Experimental asteroid hyalopathy

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The presence of asteroid bodies in the vitreous, designated asteroid hyalopathy by Rodman, Johnson, and Zimmerman (1961), is an interesting but rare condition of obscure aetiology. In spite of the presence of these vitreous opacities, there is hardly any deterioration in the acuity of vision, hence the paucity of available pathological material for study and analysis.

Many diseases have been observed to be associated with asteroid bodies from time to time, but no causal relationship has been demonstrated. A possible association with diabetes mellitus and hypercholesterolaemia has been suggested (Smith, 1958, 1965; Hatfield, Gastineau, and Rucker, 1962; Agarwal, Mohan, Khosla, and Gupta, 1963; Bard, 1964). In our series of cases, the occurrence of asteroid bodies in association with hypercholesterolaemia appeared equivocal, but the presence of vascular changes due to associated diabetes and hypertension has been recorded in many patients (Lamba and Shukla, 1970).

The aim of the present investigation was the experimental stimulation of the production of asteroid bodies in the vitreous humour by induced hypercholesterolaemia, and by local alterations in the state of the vitreous.

Materials and methods

(1) *Experimental hypercholesterolaemia*

This was produced in two groups of seven healthy albino rabbits weighing between 1.5 and 2.5 kg. All the animals were given 1 g. cholesterol (Merck) and 5 ml. olive oil daily with the standard diet for 4 to 16 weeks. The control animals received only the standard diet. Fortnightly estimations of blood cholesterol levels were carried out. When the animals were killed, the eyes were fixed in 10 per cent. formalin, and examined by paraffin and frozen sections for lipid material. The stains used were haematoxylin and eosin, Sudan III, and osmic acid.

To observe the results of mild aseptic inflammation in association with hypercholesterolaemia, a mild sterile chorioretinitis was produced by scleral diathermy in the second group of seven rabbits.

(2) *Intravitreal injection of hyaluronidase*

Twelve guinea-pigs were anaesthetized by the open ether method, and anethaine drops were instilled into the conjunctival sac. After the eye had been fixed with forceps, a 26-gauge needle on a tuberculin syringe containing 75 i.u. hyaluronidase* in 0.1 ml. of a solution of physiological saline was carefully inserted into the vitreous through the pars plana. The enzyme was injected slowly into the middle of the vitreous and the needle was prevented from touching any part of the retinal surface. When this was done properly no bleeding was seen to occur intraocularly or on the surface. The needle was then withdrawn and the animal returned to its cage. These animals were observed with the slit lamp and by ophthalmoscopy for up to 8 weeks, after which they were killed and the vitreous was aspirated and examined microscopically.

The fellow eye was treated in exactly the same manner by injecting physiological saline only, as a control.

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*Hyaluronidase BP (Ovine) Tata Fison Industries Ltd.

Observations

HYPERCHOLESTEROLAEMIA IN RABBITS

The average normal serum cholesterol level in the rabbit was 40–60 mg. per cent. In general the serum cholesterol levels showed a steady increase varying directly with the duration of cholesterol feeding, but there was considerable variation in the total blood cholesterol level, ranging from 464 to 2,040 mg. per cent., after 4 to 6 weeks of feeding. With this amount of cholesterol circulating in the blood, obvious fatty lesions could be seen in the eyes and these were proved microscopically.

The changes observed in the various ocular tissues are given below.

Cornea

There was haziness in the peripheral part with superficial vascularization. The corneal opacification occurred at the level of the superficial stroma and extended across only half the circumference. Microscopical examination showed subepithelial sudanophilia with vascularization from the limbus. The corneal thickness was normal.

Iris

Extensive lesions were common. The typical iris plaque began as white dots or flakes which enlarged and coalesced to form dense white areas. Microscopically the earliest change was the appearance of lipid-filled macrophages or foam cells. As the lesion enlarged a characteristic fatty plaque was formed under the posterior epithelium. Occasionally the plaque projected backwards into the posterior chamber covered by iris epithelium (Fig. 1).

FIG. 1 *Xanthomatous plaque in posterior surface of iris with dim outline of foamy cells. Haematoxylin and eosin. $\times 100$*

Ciliary body

Deposits were seen here in all animals, this being the first ocular change. The droplets of lipid and foam cells were located mainly in the anterior parts of the ciliary body and in the ciliary processes. Their origin showed myxoedematous changes as a reaction to the deposition of lipid.

Vitreous and retina

Fundus examination of the cholesterol-fed animals showed no significant alteration from the normal retina apart from a slight indistinctness of the retinal vessels. The vitreous

humour remained clear with no particles or bodies akin to asteroid hyalitis. Microscopical examination failed to demonstrate sudanophilia in the retinal layers, at the optic nerve head, or in the vitreous.

Diathermy to the sclera over the region of the equator was carried out to find out whether hypercholesterolaemia combined with sterile mild inflammation could induce the appearance of vitreous bodies. Ophthalmoscopy showed evidence of reaction in the choroid or retina but no bodies appeared in the vitreous.

HYALURONIDASE INJECTION IN GUINEA-PIGS

In ten guinea-pig eyes, the media became hazy after intravitreal injection of the enzyme. A few cells with plasmoid aqueous were seen in the anterior chamber within 2 to 4 hours. Vitreous liquefaction was observed 24 to 48 hours after injection, and was confined to the area injected. After about 4 days, bright, angular, discrete, refractile particles (probably asteroid bodies) were visible in the anterior part of the vitreous. The particles were static as seen with the slit lamp. The retina appeared to be normal, without holes or detachments. Posterior polar cataract developed in one animal after 7 to 8 weeks.

When examined with polarized light under the microscope, the particles exhibited birefringence of the large inner zone and a thin isotropic peripheral layer of condensed vitreous filaments (Fig. 2A and B). The particles stained intensely with a metachromatic stain, such as Alcian blue. Histopathologically the ocular coats were normal except for scanty infiltration by the inflammatory cells.

FIG. 2A *Vitreous bodies as seen under the microscope by ordinary light. $\times 72$*

B *Same field photographed with polarized light, showing birefringence of inner zone of particles. $\times 72$*

The animals were followed up clinically, and the vitreous humour was seen to begin to clear, reaching a steady state after 6 weeks when the refractile particles became few and fine.

Discussion

Experimental production of asteroid bodies may be discussed under the following heads:

- (A) Hypercholesterolaemia alone as an aetiological factor.
- (B) Hypercholesterolaemia associated with mild aseptic ocular inflammation.
- (C) Altered mucopolysaccharide metabolism of the vitreous.

(A) HYPERCHOLESTEROLAEMIA

There seems to be no direct correlation between the severity of the lesions induced in the eyes and the raised serum cholesterol levels. The length of time during which cholesterol is fed to rabbits or hypercholesterolaemia is maintained determines the amount of deposition of fat. Such an experimental model is more akin to the atherosclerosis of old age than to acute loading of the body fluids with increased lipid.

There seems to be unanimity among workers in their observations on the disposition of fatty material in the limbal area of the cornea, iris, and ciliary body after cholesterol feeding (Cogan and Kuwabara, 1959; Janes, 1964). Our findings agree with theirs. There is lack of agreement, however, concerning the deposition of fat in the retina. In our experiments and in those of Cogan and Kuwabara (1959), no sudanophilia was observed in the retina, but Janes (1964) found increased sudanophilia in the myelin of the optic nerve and nerve-fibre layer of the retina and also in the rods and cones.

The vitreous humour remained clear throughout with no appearance of particles or bodies akin to those of asteroid hyalitis, as in the previous studies of Cogan and Kuwabara (1959) and Janes (1964). Biochemical investigations in series of cases of asteroid hyalitis led Smith (1958), Agarwal and others (1963), and Bard (1964) to think that hypercholesterolaemia was a probable aetiological factor. Since the present experimental series did not demonstrate the deposition of such particles or the presence of such bodies in the vitreous, it is reasonable to suppose that hypercholesterolaemia *per se* is not the principal factor in the development of asteroid bodies. However, Rosen (1952) observed asteroid bodies in cases of primary hypercholesterolaemia.

(B) HYPERCHOLESTEROLAEMIA WITH OCULAR INFLAMMATION

Because the asteroid bodies have often been reported to be unilateral and occasionally to be associated with choroiditis, a mild aseptic ocular inflammation was produced in the experimental animals. The results differed little from those in the first group, and the intraocular inflammation did not seem to contribute to the production of asteroid bodies.

(C) ALTERED MUCOPOLYSACCHARIDE METABOLISM OF THE VITREOUS BODY

The origin of these calcium-containing lipids is uncertain. Pau (1965) thought they originated from the pigmentary epithelial cells. Considering that hyaluronic acid deficiency may assist in the precipitation of the particles (calcium ions and lipid) present in normal vitreous (Lamba and Rajan, 1970), an intravitreal injection of hyaluronidase was given to a group of animals. This injection removed the hyaluronic acid from the vitreous by upsetting the delicate balance and resulted in the precipitation of particles of a calcium-lipid complex. (The viscous polymerized hyaluronic acid is re-formed about 6 weeks after hyaluronidase injection *in vivo*.) The particles observed in the present experiments showed clinical features simulating asteroid bodies. Microscopically the particles conformed to the recently described histochemical features of vitreous bodies

seen in cases of asteroid hyalitis (Rodman, Johnson, and Zimmerman, 1961). When examined under the polarized light they appeared to be composed of fine birefringent material. They stained metachromatically with Alcian blue. The appearance of bright refractile particles in the vitreous after the injection of hyaluronidase was also observed by Pirie (1949), but their nature was not investigated.

Summary

Experiments were carried out to ascertain whether the ingestion of cholesterol by animals could produce visible vitreous bodies (asteroid hyalitis). On the basis of our investigations and the reports of earlier workers, it may be supposed that hypercholesterolaemia *per se* is not the principal factor in the development of asteroid bodies. Local causes may result in the precipitation of macroscopically visible particles of calcium and cholesterol (normal constituents of vitreous). To investigate the role of local changes in the vitreous, hyaluronidase enzyme was injected into the vitreous humour; this produced white, angular, discrete particles which were clinically and microscopically similar to those seen in asteroid hyalopathy.

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