NODULAR DYSTROPHY OF THE TRABECULAR MESHWORK*†

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THE description of the microscopic structure of individual trabecular fibres given by Salzmann (1912) has been accepted for many years, and it is only as a result of recent electron microscopic studies that his classical concepts have had to be modified. The principle modification concerns the finer structure of the "clear" zone which lies beneath the trabecular endothelium and surrounds the central collagen core. Salzmann thought this layer to be a structureless glass membrane, but Garron, Feeney, Hogan, and McEwen (1958) have shown that many fibres, resembling collagen but possessing a 1000 Å.U. banding, lie in this zone, and they believe that clumps of these fibres near the central collagen core have been mistaken in the past for elastic tissue (Ashton, Brini, and Smith, 1956). The fine structure of the trabecular fibres has been re-examined in order to correlate the findings of light and electron microscopy more accurately and to define in greater detail the third-dimensional relationships of the fibre components. In view of the numerous reports which attribute the onset of glaucoma to "sclerosis" or thickening of the fibres in the meshwork, a study has been made of variations in the trabecular fibres at different ages.

Material and Methods

Human eyes used in this study were obtained from the eye bank or were surgical specimens containing suspected malignant melanomata. Fixation was performed by injecting 10 per cent. formalin into the anterior chamber or by immersion in formalin at the time of enucleation. Eyes which came from the eye bank were used only if the interval between death and fixation was less than 12 hours. Teased fragments of the meshwork were stained with polychrome methylene blue under coverslips and studied as wet preparations. In this way the relationships between fibre bundles were preserved and the distortions of imbedding and sectioning were avoided. Findings were recorded by photographing optical sections of fibres at different levels. The eyes used in this paper to illustrate the normal trabecular structure were

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removed from a 30-year-old male who died from acute leukaemia and were fixed approximately 15 minutes after death by injections of formalin into the anterior chamber. The eyes used to illustrate degenerative changes were removed from a 53-year-old male and 55-year-old female. Both contained small posterior segment malignant melanomata, both were normotensive before removal, and both were fixed by injecting 10 per cent. formalin in saline into the anterior chambers at the time of enucleation. Several normotensive eyes from middle-aged patients, which contained small melanomata, were fixed in the same way with buffered osmic acid and used for electron microscopy.

Observations on Normal Trabecular Fibres

(1) *Uveal Meshwork*.—The width of the clear zone varied from a very thin layer beneath the endothelial cytoplasm to a thicker layer comprising a third of the diameter of the whole fibre. Those fibres with a thin clear zone consisted of many bundles of microfibrils running in the same direction as the whole fibre. The outermost fibre bundles in the clear zone formed open spirals around the inner core fibres and the transition from outer to inner fibres was frequently impossible to distinguish.

Uveal fibres which possessed a well-defined collagen core and clear zone were found to have a more recognizable fibre-bundle pattern in the outer layers (Figs 1 to 3, opposite). The fibre bundles in the clear zone varied from tiny filaments almost beyond the resolving power of the microscope to well-defined bundles approximately 0.5μ in diameter. The fine fibre bundles could not be traced far and their points of origin and termination could not be determined. Their path ran most frequently around the main fibres at right-angles to the direction of the central core. The transition from core fibre bundles to clear zone fibre bundles was also difficult to define and in certain trabecular fibres large aggregations of fibre bundles could be seen passing from one zone to another and intermingling with each other. This was particularly noticeable where branching of the main fibre had occurred (Fig. 2). The larger well-defined fibre bundle aggregations formed tight spirals around the central core and could be traced for over half the circumference of the main fibre. They were present at several levels throughout the clear zone and frequently crossed each other at acute angles. In optical sections through the centre of the trabecular fibres, the large spiralling fibre bundles appeared as rows of granules lying in the clear zone at various levels between the surface layer of cytoplasm and the central collagen core, in contrast to the fine fibre bundles which were invisible. These features were particularly clear in flat preparations obtained from the meshwork of a cat (Fig. 3) and in electron microphotographs of uveal fibres in cross-section (Fig. 4, overleaf).

FIG. 4.—Electron photomicrograph of a uveal fibre in cross-section, showing primary collagen fibrils in the core and clear zone. Human. $\times 10,500$.

(2) *Corneo-scleral Meshwork*.—The principal feature of interest was the thin clear zone surrounding the central bands of collagen which in the majority of fibres was indistinguishable from the endothelial covering (Fig. 5a). Careful focusing on the surface of broad corneoscleral lamellae revealed a complex arrangement of fibre bundles which appeared as short lines and granules (Figs 5a, b). Many of the larger granules could be resolved into twisted S-shaped structures (Fig. 5c), and in electron photomicrographs large macrocollagen bundles possessing a 1000 Å.U. banding occupied the same relative position around the collagen core (Fig. 6, opposite). These character-

FIG. 5(a-c).—Optical sections through the surface of corneo-scleral lamellae showing numerous granules and S-shaped filaments (arrow) in the clear zone (arrow) which surrounds the collagen core. Human. Polychrome methylene blue. $\times 1125$.

istic coiled fibre bundles were distributed throughout the corneoscleral meshwork. Near Schwalbe's line they were larger and appeared as granules in the clear zone at the edge of the fibres. In places where the cytoplasm formed a thick layer, the pattern of coiled fibre bundles was masked by mitochondria.

FIG. 6.—Electron photomicrograph of a corneoscleral lamella, showing in the clear zone a cluster of macrocollagen fibre bundles which possess a 1000 Å.U. banding. Human. $\times 13,500$.

Near the canal of Schlemm, where the cytoplasm may form a reticular meshwork between adjacent lamellae, it was difficult to differentiate fine strands of cytoplasm from fibre bundles crossing the trabecular spaces.

Observations on Abnormal Trabecular Fibres

(1) *Uveal Meshwork*.—The first abnormal finding noted was a diffuse thickening of the outer clear zone. This did not involve all the uveal fibres and the contour of these fibres remained regular. Optical sections through the clear zone showed thickening of the individual fibre bundles and their spiral course around the central core became more obvious because they were stained more intensely. In optical sections through the centre of the thickened fibres the spiralling fibre bundles appeared as conspicuous dark granules in the clear zone (Fig. 7*a*, overleaf). Additional thickening of the outer clear zone took the form of a series of annular rings which surrounded the central core like a column of doughnuts on a stick. In the outer layers of the rings, fibre bundles could be identified encircling the main fibre. In the inner layers could be seen one or more thin hyaline-like bands which also surrounded the central collagen core (Fig. 7*b*). Another variety of thickening in the clear zone took the form of nodular swellings or excrescences which projected into the intertrabecular spaces like beads of wax on a candlestick (Fig. 7*c*). Optical sections through the nodules revealed a laminated structure comparable to the cut surface of an onion and scattered through the core were irregular hyaline-like granules (Fig. 7*d*).

In their most advanced state the nodules resembled developing Hassal-Henle bodies (Fig. 8, overleaf).

These abnormal changes, illustrated in a composite drawing (Fig. 9, overleaf), were present in one form or another on nearly every uveal fibre in one eye, whereas only a few of the fibres showed early changes in the second eye used to demonstrate alterations in normal structure.

FIG. 7(*a-d*).—Flat preparations of uveal fibres, showing (*a*) diffuse thickening of the clear zone (arrow) and increased staining and thickening of the spiral fibre bundles, (*b*) annular thickening, and (*c,d*) nodular thickening. The nodules are laminated and some contain hyaline granules (*d*). Human. Polychrome methylene blue. $\times 1125$.

FIG. 8.—Flat preparation illustrating Hassal-Henle bodies in several stages of development. Human. Polychrome methylene blue. $\times 1125$.

FIG. 9.—Composite drawing, showing diffuse, annular, and nodular expansions of the clear zone in uveal fibres.

(2) *Corneo-scleral Meshwork*.—Certain of the corneoscleral fibres from older eyes showed a thickened clear zone which was in marked contrast to the meshwork of a young eye. Optical sections through the surface of the thickened fibres showed proliferation and enlargement of the coiled tortuous fibre bundles (Fig. 10*a*). In optical sections through the centre of the fibres, the bulk of the thickened clear zone was occupied by hyaline-like granules which in favourable locations could be resolved into S-shaped filaments. The thickening of the clear zone was generally uniform (Fig. 10*b*) but varied in degree from one fibre to another. Occasional annular (Fig. 10*c*) and fusiform (Fig. 10*d*) thickenings were noted.

FIG. 10(*a-d*).—Optical sections of corneo-scleral fibres showing proliferation and enlargement of the S-shaped filaments in the clear zone (*a*), which may result in a diffuse (*b*), annular (*c*), or fusiform (*d*) thickening of the fibre. Human. Polychrome methylene blue. $\times 1125$.

Only one of the two eyes showed abnormal changes in the corneo-scleral fibres and these were present in the inner lamellae. The outer lamellae in the region of Schlemms' canal could not be differentiated from preparations removed from the 30-year-old eye.

Discussion

Re-examination with the light microscope of both uveal and corneo-scleral trabecular fibres has provided further evidence that the outer clear zone, which was formerly regarded as a structureless glass membrane, in reality contains numerous fibre bundles. These lie in the same relative position as the clumps of "curly" collagen described by Garron in electron photomicrographs and it seems reasonable to conclude that they are identical structures. Although a single primary microfibril is probably not visible with the light microscope, a bundle consisting of several microfibrils can be seen and the

course taken by such fibres can be established. In the uveal meshwork they have a characteristic spiral configuration and in the corneo-scleral meshwork they form a complex pattern of coiled tortuous filaments which vary in size and density. The configuration of the spiralling fibre bundles resembles the construction of a twisted cable and probably confers additional strength and elasticity on the main fibre. The function of the tortuous fibre bundles surrounding the corneo-scleral fibres is, at present, obscure but they may also facilitate the stretching of the meshwork.

Ashton and others (1956) concluded their study of the trabecular meshwork by stating "no correlation was found between the histological appearances and the age of the patient". The results of this investigation indicate, however, that there may be a definite thickening of the trabecular fibres in eyes removed from older individuals. The observed thickening took place in the clear zone surrounding the central collagen core and assumed a variety of forms. In the uveal meshwork there was a diffuse thickening or a localized annular or nodular expansion of the clear zone. In the corneo-scleral meshwork there was also in some fibres a localized or diffuse expansion of the clear zone due to a proliferation and thickening of the coiled fibre bundles. The larger nodules on the uveal fibres closely resembled developing Hassal-Henle bodies. The latter occur with increasing age and it is likely that the proliferative changes taking place in the clear zone of the adjacent trabecular fibres have a similar pathogenesis. Just as the number of Hassal-Henle bodies varies from one eye to another, so the extent of the proliferative changes in the meshwork varies greatly. The process begins in the uveal meshwork and in the innermost layers of the corneoscleral meshwork near Schwalbe's line, and proceeds externally to involve the outer layers, although so far no proliferative changes have been found in the lamellae adjacent to Schlemm's canal. There have been several reports in the literature of sclerosis of trabecular fibres and thickening of the clear zone associated with chronic glaucoma (Unger and Rohen, 1960; Kornzweig, Feldstein, and Schneider, 1958; Teng, Katzin, and Chi, 1957; Garron, 1960). It is significant, however, that the abnormal trabecular changes described in this paper, which were so conspicuous in flat preparations, could not be recognized in routine paraffin sections of the same eye except for a thickened clear zone in occasional uveal fibres which were cut in cross-section. Consequently, it is difficult to decide from the photographs of paraffin sections and from the electron microscope pictures which have been published whether the pathological changes reported are similar to those described in this paper. However, in one report of a glaucoma case (Ashton, 1960), flat preparations of uveal meshwork were identical in appearance to the abnormal uveal fibres illustrated in this paper.

The eye showing the most advanced changes in the trabecular meshwork had an intra-ocular pressure of 14 mm. Hg before removal. Therefore the proliferative and degenerative changes which occurred in the inner layers of

the meshwork were not the result of increased intra-ocular pressure. Should the proliferative changes proceed throughout the meshwork one might expect the outflow of aqueous to become impaired as a result of a reduction in the calibre of the drainage channels or of an alteration in the physical properties of the fibres. Other factors which are difficult to evaluate, such as the extent of the development of drainage channels in late foetal life, the ciliary muscle tone, and the degree of trabecular pigmentation, may determine whether or not a given structural change in the meshwork will lead to glaucoma.

Conclusion

Numerous spiral and coiled fibre bundles can be identified by light microscopy in the clear zone which surrounds the central collagen core of trabecular fibres. The fibre bundles may undergo proliferative and degenerative changes in normotensive eyes which result in diffuse and localized nodular thickenings of the clear zone. The evidence suggests that these structural changes are a senile dystrophy related to the formation of Hassal-Henle bodies.

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