COATS'S DISEASE*†

III. EXPERIMENTAL STUDY OF THE EFFECT OF STERILE UVEAL INFLAMMATION ON RABBITS WITH AN INDUCED HYPERLIPAEMIA

BY

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IN a previous communication (Woods and Duke, 1963), the literature on Coats's disease was reviewed and evidence was presented which indicated that in the adult form of the disease, a hypercholesteraemia and a history of preceding uveitis were constant findings. In addition, evidence was presented (Duke and Woods, 1963) which indicated that cholesterol was the chief lipid component of the retinal and subretinal exudation in Coats's disease. These findings suggested that the insult from a previous or repeated episodes of a non-granulomatous uveitis might be the predisposing factor in the deposition of cholesterol in the external retina and subretinal space, thus producing the characteristic clinical picture of Coats's disease. The purpose of this paper is to report certain experiments in rabbits which were undertaken in the hope of lending experimental support to this hypothesis.

Design of Experiments

Experiment I.—This was a pilot study in which the objectives were:

- (a) to determine what blood lipid levels could be obtained in the breed of rabbits used in this laboratory by the various diets employed to produce a hypercholesteraemia,
- (b) to determine if a mild sterile reaction such as might be obtained by light coagulation of the retina would result in the deposition of lipids in the external retina and subretinal space of rabbits in which a hypercholesteraemia had been produced.

Experiment II.—This was somewhat more ambitious. Two series of rabbits were sensitized to bacterial and to protein antigens respectively. When a high degree of sensitivity had been produced, several animals in each sensitized group were kept on a standard Sherwood diet. The remainder of the sensitized animals in each group were placed on a hypercholesteremic diet, as determined in the pilot experiment. When a marked hypercholesteremia had been achieved, the right eyes of the hypersensitive rabbits were challenged by repeated intra-ocular injections of the specific antigen, thus producing a prolonged and sustained allergic uveitis. The left eyes remained untouched as controls. Similarly, the right eyes of the hypersensitive rabbits on the normal Sherwood diet were challenged by repeated intra-ocular injections of the specific antigen and the left eyes remained untouched as controls. All rabbits were thereafter examined at periodic intervals, and at the conclusion of the experiment all eyes of the surviving rabbits were enucleated, fixed in formalin, and prepared by appropriate techniques for the conventional haema-

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toxylin and eosin stains, the mucopolysaccharide stains, and the various lipid stains. They were then examined for any histological evidence of lipid deposition in the retina or sub-retinal space.

Production of Hypercholesteremia in the Rabbit

The early experiments of Aylward and Stott (1937) had demonstrated that when normal rabbits were fed on a diet of sunflower-seed meal, which contains 19.4 per cent. protein and 36.9 per cent. lipids, there was a deposition of lipids, chiefly in the form of cholesterol, in the various body tissues examined. This was most marked in the liver, where it reached the total of 10g. cholesterol ester in a 2-kg. rabbit. This was the basic diet used by de Queiroz, Viana, and da Silva (1958) in their investigation of possible ocular changes resulting from an experimental hypercholesteremia. To this diet de Queiroz added 2 per cent. cholesterol and was thus able to obtain levels of 1,600 to 5,000 mg. per cent. of cholesterol in the blood plasma of his experimental rabbits. However, he was unable to demonstrate any significant deposition of cholesterol in the eyes of these animals.

Cogan and Kuwabara (1959) reviewed the literature on experimental hypercholesteremia and pointed out that rabbits lacked an effective mechanism for the removal of excess cholesterol, and that a hypercholesteremia could readily be produced in them by the simple procedure of adding cholesterol to their diet, which normally contains very little lipid. This hypercholesteremia could be enhanced by the further addition of cholic acid, a substance which facilitates the absorption of dietary cholesterol. Using a normal rabbit diet, to which cholesterol was added, these workers were able to produce a hypercholesteremia and to demonstrate the deposition of cholesterol in the ocular tissue. This deposition was both intracellular (in macrophages) and extracellular and was located in the superficial layers of the corneal stroma, peripherally; as plaques in the posterior iris stroma; as fine spherules or granules beneath the ciliary epithelium; and in the deep sclera. It was specifically stated that no lipid material was present in the optic nerve, retina, or subretinal space. They also found that trauma—thermal cautery of the cornea, diathermy cautery of the sclera—enhanced the deposition of cholesterol in these sites. At the scleral site this was done “to determine whether or not a condition might be induced that simulated Coats’s disease. This was found not to be the case, but a great increase in the deposition of lipid in the sclera did occur. . . .” Cogan and Kuwabara did not state the blood lipid levels attained in their experimental rabbits nor did they comment on the time required for the production of these ocular lesions in the rabbits on the cholesterol-reinforced diet.

RESULTS

Experiment I (Pilot Study)

Following these above leads concerning the production of a hypercholesteremia in the rabbit, sixteen normal rabbits were divided into four groups of four animals each. Group I received the usual normal Sherwood diet given rabbits in this laboratory. Group II received a diet of sunflower meal only, 200 g. being offered per diem. This diet was somewhat unpalatable, the rabbits consuming only a portion of the amount offered. Group III received the sunflower meal with 2 per cent. cholesterol added. Group IV received the combination of sunflower meal plus cholesterol, with the further addition of 0.5 per cent. cholic acid.

The mortality among all four groups of animals was remarkably high. This appeared to be due chiefly to extraneous factors. Several died as a result of cage injuries, and some during a period of extraordinarily hot weather. During this

same period there was also a high mortality among other rabbits housed in the same animal room, so that the role of the high fat diet in this mortality could not be determined.

After 45 days on the respective diets during which period the light coagulation studies were performed as described below, there were two survivors in Group I, three in Group II, two in Group III, and two in Group IV. Blood from these survivors was then drawn for lipid level determinations and the animals were killed, the eyes being removed for histopathological examination.

SERUM LIPID DETERMINATIONS

Technique.—The various lipid studies on the experimental rabbits were done by Mr. J. Markowitz, under the supervision of Dr. D. A. Turner, in the Lipid Research Laboratory of the Sinai Hospital in Baltimore. While the methods employed in Dr. Turner's laboratory are slightly different from those in the Clinical Laboratory of the Johns Hopkins Hospital, the results are quite comparable to those already reported in the previous clinical communication. The techniques used by Dr. Turner may be summarized as follows:

(A) *Total Lipids.*—The method used was essentially that of Bloor (1915) (also used in the Johns Hopkins Hospital Laboratory) and represents in mg. per cent. the total extractable lipids in the blood plasma.

(B) *Cholesterol.*—The method used by Dr. Turner was that of Hanel and Dam (1955). The resulting figure, in mg. per cent., represents the sum of the free cholesterol and the cholesterol fatty acid esters.

(C) *Phospholipids.*—The method employed in Dr. Turner's laboratory was the procedure of Bartlett (1959). This gives the weight of the total extractable phosphorus in mg. per cent. This includes the phosphorus in lecithin, the phosphatides, cephalins, syringomyelins, and any phospho-glycerides which may be present. It does not include the phosphorus in the small phospho-lipoprotein fraction. To obtain the total phospholipids, the extractable phosphorous figure is multiplied by a factor of 25. This gives in mg. per cent. the figure for the phospholipids which is comparable to that reported in the previous clinical communication.

Results.—The effect on the serum lipids of the various types of hypercholesteremic diets employed is shown in Table I.

TABLE I
AVERAGE PLASMA LIPID VALUES IN RABBITS AFTER 45 DAYS ON NORMAL AND HYPER-CHOLESTERAEMIC DIETS RESPECTIVELY

Group	Diet	Mean Plasma Lipids (mg. per cent.)		
		Total Lipids	Total Cholesterol	Phospholipids
I	Normal Sherwood	1,680	145	194
II	Sunflower Meal Only	1,916	212	257
III	Sunflower Meal plus 2 per cent. Cholesterol	4,400	1,240	638
IV	Sunflower Meal plus 2 per cent. Cholesterol plus 0.5 per cent. Cholic Acid	3,019	770	358

The serum lipid values of the animals in Group II (sunflower meal only) after 45 days on this diet were only slightly raised when compared to those observed in the animals on the normal Sherwood diet. However, the animals of Group III and IV showed a marked increase in total lipids, total cholesterol, and the phospholipids after 45 days on the respective diets. Thus, the evidence of a hypercholesteremia in these groups of animals is well documented.

LIGHT COAGULATION.—This procedure was performed for the authors by Dr. Hunter Little, employing the Zeiss light coagulator. After each group of animals had been on the diet for one week the photocoagulation studies were begun. The right eyes only were utilized, the left eyes being left as controls. Four or five photocoagulations, each of about 10 sec. duration, were given to adjacent areas within one quadrant of the retina. Such photocoagulations were repeated in a different quadrant of the retina at intervals of every 2 weeks. After each quadrant had been thus treated, the surviving animals were killed and the eyes enucleated for histological study.

Clinical Observations.—Immediately after the photocoagulation, small focal spots of retinal coagulation were noted. Within 2 weeks these foci were replaced by sharply demarcated areas of scarring. In the pigmented rabbits there was sparse pigmentation of these scars. In the albino rabbits there were only flat, sharply-demarcated areas, slightly yellowish in colour. These were not raised and had none of the subretinal exudation characteristic of Coats's disease.

Histopathological Observations.—After enucleation, the eyes were fixed in formalin, and then cut into halves. One-half of each was embedded in paraffin for routine haematoxylin and eosin staining and the other half in gelatin for frozen sections and staining with oil-red-O for lipids. The results of the histological examination of these sections may be summarized as follows:

(A) *Haematoxylin and Eosin Stains.*—The lesions appeared as focal, sharply-demarcated areas of retinal thinning or scarring. In the pigmented animals there was some degree of pigment proliferation at the margin of the lesions. Within the lesion itself the retina was either unrecognizable as such or appeared as a thin strand adherent to the choroid. Nowhere was there evidence of inflammation.

(B) *Oil-Red-O Stains.*—In the control Group I, which received the normal Sherwood diet, the non-treated left eye appeared normal. The retina of the right eye, which had been the site of the photocoagulation, showed only the lipid normally present in the myelinated nerve fibres of the rabbit retina. There was no deposition of lipid at the sites of light coagulation.

In Group II, in which there was only a moderate degree of hypercholesteremia, and in Groups III and IV in which all rabbits had marked hypercholesteremia, there were in the non-treated left eyes varying and usually slight degrees of lipid deposition in the cornea, iris, ciliary body, and sclera. The localization of this lipid in the eyes of hypercholesteremic rabbits will be described in more detail below. In the treated eyes, at the sites of light coagulation, the retina showed loss of all normal structures. A thin streak of lipid was often noted lying apparently at the level of the outer nuclear layer. There was, however, no evidence of subretinal deposition of lipid. Also there was no evidence of cellular reaction or inflammation.

The histopathological findings at these sites of retinal photocoagulation are, in general, in accord with those described by previous investigators (Kissen, Delaney, and Wachtel,

1961; Okun and Collins 1962). In addition, this experiment demonstrated that, although a hypercholesteraemia could be produced in rabbits by the administration of a cholesterol-reinforced diet, this hypercholesteraemia was insufficient in itself to produce a significant degree of cholesterol deposition in the eyes of the animals during the short life of this experiment. Further, the added stimulus of photocoagulation was sufficient to produce only a mild deposition of lipid in the retina at the actual sites of retinal coagulation. If local inflammation in the presence of a hypercholesteraemia were to be considered an instrumental factor in the local deposition of cholesterol, a stimulus resulting in a more protracted state of inflammation would be necessary. To this end, Experiment II was undertaken.

Experiment II

The prime purpose of this experiment was to determine if a chronic, or repeated attacks of, allergic uveitis, in the presence of a concomitant hypercholesteraemia, would facilitate the deposition of cholesterol in the ocular tissues, and especially in the external retina and subretinal space. To explore this question, two groups of sixteen rabbits each were sensitized respectively to beta streptococci (Group A) and to bovine albumin (Group B). After sensitization was accomplished, four animals from each group were reserved as controls and were maintained on a normal diet. The remaining twelve animals in each group were placed on a high cholesterol diet. After the hypercholesteraemia had developed, the eyes were challenged by intravitreal injections of the specific antigens.

Technique of Experiment

Sensitization.—This was accomplished after the manner described by Woods, Friedenwald and Wood (1955). At the completion of the series of intravenous sensitizing injections, the sensitivity of the animals was demonstrated by intracutaneous injections of the respective antigens. All were found to be highly sensitive.

At this point blood was drawn from all animals in each group and plasma lipid levels were obtained to serve as control values (see below). Following this the animals were placed on their respective diets.

2 weeks after the initiation of these diets all sensitized rabbits (both control diet and test diet animals) were challenged by the intravitreal injection of 0.1 ml. of the specific antigen in the right eye. The left eye of each animal was left untouched to serve as a control. As the initial ocular reaction subsided, subsequent similar injections were repeated at approximately weekly intervals. In this manner the eyes were kept chronically inflamed with an allergic uveitis over a period of 7 full weeks.

Diet.—The four sensitized control animals from each group were maintained on the normal Sherwood diet throughout the experiment.

The twelve test animals of each group were offered 100 g. per day of a diet consisting of sunflower meal plus 2 per cent. cholesterol. In general, the test rabbits accepted this diet poorly. A few consumed practically all the diet offered, while others rejected the diet almost completely, preferring literally to starve to death rather than eat the cholesterol-reinforced sunflower meal. The average amount ingested daily by the streptococcus-sensitized rabbits was 40 g., and by the albumin-sensitized rabbits 34 g. As a result of this diet there was a sharp weight loss in each group and a high mortality. The weight loss at the conclusion of the experiment in the streptococcus-sensitized animals was approximately 17 per cent. of the original body weight, while that in the albumin-sensitized group was about

30 per cent. of the original body weight. The eyes of animals which died in the course of the experiment (either as a result of the diet or through accidental causes) were enucleated and reserved for histological study.

Plasma Lipid Studies.—After the animals had been sensitized but before the initiation of the special diets, blood was taken from all 32 rabbits to be used in the study and various fractions of the plasma lipids were determined (Table II). These analyses were all done in the Lipid Research Laboratory of the Sinai Hospital, using the same technique as in the pilot experiment. The average values for each lipid fraction were somewhat lower than those found in the rabbits on the normal diet in the pilot experiment.

TABLE II
AVERAGE PLASMA LIPID VALUES IN SENSITIZED RABBITS ON NORMAL AND HYPERCHOLESTERAEMIC DIETS

Status	Time on Diet (wks)	Sensitization	Mean Plasma Lipids (mg. per cent.)		
			Total Lipids	Total Cholesterol	Phospholipids
Normal Diet All Rabbits	Before Onset	None	901	57	68
Diet of Sunflower Meal plus 2 per cent. Cholesterol	3	β -Streptococcus	2,464	963	349
		Bovine Albumin	2,415	869	346
	10	β -Streptococcus	9,328	4,485	1,643
		Bovine Albumin	4,102	2,132	683
Normal Sherwood Diet	10	β -Streptococcus	781	32	108
		Bovine Albumin	813	52	107

After 3 weeks on the sunflower meal-cholesterol diet, the total plasma lipids of the test animals increased approximately two-and-a-half fold, and after 10 weeks approximately ten-fold. This increment was due chiefly to the increase in the cholesterol fraction, which increased approximately 15-fold after 3 weeks, and almost 40-fold after 10 weeks. The increase in the phospholipids was considerably less. The average values for these various fractions in the two groups of sensitized animals on the normal and on the hypercholester-aemic diets are shown in Table II. It should be noted that after similar time intervals on the hypercholester-aemic diet the lipid fractions were not so high in the albumin-sensitized group as in the streptococcus-sensitized group. However, the albumin-sensitized group seemed to find the hypercholester-aemic diet less palatable than did the streptococcus-sensitized animals and lost considerably more weight during the course of the diet. This may explain the lower lipid values in this group.

CLINICAL OBSERVATIONS.—The clinical ocular reactions to the intravitreal challenging injections of the antigen were confined chiefly to the posterior ocular segment. Exceptions to this were the development of a corneal arcus and mild to moderate degrees of iridic inflammation in some of the test animals on the cholesterol reinforced diet. The observation of any retinal changes was somewhat unsatisfactory, particularly as the experiment proceeded, due to the development of opacities in the media, *i.e.* inflammatory masses in the vitreous and cataractous changes in the lens. All these changes were somewhat less marked in the streptococcus-sensitized rabbits (Group A) than in the albumin-sensitized rabbits (Group B).

In Group A there was only a negligible or slight reaction following the first challenging intravitreal injection. After the second injection, one week later, the vitreous became so hazy in one of the control animals and in four of the test diet animals that the fundus could no longer be seen. After the third injection one week later, whitish masses appeared in the vitreous of all injected eyes in all the animals. Also, in three of the test diet animals an arcus of the cornea began to develop. Traces of fibrin were present in the anterior chambers of three of the test diet rabbits and a detached retina was noted in one. After this, the development of cataracts in all of the test diet group and in one of the control diet animals made further evaluation of fundus changes impossible.

In Group B there was an immediate reaction to the challenging intravitreal injection of bovine albumin antigen. One of the control rabbits and four of the test diet rabbits developed a marked vitreous haze. This progressed and on the following day was so intense that the fundus could no longer be seen. In another test rabbit a definite retinal exudate was noted the day after the first challenging injection. As the experiment progressed four of the test animals developed a marked arcus of the cornea, and two developed retinal detachment. Retinal detachment was also observed in one animal on the normal control diet. Heterochromia iridis (of a peculiar greenish-yellow colour) developed in the injected eye of two of the test animals, and a vitreous haemorrhage in one. At the conclusion of the experiment, all of the control and four of the eight living test rabbits had cataracts. Two of the other rabbits had developed a mass in the vitreous and one of these had developed buphthalmos as well.

HISTOPATHOLOGICAL OBSERVATIONS.—After death during the course of the experiment or at the termination of the experiment, the eyes of the rabbits were enucleated, fixed in formalin, and cut into halves. One half was embedded in paraffin and the sections stained with haematoxylin and eosin and with the various stains for the mucopolysaccharides. The other half was embedded in gelatin and stained with oil-red-O and the differential lipid stains.

(A) General Pathological Changes.—The general changes observed in the injected eyes which had been subjected to a prolonged allergic uveitis were, with one exception, comparable to those described by Woods and others (1955). These consisted of a fibrinous exudate containing numbers of mononuclear inflammatory cells on the surface of the ciliary epithelium and to a lesser extent on the anterior surface of the iris; diffuse and also focal infiltrates of similar inflammatory cells in the choroid; retinal oedema and retinal detachment with inflammatory cells in the subretinal fluid; and oedema of the optic disc with a mononuclear cellular infiltrate on its surface.

The striking exception to this conventional picture was the presence of quantities of foam-filled macrophages in the injected eyes of the rabbits with hypercholesteremia. These cells were found in the corneal stroma near the limbus, in the iris stroma, in the ciliary processes, and associated with the inflammatory exudate over the ciliary body and in the vitreous. A few such cells were present in the subretinal exudate and on the surface of the optic disc. Many were present in the suprachoroidea and inner sclera.

In the non-injected control eyes of the rabbits with hypercholesteremia similar foam-filled macrophages were also found in the cornea, iris, ciliary processes, and inner sclera. However, in these control eyes, these cells were present in much fewer numbers.

These macrophages were not found at all in the eyes of the animals on the normal Sherwood diet.

(B) Lipid Studies.—In the rabbits on the normal diet, in the non-injected left eyes, the only lipid found was that normally present in the myelinated nerve fibres of the retina. In the injected right eyes a variable amount of lipid was found in the internal retina and in the

vitreous. This appeared to be associated with necrosis of the retina or with an inflammatory reaction in the vitreous. This lipid was not cholesterol, the Schultz reaction being negative. Other differential lipid stains were also negative. Thus, the exact nature of the lipid was not determined. The probable source of this lipid, however, was the damaged myelinated nerve fibres which in the rabbit are so abundant in the retina and in the optic disc. All other structures within the eye were free of lipid.

The eyes of the rabbits on the hypercholesteræmic diet showed a similar picture in the streptococcus-sensitized albumin-sensitized groups. In the non-injected, control eye there was a deposition of lipid exactly as described previously by Cogan and Kuwabara (1959) in the hypercholesteræmic rabbit. Near the limbus of the cornea in the superficial stroma there were macrophages filled with lipid (Fig. 1). Similar lipid-filled cells were present in the deep stroma of the iris and as plaques beneath the iris pigment epithelium. In the ciliary body there were subepithelial intracellular deposits and fine extracellular deposits of lipid. Numerous lipid-filled cells were present in the suprachoroidea and in the inner sclera. No lipid was found in the retina except that normally present in the myelinated nerve fibres.

FIG. 1.—Perilimbal lipid deposition in cornea of non-injected eye of rabbit sensitized to bovine albumin on hypercholesteræmic diet. Gelatin-embedded frozen section. Oil-red-O. $\times 18$.

In the injected eyes which had been the site of a prolonged allergic uveitis, the deposition of lipid was much more intense—at least 10 to 20-fold that present in the fellow, non-injected eyes. In the cornea the lipid was not confined to the superficial stroma in the limbal area, but usually extended across the entire cornea and occupied the outer half of the stroma (Fig. 2, overleaf). The concentration of lipid in the iris stroma was much increased, the lipid infiltrating the sphincter muscle. Numerous aggregates of foam-filled cells together with extracellular lipid engorged the ciliary processes. Moderate numbers of lipid-filled macrophages were present in the subretinal exudate, but only rarely were similar macrophages noted in the retina. When present in the retina they appeared to be associated with retinal necrosis or with an inflammatory reaction. Lipid-filled cells were numerous in the exudate over the optic disc. Both choroid and sclera were heavily laden with the lipid.

When sections of these eyes were subjected to the Schultz reaction for cholesterol, there was intense staining in every area where lipid deposition had been observed (Figs 3 and 4, overleaf).

FIG. 2.—Massive lipid deposition in outer half of cornea of injected eye of rabbit sensitized to bovine albumin on hypercholesterolaemic diet. Gelatin-embedded frozen section. Oil-red-O. $\times 18$.

FIG. 3.—Cholesterol appears as black droplets in outer half of cornea of injected eye of rabbit sensitized to bovine albumin on hypercholesterolaemic diet. Gelatin-embedded unstained frozen section. Schultz reaction. $\times 18$.

FIG. 4.—Cholesterol appears as dark droplets which form plaque beneath pigment epithelium of iris of injected eye of rabbit sensitized to bovine albumin on hypercholesterolaemic diet. Gelatin-embedded unstained frozen section. Schultz reaction. $\times 30$.

The differential lipid stains for the neutral fats and the fatty acids revealed only occasional traces of these lipids. It was apparent that the great bulk of the lipid deposited in these injected eyes was cholesterol.

(C) **Mucopolysaccharide Studies.**—To ascertain what role, if any, might be played by the mucopolysaccharides in this deposition of cholesterol, representative sections of eyes from rabbits on both the normal and the hypercholesterolaemic diet were stained with the PAS stain and with colloidal iron stain. The results were as follows:

(1) *PAS Stain.*—In the injected eyes of the rabbits on the normal diet, PAS-positive retinal exudates were extremely rare, small, and insignificant. Masses or globules of PAS-positive debris were present in the vitreous. Occasionally, coarse PAS-positive granules were identified in macrophages in the vitreous or in the subretinal space. There was no thickening of the subintimal basement membrane of the retinal vessels.

In the injected eyes of both groups of rabbits on the hypercholesterolaemic diet, only one eye of one animal showed a significant deposition of PAS-positive material in the outer retina. In all the injected eyes, coarse PAS-positive granules could be identified in the macrophages present in the vitreous and in the subretinal space. Only very rarely could such PAS-positive granules be found in the other foam-filled macrophages so abundantly present elsewhere in these eyes. Thus, the locus of this intracellular PAS-positive material was quite similar in the injected eyes of the animals on the normal diet and of those on the hypercholesterolaemic diet.

(2) *Colloidal Iron Stain.*—In both the control and injected eyes of animals on a normal diet, fine acid mucopolysaccharide (AMP)-positive granules were occasionally noted in the retina. In only one injected eye were such AMP-positive granules noted within macrophages. In this instance, the macrophages were adjacent to the inner surface of the partially necrotic retina.

In the injected eyes of the rabbits on the hypercholesterolaemic diet, all the foam-filled macrophages in both the subretinal and vitreous exudates were invariably filled with a delicate reticulum of fine AMP-positive granules. In addition, the foam-filled macrophages elsewhere in the eye—in the cornea, iris, ciliary processes, choroid, and sclera—were also filled with a similar AMP-positive material. However, only a very few such macrophages could be found in the retina proper. It should be noted that the macrophages which contained this AMP-positive material were the same as those which contained the cholesterol.

DISCUSSION

Each of these two experiments failed completely to simulate the characteristic clinical and histological lesions of Coats's disease. In the initial pilot experiment the hypercholesterolaemia was of insufficient duration to produce a significant deposition of cholesterol within the eye. And the inflammatory stimulus employed—photocoagulation—did not result in any degree of prolonged inflammation. There was a minimal deposition of lipid in the retina at the sites of photocoagulation.

In the second experiment higher serum lipid levels were achieved and maintained for a more prolonged period. In addition, a posterior uveitis of protracted duration was achieved. Histopathological examination disclosed the presence of many cholesterol-filled macrophages in the subretinal space, but there were also equal numbers of these cells present in the vitreous. Of greatest significance with respect to the objective of the experiment was the failure to demonstrate to any significant degree the presence of free cholesterol and cholesterol-filled macrophages in the retina proper. Elsewhere in the eye there was a massive deposition of cholesterol. Possibly, with an inflammatory stimulus of lesser magnitude and with a less marked degree of hypercholesterolaemia the result might have been different.

In the retina of the rabbit the blood vessels are confined to the two wing-shaped areas of medullated nerve fibres spreading horizontally on either side of the optic

disc (Michaelson, 1954), the remainder of the retina being rather avascular. The larger vessels lie on the surface of the retina, and the capillary loops which extend from them penetrate only into the nerve fibre layer (Prince, Diesem, Eglitis, and Ruskell, 1960). It is conceivable that, if the retina of the animals studied had had a vascular system more nearly comparable to that in man, the result might have been different. It is notable that, in the rabbits with hypercholesteremia, the retina was actually the site least involved in the cholesterol deposition. All that can be concluded is that, although given abundant opportunity, the rabbit does not develop a condition comparable to Coats's disease in man. However, these experiments do show two things:

(1) A local inflammatory process in the eye, in the presence of a concomitant hypercholesteremia, does accelerate and augment the deposition of cholesterol in the intra-ocular tissue.

(2) This deposition of cholesterol is invariably associated with the presence of an acid mucopolysaccharide in the histiocytes which contain the cholesterol.

In this respect, the experimental deposition of cholesterol in these eyes is exactly comparable to the deposition already demonstrated in the lesions of Coats's disease in man and in human xanthomatosis. This observation supports the original hypothesis of Faber (1949) that the intermediary action of such an acid mucopolysaccharide is an instrumental factor in the deposition of cholesterol in the tissues.

CONCLUSIONS

(1) Photocoagulation of the retina in the hypercholesteremic rabbit produces a minimal deposition of lipid at the site of the retinal lesion. However, there is no subretinal lipid deposition and the histopathological picture in no way resembles that of Coats's disease in man.

(2) A prolonged allergic posterior uveitis in the hypercholesteremic rabbit does not produce changes in the retina comparable to those of Coats's disease in man.

(3) In the hypercholesteremic rabbit, this allergic uveitis does accelerate and augment the deposition of cholesterol throughout the ocular tissues. The retina, however, is virtually spared.

(4) This deposition of cholesterol in the ocular tissues is invariably associated with the presence of an acid mucopolysaccharide at the site of deposition of the cholesterol.

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