THE HISTOLOGICAL INTERPRETATION OF APPEARANCES IN THE FUNDUS OCULI

A Scheme for Methodical Investigation

BY

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ALTHOUGH, as Duke-Elder has remarked, ophthalmology has had its period of intensive ophthalmoscopic investigation and its histological phase, and must now look for greater advances in the fields of bio-physics and bio-chemistry, we must admit that our knowledge of the normal and pathological histology of the fundus oculi is far from complete and still calls for a good deal of expansion and clarification.

We frequently remind ourselves that the ophthalmologist enjoys the privilege of being in the position to study, in the living state, the retina—an extension of the brain—and its blood vessels—an important part of the peripheral circulation, closely related to the intracranial, renal and other vessels. But these facilities are perhaps not employed as fully or as systematically as they might be, for the purpose of correlating ophthalmoscopic appearances with histological findings. The limitations of our knowledge in this field are shown by the continued use of a nomenclature of eye diseases based on faulty ideas of their pathology.

In our practice and teaching we are constantly under the necessity of interpreting the details of the ophthalmoscopic picture, normal or pathological, in terms of the structures which contribute to that picture; but, glancing over the field as a whole, it is surprising to find in how few instances we have unequivocal proof of the histological conditions represented by the ophthalmoscopic picture and of the pathological processes that have led up to them.

The ophthalmoscope has been in use for ninety years, with a steady advance in its design and in its capacity for revealing detail, while the resources of histological technique have enormously increased; yet, the two aspects have been, and still are, to some extent un-co-ordinated.

The achievement of valuable results in this field demands certain conditions which are not easily fulfilled. It is desirable:

1. That the fundus should be examined by every available method and that the detailed description of the fundus under such conditions should be supported by accurate drawings or photographs, and by careful measurement of the size and position of the structures or lesions under investigation. Microscopic identification of the actual lesion is most important, but it is usually

difficult, *e.g.*, when it comes to the examination of a small haemorrhage or a selected part of a selected blood vessel.

2. That the histological material should be obtained under the most favourable conditions as regards fixation and conservation of anatomical relationships, and that all appropriate histological methods should be employed in its investigation. Here also photographic and other records can be made and correlated with those already obtained at the clinical stage.

The number of cases in the literature in which these ideal conditions have been fulfilled is surprisingly small, and it must be admitted that the difficulties are great. There are, for example, many fundus lesions which do not lead to excision of the eyeball and in which the material can only be obtained after death from intercurrent disease. These may be discovered accidentally at post-mortem examination, and there may be no record of their ante-mortem appearances. In other eyes which have been excised on account of pain, or for other reasons, a preliminary ophthalmoscopic examination may be impossible through the presence of opacity of the optical media; or, the interval between an ophthalmoscopic examination and excision of the eye may be so long that the lesion in question may have completely changed.

To these handicaps we have to add the results of post-mortem changes which destroy or greatly alter the histological material, and the chemical destruction of certain substances, such as lipid deposits, in the course of fixation and embedding.

In the Tennent Institute, with the aim of making the fullest use of the material which offers itself, the scheme here described is carried out as completely as each case allows. It is not claimed that the methods of examination referred to are new or original; but their employment in a definite sequence, and in the fullest detail, gives a completeness to the records which would otherwise be lacking.

I. Ophthalmoscopic Examination

It is not necessary to deal here with methods of ophthalmoscopy in any detail.

Indirect ophthalmoscopy, direct ophthalmoscopy with the electric ophthalmoscopes of Keeler, Hamblin, Curry and Paxton and others, and with the reflecting ophthalmoscope of Morton in conjunction with the sodium vapour and mercury vapour lamps, are our routine methods. The Cardell-Keeler polarised ophthalmoscope and the Holloway twin-beam ophthalmoscope are under trial, and have given various interesting results; but require further investigation. The binocular ophthalmoscopes of Gullstrand and of Franceschetti-Müller, and the Bausch and Lomb model, are also in use but have not been found of special value.

The method of intravital staining, advocated by Sorsby (1937, 1939) (a) and (b) has not been employed so far, nor has examination or photography by infra-red light.

Since the "normal" ophthalmoscopic picture is a two-dimensional one, it is wise to cultivate those instruments and methods which help us to ascertain the depth relationships of the structures and lesions under observation. In direct ophthalmoscopy the relative depth of such lesions as exudates or pigment deposits can be estimated by their relation to normal structures, blood vessels for example, or to other lesions whose depth is known, such as striate haemorrhages in the nerve fibre layer. Parallax movement of one structure in relation to another is sometimes useful.

Direct measurement of prominences or depressions by means of the ophthalmoscopic lenses is habitually employed in the estimation of a swollen disc or a glaucomatous cup; but in ophthalmoscopy we have no method comparable in precision with the Ulbrich drum of the corneal microscope or the micrometer adjustment of the microscope. Such measurements can be obtained at a later stage.

A very useful modification of the direct method is examination of the fundus under focal illumination. No doubt the most refined applications of this method are obtained with the apparatus of Zamenhoff (1930), Goldmann (1938-39) and others; but any electric ophthalmoscope which permits the focusing of a lamp filament or a point of light on the fundus, can give valuable results, especially when aided by controlled movement of the light image across the fundus.

So much for the ophthalmoscopic examination. There is a considerable gap between this and the histological examination as commonly understood. A broad no man's land lies between the ophthalmoscopist and the histologist; but this can be bridged by the employment of other methods. I shall refer here to two methods which fall in point of time, as well as in resolving power, between the initial ophthalmoscopic examination and the final examination of microscopic sections, and partake of the character of both methods.

The material for investigation comes to us from two sources. In the first place we have eyes excised from the living subject for various reasons. It occasionally happens that at the time of the enucleation the media are transparent and a verbal and pictorial record can be obtained of the fundus details. In the majority of cases this is impossible. The fundus may have been seen at some earlier period; but before the eye comes to excision there is usually so much opacity of the cornea, lens or vitreous, that ophthalmoscopy is impossible, and in the interval the state of the retina and other parts may have changed beyond all recognition.

It is otherwise in the case of eyes obtained in the post-mortem room. It is true that a great deal of valuable material is lost through lack of co-operation between the medical, ophthalmic and pathological departments; but where a proper liaison is maintained it is often possible to obtain accurate ante-mortem records to correlate with the post-mortem findings. In either case it is important to obtain, if possible, good illustrations of the fundus as a whole, or of important details. And here one may stress the inestimable value of coloured drawings made by one who is expert with the ophthalmoscope as well as with the brush. Fundus photography in black and white is usually a poor substitute, and even photography in colour leaves a good deal to be desired, although the beautiful examples shown by Bedell before the Ophthalmological Society (1937 and 1939) showed what can be done by the expert in this method. Photography at its best gives a faithful statement of the size, form and situation of a lesion, and is invaluable for serial illustrations of its progress. It is quick and less tiring to patient and surgeon. But the magnification is low and the finer details are often lost. A drawing, even a rough sketch, is often a more useful adjunct to a verbal description.

II. Slit Lamp Examination

For our present purpose specimens are fixed with formalin or some formalin mixture which preserves their transparency. Eyes excised from the living subject are placed directly in the fixing solution and are ready in 48 hours to be divided as convenient for the next stage of the examination.

In the case of the post-mortem material it is seldom possible to get permission for removal of the whole eyeball; but in every case where the skull is opened, the roof of the orbit can be removed, and the posterior part of the globe, with the attached optic nerve, excised after removal of the orbital tissues to the necessary extent. If the intracranial as well as the intra-orbital part of the optic nerve is to be removed, the roof of the optic canal must be chiselled away. In excising the posterior segment of the globe there is always a danger that the vitreous may escape, the sclera collapse and the retina become hopelessly detached. We have overcome this difficulty almost entirely by arranging that as soon as possible after death a few minims of ten per cent. formalin solution are injected into the eye through the sclera behind the ciliary body. If no post-mortem examination is allowed no harm is done, but when the opportunity occurs to obtain the specimen, it will be found to be in firm condition and when carefully removed the retina lies smoothly in position. In any case care must be taken in excising the specimen. With the subject in

the usual supine position the eye is looking upward. The exposed sclera is punctured by a very sharp worn Graefe knife and an incision with fine scissors carried round at or behind the equator of the eyeball. The vitreous will often be in the condition of a fairly consistent jelly and should be divided by the scissors at the same time. We should aim at keeping it in situ within the cup formed by the sclera. Before the scissors complete the scleral incision a small bottle (about 1 oz. capacity) filled with the formol

FIG. 1

Method of supporting optic nerve and posterior segment of eyeball during fixation. Both bottles are filled with the fixing fluid.

fixative is brought into position so that the optic nerve passes vertically into the bottle and when the scleral incision is completed the posterior part of the eye rests on the mouth of the bottle. In this way, spilling of the vitreous is prevented, and the retina kept in position. The small bottle with the specimen is then lowered into a larger tube or jar of the same fixing fluid (Fig. 1) and set aside for 24 hours before we proceed with the slit lamp examination. At this stage the vitreous must be removed. If it has a firm consistency and cannot be removed by irrigation it will be necessary to employ careful swabbing.

What follows applies equally to eyes excised from the living and to specimens removed after death.

It is necessary to have some form of holder to carry the eye under examination which will allow the specimen to be set in convenient positions and to "stay put." The carrier illustrated in Fig. 2 was made out of oddments but meets the requirements very well. It clips on to the forehead rest of the slit lamp stand, and an essential feature is the ball and socket joint, which formerly belonged to the "cat's whisker" of a crystal wireless set and

FIG. 2.

Appliance for holding the eye during slit-lamp examination. The sharp pins and adjustable screws grip the specimen. The ball and socket joint allows the eye to be placed and retained in any desired position.

allows of free movement of the specimen into any desired position. The eye is examined in the moist state, and it is unnecessary to place it under water until it is to be photographed.

The corneal microscope and slit-lamp give us a monochromatic view of the fundus, under magnifications of 9 to 35 diameters. In the normal eye the vessels are more or less completely filled with blood and the arteries and veins are readily distinguished from one another. The nerve fibre bundles can be easily followed. The macula presents its yellow tint and the margins of the depression are somewhat swollen. When it has been impossible, for the reasons already referred to, to make an ophthalmoscopic examination of the living eye, this slit-lamp examination provides an excellent substitute; indeed in most cases interesting features are revealed which evade observation with the ophthalmoscope.

The stereoscopic view and higher magnifications are an advantage; but much more important is the control over the direction of observation and of illumination. As in slit-lamp examination of the anterior part of the eye, we can employ the broad or narrow beam (the latter giving optical section) direct or indirect illumination, or illumination from the scleral side (retro-illumination) by the Gullstrand lamp or by any one of the familiar hand inspection lamps.

By this method we get a three-dimensional view of the fundus and can obtain micrometer measurements of the size, situation

FIG. 3.

One of a pair of stereoscopic photographs of the posterior segment of the eye. Case of subhyaloid haemorrhage.

and space relationships of the parts, thus checking and amplifying our ophthalmoscopic records.

When the object of investigation is a small lesion such as a haemorrhage, an exudate, an arterio-venous crossing or the like, we select and locate it, measure its size and position and make drawings or photographs. In larger lesions we make stereophotographs under lower magnifications, of which Fig. 3 is an example.

III. Examination of the Retina in Bulk

The next step is the excision of selected portions of the retina. Having noted the lesion in question we punch out the affected portion of the retina by means of a three or four millimetre trephine

applied at right angles to the retinal surface and pressed against this with a slight rotary movement. If necessary a binocular loupe or dissecting microscope can be employed. The small disc thus outlined can be easily lifted with an iris repositor or fine forceps. It is placed in a watch glass containing water and transferred to glycerine on a glass slide and protected with a cover glass.

The microscopic examination in this form is in some respects an extension of the ophthalmoscopic and slit-lamp examinations; but employing higher magnifications. The isolated retina, without its pigmented epithelium, is seen by transillumination, and readily reveals such details as the nerve fibres and nerve fibre bundles, the pattern of Henle's fibre layer and of other tissue elements not so fully understood, the mosaic of the rods and cones and the perforations of the external limiting membrane, the structure of the vessel walls, the relationship of arteries and veins at their crossings and the pattern of the capillary plexuses. The resolution of these and other details is assisted by the stopping down of the substage diaphragm and the occasional movement of the mirror. The micrometer indications on the fine adjustment of the microscope enable us to measure the depth relationships of normal and pathological structures with a degree of refinement which is impossible in the ophthalmoscopic and even in the slit-lamp examination.

For most purposes this stage of the examination may be completed without staining or other treatment; but some investigators, notably Shaw Dunn in Glasgow and Friedenwald in Baltimore, have employed the examination of retina "on the flat" in conjunction with scarlet red or sudan staining for the lipoids, and in the Tennent Institute Professor A. Löwenstein has elaborated and modified the technique in many ways and with fruitful results. It lends itself especially to the demonstration of the retinal capillary plexuses, both in their natural state and by the injection and staining method described by Michaelson and Campbell (1940). Naturally, the specimens at this stage also lend themselves to illustration by drawing and microphotography.

IV. Final Stage

When the possibilities of this method of examining the trephined discs of retinal tissue have been exhausted the specimens are passed through the usual stages for embedding in paraffin, and serial sections are cut for further study.

Having followed the selected lesions through the earlier stages, and having kept note of the appropriate measurements, it is not difficult to identify these in the microscopic sections. Figs. 4 to 11 show some examples of the "follow up" from one stage to another.

The eye from which these figures were taken was excised on account of acute glaucoma. Ophthalmoscopic examination was very imperfect on account of the corneal oedema, but there was extensive haemorrhage, chiefly between the vessels, with a notable blood-free zone of varying width between the haemorrhages and the vessels. Fig. 4 was a diagrammatic indication of this appearance. The vein was full and tortuous with some variation of calibre, the artery very narrow and in parts opaque. Slit-lamp examination of the posterior segment did not reveal any greater detail. A circular disc of retina, including the lower temporal vessels, was removed by trephining and mounted in glycerine without staining. Figs. 5 and 6 show the low and high power microscopic appearances and their correspondence with the ophthalmoscopic picture. The veins are not so uniformly injected as in the living eye and the arteries are for the most part represented by solid white lines. Two kinds of haemorrhages are distinguishable—the striate (A) and the punctate (B) in Fig. 6. The blood-free zone is not so sharply defined as is the pale lateral sheathing due to opacity of the vessel wall; but it is more obviously

FIG. 4.

Ophthalmoscopic examination. Case of angiosclerosis and hyperpiesis with acute secondary glaucoma. Distribution of haemorrhage in relation to retinal vessels. Semi-diagrammatic.

FIG. 5.

From same eye as Fig. 4. Selected piece of retina removed by trephine and viewed microscopically on the flat, unstained. Vein accompanied by broad blood-free zone. At some points there is narrow white sheathing due to opacity of the vein wall.

FIG. 6.

Part of Fig. 5 under higher magnification. Retinal nerve fibres outlined by effused blood. Superficial, striate, haemorrhage at (A) and deep, punctate, haemorrhage at (B).

FIG. 7.

Paraffin section at part of retina shown at (A) Fig. 6. To demonstrate situation of striate haemorrhage in nerve fibre layer.

FIG. 8.

Paraffin section of part of retina shown at (B) Fig. 6. To demonstrate situation of punctate haemorrhage in outer molecular layer.

related to the course of the vessel where the haemorrhage is of the striate variety. The micrometer of the fine adjustment of the microscope indicated that the punctate haemorrhages lay some $50\ \mu$ deeper than the striate, and the sections taken from this piece of tissue after embedding in paraffin show that the striate haemorrhage is confined to the nerve fibre and ganglion cell layers (Fig. 7) and the punctate haemorrhage to the external molecular layer. (Fig. 8.)

Fig. 9 represents a more peripheral piece of the retina prepared in the same way. At (A) the retinal haemorrhage is separated from

FIG. 9.
From the same eye as Figs. 4 to 8. Retina at periphery, removed by trephining and seen on the flat, unstained. At (A) the superficial haemorrhage is limited by the opaque vessel wall. At (B) the vessel is largely concealed by haemorrhage.

the vascular blood column by a narrow, well-defined, blood-free zone, while at (B) the vessel is largely concealed by blood. Fig. 10 shows that at (A) the haemorrhage is confined to the inner layers and the narrow pale zone or bilateral sheathing is entirely due to the vessel wall. The concealment of the vessel at (B) is seen in Fig. 11 to be due to haemorrhage in front of and behind the vessel.

Other sections showed that the blood-free zone illustrated in Figs. 4, 5 and 6, is also most typically found when the haemorrhage is confined to the inner layers (striate haemorrhage) and is separated from the blood vessels by a variable interval. The reason for this "avoidance" of the vessels has not been determined,

FIG. 10.

Paraffin section of retina shown in FIG. 9. Vessel and haemorrhage as seen at (A).

FIG. 11.

Paraffin section at retina shown in Fig. 9. Vessel and haemorrhage as seen at (B).

but in some cases this haemorrhagic pattern is very obvious. If one considers these pictures in conjunction with some of those illustrating the paper of Michaelson and Campbell (1940) the resemblance between the capillary-free zone of the injected retina and the blood-free zone in retinal haemorrhage is sufficiently striking to suggest that the latter is found in those cases where the haemorrhage has taken place from the capillary plexus internal to the inner nuclear layer.

It is outside the scope of this paper to deal with the preparation of the microscopic sections, which follows the usual lines. My purpose has been to point out that much essential information may be hidden in the gap between the ophthalmoscopic examination and the examination of paraffin or celloidin sections; and to show how complete a survey of the normal and abnormal histology of the retina can be obtained by the serial application of the methods described.

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A CASE OF BOECK'S SARCOIDOSIS

BY

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MRS. L., aged 45 years, with cataract in both eyes, with absolutely no contra-indication for immediate operation, had an extraction performed in the left eye, the right eye being quite immature. She was discharged as satisfactory within a month with correction for the operated eye. She returned a month later with signs of cyclitis in the operated eye. After getting the usual treatment for about five months she disappeared without being completely cured, but reappeared in a month's time with pain and marked loss of vision in the eye.

On examination she had well-marked ptosis of the left eyelid. Underneath, the eye was tender with deep keratitis, vascularisation obscuring deeper details as it extended nearly to the upper two-thirds of the cornea. Tension was low. The clear lower cornea