Experimental vascular occlusion in hypercholesterolemic rabbits

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Rabbits fed a diet rich in cholesterol developed hypercholesterolemia and atheromas in the cornea, sclera, iris, ciliary body, and choroid, and a few synchysis scintillans in the vitreous. The fellow eye of these rabbits, in which one nasal vortex vein was occluded, developed far more severe changes in all the above-mentioned eye tissues, especially over the sector of the eye drained by the occluded vein. In hypercholesterolemic rabbits in which a short posterior ciliary artery was occluded, the ischemic area of the choroid was gradually seen to become infiltrated with large crystalline particles. The combination of abnormal blood flow and hypercholesterolemia gives rise to an exaggerated deposition of extravascular lipid material. This experimental model is probably relevant to such human conditions as drusen and lipid deposition in the choroid and cornea and the formation of asteroid hyalosis.

Key words: hypercholesterolemia, vascular occlusion, vortex veins, atheroma, asteroid hyalosis, drusen

Experimental studies have shown that fatty lesions can be induced in the large vessels and viscera of rabbits fed a diet containing a large proportion of cholesterol.

In the eye, atheromatous lesions occur in the cornea as an arcus lipoides and also in the sclera, iris, ciliary body, and choroid. The severity of the eye lesions is related to the length of time under high cholesterol diet and to cholesterol serum levels.1

It would appear that if experimental conditions were modified in order to produce abnormal blood flow the above-mentioned metabolic alterations could probably be exaggerated to produce a model relevant to human conditions such as lipid deposits and drusen formation in the choroid, asteroid hyalosis, and lipid deposition in the corneal stroma.

The present study evaluates the influence of abnormal blood flow induced by experimental closure of a vortex vein draining the anterior segment and the choroid in the formation of fatty lesions in the eye tissues and also examines the changes induced by the closure of a short posterior ciliary artery in hypercholesterolemic rabbits.

Materials and methods

Eleven New Zealand-strain rabbits weighing between 1900 and 2100 gm were used in this study. Nine animals were fed rabbit chow containing 2% cholesterol for a period of 3 to 6 months, and two received a normal diet.

Closure of a vortex vein. Six weeks after the high-cholesterol diet was started, a nasal vortex vein exiting behind the insertion of the internal...
rectus muscle of the right eye was closed in six rabbits, and the fellow left eye was used as a control. The procedure was done after instillation of topical anesthetic drops. The conjunctiva was sectioned around the upper and lower nasal quadrants of the corneoscleral limbus, and the internal rectus muscle insertion was severed. One of the two nasal vortex veins was then closed with the help of a 7-0 silk suture tied 3 mm behind its exit site on the sclera, and the conjunctiva was sutured in place. A few minutes after the procedure, the sector of the conjunctiva and the iris drained by the occluded vein showed engorged vessels, and this event was followed by petechial hemorrhages in both tissues. The choroid in the area drained by the occluded vortex vein was seen to be congested, but no hemorrhages were present. The hemorrhages in the iris and conjunctiva and the choroidal congestion disappeared within 10 to 14 days.

In two rabbits fed a normal diet, closure of one nasal vortex vein induced similar congestive changes as those described, also resolving in about 2 weeks.

Closure of the posterior ciliary artery. In four eyes of two hypercholesterolemic rabbits and in four eyes of two rabbits fed a normal diet, one short posterior ciliary artery was cut off nasal and adjacent to the optic nerve at the entrance site into the sclera with the help of microsurgical scissors. This procedure resulted in an area of ischemia of about 1.5 disc diameter.

Sections of an eye from a rabbit fed a cholesterol diet and from an eye from a rabbit fed normally were obtained 3 months after beginning the experiment and were stained with hematoxylin-eosin for light microscopy.

Electron microscopy. A piece of choroid over the area in which a vortex vein was occluded in a hypercholesterolemic rabbit was obtained 3 months after the diet and was prepared for electron microscopic examination, together with a piece of choroid from a rabbit fed normally. For this study the tissue was fixed in a solution containing 2% glutaraldehyde in 0.1M phosphate buffer, pH 7.4, and postfixed in 2% osmium tetroxide in the same buffer. The tissue was dehydrated through graded alcohols and propylene oxide and was embedded in Epon. Thin sections were cut with diamond knives on an LKB ultramicrotome. Ultrathin sections, stained with uranyl acetate and lead citrate, were examined on a Philips EM 300 electron microscope.

Results

The serum cholesterol of the animals used in this experiment reached a level of 850 to 1800 mg/dl within 6 weeks on a diet containing 2% cholesterol, and subsequently the cholesterol level varied between 1200 and 2100 mg/dl. The level of serum cholesterol in normal rabbits of similar age ranged between 50 and 80 mg/dl. Some of the dieting rabbits
showed severe changes such as loss of hair, wrinkling of the skin, and poor weight increase within 4 to 5 weeks, but the majority showed only mild changes in appearance.

A faint deposition of whitish material occurred in the anterior layers of the cornea in the form of an arcus lipoides as early as 2 to 3 weeks after the experimental diet began. Two to 3 months after the diet began, the arcus lipoides was about 1 to 1.5 mm wide, and a few limbal blood vessels entered into the corneal fatty lesion. A clear interval, about 0.5 mm wide, was present between the fatty lesion and the limbus (Fig. 1).

In each of the six animals with vortex vein occlusion, the arcus lipoides grew rapidly in width and depth, and neovascularization of the area infiltrated by fatty tissue became dense within 25 to 30 days of the vortex vein occlusion. The area of lipoid infiltration was four to six times larger in the quadrant drained by the occluded vein than in the temporal quadrant of the same cornea or than the arcus lipoides in the fellow eye (Fig. 2).

In the iris, atheromas started to appear within 25 to 30 days in the posterior surface of the iris. The iris of rabbits with a nasal vortex vein occlusion presented with larger atheromas than the control eye.

The choroid of the eye in which vortex vein occlusion was induced showed the presence of discrete crystalline depositions in the sector of the choroid drained by the occluded vortex vein (Fig. 3), whereas the fellow eye without an occluded vein did not show such changes. It appeared that the crystals were located under the pigment epithelium, and many of them looked like the colloid bodies seen in humans. In the control eye the choroid presented a rather normal appearance.

In the two hypercholesterolemic eyes that
underwent an experimental closure of a short posterior ciliary artery, the ischemic area of the choroid was edematous for 10 to 14 days and thereafter remained unchanged for a period of 2 to 3 months. Fatty formations then developed within the affected area (Fig. 4). In the rabbits fed a normal diet the ischemic area of the choroid did not change in appearance during the 7 months of follow-up, except for some pigment proliferation at the borders of the affected area of the choroid.

The vitreous of the eyes with a vortex vein
Fig. 8. Fatty infiltration of inner scleral lamellae (arrows). (×400.)

Fig. 9. Micrograph of cholesterol-fed rabbit with vortex vein occlusion. Note small choroidal vessel with lipid droplets (L) infiltrating pericyte cell (P). E, Endothelium; C, connective tissue. (×22,000.)
Fig. 10. Micrograph of cholesterol-fed rabbit with vortex vein occlusion. Note mural cell (P) and histocyte (H) containing lipid droplets (L). Ery, Erythrocyte. (×11,200.)

Fig. 11. Micrograph of cholesterol-fed rabbit with vortex vein occlusion. Note foamy histocyte filled by lipid vacuoles (L) and cholesterol crystals (arrows) in choroidal connective tissue. Ly, Lysosomal bodies. (×24,000.)
occlusion presented with a large number of asteroid formations that began appearing 30 to 40 days after the vessel occlusion (Fig. 5). In the fellow eye, very few of these formations were found.

**Light microscopy.** The cholesterol-fed animals showed diffuse infiltration of foamy histiocytes in the cornea (Fig. 6), iris (Fig. 7), ciliary body, and inner scleral lamellae (Fig. 8). The liver showed vacuolization of the hepatocytes, and the aorta showed severe atheromatous changes.

**Electron microscopy.** The hypercholesterolemic rabbits with vortex vein occlusion showed severe fatty deposits. In the small vessels of the choroid the pericytes were filled with numerous fatty droplets (Fig. 9). The connective tissue of the choroid contained free droplets, but more frequently the fatty vacuoles were seen in foamy histiocytes (Fig. 10). These large macrophages contained, in addition to lipid material, cholesterol crystals and lysosomal bodies (Fig. 11).

Rabbits fed a normal diet did not show any such degenerative changes in the choroidal microvessels and connective tissue (Fig. 12).

**Discussion**

The eye findings in this group of rabbits fed with 2% cholesterol are similar to those found by Janes,1 except for the fact that we
could detect the presence of a few asteroid bodies in the vitreous.

However, where blood flow stagnation is induced by closure of a vortex vein, a marked increase in the extent and severity of atheromatous lesions occurs in the cornea, iris, ciliary body, and choroid, especially in the sector of the eye in which venous stasis was induced, and a large number of asteroid bodies is seen in the vitreous of this group of eyes. It appears from this study that venous stasis has a marked sectorial effect in deposition of fatty material in the eye tissues.

It is also of interest that in an area of choroid deprived of arterial blood supply a gradual and discrete infiltration of lipid material takes place after an interval of time. This material probably originates from a leakage in the surrounding choroidal vessels. The ischemic area of the choroid is the site of deposition of lipid material in the affected tissue, a fact that might well have implications in the understanding of common degenerative conditions in the fundus of the eye.

It is well known that the permeability of endothelial cells is increased in hypercholesterolemia. This fact has been explained on the basis of increased junctional passage and vesicular transport through plasmalemmal vesicles. The pericytes are especially permeable cells. A similar picture of foamy cell pericytes was found in the conjunctival vessels of a patient with high-density lipoprotein deficiency. It is probable that the local leakage of lipovesicles in the connective tissue is highly increased in the area affected by circulatory stasis, whether this is produced by vein or artery occlusion. Subsequently, phagocytosis of the fatty material by the surrounding macrophages will take place.

The finding of lipid material in the small choroidal vessels is not consistent with the results of a previous study of small vessels in the brain of cholesterol-fed rabbits; those vessels did not appear to differ from control microvessels in spite of severe accumulation of lipids in the aortic muscle cells.

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REFERENCES