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POLARIZED LIGHT MICROSCOPY AS A TOOL OF DIAGNOSTIC PATHOLOGY

A REVIEW

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Diagnostic pathologists as a group might be regarded as a conservative group of physicians. Although changes and new procedures have been and are being introduced all the time, the lag period between introduction of new diagnostic procedures in experimental studies on animals and their application to human pathology is often long. Accordingly, the present review will include observations made on experimental animals which seem to be applicable to human diagnostic pathology, in addition to observations actually made on human material in which polarized light microscopy was of direct help in establishing the diagnosis. Following this line of reasoning the review will also include observations made on human or animal tissues which help recognize normal tissue elements, and thereby might help diagnosis.

THEORETICAL BASIS AND SOME PRACTICAL HINTS

Polarized light microscopy can selectively visualize anisotropic compounds or structures, and with good optics and proper alignment these objects appear bright and shining on a dark field. Anisotropic objects may exhibit a number of phenomena, one of which, birefringence, will be mainly discussed in the present review. Birefringence is the characteristic of an object to transmit plane-polarized light at different velocities at different azimuths. In other words, the velocity of a ray which is polarized and runs in the vertical plane significantly differs from that of a ray which runs through the same trajectory but is polarized in a horizontal plane. The physical nature of the phenomenon, its laws and the equipment used have been described in a number of publications (17, 150, 198, 215) and recently the subject has been discussed in rather simple terms (252) for persons who are not familiar with the mathematical-physical approach.

The presence of anisotropy indicates polarity and order. Directional movement, pressure or stretching often induce parallel alignment of molecules and, consequently, birefringence. Relaxation, heating and other factors causing random arrangement of molecules usually cause loss of anisotropy. It should be realized that birefringence has a sign. If the refractive index (RI) of the polarized ray which runs parallel to the length of a fiber is greater than that of the ray polarized in a plane perpendicular to the axis, the fiber exhibits a positive birefringence. Positive birefringence in a sphere means that the RI of the radius is greater than that of the tangent.

Birefringent objects are seen in the polarizing microscope ("under crossed polars") as shining bodies on a dark background. Different objects vary in their brightness, *i.e.*, in the intensity of birefringence. Two objects having the same thickness differ in the intensity of birefringence in accordance with their specific retardation: the object in which the difference between the two refractive indices is greater appears brighter than the object with a smaller difference. Another factor which determines the intensity of birefringence is the thickness of the structure along the path of the light. When two parallel fibers of identical constitution and structure which lie in the plane of the tissue section are examined together, the fiber which is twice as thick as the other one will exhibit double the intensity of birefringence.

The brightness of the image under crossed polars also depends on other factors. The intensity is greatest when the object is aligned at an angle of 45° to the polars. Alignment of the object parallel to one of the two planes of polarization (that of the polarizer or that of the

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analyzer plates) renders the object invisible. Lastly, most anisotropic biologic objects are birefringent and exhibit differences in their refractive indices along two of the three spatial axes. For example, a birefringent fiber (for example, of collagen) appears brightly illuminated when lying in the NE-SW and in the NW-SE directions (assuming that the polarizer and analyzer polarization planes are North-South and East-West), as has been pointed out in the preceding paragraph. The same fiber appears dark when cut transversely, as the refractive indices of polarized light in all the planes which pass through the fiber axis are equal. Tilted fibers appear brighter in accordance with the size at the angle between the fiber and the optical axis of the microscope.

The bright image of an object examined under crossed polars may be due to one or both of the following: intrinsic and form birefringence. While intrinsic birefringence is related to the spatial arrangement of atomic groups and molecules, form birefringence is due to the spatial arrangement of objects (rods or plates) submerged in a medium of a different RI. The intensity of form birefringence varies proportionally to the difference between the two refractive indices.

The effect on the compound birefringence (that observed between crossed polars) of the interplay of the two forms of birefringence was described over half a century ago (3) and may be summarized as follows. Whenever the intrinsic and the form birefringence are of the same sign, their effects are additive. Changing the mounting medium so that the difference between the RI of the studied object and that of the medium becomes greater will increase the intensity of birefringence in such a case. In those cases where form and intrinsic birefringence have opposite signs, the compound birefringence will have the sign of the more intense of the two, and its intensity will represent the algebraic sum of the two effects. Abolition of form birefringence can be obtained by using a mounting medium of similar RI to that of the object studied.

The interplay between form and intrinsic birefringence is of practical importance as in many instances weak and hardly observable birefringence may be made easily discernible by changing the nature of the mounting medium. Another way in which the weak birefringence of certain structures can be markedly intensified is in the staining of certain structures by some procedures. Impregnation with metals and even certain stages of some enzyme histochemical reactions result in the orderly alignment of the dyes or reaction products on a surface. The orderly deposition may result in the appearance or increase of birefringence. Such procedures often result in the appearance of dichroism. This phenomenon, which is another form of anisotropy, consists of different absorption curves (or different colors) of the two plane-polarized rays perpendicular to each other, the one parallel and the other perpendicular to the axis of the observed structure. As will be seen in the subsequent sections of this review, agents which increase birefringence or induce dichroism are often useful for diagnostic purposes. In addition, orderly alignment of the dye molecules (or of other molecules which increase anisotropy) indicates that the chemical moieties to which they are attached are aligned in a certain definite order within a single plane.

It is not clear whether the observations that formazan deposits obtained in the histochemical succinic dehydrogenase reaction are birefringent (55), and that the same is true for peroxidase, phosphatase and esterase techniques which employ benzidine and azo dyes, respectively, (92) indicate a definite spatial arrangement of the enzymes, or rather crystallinity of the products.

Many commonly used dyes exhibit dichroism and anomalous polarization colors (43). The enhancement of anisotropy by dyes and other agents (for example, metals in impregnation procedures) has a similar histochemical meaning to that of metachromasia (177, 189, 204), which also depends on orderly arrangement and spacing of certain reactive groups.

The importance of good, possibly strain-free optics and other requirements for effective polarized light microscopy will not be discussed here. Mention should be made, however, of the importance of proper dewaxing of paraffin-embedded material. Incomplete extraction of paraffin often occurs in sections treated by a repeatedly used xylene (or other dewaxing fluid), or when the laboratory temperature is low. Paraffin is extracted more rapidly from some structures than others and the artifactual birefringence of nuclei in incompletely dewaxed sections has been described by Nedzel (143).

Figure 1 shows the appearance of incompletely extracted paraffin section.

Weak anisotropy has been made easier to detect by any of the following procedures. In some studies tissue sections were left to dry in air so that air (RI = 1.00 versus about 1.53 of glass and of most good mounting media) was the "mounting medium." In other studies water was used (RI = 1.33). These procedures were effective mainly when form and intrinsic birefringence were of the same sign. Staining by routinely used procedures often increased anisotropy. As has been explained above, some stains and impregnations were found to be particularly effective in this respect. The procedure of Allen and Nakajima (2) for observing (and measuring) weak birefringence by two photographic exposures of the same field with compensatory settings off the optimum in two opposite directions has not been used in diagnostic pathology and might not be of use in this field.

The sign of birefringence can be best determined with a compensator. For routine work, however, a gypsum plate allows an easy determination of the sign. Intensely birefringent structures may appear yellow and blue, respectively, in the two diagonal directions. Comparison with collagen, which is positively birefringent, allows the identification of the sign in the studied structures.

It is important to keep in mind that the microscopic detection of birefringence depends on two independent factors. (*a*) The object must exhibit birefringence of sufficient intensity for detection under the given conditions of observation. This birefringence may be of form, intrinsic or compound. Whenever the form and the intrinsic birefringence are of opposite signs, they can cancel each other and the compound birefringence may be nil. (*b*) The object must be aligned at 45° to the planes of the two polars. Alignment in a parallel plane to one or the other polar renders objects invisible. Thus, the appearance of dark objects under crossed polars does not necessarily indicate absence of anisotropy.

Two procedures were proposed for demonstrating birefringence independently of the objects' alignment. The one procedure stems from the usual trick used by many microscopists: after rotating both polars by 45°, lack of change indicates that the object which remained dark is isotropic. Gustafson (68) built a polarizing microscope with a bar connecting both polars. By rotating both by 135° during photographic exposure, each birefringent object is twice lighted and twice dark. The photograph shows birefringent objects, whatever their alignment. Another more elegant procedure is the use of circular polarized light as proposed by Schnabel (203).

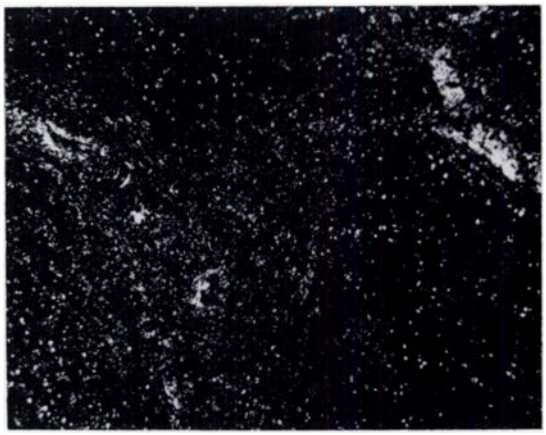

FIG. 1. Amyloidotic spleen stained with Congo red photographed under crossed polars. Because of incomplete deparaffinization nuclei are intensely birefringent. Amyloid in arteries is seen below center and near both upper corners. The importance of complete dewaxing of sections for good diagnostic work is obvious.

Dichroism, including that obtained after staining by dichroic dyes, can be observed with only one polarizing plate. An increased effect can be obtained in some instances by using both polars and rotating the analyzer by some degrees off the perpendicular to the polarizer. In this way additive (or subtractive) colors may enhance the dichroic effect. Schnabel (202) used such a procedure in a study of dichroism of glia fibers in normal tissue and in glial tumors stained by different procedures. A deflection of about 10° from the East-West direction produced best results.

NORMAL AND PATHOLOGIC MESODERMAL ELEMENTS

Collagen: The birefringence of collagen can be used as a quick way of differentiating collagen, as for example in scar tissue, from necrosis. Even in rapid diagnostic procedures, based on frozen or cryostat sections, polarized light microscopy allows simple differentiation between an active and a scarred tuberculous focus, as in the last the collagen network is intensely birefringent while necrotic tissue is not. Polarized

light has been suggested (252) as a useful procedure for studying hyalin change. Under crossed polars hyalin is often shown to contain numerous collagen bundles (Fig. 2). Cooper, Haq and Bagnell (31) have shown that the splenic arterial and peripheral follicular hyalin is not birefringent, while central follicular hyalin is intensely birefringent. Mounting in phenol decreased the intensity of birefringence of the central hyalin, while the other two acquired birefringence by this treatment. It is probable that all the findings are related mainly to the presence of collagen fibers in the hyalins with a decrease in the intense positivity of the central follicular hyalin by phenol, and with a change from weak hardly discernible birefringence in the other two hyalins into a more intense negative birefringence. It is also possible that fibrin played a role in the total birefringence.

In routinely stained or unstained sections, collagen fibers can be easily distinguished from striated muscle (which shows birefringent transverse striation), smooth muscle (which is weakly birefringent) and elastic fibers (also weakly or not birefringent). Collagen is positively birefringent in relation to the length of the fibers (23, 196). The birefringence is due to a positive intrinsic and form birefringence of the fibers. The intensity of birefringence may be increased and dichroism induced by metallic impregnations with gold, copper or silver (117, 195). Specificity may be obtained by the von Ebner reaction (194, 198) or by examination under crossed polars of sections stained with picrosirius red F3BA (29).

The intensity of birefringence of collagen depends on a number of factors which are of considerable importance in diagnostic pathology. Young collagen, the fibrils of which are more hydrated and less perfectly aligned than those of mature collagen, is also less or not at all anisotropic. According to Puett, Ciferri and Rajagh (166), cross-links between fibrils determine the intensity of birefringence. Elden (51) concluded from a review of available information that fiber-fiber bonds form by displacing fiber-water bonds. Tonna (233), Tonna and Hatzel (236) and many other authors noted that the intensity of birefringence of collagen increases with age. Engel and Catchpole (52)

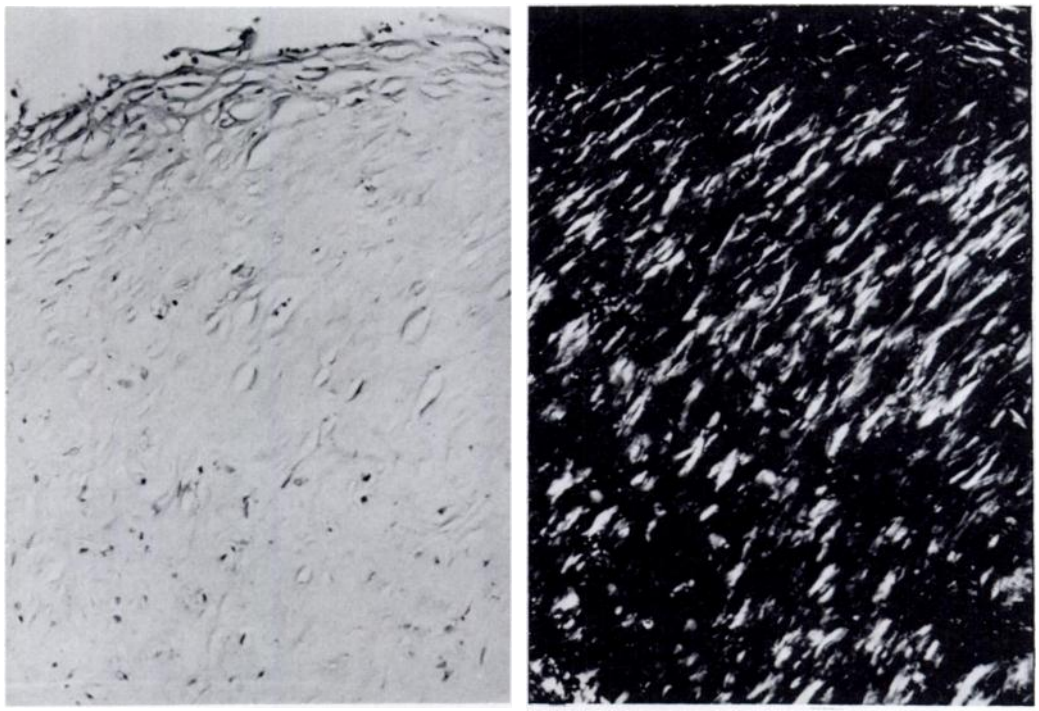

FIG. 2. Old hyalinized tuberculous scar in pleura. Photographs of the same area in a hematoxylin and eosin-stained section. Left, under ordinary illumination the hyalinized collagen appears amorphous; right, under crossed polars the tissue is seen to contain numerous collagen fibers.

found that the loss of birefringence by heating occurs more rapidly in young than in old animals. Changes in birefringence of collagen in the course of aging occur slowly during months and years, while in healing wounds such changes take days. Wolman and Gillman (255) found that the changes involved mostly form birefringence. Intrinsic birefringence was almost zero in healing wounds of 1 month or less. It appears, therefore, that in sections mounted in glycerogel, for example (the RI of which is near the RI of collagen), scars of wounds can be easily discerned as weakly birefringent or isotropic bands, even months after healing began. Laufer *et al.* (101) have further shown the usefulness of polarized light microscopy in studying the orientation of collagen in healing wounds. Collagen fibers in tendons and aponeuroses are always well aligned as they are constantly exposed to mechanical forces. The importance of these forces in determining the degree of order and the intensity of birefringence can be deduced from the experiments of Rollhäuser (173). This author forced guinea pigs to run daily. After 3 days of training, the intensity of birefringence of the tendon and its resistance to tear were reduced in comparison to normal. From the 7th day of exercise, both these parameters progressively increased over normal. De Campos Vidal (40) reported that detachment of tendons, with the consequent loss of tension, caused molecular disorientation of the ground substance mucopolysaccharides which could be gauged by loss of anisotropy followed by decrease in the orientation of collagen fibers. Figure 3 shows how polarized light microscopy can conclusively demonstrate the abnormal alignment of collagen in cardiomyopathy.

The above mentioned von Ebner (phenol) reaction of collagen was found to be useful in studying pathologic changes in collagen. Missmahl (125) standardized the procedure on formalin-fixed frozen sections and developed a formula representing the change in birefringence caused by the phenol treatment:

$$\frac{\text{Retardation of untreated collagen} + \text{retardation of same fiber treated by phenol}}{\text{Retardation of untreated collagen}}$$

The values obtained in various animals differed to a great extent. In patients with Addison's disease the values of dermal collagen fibers were appreciably higher than normal and the values returned to normal after corticosteroid treatment. The author proposed that the increased effect of phenol indicates that the collagen of Addison patients is immature and richer than normal in procollagen. In fact, the same change was found in the dermal collagen of children. This possibility of diagnosing Addison's disease by a skin biopsy does not seem to have been further tested.

It has been noted that form birefringence depends on the RI of the mounting medium. In the case of collagen the nature of the mounting medium partly depends on the constitution of the interfibrillar matrix. De Campos Vidal (39) noted that the ground substance acid mucopolysaccharides (AMP) plays an important role in the form birefringence of collagen. The orderly alignment of the AMP of the ground substance of connective tissue could be also deduced from the intense birefringence of the AMP in sections stained by dyes mostly used as fluorochromes, such as acridine orange, trypoflavin and rivanol (187).

Módis, Módis-Süveges and Conti (133), studying the birefringence of ground substance of cartilage stained with thiazine dyes, also found that these AMP are anisotropic. In the cornea of the eye, wound healing seems to proceed somewhat differently from the process in the skin. Varga and Fehér (238) found that following experimental perforating wounds in the corneas of rabbits, the ground substance swelled but the birefringence of the collagen fibers remained unchanged. For some unknown reason the keratocytes, although unchanged in size and shape, became more intensely birefringent. François and Fehér (57) found that polarized light microscopy allowed them to detect the two changes which are characteristic for macular dystrophy of the cornea. The one change occurs in the keratocytes which become full of birefringent granules of AMP and protein. The second change occurs within and between the collagen fibers, where negatively birefringent crystal-like structures can be seen. These structures are not collagen, as they are negatively birefringent and are not affected by phenol treatment.

Neumark (144) observed that the intensity of the phenol effect was reduced in collagen surrounded by ground substance rich in AMP, as for example in cartilage. This observation might

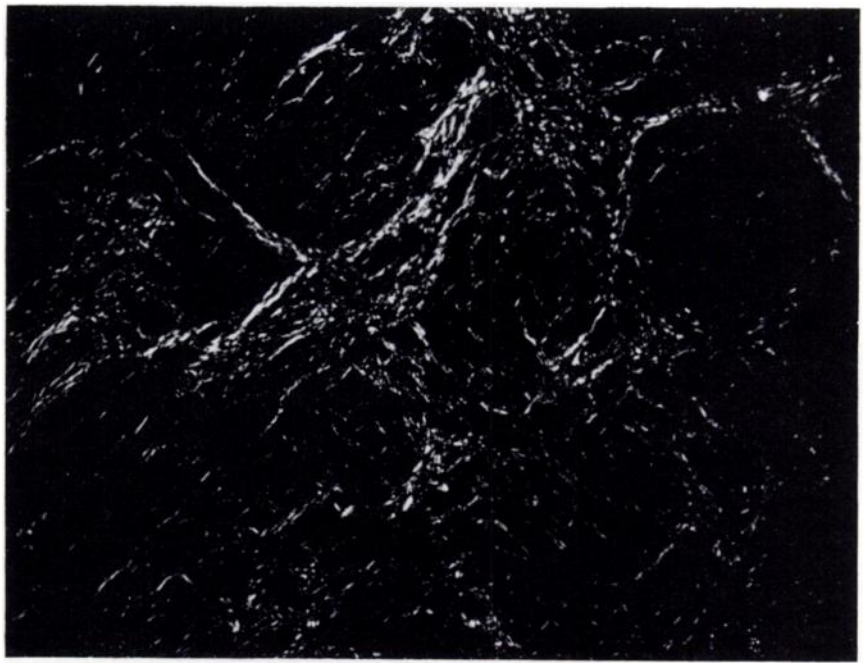

FIG. 3. Irregular fibrosis in the myocardium of a patient with cardiomyopathy. The organization of the collagen network is haphazard with muscle bundles and their thickened sheaths oriented in different directions.

have some interesting applications in human pathology, mainly in processes associated with changes in the ground substance constitution. Katenkamp (93) has, in fact, found that in rheumatoid arthritis there is destruction and formation of new collagen fibers in the synovial membrane. This process is associated with a marked increase of AMP and with a marked decrease in phenol effect on the collagen.

In the elastotic (basophilic) degeneration of collagen it can be easily seen that the positive birefringence of collagen is lost. Therefore, the elastotic fibers, although refractile, differ under crossed polars from both collagen and elastin fibers. Hutschenreiter and Scheuner (83) studied the intrinsic birefringence of the zonula ciliaris of the eyes of cattle and found that the fibers differed in essential points from collagen.

Basal membranes: These are known to be of different types and often contain collagen. Niessing and Rollhäuser (145) studied the birefringence of cerebral capillaries and observed only form birefringence. Scheuner and Hutschenreiter (188) studied the birefringence of basement membranes in paraffin-embedded formalin-fixed autopsy material. They found that the basement membranes of capillaries and other small blood vessels in the intestinal submucosa and in the placenta were negatively birefringent in respect to the length of the vessel. Phenol treatment had no effect on the birefringence. The basement membranes of the intestinal epithelium and of the amnion, chorion and renal tubules were also negatively birefringent, but their negativity was inverted by treatment with phenol. This indicated that the basement membranes which separate epithelial from mesenchymal elements contain collagen fibers aligned perpendicular to their length.

Reticulin: Polarization microscopy can be used as a means of differentiating reticulin from collagen and of studying reticulin fibers. Missmahl and Hartwig (128) observed that the positive birefringence of reticulin can be changed into negative birefringence by treating histologic sections with glycerin, or by mounting in glycerin-gelatin. The authors found that the effect is specific for reticulin and that it may be abolished by treatment with lipid solvents. Brewer (23) confirmed the presence of lipid chains arranged perpendicular to the length of reticulin fibers. Missmahl (122) observed that quantitative polarized light microscopy could be used to detect pathologic changes in these fibers. Treatment with cortisone decreased the

lipid content of these fibers (the intensity of the negative birefringence in sections mounted in glycerin) in the liver, spleen and kidney. Administration of a bacterial extract caused an increase in the amount of fibers without change in their lipid content.

Elastin: The structure of the elastic fiber has also been studied by polarized light. Cruse (32) found that aortic elastin contains a birefringent component which is present in sheets, while in the ligamentum nuchae the elastin appears different and is vitreous and fibrillar. Other studies (97, 176, 178) indicate that the elastic fiber is made of an axial component with positive intrinsic birefringence in fibers studied in longitudinal sections (indicating a longitudinal arrangement of the proteins), separated by a thin isotropic band from an outer sheath with an annular micellar arrangement producing a negative intrinsic birefringence. The birefringence of the outer layer of human arterial elastica seems to increase with age.

Bone and dentin: In bone both the organic matrix and the apatite crystals are anisotropic so that birefringence can be studied either in undecalcified ground sections or in decalcified and mounted bone sections. In decalcified bone and in cartilage the main anisotropic component is collagen. Figure 4 shows that mature noncalcified osteoid is also intensely birefringent. The studies of Tonna (234, 235) indicate that the healing of bone fractures is accompanied by progressive increase in birefringence of newly formed collagen, and that also in bone the collagen of old animals is more intensely birefringent than that of young animals. Modis *et al.* (132) studied the birefringence of experimentally induced callus formation in tissue sections stained with toluidine blue. Treatment with vasopressin was shown to increase the orientation of the acid mucopolysaccharide-protein complexes. These complexes were again shown to play a role in callus formation.

In dentin the situation is similar to that prevailing in bone: both the organic network and the hydroxyapatite crystals contribute to the total birefringence. Spreter (226) has shown that in dentin with deficient mineralization the changes in birefringence are mostly due to altered collagen.

Amyloid: The moderately intense positive birefringence of amyloid may be strengthened by various staining procedures. Divry and Florkin (45) found that the birefringence of human and experimental mouse amyloid could be enhanced by staining with Lugol's solution and with Congo red. Romhányi (175) and Missmahl and Hartwig (126) found that the birefringence of amyloid consists of both intrinsic and form positive components. The last mentioned authors also found that the sign of the birefringence could be changed by treatment with

FIG. 4. Intense birefringence of osteoid laid down by the proliferating cells of an osteogenic sarcoma.

phenolic compounds. The green polarization color of amyloid stained by Congo red was found to indicate an orderly alignment of the dye molecules on the amyloid fibrils (121), and the cause of this phenomenon has been explained by Wolman and Bubis (254). Figures 5 and 6 show the appearance of amyloidotic tissues stained with Congo red under crossed polars. The orderly arrangement of molecules of different dyes attached to amyloid fibrils and the consequent dichroism and anomalous polarization colors have been repeatedly observed with various dyes (42, 123, 124, 209).

One of the most commonly used procedures for diagnosing amyloid deposits was the study of birefringence in Congo red-stained sections; the detection of the green polarization color was considered pathognomonic. It is common experience that the results of this procedure sometimes leave the observer in doubt, and Klatskin (95) showed that false positive results are not uncommon. In view of the need to establish a restrictive standard which will eliminate false positive results, Wolman (253) proposed a procedure for staining of amyloid with toluidine blue and examination under polarized light. This procedure (STB) is less sensitive but more selective than other currently used techniques for diagnosing amyloid. The procedure allows easy elimination of substances which, although obviously not amyloid, are demonstrated by Congo red and other amyloid procedures. The weak sensitivity of the method renders it unsuitable for detection of tiny or thin amyloid deposits which are better demonstrated by other procedures once the diagnosis of amyloidosis has been definitely established.

Romhányi (180, 181) recently published further tests for differentiating amyloid from collagen and for differentiating primary from secondary amyloid. The author found that in polar mounting media, specifically in gum arabic, amyloid stained by Congo red is positively birefringent, while collagen stained similarly is negatively birefringent. The difference between the two structures could be increased by pretreatment of the tissue sections with proteolytic enzymes. The birefringence of amyloid stained by Congo red became more intensely positive and that of collagen more intensely negative after the digestion. The author also found that secondary amyloid deposits were digested by trypsin after a mild permanganate oxidation. Deposits of nonsecondary amyloid (primary

FIG. 5. Rectal biopsy of a patient suffering from amyloidosis. On the right, mucosa with fine amyloid deposits on the gland basement membranes. On the left diffuse, amyloid deposits situated mainly in the blood vessels. Congo red under crossed polars.

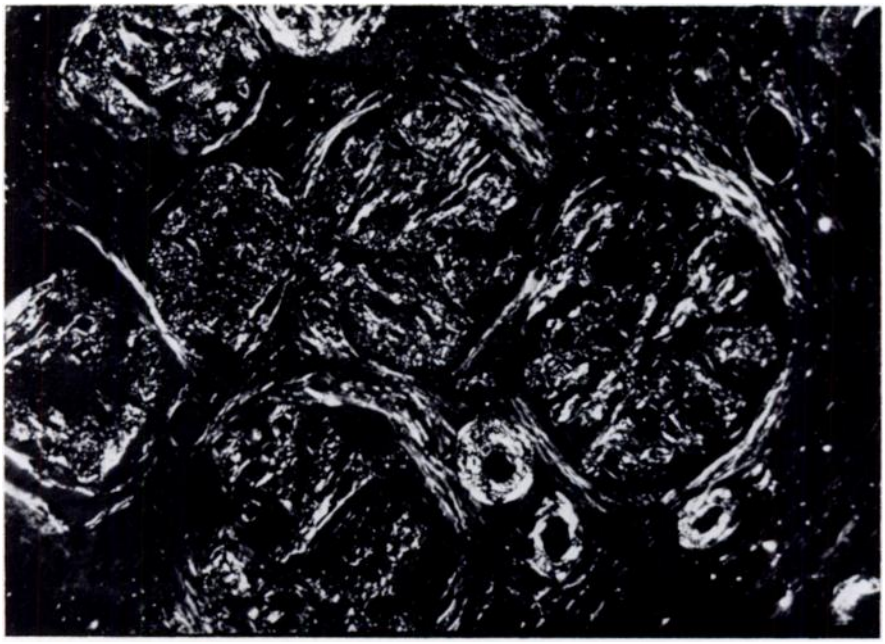

FIG. 6. Renal amyloidosis in a patient suffering from familial Mediterranean fever. Glomerular capillaries and capsules, arterioles and arteries are severely involved. Congo red under crossed polars.

generalized amyloidosis, myeloma, tumor or localized senile deposits) were not digested by trypsin after oxidation and preserved their green polarization color after staining with Congo red.

Ectodermal amyloids: Corpora amylacea and localized amyloid deposits are known to occur in the nervous system, ocular structures, endocrine tissues, prostate and the lung. Although they do not belong in this section, they will be described here. Divry and Florkin (45) have already found that the core of senile plaques in the brain is amyloid which gives green birefringence after staining with Congo red. Missmahl and Hartwig (121) described similarly demonstrable amyloid in the fibrillar change of brains of patients with presenile dementia and in small foci in the adrenal and other organs. According to Katenkamp and Stiller (94), who used polarization microscopy after a variety of histochemical tests, the Alzheimer fibrils and the senile plaques differ from primary and secondary amyloids. Cerebral corpora amylacea are not usually demonstrated by the polarized light amyloid procedures (88). Amyloid bodies of the prostate have been variously reported in the literature as anisotropic (136) or isotropic (208). As these bodies represent secretion inspissated and compressed to variable degrees, the intensity of their birefringence varies; this explains the negative findings of some authors. The present author found that well formed prostatic amyloid bodies exhibit all the reactions which are typical for amyloid (see Fig. 7); the same is also true of the less common amyloid bodies of pulmonary alveoli, and in veterinary pathology of the corpora amylacea of the bovine mammary gland (167).

Smooth and striated muscle: The positive birefringence of smooth and striated muscle has been known for many years (193). This is a compound birefringence and both the intrinsic and the form birefringence contribute to the positivity which is weaker than that of collagen (Fig. 8). According to Fischer (54), stretching markedly increases the birefringence of smooth muscle, while the effect on birefringence of striated muscle is negligible. The observation of Conti, Módis and Gotzos (30) indicates that stretched muscle cells in the wall of arteries exhibit a more intense birefringence than apparently relaxed cells.

Atrophy and loss of functional activity in muscle markedly affects the intensity of its birefringence. Fischer (54) found reduced birefringence in degeneration atrophy of striated

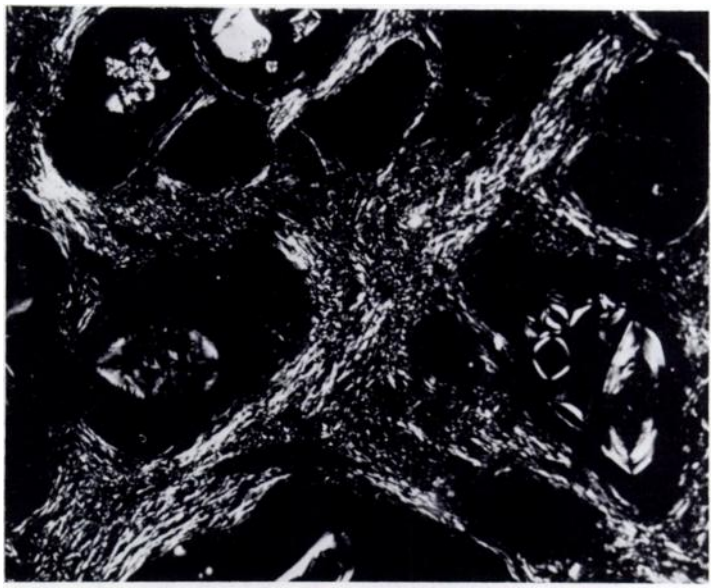

FIG. 7. Amyloid bodies within dilated lumina of prostatic glands. The amyloid bodies vary in the intensity of birefringence and in their structural pattern. The bodies on the right show Maltese crosses and appear laminated. The amyloid body on the left of center and the numerous small bodies in the upper left appear granular or of radial pattern. The birefringent fibers in the septa are collagen.

FIG. 8. Wall of human ileum. The thick intensely birefringent bundles on the right are collagen. The weakly birefringent bundle of loose fibers running through the center of the figure is smooth muscle.

muscle. The possibility of differentiating leiomyomata and leiomyosarcomata from the fibroblastic counterparts by demonstrating the weak birefringence of the smooth muscle cell cytoplasm has been repeatedly suggested, but it has never gained acceptance. On the other hand, Orban and Romhányi (149) have successfully used polarized light microscopy for detecting myoblasts in Wilms' tumor.

Myocardial cells mostly exhibit positive birefringence, but Missmahl (120) found that some fibers show negative birefringence. This phenomenon was found to be caused by the presence in the fibers of lipids oriented perpendicular to the fiber length. A number of scientists used polarization microscopy for studying pathologic changes in myocardial fibers. Orban and Romhányi (149) found that such study of toluidine blue-stained sections can be used to detect early ischemic changes in the myocardium. Soviet pathologists described changes in birefringence of myocardial cells in pathologic conditions. Tsellarius (237) observed loss of birefringence caused by lysis or contraction of myofibrils in myocardial cells of rats given intravenous injections of epinephrine. The changes in birefringence could be observed within 15 min of the injection. Revzis (168) found by polarization microscopy that the fibrinoid found in the auricular appendage of the heart in cases of rheumatic carditis contains fibrin fibers.

The changes caused by ischemia in birefrin-

gence of myocardial cells seem to be biphasic, or possibly of two types. In an experimental study on rats treated with isoproterenol, Pilny, Kiefer and Sandritter (159) found that in addition to frankly necrotic cells in which no striation or birefringence could be observed, there were two other types of changes: (*a*) eosinophilic cells in which cross-striation was preserved and the birefringence was increased by about 50% over normal, and (*b*) eosinophilic cells with loss of striation but with increased birefringence.

Fibrin: The weak birefringence of fibrin and of fibrinoid deposits helps to differentiate them from collagen fibers and in some instances from fungal micelia. The birefringence is positive in respect to the length of the fibrils, consists of both form and intrinsic components and is more pronounced in stretched fibers. Von Dungern (244) described these phenomena and observed dichroism in trypan blue-stained fibrin.

NORMAL AND PATHOLOGIC ECTODERMAL ELEMENTS

Epidermis, keratin and hair: The epidermis of humans and of many other animal species contains a number of birefringent structures, the most conspicuous of which are the tonofibrils and keratin (196). An extensive study of the birefringence of human hair was published many years ago by Schmidt (192), who later described lipid deposits in the medulla of the hair of many humans (200). At present, detection of the birefringence of hair and keratin is not of great practical importance in diagnostic pathology except for conditions in which the identification of keratin squames or of hair allows the establishment of a correct diagnosis. Such cases are aspiration of amniotic fluid in infants and amniotic fluid embolization in mothers—both instances in which birefringent squames are found in the lungs and pilonidal cyst, in which an ectopic hair is found in the center of the lesion.

Enamel: In dental tissues, polarization microscopy played an important role in the study of a number of diseases, mainly caries. Study of the birefringence of dental enamel is over 100 years old. In 1862 Hoppe (81) noted that enamel is more intensely birefringent than dentin and cement; when mature it is negative in respect to the length of the prisms. Developing cement and cement heated to 800°C were also found by Hoppe to be positive.

In modern times a number of authors noted that the development of dental caries is associated with changes in birefringence. Darling (35) found that the intrinsic birefringence of carious enamel is lower than that of the normal tissue. In an extensive study Gustafson and Gustafson (69) showed that polarized light microscopy can help in a number of dental diseases. In cases of hereditary amelogenesis imperfecta, one of the authors with co-workers had previously shown that the enamel exhibited positive birefringence. The authors found that the carious lesion is sometimes surrounded by a zone of increased negative birefringence. Inside this zone there are two areas of partial and complete demineralization associated with isotropy. Carlström (25) reviewed the subject and noted that negative intrinsic birefringence of mature enamel was caused by the apatite crystals. The study of Angmar, Carlström and Glas (5) has shown, however, that mineral content did not determine the intensity of birefringence. The intensity of birefringence was found to vary in different areas.

In developing enamel the tissue (with the possible exception of a thin layer at the dentinoenamel junction) is positively birefringent, the effect being mainly caused by form birefringence. Fluorosis and incipient caries are associated with changes in the birefringence of enamel. Angmar (4) found that the intrinsic birefringence of enamel, even after correction of the data for effects of the imperfect alignment of the apatite crystals and of the different mineral content, varied in different areas. These observations were believed to be caused by differences in packing so that some immersion media used for determining intrinsic birefringence could not penetrate the denser areas. In further studies (6), the author found that the intrinsic birefringence of the organic matrix of enamel is extremely low, indicating that the protein micelles are not aligned in an ordered fashion. The differences in form birefringence between various areas has also been noted by Houwink (82) who found that the differences are also present in unerupted or recently erupted teeth. Confirmation of the variability in density and degree of order in various parts of single teeth has also been obtained by studies on acoustic anisotropy (104).

The observation of differences in form birefringence in the enamel of a single tooth, associated with differences in permeability to

immersion media, may be of great practical importance. Those areas which are less packed and more permeable than others can be expected to be also more accessible to penetration by various noxious agents and may be regarded as loci of lowered resistance or areas in which primary damage has already occurred.

Other surface epithelia: Fine anisotropic granules were found (57) in the corneal epithelium of patients with macular dystrophy of the cornea. Vidal and Bozzo (240) observed dichroic changes in gingival epithelial cells stained with Sudan black, indicating that sudanophilic lipids are arranged perpendicular to the gingival surface.

Secreting and absorbing epithelia: Hillarp and Olivecrona (77) found that various epithelial cells appear isotropic in water, but are anisotropic when mounted in glycerin. The birefringence was negative in relation to the direction of the lumen to basal membrane. In the epithelial cells of the intestine, prostate, seminal vesicle, endometrium and tube the whole cytoplasm was birefringent. In salivary glands secretory duct cells were anisotropic while secreting cells were negative. In the exocrine pancreas weak birefringence was found in the basal part of secreting cells. In the kidneys some tubular cells exhibited negatively birefringent striae in their basal parts and in the brush borders. The epithelial cells of the thyroid, choroid plexus, liver, gastric mucosa and cumulus oophorus exhibited anisotropy both in water and in glycerin. The authors found that the birefringence which could be detected only in glycerin-mounted sections was due to lipids oriented perpendicular to the apex-base axis, as it could be eliminated by treatment with lipid solvents. Rollhäuser (174) extended the findings in renal structures and correlated them with electron microscopic observations and function. Jahn, Scheuner and Hutschenreiter (87) studied the birefringence of the apical part of the small intestine epithelial cells of rats. In addition to the birefringence caused by the microvilli, the authors found a deeper birefringent layer in which the anisotropy was caused by the terminal web. The birefringence of this layer was caused by the presence of axially oriented protein filaments with perpendicular lipids. It is regrettable that these observations have not been properly utilized in diagnostic pathology.

In steroid-secreting organs, the appearance of birefringent crystals or liquid crystals is part of normal functional activity. The birefringence of the lipid droplets in adrenocortical cells had been discovered by Kaiserling in 1895, and Kaiserling and Orgler (91) extended this observation. The authors noted that in severe infections and poisoning the adrenal lipids lose their birefringence. Birefringent lipids were also found in corpora lutea of the ovary and in hypernephromata. Similar observations on the adrenals were made by Herrmann (75). Mulon (137), Pickard, Wacker and Hueck (157) and Weltmann (248) reported on the adrenals of frogs, rabbits, dogs and humans in which excessive muscular exercise and severe infections caused depletion of birefringent lipids. The classical studies of Bennett (16) and later of Deane and Greep (38), Symington, Duguid and Davidson (231) and others indicated that the presence of birefringent lipids in the adrenal cortex is a good indication of the functional state of the cells. In fact, these crystals represent mostly precursors and their presence indicates that the cells contain a functional reserve and are not exhausted.

Also, in the ovary (26, 41), in ovarian tumors (61, 116), in placental villi (250) and in some cells of the testes (135) the presence of birefringent lipids in the secreting cells is related to functional activity and is believed to indicate the accumulation of steroid precursors.

Sinapius, Avenarius and Gunkel (216) reported on the presence of anisotropic fat crystals in formalin-fixed hepatic tissue obtained from autopsies and surgical biopsies. Two types of lipid crystals were observed in hepatocytes. The first are needle-like long crystals (3-40 μ), often arranged in bundles, situated within fat droplets. These crystals were shown to consist of triglycerides and their appearance seemed to be enhanced by formalin fixation. The second type of anisotropic crystals were in the form of needles, granules or spherocrystals (see pages 33-35) and resulted from lipolytic processes occurring during autolysis. These crystals appeared to consist of fatty acids and other polar lipids.

Studies have been published on birefringence of spermatozoa (15). The possibilities inherent in these observations of distinguishing organized sperm heads from disorganized ones in the study of abnormal semen and male sterility seem not yet to have been exploited.

NORMAL AND PATHOLOGIC NERVOUS TISSUE ELEMENTS

Some processes involving the nervous system are not included in the present heading. The first, deposition of amyloid, is discussed on pages 23-26. The second, lipid storage diseases in the nervous system, is presented on pages 38 and 39. Maple syrup disease is discussed on page 40.

Nerve fibers: It has been known for over 100 years (96) that the myelin sheath of peripheral and central nervous tissue is negatively birefringent in respect to the length of the fiber. The negative birefringence is caused by lipids which are radially oriented so that in lipid-extracted material, as in paraffin sections, peripheral nerves exhibit weak positive birefringence (66, 201) which is caused by the axial alignment of the protein constituents. In frozen sections the intense birefringence of myelin together with its straight course (different from the wavy course of collagen), tubular structure and negativity in respect to length provide a rapid means of identifying nerve fibers. Schnabel (203) used circular polarized light for the study of normal and damaged nervous system structures. Figure 9 shows the appearance of myelin in transverse sections in linear polarized light and in circular polarized light. The identification of myelin by polarization microscopy is important in instances of myelinated nerve emboli and of artifactual squeezing of nerve myelin into surrounding tissues (27).

Minor damage, which is often reversible, may be detected by polarized light microscopy: pressure or tension may cause changes in the intensity and sign of birefringence of myelin constituents (66). It has been found, however, (242) that this effect is obtained in axons and Schwann cells but not in myelin. Spiegel (224) observed reduced birefringence in teased nerves and in nerves *in vivo* during narcosis, and Baldi (10) found fragmentation of myelin when a peripheral nerve was cooled *in vivo*.

Demyelination, whether as an autonomous process or as part of nervous tissue destruction, is associated with the breakdown of myelin into globules and myelin figures and eventually with loss of the negative birefringence. Spiegel (225) found that changes in the birefringence of myelin occur at an early stage of Wallerian degeneration and result, with time, in reversal of the sign. Polarized light studies of the progress of demyelination after experimental transection of nerves have been published by numerous authors (48, 78, 146, 147, 163, 214). Figure 10 shows the appearance with crossed polars of a peripheral nerve undergoing Wallerian degeneration in comparison to normal nerve fibers. Similar fragmentation of myelin into birefringent globules and ovoids with consequent disappearance of the negative birefringence of myelin has been described in sudanophilic leukodystrophies, in multiple sclerosis (42, 115, 153, 154) and in ischemic changes in the brain (152, 153). Bozzo and Almeida (20) described weakening of birefringence in peripheral nerves of inflamed dental pulp.

It has been repeatedly suggested that the appearance of myelin breakdown products under crossed polars can be used as a means for positive identification of the compounds. It should be stressed that the tacit assumptions that birefringent acicular crystals in the tissue are cholesterol or fatty acid and that spherocrystals giving Maltese crosses under crossed polars are cholesterol esters are completely wrong. Acicular birefringent crystals of endogenous material which are soluble in lipid solvents may consist of cholesterol, fatty acids or triglycerides. Spherocrystals which appear as Maltese crosses in polarized light may be made of mixtures of different lipid substances (141, 142, 171, 197). Formation of spherocrystals depends on the presence of both hydrophilic and hydrophobic constituents, as triglycerides and esterified sterols often play an important role in their constitution. Positive identification of free cholesterol with polarized light microscopy is possible by using the digitonin reaction as proposed by Lison (110).

Neurons and glial cells: The existence of myelinated ganglion cells which can be easily detected by polarization microscopy is well known in zoology, but few workers have studied these cells in human material (186, see also 112). Another type of normal but specialized neuron which can be demonstrated by polarization microscopy is the neurosecretory cell, or rather its specific granules. Scheuner and Weiss (190) proposed a procedure which, like the ordinary light microscopic method for demonstrating these granules, is based on oxidation of the sulfur moieties of SO_3H^- groups. After oxidation with acid $KMnO_4$, the granules are stained by a pseudocyanin dye. These dyes can

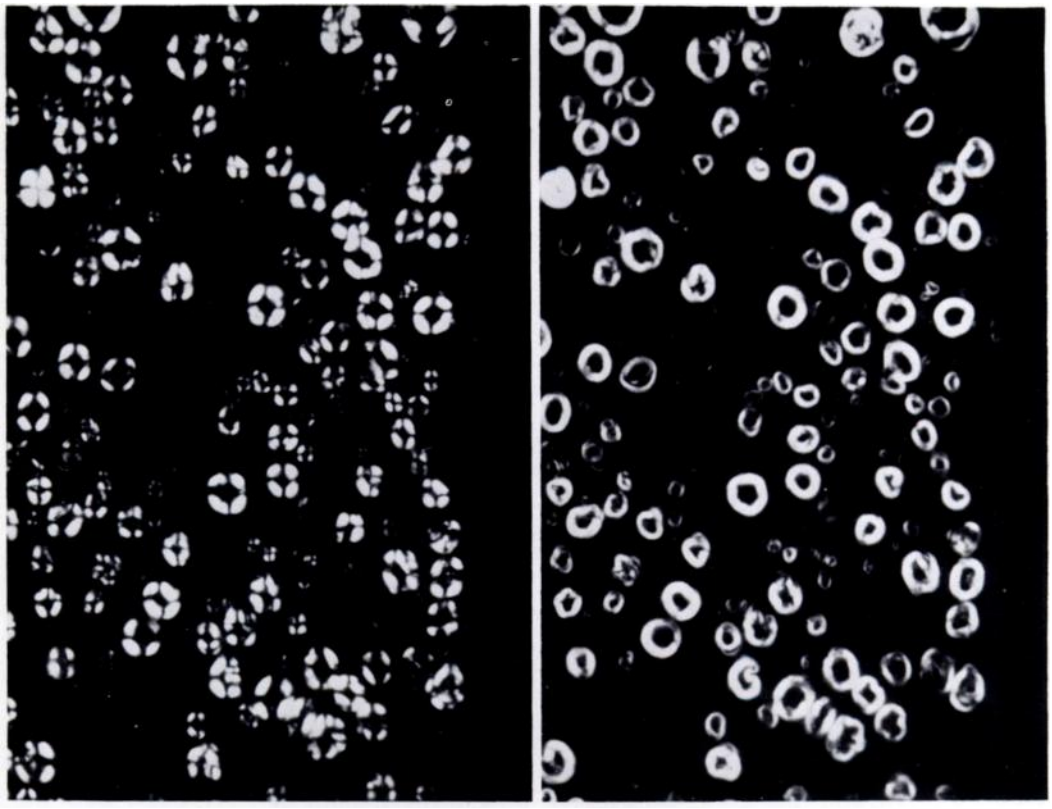

FIG. 9. Myelinated nerve fibers of peripheral nerve of a rat sectioned transversely and not stained. Left, myelin sheaths have the Maltese cross appearance; right, birefringence is homogeneous. By courtesy of Dr. R. Schnabel of Jena, German Democratic Republic (203).

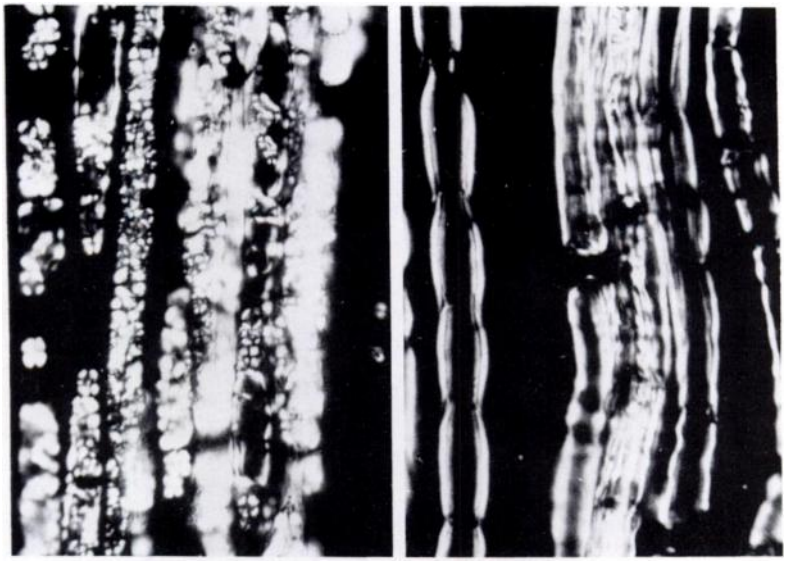

FIG. 10. Rat sciatic nerve teased in Ringer's solution. Left, fibers of a nerve undergoing Wallerian degeneration 10 days after transection. The continuity is broken and myelin is transformed into globules and ellipsoids; right, the normal contralateral nerve with preservation of structure.

be shown by their fluorescence. They also exhibit metachromasia, however, because of the orderly alignment of the SO_3H^- groups and consequently the dye molecules. This orderly alignment allows their easy demonstration with polarized light.

It is known (195) that astrocyte processes are positively birefringent in respect to their length, and this positivity is not affected by the phenol reaction. Schnabel (202) showed that these processes exhibit an intense dichroism after a number of procedures: trypan blue or Congo red staining, gold impregnation, and most clearly, staining by the Kanzler procedure with gentian violet or methyl violet. Schnabel enhanced the dichroitic effect of staining by rotating the analyzer by 10° from the perpendicular to the polarizer and obtained intense colors in the cell processes of a glioblastoma. It is believed that the enhancement was caused by the production of additive polarization colors, but the possibility of a Cotton effect cannot be ruled out.

The outer segments of the retinal receptor cells are anisotropic and the sign of birefringence changes when the cells are damaged (172, 241). These phenomena, although of great theoretical importance, do not seem to be applicable to diagnostic pathology.

Myoclonus body disease: In this process many neurons contain large spherical bodies. Seitelberger *et al.* (212) and Seitelberger (211), in extensive reviews, reported that the central core of these bodies gives the typical polarized light appearance of a spherocrystal, while the peripheral part contains birefringent radial bars. Deposits of a not quite similar nature were found in cardiac and hepatic cells of the same patients. These deposits were not birefringent.

BIREFRINGENCE OF MEMBRANES, CELL STRUCTURES AND SECRETIONS

Although all cellular membranes are highly ordered structures, except when they are arranged in multiple layers, their birefringence is usually too weak for detection. The anisotropy may be usefully increased by staining with substances which become aligned.

Organellar and plasma membranes and specialized cellular structures: Baud (13) showed the nuclear envelope by staining the cells with sodium sulfoantimonate. It is interesting that this procedure shows the ordered arrangement of protein rather than lipid moieties, as the birefringence is not affected by pretreatment with lipid solvents. The birefringence of secreting and absorbing cells (see pages 31 and 32) is probably caused by parallel alignment of membranous structures in them.

Romhányi and Deák (182, 183) proposed the following procedure for increasing the birefringence of cellular membranes. After fixation, during which autooxidative changes were avoided, sections were stained with either toluidine blue or rivanol, with subsequent precipitation of the dye-substrate complex by potassium ferricyanide (alone or together with KI) and mounting in gum arabic. Plasma membranes and those of the endoplasmic reticulum were well demonstrated. Interestingly, oxidation during fixation destroyed the birefringence and presumably, therefore, the ordered alignment of the membrane lipids.

The erythrocyte membrane has been the subject of numerous studies including examination under crossed polars. It is common knowledge that in air-dried smears of peripheral blood, the erythrocyte membranes exhibit intense birefringence. Mitchison (131) studied the birefringence of erythrocyte ghosts and found that this was mainly form birefringence.

Csillik (33) has shown that the postsynaptic membrane of the myoneural junction can be studied by polarized light microscopy. Changes in the intrinsic birefringence of the membrane were found after prolonged tetanic stimulation and after administration of acetylcholine. Both types of birefringence changed after denervation.

Birefringence of microfilaments of the mitotic spindle has been shown by Inoué and Sato (86). Goldman and Follett (63) showed anisotropic bundles of filaments in cultured fibroblasts. These structures seemed to play a role in movement of the cells.

Missmahl and Riethmüller (130) showed that the granules of polymorphonuclear leukocytes of rabbits, believed to represent lysosomes, are birefringent when stained with toluidine blue.

Nucleoprotein- and mucoprotein-containing structures: These can be demonstrated by polarized light microscopy as the negative charges on the molecules mostly cause stretching and a tendency for parallel alignment. It has been known for some time that fibrils of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) prepared or stretched *in vitro* exhibit negative birefringence (169). Romhányi

and Jobst (184) were the pioneers of study of DNA in tissue sections by polarized light microscopy. The possibilities inherent in the study of changes in DNA by polarization microscopy have apparently been seldom used. Sugar (230) studied the effect of antimitotic drugs used in the treatment of human neoplasms on the birefringence of nucleic acids in cancer cells. The author used two types of tumors (Ehrlich ascites carcinoma and NK/LY ascites lymphoma) and treated them with a mustard drug and a Vinca alkaloid. The birefringence was studied and measured after staining with rivanol. In treated cells the birefringence of both DNA and RNA was decreased. These studies open up new possibilities of studying changes in the regular alignment of nucleoprotein molecules in naturally occurring and induced processes associated with the disorganization of genetic material.

Romhányi (179) used toluidine blue staining followed by treatment with ferricyanide for a polarized light study of chromatin. The author found that interphase nuclei could only be demonstrated after tryptic digestion of histones, and that this effect was not obtained in a renal infarct.

White, Elmes and Walsh (249) found that the thick mucous secretions of chronic bronchitis and asthma were positively birefringent in respect to the length of the threads. Musy *et al.* (139) and Musy and Gotzos (138) confirmed the birefringence of mucus droplets in a study of mucus-secreting cells in the colon and in mixed salivary gland tumors. The authors used Alcian Blue, Alcian Blue 8GS and Alcian Green 8GX in addition to toluidine blue in order to increase the intensity of birefringence. These authors and Gyenge *et al.* (70) before them observed the same phenomena in the acid mucopolysaccharide-containing granules of mast cells.

DEPOSITION OF BIREFRINGENT LIPIDS IN THE TISSUES

Crystallization of tissue lipids: Frozen sections of various lipid-containing tissues, including normal adipose tissue, often show birefringent acicular crystals sometimes situated within fat droplets. Lison (110) justly stressed that the assumption that crystallization of fats indicates breakdown with formation of fatty acids and soaps is completely wrong. With lowering of the temperature, tripalmitin and tristearin can crystallize and partly separate from the still fluid triolein. This artifactual crystallization due to cold has its counterpart in human pathology.

Many years ago Harrison and McNee (73), Lemež (105), Siwe (217) and others observed that in the disease of newborns called sclerodema neonatorum or adiponecrosis subcutanea, the inflammation in the adipose tissue was associated with the appearance of birefringent acicular crystals. The authors found that these crystals consisted mainly of triglycerides admixed with some cholesterol esters. It is probable that this crystallization is caused by excessive lowering of the body temperature in relation to the melting point of the body fats. In fact, Collins, Stahlman and Scott (28) observed similar changes in the subcutaneous fat of an infant who had been operated on under hypothermia. The hydrolysis of fats, shown by the presence of fatty acids and soaps in many instances (164, 165), might represent a secondary change.

Crystallization of triglycerides in the tissues might be caused by a diet rich in saturated fats (76, 222, 223). Crystallization of fatty acids and soaps (products of hydrolysis of fats) occurs in various conditions associated with fat necrosis or hydrolysis as a result of traumatic, enzymic and other agents capable of breaking down adipose tissue cells or the constituent fats (220).

Atheromatosis: The presence of birefringent crystals consisting mainly of cholesterol esters had been known for years as an important feature of this disease process (74, 91, 98, 102). Figure 11 shows the appearance of these crystals under polarized light. Also in the experimental disease produced in susceptible animals by a diet rich in cholesterol, deposition of anisotropic lipid crystals in the aorta was noted by early observers (7). In some animals susceptible to atheromatosis, such as chicks, and in animals which are not very susceptible to this process, such as dogs and rats, the occurrence of birefringent crystals in experimental atheromata is not a constant feature (21, 108, 109). Some authors (213) considered the presence of birefringent lipids as a distinctive sign of atheromatosis, without which the process in the arteries was considered similar to but not identical with atherosclerosis.

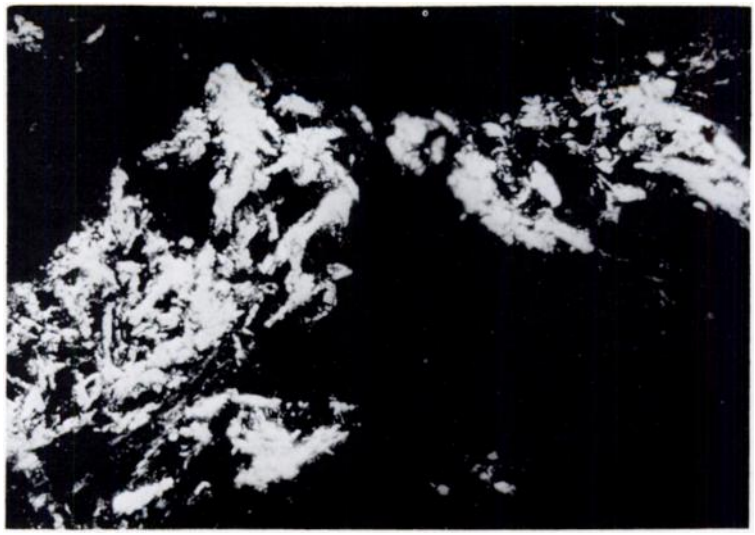

FIG. 11. Atheromatous deposit (mainly cholesterol ester) in the intima of a human aorta. Masses of intensely birefringent acicular crystals and tiny spherocrystals. Unstained frozen section.

The detection of birefringent crystals in atheromata acquired major importance after atheromatous embolization had become recognized as a not uncommon clinical entity (134, 170). The occurrence of cholesterol emboli in small arteries because of erosion of atheromatous plaques, appearance of the lesions, the importance of polarized light examination for diagnosing the process and the changes caused by these emboli have been repeatedly described (65, 72, 89, 90, 118, 205). David *et al.* (36) reported on fundoscopic observation of shiny yellow plaques in retinal arterioles of a patient. Postmortem examination revealed the birefringence of the retinal bright plaques. It is obviously feasible, but apparently has never been accomplished, to diagnose *in vivo* atheromatous emboli by fundoscopy of the eye with polarized light.

Xanthomatosis: This term denotes foamy transformation of histiocytic cells caused by deposition of multiple discrete droplets consisting mainly of nonpolar lipids, especially cholesterol esters and triglycerides. Xanthomatous change may occur in chronic inflammatory processes, in tumors and in apparently normal tissue in the presence of high concentrations of lipids in the milieu (as for example in the histiocytes of the gallbladder mucosa in cholesterolosis, or in histiocytes of different organs in some cases of hyperlipidemia), or without any evidence of increased lipid supply to the cells. Xanthomatous change may be localized (*e.g.*, in the kidney, skin or spleen only) or disseminated in many organs. It may be primary, as in Wolman's disease, or secondary, as in diabetic hyperlipemia.

In the first decade of this century the basic knowledge about localized, generalized, primary and secondary xanthomatous changes was available and scientists made an effective use of polarization microscopy (156, 160, 161). The authors also studied the effect of warming the microscopic sections in order to determine the point at which birefringence is lost in an attempt to better define the nature of the anisotropic substance.

Recently Ferrans *et al.* (53) have shown that in type II hyperlipoproteinemia the xanthoma cells exhibit intense birefringence at an early stage. With time the lipid droplets are progressively being transformed into chromolipid (pigment) granules and they lose the birefringence.

The presence of anisotropic cholesterol-triglyceride mixtures has been reported in a variety of xanthomatous processes: (*a*) in localized and generalized cutaneous xanthomata of different kinds (148, 160, 227, 251); (*b*) in the Hand-Schüller-Christian disease and in other systemic processes undergoing secondary xanthomatous transformation (22, 114, 119, 228); (*c*) in localized chronic inflammations with xanthomatous change (12, 62, 106); (*d*) in the cerebrotendinous xanthomatosis (239); (*e*) in a

group of primary xanthomatoses including Tangier disease (11), Wolman's disease (111, 256) and cholesterol ester storage disease (151, 191); and (*f*) in experimental xanthomatosis (84, 247).

The literature dealing with xanthomatous processes is full of apparent inconsistencies in respect to the presence of birefringent lipids in the storing cells. In any one of the various processes associated with xanthomatous change, some cases, some organs or some areas are free or almost free of anisotropic lipids, while others are full of them. For example, in the report of Lane and Smith (99) on the findings in four cases of Hand-Schüller-Christian disease, some histiocytes contained very few anisotropic droplets while in other sites and cases birefringent droplets were numerous. These differences are not believed to have any deep significance. In the presence of a sufficiently high proportion of polar lipids and when the temperature is not high enough to disrupt orderly alignment, the lipids are arranged in lamellae (myelin figures) so that the whole structure is birefringent. In the presence of a low proportion of polar lipids or in other conditions allowing fluidity, a similar mixture or triglycerides and cholesterol esters may exhibit no birefringence except for the weak effect of the external surface of the droplet, as shown in Figure 12.

Reference might be made to chronic pneumonia with lipid crystal deposits, so-called endogenous lipid pneumonia or cholesterol pneumonia, in which birefringent crystals of cholesterol esters are found in the lung (245). Another group of processes in which polarized light microscopy can greatly help the diagnosis consists of inflammatory exudative processes with cholesterol crystals in the exudate, for example in the pericardium (34, 206, 229).

Gaucher's disease: In this process the lipid birefringence is weak so that some authors considered the presence of birefringence in a storing cell a definite sign against this diagnosis. Missmahl and Kübler (129) described the presence of intense positive form and negative intrinsic birefringence of the fibrils of Gaucher cells. In addition to the birefringence of the fibrils, some cells were found to contain spherocrystals, presumably caused by separation of the lipid moieties from the fibrillar proteins.

Niemann-Pick disease: Smetana (219) observed large amounts of birefringent crystals in the thymus, bone marrow and lymph nodes in a case of this disease, while none were found in the liver and spleen. According to Diezel (42) the amount of birefringent material in storing neurons in this disease is rather small, while in hepatic Kupffer cells the amounts are large. Also, Seitelberger (210) and others reported on the presence of spherocrystals giving Maltese cross appearance under crossed polars in Niemann-Pick neurons.

Infantile amaurotic idiocy (Tay-Sachs disease): The storing neurons of this disease give intense birefringence (42). This fact corresponds to the electron microscopic observation on the lamellar structure of the Tay-Sachs granules (232). Also in amaurotic idiocy of dogs (G_{M2}-type) the storage granules are birefringent (19). It has been noted already by Diezel (42) that the storage granules of the late forms of amaurotic idiocy are isotropic. This corresponds to their chromolipid nature (258), as in most of these pigment granules polymerization through numerous cross-links produces an amorphous mass.

Sulfatide lipidosis (metachromatic leukodystrophy): The metachromatic deposits found in this disease in the nervous system and in other organs are intensely birefringent (50). It is not known whether the metachromatic urinary deposits are birefringent, a point which might possibly be of practical importance for diagnosis. Dayan (37) reported that the Hirsch and Peiffer cresyl violet procedure for sulfatides, which has been successfully used for diagnosing this disease, gives a golden brown polarization color under crossed polars and exhibits distinct dichroism when examined with one polarization plate.

Fabry's disease (angiokeratoma corporis diffusum): This is a process in which the finding of birefringent lipids in endothelial, muscle and some epithelial cells as well as in the urinary sediment has often been helpful in establishing the correct diagnosis (56, 60, 162, 207). The birefringent glycolipid is often arranged in spherocrystals appearing as Maltese crosses under crossed polars.

DEPOSITION OF ENDOGENOUS NONLIPIDIC SUBSTANCES IN THE TISSUES

Urates: In gout and in other conditions associated with increased purine base release,

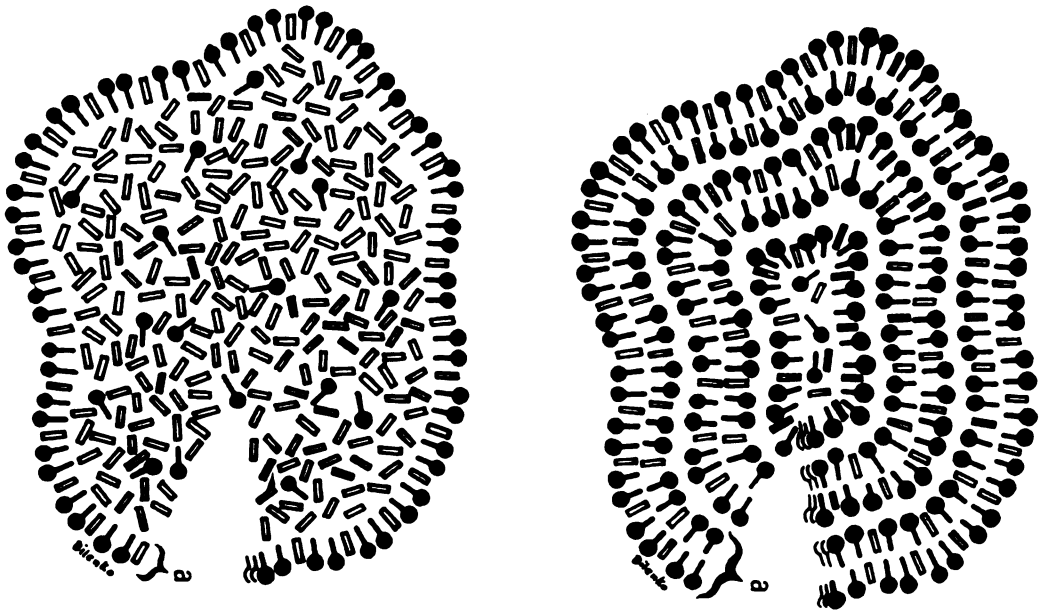

FIG. 12. Illustration of the difference between an anisotropic myelin figure and an isotropic lipid droplet. □, molecules of nonpolar lipids such as cholesterol ester or triglyceride; ●, polar lipids such as phospholipids or fatty acids. Left, a lipid droplet, all the molecules except the surface layer arranged in haphazard fashion with consequent isotropy except for the weak effect of the thin surface layer; right, the molecules of both types arranged in alternating hydrophobic layers (the rods of the polar lipids and the nonpolar lipids) and hydrophilic layers (between the bulbs of the polar lipids).

urate crystals may appear in some articulations, in the kidneys and occasionally in other tissues. Urate crystals are highly soluble and are mostly lost in material fixed or treated by aqueous reagents. Occasionally, probably because of binding to proteins, urate crystals are preserved in tissues fixed in formalin and the positive birefringence in respect to length of the crystals can be shown.

Waldmann (246) suggested the use of a simple inverted polarizing microscope for identifying various crystalline deposits in tissue sections. As this instrument allows the use of high temperatures, the author could determine the effects of temperature, solvents and various reagents on the crystals and thus identify urates, oxalates and various inorganic compounds. Sorger and Bausch (221) also recommended the use of crystallographic polarization techniques for identifying urinary sediment crystals.

Oxalates: These compounds can be found in the urinary tract and occasionally elsewhere in a number of conditions. They were found by their birefringence in the kidneys of rats given high doses of ammonium chloride and oxalate (46), in humans poisoned by ethylene glycol and some other poisons (1, 259), in cases of endogenous oxalosis (100, 158) and in various conditions associated with uremia (14). Voigt (243) introduced a new procedure for identifying calcium oxalate: the sections are treated by a solution of sodium naphthalhydroxamate which produces brownish yellow crystals which appear bright yellow under crossed polars. Macaluso and Berg (113) found this procedure useful in identifying calcium oxalate in autopsies of nephrotic patients. Figure 13 shows the appearance of calcium oxalate crystals under incompletely crossed polars in the kidney, and Figure 14 shows such crystals within the epiphyseal cartilage in human autopsy material.

Cystinosis: In this disease birefringent crystals of cystine can be found in various organs (Fig. 15). Roulet (185) gave an extensive description of the pathologic changes and the crystals and pointed out the ease with which the crystals are dissolved out of the tissues. The author advocated alcohol fixation and no staining. Bürki (24) found cystine crystals in such patients also in ocular tissues. In a critical review Eberlein (49) sharply separated cystino-

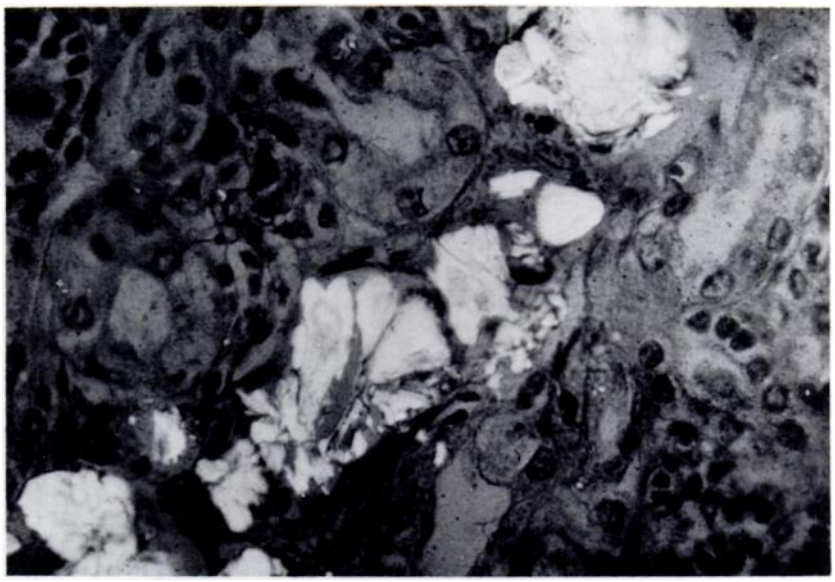

FIG. 13. Crystals of calcium oxalate in the kidney of a patient suffering from endogenous oxalosis under incompletely crossed polars. (Courtesy of Dr. E. Liban of the Beilinson Hospital, Petah Tiqvah.)

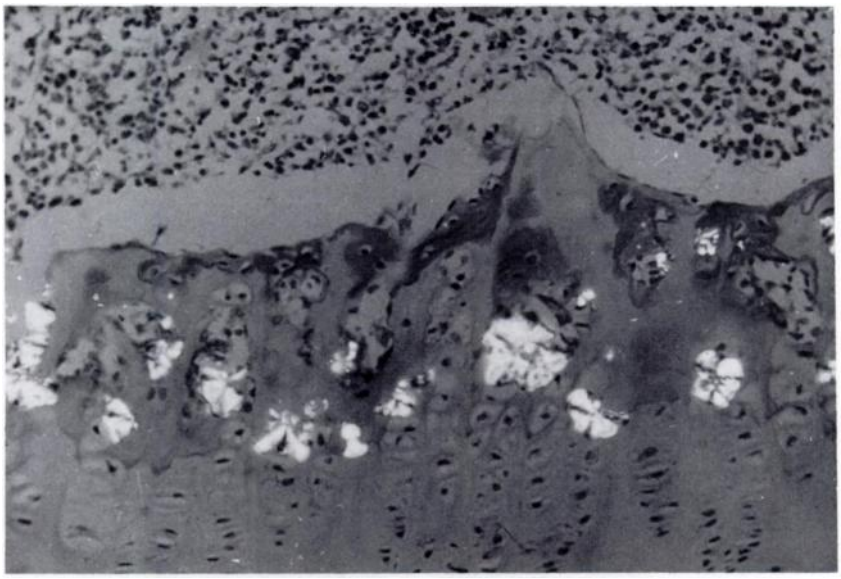

FIG. 14. Crystals of calcium oxalate within the epiphyseal cartilage of the same patient as in Figure 11. (Courtesy of Dr. E. Liban of the Beilinson Hospital, Petah Tiqvah.)

sis from cystinuria and stressed the importance of polarized light microscopy for visualizing and recognizing the crystals. Frazier and Wong (59) again described the crystals in the ocular tissues but in the case of ocular cystinosis no study of the detection of these crystals *in vivo* with polarized light seems to have been performed to date.

Maple syrup disease: In this disease of amino acid metabolism, Diezel and Martin (44) and Hooft *et al.* (79) reported on the presence of birefringent crystals in various organs. The crystals could be found in fresh-frozen sections or in sections prepared from ethanol-fixed material. The crystalline material seemed to be an amino acid, peptide or protein which is soluble in aqueous fixatives. Birefringent crystals were found in glial cells and interstitially in the

brain, in liver cells, in tubular lining cells and in Bowman's capsule in the kidneys, in the adrenal medulla, bone marrow and in many other organs both within epithelia and in stromal and reticuloendothelial cells.

Other diseases of amino acid metabolism: In some of these processes only a limited number of autopsy- or biopsy-based studies were made. It is possible that crystals might be detected by polarization microscopy in some of these processes when tissues are examined without exposure to aqueous reagents, or preferably to any liquid reagents whatsoever. Study of fresh-frozen sections cut in the cryostat and examined under crossed polars in the dry state might be of diagnostic importance in some instances.

Calcium pyrophosphate dihydrate deposition (pseudogout): This is a condition which is often diagnosed by examination of synovial fluid (or eventually of the synovial membrane) by polarized light (155, 218). The crystals exhibit weakly positive birefringence and the examination under crossed polars of a wet smear allows to differentiate them from crystals of urates and cholesterol. Figure 16 shows the appearance of the synovia in such a case.

PRESENCE OF EXOGENOUS NONLIPIDIC BIREFRINGENT BODIES IN THE TISSUES

Formalin pigment: This is in reality an artifact formed in blood-rich organs fixed in formalin. This pigment appears as birefringent needles or rhomboids (107, p 391).

Parasitic organisms: The cuticle and other outer layers of various fungi, helminths and some ectoparasites exhibit anisotropy. For example, chitin (196, 199) and cellulose (107, p 503) are birefringent.

Schmidt (197) pointed out that the proteinaceous outer cuticle of some arthropods changes the sign of birefringence during maturation. The lamellar orientation of the proteins results in positive birefringence which changes into negativity with maturation. The change of sign is due to quinone tanning and the effect of the tanning is similar to that of von Ebner's reaction on collagen.

Figure 17 shows the birefringence of the cuticle of an oxyuris lying in the lumen of a human appendix. Figure 18 shows scolices of echinococcus obtained from the sediment of the cyst fluid.

Crystalline drugs: Sulfonamides can be shown by polarized light, as, for example, by Fransden and Pindborg (58). This methodology can even be used for positively identifying them in tissue sections (246).

Silicosis: This condition can be diagnosed by the examination of sections of affected lungs or lymph nodes under crossed polars (64). Identification of the birefringent material as silica

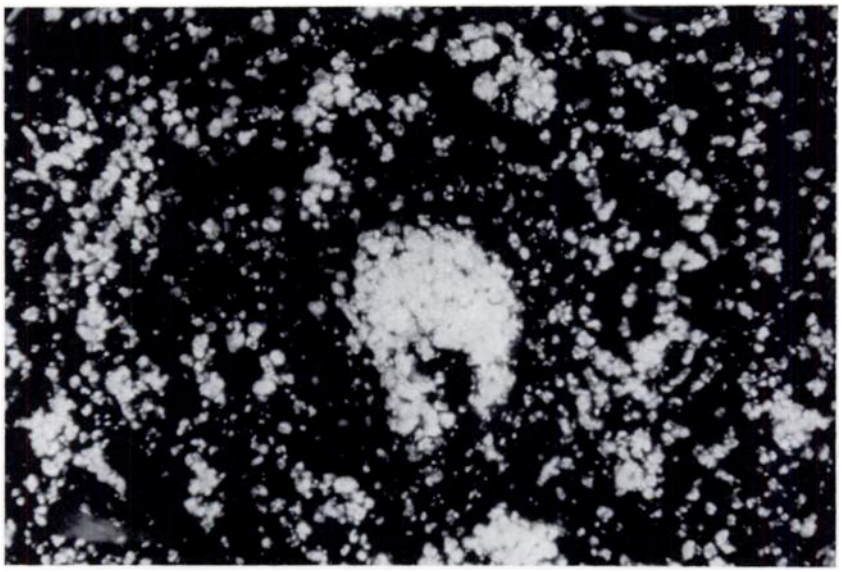

FIG. 15. Cystine crystals in the spleen of a cystinosis patient. Unstained cryostat section examined under crossed polars. (Courtesy of Dr. E. Liban of the Beilinson Hospital, Petah Tiqvah).

FIG. 16. Calcium pyrophosphate dihydrate crystals within the synovia of a pseudogout patient. Unstained cryostat section under crossed polars.

FIG. 17. Oxyuris worm within the lumen of an appendix. The cuticle of the worm is birefringent.

depends on pretreatment. The commonly used procedure is to incinerate paraffin sections for about 2–3 hr at 550–600°C (67). Birefringent crystals which withstand the incineration may be presumed to consist of SiO_2.

Asbestosis: The diagnosis of asbestosis can usually be made on tissue sections without recourse to polarized light microscopy. Asbestos fibers, as such, exhibit weak birefringence and after some time in the body they become coated with tissue constituents and often become isotropic (18). Some authorities prefer, in fact, to use phase microscopy for identifying asbestos fibers (8). Study under crossed polars is of importance, however, in the screening of dusts for their asbestos content (140).

Extraneous artificially introduced materials: Experimental animals and also human beings are often injected with solutions containing formed impurities. These formed contaminants may cause clinical or subclinical embolization and in many instances they can be detected by polarized light microscopy. Innes, Donati and Yevich (85) found hair emboli in mice used in toxicity tests. The hair was obviously birefringent and this characteristic helped their detection. Some drug addicts inject themselves intravenously with tablets intended for oral administration suspended in water. The occurrence of talc and starch emboli in the lung and in parenchymatous organs, as well as at the site of injection, has been described in patients injecting themselves with dissolved methylphenidate tablets (71, 80) and possibly tablets of other drugs (47, 103). Atlee (9) described a peculiar retinopathy in patients addicted to methylphenidate and at autopsy the retina of one patient was found to contain starch emboli.

In firearm wounds and in various other instances of trauma, birefringent crystalline material, such as pieces of calcareous rock, pieces

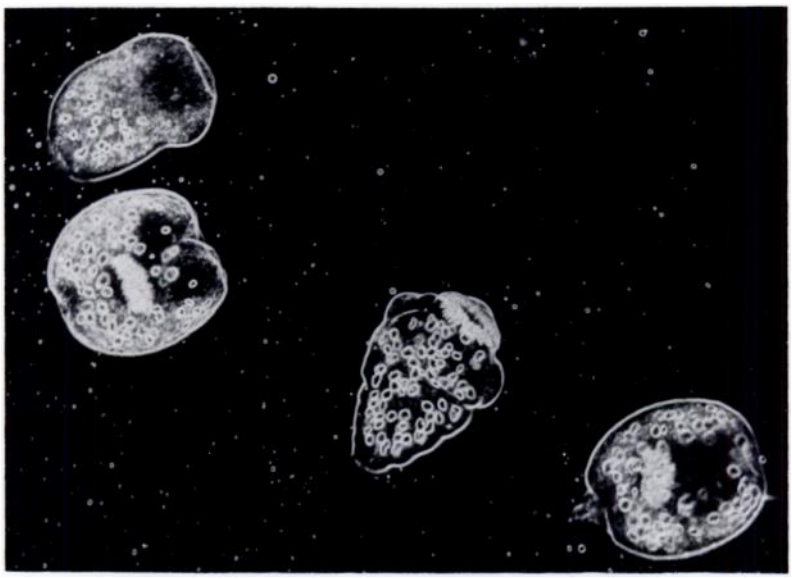

FIG. 18. Scolices of Taenia echinococcus obtained from the sediment of cyst fluid. The outer cuticle and inner structures are intensely birefringent. Wet smear under crossed polars.

of clothing and vegetal matter, can be found in the tissue. Polarized light microscopy is useful in detecting these, often unstained, materials. For example, Wolter and Tibble (257) reported on a case of long standing conjunctivitis in which birefringent threadlike foreign bodies were detected by polarization microscopy.

Talcum powder and starch are commonly used by surgeons for lubricating gloves. Tears and other accidents occasionally cause spillage of these substances into the patients' tissues. Both talcum and starch may produce chronic granulomatous reactions, often with foreign body giant cells. In surgical operations dealing with exposed serosal surfaces, adhesions, lymph stasis and other complications of grave import may ensue. I have recently examined a piece of bowel from a patient where a tentative diagnosis of regional enteritis would have been made on microscopic examination, but polarization microscopy revealed the presence of a few talcum grains from a past operation.

In conclusion, it is suggested that the use of polarized light microscopy can help pathologists detect substances which might have escaped detection in ordinary light microscopic examination and establish the chemical and physical nature of some anisotropic structures and deposits. The use of polarization microscopy on unfixed cryostat sections might help detect deposits of water-soluble crystals in some metabolic disease processes. It is further suggested that polarization optics might be useful in ocular fundoscopic examinations.

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