously seen by me, and the position of which made me already sure that it was the optogram. Every one of the witnesses recognized the spot as being in the same place. The eye was removed from its support, and for my own satisfaction I tried to find on the sclerotic from behind the previously observed position of the image. In this I was completely successful, thanks to the help given by the small remains of the ocular muscles, the position of which in reference to the position of the image had previously been observed. I then thrust a needle through from behind, and the exclamations of surprise from the bystanders told me at once that I had hit the right point, for the needle went straight through the pale spot. In a similar manner some thirty experiments were carried out, most of them with worse, scarcely any with better results, in spite of the most varied changes in the object, the focal distance, the time after death, the intensity of the light, and the duration of exposure. In no case did we succeed in obtaining a regular image: all we got was a mere spot or patch. In my notes, out of all this series of experiments, I only find one which would justify me in asserting the possibility of optography. This was the case of an eye in which, 45 minutes after death, an image of a magnesium flame behind red and yellow glasses was thrown upon the eye at a distance of 30 cm. After 15 sec. exposure to the uncovered flame, there was visible on the deep red ground of the retina a white patch, the form of which was so like that of the burning magnesium tape, and was accompanied moreover by two bright dots, which corresponded so well with the pieces of metal continuing to burn on the table after they had dropped off, that myself excepted every one else was free from doubt about it.

These first not altogether satisfactory results naturally led us to consider and experimentally to test all the possible obstacles which might interfere with the desired result; meanwhile I succeeded—accidentally, to tell the truth, and without the intervention of any human art—in obtaining the most beautiful optograms. I had not thought of the simplest method of all. I had not remembered that the retinal surface turned towards the vitreous humour, which alone I invariably looked at, is not the red surface, and that the anterior layers of the retina, becoming opaque in death, would naturally throw a veil over the real purple surfaces which would allow one to distinguish very well pale from coloured parts, but would not permit one to distinguish the outlines and individual features of the images. And when the eyes used were so fresh, and the operations conducted so rapidly, that the retina might still be considered sufficiently transparent to give good results, other difficulties interfered with the satisfactory observation of the images from the front—difficulties which arose from the creased condition of every fundus everted in the manner previously described, and from its moist and glittering surfaces. I have since indeed seen in eyes of oxen that the difficulties just mentioned are sufficient to render perfectly indistinct all the images occurring in the layer of rods.

Difficult as it is to avoid tearing the retina of a rabbit's eye in its fresh and unprepared condition, a very simple artifice enables one in the happiest and easiest manner to effect that removal and inversion of the retina which has become necessary for these optographic purposes. After trying a variety of substances which I knew did not injure the visual purple, and yet might be trusted as hardening reagents for the retina, I found a solution of 4 per cent. potash-alum the most suitable for giving the membrane its desired consistency. In using this substance I neither desired nor reached any other end; and I say this expressly in order to correct the opinion, which has been widely spread, that the alum is used as a kind of photographic developer of the image. The eye, opened and thoroughly freed from the vitreous humour, was, immediately after receiving the image, thrown into the alum solution and allowed to remain in it in the dark for 24 hours. My original method of inverting the eye and drawing the retina over the anterior half before laying it in the solution, I no longer recommend, for I have since learned the better plan of punching out the papilla from the inside. I therefore now recommend that all the more care should be taken to prevent the eye collapsing or becoming folded in the alum, for the solution does not make the retina so tough but that it readily breaks at any creases which have arisen during the hardening. After the action of the alum is completed the eye is placed under water upon a leaden support, the optic entrance punched out, and the membrane seized at a point above the optic disk, that is to say, in the more faintly red area on which the images seldom fall. If the operation is carried out without a mistake, the retina can often, with a slight jerk, be pulled out of the solid sclerotic cup like a delicate shell, without its collapsing or creasing at all. The inner surface now looks perfectly white and opaque, it is only the convex posterior surface which is red or rose-coloured, like a rose petal. I then bring under the water, upside down, a tiny porcelain capsule hardly bigger than half a rabbit's eye, slip under it a suitably bent strip of lead (by means of which I can afterwards raise it into a horizontal position) and allow the retina to sink slowly with its concave anterior surface upon the convex outside of the porcelain capsule. Sometimes it is possible to lift the membrane out of the water, with every part of it lying smooth on its little porcelain support. Still it will be as well in most cases to snip it a little at the edge (taking care of course to avoid the image, the position of which can be rapidly determined by the light of a match), and to let it float slowly upon its support until it becomes spread out, as free from folds as possible. I need hardly say that the optogram is best seen when the retina is allowed to swim without any change in its curvature. The depth of fluid in which the above operation is carried on should not be too great, for it is very remarkable to what an extent the purple colour is dissolved out by water—at least this is the case in alum preparations. In the ox's eye the matter is simple beyond all expectation, for in this, after the action of the alum, the retina comes out almost as easily as if one had to deal with a leathern sack. The method of exposing the retina of this animal, when fresh, in saline solution has been already described (p. 44). If it is desired to see optograms on it, it must be allowed to float. I prefer, however, the alum preparations to all others, because in them the red and the white are most sharply defined both in the moist and in the dry condition, whereas in the fresh ox-retina placed in saline solution the optograms look very much as if the colourless parts had been cut out in a pale red etching glass. When dried these images are more distinct, although not so good as the dried, and far inferior to the wet alum preparations.

The question of fixing the visual purple is of scientific interest only so far as the methods which effect it may throw light on the chemical characters of this body, or of technical value, inasmuch as it is very desirable that, in forming a judgment on the effects of the exposure of the retina to light, we should not be limited to the short period during which the light is actually falling on the eye. Every light that would enable us to see anything whatever of the retinal colour will, from the very beginning, give rise to changes in the retina, and these must of necessity interfere with the exactness of the observation, all the more so because they are not supposed to be taking place.

Here, again, it was an accident which led to the first approximation towards a successful issue. I had dried the retinas of oxen in the dark on strips of milk-white glass, in order to be provided with material for spectral experiments when the sun should shine again. In the case of frogs I had observed that retinas which were simply dried in the air could be completely bleached, especially in sunlight, although the action took place more slowly than usual. When at last, after some weeks, the wished-for sunlight arrived and remained constant for some time, the dried ox-retinas were placed in the spectrum and allowed to remain there a long time. To my surprise no diminution whatever of colour could be observed. I laid them in the open air in direct sunlight, nevertheless the beautiful orange colour remained indestructible. The glass slips were then laid in water until the dried retinas had become soft, and these while still moist were exposed during the next two days to the sun, which, however, only came out at intervals. The colour suffered somewhat perhaps in depth, but no real bleaching took place. This suggested a method of preserving optograms, by bringing the porcelain capsules with the retinas lying upon them into a sulphuric acid desiccator, and leaving them there in the dark. How long they should be kept in this condition I have not yet been able to determine exactly. It is desirable, however, that the time should be as long as possible, for the influence of the length of the dried condition is most remarkably great. Small retinas of frogs and rabbits of course dry very rapidly. In an ordinary desiccator, in 24 hours they are as completely dry as it is possible to make them by means of sulphuric acid, especially if, during the drying, they are lifted up from their porcelain supports. In a perfectly dry chamber I have found such retinas wholly resist exposure to sunshine even for hours, at least nothing could be seen but a certain diminution of the purely purple colour, so that an orange tint became more prominent. It is sufficient to bring them into ordinary moist air in order to render them fairly sensitive to light; they may even become completely bleached, just like those retinas which have been simply dried in ordinary air. When they have been softened with water they bleach at once. When, on the other hand, they are kept in the desiccator for several days, for instance a week or more, the power of bleaching upon being moistened diminishes more and more, so that one can speak of the visual purple in this condition in the same way as one does of so many of the colours employed in manufactures, and say that it is "fast." I have not yet had a sufficiently long experience to be able to say to what extent this durability may be carried. To myself and others, however, it is a matter of importance to have discovered a simple method, only needing patience, which renders possible the most leisurely observation of optograms. Any one who is unwilling to wait for the action of a long drying can make observations by means of the desiccator.

While still in the moist condition optograms may be made durable for about a two-days' exposure by laying the alum preparation in a weak solution of corrosive sublimate; in this the purple passes, even in the dark, into a very light yellow, which, particularly in frogs and oxen, less so in rabbits, resists the action of light to a markedly greater extent than the natural yellow, which in the normal action of light is the forerunner of the completely bleached condition. It is not pleasant, however, to see a beautiful purple or orange-red optogram assume such a washed-out appearance.

The changes in their behaviour towards light thus witnessed in fresh retinas, or in those which had been dried in the desiccator after treatment with alum, I have also observed, as the result of a long-continued drying, in isolated visual purple mixed with some amount of the retinal neurokeratin. A filter covered with this substance, after it had been dried for a considerable time, no longer bleached in the sun when moistened again; and it was evident in this case that the indifference was due not to the mere fact of the preparation being old, but that it had been kept dry, since undried preparations of visual purple mixed with keratin—which if kept in the dark under pure water remain unchanged for a week at a time without undergoing decomposition—bleach very well when exposed to light, however long they may have been thus kept.

Although I had thus attained my immediate object, I nevertheless undertook several experiments with a view of improving the optograms. In these I attempted to follow the steps of the photographers, who use the first traces of the products of photo-chemical decomposition in order by means of them to accumulate new precipitates on the lights of their pictures, either through the reduction of the materials in the bath, or through the fixation of precipitates already present. This however is a development, not a fixation, and is worth attempting to produce on the retina, on account of the interest attached to the question whether bleached or genuine visual purple possesses reducing properties. Gold, silver, and iron compounds proved in this respect of no use. The use of osmic acid brought to light the interesting fact, that the frog's retina can remain in a 1 p. c. solution of this substance for half-an-hour in the dark without destruction of the visual purple. The rods it is true become very dark, but their brown is distinctly a reddish-brown, which when exposed to light becomes markedly paler, and can be very easily distinguished from the colour assumed by a retina which had been bleached by exposure to light before it was submitted to the action of the osmic acid. Pyrogallic acid gave rise to some amount of browning of the alkaline membrane, but there was no destruction of the purple or of its sensitiveness to light. Permanganate of potash coloured the retina brown, the tint being of course less deep when the solution was mixed with 2 p. c. of acetic acid. In the mixture the purple remained constant in a wonderful way for several hours, bleaching as usual on exposure to light, with the intermediate stages of orange and chamois. My efforts in the inverse direction, namely, to find energetic reducing agents, which would decolourize the visual purple or prevent it bleaching on exposure to light, were equally unsuccessful. I made use of the mixture of ferrous sulphate or stannous chloride, with tartaric acid and an excess of ammonia, employed by Stokes for the reduction of hæmoglobin, but obtained no change of the visual purple and no return of colour in a retina bleached by light. I was equally unsuccessful with ammonium sulphide or hydric sulphide. After such a behaviour of the purple, no surprise could be felt on finding that the changes in it are quite independent of oxygen, that for instance it behaves, when exposed to light in a stream of pure carbonic acid, exactly the same as it does in air, in water, in blood serum.

In ordinary photography, fixing, as is well known, means, in the majority of cases, the removal of the excess of the undecomposed substances still sensitive to light, and in this sense in optography there can be no fixing, because the removal of the still sensitive purple in the optogram would simply amount to washing out the image. The dry method of fixing the optogram is something quite different from this, inasmuch as it consists in the transformation of the materials still sensitive to light into those which are insensitive, the substance itself and its colour remaining intact. There is also another difference, since the optogram is not a negative but a positive, if, as there is some reason for doing, the ground colour of an image marked out on the red be spoken of as the dark part. On the other hand, the optogram agrees with a direct negative photogram, inasmuch as the light parts are those which have undergone decomposition and are no longer sensitive, while the red parts correspond to those, the decomposition of which, unless it were arrested, would obliterate the picture. The choice of a method for fixing the image is determined however not by the last-named agreement, real as it is, but by the first-named difference; for the object we have in view is the preservation of the image, and consequently our efforts are directed towards a point which may be neglected in the photogram, but which must be secured in the optogram, namely, the bringing the material into a stable condition without robbing it of its colour.

The foregoing remarks have, I trust, sufficiently explained the methods necessary for optography, as far as the mechanical and chemical manipulations are concerned, to enable any one who so desires to obtain results for himself. I pass on accordingly to some of the experiments themselves, in order to afford proof of the assertions which I have made, and to keep more strictly to the optical and physiological questions.

The alum method which has been previously described, and which is so indispensable in dealing with rabbits' eyes, was in the first instance accidentally employed in an experiment, which I may well call the 'despair' experiment. After again and again failing to bring out any distinct image on the unhardened retina, and when I began to fear that I must have exaggerated the importance of the visual purple for the act of vision, I determined to conduct the experiment just in such a way as it would be conducted by any one who undertook the task for the first time, without any preconceived ideas. I fastened a living rabbit in such a position in the holder that the head was fixed immoveable with one eye directed against one of the many windows of the laboratory. The bulb was made perfectly fast by means of a thread passed through the conjunctiva, the lids were kept open by means of a spring holder, in which a piece of black paper with a hole about 4 mm. wide, serving as a diaphragm, was placed immediately before the pupil. The head thus prepared was covered for about ten minutes with a black cloth, this was then removed, and after two minutes the head was separated from the trunk, the eye being closely covered with the hand, the ball was extirpated and opened in the dark, and immediately placed in a 5 p. c. alum solution. On the third day afterwards I had the pleasure of recognizing in the removed retina the image of the window to which it had been exposed, its arched ends appearing as white silhouettes on a red ground, and between them several smaller clear fields. There was only wanting a distinct reproduction of the cross sashes. After this we proceeded to further experiments, a short account of which has been already given (*Centrlb.* l. c.), but may here be repeated with some slight additions.

The plan of optography in the living eye which I had formed before I came to make the experiment just recorded, was framed in accordance with the following considerations. Since normal vision is evidently only possible so long as a balance is constantly maintained between the bleaching of the visual purple of the rods on the one hand, and the purpurogenous activity of the retinal epithelium on the other, it is obvious that one can only expect to obtain permanent optograms when that balance is destroyed, either by the epithelium, in spite of its continued functional activity, being insufficient again to colour the rods, or in consequence of circumstances which prevent the epithelium from performing its functions.

This last cause might be expected to come into operation in the eye some minutes after death, but I failed to obtain optograms under those circumstances, for reasons which were at that time not clear to me. I accordingly fell back upon the assumption that the dead eye, and especially the anterior layers of the retina, became after death impervious to those rays which have the greatest chemical activity. This is probably the case, but however does not come into play until an hour or an hour and a half after death. This view of the post-mortem loss of transparency led me to suppose that the experiment must always be conducted on living eyes, but I feared occurrence of regeneration, which, as I well knew, even in the mammalian eye was sufficiently powerful to obliterate the image in the short period between the decapitation of the animal and the contact of the retina with the alum solution; this latter appearing to be the best means of rapidly killing the epithelium, while it did not affect the colour. The foregoing experiment was accordingly repeated on a curarised dog with artificial respiration, with the difference that it took place in a room with one window, and that I took care to saturate the eye with alum after the conclusion of the experiment.

For this purpose, I had previously connected the carotid of the same side with an injection apparatus, in order that I might drive a rapid stream of warm alum solution into the head and into the eye. The vessels of the neck, which upon the separation of the head allowed the injection material to flow back, were as quickly as possible clamped. One experiment only was carried out on this plan, because a repetition of it was meanwhile shewn to be unnecessary for my immediate purpose by further experiments on rabbits. It will however be worth while to repeat it when it is desired to study more fully the changes which occur in the equilibrium existing between the processes taking place in the rods on the one hand and those in the epithelium on the other.

Meanwhile the already mentioned image of the window was obtained, and thus all fears were rendered unnecessary, and the doubts which had arisen expelled. The condition of disturbed equilibrium between the function of the epithelium and the bleaching of the purple, which hitherto had been put forward as a suspicion only, must therefore be considered really to exist, and I imagine it will be thought not only not absurd, but even altogether natural. We must suppose it to occur in all cases where our vision-power is either diminished or annulled in consequence of exposure to light, and how easily that may occur is known to every one who is versed in after-images, and the fixation of sight. I might maintain that we cannot fix our sight, without winking, for 30 seconds on a large bright light without becoming incapable of seeing it, and I find nothing wonderful in the fact that the mammalian eye, after a minute's constant exposure to moderate light, is blinded in the spots where in the visual image the light fell on the retina, indeed is so far blinded that the purple either no longer exists there or is visibly decolourized, and has diminished so much, that a considerable time is necessary before such regeneration can take place as to render it again visible. In such cases we must suppose the existence of, and indeed on examination shall discover the presence of, an optogram that is an after-image in the proper sense of the word.

It may be urged that optography depends on changes in the rods only, not in the purple free cones, and therefore on processes taking place in a visual apparatus undoubtedly far less perfect than that of the cones, or that which exists in the region of distinct vision; and it might further be urged that the imperfection of our peripheral vision, in spite of the existence of the scattered cones which exist in the anterior region of the retina, is in many respects remarkable, and especially so because the functional activity of the rods is therein involved. Under this view, optographic methods which depend principally on an incompleteness of the regeneration processes might well be expected to indicate the most varied acts of vision. It is well known how rapidly objects seen by indirect vision disappear when the eye is well fixed; and this would seem to shew, even more strongly than does the persistence of after-images, that the regeneration of the purple in the rods is a slow process, and has therefore the same significance as the photophobia so characteristic of creatures in whose retinas the cones are scanty or absent. When these animals lose their visual purple they remain blind until the epithelium has furnished a fresh supply, while animals which are provided with cones continue, under the same circumstances, to be able to see by means of a second more perfect visual apparatus which, in all probability, is the sole organ of specific colour sensations—I mean the cones. I have convinced myself[1] that frogs, whose retinas had been so completely bleached by exposure to the sun that a reddish tinge did not return till they had been kept 30 minutes in the dark, could see thoroughly well, and I hope hereafter to give a satisfactory account of their ability under these circumstances to distinguish colours. As far as our knowledge goes at present, it is extremely improbable that the visual purple has any share in colour vision, although of course it must be admitted that the visual purple and the rods (apart from the cones) enable us not only to see the spectrum, but also to perceive it as consisting of a series of various tints of grey, very much as it appears, in all probability, to the colour blind. Our results concerning the occurrence and relations of the visual purple are in such close accordance with M. Schultze's hypothesis of the significance of the cones and rods in reference to colour vision, that it is sufficient to refer to it in order to check at once all hopes of finding a correspondence between optographic results and specific colour perceptions. If, however, we succeed, as in ordinary photography, in applying photographic developers to the red rods at the moment when the very first steps of the decolourisation of the visual purple have begun, we must in the end succeed in reproducing optographically any thing which we see as dark and light in indirect vision, if we could disentangle in the optogram the numerous after-images which in daily life are so completely woven together. It remains indeed as wonderful as before how it is that the inner limbs of the rods, by a chemical touch as it were, can feel such minute decomposition of the purple; but that is, after all, nothing more wonderful than what is taking place in our olfactory cells every day of our lives.

In spite of regeneration of the purple during life as after

[1] See Appendix, note E.

death, optograms are possible even during life; here is the proof. After we had obtained the above-mentioned image of the window, whieh, though suffieient of itself to prove the point, happened to have been seen by two persons only, a eoloured rabbit was, on the 16th of January at 11.40 in the morning, plaeed in position, with the head well fixed and the right eye exposed at a distanee of 1·5 meters to a square opening in the shutter 23 cm. high, 27 cm. broad. The eye was not fixed with threads, for it was found that rabbits, after the introduetion of the eyelid-holder, usually remained perfectly quiet unless a noise happened to be made. The opening in the shutter was at the level of the lowest row of panes, and eovered with ordinary window-glass, the rabbit, with its holder, being plaeed at a somewhat lower level on a ehair. The direetion of the eye was arranged as follows: standing with the baek of our head against the opening in the shutter and looking towards the eornea, we shifted the position of the rabbit until the eentre of the image of the sky on the cornea eoineided with that of the eornea itself. No other light entered the ehamber. The eye was eovered for five minutes with a blaek eloth, then exposed for three minutes, whereupon the head was severed, the eyeball extirpated as rapidly as possible by the light of a sodium flame in an adjoining dark chamber, opened, and immediately plaeed in a 4 per eent. alum solution. All this was effected so rapidly that Dr Ewald, within two minutes after the exposure of the living eye was finished, was able to repeat a similar experiment on the left eye of the same head. For the proper position of the eye in relation to the objeet one was obliged in this ease to trust to a lucky hit.

When on the following morning the milk-white and softened retinas were earefully isolated over the whole of their areas, separated from the optie nerve and spread out, eaeh of them shewed on a beautiful rosy-red ground a sharply defined almost square elear image, whieh in the seeond eye was quite white, and sharply defined as if drawn with a ruler, but in the first eye was still of a pale rose, with the outlines less sharply defined. The dimensions of the images whieh in both eases were thrown on the red retinal areas amounted to more than a square millimeter; they of course disappeared in proportion as the ground-colour bleached while they were being observed in daylight, still their disappearance was slow enough to enable me to shew them to several competent witnesses. The first account (*l. c.*) of this experiment requires a slight correction, since, through an error of measurement, the orifice of illumination was then given as a square with a side of 30 cm.; it was not until the error was discerned that the aberration of the optogram from a square figure became intelligible, and I remembered distinctly that the longer side of the image lay in the direction of the red line of partition of which I have spoken before, and the position of which in the retina was horizontal. Later on an isolated rabbit's eye happened to be exposed before the same object under quite similar circumstances, and in this an image was visible altogether corresponding to the one I have described.

In order to shew more clearly that the optograms thus obtained were really optograms, that is, were true images of the objects selected to give rise to them, slight changes were made in the latter. The window of my optical chamber seemed suitable for the purpose; in order to make the bars more distinct I increased their width to about 22 cm. by nailing boards to them. Having replaced the lowest row of panes with yellow glass, I fastened an albino rabbit, whose eye was only covered with a diaphragm, in such a position in front of and below the window, that the distance of the cornea from the first colourless pane amounted to 1·75 m. If one placed one's own eye in the position of the head of the rabbit, one saw in looking obliquely upwards nothing but the sky through all parts of the window. The distance to the key-stone of the arch of the window amounted to more than three meters. The animal was covered for a few minutes with a cloth, after removing which it was exposed for three minutes to the very cloudy and extremely dull sky (11 a.m.) and then decapitated. The eye was immediately extirpated and laid open in alum. Two minutes later the other eye remaining in the head was treated in the same manner. In this second eye upon a rapid look at the everted retina in daylight, no image was visible upon its beautifully rose-coloured glistening surface. All the more striking was its appearance after it had lain twenty-four hours in alum. The posterior surface of the retina of the eye which had been exposed during death exhibited the most complete image of the window, with two rows of square panes surmounted by a semilunar space, all white on a red ground, with sharp red crosses. The image began at the red line of partition of the retina, and shewed in its lower part strong perspective foreshortening, especially of the upper row of panes and of the arch. Since the head had been laid in a natural position, resting on the lower jaw, the upper part of the window would of course be reflected into the lower part of the eye, just as it appeared in the image. The strong foreshortening in the figure is intelligible when the distances of the lower and upper window-edge from the eye are borne in mind, and an albino eye, which for the sake of comparison was placed in the same position in reference to the window, exhibited an image of exactly the same character, and so afforded a corroboration of the correctness of the optogram.

The experiment shewed at the same time in a very striking manner what excellent optograms may be present in the layer of the rods without their being visible when the retina is looked at from the front. For in this albino eye, when the retina was simply everted, though the most beautiful purple colour could be seen on it, no difference of tint could be recognized which would enable one to infer the existence of an image, certainly at least not of so sharp and large an image as really existed there.

In the case of the first eye, which had been exposed to light during life, the posterior surface of the retina shewed in the experiment no regular optogram, but only a hardly perceptible spotty bleaching, leaving it a matter of doubt whether it corresponded in size and arrangement to the image which had been formed post mortem in the other eye. The intensity of the light of the dull sky remained the same as far as the eye could judge in both experiments. The weather indeed was as bad as possible, therefore all the more suitable for shewing the difference between an intra vitam and post mortem action of light. The reason why the optography was unsuccessful in the first case must lie in the fact, that during life the processes of regeneration are more powerful than after death, and the intensity of the illumination and time of exposure being such as they were, these processes were able to replace the decolourized purple by new material. Since this experiment I have made several others in a similar manner on living eyes, but I must in this place limit myself to the general assertion, that one must use good light if one may hope for sharp and colourless optograms, when the exposure for many reasons is limited—as when one is experimenting on unnarcotized animals—to a short time, for instance, three to five minutes. The further development of optography in the living eye, however, must depend on the discovery of methods of following out the photo-chemical processes of the act of vision, and therefore requires more careful treatment than I can at present devote to it.

Post-mortem optography, on the other hand, as is clear from what has gone before, offers no real difficulty. I myself, unfortunately, had for a long time no idea that the experiment, which will probably be classed among the lecture experiments of the future, is so simple as it really is. One needs for it only a fresh eye and a suitable object placed between it and the sky. Daylight is always sufficient, even in the worst of weather. Whoever has a skylight at his disposal needs either no apparatus whatever, or at most only a dark box, the cover of which is formed by the object. When the head, eye, and object have to be directed obliquely towards the sky, a suitable arrangement of the most simple character is desirable.

Of the rooms here at my disposal, I prefer to make use of a large chamber of eighteen meters deep, which receives light from two opposite sides, viz. by three large windows on each side, and from above by two horizontal windows in the middle of the roof. The skylight, which serves as an object, is of ground-glass and is illuminated by an external glass roof of considerable size, having a slight slope and looking to the north. The iron frame-work of this window, composed of very thin bars, is 3·16 by 2·7 meters in size, and is at a distance of 3·98 meters from an eye placed on the table which is generally used for the purpose. Parallel with the short side of the skylight, boards 60 cm. broad were placed over the panes at equal distances from the middle, in order to obtain distinctive points in the optogram. In a rabbit's eye, I obtained in this manner white tolerably rectangular areas of 6 by 4 mm. in size, with two red streaks of 1 to 1·3 mm. in length, crossing each other at right angles; at least this was the case when the eye was placed so that the visual axis was directed nearly at right angles to the middle of the illuminated space, which when the bulb only was used was easily arranged, by placing it in the mouth of a test tube, and when the head was used by employing a soft folded towel as a support. When the image comes out thoroughly sharp in a deep-red retina, there may be seen by its side an indication of the second, exterior, skylight in the form of a narrow trapezium, perspectively shortened. Often have I even seen on one side traces of three streaks of light, reflected by the side windows on to the roof. In the eye of an ox I obtained under similar circumstances images of 17 to 18 mm. in length in their largest dimension, that is to say, three times greater than in the rabbit's eye. In order to protect the eye before the exposure to light, any covering which comes to hand is sufficient, a box, a black cloth, &c. Although it is not absolutely necessary, it is advisable in order to obtain quite sharp images, to place the eye in the middle of a blackened card-board cylinder. I generally use one about 40 cm. wide and 40 cm. high.

The large number of experiments which I have made in this way might have been expected to have led to an exact knowledge of the proper time of exposure and the necessary freshness of the eyes employed; unfortunately, however, I can state very little concerning the first point. As far as I can understand, even an experienced photographer is in this respect no better off. He acquires a certain eye as to the state of the sky, and regulates the exposure by that, without being able to give any precise rules. I have obtained optograms in all sorts of weathers, in rain, snow, and hail, and after an exposure varying from one to twenty minutes. From eleven to two o'clock has always seemed to me the best time of day, the afternoon being invariably inferior to the morning. The more intense the light and the shorter the necessary exposure, so much the better were the optograms. As far as the time after death is concerned, the following rule proved general: that the exposure must first of all be long, then shorter, then after a while longer again. If, for example, one eye immediately after decapitation requires five minutes' exposure, the other eye taken immediately afterwards will give in three minutes an image of equal sharpness on a ground of equally deep purple; while under the same light the eye thirty minutes after decapitation requires from five to seven minutes, and in an hour or an hour and a half a still longer time to bring out an optogram. I am not prepared to say that eyes which have remained in the head an hour or more after decapitation will no longer give satisfactory optograms; indeed, the limit for obtaining a good image seems to be in rabbits from about sixty to ninety minutes, while the eyes of oxen seem to be useless after one hour. Still I have never worked with the latter otherwise than after removal from the head, which I find in the case of rabbits shortens the period during which they are available. Most probably this point depends largely upon temperature. To save others trouble and time, I may as well add, that when the severed head manifests snapping movements, and spasms develope in the ocular muscles, it is well to destroy the brain completely with a gum catheter, in order to prevent the optogram from being spoiled. The reason why it is in all cases necessary to make the exposure longer immediately after death than when the experiment is conducted somewhat later, seems to me to lie in the maintenance of the power of regeneration, which apparently continues the longer the less the eye is touched, and therefore lasts longer in the head than in the isolated bulb, and is shortest in the opened eye. The necessity on the other hand which is felt of increasing the time of exposure again at a still later period, I would explain by the assumption that in such cases the increasing turbidity of the various media of the eye, as well as of the retina, allows chiefly the less refractive rays to pass through, which act much more slowly on the purple. There seems to be no other explanation possible, for when eyes which have been dead some time and no longer give an optogram are examined, there may be seen on the posterior surface of the sclerotic retinal images hardly inferior to those witnessed in quite fresh eyes. That the matter is essentially due to the retina becoming cloudy I do not at all doubt, for when one uses retinas which are more than an hour old, it can readily be seen that they bleach much more rapidly on a porcelain plate when they are laid with the bacillary layer towards the light, than when the other surface is uppermost—a difference which is hardly visible in fresh mammalian retinas, or in the retinas of frogs.

Sometimes one obtains optograms which are either improved or spoiled by a peculiar circumstance. In the alum preparation, especially after long exposure, very frequently black pigment flakes come away with the retina, and these cannot be removed, and so either cover the image to a certain extent, or, on the other hand, range themselves so sharply at the sides of the figure that the picture seems to be sharpened either in a positive or negative sense. This result has several times occurred to me in optograms taken during life, and in these instances possesses double interest, because it seems to depend on those movements of the pigment between the rods which was first pointed out by Czerny, and which I can now say stand in a definite relation to the exposure to light and to the subsequent restoration processes *. The occurrence of this imposed on me the duty of investigating whether optograms could not arise, so to speak, in a reverse manner, by the rods being torn away during the separation of the retina from the epithelium and left behind in the eye, where their colour would be overlooked in the midst of the dark pigment, while the corresponding portions of the retina would be colourless. On all occasions, however, when I have snipped off patches of the posterior retinal surface in the white portions of an optogram, I have never missed the rods, these remaining most distinctly recognizable in alum solution. Everywhere were the rows of these structures found continuous, in the white parts quite as much as in the red.

* See Appendix, Note F.

Far more striking and more delicate pictures than those already mentioned I have obtained when very near objects were brought before the eye by means of the pasteboard cylinder just described. This consisted of two tubes sliding one within the other, so that the distance between the eye and the cover which bore the objects, and which consisted of a ground-glass table, or an oil-paper stretched on a frame, could be varied from 18 to 30 cm. With this very primitive apparatus I have procured a large number of optograms, generally under the open sky. Objects were formed on the transparent cover by means of strips of black opaque paper, about 4 cm. broad, and placed at the same distance from each other. Over these strips were placed card-board discs, cut out in the centre into a square, triangular or circular figure, so that by varying the position of the angular diaphragm thus formed to the direction of the stripes, or by turning the whole object round the axis of the animal's head, the most varied pictures could be formed. It would be useless to describe all the optograms so obtained, but they all agreed in this, that they afforded diminished copies, such as might be expected, of simple geometric figures. When the eyes were placed in an oblique position the optograms were correspondingly distorted.

It is not the duty of physiological optics to bring optography to such a perfection as it might acquire in the skilled hands of a professional photographer. I could not, however, deny myself the pleasure of treating optographically a few complicated objects, such as the garden side of the laboratory here and the portrait of a man. Both as yet leave much to be desired. In the case of the house the row of windows was unmistakeable, in the head (the object was the very large glass photograph spoken of in page 70) one could recognize only the outline, the limits of the hair, the beard and the shirt collar. Whoever took the trouble would probably be still more successful, and by means of such objects might approximately determine to what limits the photo-chemical destruction of the visual purple runs parallel to difference in the intensity of light.

APPENDIX.

Since the foregoing text was published, and during the time the translation has been in preparation, investigations have been earnestly carried on by Prof. Kühne and his school, the results of which have been published in the *Untersuchungen aus dem Physiologischen Institute der Universität Heidelberg*. Heidelberg, Carl. Winter, 1877—1878. To translate the whole of these would have made this little treatise far too bulky. I have therefore added, in the shape of notes, brief accounts of some of the most striking results of these inquiries. They will serve it is hoped to fill up gaps in the text, as well as to indicate the directions in which progress continues to be made; in some instances, it will be seen that further inquiry has not corroborated the impressions first made; but in many other instances, the later experimental confirmation of early guesses is very happy.

M. F.

Note A. p. 5 *.

Chamois.

This colour, the name of which, derived from its being one of the tints visible in the skin of the chamois, is probably unfamiliar to the English reader, deserves some little attention. Like purple it is not a spectral colour, but it has the same claims as purple to be recognized as a distinct sensation.

Chamois is a mixture of violet (or purple) with yellow; and various shades of chamois may be obtained by varying the admixture of white light with the above two colours. Violet and pure

* *Untersuch.* Bd. I. Hft. 4, p. 463.

yellow (that is, yellow free from green) are not complementary; their mixture therefore does not give rise to white; but the resulting sensation is, as in the case of mixing red and violet, something *sui generis;* it contains no shade of green or blue, however much either of the two component colours, the violet or the yellow, may preponderate; it is simply chamois, just as purple is simply purple.

It may perhaps be best seen when pure spectral violet is looked at in the light of a sodium flame, if a varying amount of white light be admitted at the same time as the various shades of chamois are obtained. Purple obtained by mixing the two ends of the spectrum also becomes chamois when further mixed with white light. This result happens because the pure red of the spectrum diluted with white light becomes yellow, while the violet similarly diluted becomes lilac, and lilac mixed with yellow forms a light chamois. Such a conversion of purple into chamois by mixture with white light takes place the more readily the more yellow the tint of the red employed. If the violet preponderates over the red in the purple, the result of the admixture of white light becomes more and more a lilac; Helmholtz * speaks of the mixture of yellow and violet as a whitish rose, but the exact tint of the rose colour thus produced will depend on the amount or character of the red present at the same time and vary from lilac to chamois.

Mixed with grey, chamois becomes a distinctive brown, in which a violet tint may be readily recognized.

Chamois occurs in various shades in many tea roses; many flesh-tints are also in reality chamois, and the tints known as "Isabel Yellow," "Nankeen" and "Havannah" are so many various shades of chamois.

NOTE B. pp. 6, 11, 56.

Visual Yellow and Visual White.

The view expressed on p. 18, that visual purple is not the only photo-chemical substance present in the retina has already been realized. Kühne and Ewald † have shewn reasons for thinking that the visual purple in being bleached becomes converted into

* *Physiol. Optik.* p. 279.

† "Untersuchungen über den Sehpurpur." *Untersuch. a. d. Physiol. Inst. d. Universität Heidelberg.* Bd. I. Hft. 1—4.

a substance which they have called "visual yellow," which in turn by photo-chemical action is further changed into "visual white."

Visual purple during bleaching becomes chamois and then orange and yellow before finally becoming colourless. Very frequently (p. 11) attention is arrested by the distinctly orange or yellow colour of the retina. Now true unbleached visual purple contains no yellow; the red factor forming its hue has little or no yellow element. Hence when pure visual purple is diluted, it becomes rose-coloured or lilac, growing fainter and fainter, the greater the dilution, without ever shewing any tinge of yellow. By dilution it does not become chamois or orange. It is only when photo-chemical action intervenes that the yellow tint is visible. And this is true both of the visual purple in solution and of the natural visual purple as existing in the rods.

From this it is inferred that the passage (during the action of light) of the purple through chamois to orange and yellow indicates that the optical effects of the disappearance of the purple are modified by the appearance of a second yellow colour, a product of the decomposition of the purple. And this view is borne out by the changes in the absorptive power of the purple as it is becoming bleached. As is stated in the text (p. 47) with unchanged normal visual purple, absorption commences very faintly at Frauenhofer's line C, begins to increase very rapidly beyond D, reaching its maximum between D and E, and then declines, though the diminution hardly becomes marked till beyond F, with a very rapid decrease beyond G. When a solution of visual purple is brought before the spectroscope at the stage where by the action of light it has become a pure yellow, the absorption is very different. There is no absorption at all until about midway between D and E, and no marked increase until about midway between *b* and F, just where the absorption of the purple itself is beginning to diminish. It increases very rapidly at F and reaches its maximum a little before G, from which point to the end of the visible spectrum the decrease is very slight. The absorption-features of the two things therefore, visual purple and visual yellow, are, corresponding to their hues, entirely different.

The evidence of the existence of a "visual white" is as follows.

It has been shewn by Helmholtz and others that the retina is fluorescent: examined in the ultra-violet rays it gives a blueish fluorescence. According to Kühne and Ewald this fluorescence belongs to all the layers of the retina and is visible in the retina under all circumstances. When however a retina has been completely bleached, brought to a thoroughly colourless condition, the fluorescence is not only increased but differs in character from that of the unbleached retina. In the ultra-violet rays the fluorescence is not blueish but greenish. The cause of this new fluorescence is confined to the bacillary layer, in fact to the outer limbs of the rods, for if these be brushed off, the greenish fluorescence disappears, and isolated bleached outer rods when seen together in masses exhibit the same greenish fluorescence.

Thus there seem to exist three visual colour substances; visual purple or Rhodopsin, visual yellow or Xanthopsin and visual white or Leucopsin, the first passing by the action of light into the third through the intermediate stage of the second. Visual yellow is very apt under certain circumstances, as when the retina is dried, to become indolent and to resist the further action of light. The orange retina of the rabbit, mentioned at p. 11, was evidently one in which the visual purple had been largely changed into visual yellow.

If under the action of light, by the conversion of visual purple into visual yellow, the absorptive power of the retina (or of the solution of visual purple) is thus itself changed, one might fairly expect that photo-chemical action would also vary in correspondence to the changes in absorption. This Kühne and Ewald have found to be the case.

It has been stated, p. 54, that the rays most effective in bleaching the visual purple are the greenish-yellow and yellowish-green, *i.e.* precisely those rays which are absorbed with the greatest readiness by the visual purple. But this is only true so far as the conversion of visual purple into visual yellow is concerned. Kühne and Ewald have shewn that the conversion of visual yellow into visual white is most readily effected not by the yellow-green but by the more refrangible rays, in other words by those rays which are most readily absorbed by the visual yellow itself. The less refrangible yellow-green rays, while they rapidly convert the visual purple into visual yellow, act very slowly in carrying out the further change into visual white, while the more refrangible blue rays, though slow to convert visual purple into visual yellow, rapidly change the already formed visual yellow into visual white. Thus under blue light the retina or purple solution becomes colourless more easily than under yellow-green light, while the initial partial bleaching into chamois or yellow is most rapid under the latter kind of light. Kühne and Ewald have formulated their results as follows:—

1. All visible light decomposes visual purple; but, intensity remaining the same, in very different times, viz. in times proportional to the absorption of monochromatic light.

2. Rays of such wave-lengths that they most rapidly convert visual purple into visual yellow act most slowly on the latter: those which most easily change the visual yellow into visual white, and which are most readily absorbed by the visual yellow have, as a rule, a weaker action on the visual purple itself.

These separate actions on visual yellow and visual white explain the difficulties met with in determining the bleaching power of the different rays, before the distinction was appreciated.

NOTE C. pp. 31, 33.

Visual Purple in the Human Eye.

Since the paragraph in the text was written Prof. Kühne has had opportunities of extending and corroborating the observations there recorded*. He finds that the purple is present in the rods only, being entirely absent from the cones. To the naked eye the fovea and even the macula appear colourless, while in a zone of about 2 mm. round the macula the colour is extremely faint. Under the microscope, however, the rods in the outer margin of the macula are seen to possess purple.

At the peripheral margin of the retina, a zone of about 2 mm. in width lying immediately behind the ova serrata is completely colourless, and here the want of colour is due to its absence from the rods, and not merely to the scantiness or absence of the rods themselves.

Kühne's observations have, in the main, been corroborated by

* "Ueber die Verbreitung des Sehpurpurs im menschlichen Auge. Weitere Beobachtungen über den Sehpurpur des Menschen." *Untersuch.* Bd. I. Hft. 2. "Nachträge zu den Abhandlungen über Sehpurpur." *Ibid.* Hft. 4.

other observers, including Donders*. In many cases, it is true, the attempt to discover visual purple, even in eyes free from disease, examined apparently with all proper precautions, has failed †; and it is of course possible that we have not at present mastered all the conditions determining the presence or absence of visual purple under various circumstances; but on the whole it seems more probable that these exceptions were after all due to defective manipulation. Kühne points out a possible source of error in the rods being so closely attached to the pigment epithelium that their outer limbs remain in the fundus when the retina is removed; in such a case of course the retina would possess no purple.

It is more than probable that individual variations in the intensity and even in the distribution of the purple may be common in man; but Kühne denies that the intensity of the colour itself, allowance being made for the less saturation due to the abundance of cones in the human retina, is less in man than in the rabbit.

Note D. p. 52.

Auto-regeneration of the Visual Purple.

Kühne and Ewald‡ have shewn that a bleached frog's retina may regain its purple even in the absence of the retinal epithelium, by a process which may be spoken of as "auto-regeneration." If a retina bleached until it has become quite colourless be kept in the dark for some time it will to a large extent regain its purple, becoming first yellow, then chamois, and finally of a distinct rose or lilac colour. This colour will disappear when exposed to light, and reappear once more in the dark. In fact the bleaching and regeneration may be repeated several times, the revived colour becoming of course fainter on each occasion. The same "auto-regeneration" may be observed in solutions of visual purple prepared with solutions of bile salts free from ether. These facts suggest the idea that the visual purple may be formed out of some substance in the retina, by an action analogous to that of a ferment, and the view at once readily occurs that the body which is thus converted by a ferment action into visual purple is in reality the visual white. This view is supported by the facts (1) that

* *Beilageheft d. Klin. Monatsbl. f. Augenheilk.* xv. Jhrg. 5, 156.

† Cf. Michel. *Centralbt. f. Med. Wiss.* 1877, p. 433.

‡ *Untersuch.* Bd. I. Hft. 2.

the isolated retina becoming fluorescent (see note B, p. 97) as it bleaches, loses its fluorescence as it becomes once more purple: that is to say, the return of the visual purple is accompanied by the disappearance of the visual white; (2) that the retina bleached *in situ* during life is found when removed to be incapable of auto-regeneration, and is at the same time devoid of fluorescence,—that is to say, when the retina is bleached *in situ* the visual white appears to be removed from the rods as it is formed. It is no great stretch to suppose that it is taken up by the retinal epithelium, and there, in the epithelium cells, reconverted into visual purple. And this further view is again supported by the facts that a solution of the retinal epithelium in bile-salt solution, free from ether, is rose-coloured in the dark, becomes colourless when exposed to light, and rose-coloured once more when returned to the dark. The best results, however, are obtained when a solution of the epithelium and the rods mixed together is employed.

These facts would seem to indicate the existence of a continual intercourse between the epithelium and the rods, the former absorbing from the latter the visual white arising from the action of the light on the visual purple, and by the action of a ferment body (to which the provisional name of Rhodophyllin might be given), reconverting it into visual purple, and returning it to the rods; in the isolated retina, however, one must suppose the Rhodophyllin to be present in the rods themselves. Further, one might readily imagine that such a secretory activity of the retinal epithelium would be placed under nervous guidance. But the whole of this part of the subject needs to be elucidated by further researches.

NOTE E. p. 86.

Vision without Visual Purple.

The facts that visual purple is never present in the cones, that it is absent from the macula lutea and fovea centralis, and that some animals (and these not all nocturnal, *ex. gr.* the pigeon and the fowl) possess no visual purple at all, prove very conclusively that the presence of this substance is not essential to even the most accurate vision. It is impossible, however, to suppose that the changes of the visual purple have no relation whatever to vision; and the interesting question arises, what differences in respect to vision can be detected in animals when their visual purple has been completely bleached as compared with the same animals when their visual purple is intact? Prof. Kühne* has attempted to answer this question as regards frogs; and though the results are negative, they are worthy of attention.

The retinas of living frogs may be completely bleached by direct sunlight (in summer) in about fifteen minutes, the colour returning in about an hour, the first traces being visible in about thirty minutes. Frogs, therefore, may be obtained which for about half an hour possess absolutely no visual purple at all. Kühne satisfied himself that during this half hour the frogs were able, as far as could be ascertained, to see as usual. They fled like ordinary frogs when a hand was stretched out to seize them. They caught flies with the nimbleness of an ordinary frog. Like ordinary frogs they chose the shade, overcoming all manner of difficulties, in order to reach a shady spot. In a word, they shewed no deficiency of vision whatever, and in all their behaviour exhibited the strongest contrast to frogs which had been really blinded by extirpation of the eye-ball or otherwise. It need hardly be added that care was taken to avoid any errors due to the frogs being guided by the heat as distinguished from the light of the sun.

The idea naturally suggested itself that the frogs with bleached retinas, though not blind as regards the amount and intensity of light as a whole, might still be colour-blind. But an ingenious experiment proved this idea to be baseless. Kühne finds that the vast majority of frogs (a few individual exceptions being from time to time met with) shew a most decided preference for green as compared with blue light. When a number of frogs are placed in a box, half of which is covered by green glass, and the other half with blue glass, they will in a very short time be all found huddled together under the green glass. Of course the experiment must be conducted in such a way that the illuminating power of the light and the temperature are nearly as possible the same in the two kinds of light. Frogs whose retinas have been bleached congregate under the green glass just in the same way as those whose retinas are in a normal condition. Very marked here also is the contrast between frogs with bleached retinas and really blind frogs. Of course in all these experiments care was taken, by repeated examination of specimens, to make sure that no regeneration of purple took place during the experiments.

The foregoing experiments prove at all events that, in frogs at

* "Sehen ohne Sehpurpur." *Untersuch.* Bd. I. Hft.

least, the presence of a store of visual purple in the rods is not essential to the appreciation of differences in colour. They are, it may be remarked, quite consistent with the favourite hypothesis that the cones only, and not the rods, are concerned in colour vision; and they are at least highly suggestive of future researches. It would for instance be very interesting to ascertain the behaviour, when their retinas are bleached, of those animals, if there be any, which possess no cones, or rather in which all the terminal organs of the retina are coloured with the purple.

NOTE F. p. 93.

The Retinal Epithelium.

The necessity in their various experiments of obtaining the retina free from the pigment epithelium has led Kühne and Ewald † to pay particular attention to the behaviour of the latter structure under the influence of light and other circumstances. These results are briefly as follows.

In the eyes of frogs kept in the dark the pigment is for the most part concentrated in the body of the cell, a small quantity only being seated between the rods and reaching down for not more than about one-third the length of the outer limbs. After the animal has been exposed to light, the pigment is found, to a very large extent, arranged in strata between the rods. There is a tolerably large deposit between the posterior ends of the outer limbs; then follows a gap tolerably free from pigment, in front of which, between the anterior ends of the outer limbs, there is again a large deposit. From this it would appear that light acts as a stimulus to the cells of the retinal epithelium very much in the same way as it does to the cutaneous pigment-cells of the chameleon: they are by it excited to throw out pseudopodic processes which pass down between the outer limbs of the rods. Upon the removal of the light, the processes are withdrawn towards or into the body of cell. Under red light, singularly enough, the forward transit of the pigment is even greater than in white light, a very large amount indeed of the pigment becoming heaped up at the front ends of the rods, the epithelium in consequence adhering very pertinaciously to the retina, so that the two are separated with the greatest difficulty.

In general, exposure to light increases, and the withdrawal of light diminishes, the adhesion of the epithelium to the retina; but

† *Untersuch.* Bd. I. Hft. 4, p. 411.

the absence or presence of light is not the only conditioning factor. Urari, apparently by giving rise to an œdema, favours the separation of the epithelium, while a low temperature produces the same effect as light, a high temperature having of course the opposite effect. A high temperature (37° C.), combined with urari poisoning, is sufficiently efficacious to overcome even the action of light; and such a treatment is found very useful in experiments where it is desired to obtain retinas free from epithelium.

NOTE G. p. 36.

Action of Light on the Structure of the Rods.

Kühne and von Hornbostel* have satisfied themselves that in the frog light does produce an effect on the rods themselves. These swell up under the influence of light during life, and thus, by "jamming up," so to speak, the processes of the retinal epithelium between, help to increase the cohesion of the retina to the epithelium.

NOTE H. p. 46.

Chemical Characters of Visual Purple.

With regard to the chemical composition of visual purple we at present know little. Kühne† has failed to discover in it any satisfactory evidence of iron being present in it, and it is not diffusable. It is not soluble in urea solutions or in melted paraffin; and though easily destroyed by chlorine, nitrous acid, &c. it resists largely the action of ozone, permanganate of potash, &c.

When exposed, in the dark; to a temperature of 50° or 52° C., bleaching begins, becoming very rapid as the temperature rises to 70°, taking place indeed almost instantaneously at 74° C. The bleaching seems to be identical with that produced by light, in so far at least as the visible changes are the same, the purple passing through chamois and yellow to white.

Temperature has also a remarkable influence on the rapidity with which bleaching is effected by light, the purple becoming remarkably sensitive at about 45°.

It is possible that the change of the purple into yellow, and of the yellow into white, may be instances of dehydration.

There are some facts which indicate that the visual purple, like hæmoglobin, differs somewhat in nature in different animals.

* *Untersuch.* Bd. I. Hft. 4. † *Untersuch.* Bd. I. Hft. 4, p. 438.

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