veloped well in spite of inadequate blood supply. The inadequacy of the blood supply was proven by delayed and decreased entrance of dye into the extremity and by the persistent lowering of the skin temperature. The ischemic extremity, however, received some blood as evidenced histologically by the lack of necrosis. The limb may have been kept viable by collaterals from the pelvic arteries and skin vessels. In these experiments it was shown that granulation tissue developed well in spite of inadequate blood supply. This seems to rule out ischemia as a cause for inhibition of fibroblastic proliferation by cortisone.

In our experiments, in which the sciatic nerve was transsected, the action of cortisone was evident in the denervated as well as in the intact extremity. The damage induced to motor and sensory fibers by transsection of the sciatic nerve of the rat has not been adequately studied and the amount of sympathetic fibers destroyed by this procedure is unknown. It was thought advisable therefore to perform a more radical denervation to corroborate these results. In animals with cord transsection and lumbar sympathectomy granulation tissue in denervated areas was affected by cortisone to the same degree as in intact areas.

The mechanism of the inhibitory effect of cortisone upon granulation tissue remains unknown. As each of the possibilities discussed above is ruled out, it becomes more likely that the effect is direct and locally exerted; presumably upon the growing fibroblasts and/or the matrix in which these cells develop.

Summary. Granulation tissue developing around turpentine abscesses in rats is inhibited if cortisone is suspended in the turpentine. This effect is a local one, because in the same animal a simultaneously developing abscess caused by turpentine in which cholesterol is suspended showed no such inhibition. Moderate ischemia does not inhibit granulation tissue formation and presumably does not underlie the inhibitory action of cortisone upon granulation tissue. Denervation does not interfere with this effect of cortisone. The implication of these findings on the possibility of a direct cortisone action upon granulation tissues is discussed.

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A Study of the Fibrous Components of the Vitreous Body of the Electron Microscope.* (18655)

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(Introduced by Chester N. Frazier)

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The vitreous humor of the mammalian eye has been described by Robertson and Duke-Elder(1) as "a gel composed of a meshwork of elastic fibrillae suspended in a viscous fluid." Young(2) and Mörner(3) considered the fibrous residue of the vitreous humor to be collagenous since it dissolved in boiling water and gelled on cooling. Pirie, Schmidt and Waters(4) extracted ox vitreous body with saline, solubilized the fibrous residue

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1. Robertson, E. B., and Duke-Elder, W. S., *Proc. Roy. Soc. Lond. B.*, 1933, v112, 215.
2. Young, R. A., *J. Physiol.*, 1894, v16, 325.
3. Mörner, C. T., *Z. f. Physiol. Chem.*, 1894, v18, 233.
4. Pirie, A., Schmidt, G., and Waters, J. W., *Brit. J. Ophthal.*, 1948, v32, 321.

with hot hydrochloric acid and studied this substance by means of X-ray diffraction, chromatography of the hydrolysate and swelling phenomena. The wide angle X-ray diffraction pattern of the stretched fibrous material was typical of the fiber diagram of the collagen class of proteins(5). Paper chromatography revealed glycine, proline, hydroxyproline, alanine, asparate and glutamate in amounts comparable to those found on the chromatographs of hydrolysed gelatin. From these data and the shrinkage and swelling properties they concluded that the vitreous body consists of a network of collagen-like fibrils imbedded in a jelly consisting of hyaluronic acid and protein. In a recent paper published during the course of the present investigation Schuchardt and Knoch(6) described a meshwork of thin ill-defined fibrils, in formalin fixed vitreous body, observed in the electron microscope.

This paper describes the results of a preliminary study with the electron microscope of the fibrous components of the vitreous body.

Methods. Cattle eyes obtained 3-6 hours after death were used. The vitreous humor was removed intact from the eye and the adhering tissue fragments were carefully dissected away. Fifteen to 20 vitreous bodies were fragmented in the Waring blendor for 5-10 minutes. Foaming occurred during this period, but subsided spontaneously within 20-30 minutes. The resulting fluid was quite viscous. A small amount of sediment produced by centrifugation at 3,000 r.p.m. for 20 minutes showed large fibers, broken membranes and granular debris in the light microscope. The water clear supernatant was then centrifuged at 18,000 r.p.m. for 30-40 minutes and a white, sticky sediment was obtained, which when dried accounted for 0.0028% of the fresh vitreous body by weight. The sediment was then resuspended in distilled water. Similar preparations were made from the central portion of the vitreous body and from the peripheral regions. The periphery was arbitrarily defined as the outer third.

All operations were performed at temperatures of 0-5°C. The above-described high-speed sediments were prepared for electron microscopy by placing a drop of the well-stirred water suspension on the collodion-supporting film of the specimen grid. The preparations were drained and shadowed with 6-8 mg of chromium at 20 cm filament to specimen distance at angles of 6, 8 or 10 degrees. The suspensions were necessarily diluted with distilled water to reduce the amount of material deposited on the film. Some preparations were stained with a 1.0% solution of phosphotungstic acid and were examined directly or shadowed in addition. PTA tends to clean up the background considerably. An RCA type EMU electron microscope in the Department of Biology, Massachusetts Institute of Technology, was used for these studies.

Results. Electron micrographs of aqueous suspensions of fragmented whole bovine vitreous sediment revealed three distinct types of fibrils and a moderate amount of a fine amorphous material. The most abundant form was represented by a long thin unbranched fibril, uniform in width and curving

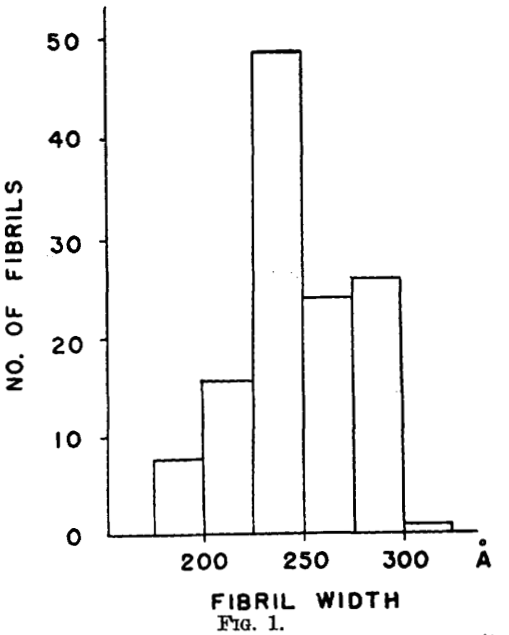

FIG. 1.

Distribution of fibril widths in Angstrom units for smooth fibrils.

5. Astbury, W. T., *J. Int. Soc. Leather Trades Chem.*, 1940, v24, 69.

6. Schuchardt, E., and Knoch, M., *Naturwissenshaften*, 1950, v18, 426.

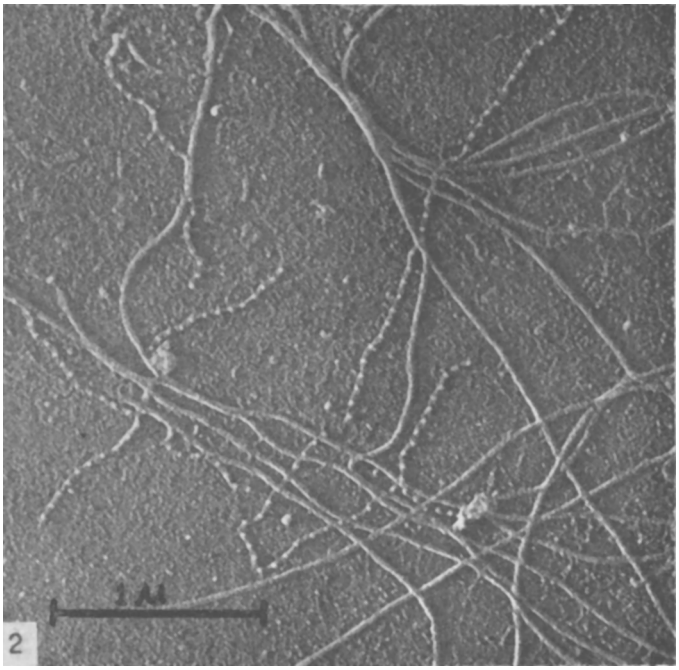

FIG. 2.
Electron micrograph of fragmented diluted sediment of fresh cattle vitreous showing two distinct fibril forms. Chromium shadowed. 29000×.

in relatively smooth arcs. Fibril widths ranged from 150-300 Å, averaging 250 Å (Fig. 1). Occasionally flattened fibrils were also observed which often frayed into extremely thin filaments. There was some suggestion of a very fine axial repeating period in some micrographs, but this was not resolved adequately or consistently enough to warrant determination of period at this time (Fig. 2).

The second type of fibril present in moderate number as compared to that described above, exhibited wide dispersion of length, was more tortuously bent and was clearly striated. The width of the fibrils averaged about 150-200 Å. No branching or fraying was observed. The axial periodicity varied between 500-850 Å, averaging 610 Å (Fig. 3). Resolution has not been adequate to reveal more than a suggestion of fine structure within the axial repeating period. The relative number of these thin striated fibrils varied greatly from preparation to preparation and in general were much less numerous than the nonstriated form.

In addition to the new fibrous type described above, small numbers of striated fibrils about 500-800 Å in width, characteristic of collagen, have been seen in most preparations.

Examinations of sediments prepared from the peripheral and central parts of the vitreous body revealed all three types of fibrils to be present in each instance.

Discussion. The presence of a fibrous structural element in the vitreous body was suspected at least as far back as 1894 and was termed "residual protein" at that time by Mörner. Examination of the fresh vitreous body by light microscopy in the living state and after removal from the eye revealed the presence of membranes and fibers(7). In this study membranes were only observed in the low-speed sediment by light microscopy. Schwarz and Schuchardt(8) and Grignolo (unpublished), using the phase contrast micro-

7. Duke-Elder, W. S., *Br. J. Ophthal.*, 1930, Suppl. 4.

8. Schwarz, W., and Schuchardt, E., *Z. f. Zellforsch. u Mikr. Anat.*, 1950, v35, 292.

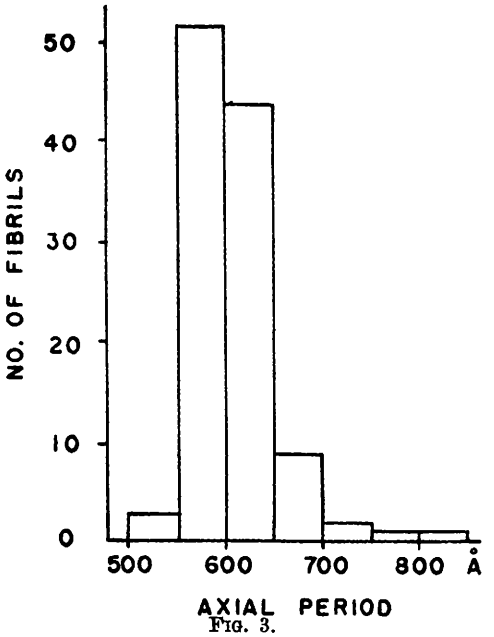

FIG. 3.

Distribution of axial period in Angstrom units for headed fibrils.

scope, observed large fiber bundles which frayed into finer fibrils in the fresh vitreous body. Whether or not these large fibers represent bundles of the finer elements observed in the electron microscope will remain uncertain until they can be examined directly after microdissection.

There is no information at this time as to whether the three fibrous elements are uniformly distributed throughout the vitreous body or are individually localized to specific regions or structures.

It is reasonable to presume that the small fraction of collagen fibrils is responsible for the wide angle collagen pattern obtained by Pirie *et al.*(4). However, it is also quite possible that the other two fibrous units may belong to the collagen class of proteins and also contribute to the X-ray diagram. The periodicity of the fine, striated fibrils is sufficiently close to 640 Å, characteristic of collagen, to suggest a close relationship to collagen.

However, Krause(9) and Schaeffer and Murray(10) have reported significant amounts of tryptophane in vitreous hydrolysates, an amino acid characteristically absent from collagen, suggesting the presence of non-collagenous protein.

By checking the morphology of the elements separated from the vitreous body with the electron microscope it is hoped to produce homogeneous fractions of the different structural elements thereby permitting more definitive chemical analyses.

Summary. 1. Electron micrographs have been obtained of a fibrous fraction separated from fragmented fresh vitreous humor of cattle eyes by centrifugation, which reveal the presence of three morphologically distinct fibril types. 2. The least numerous species is represented by a fibril 500-800 Å in width having an axial periodicity of about 640 Å and intraperiod structure characteristic of collagen. 3. A second fibrous component measuring 150-200 Å in width is characterized by a repeating axial period averaging about 610 Å. 4. A third and most abundant element is a fibril averaging 250 Å in width exhibiting only a suggestion of a very fine axial repeating period.

9. Krause, A. C., *Biochemistry of the Eye,* 1934, The Johns Hopkins Press, Baltimore.

10. Schaeffer, A. J., and Murray, J. D., *Arch. Ophthal.,* 1950, v44, 833.

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