THE ROLE OF CHOLESTEROL IN THE PATHOGENESIS OF COATS' DISEASE

BY *James R. Duke*, M.D.*

THE PURPOSE OF THIS THESIS is to report certain clinical and histopathologic observations, biochemical determinations, histochemical reactions, and differential lipid studies, and, finally, the results of animal experimentation, all relative to Coats' disease. These studies taken as a whole appear to establish Coats' disease as a definite clinical and pathologic entity, with a quite probable pathogenesis. In this thesis the various phases of this composite study are reported in three sections. Part I consists of a digest of the pertinent literature on Coats' disease, especially as concerns a lipid deposition, together with clinical and biochemical observations. Part II is a full report of various studies concerned with histochemical reactions and differential lipid stains. Part III concerns the animal experiments. Parts I and III, in which there was an associate investigator, will be reported elsewhere in full detail[1,28] and are reported here in summary only. Part II, which was the full responsibility of the present author, forms the chief burden of this thesis and is reported here in full detail. In this phase of the work my associate acted only in an advisory capacity. Summaries of Parts I and III are included here for the purpose of thus presenting a full picture of the entire investigation and providing a rationale for the various conclusions.

INTRODUCTION

The stimuli which prompted these investigations were observations on two adult patients who were first seen in 1958 and 1960. Both of these patients showed the ophthalmoscopic picture of classical Coats' disease. Both had authenticated histories of repeated attacks of nongranulomatous uveitis prior to the development of the present fundus

*From the Wilmer Ophthalmological Institute, Johns Hopkins Hospital and University, Baltimore 5, Maryland.

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picture. On diagnostic survey both patients showed a marked disturbance in their lipid metabolism, especially a hypercholesteremia. These findings suggested that in some adults the repeated attacks of uveitis might in some way be the trigger mechanism responsible for the later development of the Coats' symptomatology and thus be concerned in the pathogenesis of the disease.

PART I

In the exploration of the above idea, the voluminous and often repetitive literature on Coats' disease, especially that concerning lipid deposition, was first reviewed. Thereafter, all patients in the clinical diagnostic file of the Wilmer Institute were, as far as possible, recalled and re-evaluated, and plasma lipid studies were made on their sera. The histologic material on Coats' disease, available in the pathological laboratory was then exhumed, restained, and restudied. The following is a summary of these various studies.

A. LITERATURE

In 1908 Coats[2] described three forms of a rare retinal disease with massive exudation. He divided his reported cases into three groups. Groups I and II have since then been known as "Coats' disease." Group III, which had been previously described, was soon recognized to be a separate clinical and pathologic entity, and is now known as "von Hippel's" or the "von Hippel-Lindau" disease. This Group is not concerned in the present studies or discussions.

Groups I and II had the common clinical denominators of a massive yellowish or yellowish-white exudation in the external retina and in the subretinal space. This exudation resulted in a localized or diffuse elevation of the retina which at times might resemble a solid detachment. The retinal vessels coursed cleanly over the masses of exudation and were not obscured. The difference between the cases of Group I and those of Group II lay in the vascular changes. In the Group I cases there were no significant retinal hemorrhages or vascular changes. In the Group II cases, there were either fresh or old retinal hemorrhages and also marked retinal vascular changes, which Coats described as fusiform or beaded dilatations with "loops, kinks, and small spiral tortuosities, brushes and glomeruli, formed by the growth of new vessels." Histologically, both groups were characterized by the presence of massive exudates in the retina and subretinal space. Associated with this exudation were numerous large empty cells which

were variously designated as "foam," "ghost," or "balloon" cells, also many elliptical, empty spaces which are commonly termed "cholesterol clefts." In a number of his cases Coats spoke of crystals which he apparently believed were actually cholesterol. He described the tendency of the subretinal exudate to become organized and noted minor inflammatory changes at the points of chorioretinal adhesions. Otherwise, the reported inflammatory changes in the choroid were minimal.

In discussing the possible etiology of this condition, Coats considered but discarded syphilis, tuberculosis, and bacterial metastases. Acting on the premise that the presence of cholesterol crystals in a lesion was *prima facie* evidence of a preceding hemorrhage, he came to the conclusion that the organization of hemorrhage was the probable cause of the subretinal mass. He admitted, however, that the hemorrhage might well be secondary to some infectious process.

In the half century which has followed Coats' clear and convincing paper, the disease as originally described by him has been subjected to various additions, embellishments, and suggested etiologies. The net result of this has been that the term "Coats' disease" has finally become what several distinguished ophthalmologists have alluded to as a "scrap-basket." As a result, this syndrome has become increasingly ill-defined. The repetitive, argumentative, and often conflicting literature which has produced this chaos has centered upon six principal issues. These issues and the more pertinent literature relating to them may be summarized as follows.

ARE THE CLINICAL PICTURE AND THE CHARACTERISTIC HISTOLOGIC FINDINGS IN THE EXTERNAL RETINA AND THE SUBRETINAL SPACE THE RESULT OF AN INFLAMMATORY PROCESS OR ARE THEY SECONDARY TO THE VASCULAR CHANGES WHICH ARE SO FREQUENTLY AN OUTSTANDING FEATURE OF THE DISEASE? An inflammatory etiology was first championed by von Hippel[3] (1913) who reported a case of Coats' disease which histologically showed a marked chorioretinitis. He considered any hemorrhage secondary to the inflammation. This viewpoint was echoed by Leber[4a] (1916), by Hanssen[5] (1920), and by Meller[6] (1922). In more recent years a specific variation of the inflammatory etiology has been suggested. Thus, François and his associates[7] (1956) have suggested that a *toxoplasma* uveitis might occasionally be the "trigger mechanism" which initiates the primary subretinal exudation and consequent separation of retina and choroid. In 1960 Rieger[8] described a group of adult cases of external central exudative chorioretinitis which he termed "Adult Coats' Disease of the Macula Area." A high percentage of the patients with this fundus picture gave positive

Sabin-Feldman dye tests for toxoplasmosis. A similar idea of a relation between Coats' disease and toxoplasmosis was endorsed by Berengo and Frezzotti[9] in 1962.

The concept of an inflammatory etiology was strongly questioned by Berg[10] (1919) who reported four cases of probable Coats' disease and cited somewhat similar cases from the literature. He noted the salient vascular changes in these cases—capillary endothelial proliferation, thrombosis, neovascularization, the formation of miliary aneurysms. He believed these to be the cause of the retinal and subretinal exudation. From 1920 to 1962 there have been numerous reports endorsing the view that the external retinal and subretinal exudation are either directly dependent on the vascular changes or are related to these changes. Notable among such papers is that of Reese[11] in 1956. He reported Coats' disease associated with telangiectasis of the retina. He described the characteristic ophthalmoscopic picture of the telangiectasis and noted that similar vascular lesions might occur in other conditions. He identified these telangiectases with the multiple retinal aneurysms first described by Leber.[4b] He reported two cases of telangiectasis of the retina which he observed to progress from the early stages of telangiectasis to the final clinical and histologic picture of Coats' disease.

Without going into further details of this polemic of an inflammatory *versus* a vascular etiology it is a fair statement that, while no convincing evidence has been produced which would establish either viewpoint as correct, it is now generally accepted that through one mechanism or another the clinical and histological features of Coats' disease are related to a vascular disturbance.

WHAT IS THE PATHOGENESIS OF THE TELANGIECTASES AND VASCULAR CHANGES WHICH ARE SUPPOSED TO PLAY A ROLE IN COATS' DISEASE? It has already been noted that the advocates of a vascular or hemorrhagic etiology for Coats' disease believe that the telangiectases are the primary lesion and that the evolution of the typical Coats' symptomatology with the development of the subretinal mass is due to exudation and hemorrhage with subsequent organization within the subretinal space. This viewpoint has recently been sharply challenged by Wise[12] (1957, 1961). Wise studied the development of retinal neovascularization and telangiectasia in a variety of types of retinopathy (diabetes, retrolental fibroplasia, Coats' disease) and concluded that this particular type of vascular response resulted from activation of Michaelson's unknown vasoproliferative factor[13] in the anoxic retina. Thus, this neovascularization could occur in either the external or internal retina,

wherever the relative degree of anoxia was the greater. His histologic evidence on this point is highly suggestive. Therefore, in whatever form of retinopathy that might occur, these vascular proliferations and telangiectases would be a secondary factor to the primary or etiologic factor which causes the anoxia, rather than be *per se* the actual cause of the retinopathy. As concerns Coats' disease, this offers a ready explanation of the cases in Coats' Group I, where the typical symptomatology of external retinopathy and a subretinal exudation may occur without the sufficient degree of retinal capillary thrombosis or venous obstruction (and consequent retinal anoxia) to activate the vasoproliferative factor. Thus, the lesion presents as a purely exudative retinopathy without the presence of retinal telangiectasia, a situation which those who advocate telangiectasia of the retina as the etiology of the disease tend to ignore.

WHAT IS THE ORIGIN AND THE ROLE OF THE FOAM CELLS IN THE PATHOGENESIS OF COATS' DISEASE? Leber believed the foam cells arose from a proliferation of the cells of the pigment epithelium and were concerned in the phagocytosis of some lipid. He was the first to point out that any lipid studies done on paraffin- or celloidin-embedded sections, which in their preparation had of necessity been exposed to fat solvents, were utterly worthless. While subsequent investigations have shown that Leber was undoubtedly correct in his conclusions concerning the role of the foam cells in lipid phagocytosis, he was almost certainly wrong in his idea that these cells originated from the pigment epithelium. He was apparently unaware that Anitschkow[14] had already demonstrated that in the various xanthomatoses the foam cells arose from the histiocytes of the reticulo-endothelial system. In 1933, Marshall and Michaelson[15] demonstrated this same origin for the foam cells found in the lesions of Coats' disease. This view is now generally accepted and is the basis for the grouping of Coats' disease under the generic title of "ocular lipid histiocytoses," a term introduced by Heath[16] (1932).

IS COATS' DISEASE A VARIANT OF THE OTHER LIPID RETINOPATHIES? Heath's inclusion of Coats' disease under the generic classification of "ocular lipid histiocytoses," while technically correct, nevertheless had the result of grouping Coats' disease with the retinopathies of Tay-Sachs disease, Niemann-Pick disease, von Hippel-Lindau disease, Junius-Kuhnt disciform degeneration of the macula, atherosclerosis, diabetes, various types of chorioretinal inflammation, and other forms of retinal degeneration. In 1941, Duke-Elder[17] in his classic textbook listed under one classification six different retinopathies (including

Coats' disease), all with the common denominator of exudation in the external retina and subretinal space.

Although Heath's term was apparently never widely adopted, gradually these various retinopathies therein included were often spoken of or accepted as forms of Coats' disease. Such a blanket grouping of Coats' disease with other types of retinal degenerations is exemplified in the paper by Sugar[18] in 1958. He reported eleven cases under the generic title of Coats' disease, in all of which the one common denominator was telangiectasis of the retina. On scrutiny, only three of these cases showed the external retinal exudation and the subretinal mass described by Coats. The remainder were apparently all cases of von Hippel-Lindau disease, diabetic retinopathy, or the Junius-Kuhnt or other forms of macular degeneration.

In several of these ocular lipid histiocytoses specific lipids or classes of lipids have been incriminated as responsible for the retinal changes and exudates. Thus, a cerebroside is thought to be responsible in Tay-Sachs disease, sphingomyelin in Niemann-Pick disease, the neutral fats (probably) in diabetic retinopathy and in the occasional retinal exudates observed in idiopathic hyperlipemia.

There is little to be gained in the grouping of these several disease processes, each with diverse clinical pictures and with varied types of lipids concerned in their pathogenesis, under the term "ocular lipid histiocytoses." Coats' original and clear cut description of the Groups I and II, and his apparent recognition that cholesterol played some role, appear to have been largely forgotten. The confusion in the literature is rampant; and the references to the term "Coats' disease" as a "scrap-basket" appear, at this point, to be fully justified.

IS COATS' DISEASE RELATED TO A DISORDER OF THE SYSTEMIC LIPID METABOLISM? In his original papers, Coats made frequent allusions to "cholesterol clefts" and "cholesterol crystals" which he believed were the result of preceding hemorrhage. Although later students heeded Leber's admonition that all lipid studies on paraffin sections were worthless, and although there are numerous reports of studies in which the presence of a lipid in the organized subretinal mass has been demonstrated in frozen sections, there has apparently been no actual evidence or proof that the lipid involved was actually cholesterol.

In 1950, Lewis[19a] reported the ophthalmologic findings in an adult with primary essential xanthomatosis of the hypercholesteremic type and with xanthomatosis biliary cirrhosis. She described "widely scattered yellowish areas involving all parts of the fundus. These yellowish patches are deep to the retinal vessels which appear normal except

where displaced forward by the larger collections." In 1952[19b] she described the histopathologic findings in these eyes. A connective tissue mass separated retina from choroid. Xanthoma cells which were positive for lipid with Sudan III were present in the retina, in the subretinal mass, and in the choroid. No positive identification of the nature of the lipid is reported although the author comments on masses of cholesterol crystals. The clinical and histologic pictures described by Lewis in these eyes were identical with those of Coats' disease. This is apparently the only case known in which a clinicopathologic picture indistinguishable from Coats' disease has been reported as a complication of a systemic lipidosis.

WHAT FACTORS MAY INITIATE OR FACILITATE THE DEPOSITION OF CHOLESTEROL IN THE RETINA? It is recognized (Thannhauser,[20] 1958) that the deposition of cholesterol in the tissues may occur in different stages of the various xanthomatoses, and in any tissue which has been the site of a prolonged, chronic inflammation. In the latter situation, such deposition is greatly heightened by the presence of a hypercholesteremia. He also mentions the possibility of the local synthesis of cholesterol at the site of inflammation.

There are two additional reports which deserve especial mention in relation to the deposition of cholesterol in the lesions of Coats' disease. The first of these is by Faber[21] in 1949. Faber studied the deposition of cholesterol in the human aorta. He found that such deposition occurred only in those aortas which showed an ethanol resistant metachromasia when stained with Toluidine blue. An increase in the amount of metachromatic material in the aorta was paralleled by increase in the deposition of cholesterol in the tissue. In these aortas such deposition might occur in the presence of a normal serum cholesterol level, but it was greatly accelerated in individuals with a concomitant hypercholesteremia. The metachromasia, he found, was due to a polymerized acid mucopolysaccharide which was identified as probably being chondroitin acid sulfate. He believed that this acid mucopolysaccharide entered into a molecular interchange with the lipoprotein molecule of the blood plasma and so freed the cholesterol for deposition in the tissues. Faber supported this hypothesis by both animal experimentation and by histopathologic and histochemical studies of the human aorta.

The second report embodies a somewhat similar suggestion made by Reese in 1956. In his histopathologic study of two eyes with telangiectasis of the retina which subsequently developed Coats' disease, he described, using the periodic acid-Schiff reaction (PAS), a brilliant

subintimal basement membrane in the retinal capillaries and telangiectases. This deposition of mucopolysaccharide in many instances greatly thickened the wall of the vessels, reducing or even obliterating their lumina. He believed that this material was concerned in the ultimate development of the typical Coats' symptomatology, although he hazarded no guess on the actual mechanism involved.

This review of the literature clearly shows that there is no general acceptance of any definite or rigid criteria for either a clinical or histologic diagnosis of Coats' disease. Likewise, it raises the question of the validity of certain generally accepted clinical concepts. Furthermore, it reveals that there is no information in the literature concerning the possible role of a hypercholesteremia in the pathogenesis of Coats' disease. A study of the material available for the present investigation throws considerable light on these points.

B. DIAGNOSTIC CRITERIA

The material on which the clinical and biochemical aspects of this study are based consists of eighteen cases of juvenile Coats' disease in children sixteen years of age or less and five adult cases, all over thirty years of age. Nine of the juvenile and two of the adult eyes had been enucleated and were thus available for histologic examination. The selection of this material was based on the following criteria. These criteria closely follow those outlined by Coats in his original paper. These may be summarized as follows.

CLINICAL DIAGNOSIS. The basic criterion for the clinical diagnosis was the presence of a massive yellowish or yellowish-white exudate in and beneath the external retina, over which the retinal vessels usually passed without obscuration, and which had produced a localized elevation of the retina. In addition to the massive exudation there might also be isolated, focal exudates. However, all cases in which the external retinopathy had not produced an actual elevation of the retina were excluded. Similarly excluded were all cases in which there were other clinical findings to which the elevation of the retina could be attributed: that is, an actual hole in the retina, inflammatory changes in the vitreous, cyclitic membranes, or other allied conditions. However, cases in which the eyes showed anterior segment changes indicative of a former or present iritis were accepted provided they fulfilled the other diagnostic criteria. Retinal vascular changes—telangiectases, dilated vessels, abnormally twisted vessels, foci of neovascularization, and hemorrhages—were observed in a number of the accepted cases.

In others, however, such vascular changes were not noted, it being specifically recorded in the histories that the retinal vascular pattern appeared normal.

HISTOLOGIC DIAGNOSIS. The basic criterion for a histopathologic diagnosis was the presence of a massive eosinophilic exudate in the external retina and in the subretinal space. This exudate invariably contained numerous "foam" cells with a finely vacuolated cytoplasm. In all except one of the cases here reported, the subretinal exudate was in part organized into a fibrous tissue plaque which contained cholesterol clefts, aggregates of foam-filled cells, and hemosiderin pigment. The one exception was the eye of an infant of seventeen months of age in whom it was believed there had been insufficient time for organization of the subretinal exudate to occur. Obliterative retinal vascular changes, perivasculitis, and retinal vascular abnormalities such as telangiectases were often present but were not prerequisite criteria for the diagnosis. Occasional focal mononuclear infiltrates in the choroid were noted but in no instance were these inflammatory changes of such a degree that they suggested a choroiditis as the basic cause of the subretinal exudation. A few of the accepted cases showed evidence of minimal low-grade inflammation of the anterior uvea. All cases in which the eyes showed a retinal hole or disinsertion, marked inflammatory foci in the uveal tract, a vitreous abscess or traction bands in the vitreous, cyclitic membranes, or other pathologic changes which might conceivably account for retinal detachment were rejected for this study.

C. AGE AND SEX INCIDENCE

A review of the cases from the literature in which the age and sex of the patient are given, and of the cases concerned in the present study, lends negligible support to the prevailing and widely held view that Coats' disease is almost invariably unilateral and occurs predominantly in young children of the male sex. It is true that in the twenty original cases reported by Coats the disease was unilateral in all; twelve of the patients were nineteen years of age or less and fifteen were males. In the present study, as already noted, there are eighteen juvenile cases (age sixteen or less) and five adult cases (all over thirty). Seventeen cases are unilateral, six are bilateral. Thirteen patients are males and ten are females. These figures, combined with those of Coats and those available in the literature provide a total of seventy-eight cases. Analysis discloses that sixty-eight of this total were unilateral and ten were bilateral—a ratio of 7:1. Sixty-two would be classed as juveniles

(nineteen years of age or less), and sixteen as adults—a ratio of 4:1. Fifty-one were males and twenty-seven were females—a ratio of less than 2:1. Thus, there is no reason to exclude Coats' disease in the differential diagnosis of an external retinopathy if (a) there is bilateral involvement; (b) the patient is an adult; (c) the patient is a female.

D. IDENTITY OF THE JUVENILE AND ADULT FORMS OF THE DISEASE

Ophthalmoscopic examination revealed no significant or essential clinical difference between the juvenile and the adult cases. The observed histopathologic changes were the same in both the juvenile and in the adult cases.

E. THE POSSIBLE INFLUENCE OF SYSTEMIC OR LOCAL INFLAMMATION

While there were no differences in the clinical appearance and histopathology of the adult and juvenile cases there were definite differences in the histories and ancillary findings in these two groups. These differences may be outlined as follows.

JUVENILE COATS' DISEASE. Two of these eighteen patients showed organic evidence of a preceding iritis; one had an active iritis at the time of initial examination; and one developed a recurrent, non-granulomatous iridocyclitis with band keratopathy five years after the onset of the retinopathy. Diagnostic surveys were done in fourteen of these patients. Two patients gave a history of an immediately antecedent illness, undiagnosed in one case, and chicken pox in the other. One child had multiple telangiectasia of the skin similar to the vascular changes in the retina. Two others, both with vascular changes and hemorrhages in the retina, had low prothrombin titers in the blood serum. One child had nematode larvae in the stool but the enucleated eye disclosed no evidence whatsoever of nematode endophthalmitis. Sabin-Feldman dye tests had been done in ten of these eighteen patients and were negative in nine instances and positive in one. In eight patients both the histories and the surveys were negative.

ADULT COATS' DISEASE. In all of these five patients there was an authenticated history of a preceding uveitis—non-granulomatous in three, granulomatous in one, and post-traumatic in one. In three cases the uveitis had been bilateral, and in two of these the Coats' disease was likewise bilateral. In the third case of bilateral uveitis, the presence of Coats' disease in the second eye could not be determined because a cataract was present. In two cases the preceding uveitis had been unilateral and the Coats' disease occurred in the eye which had been the site of the preceding uveal inflammation.

One of the patients with a history of non-granulomatous uveitis had a positive Sabin-Feldman dye test for toxoplasmosis. Another patient with a history of a preceding granulomatous chorioretinitis had a positive Sabin-Feldman dye test and a cutaneous reaction to toxoplasmin. In two patients these tests were negative and in one no studies for toxoplasmosis were performed.

F. PLASMA LIPID STUDIES

The chief lipid components of the blood plasma are the total cholesterol (the free cholesterol plus the cholesterol esters), the phospholipids, and the neutral fats. The sum of these lipids, with other minor fractions, is known as the "total plasma lipids." The following are the results of the plasma lipid studies in the juvenile and the adult cases.

JUVENILE COATS' DISEASE. The plasma lipids were determined in nine of the juvenile cases. In every case the plasma lipids were within normal limits, and, specifically, the serum cholesterol was below 250 mgm. percent.

ADULT COATS' DISEASE. The plasma lipids were determined in four of the adult cases. In each of these four patients the total cholesterol was elevated to an abnormal level—an average of 340 mgm. percent against a high normal of 260–300 mgm. percent. In these four patients there was a slightly less marked elevation of the phospholipids—an average of 310 mgm. percent against a high normal of 260 mgm. percent. The average value of the neutral fats was 255 mgm. percent, well below the possible high normal given by Thannhauser.

In order to evaluate the significance of this observation that a hypercholesteremia was a constant finding in these adult patients who had either a history or objective findings of uveal inflammation and who subsequently developed Coats' disease, cholesterol determinations were made on the serum of thirty-five consecutive adult patients with repeated or protracted attacks of uveitis but who showed no evidence of any symptomatology suggestive of Coats' disease. In thirty-one of these patients (89 percent) the blood cholesterol was within normal limits, and in only four (11 percent) was it elevated. This ratio is not unusual for a hypercholesteremia in adults.

RÉSUMÉ

The review of the literature suggests that it has been suspected that the deposition of cholesterol in the lesions is a salient feature of Coats'

disease. However, such an assumption has never been proven. The clinical and histopathologic changes observed by Coats and those observed in the cases here reported indicate that Coats' disease presents an identical picture in both adults and children. In all the adult cases here reported there was, however, a history of some preceding uveal inflammation and the constant finding of a hypercholesteremia. In children, such a history could only rarely be obtained and the plasma lipids were always within normal limits.

The constant findings of a preceding uveitis and a hypercholesteremia in the adult patients confirm the original idea which prompted this study, that is, that the pathogenesis of the disease in the adult is related to local inflammation in the presence of hypercholesteremia. However, this explanation can not apply to the juvenile form of the disease. Uveal inflammation and hypercholesteremia obviously play no role in the pathogenesis of the disease in children. Since the clinical and histologic pictures of the disease are identical in adults and children it is probable that the intermediary action of some tissue factor is also necessary for the actual deposition of lipid in the tissues in both adults and children. Thus, it is necessary to explore the nature of the hypothesized tissue factor.

PART II

The specific objectives of the histopathologic and the histochemical studies here reported may be stated as follows.

1. To determine whether the proteinaceous exudate in the external retina and subretinal space contains a lipid component.

2. To establish the nature of any lipid fraction which may be so present.

3. To explore the hypothesis that the intermediary action of some mucopolysaccharide may be concerned in the deposition of cholesterol in eyes with Coats' disease. Such a mucopolysaccharide might be either the PAS positive subintimal membrane found in the retinal telangiectasis by Reese[11] in 1956, or the acid mucopolysaccharide described by Faber[21] in 1949, which he believed was responsible for the deposition of cholesterol in the human aorta.

MATERIAL

The material on which these studies is based consists of (a) the cases described in Part I, that is, two adult eyes and nine juvenile eyes;

one-half of one of the juvenile eyes had been reserved and was embedded in gelatin for this study; (b) one, recently acquired, juvenile eye, available for paraffin and gelatin embedding; (c) two juvenile eyes received from Dr. Norman Ashton of the Institute of Ophthalmology in London and each suitable for both paraffin and gelatin embedding. Thus, the total available material consists of calottes of twelve juvenile and two adult eyes embedded in paraffin, and calottes of four juvenile eyes embedded in gelatin.

THE IDENTIFICATION OF THE NATURE OF THE RETINAL AND SUBRETINAL EXUDATE

1. The Demonstration of a Lipid Component in the Exudates of Coats' Disease

In order to demonstrate the presence of a lipid component in these exudates broad spectrum lipid stains, with an affinity for any and all lipids present in the tissues, were employed.

STUDIES ON PARAFFIN-EMBEDDED MATERIAL. Sections of all twelve of the paraffin-embedded eyes with a clinical and histologic diagnosis of Coats' disease were stained with Oil-Red-O, a broad spectrum lipid stain. Paraffin sections of nine of these eyes disclosed no lipid whatsoever when stained with Oil-Red-O. However, one eye showed a trace, and two showed appreciable amounts of lipid in the subretinal exudate and within the organized fibrous tissue plaques in the subretinal space (Figure 1). This lipid material appeared to be in macrophages in the subretinal exudate. Similarly, some of it had also been ingested by macrophages in the organized fibrous plaque and the remainder was extracellular and incorporated within the scar tissue.

When sections of these same three paraffin-embedded eyes were examined with the Schultz reaction for cholesterol or were stained with the differential fat stains believed to be specific for the neutral fats and the fatty acids, in no case was a positive reaction found. It is notable that these eyes had originally been fixed in formalin, and it is known that formalin fixation alone will, to a slight extent, fix certain lipids and alter their solubility. Pearse[22] points out that these formol-fixed lipids can be demonstrated with the Sudan black stain and that they are usually cerebrosides or phosphatides. When sections of these paraffin-embedded eyes, containing Oil-Red-O positive material were now stained with Sudan black, the result was positive (Figure 2). Thus, there is indirect evidence that the persisting lipid, occasionally found in formol-fixed and paraffin-embedded eyes may be, at least in

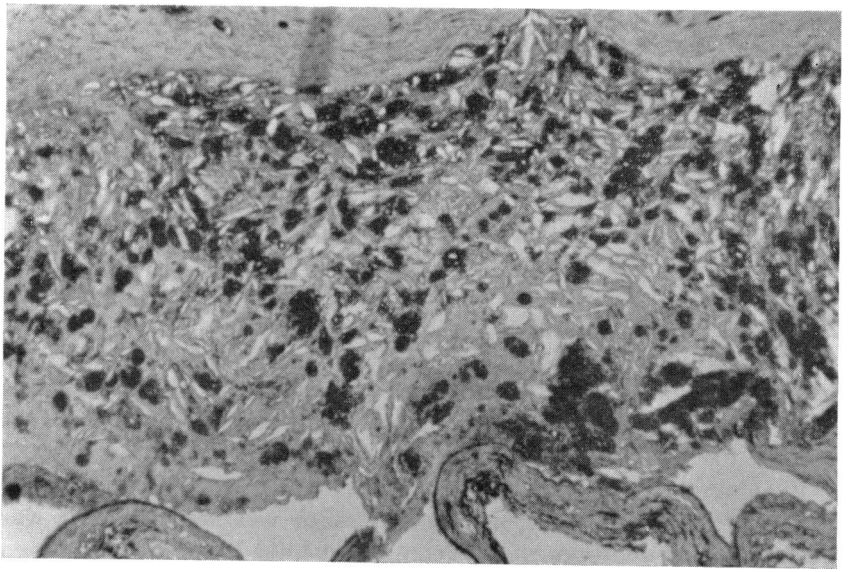

FIGURE 1. MASSES OF RESIDUAL LIPID REMAINING IN SUBRETINAL EXUDATE IN PARAFFIN-EMBEDDED EYE WITH COATS' DISEASE (ADULT CASE)
A portion of the organized subretinal plaque is seen above; the pigment epithelium and choroid below. Many cholesterol clefts are also present in the exudate. Oil-Red-O; ×25.

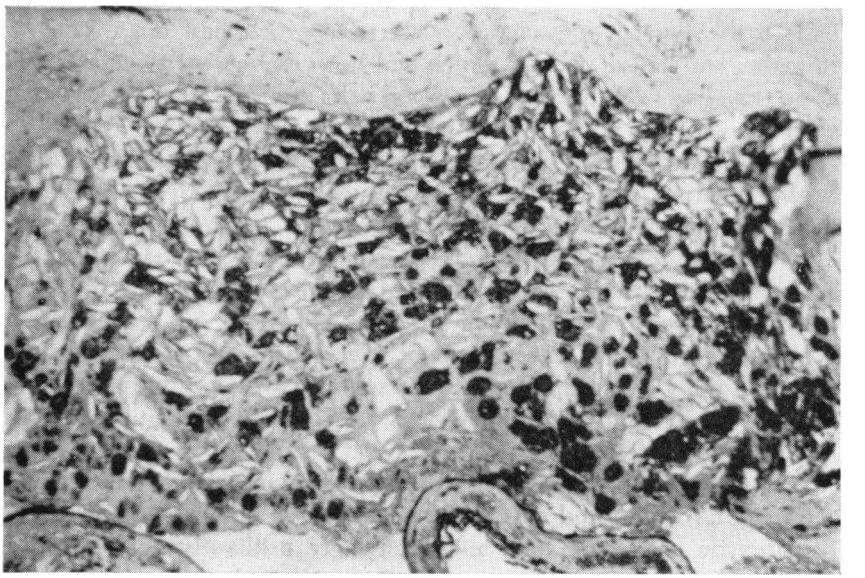

FIGURE 2. RESIDUAL LIPID REMAINING IN SUBRETINAL EXUDATE IN PARAFFIN-EMBEDDED EYE WITH COATS' DISEASE (ADULT CASE)
The orientation is the same as that in Figure 1. Sudan black; ×25.

part, a cerebroside or phosphatide complex. There are two possible sources of this Sudan black positive lipid. Streeten[23] has pointed out that the retinal pigment epithelium contains sudanophilic granules and that "Histochemical tests indicate the presence of unsaturated fatty acids in the granules, possibly of a phosphatide complex." Another, and probably more abundant, source of phospholipids is the envelope of the red blood cells. The frequent hemorrhages which may characterize Coats' disease and the subsequent breakdown of the red cells may liberate quantities of phospholipids, some of which is picked up in macrophages or is encased in scar tissue. Thus, the retinal pigment epithelium (which in these cases always shows varying degrees of proliferation and destruction) or the extravasated red blood cells may be the source of this formol-fixed lipid material.

In any event, taken as a whole, the amount of residual lipid material in these sections of paraffin-embedded eyes was small, and it was evident that if any appreciable quantity of lipid had previously been present that the great bulk of this had been extracted during the processing of the material. Leber was clearly correct in his conclusion that such eyes were unsuited for valid lipid stains.

STUDIES ON GELATIN-EMBEDDED MATERIAL. To study properly the retinal and subretinal exudate for any lipid component, resource was had to the four eyes with Coats' disease which had been fixed in formalin, one-half of each of which had been embedded in gelatin and at no time had been exposed to any fat solvents. Frozen sections cut at a thickness of ten micra were prepared from these eyes.

The Oil-Red-O stain and the Lorrain-Smith-Dietrich stain, another broad spectrum, non-specific lipid stain, were employed. In sections of these four gelatin-embedded eyes both stains gave identical results. The external retina and subretinal exudate stained vividly. In the external retina there were abundant deposits of positive staining material, concentrated chiefly in the outer plexiform and outer nuclear layers (Figure 3). In the subretinal space the material appeared as large, round, globules, probably representing swollen, lipid-filled cells (Figure 4). Very little lipid material was seen in the organized fibrous tissue of the subretinal space.

It is quite possible that much of the homogenous staining exudate in the external retina and subretinal space, seen with the conventional hematoxylin and eosin stain and even more brilliantly with the PAS stain, may be proteinaceous in nature. Nevertheless, the massive staining demonstrable with the broad spectrum fat stains leaves little doubt that the exudate in Coats' disease is chiefly lipid in nature.

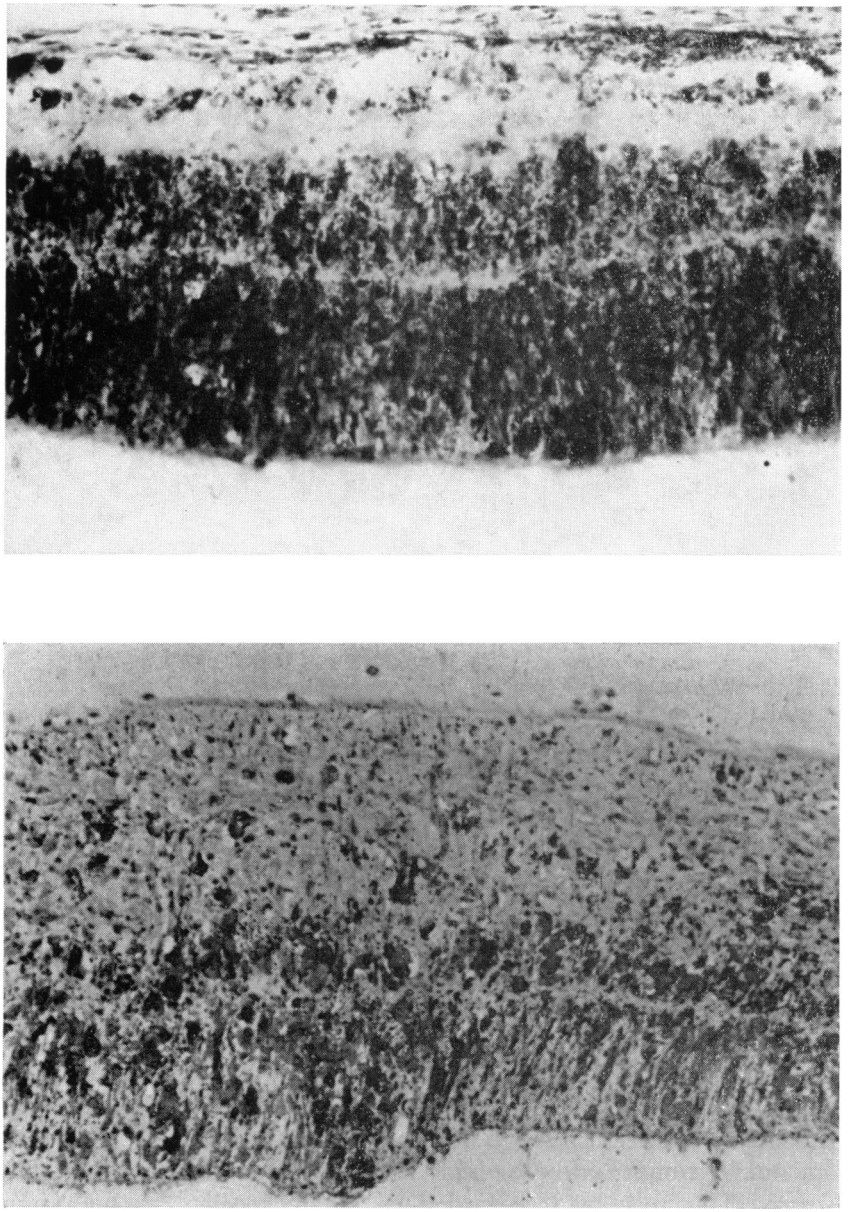

FIGURE 3. MASSIVE DEPOSITION OF LIPID IN THE RETINA IN TWO CASES OF JUVENILE COATS' DISEASE

The subretinal space is below and rods and cones are absent. Gelatin-embedded, frozen section. Oil-Red-O; ×40.

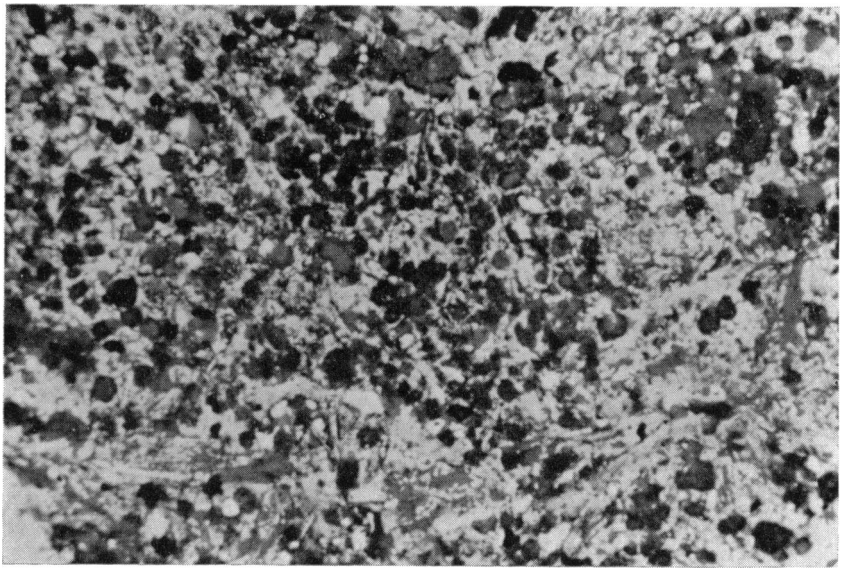

FIGURE 4. LIPID DEPOSITION IN SUBRETINAL SPACE IN JUVENILE COATS' DISEASE
Gelatin-embedded, frozen section. Oil-Red-O; ×25.

2. The Nature of the Lipid in the Exudate of Coats' Disease

To identify the nature of this lipid in the retina and subretinal space, crystalography and histochemical reactions were employed.

CRYSTALOGRAPHY. Unstained frozen sections were first examined under polarized light. Masses of doubly-refractile crystals were clearly seen in both the external retina (Figure 5), and in the subretinal exudate (Figure 6). In many places these crystals had the long needle form of cholesterol (Figure 7). In the hope of identifying the exact nature of these crystals, various sections were submitted to Dr. J. D. Donnay, Professor of Crystalography and Mineralogy in the Johns Hopkins University, who kindly examined them. While there was sufficient material to determine the index of refraction of these crystals, there was insufficient to permit X-ray spectrography or similar studies which would have been necessary for their absolute identification. Resource was therefore had to histochemical reactions.

SCHULTZ MODIFICATION OF THE LIEBERMAN-BURCHARDT STEROL REACTION. This reaction is regarded as highly specific for both free cholesterol and the cholesterol esters.[22] Unstained sections are first oxidized and mordanted with 2.5% iron alum for three days. Then they are submitted to a mixture of sulfuric and glacial acetic acids. In the

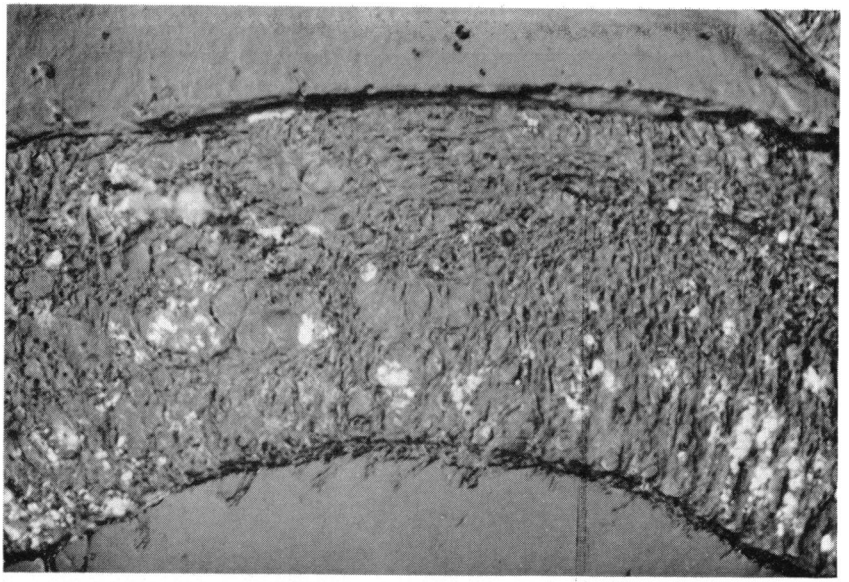

FIGURE 5. DOUBLY REFRACTILE CRYSTALS IN THE RETINA OBSERVED WITH POLARIZED LIGHT; JUVENILE COATS' DISEASE
The vitreous space is above, subretinal space below. Gelatin-embedded, unstained, frozen section; ×40.

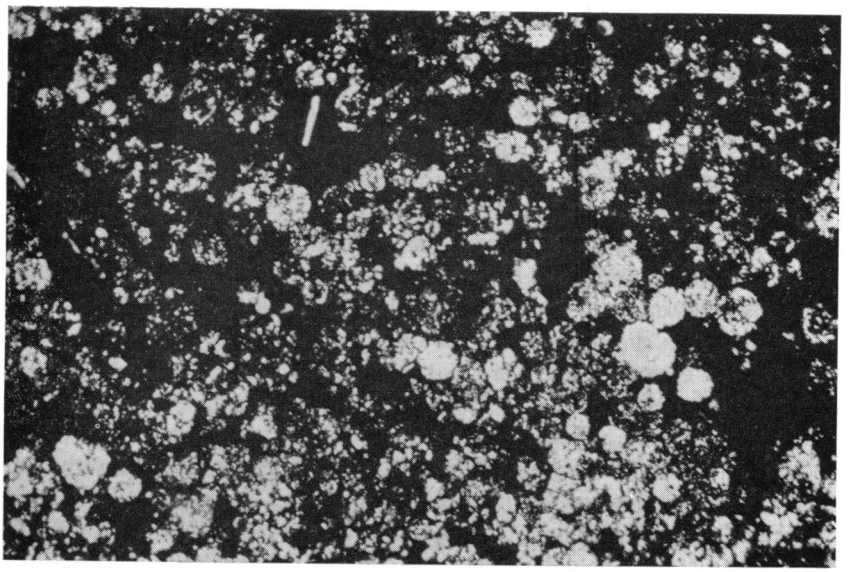

FIGURE 6. DOUBLY REFRACTILE CRYSTALS WITHIN FOAM CELLS IN SUBRETINAL SPACE OBSERVED WITH POLARIZED LIGHT, JUVENILE COATS' DISEASE
Gelatin-embedded, unstained, frozen section; ×40.

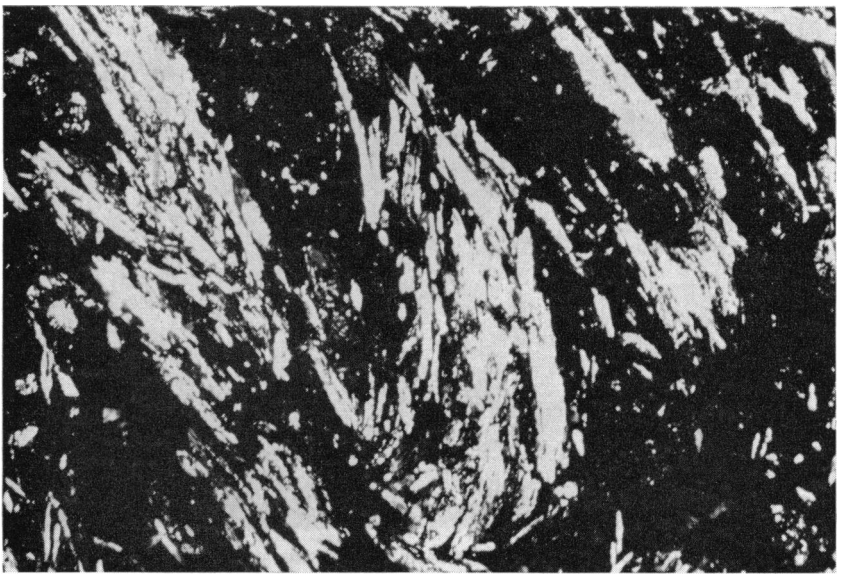

FIGURE 7. DOUBLY REFRACTILE NEEDLE-LIKE CRYSTALS LYING FREE IN THE SUBRETINAL SPACE, OBSERVED WITH POLARIZED LIGHT; JUVENILE COATS' DISEASE
Gelatin-embedded, unstained, frozen section; ×40.

presence of free cholesterol and the cholesterol esters, the sections take on a bluish-green color which persists for 30–60 minutes and then rapidly fades. The unstained sections of the Coats' disease eyes, submitted to this reaction, gave strongly positive results in both the external retina (Figure 8) and the subretinal space (Figure 9) indicating that free cholesterol or the cholesterol esters were an important lipid component in the exudative process.

WINDAUS DIGITONIN REACTION. This reaction is employed to differentiate free cholesterol from the usual cholesterol esters. When unstained sections containing free cholesterol are treated with digitonin, the free sterol and the digitonin form an insoluble cholesterol-digitonin complex which is precipitated in the tissues in crystalline form, that is, in long doubly refractive needles and rosettes, similar to the usual cholesterol esters. These are readily visible under polarized light. When such digitonin-treated sections are counterstained with Oil-Red-O, the cholesterol-digitonin complex does not accept the stain; the needles remain clearly visible, while the usual cholesterol esters do accept the stain, are colored a deep red, and lose their characteristic crystalline appearance. Further, when digitonin-treated slides are exposed to cold acetone, the insoluble cholesterol-digitonin crystals

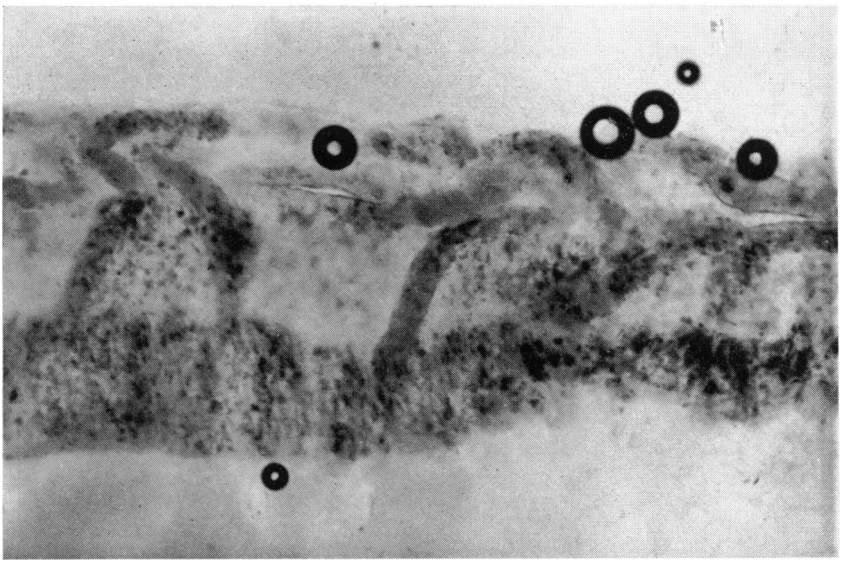

FIGURE 8. THE GREENISH AREAS (DARK GREY IN THIS PHOTOGRAPH) INDICATE SITES OF CHOLESTEROL DEPOSITION IN THE RETINA; JUVENILE COATS' DISEASE

The subretinal space is below. The air bubbles are artifact. Gelatin-embedded, unstained, frozen section. Schultz reaction; ×40.

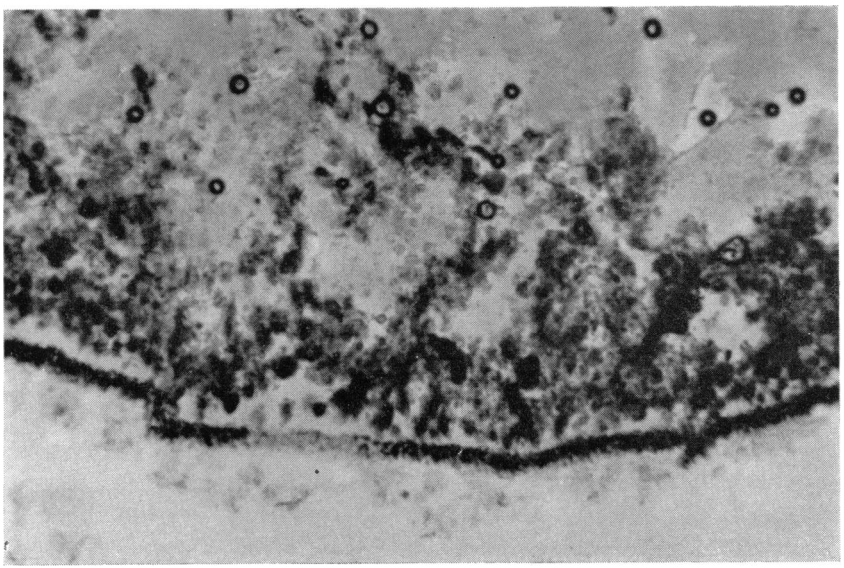

FIGURE 9. MASSES OF CHOLESTEROL-FILLED FOAM CELLS IN THE SUBRETINAL SPACE; JUVENILE COATS' DISEASE

The pigment epithelium is seen below. Gelatin-embedded, unstained, frozen section. Schultz reaction; ×25.

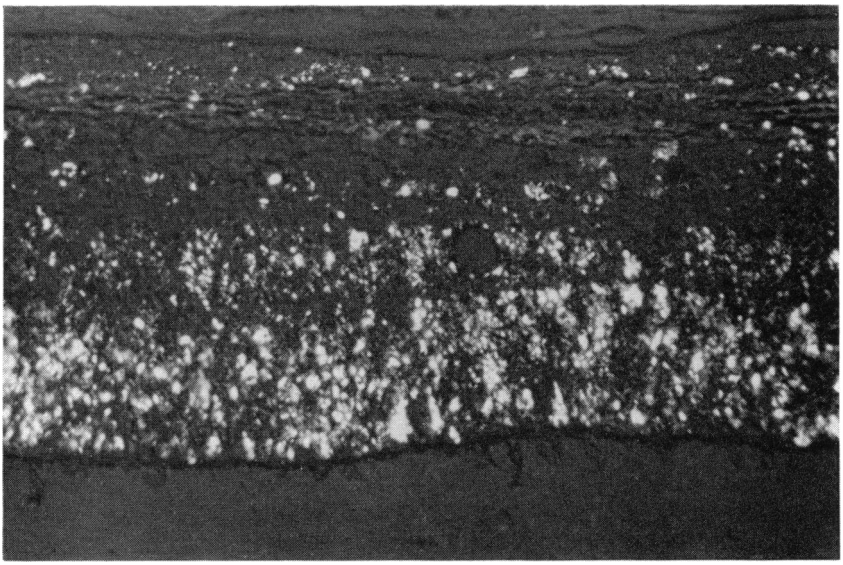

FIGURE 10. DOUBLY REFRACTILE CRYSTALS IN RETINA AFTER TREATMENT WITH DIGITONIN 0.5%; JUVENILE COATS' DISEASE

Polarized light. The concentration of crystals is chiefly in the external retina. Subretinal space is below. Gelatin-embedded, unstained, frozen section. Windaus Digitonin reaction; ×40.

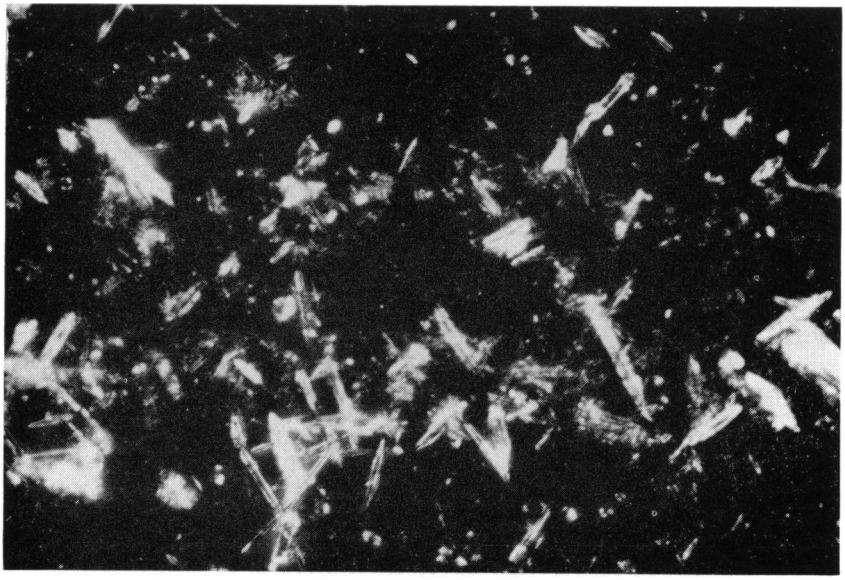

FIGURE 11. PERSISTENCE OF CRYSTALS OF CHOLESTEROL-DIGITONIN COMPLEX IN SUBRETINAL SPACE AFTER TREATMENT WITH COLD ACETONE; JUVENILE COATS' DISEASE

Polarized light. Gelatin-embedded, unstained, frozen section. Windaus Digitonin reaction; ×40.

remain clearly visible, while the usual cholesterol esters go into solution and disappear.

When unstained sections of the eyes with Coats' disease were treated with digitonin and re-examined under polarized light, a great increase in the crystals was immediately evident. This was especially marked in the subretinal space where the entire subretinal exudate appeared to be a mass of crystals. This same increase was also evident, but to a lesser extent, in the external retina (Figure 10). When these slides were now stained with Oil-Red-O, many of these crystals took the stain and could no longer be identified while other crystals remained unchanged. When unstained, digitonin-treated sections were exposed to cold acetone, there was a moderate decrease in the doubly refractile material, the usual cholesterol esters going into solution, while the insoluble needles and crystals of the cholesterol-digitonin complex remained unchanged (Figure 11).

Thus, there was conclusive evidence that both free cholesterol and the cholesterol esters were present in the exudate, the free sterol apparently being somewhat more abundant in the subretinal space.

3. The Role of Other Lipids in the Exudate of Coats' Disease

To explore the possible role other lipid fractions might play in the histopathology of Coats' disease, sections of the gelatin-embedded eyes were stained with the Nile blue sulfate stain for neutral fats, with Fischler's fatty acid stain for the fatty acids, and were examined with Baker's acid hematin reaction for the phospholipids. Primarily, it may be stated that the absolute specificity of these stains for the lipid fractions is not fully accepted by all histologists. Be that as it may, the results obtained with these various stains on the sections of eyes with the classical picture of Coats' disease, as previously defined, were as follows.

THE NEUTRAL FATS. With the Nile blue sulfate stain, the neutral fats are supposed to stain with a pink color while all other lipids (cholesterol, the fatty acids, and the phospholipids) stain a bluish-purple. In the eyes with Coats' disease, the greater portion of the material in the subretinal space stained bluish-purple, while a small amount only stained with a pink color (Figure 12). In the external retina, all the lipid material stained bluish-purple, and no pinkish-staining material could be detected (Figure 13). If this staining reaction is valid, this finding would indicate that there is a small admixture of neutral fat in the subretinal exudate, and none in the external retina.

THE FATTY ACIDS. Fischler's fatty acid stain is believed to be specific

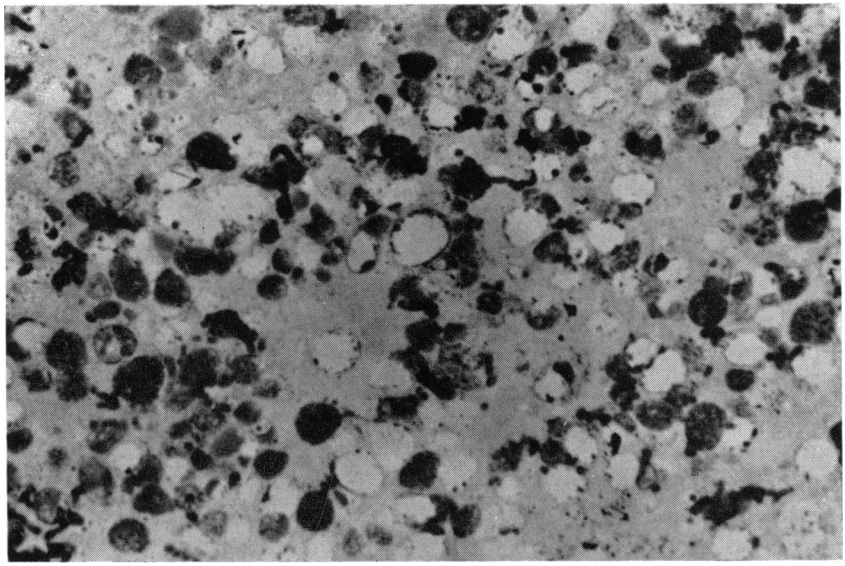

FIGURE 12. ADMIXTURE OF NEUTRAL FATS (GREY) AND OTHER LIPIDS (BLACK) IN FOAM CELLS IN SUBRETINAL EXUDATE; JUVENILE COATS' DISEASE
Gelatin-embedded, frozen section. Nile blue sulfate; ×40.

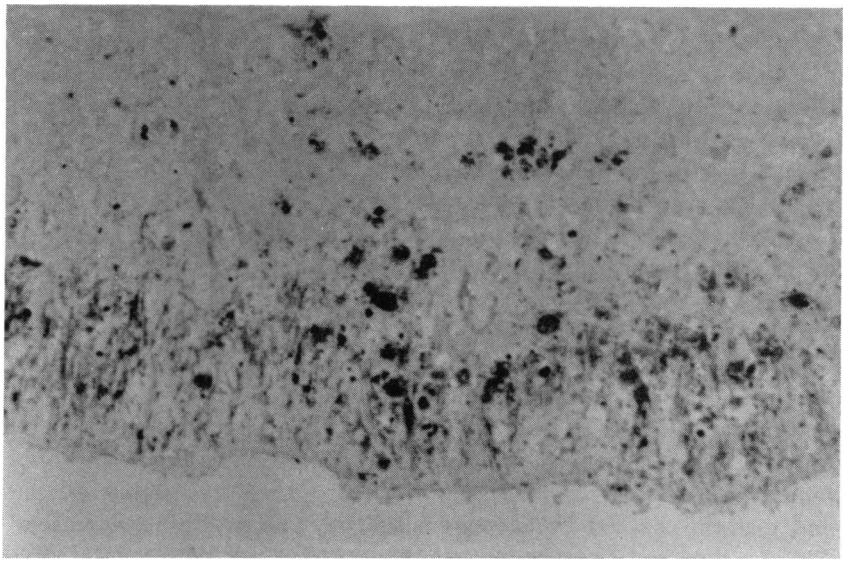

FIGURE 13. BLUISH-PURPLE (BLACK IN THIS PHOTOGRAPH) STAINING LIPID DEPOSITS, CHIEFLY IN OUTER RETINA; NO NEUTRAL FATS ARE DEMONSTRATED; JUVENILE COATS' DISEASE
Gelatin-embedded, frozen section. Nile blue sulfate; ×40.

for the fatty acids, and is not accepted by other lipid fractions. The fatty acids stain grey to black, while the free cholesterol, the cholesterol esters, the phospholipids, and the neutral fats remain unstained. When the frozen sections of the eyes with Coats' disease were stained with this stain, there was no staining whatsoever in the external retina, while the exudate in the subretinal space stained deeply (Figure 14). This finding, taken in conjunction with the results observed with the Nile blue sulfate stain, would indicate that neither fatty acids nor neutral fats are components of the lipid deposits in the external retina, while in the subretinal exudate there is a considerable amount of the unsaturated fatty acids, with a small admixture of the neutral fats.

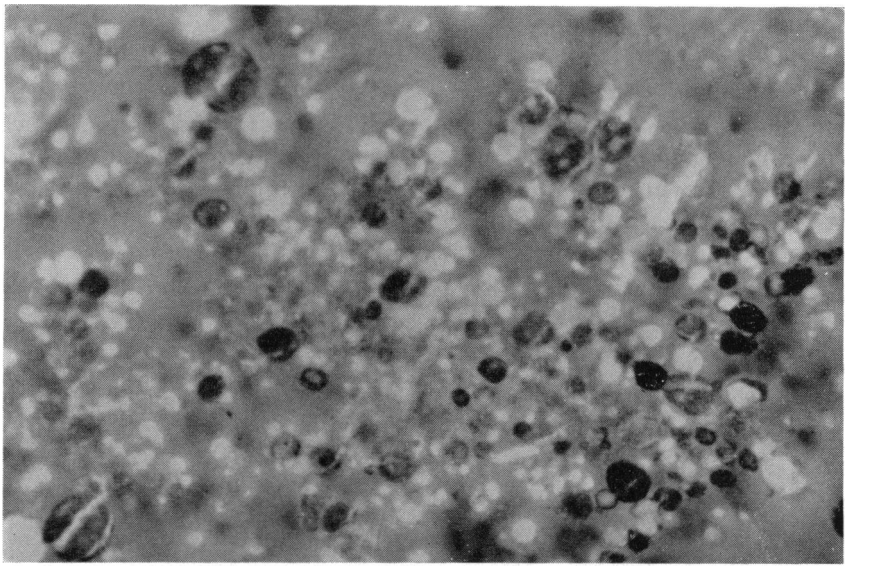

FIGURE 14. THE GREY AND BLACK STAINING FOAM CELLS IN THE SUBRETINAL EXUDATE CONTAIN FATTY ACIDS; JUVENILE COATS' DISEASE
Gelatin-embedded, frozen section. Fischler's fatty acid stain; ×40.

THE PHOSPHOLIPIDS. Baker's acid hematin reaction, a lengthy and complicated procedure, is employed to demonstrate the presence of any phospholipids in tissues. The material to be examined is fixed in formol-calcium, the calcium restraining the phospholipids from going into solution. After fixation, the material is embedded in gelatin and frozen sections are subjected to prolonged chromation, first at 22° and then at 60°. Thereafter, they are stained with a freshly prepared, oxidized acid hematin solution. Differentiation is carried out with a

borax-ferricyanide mixture. Since the material available for this study had originally been fixed in formalin, it was necessary to refix it in formol-calcium before proceeding to the chromation, staining, and differentiation.

Employing this method, but with the added step of refixation, no trace of phospholipids could be demonstrated in either the retina or the subretinal exudate in the gelatin embedded eyes with Coats' disease. However, the lack of initial formol-calcium fixation of these tissues may qualify the validity of these negative findings.

UNIDENTIFIED CRYSTALLINES. In all four of the gelatin-embedded eyes studied, there remained small to moderate amounts of doubly refractile crystals, both in the retina and in the subretinal space, which could not be positively identified. These crystals did not stain with the broad spectrum stains (Oil-Red-O or the Lorrain-Smith-Dietrich) or with the specific differential lipid stains (Nile blue sulfate and Fischler's fatty acid). They did not react to the Schultz test for cholesterol. They also failed to accept the Sudan black stain, eliminating the possibility that they were phospholipids derived from the pigment epithelial cells or from the broken down red cells (Figure 15).

The probable explanation for these "unidentified crystals" is that

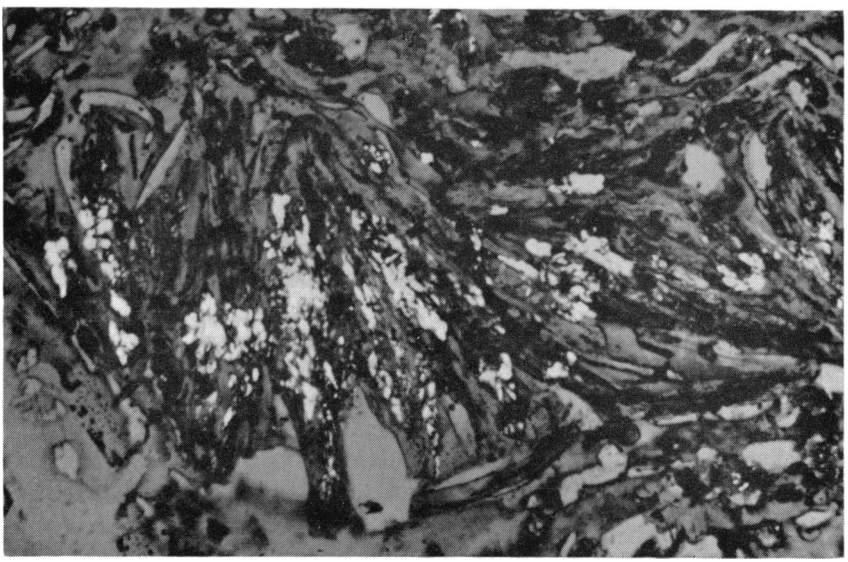

FIGURE 15. DOUBLY REFRACTILE "UNIDENTIFIED CRYSTALLINES" (HERE THEY APPEAR WHITE) IN THE SUBRETINAL SPACE, WHICH FAILED TO ACCEPT LIPID STAINS; JUVENILE COATS' DISEASE

Gelatin-embedded, frozen section, partially polarized light. Sudan black; ×100.

they were originally cholesterol or some other lipid fraction which through a molecular transfer has entered into combination with some intermediary factor forming a new crystalline complex which lacks the specific staining affinity and chemical reactions of the original lipid. Thus, these "unidentified crystals" would be analogous to the free cholesterol-digitonin complex formed in the Windaus reaction, in which the free cholesterol has lost its specific staining and reactive properties. The hypothetical intermediary factor predicated here may well be analogous to the metachromatic acid mucopolysaccharide found by Faber in the human aortas in which cholesterol has been deposited. An acid mucopolysaccharide conceivably available for this role, is found associated with the rods and cones and pigment epithelium of the normal eye. This possibility is further discussed below.

It is obvious from these various studies, taken as a whole, that in Coats' disease the exudates in the external retina are almost entirely free cholesterol and the cholesterol esters with a small admixture of the "unidentified crystalline." Neutral fats and the fatty acids are not deposited in the retina proper. In the subretinal exudate the chief lipids are again free cholesterol and the cholesterol esters together with the "unidentified crystalline," fatty acids, and possibly a small admixture of neutral fats. Certainly in both the external retina and the subretinal exudate free cholesterol and the cholesterol esters are the predominating lipids.

The immediate question which arises is whether this massive deposition of cholesterol and the cholesterol esters in these locations is peculiar to Coats' disease *per se* or whether it is (a) a common characteristic of the so-called "ocular lipid histiocytoses;" or (b) a non-specific consequence of prolonged or repeated hemorrhages in the retina or subretinal space; or (c) a non-specific consequence of prolonged retinal detachment *per se*.

CONTROL STUDIES

To explore these questions, control studies were made to determine the nature of any lipids which might be present in the fundus lesions of: (a) eyes which have been classified as belonging to the generic group of "ocular lipid histiocytoses," that is, arteriosclerotic retinopathy, diabetic retinopathy, disciform degeneration of the macula (Junius-Kuhnt), Tay-Sachs disease, and Niemann-Pick disease; (b) eyes which have been the site of repeated retinal hemorrhages; (c) eyes with long-standing detachment of the retina. The results of these control studies are as follows.

A. Ocular Lipid Histiocytoses

ARTERIOSCLEROTIC RETINOPATHY. The posterior halves of fifteen eyes from individuals dying with advanced general and cerebral arteriosclerosis were obtained from the autopsy room. These eyes were admittedly not in an ideal condition for study, the retinas being billowed and folded together. However, the five most promising ones were selected, although in these no gross retinal lesions had been identified. These five eyes were fixed in formalin, embedded in gelatin, and sectioned. They were then stained with Oil-Red-O. No lipid could anywhere be demonstrated.

DIABETIC RETINOPATHY. Two eyes with advanced diabetic retinopathy were obtained from the autopsy room. These eyes were fixed in formalin, embedded in gelatin, sectioned, and stained with Oil-Red-O. Both eyes showed brilliantly staining, circumscribed deposits deep in the retina (Figure 16). When stained with Fischler's fatty acid stain, the result was negative. When stained with Nile blue sulfate, the reaction indicated a predominance of neutral fats. In one of these eyes the Schultz reaction for cholesterol was doubtfully positive in one small area of the retinal exudation. If cholesterol was present in this lesion

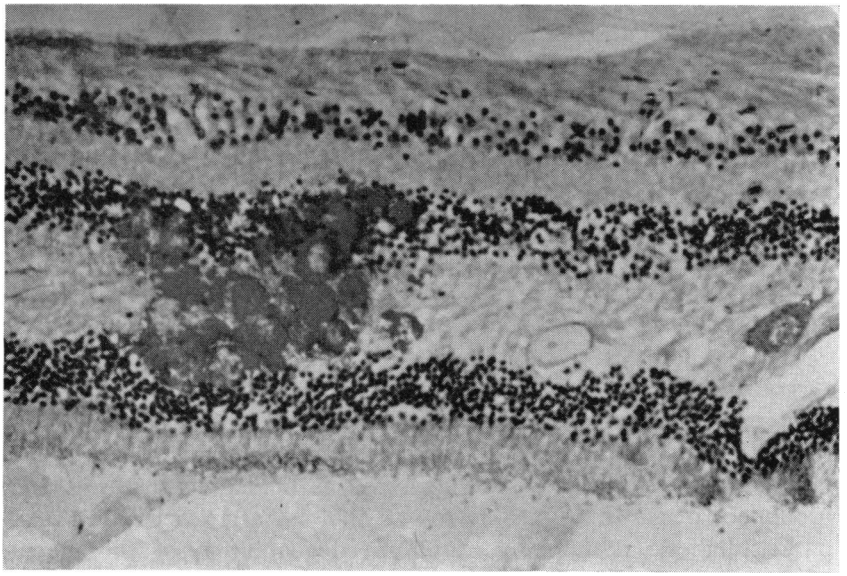

FIGURE 16. LIPID EXUDATES IN THE RETINA IN DIABETES, THE MAJORITY BEING IN THE OUTER PLEXIFORM LAYER
Gelatin-embedded, frozen section. Oil-Red-O; ×40.

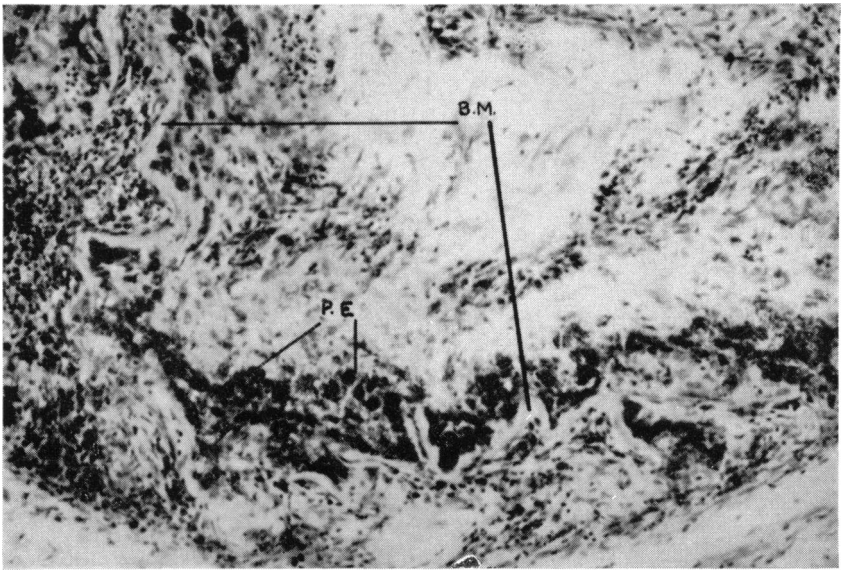

FIGURE 17. PROLIFERATION OF RETINAL PIGMENT EPITHELIUM (P.E.) AND REDUPLICATION OF BRUCH'S MEMBRANE (B.M.) IN JUNIUS-KUHNT DISCIFORM DEGENERATION OF THE MACULA
Celloidin embedding; hematoxylin and eosin; ×40.

it was there in only trace amounts. In the second eye, the lipid-positive exudates were entirely negative for cholesterol. Thus, the chief component of these lipid deposits clearly appeared to be neutral fat. A third eye from a diabetic was also obtained at autopsy. No evidence of retinopathy was noted on gross examination and no lipid was identified with the Oil-Red-O stain.

JUNIUS-KUHNT DISCIFORM DEGENERATION OF THE MACULA. Formalin-fixed sections of a typical case of disciform degeneration of the macula were obtained from the Pathologic Laboratory of the Institute of Ophthalmology of London through the courtesy of Dr. Norman Ashton.

The usual hematoxylin and eosin stain showed the retina in the macula region was elevated by a dense fibrous tissue plaque. The pigment epithelium beneath this plaque had been partially destroyed, but within the plaque itself there was proliferation of pigment epithelium with a reduplication of Bruch's membrane (Figure 17). In addition, there were fresh hemorrhage and a few foci of lymphocytes within this plaque near its periphery. The retina over this lesion was intact.

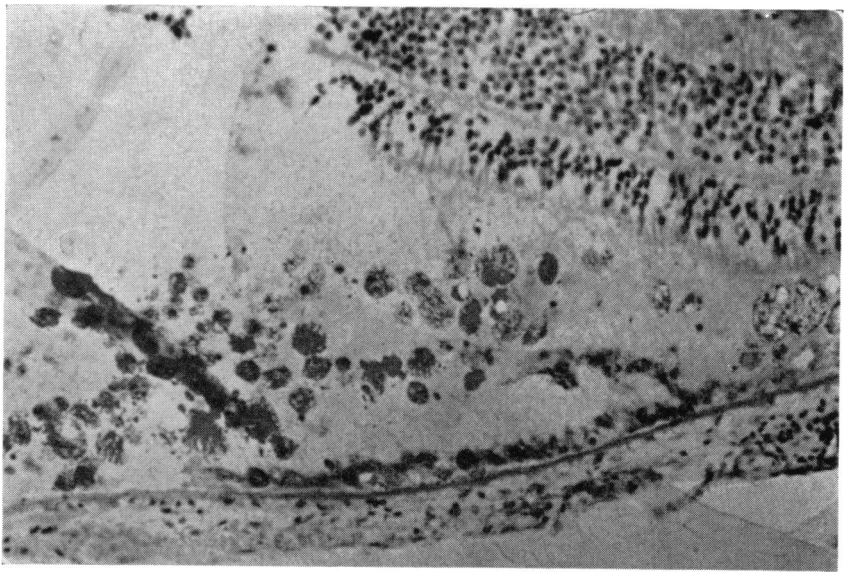

FIGURE 19. LARGE LIPID LADEN CELLS IN SUBRETINAL SPACE ADJACENT TO PIGMENT EPITHELIUM AND BRUCH'S MEMBRANE IN JUNIUS-KUHNT DISCIFORM DEGENERATION OF THE MACULA. STAINING OF BRUCH'S MEMBRANE IS PROMINENT.
Gelatin-embedded, frozen section. Oil-Red-O; ×50.

Unstained, gelatin-embedded sections examined under polarized light disclosed a few doubly refractile crystals in the area of fresh hemorrhage. These lay within hemosiderin-laden macrophages and were apparently related to the breakdown of the red blood cells. No other doubly refractile crystals were seen at all in the lesion.

OIL-RED-O—the retina was free of lipid. Bruch's membrane, including its reduplication, stained intensely (Figure 18*). A few large cells located in the subretinal space and adjacent to Bruch's membrane and the pigment epithelium also contained droplets of positive staining material (Figure 19). These were assumed to be either macrophages which had ingested fragments of the lipid-filled Bruch's membrane or altered pigment epithelial cells.

SUDAN BLACK—the results obtained were similar to those observed with Oil-Red-O: that is, intense staining of Bruch's membrane and its reduplication, as well as the staining of a few large macrophage-like cells in the subretinal space. The doubly refractile crystals noted in

*Figure 18, a color illustration, unfortunately could not be adequately reproduced in black and white and has therefore been omitted.

unstained sections within the area of hemorrhage did not accept the lipid stain.

NILE BLUE SULFATE—Bruch's membrane failed to accept this stain at all, but traces of neutral fats were observed in a few of the large cells in the subretinal space.

Fischler's fatty acid stain and the Schultz reaction for cholesterol were likewise entirely negative.

In summary, in this one example of Junius-Kuhnt macular degeneration, Bruch's membrane and its reduplication accepted the broad spectrum lipid stains but failed to accept any of the differential lipid stains. Thus, the exact nature of this lipid remains unknown.

Lack of the proper gelatin-embedded material has thus far precluded study of eyes with the other so-called "ocular lipid histiocytoses"—Tay-Sachs disease and Niemann-Pick disease.

B. Repeated Episodes of Retinal Hemorrhages

HYPERTENSIVE RETINOPATHY. Two eyes with severe hypertensive retinopathy were obtained at autopsy and prepared in the manner described above. Massive hemorrhages within the retina and in the subretinal space were present. The Oil-Red-O stain revealed no lipid component associated with these hemorrhages and the Schultz reaction for cholesterol was similarly negative.

HEMORRHAGIC GLAUCOMA. An eye from a 35-year-old female (nondiabetic) with hemorrhagic glaucoma of several months' duration was studied in a similar manner. The Oil-Red-O stain demonstrated no evidence of lipid deposition in the retina and the Schultz reaction for cholesterol was negative.

C. Long-Standing Retinal Detachment

ABSOLUTE GLAUCOMA AND LONG-STANDING RETINAL DETACHMENT IN A 72-YEAR-OLD MALE. The eye was embedded in gelatin and prepared for lipid studies in the usual manner. Unstained sections examined under polarized light showed no doubly refractile crystals. Oil-Red-O, Fischler's fatty acid, and the Nile blue sulfate stains all gave negative results. The Schultz reaction for cholesterol was negative.

ABSOLUTE GLAUCOMA AND LONG-STANDING DETACHMENT IN A 19-YEAR-OLD MALE WITH A HISTORY OF ATOPIC DERMATITIS, CATARACT, AND CATARACT EXTRACTION. Unstained sections examined under polarized light showed no crystals in the retina proper and only an occasional one in the subretinal space. The Oil-Red-O stain showed a moderate

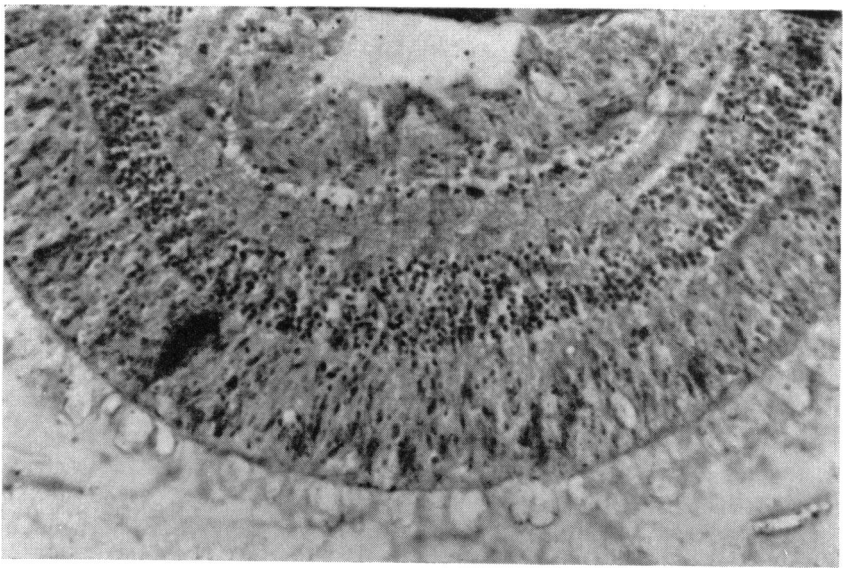

FIGURE 20. A SITE OF MAXIMUM LIPID DEPOSITION IN RETINA WITH DETACHMENT OF LONG DURATION IN A 19-YEAR-OLD MALE WITH HISTORY OF ATOPIC DERMATITIS, CATARACT, AND CATARACT EXTRACTION; THE MAJORITY OF THE LIPID IS IN THE EXTERNAL RETINA
Gelatin-embedded, frozen section. Oil-Red-O; ×40.

amount of lipid in the outer plexiform layer of the retina and in the rod and cone layer (Figure 20). The Nile blue sulfate stain identified these lipids as neutral fats in the outer plexiform layer. Fischler's fatty acid stain was negative. The Schultz reaction for cholesterol was negative.

LONG-STANDING DETACHMENT DUE TO RETROLENTIL FIBROPLASIA IN A 9-YEAR-OLD MALE. Again the eye was prepared in the usual manner for lipid studies. Unstained sections examined under polarized light showed moderate numbers of doubly refractile crystals in the vitreous cavity. The Oil-Red-O stain showed masses of lipid free in the vitreous space and lesser amounts within an organized subretinal plaque. There was a small quantity of lipid in the rod and cone layer as well. Fischler's fatty acid stain showed traces of fatty acids also in the vitreous space. This lipid for the most part was contained in large phagocytic cells which also contained hemosiderin pigment. The Nile blue sulfate stain showed neutral fats in the vitreous space in small quantities. The Schultz reaction showed traces of cholesterol in the

vitreous cavity and in the subretinal space but the retina itself was free of cholesterol. Paraffin-embedded sections of this same eye were then stained with hematoxylin and eosin. With this stain it was immediately evident that most of this lipid material had been associated with recent and old hemorrhage in the vitreous. Acting on the supposition that this lipid might be derived from the phospholipid complexes in the fragmented and broken down red blood cell envelopes, these sections were then stained with Sudan black. This stain disclosed a great quantity of formol-fixed lipid remaining in these paraffin sections. This material was found in abundance in the vitreous within macrophages, which were also laden with hemosiderin pigment. Similar Sudan black positive macrophages were found in areas of organizing hemorrhage and fibrous plaque formation in the subretinal space (Figure 21). Thus, there is strongly suggestive evidence that the majority of the lipid demonstrated by the differential stains in the gelatin-embedded sections of this eye with retrolentil fibroplasia was derived from the breakdown of red blood cells with the attendant release of their phospholipid complexes.

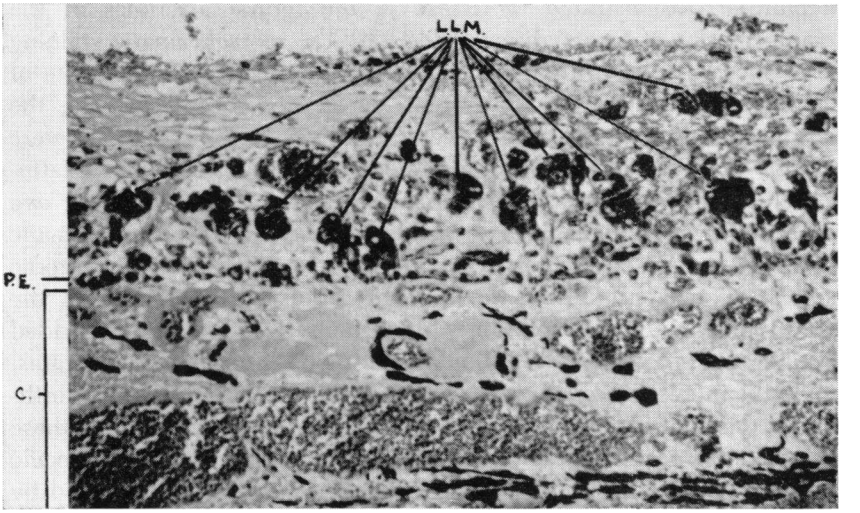

FIGURE 21. AN EYE WITH LONG-STANDING RETINAL DETACHMENT, SECONDARY TO RETROLENTIL FIBROPLASIA

Residual "formol-fixed" lipid present in lipid-laden macrophages (L.L.M.) within organized subretinal plaque. This lipid has persisted after dehydration through alcohols. The pigment epithelium (P.E.) and choroid (C) are identified. Paraffin embedding. Sudan black; ×40.

Summary of Control Studies

The information thus far adduced from these control studies indicates that lipids are an important component of the exudate in diabetic retinopathy. Differential lipid stains indicate that this lipid is neutral fat. In hypertensive retinopathy there is little if any lipid deposited in the retina, either independent of or in association with the retinal hemorrhages. Old hemorrhages in the vitreous may release phospholipid complexes from the breakdown of the red cell membrane. In addition, there may be traces of cholesterol present. Cholesterol was not present, however, in significant quantities and was never found deposited within the retina. In disciform degeneration of the macula there is a heavy lipid deposition in Bruch's membrane. The nature of this lipid is unclear. It accepts none of the differential lipid stains. A retina which has been detached for a prolonged period may show small deposits of lipid in the outer layers; however, this is not cholesterol.

MUCOPOLYSACCHARIDE STUDIES

These studies were undertaken to explore the possible role in the pathogenesis of Coats' disease of (a) the intimal mucopolysaccharide membrane demonstrated by Reese in the retinal arterioles of the telangiectasias of Coats' disease; and (b) the metachromatic staining acid mucopolysaccharide described by Faber and others at the site of cholesterol deposition in the human aorta. For these purposes, the paraffin-embedded eyes, which were worthless for lipid studies, were entirely valid and indeed in some respects were superior to the gelatin-embedded eyes. Therefore, in these studies paraffin sections of two adult and twelve juvenile eyes, and gelatin sections of four juvenile eyes were used. Representative sections were stained with the periodic acid Schiff reagent (PAS), the Rinehart-Abul-Haj colloidal iron, the Alcian blue, and the Toluidine blue stains. The gelatin-embedded sections did not accept the colloidal iron or the Toluidine blue stains; while the intense staining of the gelatin by PAS made this stain worthless on this type of material. The paraffin sections accepted all of these stains. As the results obtained on each of these eyes with each specific stain varied only in degree (taking note of the limitations imposed by the embedding media) they are, therefore, reported collectively as a unit for each stain, and not individually.

PAS STAIN. The basement membrane of the retinal vessels possesses two (and possibly more) components which may be identified by differential stains. The most characteristic component is a glycoprotein (that is, a protein containing a carbohydrate moiety as an integral

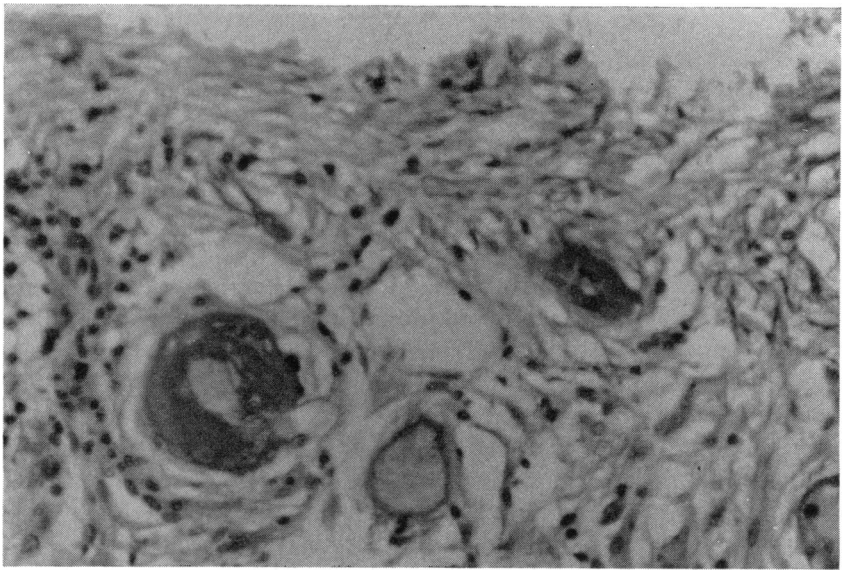

FIGURE 22. MARKED SUBINTIMAL THICKENING OF BASEMENT MEMBRANE OF RETINAL VESSELS IN JUVENILE COATS' DISEASE
Paraffin embedding, PAS-hematoxylin; ×100.

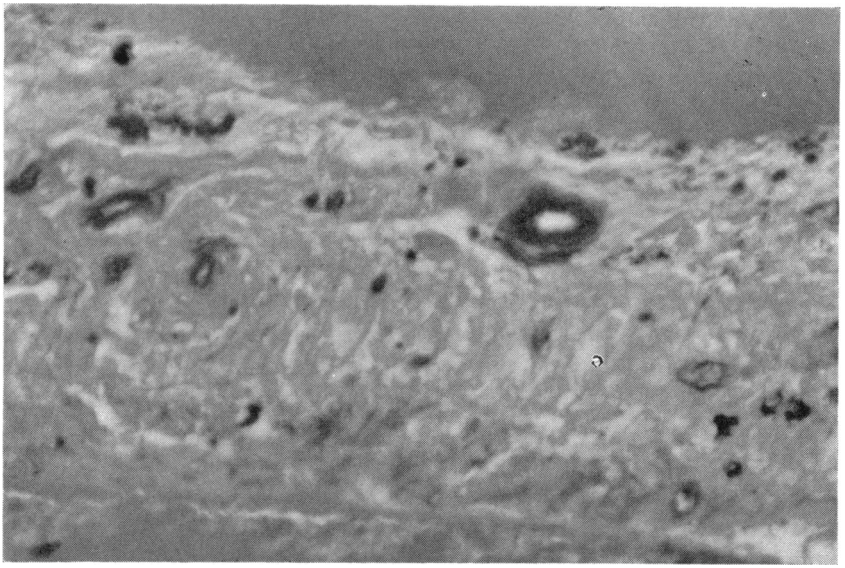

FIGURE 23. MARKED SUBINTIMAL THICKENING OF BASEMENT MEMBRANE OF A RETINAL VESSEL IN AN EYE WITH LONG-STANDING RETINAL DETACHMENT AND WITH NO EVIDENCE OF COATS' DISEASE
Paraffin embedding; PAS-hematoxylin; ×100.

part of its structures). Any metachromatic component in the basement membrane is an acid mucopolysaccharide (hyaluronic acid, heparin, or chondroitin sulfuric acid), which does not stain with PAS. Flat preparations of the normal retina stained with PAS clearly demonstrate that there is present a PAS positive subintimal basement membrane which is usually sufficient to delineate the entire retinal vascular pattern.[24]

In the eyes with Coats' disease in which paraffin sections were available, the PAS stain showed this subintimal membrane. It frequently appeared to be greatly thickened, as described by Reese, and in one of the adult and two of the juvenile eyes, this membrane was so thickened that it produced a partial to almost complete occlusion of the lumen of the affected vessels (Figure 22). In addition, brilliantly staining PAS positive exudates were found in the external retina, and coarse PAS positive granules were noted in the foam cells lying in the external retina and in the subretinal exudate.

To evaluate the possible presence of the PAS positive subintimal membrane in conditions other than Coats' disease, five juvenile eyes and four adult eyes with long-standing detachment of the retina, but in which there was no suggestion or evidence of Coats' disease, were studied with the PAS stain. The PAS positive subintimal membrane was present in all these eyes and markedly thickened in four of the juvenile eyes and in two of the adult eyes, resulting in partial occlusion of the lumina of the vessels (Figure 23). In addition, PAS positive exudates were found in the outer retina of four of the juvenile and one of the adult eyes. Occasionally, macrophages filled with PAS positive granules were noted in the subretinal exudate. It is, therefore, obvious that the presence and thickening of this PAS positive subintimal membrane is not specific for Coats' disease and is, therefore, probably not concerned in its pathogenesis.

COLLOIDAL IRON AND ALCIAN BLUE STAINS. These stains are believed to be specific for the acid mucopolysaccharides—chondroitin sulfuric acid, heparin, and hyaluronic acid. They are not metachromatic stains.

In paraffin-embedded sections of the Coats' disease eyes both of these stains occasionally demonstrated a small amount of acid mucopolysaccharide material beneath the intima of a retinal capillary or cuffing a retinal vessel (Figure 24). The foam cells of the external retina occasionally contained numerous, fine acid mucopolysaccharide positive granules, while the foam cells of the subretinal exudate contained a considerable quantity of this same material (Figure 25).

In the gelatin-embedded eyes these acid mucopolysaccharide granules

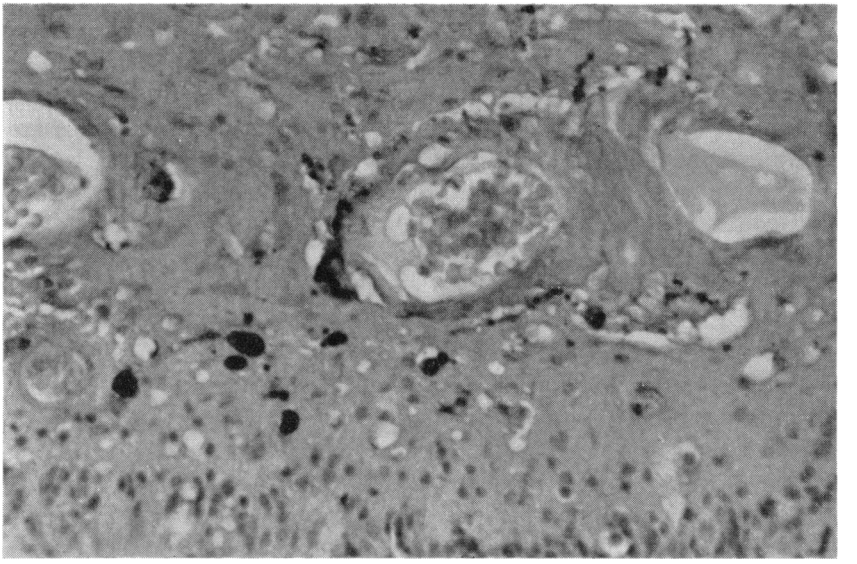

FIGURE 24. DEPOSITION OF AN ACID MUCOPOLYSACCHARIDE (BLACK IN THIS PHOTOGRAPH) ABOUT RETINAL VESSELS IN JUVENILE COATS' DISEASE
Paraffin embedding; colloidal iron; ×100.

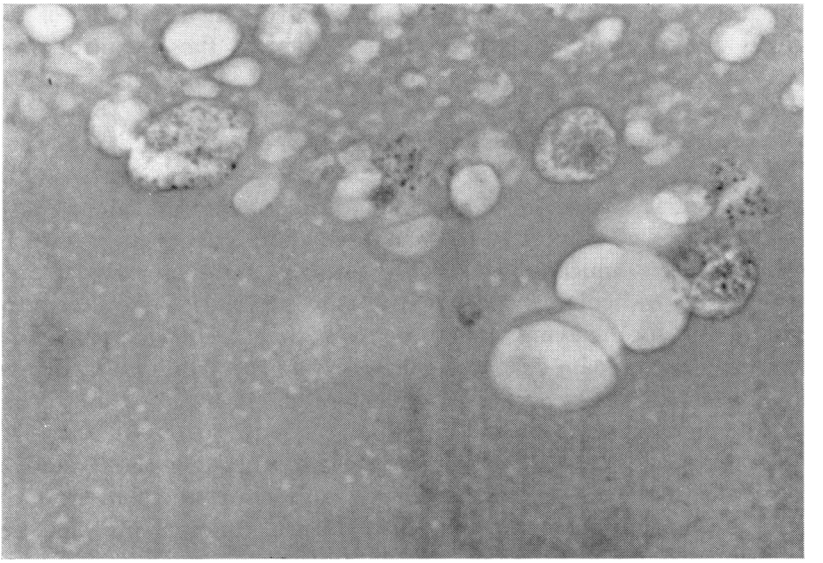

FIGURE 25. NUMEROUS FINE ACID MUCOPOLYSACCHARIDE POSITIVE GRANULES (GREY IN THIS PHOTOGRAPH) WITHIN FOAM CELLS IN SUBRETINAL EXUDATE IN EYE WITH JUVENILE COATS' DISEASE
Paraffin embedding; colloidal iron; ×160.

within the foam cells could be demonstrated only with the Alcian blue stain, but in these sections and with this stain, they were present in abundance. When these same Alcian blue-stained sections were counterstained with Oil-Red-O, masses of lipid were found within these same cells in which the acid mucopolysaccharide had been demonstrated. In fact, this intracellular lipid was so intense that the acid mucopolysaccharide positive material was completely masked.

The heavy subintimal membrane which was so clearly demonstrated with the PAS stain, and the intensely PAS positive retinal exudate, did not accept either of these stains. This was to be expected and clearly illustrates the different staining affinities of the glycoprotein mucopolysaccharides and the acid mucopolysaccharides.

It is known that in normal eyes an acid mucopolysaccharide is present in the interstices of the rods and cones.[25] In control eyes with long-standing detachment of the retina and in which the rods and cones have degenerated, considerable quantities of this material were also seen on the inner surface of the retinal pigment epithelial cells. In several such control eyes, some of this material had been phagocytosed and was present in large phagocytic cells in the subretinal space. In these cells, however, there was no lipid present, and the acid mucopolysaccharide within the cell was apparently only the result of the normal phagocytosis of detritus. In addition, in these control eyes, there was occasional cuffing of a retinal vessel with similar staining material.

The particular acid mucopolysaccharide associated with the rods and cones and with the pigment epithelium, and which is present in normal eyes, is known to be resistant to the action of hyaluronidase. When sections of the Coats' disease eyes which contained foam cells filled with acid mucopolysaccharide positive granules were treated with bovine testicular hyaluronidase* these granules proved to be resistant to the enzyme. This suggests that the acid mucopolysaccharide in the foam cells of Coats' disease is probably derived from the acid mucopolysaccharide normally present in the rods and cones or in the pigment epithelium.

TOLUIDINE BLUE STAIN. In the studies here reported, the Toluidine blue stain employed in both alcoholic and aqueous solutions gave decidedly less satisfactory results than did the colloidal iron and the Alcian blue stains. It failed completely in the gelatin-embedded eyes. In four of the paraffin-embedded Coats' disease eyes and in one retinal

*Wydase, Wyeth Laboratories, Philadelphia.

detachment control eye an occasional retinal blood vessel showed metachromasia of the vessel wall. Also in one control eye fine, pink coloured metachromatic granules were noted within macrophages in the subretinal space. However, this was an exception. The fine acid mucopolysaccharide granules so well demonstrated in the foam cells of the Coats' disease eye with colloidal iron and the Alcian blue stains could not consistently be demonstrated with the metachromatic Toluidine blue stain.

These results obtained with the colloidal iron and Alcian blue stains are in accord with Faber's hypothesis that the deposition of cholesterol in the tissues is mediated by an acid mucopolysaccharide. The finding of hyaluronidase-resistant acid mucopolysaccharide granules on the retinal pigment epithelium and the presence of granules with similar characteristics together with cholesterol within the foam cells of Coats' disease certainly suggests that this material may play some role and may be the tissue factor responsible for the deposition of cholesterol in the tissues.

XANTHELASMA STUDIES

In view of the above findings it appeared worthwhile to investigate the occurrence of such an acid mucopolysaccharide in other lesions in which the deposition of cholesterol in the tissues is the salient feature. To this end, the same techniques were applied to the study of skin xanthelasma. Xanthelasma lid lesions from three patients were fixed in formalin. One-half of each lesion was then sectioned on the freezing microtome. The results were identical in each case and were as follows:

UNSTAINED SECTIONS. With polarized light, masses of doubly refractile crystals were seen in the dermis within the foam cells (Figure 26).

OIL-RED-O STAIN. There was intense staining of all the foam cells in the dermis (Figure 27).

SCHULTZ REACTION FOR CHOLESTEROL. There was intense greenish staining of the foam cells (Figure 28).

WINDAUS DIGITONIN REACTION. The majority of the cholesterol was present as free cholesterol. The cholesterol esters were present in minimal amounts.

FISCHLER'S FATTY ACID STAIN. This was entirely negative, there being no staining of the section at all.

NILE BLUE SULFATE STAIN. This was also entirely negative.

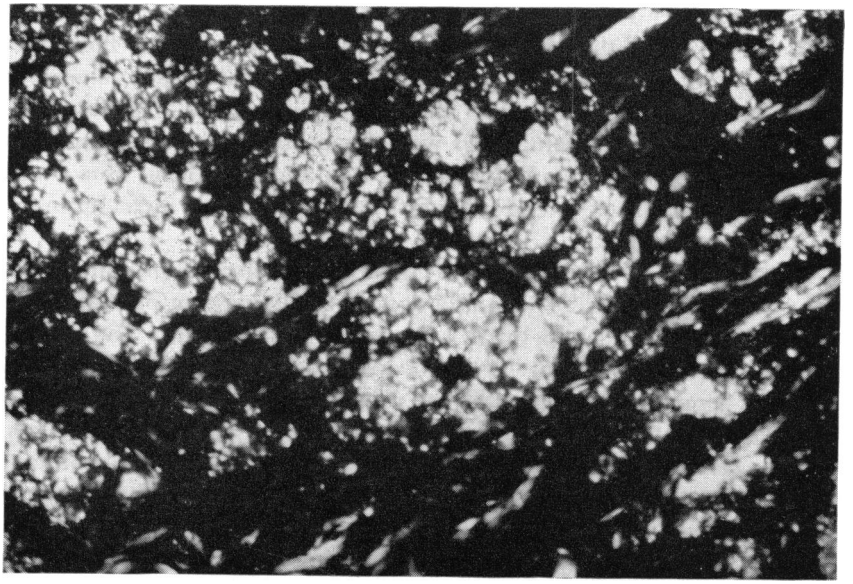

FIGURE 26. MASSES OF DOUBLY REFRACTILE CRYSTALS IN THE DERMIS IN XANTHELASMA
Frozen, unstained section. Polarized light; ×100.

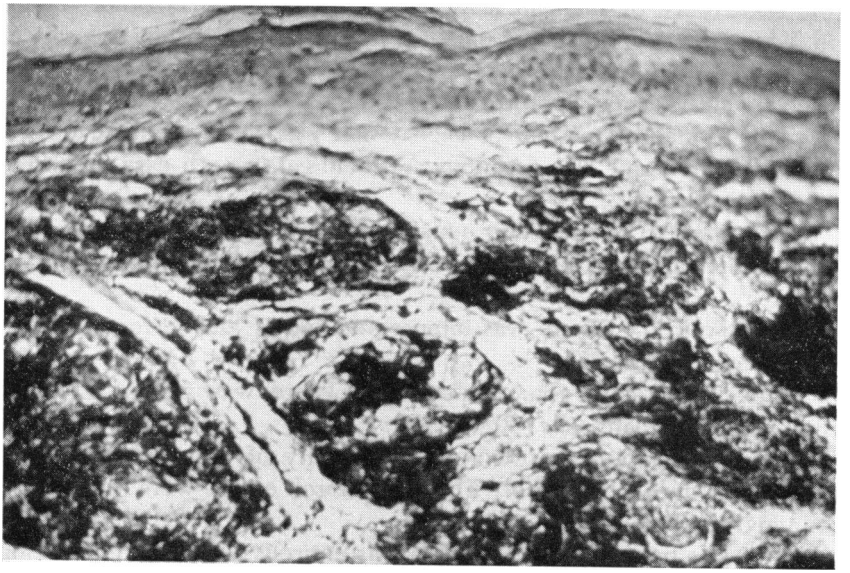

FIGURE 27. DEMONSTRATION OF THE LIPID NATURE OF THE DEPOSITIONS (AGGREGATES OF GREYISH-BLACK MATERIAL IN THIS PHOTOGRAPH) IN THE DERMIS IN XANTHELASMA
Frozen section. Oil-Red-O; ×40.

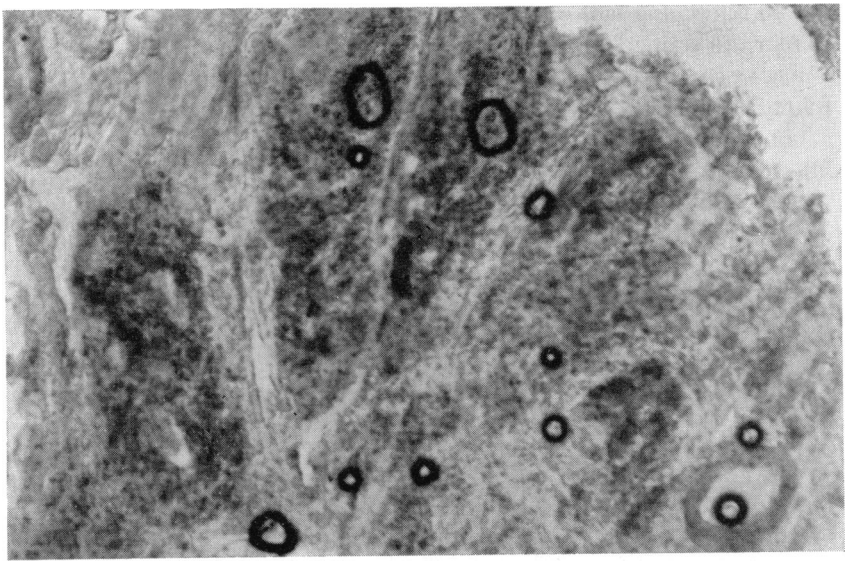

FIGURE 28. THE DARKISH AREAS AND DARK DROPLETS (BOTH GREEN IN ACTUAL COLOUR) REPRESENT SITES OF CHOLESTEROL DEPOSITION IN THE DERMIS IN XANTHELASMA

The air bubbles are artifact. Frozen, unstained section. Schultz reaction; ×40.

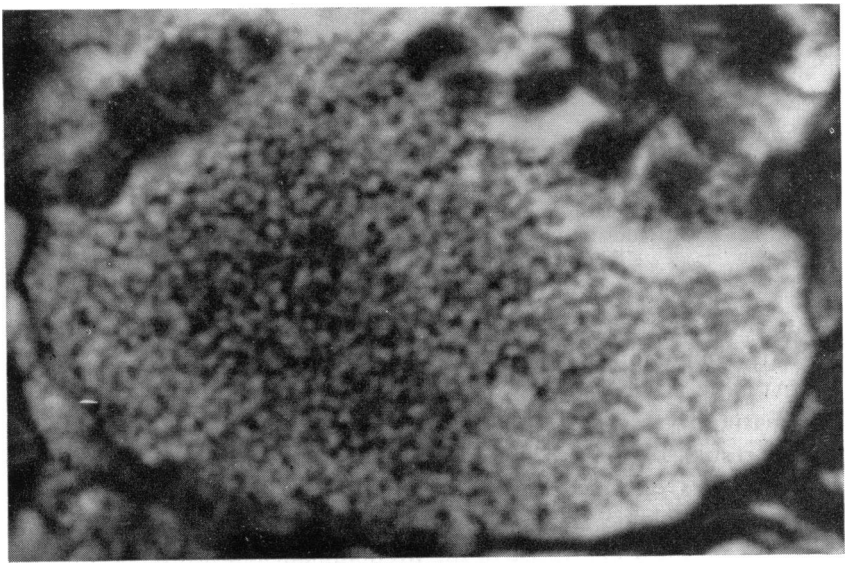

FIGURE 29. RATHER COARSE PAS-POSITIVE GRANULES PRESENT IN A HISTIOCYTE WHICH ALSO CONTAINS THE LIPID IN XANTHELASMA

Paraffin embedding; PAS-light green; ×256.

The remaining half of each lesion was embedded in paraffin and sections were stained as follows:

PAS STAIN. Coarse PAS positive granules were seen in some of the foam cells. Others were free of granules (Figure 29).

COLLOIDAL IRON STAIN. The great majority of the foam cells were filled with fine blue granules arranged in a delicate meshwork (Figure 30). The appearance of these was identical to that of the granules in the foam cells in the Coats' disease cases.

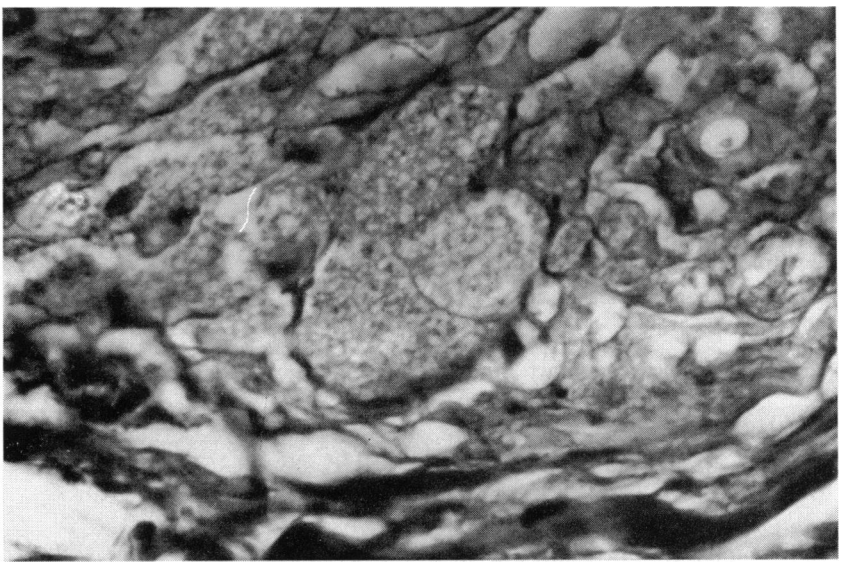

FIGURE 30. HEAVY DEPOSITION OF ACID MUCOPOLYSACCHARIDE POSITIVE GRANULES (GREYISH INTRACELLULAR MATERIAL IN THIS PHOTOGRAPH) IN THE HISTIOCYTES WHICH ALSO CONTAIN THE LIPID IN XANTHELASMA
Paraffin embedding; colloidal iron; ×256.

ALCIAN BLUE STAIN. The results were exactly similar to those obtained with the colloidal iron stain.

TOLUIDINE BLUE STAIN. (Aqueous and alcoholic solutions.) No metachromasia of vessel walls or of the foam cells could be demonstrated.

The results of these differential lipid stains and reactions and the various types of mucopolysaccharide stains make apparent at once the great histologic similarity between the lesions of Coats' disease and skin xanthelasma. In each, the predominant feature is the massive deposition of cholesterol in the tissues; combined with this, there is,

in each, an acid mucopolysaccharide in the same foam cells which contain the cholesterol. It should be noted, however, that in xanthelasma the cholesterol deposition appears invariably to be within histiocytes. In Coats' disease the deposition of cholesterol appears to be both intracellular (within foam cells) and extracellular (free in the retina and subretinal space). This extracellular deposition in Coats' disease may possibly be related either to the rate of deposition of the material or to the relative scarcity of tissue macrophages in the retina when compared to their abundance in the skin. Also, hemorrhage *per se* is never associated with xanthelasma and while it may or may not occur in Coats' disease, it (together with the serous exudation) is a secondary phenomenon. These factors of hemorrhage and serous exudation may account for the one demonstrable histochemical difference between these two conditions, that is, the presence in Coats' disease of an admixture of fatty acids and a trace of neutral fats in the subretinal exudate. In xanthelasma, cholesterol is the only lipid fraction involved.

COMMENT

Primarily, it should be noted that the lipid studies here reported are only on eyes with the juvenile form of Coats' disease. Efforts to obtain a gelatin-embedded eye with the adult form of Coats' disease have been unsuccessful, and it will be only by some fortuitous chance that one will ever become available. However, as pointed out in Part I of this thesis, the clinical pictures of the adult and juvenile forms of the disease are identical. Likewise, with the ordinary hematoxylin and eosin stains of paraffin sections the histopathology of the two forms is the same. Foam cells containing the fine bluish granules of an acid mucopolysaccharide are present in both the adult and juvenile types. There is no reason to believe there would be any difference in the lipid fractions involved. This is the premise assumed in this report.

As already stated, subject to the reservation that the staining techniques and histochemical reactions here employed may not be entirely specific for the lipid under scrutiny, these observations clearly demonstrate that in Coats' disease the salient feature is the extravasation of free cholesterol and the cholesterol esters. In the retina proper these are the only lipids involved with the single exception of small quantities of "unidentified crystals," which do not accept any lipid stains. This has already been commented upon. In the subretinal exudate,

while free cholesterol and the cholesterol esters were again the chief lipid constituents, there were also moderate amounts of fatty acids, of the "unidentified crystals," and possibly traces of neutral fats and the phospholipids.

The presence of the fatty acids in the subretinal space is most interesting. As pointed out in the first communication (of which Part I of this thesis is a short summary), there is excellent evidence[26,27] that while heparin has no direct clearing action on the triglycerides of the blood it does activate a lipoprotein lipase. The heparin itself apparently enters into a combination with the lipoprotein molecules of the blood serum forming a substrate on which the activated lipase acts with a resultant hydrolysis of the heparin-lipoprotein complex. Since heparin and chondroitin sulfuric acid are both acid mucopolysaccharides and have a similar chemical structure, it may well be that they have a similar action with the lipoprotein. Thus, the presence of fatty acids in the subretinal space may well be the result of a hydrolysis of a chrondroitin acid sulfate-lipoprotein complex with the resultant reformation of fatty acids and cholesterol. The fatty acids are extravasated in the subretinal space, while the cholesterol fraction is deposited both in the retina and in the subretinal space.

The most interesting question arising from these observations concerns the pathogenesis of the disease. Since the clinical and histologic pictures of the adult and the juvenile forms of the disease appear identical, it is probable there is the same pathogenesis for the disease in the two age groups. If the hypothesis of a molecular transfer between an acid mucopolysaccharide and the lipoprotein molecules of the blood plasma is accepted as the cause of the cholesterol deposition in the tissues, the salient question as concerns Coats' disease is what factor promotes the liberation of the acid mucopolysaccharide for the molecular transfer. In the adult cases, the answer is easy. It clearly appears to be a preceding uveitis in the presence of a concomitant hypercholesteremia. This is in accord with the known facts, and with the generally accepted views on the role of inflammation and a hypercholesteremia in the systemic xanthomatoses. In the juvenile cases there is a normal serum cholesterol, and the existence of a trigger mechanism comparable to the antecedent uveitis in the adult cases has not been demonstrated. Consequently, in the juvenile cases it appears probable that the intermediary action of the acid mucopolysaccharide is the dominant factor which leads to the deposition of cholesterol in the tissues.

What circumstances or conditions initiate the intermediary action of the acid mucopolysaccharide in children? It may be that the fixation of the acid mucopolysaccharide between the rods and cones and in the pigment epithelial cells of infant and young children is less stable than in adults. It may be some low-grade and undetected minor traumatism. The usual unilaterality of the juvenile form enhances this latter possibility. It may conceivably be a vascular anomaly with a slow leaking of serum. These are all imponderables still to be resolved, if indeed they are capable of resolution. Likewise, the possible role of a lipoprotein lipase is undetermined.

PART III. EXPERIMENTAL OBSERVATIONS

The experiments here reported were undertaken with the dual objectives of studying the effect of a sterile uveal inflammation on the deposition of cholesterol in the eyes of hypercholesteremic rabbits, and in the hopes of producing ocular lesions which might be comparable to those of Coats' disease in humans. To this end two experiments were undertaken. They will be reported in detail elsewhere[28] and are here reported in summary.

EXPERIMENT I (PILOT STUDY)

Four groups of four rabbits each were placed respectively on the following diets: Group I—normal Sherwood diet; Group II—sunflower meal; Group III—sunflower meal plus 2 percent cholesterol; Group IV—sunflower meal plus 2 percent cholesterol, plus 0.5 percent cholic acid. With these diets there was, in all groups except Group I, a heavy mortality, there being a total of only seven survivors in the other three groups at the end of the experiment. The survivors showed the following average blood cholesterol levels: Group I—145 mgm. percent; Group II—212 mgm. percent; Group III—1240 mgm. percent; Group IV—782 mgm. percent.

After this degree of hypercholesteremia had been attained the right eyes of all surviving rabbits were subjected to photocoagulation with the Zeiss light coagulator. Four or five photocoagulations 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 two weeks. After each quadrant had been thus treated the animals were sacrificed and the eyes enucleated for histologic study.

CLINICAL OBSERVATIONS

The immediate results observed in these rabbits were comparable to those observed in humans who have received similar treatment—small focal areas of retinal coagulation and necrosis. Within two 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 color. These were not elevated and had none of the subretinal exudation characteristic of Coats' disease.

HISTOPATHOLOGIC OBSERVATIONS

After enucleation, the eyes were fixed in formalin and then cut into halves. One-half was embedded in paraffin for routine hematoxylin and eosin staining and the other half embedded in gelatin for frozen sections and staining with Oil-Red-O.

HEMATOXYLIN AND EOSIN STAINS. The lesions appeared as focal, sharply demarcated areas of retinal thinning or scarring. In the pigmented animals there was some 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 choroid. Nowhere was there evidence of frank inflammation. The picture closely resembled that recently described by Okun and Collins.[29]

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 fibers of the retina. There was no appreciable deposition of lipid at the sites of light coagulation.

In Group II, in which there was only a moderately elevated hypercholesteremia and in Groups III and IV, in which all rabbits had a 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 structure and a thin streak of lipid lying apparently at the level of the outer nuclear layer. There was no evidence of cellular reaction or inflammation. No lipid was present in the subretinal space. Nowhere were there any lesions even faintly reminiscent of Coats' disease.

This experiment demonstrated that while a hypercholesteremia

could be produced in rabbits by the administration of a cholesterol-reinforced diet, that this hypercholesteremia was insufficient in itself to produce a significant degree of cholesterol deposition in the eyes 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 hypercholesteremia were to be considered an instrumental factor in the local deposition of cholesterol, certainly a stimulus resulting in a more protracted state of inflammation was necessary. To this end Experiment II was undertaken.

EXPERIMENT II

In this experiment sixteen normal rabbits were sensitized to killed beta streptococci, and sixteen others were sensitized to bovine albumin. The technique of this sensitization was that described by Woods, *et al.*[30] in 1955. After a high degree of sensitivity had been produced the rabbits were divided into three groups as follows.

GROUP I. Eight rabbits, four sensitized to beta strepticocci and four to bovine albumin. These rabbits were maintained on the normal Sherwood diet during the course of the experiment.

GROUP II. Twelve rabbits, all sensitized to beta streptococci, maintained on a cholesterol-reinforced diet during the course of the experiment.

GROUP III. Twelve rabbits, all sensitized to bovine albumin, maintained on a cholesterol-reinforced diet during the course of the experiment.

Blood was drawn from each rabbit to determine their normal lipid levels, prior to placing them on the cholesterol-reinforced diet. Thereafter, blood was drawn from all surviving rabbits after three weeks' maintenance on this diet, again after ten weeks on the diet, and finally at the close of the experiment before the animals were sacrificed. The serum plasma lipids in the Group I rabbits remained essentially unchanged, while in the Group II and Group III rabbits they became greatly elevated; for example, at the end of the experiment the average cholesterol level in the Group II rabbits was over 4000 mgm. percent and in the Group III rabbits over 2000 mgm. percent. Again, there was a high mortality rate in these animals, and at the end of the experiment there were only five survivors in Group I, five in Group II, and nine in Group III.

After the animals of Groups II and III had been on the cholesterol-reinforced diet for three weeks and a marked hypercholesteremia had

been attained, the right eyes of all animals (Groups I, II, and III) were challenged by intravitreal injections of the specific antigens, using the same technique employed by Woods, *et al.* Thereafter, the intravitreal challenging injections were repeated whenever the resulting allergic inflammation subsided. By this means the right eyes of all surviving rabbits were kept chronically inflammed over a full period of ten weeks. The left eyes were left undisturbed to serve as controls.

CLINICAL OBSERVATIONS

A ciliary flush in the injected eyes was common. In some animals fibrin appeared in the anterior chamber. In the hypercholesteremic animals annular limbal opacities of the cornea resembling arcus senilis were common and were much more prominent in the injected eye. Owing to rapidly developing opacities in the vitreous, to the clouding of the corneas, and to the development of secondary or traumatic cataracts the ophthalmoscopic observations were generally unsatisfactory. However, in many of the injected eyes retinal detachments were observed.

HISTOPATHOLOGIC 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 hematoxylin and eosin to study the general histopathologic changes. The second half of each was embedded in gelatin and stained with Oil-Red-O and the differential lipid stains.

GENERAL PATHOLOGIC CHANGES. The general changes observed in the injected eyes which had been subjected to a prolonged allergic uveitis were, with one exception, exactly similar to those described by Woods, *et al.*—a fibrinous exudate containing numbers of mononuclear inflammatory cells on the surface of the ciliary epithelium; diffuse and focal, frequently nodular, infiltrates of similar inflammatory cells in the choroid; retinal edema and retinal detachment with inflammatory cells in the subretinal fluid; edema of the optic disc with cellular infiltrates on its surface.

The one exception to this conventional picture was the presence of quantities of foam-filled macrophages in the injected eyes of the rabbits with the hypercholesteremia. These cells were found in the corneal stroma near the limbus, in the iris stroma, in the ciliary pro-

cesses, 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.

LIPID STUDIES. In Group I rabbits on the normal diet, in the non-injected left eyes, the only lipid found was that normally present in the myelinated nerve fibers 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, and the other differential lipid stains were negative as well. The nature of the lipid was therefore unclear. The probable source of this lipid, however, was the damaged myelinated nerve fibers 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 Group II and Group III rabbits showed identical pictures. In the uninjected, control eye there was a deposition of lipid exactly as described previously by Cogan and Kuwabara[31] in the hypercholesteremic rabbit. Near the limbus of the cornea there were superficial intracellular lipid deposits. Similar lipid-filled histiocytes 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 droplets of lipid. No lipid was found in the retina except that normally present in the myelinated nerve fibers.

In the injected eyes which had been the site of a prolonged allergic uveitis, the deposition of lipid was much more intense—at least ten- to twentyfold 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. The concentration of lipid in the iris stroma was greatly increased and infiltrated 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 within the retina. When present in the retina, they appeared to be associated with retinal necrosis or with an inflammatory reaction. These lipid-filled cells were almost constantly present 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 and heavy staining in every area where lipid deposition had been observed. 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, if not all, of the lipid deposited in these injected eyes was cholesterol.

MUCOPOLYSACCHARIDE STUDIES. To ascertain what role, if any, might be played by the mucopolysaccharides in this deposition of cholesterol, representative sections of eyes from these three groups of rabbits were stained with the PAS stain and with the colloidal iron stain. The results were as follows.

1. PAS stain. In the injected eyes of the Group I (normal diet) rabbits, PAS positive retinal exudates were extremely rare, small and insignificant. More frequent were masses or globules of PAS positive debris in the vitreous. Occasionally, coarse PAS positive granules were identified in macrophages in the vitreous or subretinal space. There was no thickening of the subintimal basement membrane of the retinal vessels. In the injected eyes of the Groups II and III (hypercholesteremic diet) rabbits, only one eye showed a significant deposition of PAS positive material in the outer retina adjacent to the disc. In all of 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. In respect to the locus of this intracellular PAS positive material, the injected eyes of Group I (normal diet) and the injected eyes of Groups II and III (hypercholesteremic diet) were similar.

2. Colloidal iron stain. In both the normal and in the injected eyes of the Group I rabbits 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 Groups II and III rabbits, all the foamy macrophages in both the subretinal and vitreous exudates were invariably filled with a delicate recticulum of fine AMP positive granules. Similarly, the foamy macrophages elsewhere in the eye—in the cornea, iris, ciliary processes, choroid, and sclera—were likewise filled with this same AMP positive material. However, only a very few such macrophages could be found in the retina proper. It is notable that with the lipid stains these same AMP filled macrophages were observed to be laden with cholesterol.

Summary of Experimental Studies

These experiments, briefly reported here, failed completely to simulate the characteristic clinical and histologic lesions of Coats' disease. While there were many cholesterol-filled macrophages in the subretinal space, there were equally many present in the vitreous, and their presence in the retina proper was only rarely noted. These lipid deposits appeared to be only part and parcel of the massive cholesterol deposition throughout the eye. Possibly, with an inflammatory stimulus of lesser magnitude and with a less marked degree of hypercholesteremia, the result might have been different. Also, if the retina of the animals studied had had a vascular system more nearly comparable to that of the human retina the results might have been different. However, in these rabbits with a hypercholesteremia it is notable that the retina was actually the site of least involvement in the cholesterol deposition. All that can be concluded is that, although given abundant opportunity, the rabbit does not develop Coats' disease!

However, these experiments do show two things. The first is that a local inflammatory process in the eye, in the presence of a concomitant hypercholesteremia, does accelerate and augment the deposition of cholesterol in the intraocular tissues. Second, that 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' disease in humans and in human xanthomatoses. This observation gives further support to Faber's original 1949 hypothesis that the intermediary action of such an acid mucopolysaccharide is an instrumental factor in the deposition of cholesterol in the tissues.

CONCLUSIONS

For the present, subject to the reservations of the possible nonspecificity of the techniques here employed and the improbability that such a massive deposition of cholesterol may be demonstrated in some ocular lipid histiocytosis other than Coats' disease, the following conclusions are justified.

1. Coats' disease (Groups I and II as described by him in 1908) is a clinical and histologic entity. The clinical and histologic pictures are the same in both the adult and juvenile forms of the disease.

2. In the adult cases there is a hypercholesteremia and a history and physical findings of a repeated or protracted prior uveitis.

3. In the juvenile cases there is usually no evidence of uveal inflammation and the plasma lipid levels are normal.

4. The essential pathologic feature of Coats' disease is the deposition of free cholesterol, the cholesterol esters, and an unidentified crystalline in the external retina, and of these same lipids together with fatty acids in the subretinal space. Other lipids play a negligible or insignificant role in the disease. In this respect Coats' disease appears to differ profoundly from the other ocular histiocytoses where cholesterol is not the lipid concerned. For example, the specific lipid stains employed in this study indicate that the lipid deposits in diabetic retinopathy are almost entirely neutral fats.

5. It is clear that the intimal and subintimal deposition of a PAS positive mucopolysaccharide in the retinal arterioles and telangiectases plays no role in the pathogenesis of the disease.

6. In the adult cases the trigger mechanism initiating the deposition of cholesterol in the tissues appears to be the insult of a previous uveal inflammation in the presence of a hypercholesteremia. For this actual deposition of cholesterol to occur the intermediary action of some tissue factor is almost certainly necessary.

7. In the juvenile form of the disease in which there is no evidence of uveal inflammation and in which plasma lipid levels are normal, the intermediary role of the tissue factor must be dominant.

8. There is highly suggestive evidence that the tissue factor involved is an acid mucopolysaccharide which acts as an intermediary factor, possibly as a catalytic agent or, more probably, by entering into a combination with the lipoproteins of the blood plasma, thus forming a new acid mucopolysaccharide-lipoprotein complex. The resulting hydrolysis of this complex would free the cholesterol for deposition in the external retina and subretinal space, while the fatty acids are extravasated into the subretinal space.

9. The experiments designed to produce ocular lesions in the experimental rabbit comparable to those of Coats' disease in humans did not achieve this objective. However, they did show that in the experimental rabbit, a local inflammatory process in the presence of a concomitant hypercholesteremia accelerates and augments the deposition of cholesterol in the ocular tissues. This deposition of cholesterol is invariably associated with the presence of an acid mucopolysaccharide at the locus of deposition of cholesterol in the tissues.

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