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Double Staining for Bulk Specimens of Retina and Choroid*

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Publications from the Tennent Institute (A. J. Ballantyne, 1941) have shown that in the anatomical investigation of pathological fundus changes, unless we follow a certain routine we run the risk of missing essentials. Slit-lamp examination of the optic nerve and retina is the first step. Ample use of the clearing method of retinal tissue is the second in the scheme described by Loewenstein, 1942.

This method made possible the discovery of microaneurysms in the retina of diabetics with no signs of retinopathy (A. J. Ballantyne and A. Loewenstein, 1944, a and b).

Study of the cleared unstained retina in bulk was a great help in the investigation of hypertensive retinopathy, of new formed intra-, pre- and retro-retinal vessels, of thrombotic changes, of vasculitis retinae, and many other diseases of the eye. It also led the way to the discovery of “cushion cells” in the capillaries, and of intramural vessels in the retinal vascular system (Loewenstein, 1946 and 1948).

The cleared unstained retina and choroid in bulk revealed a multitude of details.

But one of the greatest advantages of this method, which could be called the “simplest type of microscopical investigation,” is undoubtedly the survew it provides of different pathological appearances, without inhibiting the study of the finest details. Thus, the close linkage of several changes became manifest in this simple manner, a linkage which could only have been proved otherwise by painstaking reconstruction of serial sections.

The range of detail we are able to perceive in unstained cleared specimens is increased if we make ample use of reduced illumination by closing down the iris diaphragm, and by dark adaptation of the observer. On the other hand, some parts of the specimen may demand more light. Microscopy of unstained bulk specimens cannot be approached in the “static” manner reserved for stained sections. It has to be performed “dynamically,” one hand always being on the iris diaphragm.

And, last but not least, patience and far more time are required for a single specimen, as we are dealing with a tissue of considerable

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thickness. We therefore focus different layers. If we assume the thickness of a normal histological section to be 10µ we are investigating a tissue of approximately ten times the usual thickness, and each layer of about 10–20µ thickness has to be studied separately. Our work is three dimensional in contrast to the usual two dimensional routine microscopy. The use of a binocular eye-piece is recommended. Use of the phase microscope promising.

In spite of the improvements achieved, I soon felt that staining might increase contrasts, and add something to the elucidation of retinal anatomy. I have used a great many stains since I started this work eight years ago, without the desired result. Recently, more satisfactory results were obtained since I returned to haematoxylin and used a prolonged overstaining solution, and washed in tapwater for ten minutes after careful removal of the vitreous with cotton wool. The specimen is placed into a stain consisting of 2–4 drops of the stock solution of haematoxylin in 5 ml. tapwater, and left in this solution 4–6 hours or longer at room temperature. Control under the microscope until overstaining is achieved. The specimen must appear dark bluish in water, and not translucent enough to distinguish details.

This staining sufficed to show beautifully the dark concentric lines in a case of pressure folds caused by a retrobulbar metastatic tumour. It shows ganglion cell distribution of the macular area and the nerve fibre pattern perfectly.

After rinsing in water, fat staining of the specimen is performed with Sudan IV. I now use the Kay and Whitehead technique with a stock solution in which 2 g. Sudan IV powder is dissolved in one litre of absolute ethyl alcohol at room temperature. It is boiled gently till all the powder is dissolved.

To 7 ml. of this stock solution, 9 ml. 50 per cent. alcohol is added. It is left at room temperature for an hour and filtered. The specimen is put into the filtrate and kept forty-five minutes in the incubator at 37°C. The specimen is washed in distilled water and examined in 50 per cent. glycerine.

I have abandoned the earlier used Spielmeyer technique, in which the specimen is heated till vapours rise. This higher temperature might do harm to the fine retinal structure, and specially to the distribution of fat.

If it is to be kept permanently, it can be framed with tempera or mounted in glycerine jelly to which several crystals of carbolic acid are added to keep off mould infection. This may still occur, unfortunately, after some years, in spite of carbolic acid addition.

The nuclei appear dark purplish, the whole vascular system standing out clearly in the purple mass of ganglion cells, and
nuclei of the inner nuclear layer. If we want to study the deepest retinal layers we turn the specimen upside down. We may achieve the same effect with a dry specimen turning the whole slide. The use of the highest powers of the microscope will depend then on the thickness of the used glass slide, and the focal distance of the front lens in the microscopic objective.

The capillaries are seen in their course, both the superficial network and the deeper one. It is interesting to observe the linkage between the two systems.

All calibre variations are sharply visible, spherical and spindle-shaped dilation stand out clearly (Fig. 1). They are of enormous frequency in different vascular retinal diseases.

Photomicros are unable so far to reproduce the fullness of the stereoscopic impression; sketches combining the images of different focus are more suitable.

Fatty changes of the vessel walls present a lively contrast shining red against the dark blue of the nuclei. Fatty aneurysms are frequent—they are convincing if we find them linked with a fatty capillary like a grape (Fig. 2). We found fatty aneurysms in diabetic eyes, sometimes with blood-filled dilatation of the venules, sometimes without "red" aneurysms. They are even more frequent in cases of thrombotic occlusion of the central vein. Sub-endothelial fatty necrosis is frequent as well, and is easily recognised in arteriosclerosis and atheromatosis, often found side by side in the same retina.
Diabetic and hypertensive retinopathy. ×600. Haemat. + Sudan IV. Fatty aneurysm in connection with fatty capillary.

Diabetic + hypertensive retinopathy. Haemat. + Sudan IV. ×150. Note the blood-filled vessel α with two fatty tributaries. (There are many others of the same type).
It is interesting to find that in some cases of hypertension a certain type of vessel exclusively is fatty (Fig. 3), neither the smaller of capillary size nor the bigger ones. I feel that the significance of this appearance needs explanation, which I have not found so far.

We recognise the fatty droplets in senile and degenerative ganglion cells (Loewenstein and MacGregor, 1943). The fat infiltration of the retinal ganglion cells in relatively young people (after thirty) is surprising and corresponds to similar changes in the brain substance. We are able to judge shape and position of fatty exudates and to decide definitely that the star-shaped exudate in the macular area is found in Henle's fibre layer.

If the specimen needs cutting we might decide to cut it with the fat reaction preserved, and we embed it, therefore, in gelatine, and section it with the freezing microtome, which makes serial investigation difficult.

No additional staining is necessary. Nuclear and fat staining show well in the section, or we embed in paraffin and section it serially, in which case, of course, there is no fat staining.

The technique of bulk investigation of choroidal tissue is similar. The hexagonal cells are so densely pigmented that they hinder the examination of the choriocapillaris. Depigmentation with potassium permanganate and oxalic acid must be carried out for a longer period than with sections. The retinal pigment is more resistant to oxdation than the branched chromatophores.

A very good view of the choriocapillaris is achieved by brushing off the hexagonal cells of their pigment content.

In such bulk specimens, stained with haematoxylin and eosin, I found in the lumen of the capillaries of the choriocapillaris large, prominent, endothelial cells, which might correspond to the "swell"' cells I have described in the venules of the retina (Loewenstein, 1946).

Fatty sub-endothelial droplets in the vessels of the choriocapillaris are frequent. They are liable to escape routine examination. While investigating a considerable part of the choroid in bulk of hypertensive cases, I found only one or two bigger vessels with extensive fatty infiltrates, and the remaining choroid completely normal. I do not understand the significance of sclerotic changes of isolated choroidal arteries.

I found in different cases of uveitis studied in bulk and stained with haematoxylin—Sudan IV, masses of chromatophores filled with shining red droplets of different size, especially if the choroid has been placed outer layer upwards.

It seems that these branched chromatophores are reticulum cells which have phagocytosed fatty droplets. They mostly contain
besides fatty droplets, a granular light brown pigment. It seems that these reticulum cells have engulfed pigment granules produced by other pigment cells, the melanoblasts.

Summary

Double staining with haematoxylin and Sudan IV with clearing of the tissue, is recommended for bulk specimens of retina and choroid. It offers a better chance of discovering such vascular anomalies as pathological anastomoses, aneurysms, sub-endothelial necrosis, and exudates in the retina, especially if fat plays a part.

The method allows a careful study of the choriocapillaris and sometimes shows fatty changes in isolated choroidal vessels. The branched chromatophores in the outer choroidal layers are frequently filled with fat, in cases of uveitis, and they are considered to be phagocytic reticulum cells.

The technique recommended is very simple, and does not presume histological experience. Even a busy practitioner may learn to do the work unsupported by a technician.

REFERENCES

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ANTERIOR FLAP SCLEROTOMY WITH BASAL IRIDENCELEISIS

(A Preliminary Note)

BY

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LONDON

RECENTLY I have tried a combined operation for glaucoma which I think possesses the merits of several of the accepted surgical procedures for this disorder. The operation consists in reflecting a conjunctival flap, fashioning a scleral flap hinged on the corneoscleral junction, a limited cycloclialysis and the inclusion of a basal tongue of iris between the lips of the sclera leaving the sphincter pupillae intact. (See Fig. 1.) The results to date in 29 cases of chronic glaucoma and two of acute congestive glaucoma have been encouraging.
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