COMMUNICATIONS

OBSERVATIONS ON THE CHOROIDAL CIRCULATION*

BY

NORMAN ASHTON

Department of Pathology, Institute of Ophthalmology, London

THE discovery of aqueous veins and subsequent studies of their significance in health and disease have been responsible in no small measure for a new impetus to research work in the aetiology of glaucoma, which in turn has necessitated a re-examination of our fundamental knowledge of the anatomy and physiology of the ocular circulation and the drainage of the aqueous humour, with particular reference to the ocular tension. Amid the abundant recent literature on these subjects are a few papers directing attention towards the minute anatomy of the choroidal circulation, and the findings reported would be of very great importance if the evidence could be regarded as conclusive. It is the purpose of this paper to discuss these reports in the light of our own experience, and to introduce a technique which is likely to prove of value in future work in this field.

The anatomy of the small vessels in such a structure as the choroid cannot be studied adequately by the methods at present in use. It is impossible to reconstruct complicated minute branches and anastomoses from serial sections; dye injection preparations, while of much greater value, are not suitable for dissection and are, therefore, prone to misinterpretation. The earlier investigators, using an injection mass of vermilion and size, were much nearer to the ideal technique. In a previous publication, the value of Neoprene latex in the study of Schlemm's canal and aqueous veins has already been demonstrated (Ashton, 1951, 1952) and we have now applied this technique to a study of the choroidal circulation. We are using Neoprene casts, together with flat sections and bulk preparations, to investigate the possible existence of arterio-venous anastomoses, with or without a glomus-like apparatus, in the choroidal circulation both in normal and pathological conditions.

Methods

Neoprene Casts of the Choroid.—At *post mortem*, after the brain has been taken for examination, the orbital contents are separated from the bony margin, from all sides up to the apex of the orbit, and the orbital roof is removed from the interior of the skull. A sagittal incision is made through the bone of the sella turcica and, by chiselling through the sphenoid bone under the cavernous sinus, the whole of the orbital contents, together with the optic nerve and the cavernous portion of the internal carotid artery, may be removed *in toto*.

The specimen is taken direct to the laboratory, where the internal carotid artery is slit down to reveal the opening of the ophthalmic artery. A glass cannula is inserted into this opening, and the vessels are then irrigated with tap water, any large leaking vessels

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FIG. 1.—White Neoprene cast of choroid viewed from without, showing layer of large and medium vessels and chorio-capillaris beyond. Equatorial region. $\times 66$.

being secured with artery forceps and ligatured. After irrigation has been carried out for one hour, the specimen is placed in the refrigerator overnight to promote haemolysis in any remaining clots. The next morning the vessels are again irrigated for about 15 minutes.

Neoprene latex 572 diluted 1 in 3, either white or coloured red,* is injected from a Wolff's bottle, under 10 lb. pressure from an electric pump. In order to force the Neoprene into the finest vessels it is important to make the initial injection under maximum pressure. This can be achieved by clamping the tubing on to the cannula side of the Wolff's bottle, switching on the pump, and then releasing the clamp after a few seconds.

When the injection is complete the specimen is fixed in 10 per cent. formal saline; the eye is then cut equatorially through the ora serrata, the vitreous and retina are removed, and the choroid is carefully dissected out by severing its vascular and nervous connections to the sclera. The injected choroid is then floated out of the sclera and bleached in 2 per cent. potassium oxalate for 15–30 min., followed by washing and 1 per cent. oxalic acid.

If the injection has been successful, the whole choroidal circulation may be seen perfectly, even to its finest detail (Figs 1 and 2); it may be studied whole in a glass sphere the same size as the sclera, or cut radially and mounted flat—a more

* As supplied from B.B. Chemical Co., Ltd., Ulverscroft Road, Leicester, England.

FIG. 2.—Another view of cast shown in Fig. 1. The vessels may be studied by microdissection. The complexity of the vascular network, as shown in the cast, demonstrates the futility of attempting to determine their connections by serial sections. Equatorial region. $\times 45$.

satisfactory preparation for photographic purposes. If the vessels have been injected with red Neoprene the arteries will appear an intense red and the veins a delicate pink; a differentiation most useful for purposes of identification (Figs 3 and 4, overleaf). It is not clear why this occurs; presumably the pressure propels the Neoprene further than the vermillion granules so that the more proximal vessels tend to contain a higher proportion of pigment. Even with white Neoprene some differentiation is possible, because the thicker arterial walls impart a dense white appearance to the cast, whereas the thin-walled veins appear greyish-white.

It is occasionally of value to digest the tissue in pepsin and trypsin, as described in the technique for preparing casts of Schlemm's canal; this allows complete dissection of the choroidal vessels, and the observer may decide with certainty at which points the vessels anastomose. On the other hand, if the bleached tissue is allowed to remain, the vessels are retained in their correct anatomical relationship.

Histological Techniques

Flat Bulk Preparations :

- Bleached choroid stained with haematoxylin and eosin, cleared and mounted in polystyrene.
- Bleached choroid stained with haemalum, cleared and mounted in polystyrene.
- Bleached choroid stained by Holmes's silver method, cleared and mounted in polystyrene.
- Bleached choroid stained with haemalum and mounted in glycerine.

FIG. 3.—Red Neoprene cast of choroid viewed from without. The arteries appeared in red and the veins in pink. Note the characteristic loop at the origin of a vein. The direct capillary connections between artery and vein are well shown. Anterior region. $\times 28$.

FIG. 4.—Red Neoprene cast of choroid viewed from without. The contrast between arteries and veins and the dense arborization in the chorio-capillaris are well shown. Anterior region. $\times 28$.

Flat Serial Sections of the Choroid :

Bleached choroid sections stained with Mallory's phosphotungstic haematoxylin.

Bleached choroid sections stained with Holmes's silver method.

Unbleached choroid sections stained with haematoxylin and eosin.

Unbleached choroid (Zenker fixation) sections stained with Mallory's phosphotungstic haematoxylin.

Unbleached choroid sections stained with Masson's trichrome stain.

Unbleached choroid sections stained with iron haematoxylin and Van Gieson.

Results and Discussion

While we were engaged on our investigation, Loewenstein (1949a, b) reported the presence of large, round polyhedral cells in the periphery of large and medium-sized choroidal arteries in the posterior choroid; they are 10–20 microns in diameter and have a central, round, dark nucleus, a clear cytoplasm, and a smooth cell membrane. He stated that these correspond to epithelioid muscle cells or glomus cells; the latter have been described as the basic elements of arterio-venous anastomoses, which he therefore believes must be present in the posterior segment of the human choroid. He regards these glomera as an essential part of the mechanism for the maintenance of the intra-ocular pressure. Curiously enough Loewenstein found the “choroidal glomus cells” more frequently in hypertensive cases than in normal eyes, and they were particularly abundant in a case of retrobulbar tumour. More recently Loewenstein’s theory has been taken a stage further by Orbán (1951) and by Kiss and Orbán (1952), who described localized dilatations upon the tributaries of the vortex veins, which they termed “bulbiculi”. By analogy with the work of Clara (1939), and reinforced by the findings of Loewenstein, these workers concluded that these “bulbiculi” are the sites of arterio-venous anastomoses and that the choroidal circulation is controlled through their agency.

As is well known, however, the choroid contains a profusion of cells which may vary in appearance according to the normality or morbidity of the tissue, the method of fixation, the stains employed, and the plane in which the sections are cut. Thus, before deciding upon the presence or absence of glomera in the choroid, one must first define the minimal requirements necessary to establish their existence beyond reasonable doubt. Essentially, a glomus consists of an afferent artery, the Sucquet-Hoyer canal or shunt, and an efferent vein.

The first requirement, therefore, is an arterio-venous anastomosis. The shunt is surrounded by *several layers* of small polyhedral, epithelium-like cells, uniform in size with a well-defined cell membrane and having central, rounded, darkly-staining nuclei 7–10 microns in diameter. They superficially resemble naevus cells and are intimately associated with non-medullated nerve fibres; through their contractility they control the blood flow through the shunt. The second requirement, therefore, is an agglomeration of these characteristic glomus cells around the arterio-venous anastomosis. These two aspects of the problem will be considered separately:

Arterio-Venous Anastomoses.—The widespread existence in the normal circulation of arterio-venous anastomoses, which, under certain conditions, serve to short-circuit the blood from arterioles to venules, has been known since the latter part of the 19th century.

They have been described in the skin of man and are particularly abundant in the fingers, toes, nose, and lips (Sucquet, 1862; Hoyer, 1877; Bourceret, 1885; Grosser,

1902), in the glomus coccygeum (Schumacher, 1908), in the corpora cavernosa of the penis (Hoyer, 1877), in the thyroid gland (Modell, 1933), in the kidney capsule (Geberg, 1885; Golubew, 1893), in the membrane of the gastro-intestinal tract (Spanner, 1931), and in the submucous layer of the human stomach (Barclay and Bentley, 1949; Barlow, 1951; Walder, 1952).

In animals, arterio-venous anastomoses have been found in the rabbit's ear and in the toes of birds (Grant, 1930; Grant and Bland, 1931; Hoyer, 1877), in the fingers and toes of mammals (Vastarini-Cresi, 1902), in the tongue (Brown, 1937) and sympathetic chain ganglia of the dog (Nonidez, 1942), in the posterior abdominal region in cats (Miller and Godfrey, 1917), and particularly large ones in the wings of bats (Grosser, 1902). The existence of anastomoses in the living animal was established by the work of Clark and Clark (1930; 1934a, b), who studied their structure and function in vascularized transparent rabbit-ear chambers.

The recognition of the importance of arterio-venous anastomoses, however, was due largely to the classical work of Masson (1924; 1927; 1935; 1937) and Masson and Géry (1927), who made a detailed study of anastomoses occurring in the pulp of the fingers and toes in man; he applied to these structures the term neuro-vascular glomus, and his observation that they may occasionally give rise to tumours stimulated great interest in them. Their histology has been studied in detail by Clara (1927, 1939) and by Masson (1937). Both authors describe epithelioid cells in the outer wall of the arterial segment, and it is believed that they are modified smooth muscle cells: stimulation of the non-medullated nerve fibres, which form a network around them, gives rise to vigorous contraction in the anastomotic wall which also shows an independent rhythmic contractility.

The function of arterio-venous anastomoses is not completely understood, but when dilated they clearly permit a large amount of blood to pass directly from artery to vein without passing through the capillaries, and it is obvious that the amount of blood which may be by-passed through the anastomosis is enormous as compared with the normal capillary flow. Those found in the skin appear to play a part in temperature control, as probably do those in the dog's tongue and the bat's wing, but it is difficult to assume a similar function for those situated elsewhere; Clark (1938) suggested that they may in fact serve no useful function, being merely an unfortunate type of reaction to special circulatory conditions. This view receives some support from his finding that the arterio-venous anastomoses were nearly twice as frequent in a rabbit-ear chamber subjected to sudden temperature changes and infection. In this connection it would be of great importance if they could be shown to develop in the choroid in some types of glaucoma; indeed Cristini (1950) suggests that a reduction in the capillary bed may lead to shunts between the afferent and larger efferent vessels of the choroid.

By the micro-dissection of our Neoprene casts of the *normal* choroid we have not yet succeeded in demonstrating vascular connections which could be interpreted as arterio-venous anastomoses; certainly there are no such shunts associated with the large and medium-sized choroidal arteries. Nor was there evidence of any such structures in the flat bulk preparations or serial sections, although, as already stated, we would not expect such techniques to reveal them. In a few cast preparations the injection was incomplete and the Neoprene was seen to be entirely confined to the arterial side of the circulation: a strong point against the existence of short-circuit connections within the normal choroidal vascular system.

Neoprene casts demonstrate perfectly the "bulbiculi" of the vortex veins

FIG. 5.—Red Neoprene cast of choroid viewed from without, showing tributaries of a vortex vein passing over and under the choroidal arteries. At the point of crossing the vein is flattened and distorted. These swellings correspond to the "bulbculi" of Kiss and Orbán (1951). Anterior region. $\times 33$.

FIG. 6.—Neoprene cast of a vortex vein. Bulbous swellings may be seen on the venous tributaries, where they cross the horizontal artery seen above the mid-line. Anterior region. $\times 10$.

(Fig. 5) described by Orbán (1951) and by Kiss and Orbán (1951), but dissection of the cast proves conclusively that there is no vascular channel connecting the artery and vein at this site. It can be stated with complete confidence, therefore, that these bulbous swellings do not represent arterio-venous anastomoses. They result from simple compression of the vein by the overlying artery, as the two vessels cross in the restricted space of the choroid, bounded by the intra-ocular pressure on the one side and the unyielding sclera on the other. This point will be readily appreciated by reference to Figs 5 and 6. An exactly similar distortion

FIG. 7.—Vessels seen in a rabbit-ear chamber. There is a bulbous swelling of the vein at the point where it is crossed and compressed by an artery. $\times 88$.

FIG. 8.—Neoprene cast of choroid showing a terminal arteriole. Note that the transition into capillaries is strikingly abrupt. Transmitted light. $\times 49$.

of a vein by a traversing artery is seen in like circumstances in a vascularized rabbit-ear chamber (Fig. 7).

As one traces each minute vessel throughout the highly complex network in the choroidal cast, the absence of arterio-venous anastomoses becomes increasingly remarkable. Although the arterioles and venules intimately intertwine, there always appears to be a capillary subdivision, be it large or small, between their ultimate junction. There is, however, no gradual narrowing of the arterioles to capillary calibre as elsewhere in the body, for the transition into capillary subdivisions is unusually abrupt, as also is the formation of the venules (Fig. 8).

FIG. 9.—White Neoprene cast of choroid. Owing to partial filling Neoprene is largely confined to the arterial system. Note density of arterial anastomoses. Equatorial region. $\times 28$.

FIG. 10.—Red Neoprene cast of choroid. Note directness of capillary connections between artery and vein. Anterior region. $\times 50$.

The directness of the capillary communications between arteries and between arteries and veins (Figs 9 and 10) is also a noteworthy point; one can easily imagine that atrophy of the capillary bed in disease might give rise to a compensatory dilatation of the remaining capillaries, with the formation of channels which would function in effect as arterio-venous shunts, thus producing a rise in venous pressure. Such a theory is purely speculative and must remain so until the opportunity arises of examining the choroid by this method, in cases of chronic glaucoma.

As will be readily appreciated on examination of these Neoprene casts, the choroidal vascular network is so exceedingly delicate and complicated that a detailed study of a sufficient number of specimens will take a considerable time.

We are not, therefore, at this stage of our investigation, prepared to state that arterio-venous anastomoses do not occur in the finest branches of the normal choroidal vasculature. Nevertheless, it can be stated that the only type of method likely to demonstrate them has so far failed to do so, and that there is no convincing evidence in the literature to warrant the assumption that they exist.

Choroidal Glomus Cells.—Turning now to consider the reported presence of glomus cells in the periphery of large and medium-sized choroidal arteries, our findings in the normal and pathological choroid will be discussed together.

It should be stated at the outset that horizontal and sagittal sections of some 500 eyes coming to the department for routine reporting, together with serial flat sections and bulk preparations stained by a variety of methods as already indicated, failed to reveal typical agglomerations of glomus cells in the walls of the choroidal arteries. Some preparations, however, show a number of different kinds of cells in relation to the arterial wall which may have been so described. Their characteristics and possible nature will be considered individually.

Ganglion Cells.—It has long been known that the ciliary nerves give off many finer branches, which subdivide into still finer plexuses in the inner layers of the suprachoroid and further on into the choroidal stroma, where they may consist of only a few or a single non-medullated fibre. On the nodal points of these plexuses and scattered along the course of the nerve branches, larger ganglion cells are interposed (Bietti, 1897; Salzmann, 1912). The fibres end in the choroidal vessels and presumably serve a vasomotor function. A typical choroidal ganglion cell is shown in Fig. 11. The significance of these cells in our present discussion is that they may occasionally be seen close to an arterial wall (Fig. 12), and it is important to bear their existence in mind when studying cells in relation to the circulatory system.

FIG. 11.—Choroidal ganglion cell situated at a nodal point in a fine nervous plexus. Flat section of choroid. Holmes's silver $\times 330$.

FIG. 12.—Ganglion cells situated in close relation to an arterial wall. Flat section of choroid. Haematoxylin and eosin. $\times 330$.

Although ganglion cells are considerably larger than glomus cells, being about 20 microns in diameter, they very closely resemble the large clear cells depicted by Loewenstein in his photomicrographs, but they differ in possessing a pale basophilic cytoplasm.

Endothelial Cells.—In sections which cut the choroidal vessels obliquely, a normal endothelial cell may occasionally appear flat and present as a large clear cell with a pale nucleus in the vessel wall; if it is borne in mind, this unusual appearance will create little difficulty. In malignant hypertension and atherosclerosis, however, the endothelial cells may undergo fatty degeneration and appear as large foamy cells in the vessel wall (Fig. 13). Loewenstein pointed out that these cells may be confused with the glomus cells he described, but it is to be remembered that his cells also had an increased frequency in hypertension and atheroma, and that his differentiation was based almost entirely upon the granularity of the cytoplasm. He states that fat staining makes the differentiation complete, but he does not say whether this investigation was, in fact, carried out. Indeed, it would be necessary to cut frozen or carbowax sections for such a purpose, and it would be impossible to ascertain whether or not the cells he depicts in dehydrated material originally contained fat.

In any event, the normal flat endothelial cell, the endothelial cell undergoing fatty degeneration, and the cells which Loewenstein has demonstrated in his photomicrographs, do not, in our opinion resemble glomus cells.

Smooth Muscle Cells.—As elsewhere in the body, the tunica media of the small arteries of the choroid consists of fusiform smooth muscle cells which are always orientated transversely to the length of the vessel, so that in longitudinal or oblique sections they appear as round cells, which, according to the site of section through the spindle shaped cell, will appear large and nucleated or small and non-nucleated. In the choroid, these muscle cells when cut transversely, that is in a longitudinal section of the artery, show a particularly ample cytoplasm. They appear to be somewhat fatter cells than are general elsewhere, and this feature is particularly noteworthy in the posterior choroid (Figs 14 and 15, overleaf) because the arteries are larger here and have a thicker muscular coat; indeed, they are even more obvious and numerous in the ciliary arteries. We believe that these are the cells which Loewenstein has demonstrated in his drawings; they differ, however, from his description in that their average size is only 7–10 microns, whereas he speaks of 10–20 microns, but there may have been some confusion between these cells and the endothelial type of cell seen in transverse sections of the artery.

More recently, similar cells have been noted by Sautter (1952) in the central artery in the region of the lamina cribrosa, and he compares them with those described by Loewenstein and also concludes that they are glomus cells. Schumacher (1938) reported that he had found epithelioid muscle cells apart from anastomotic vessels in the small arteries of several organs, namely in the vasa efferentia of the glomeruli of the kidney and in the small arteries of the thyroid.

FIG. 13.—Large foamy endothelial cells in a choroidal arterial wall in a case of malignant hypertension. The average size of these cells is 18 microns. Haematoxylin and eosin. $\times 650$.

FIG. 14.—Normal choroidal artery showing smooth muscle cells, cut transversely, in the vessel wall. Their average size is 7–10 microns. Flat section of choroid. Haematoxylin and eosin. $\times 380$.

FIG. 15.—High-power view of Fig. 14. Note ample cytoplasm and well-defined cell margins. Haematoxylin and eosin. $\times 800$.

As has already been stated briefly, it is generally agreed among those who have particularly studied the normal glomus, that the cells investing the anastomosis derive from typical smooth muscle cells and pass through a series of transitional forms to reach the characteristic epithelioid or glomus cell, which is the final stage of the process. Murray and Stout (1942) have drawn an analogy between this transition and the cellular investiture of capillaries seen in various organs, as described by Zimmerman (1923), and they have advanced strong evidence that these epithelioid cells are in fact identical with Zimmerman's capillary pericytes. It is known that such cells occur throughout the vascular system, even where no arterio-venous shunts have been found, and, as Murray and Stout have stated, this offers a satisfactory explanation for the occurrence of glomus tumours in the absence of normal glomera.

If we are correct in assuming that Loewenstein has described these large smooth muscle cells, then there is a certain amount of evidence, as indicated above, to support his contention that they may be of the nature of glomus cells. Since, however, the vasomotor nerves terminate, as far as is known, in the ordinary smooth muscle cells, which must ultimately be responsible for the control of blood flow, it is difficult to see, in the present state of our knowledge, what greater significance these larger muscle cells could have, unless, together with an arterio-venous shunt, they form a special apparatus. The choroidal vessels may contain isolated cells or groups of cells which depend for their stimulation upon mechanical or circulating chemical factors rather than upon nervous impulses, but this is not what is generally understood by glomus cells.

In conclusion, therefore, it is felt that the term glomus cells is better reserved to denote those epithelioid cells found in association with arterio-venous anastomoses, and that, until such structures have been demonstrated, it is neither justifiable nor useful to apply the term to the smooth muscle cells of the arteries of the normal choroid.

Cells of Unknown Origin.—On rare occasions, in pathological conditions, large cells may be seen either in the walls of choroidal arteries or scattered sparsely throughout the uveal stroma. They are of striking appearance, having a clear-cut cell membrane, a round or oval nucleus, and an abundant, faintly granular, refractile cytoplasm. They usually appear singly but occasionally in groups, and they vary in size from 10 to 15 microns, in proportion to the amount of cytoplasm present.

Their exact origin has not yet been determined, but they appear to be degenerating cells and are generally associated with inflammatory or degenerative processes. Those shown in Figs 16 and 17 (overleaf) were found in the choroid in a case of central choroidal sclerosis with hypertension. They may be macrophages or plasma cells containing pathological muco-protein secretions, that is, they may be of the nature of Russell bodies, with which, indeed, they may sometimes be associated. A point in favour of this latter view is that the cytoplasm of some of these cells was found to stain positively with the periodic-acid-Schiff stain. Alternatively they may be degenerate muscle cells.

Another possible explanation, although a highly speculative one, is to be found in the work of Goormaghtigh and Grimson (1939) and Goormaghtigh (1939, 1940, 1942, 1943, 1944, 1945, and 1947), who described large afibrillar and granular cells in the juxtaglomerular apparatus and in the media of the pre-glomerular arterioles of the kidney; these acquire a glandular activity which is believed to be the source of the pressor substance in hypertension. These cells were never found in the renal arterioles unless there was hypertensive disease, a condition of anæmia, or serious liver damage. Dunihue and Candon (1940) confirmed the original observations of Goormaghtigh and suggested that they originate from smooth muscle cells. Although Goormaghtigh cells have not been described in the vessels of the eye, it is not improbable that these choroidal cells may be of the same or similar origin.

Their importance in our present investigation lies in the fact that they also very closely resemble the cells depicted in some of Loewenstein's photomicrographs. The fact that his cells occurred in hypertensive disease, and when a malignant growth pressed on the posterior portion of the sclera, is additional evidence of their possible identity.

The nature and origin of these cells require further investigation, but the fact that we have not found them in the normal eye indicates that they are probably not part of a physiological mechanism for the control of blood flow. Furthermore, since they are apparently pathological cells and only rarely occur in layers or large groups around vessels, it is justifiable to assume that they are not of the nature of glomus cells.

In describing these various cells and in attributing to them a glomus-like function, Loewenstein supported his argument by an analogy with the sphincteric contraction which he had described in the retinal and conjunctival vessels (Loewenstein, 1949b; 1951). A similar sphincteric action was postulated by Evans (1947) to

FIG. 16.—Cell of unknown origin in wall of choroidal artery. Note clear-cut cell membrane and refractile material in cytoplasm. Longitudinal section. Haematoxylin and eosin. $\times 1570$.

FIG. 17.—Cell in choroidal artery wall similar to that shown in Fig. 16 but larger, due to a greater quantity of refractile material in cytoplasm. Flat section. Haematoxylin and eosin. $\times 1520$.

explain the narrowing of vessel junctions which he noted in his *post-mortem* preparations of the retina and choroid. While these deductions may well be correct, for there is indirect pathological evidence to support them, it is to be remembered that one of the pitfalls of examining *post-mortem* material, whether in bulk preparations or in sections, is the misinterpretation of artefacts. In sectioning vessels which are as involved and tortuous as those of the choroid, one cannot accept a narrowing of the calibre as definite evidence of a constriction or spasm which existed in life, although it may have so arisen. Similarly, to point to narrowing of the vessel junctions as unequivocal evidence of sphincteric action in

a piece of *post-mortem* retina or conjunctiva, which has been fixed in formalin, cut radially, and mounted flat, is to be unmindful of the limitations of the technique. Indeed, in the case of the retina, it can be shown quite readily that when a *post-mortem* specimen is laid flat a pull is exerted on the vessel junctions where narrowing may then appear; furthermore, if the supporting tissue is partially destroyed by digestion, as may occur in *post-mortem* autolysis, these appearances may be further exaggerated (Fig. 18). While the theories of Evans and Loewenstein are most acceptable, their work is open to criticism on the above grounds and the correctness of their arguments still remains to be proved. Owing to the great difficulty of interpreting *post-mortem* appearances in terms of function, this confirmation is likely to emerge only through the examination of living tissues.

FIG. 18.—Retina injected with Indian Ink and partially digested. The tension, arising in a flat preparation of retina, is exerted at the vascular junction and the narrowing is thus due to artefact. Flat retina. $\times 95$.

FIG. 19.—Choroidal circulation in a living rabbit as seen through a Perspex window in the sclera.

An attempt along these lines has recently been made in this department. Mr. Charles Cook has inserted small circular perspex windows into the sclera of living rabbits and thus, with a light source directed into the eye, it is possible to study the living choroidal circulation. The blood flow through the large vessel layer of the choroid can be readily seen, but the slight head movements of the anaesthetized animal make it difficult to obtain photographic records, which are an essential part of such a study. However, Fig. 19 gives an indication of the results obtained. So far we have not observed any evidence of shunt mechanisms or sphincteric action in the choroids of these animals.

In concluding this paper, it may be recalled that many of the difficulties still existing in our understanding of normal and pathological processes in the eye depend to a great extent for their ultimate elucidation upon an exact knowledge of its minute anatomy. After more than two centuries of careful

research it is remarkable that our information upon some of the essential points should remain so inadequate. It would appear that new techniques are necessary to obtain the accurate evidence required, for the gaps in our knowledge are not to be bridged by speculation. It is hoped, therefore, that the potentialities of these microscopical Neoprene casts when fully explored will help to provide some of the necessary information by which old theories may be tested and new ones constructed.

Summary

(1) A technique for preparing Neoprene casts of the choroidal vessels is described and the advantages of the method indicated.

(2) A detailed study of the choroidal vessels in the normal and abnormal subject is at present in progress at the Institute of Ophthalmology. The above technique, in conjunction with flat bulk preparations and flat serial sections of the choroid, together with observation of the choroid in the living animal, is being used. An account is given of the findings so far obtained.

(3) Other workers have reported the presence of glomus cells and arterio-venous anastomoses in the normal choroid. These findings have not been confirmed by our investigation and the evidence advanced in their reports is critically discussed.

(4) The proposition that arterio-venous anastomoses may develop in the choroidal circulation in pathological conditions is thought to be a feasible one. In at least some cases of glaucoma, these anastomoses might be the anatomical basis of the rise in ocular tension. The Neoprene cast technique should be of value in investigating the choroidal vessels in glaucoma, and the method will be utilized in future studies, when suitable material becomes available.

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