COATS'S DISEASE*

II. STUDIES ON THE IDENTITY OF THE LIPIDS CONCERNED, AND THE PROBABLE ROLE OF MUCOPOLYSACCHARIDES IN ITS PATHOGENESIS†

BY

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IN a previous communication (Woods and Duke, 1963), the literature on Coats's disease, especially as concerns the deposition of lipid in the sub-retinal space and the external retina, was reviewed and evaluated. From this review it appeared clear that, while the presence of foam cells and cholesterol clefts had indicated to previous students the probable deposition of cholesterol in the involved ocular tissues, no actual proof of this assumption had ever been presented. In this same communication clinical, histological, and blood chemistry studies were also presented. These studies indicated that Coats's disease presented the same pictures in both adults and children. In adults, however, there was invariably a history of some preceding uveal inflammation and the constant finding of a hypercholesteraemia. In children such a history could only rarely be obtained, and the plasma lipids were always within normal limits.

These findings of a preceding ocular inflammation and a hypercholesteraemia made it possible to formulate a reasonable explanation for the pathogenesis of the disease in adults, *i.e.* local inflammation in the presence of a hypercholesteraemia. However, it is probable that the intermediary action of some tissue factor is also necessary for the actual deposition of the lipid in the tissues. Uveal inflammation and hypercholesteraemia obviously play no part in the pathogenesis of the juvenile form of the disease. Here it becomes imperative to explore the nature of the hypothesized tissue factor.

The most plausible suggestion on the nature of this factor was that made by Faber (1949), who produced evidence which indicated that a metachromatic acid-mucopolysaccharide, which could be demonstrated in the subintima and medial intima of human aortas, was the tissue factor responsible for the later deposition of cholesterol in the same locus. Reese (1956) quite independently suggested that the periodic acid-Schiff (PAS)-positive subintimal membrane which he demonstrated in the retinal telangiectasis of Coats's disease might serve this same function.

The histopathological studies here reported were undertaken in the hope of resolving some of these unknowns. The specific objectives may be stated as follows:

I. To determine whether the proteinaceous exudate in the subretinal space and external retina contains a lipid component, and to establish the nature of any lipid fraction which may be so present.

II. To explore the hypothesis that the intermediary action of some mucopolysaccharide may be concerned in the deposition of cholesterol in eyes with Coats's disease. Such a mucopolysaccharide may be either the PAS-positive subintimal membrane found in the retinal telangiectasis by Reese (1956), or the acid-mucopolysaccharide described by Faber (1949) and believed by him to be 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: *i.e.* 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 Prof. Norman Ashton of the Institute of Ophthalmology, London, each of which was 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.

I. IDENTIFICATION OF THE NATURE OF THE RETINAL AND SUBRETINAL EXUDATE

(1) Demonstration of a Lipid Component in the Exudate of Coats's 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.

(*A*) *Studies on Paraffin-Embedded Material.*—Before 1960, all the histological material in the Pathological Laboratory of the Wilmer Institute in which the clinical diagnosis of Coats's disease had been made, had routinely been embedded in paraffin. As Leber (1916) had pointed out, such material had been exposed to fat-dissolving alcohols in the course of preparation, and was therefore probably invalid for accurate lipid studies. Primarily, it was necessary to settle this point definitely. To this end, sections of twelve of the paraffin-embedded eyes (ten juvenile and two adult) with a clinical and histological diagnosis of Coats's 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 (Fig. 1, overleaf). This lipid material appeared to exist 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, no positive reaction was 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 (1961) 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. Thus, there is indirect evidence that the persisting lipid occasionally found in these formol-fixed and paraffin-embedded eyes may be, at least in part, a cerebroside or phosphatide complex. There are two possible sources of this Sudan black-positive lipid. Streeten (1961) 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 haemorrhages which may characterize Coats's disease and the subsequent breakdown of the red cells, may liberate quantities of phospholipids, some of which are picked up in macrophages or 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, the great bulk of this had been extracted during the processing of the material. Leber (1916) was thus clearly correct in his conclusion that such eyes were unsuited for valid lipid stains.

(B) Studies on Gelatin-Embedded Material.—To study properly the retinal and subretinal exudate for any lipid component, recourse was had to the four eyes with Coats's disease which had been fixed in formalin, one-half of each of which had been embedded in gelatin and at no time exposed to any fat

solvent.   Frozen sections cut at a thickness of 10μ were prepared from these eyes.

The Oil-red-O stain and the Lorraine–Smith–Dietrich stain, another broad-spectrum nonspecific 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 (Fig. 2, opposite).   In the subretinal space the material appeared as large round globules, probably representing swollen, lipid-filled cells (Fig. 3, opposite).   Very little lipid material was seen in the organized fibrous tissue of the subretinal space.

It is quite possible that much of the homogeneous staining exudate in the external retina and subretinal space, seen with the conventional haematoxylin 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's disease is chiefly lipid in nature.

(2) Nature of the Lipid in the Exudate of Coats's Disease

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

(A) Crystallography.—Unstained frozen sections were first examined under polarized light.   Masses of doubly-refractile crystals were clearly

FIG. 1.—Masses of residual lipid remaining in subretinal exudate in paraffin-embedded eye with Coats's 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.

FIG. 2.—Massive deposition of lipid in retina in a juvenile case of Coats's disease.   The subretinal space is below and rods and cones are absent.   Gelatin-embedded frozen section, Oil-red-O. ×40

FIG. 3.—Lipid deposition in subretinal space in a juvenile case of Coats's disease.   Gelatin-embedded, frozen section, Oil-red-O.   ×25.

FIG. 7.—Greenish areas indicate sites of cholesterol deposition in the retina in a juvenile case of Coats's disease.   The subretinal space is below.   The air bubbles are artefacts.   Gelatin-embedded unstained frozen section, Schultz reaction.   ×40.

FIG. 8.—Masses of cholesterol-filled foam cells in subretinal space in a juvenile case of Coats's disease.   The pigment epithelium is seen below.   Gelatin-embedded unstained frozen section, Schultz reaction.   ×25.

FIG. 11.—Admixture of neutral fats (pink) and other lipids (purple or lavender) in foam cells in subretinal exudate in a juvenile case of Coats's disease.   Gelatin-embedded frozen section, Nile blue sulphate.   ×40.

(1)

(2)

(3)

(7)

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(11)
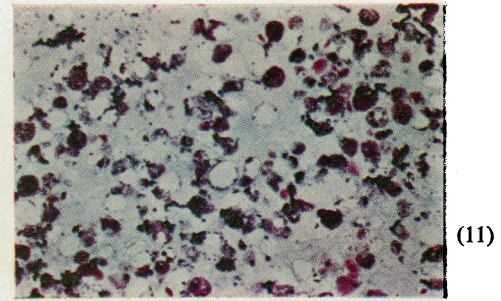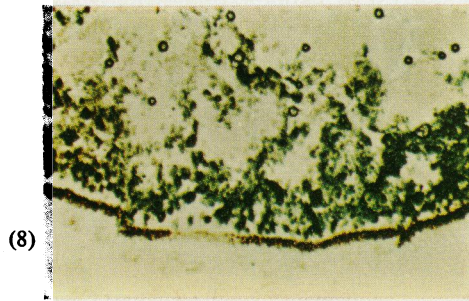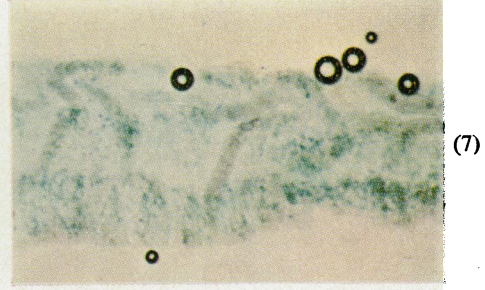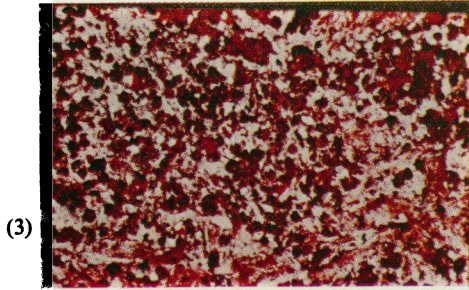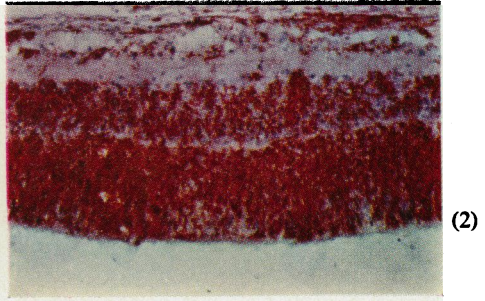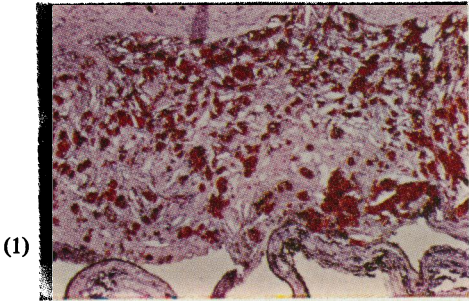
seen both in the external retina (Fig. 4), and in the subretinal exudate (Fig. 5).
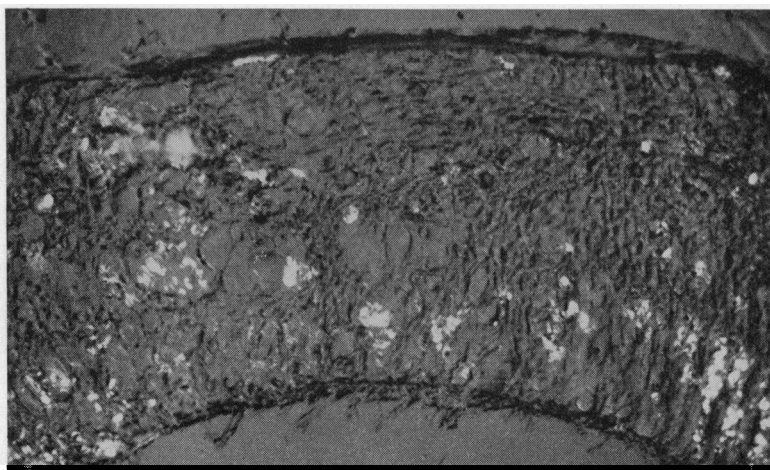

FIG. 4.—Doubly-refractile crystals in the retina observed with polarized light in a juvenile case of Coats's disease. The vitreous space is above, subretinal space below. Gelatin-embedded unstained frozen section. × 32.
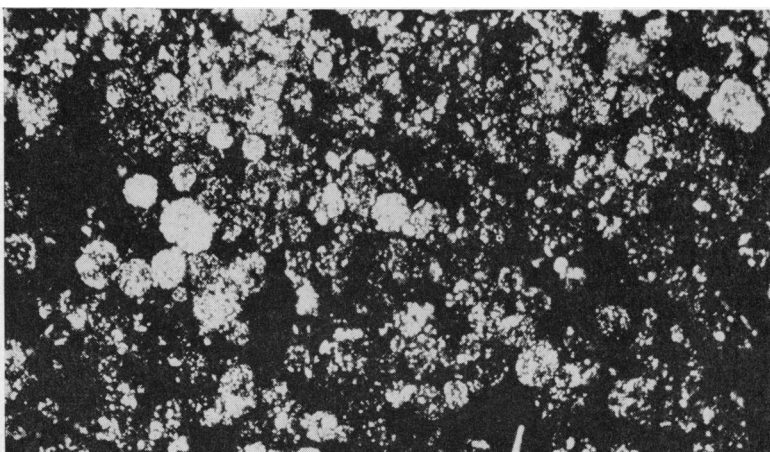

FIG. 5.—Doubly-refractile crystals within foam cells in subretinal space observed with polarized light, in a juvenile case of Coats's disease. Gelatin-embedded unstained frozen section. × 32.

Some crystals had the long needle form of cholesterol (Fig. 6, overleaf).

In the hope of identifying the exact nature of these crystals, various sections were submitted to Dr. J. D. Donnay, Professor of Crystallography 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. Recourse was therefore had to histochemical reactions.
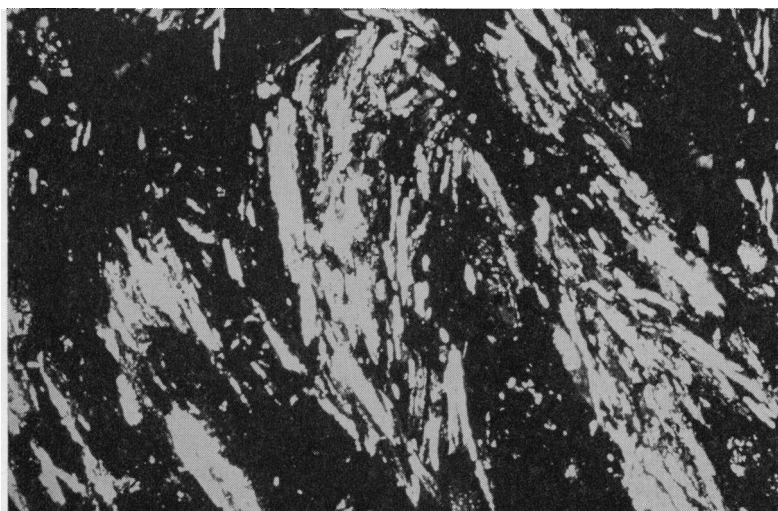
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FIG. 6.—Doubly-refractile, needle-like crystals lying free in the subretinal space, observed with polarized light, in a juvenile case of Coats's disease. Gelatin-embedded unstained frozen section. × 32.

(B) *Schultz Modification of the Lieberman–Burchardt Sterol Reaction.*— This reaction is regarded as highly specific for both free cholesterol and the cholesterol esters (Pearse, 1961). Unstained sections are first oxidized and mordanted with 2·5 per cent. iron alum for 3 days. They are then submitted to a mixture of sulphuric and glacial acetic acids. In the presence of free cholesterol and the cholesterol esters, the sections take on a bluish-green colour which persists for 30 to 60 minutes and then rapidly fades.

The unstained sections of the eyes with Coats's disease submitted to this reaction gave strongly positive results in both the external retina and the subretinal space, indicating that free cholesterol or the cholesterol esters were an important lipid component in the exudative process (Figs 7 and 8, col. plate I).

(C) *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, *i.e.* 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 thus remain clearly visible, while the usual cholesterol esters do accept the stain, are coloured a deep red, and lose their characteristic crystalline appearance. Further, when digitonin-treated slides are exposed to cold acetone, the insoluble cholesterol-digitonin crystals

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

When unstained sections of the eyes with Coats's 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 sub-retinal 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 (Fig .9).
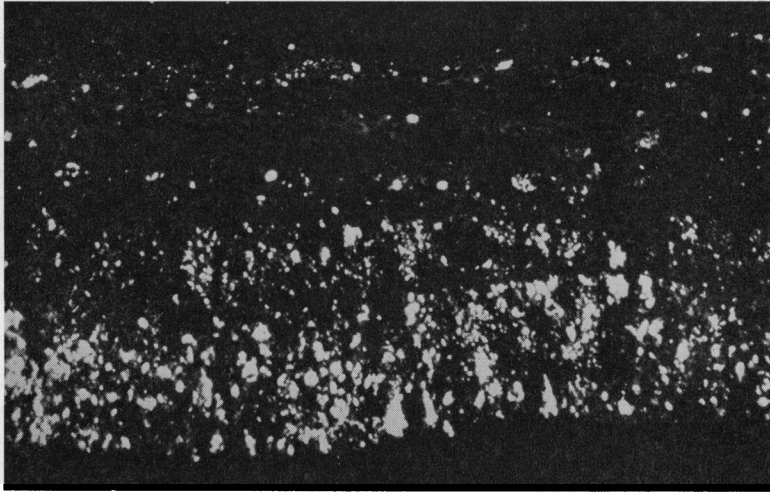

FIG. 9.—Doubly-refractile crystals in retina after treatment with digitonin 0·5 per cent. in a juvenile case of Coats's disease observed with polarized light. The concentration of crystals is chiefly in the external retina. Subretinal space is below. Gelatin-embedded unstained frozen section, Windaus digitonin reaction. × 32.

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 (Fig. 10, overleaf).

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

(3) Role of Other Lipids in the Exudate of Coats's Disease

To explore the possible role other lipid fractions might play in the histopathology of Coats's disease, sections of the gelatin-embedded eyes were stained with the Nile-blue-sulphate stain for the neutral fats and with Fischler's fatty acid stain for the fatty acids, and were examined with Baker's acid haematin reaction for the phospholipids. Primarily, it may be stated
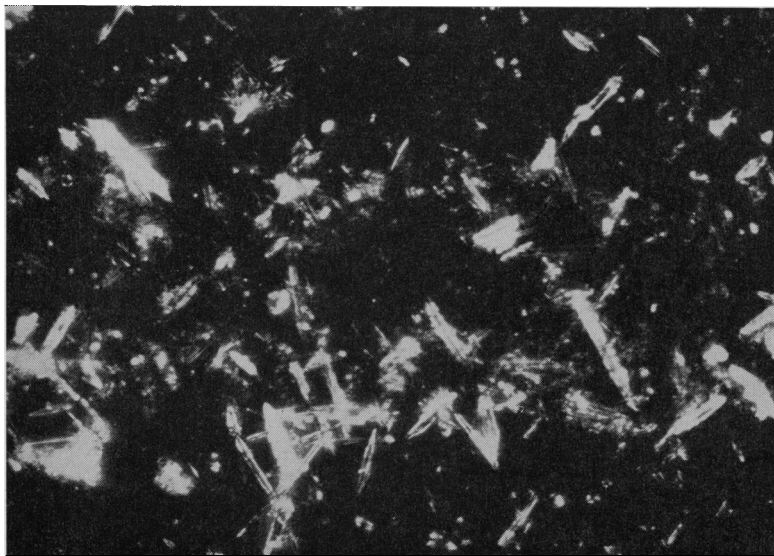
FIG. 10.—Persistence of crystals of cholesterol-digitonin complex in subretinal space after treatment with cold acetone in a juvenile case of Coats's disease observed with polarized light.   Gelatin-embedded unstained frozen section, Windaus digitonin reaction.   × 32.

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's disease, as previously defined, were as follows:

(*A*) *Neutral Fats.*—With the Nile-blue-sulphate stain, the neutral fats are supposed to stain pink while all other lipids (cholesterol, the fatty acids, and the phospholipids) stain bluish-purple.   In the eyes with Coats's disease, the greater portion of the material in the subretinal space stained bluish-purple, while only a small amount stained pink (Fig. 11, col. plate I).   In the external retina, all the lipid material stained bluish-purple, and no pink-staining material could be detected (Fig. 12, opposite).   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.

(*B*) *Fatty Acids.*—Fischler's fatty acid stain is believed to be specific 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's disease were stained with this stain, no stain whatsoever appeared in the external retina, while the exudate in the subretinal space stained deeply (Fig. 13, opposite).

This finding, taken in conjunction with the results observed with the Nile-blue-sulphate stain, would indicate that neither fatty acids nor neutral fats are components of the lipid deposits in the external retina, while in the
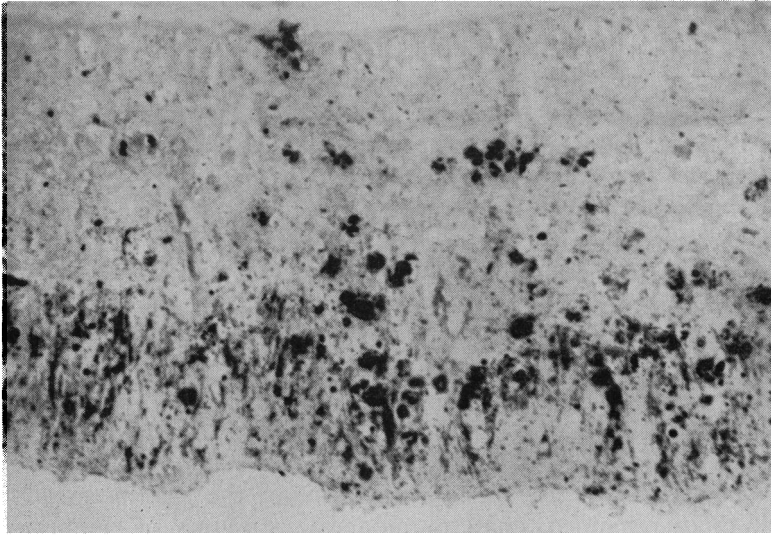
FIG. 12.—Bluish-purple staining lipid deposits, chiefly in outer retina in a juvenile case of Coats's disease. No neutral fats are demonstrated. Gelatin-embedded frozen section, Nile blue sulphate. × 32.
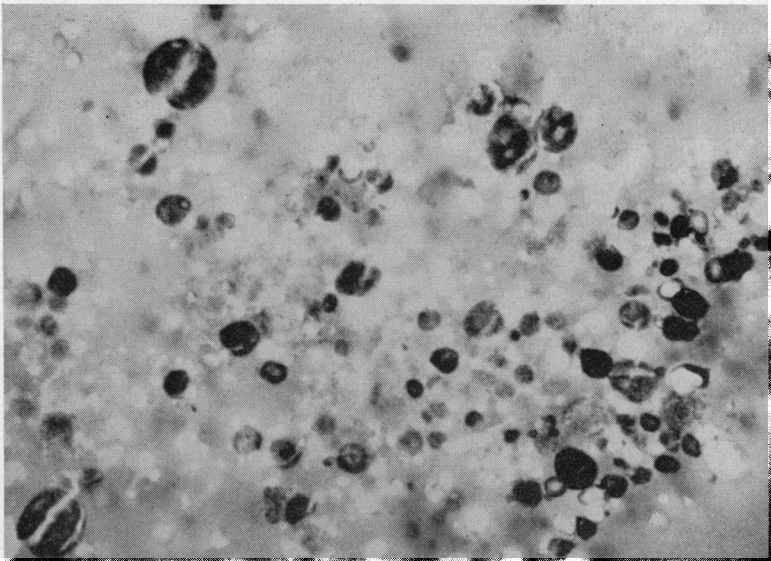
FIG. 13.—Grey and bluish-black-staining foam cells in subretinal exudate containing fatty acids in a juvenile case of Coats's disease. Gelatin-embedded frozen section, Fischler's fatty acid stain. × 32.

subretinal exudate there is a considerable amount of the unsaturated fatty acids, with a small admixture of the neutral fats.

(C) *Phospholipids.*—Baker's acid haematin 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 haematin 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 proceding 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's disease. However, the lack of initial formol-calcium fixation of these tissues may qualify the validity of these negative findings.

(D) *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-sulphate 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 (Fig. 14).
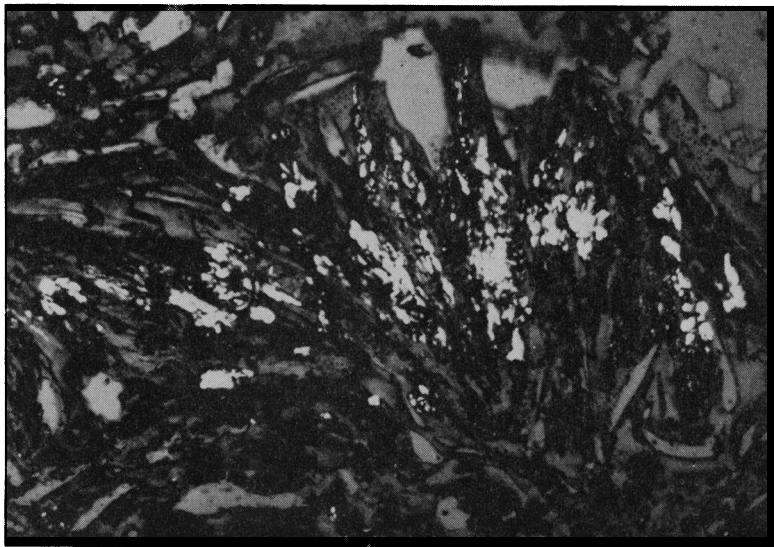

Fig. 14.—Doubly-refractile "unidentified crystallines" (here they appear white), which failed to accept lipid stains, in the subretinal space in a juvenile case of Coats's disease. Gelatin-embedded frozen section observed with partially polarized light, Sudan black.　× 80.

The probable explanation for these "unidentified crystals" is that 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 (1949) in 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's disease the exudates in the external retina and in the subretinal space 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's disease *per se* or whether it is a common characteristic of the so-called "ocular lipid histiocytoses" (Heath, 1932), a nonspecific consequence of prolonged or repeated haemorrhages in the retina or subretinal space, or a nonspecific consequence of prolonged retinal detachment *per se*.

(4) 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", *i.e.* 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 haemorrhage;

(*c*) Eyes with long-standing detachment of the retina.

The results of these control studies are as follows:

(*A*) *Ocular Lipid Histiocytoses*

(1) *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.

(2) *Diabetic Retinopathy.*—Two eyes with advanced diabetic retinopathy were obtained from the autopsy room, fixed in formalin, embedded in gelatin, sectioned, and stained with Oil-red-O.   Both eyes showed brilliantly-staining, circumscribed deposits, deep in the retina (Fig. 15, col. plate, overleaf).

When they were stained with Fischler's fatty acid stain, the result was negative. When they were stained with Nile-blue-sulphate, the reaction indicated a pre-dominance 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 were present in this lesion 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 Oil-red-O stain.

(3) *Junius-Kuhnt Disciform Degeneration of the Macula.*—Formalin-fixed sections of a typical case were obtained from the Pathological Laboratory of the Institute of Ophthalmology of London through the courtesy of Prof. Norman Ashton.

Haematoxylin and Eosin Stain.—The retina in the macular region was elevated by a dense plaque of fibrous tissue.   The pigment epithelium beneath this plaque had been partially destroyed, but within the plaque itself there was proliferation of pigment epi-thelium with a reduplication of Bruch's membrane.   In addition, there was fresh hae-morrhage and a few foci of lymphocytes were seen within this plaque near its periphery. The retina over this lesion was intact.

Unstained Gelatin-embedded Sections.—When these were examined under polarized light a few doubly-refractile crystals in the area of fresh haemorrhage were disclosed. These lay within haemosiderin-laden macrophages and were apparently related to the breakdown of the red blood cells.   No other doubly-refractile crystals were seen in the lesion.

Oil-red-O.—The retina was free of lipid.   Bruch's membrane, including its redupli-cation, stained intensely (Fig. 16, col. plate II, overleaf).   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 (Fig. 17, col. plate II, overleaf).   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, *i.e.* intense staining of Bruch's membrane and its reduplication, and staining of a few large macrophage-like cells in the subretinal space.   The doubly-refractile crystals noted in the unstained sections within the area of haemorrhage did not accept the lipid stain.

Nile-blue-sulphate.—Bruch's membrane failed to accept this stain, but traces of neutral fats were observed in a few of the large cells in the subretinal space.

Fischler's Fatty Acid Stain and Schultz Reaction for Cholesterol.—These were like-wise 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 the study of eyes with the other so-called "ocular lipid histiocytoses"—Tay–Sachs disease and Niemann–Pick disease.

(B) Repeated Episodes of Retinal Haemorrhage

(1) *Hypertensive Retinopathy.*—Two eyes with severe hypertensive retino-pathy were obtained at autopsy and prepared in the manner described above. Massive haemorrhages within the retina and in the subretinal space were present. The Oil-red-O stain revealed no lipid component associated with these haemor-rhages and the Schultz reaction for cholesterol was similarly negative.

(2) *Haemorrhagic Glaucoma.*—An eye from a 35-year-old female (non-diabetic) with haemorrhagic 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

(1) *Absolute Glaucoma and Long-standing Retinal Detachment in a 72-year-old Man.*—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. The Oil-red-O, Fischler's fatty acid, and Nile-blue-sulphate stains and the Schultz reaction for cholesterol were all negative.

(2) *Absolute Glaucoma and Long-standing Retinal Detachment in a 19-year-old Man 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 minimal amount of lipid in the outer plexiform layer of the retina and in the rod and cone layer. The Nile-blue-sulphate stain identified these lipids as neutral fats in the outer plexiform layer. Fischler's fatty acid stain and the Schultz reaction for cholesterol were negative.

(3) *Long-standing Retinal Detachment due to Retrolental Fibroplasia in a 9-year-old boy.*—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 also a small quantity of lipid in the rod and cone layer. 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 haemosiderin pigment. The Nile-blue-sulphate 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 haematoxylin and eosin, and with this stain it was immediately evident that most of this lipid material had been associated with recent and old haemorrhage 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, which 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 haemosiderin pigment. Similar Sudan black-positive macrophages were found in areas of organizing haemorrhage and fibrous plaque formation in the subretinal space. 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 retrolental fibroplasia was derived from the break-down of red blood cells with the attendant release of their phospholipid complexes.

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, and differential stains indicate that this lipid is chiefly neutral fat.

In hypertensive retinopathy little if any lipid is deposited in the retina, either independently of or in association with the retinal haemorrhages. Old haemorrhages in the vitreous may release phospholipid complexes from the break-down of the red cell membrane. In addition traces of cholesterol may be 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, but its nature is unclear, since it accepts none of the specific lipid stains.

A retina which has been detached for a prolonged period may show small deposits of lipid in the outer layers, but this lipid is not cholesterol.

II. MUCOPOLYSACCHARIDE STUDIES

These studies were undertaken to explore the possible role in the pathogenesis of Coats's disease of (*a*) the intimal polysaccharide membrane demonstrated by Reese (1956) in the retinal arterioles of the telangiectasis of Coats's disease, and (*b*) the metachromatic-staining acid-mucopolysaccharide described by Faber (1949) and other workers 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 were indeed in some respects superior to the gelatin-embedded eyes. Therefore, paraffin sections of two adult and twelve juvenile eyes and gelatin sections of four juvenile eyes were used in these studies. Representative sections

were stained with the periodic acid-Schiff (PAS) reagent, the Rinehart–Abul-Haj colloidal iron, the alcian blue, and the toluidine blue stains. In our hands, 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 reported collectively as a unit for each stain, and not individually.

(A) 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 (*i.e.* a protein containing a carbohydrate moiety as an integral part of its structures). Any metachromatic component in the basement membrane is an acid-mucopoly-saccharide (hyaluronic acid, heparin, or chondroitin sulphuric acid), which does not stain with PAS. Flat preparations of the normal retina stained with PAS clearly demonstrate the presence of a PAS-positive subintimal basement membrane which is usually sufficient to delineate the entire retinal vascular pattern (Friedenwald, 1948).

In the eyes with Coats's 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 was so thickened as to cause a partial to almost complete occlusion of the lumina of the affected vessels (Fig. 18).
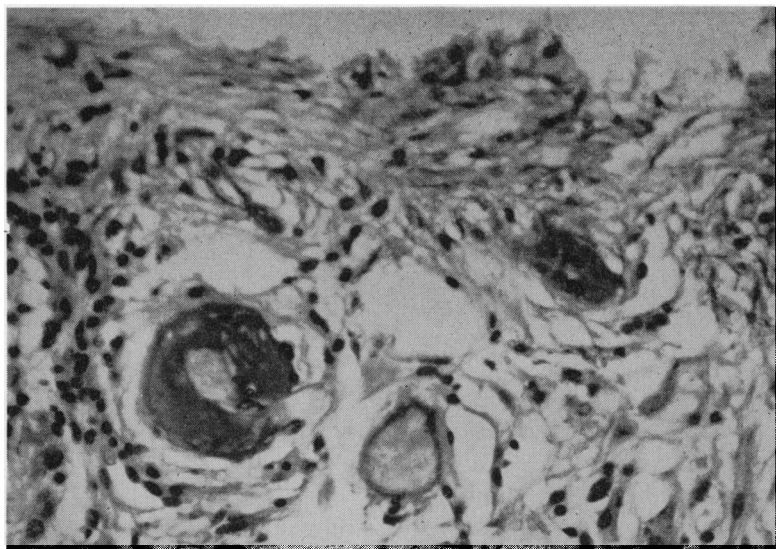

Fig. 18.—Marked subintimal thickening of basement membrane of retinal vessels in a juvenile case of Coats's disease. Paraffin-embedded, PAS-haematoxylin stain. × 80.

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's disease, five juvenile eyes and four adult eyes with long-standing retinal detachment but no suggestion or evidence of Coats's disease were studied with the PAS stain. The PAS-positive subintimal membrane was present in all these eyes, and was markedly thickened in four juvenile and two adult eyes, causing partial occlusion of the lumina of the vessels (Fig. 19). In addition, PAS-positive exudates were found in the outer retina of four juvenile eyes and one adult eye. 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's disease. and is, consequently, probably not concerned in its pathogenesis.
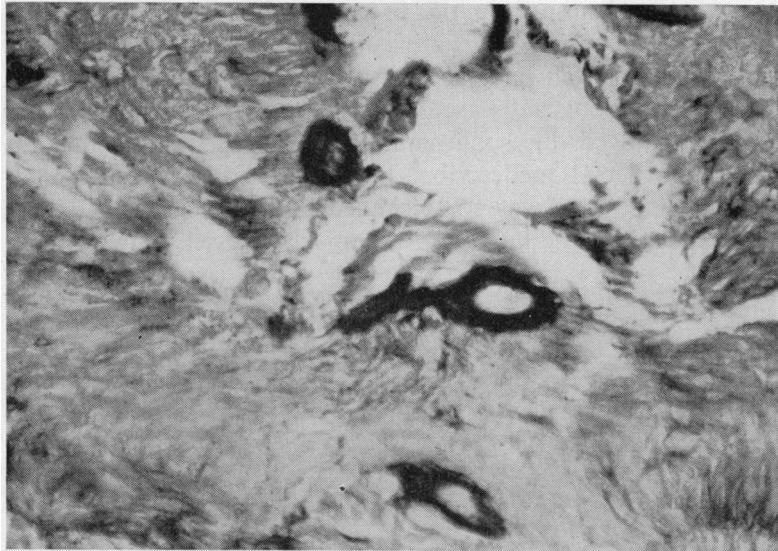

Fig. 19.—Marked subintimal thickening of basement membrane of a retinal vessel in an eye with long-standing retinal detachment and with no evidence of Coats's disease. Paraffin-embedded, PAS-haematoxylin stain. × 80.

(B) Colloidal Iron and Alcian Blue Stains.—These are believed to be specific for the acid-mucopolysaccharides—chondroitin sulphuric acid, heparin, and hyaluronic acid. They are not metachromatic.

In paraffin-embedded sections of eyes with Coats's disease, both stains occasionally demonstrated a small amount of acid-mucopolysaccharide material beneath the intima of a retinal capillary or cuffing a retinal vessel. The foam cells in the external retina occasionally contained numerous fine

acid-mucopolysaccharide-positive granules, while the foam cells in the subretinal exudate contained a considerable quantity of this same material (Fig. 20, col. plate II, overleaf).

In the gelatin-embedded eyes these acid-mucopolysaccharide granules 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 the 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 intense PAS-positive retinal exudate, did not accept either of these stains. This was to be expected, and it clearly illustrates the different staining affinities of the glycoprotein mucopolysaccharide and the acid-mucopolysaccharides.

It is known that in normal eyes an acid-mucopolysaccharide is present in the interstices of the rods and cones (Zimmerman, 1957, 1958). In control eyes with long-standing detachment of the retina in which the rods and cones had 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, no lipid was present, and the acid-mucopolysaccharide within the cell apparently resulted merely from the normal phagocytosis of detritus. In addition, in these control eyes, there was occasional cuffing of a retinal vessel with similar positive-staining material.

The particular acid-mucopolysaccharide associated with the rods and cones and with the pigment epithelium, which is present in normal eyes, is known to be resistant to the action of hyaluronidase. When sections of eyes with Coats's disease 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's disease is probably derived from the acid-mucopolysaccharide normally present in the rods and cones or in the pigment epithelium.

(C) **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 alcian blue stains. It failed completely in the gelatin-embedded eyes. In four of the paraffin-embedded eyes with Coats's disease and in one control eye with retinal detachment an occasional retinal blood vessel showed metachromasia of the vessel wall. Also in one control eye fine pink-coloured metachromatic

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* Wydase, Wyeth Laboratories, Philadelphia.

granules were noted within macrophages in the subretinal space.   However, this was an exception.   In our hands, the fine acid-mucopolysaccharide granules so well demonstrated in the foam cells of the eye with Coats's disease with colloidal iron and alcian blue stains, could not be consistently demonstrated with the metachromatic toluidine blue stain.

These results obtained with the colloidal iron and alcian blue stains are in full 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's disease certainly suggests that this material may play some role and is probably 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 which were identical in each case, were as follows:

Unstained Sections.—With polarized light, masses of doubly-refractile crystals were seen in the dermis within the foam cells (Fig. 21).
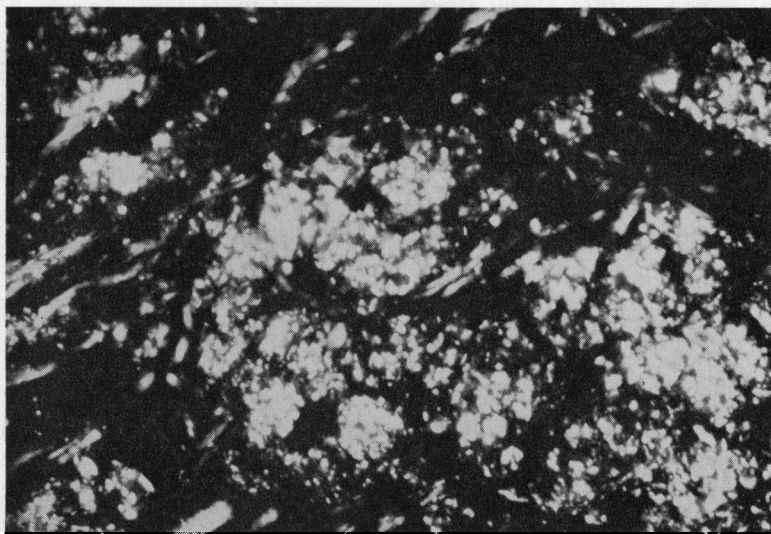

FIG. 21.—Masses of doubly-refractile crystals in the dermis in a case of xanthelasma.   Frozen unstained section, observed with polarized light.   × 80.

Oil-red-O.—There was intense staining of all the foam cells in the dermis.

Schultz Reaction for Cholesterol.—There was intense greenish staining of the foam cells (Fig. 22, col. plate II, overleaf).

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-sulphate Stain.—This was also entirely negative.

The remaining half of each lesion was then 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.

Colloidal Iron Stain.—The great majority of the foam cells was filled with fine blue granules arranged in a delicate meshwork (Fig. 23, col. plate II, overleaf). The appearance of these was identical with that of the granules in the foam cells in the cases of Coats's disease.

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 of the various types of mucopolysaccharide stain make apparent at once the great histological similarity between the lesions of Coats's disease and skin xanthelasma. In each, the predominant feature is the massive deposition of cholesterol in the tissues; and there is also an acid-mucopolysaccharide in the same foam cells as those which contain the cholesterol. It should be noted, however, that in xanthelasma the cholesterol deposition invariably appears to be within the histiocytes. In Coats's 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's 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 compared with their abundance in the skin. Also, haemorrhage *per se* is never associated with xanthelasma and, while it may or may not occur in Coats's disease, it (together with the serous exudation) is a secondary phenomenon. These factors of haemorrhage and serous exudation may account for the one demonstrable histochemical difference between these two conditions: *i.e.* the presence in Coats's 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

It should be noted that the lipid studies here reported were carried out only on eyes with the juvenile form of Coats's disease. Efforts to obtain a gelatin-embedded eye with the adult form of Coats's disease have been unsuccessful, and it may be only by some fortuitous chance that one will ever become available. However, as pointed out in the previous paper, the clinical pictures of the adult and juvenile forms of the disease are identical.

Likewise with the ordinary haematoxylin and eosin stains of paraffin sections the histology 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's 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 stain. 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 and "unidentified crystals", and possibly traces of neutral fats and the phospholipids.

The presence of fatty acids in the subretinal space is most interesting. As pointed out in the previous communication (Woods and Duke, 1963), there is excellent evidence (Bragdon and Havel, 1954; Korn, 1955) that, while heparin has no direct clearing action on the triglycerides of the blood, it does activate a lipoprotein lipase. The heparin itself apparently combines with the lipoprotein molecules of the blood serum forming a substrate on which the activated lipase acts with resultant hydrolysis of the heparin-lipoprotein complex. Since heparin and chondroitin sulphuric 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

FIG. 15.—Lipid exudates in retina in a case of diabetes. Most of the exudates are in the outer plexiform layer. Gelatin-embedded frozen section, Oil-red-O. × 40.

FIG. 16.—Lipid deposition in Bruch's membrane (red line below) and in its reduplication (wavy red line above) in a case of Junius–Kuhnt disciform degeneration of the macula. The reduplication and the proliferating pigment epithelium are surrounded by fibrous scar tissue and some fresh haemorrhage. Gelatin-embedded frozen section, Oil-red-O. × 25.

FIG. 17.—Large lipid-laden cells in subretinal space adjacent to pigment epithelium and Bruch's membrane in a case of Junius–Kuhnt disciform degeneration of the macula. Gelatin-embedded frozen section, Oil-red-O. × 50.

FIG. 20.—Numerous fine acid mucopolysaccharide-positive granules within foam cells in subretinal exudate in eye with Coats's disease (juvenile case). Paraffin-embedded, Colloidal iron. × 160.

FIG. 22.—Greenish areas and green droplets represent sites of cholesterol deposition in the dermis in a case of xanthelasma. The air bubbles are artefacts. Frozen unstained section, Schultz reaction. × 40.

FIG. 23.—Heavy deposition of acid mucopolysaccharide-positive granules in histiocytes which also contain the lipid in a case of xanthelasma. Paraffin-embedded, Colloidal iron. × 256.

(15)

(16)

(17)

(20)

(22)

(23)
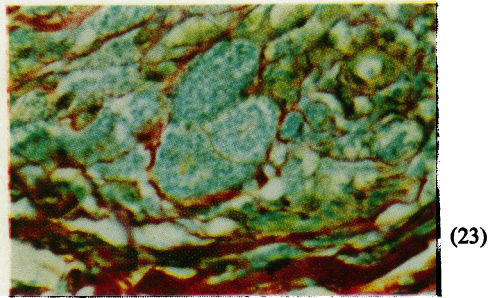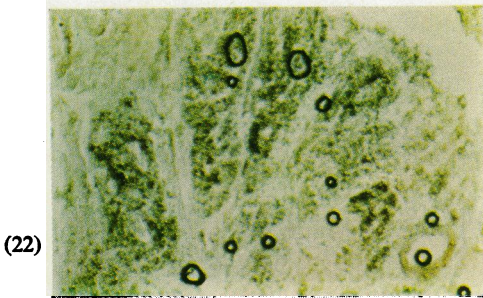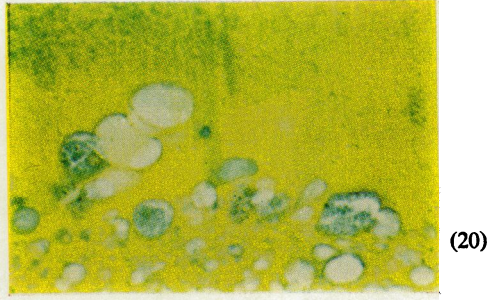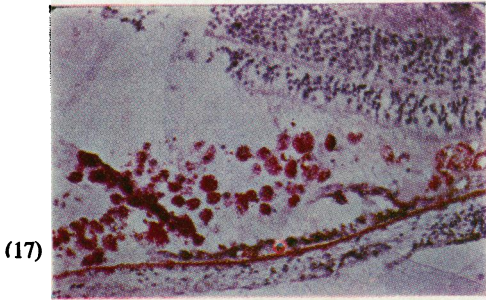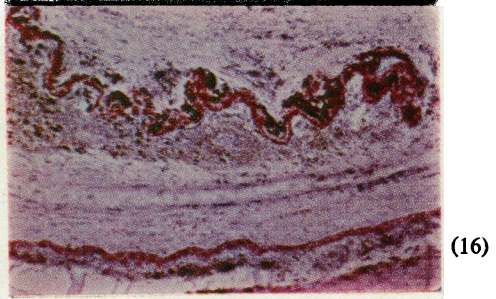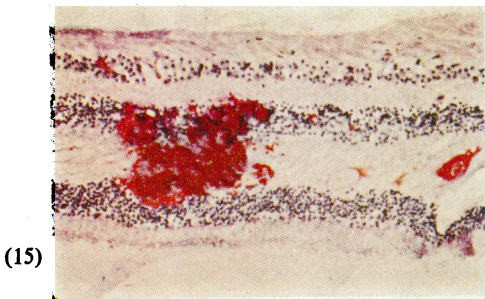
presence of fatty acids in the subretinal space may well be the result of a hydrolysis of a chondroitin acid sulphate-lipoprotein complex with the consequent reformation of fatty acids and cholesterol. The fatty acids are extravasated in the subretinal space, while the cholesterol fraction is deposited 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 histological pictures of the adult and the juvenile forms of the disease appear to be identical, it is probable the pathogenesis of the disease in the two age groups is the same. 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 is the factor which promotes the liberation of the acid-mucopolysaccharide for the molecular transfer. In the adult cases, the answer is easy: a preceding uveitis in the presence of a concomitant hypercholesteraemia. This is in accord with the known facts and with generally accepted views on the role of inflammation and of hypercholesteraemia in the systemic xanthomatoses. In the juvenile cases the serum cholesterol is normal 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 leading 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 infants and young children is less stable than in adults. It may be some low-grade and undetected minor trauma. The usual unilaterality of the juvenile form enhances this latter possibility. The fault 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.

CONCLUSIONS

For the present, subject to the reservations of the possible non-specificity of the techniques here employed and the improbability that such a massive deposition of cholesterol may be demonstrated in some ocular histiocytosis other than Coats's disease, the following conclusions are justified from the clinical observations and plasma lipid studies reported in the first communication and from the histochemical studies reported here:

(1) The essential pathological feature of Coats's 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

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subretinal space. Other lipids play a negligible or insignificant role in the disease. In this respect Coats's disease appears to differ profoundly from the other ocular histiocytoses in which 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.

(2) 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.

(3) In adults 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 hypercholesteraemia. For this deposition of cholesterol to occur, the intermediary action of some tissue factor is almost certainly necessary.

(4) 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.

(5) 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-mucopoly-saccharide-lipoprotein complex. The resulting hydrolysis of this complex would free the cholesterol for deposition in the external retina and sub-retinal space, while the fatty acids are extravasated into the subretinal space.

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