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Hereditary crystalline stromal dystrophy of Schnyder

II. Histopathology and ultrastructure

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Although first described almost half a century ago (van Went and Wibaut, 1924) and firmly established as a distinct entity by Schnyder (1929), proof that the crystalline deposits in this disorder are cholesterol was not forthcoming until quite recently (Delleman and Winkelman, 1968). Nearly 40 years ago Bonnet, Paufique, and Bonamour (1934) demonstrated the presence of crystals which were birefringent and soluble in ether, and Sédan and Vallès (1946) carried out specific histochemical staining for cholesterol, but in neither of these reports was the hereditary nature of the lesion established. Reports of *bona fide* cases of hereditary crystalline dystrophy examined histologically are scarce (Malbrán, Paunessa, and Vidal, 1953; Paufique, Ravault, Bonnet, and Laurent, 1964; Delleman and Winkelman, 1968) and, apart from the case described by Offret, Payrau, Pouliquen, Faure, and Bisson (1966), which must be accepted with some reservation since cholesterol crystals were not demonstrated, there is no account of the ultrastructural appearances in this condition.

In this report we present the combined light and electron microscopical findings in an attested case, the clinical, genetic, and biochemical aspects of which have been described in the preceding paper (Bron, Williams, and Carruthers, 1972).

Material and methods

A partial-thickness disc of cornea was removed from the left eye of a 47-year-old man with bilateral crystalline dystrophy of the Schnyder type (see Bron and others, 1972 for clinical details).

The specimen received in buffered glutaraldehyde was divided into three portions, one for paraffin-embedded section, one for frozen section, and one for electron microscopy.

In addition to conventional staining with haematoxylin and eosin, sections were also examined for protein by the coupled tetrazonium method, for glycogen by the periodic acid—Schiff, Grocott-Gomori, and Best's carmine staining reactions (using diastase digested controls), and for acid polysaccharide by the Alcian blue method. Staining for fat was performed on frozen sections by the oil red O and Sudan black B techniques and specifically for cholesterol by the Schultz and Okamoto methods. The techniques employed were in most instances as described by Pearse (1968).

The specimen for electron microscopy was postfixed in Zetterquist's osmium tetroxide and embedded in araldite. Thick sections were cut and stained with toluidine blue for the purpose of orientation by light microscopy, after which sections for electron microscopy were cut on an LKB III ultratome at 500 to 800 Å, stained with uranyl acetate and lead citrate, and examined with an AEI-EM6 microscope.

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Results

LIGHT MICROSCOPY

The epithelium which was of generally normal thickness showed a moderate increase in dark cells in the basal layer—a feature most readily appreciated in araldite-embedded sections (Fig. 1). In places the epithelium was lifted away from the basement membrane by focal accumulations of an eosinophilic granular material which gave the appropriate staining reactions for glycogen (Fig. 2). Increased glycogen was also demonstrable in

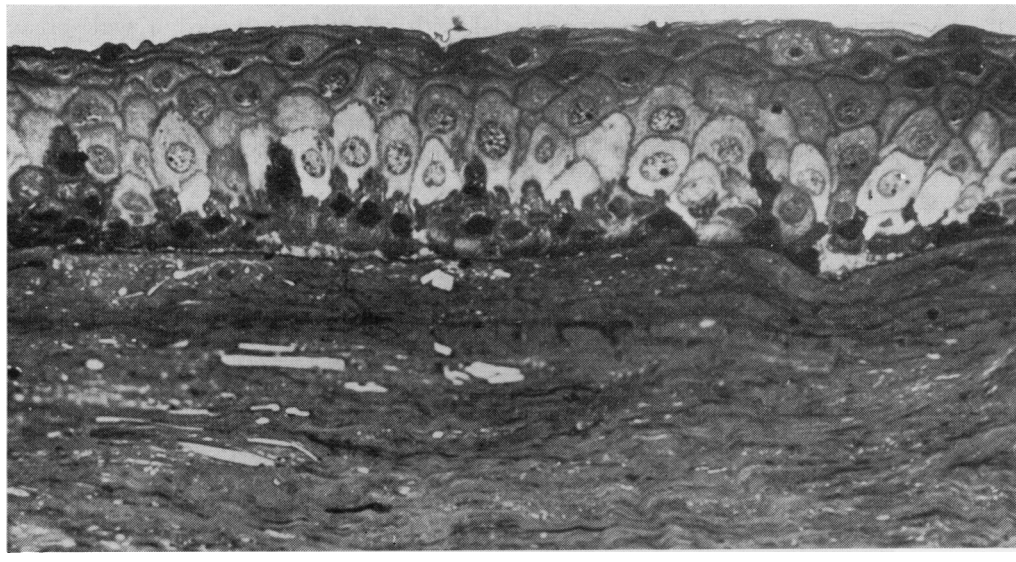

FIG. 1 *Araldite-embedded sections, showing marked increase in dark cells in basal layer of epithelium, absence of Bowman's membrane, and both rectangular and needle-shaped spaces in the superficial stroma. Toluidine blue.* ×440

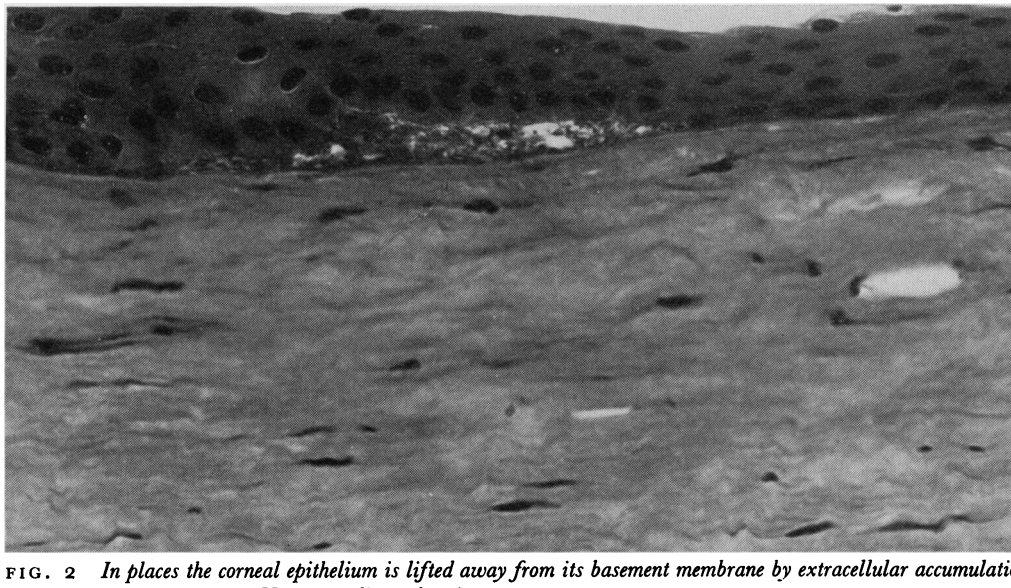

FIG. 2 *In places the corneal epithelium is lifted away from its basement membrane by extracellular accumulation of glycogen-rich material. Haematoxylin and eosin.* ×440

the intercellular spaces of parts of the epithelium as well as within some of the basal cells.

Bowman's membrane was destroyed except for a remnant at the periphery of the disc, while the anterior lamellae of the substantia propria contained multiple crystalline deposits which, though merely represented by empty spaces in paraffin and araldite-embedded material, were clearly recognizable as birefringent structures in frozen sections (Fig. 3). The crystals presented either as rectangular plates, which in some instances were notched, or as needle-shaped clefts, indicating that the crystalline material might be cholesterol, and this was supported by positive Schultz and Okamoto reactions in frozen tissue. Staining of frozen material with oil red O showed a widespread particulate distribution of neutral fat both in the corneal stroma and in the remnant of Bowman's membrane. Cholesterol was also demonstrable in this latter structure by histochemical techniques although no crystalline structures were seen. There was no evidence in the corneal stroma of either abnormal protein deposition or excessive mucopolysaccharide accumulation.

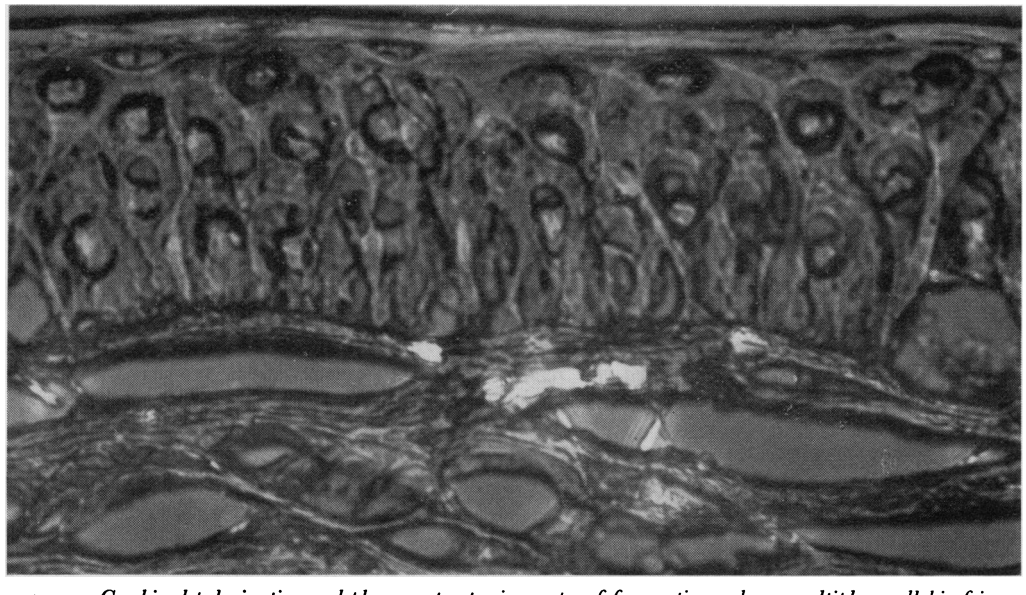

FIG. 3 *Combined polarization and phase contrast microscopy of frozen tissue shows multiple small birefringent structures in the superficial corneal lamellae. Unstained preparation.* ×700

ELECTRON MICROSCOPY

The anterior layers of the corneal stroma showed some disruption of the normal lamellar architecture and contained multiple empty spaces which were either needle-shaped or approximately rectangular with notching at the corners (Figs 4 and 5). These spaces were in places in direct contact with the epithelial basement membrane, Bowman's membrane being absent. There were also numerous rounded spaces of smaller dimension and, while some contained membrane-bound structures suggestive of keratocyte processes or organelles, the majority were empty: the spaces themselves had no limiting membrane. A third unusual feature in the substantia propria was the presence of ill-defined areas of a relatively electron dense material which, though in the main granular, sometimes exhibited a fine fibrillar organization (Fig. 6).

The basal layer of the epithelium included a large proportion of dark cells characterized

by a dense somewhat shrunken nucleus with an irregular wrinkled outline and cytoplasm of increased electron density attributable to condensation of its tonofibrils and accumulation

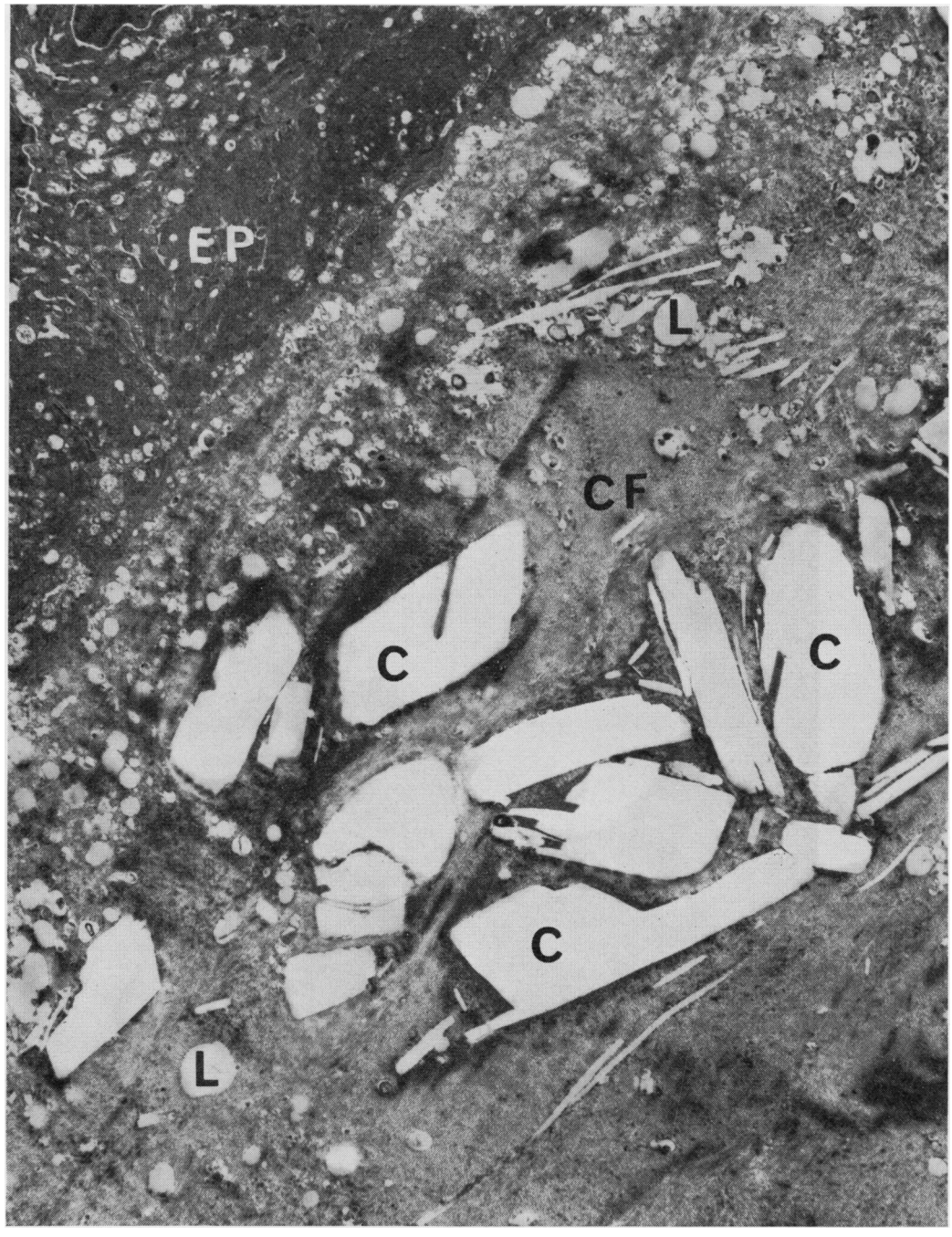

FIG. 4 *Electron micrograph of superficial corneal stroma, showing electron transparent areas having the outline of dissolved cholesterol crystals (C) and lipid droplets (L). CF = Collagen fibrils of corneal stroma. EP = Epithelium.* ×9,600

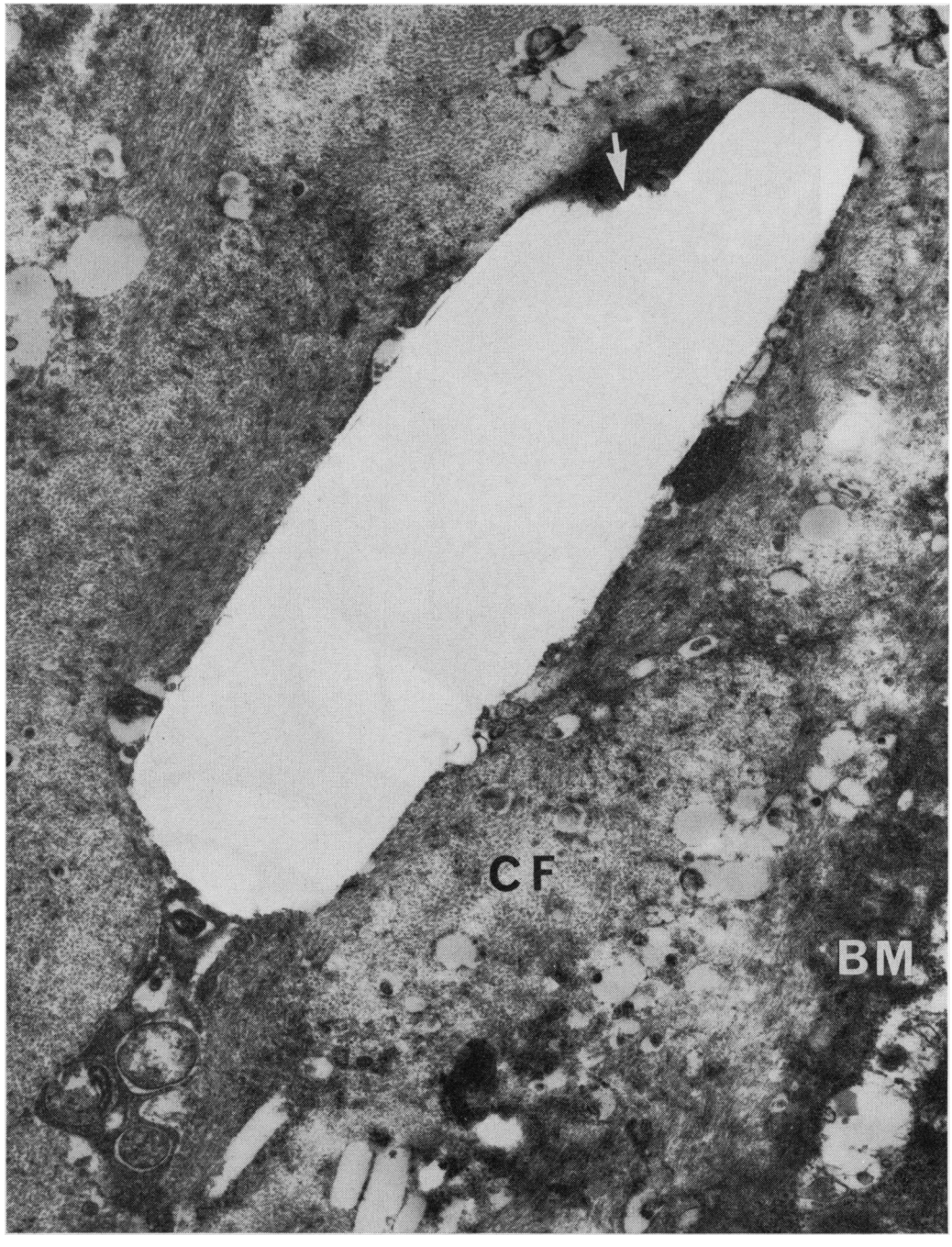

FIG. 5 *Outline of an electron transparent structure, showing typical notch (arrowed) of a cholesterol crystal. CF = Collagen fibrils. BM = Basement membrane of epithelium.* ×24,000

of glycogen granules (Figs 7 and 8). An additional feature was the presence of mitochondria showing cystic swelling. The basement membrane of the epithelium was in some

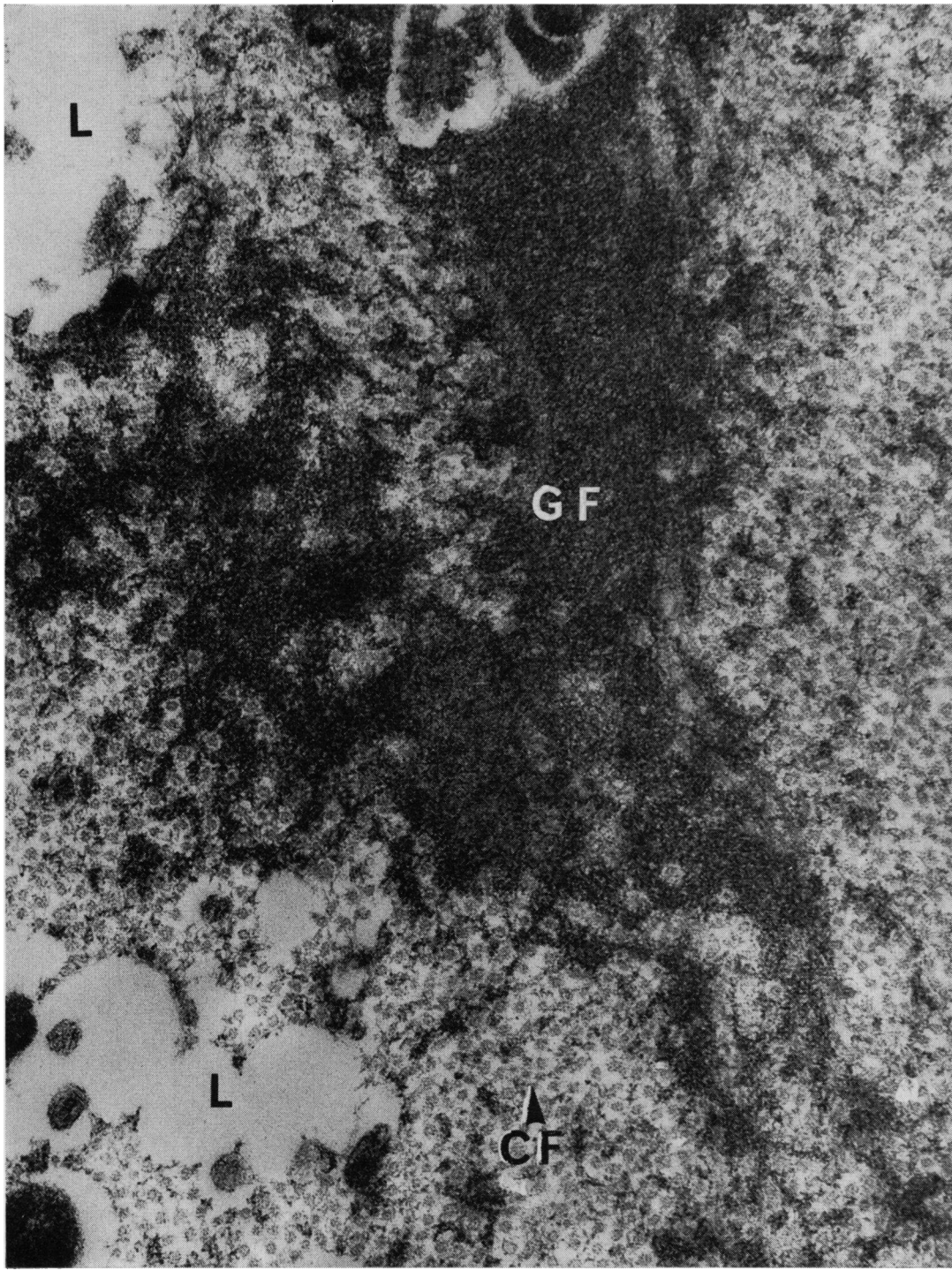

FIG. 6 *Electron micrograph of granulo-fibrillar (GF) deposits in corneal stroma. Note also outline of dissolved lipid deposits (L). CF = Collagen fibrils cut transversely.* ×75,000

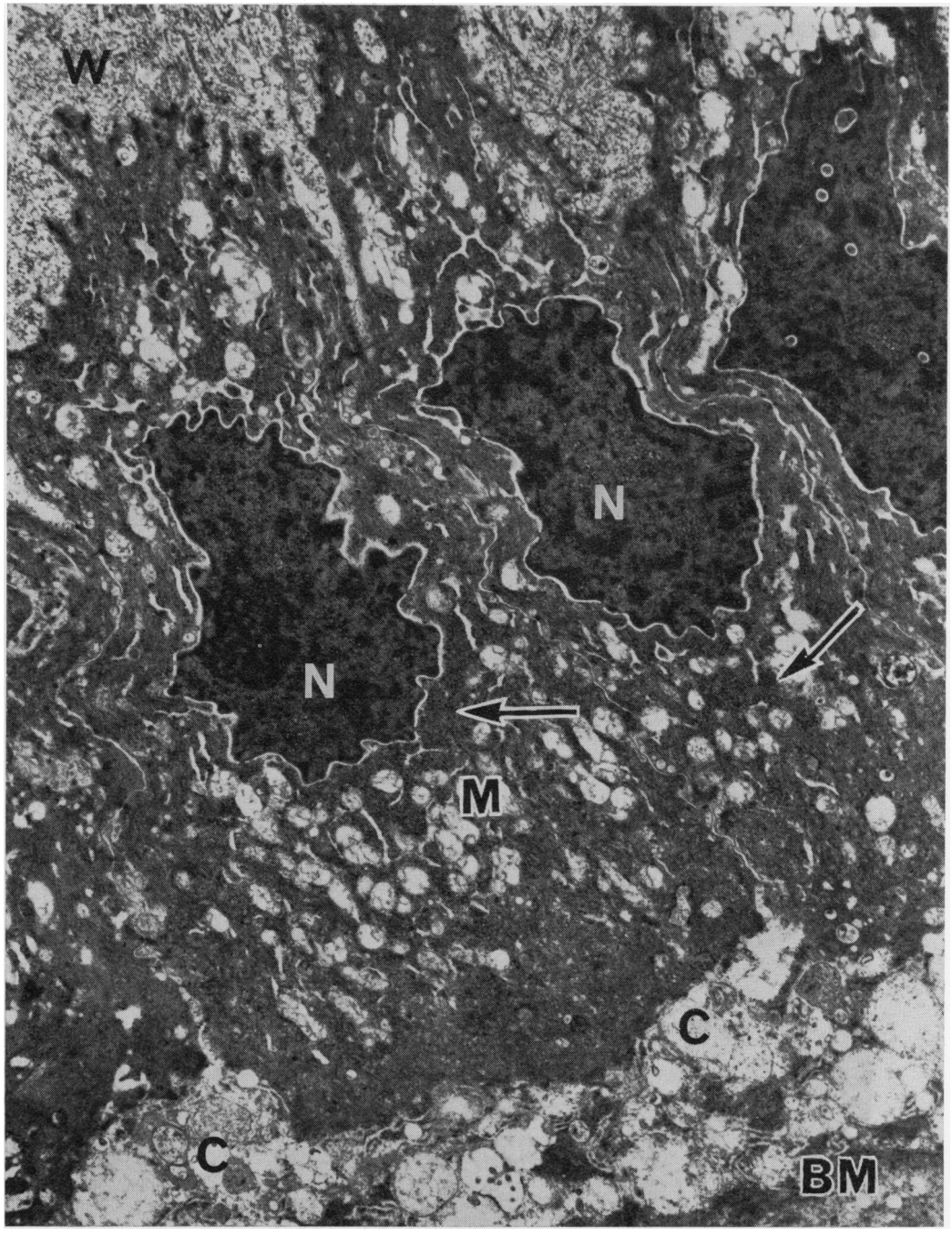

FIG. 7 *Survey electron micrograph of "dark" cells in basal epithelium, showing dense nuclei (N) having a wrinkled outline, cystic swelling of mitochondria (M), and dense deposits of glycogen molecules (arrowed). Compare the density of these cells with the normal "wing" cells (W) above. C = Degenerating cell organelles between basal cells and basement membrane (BM).* ×10,000

places disrupted and in others separated from the epithelium by focal aggregations of glycogen and degenerate cell organelles.

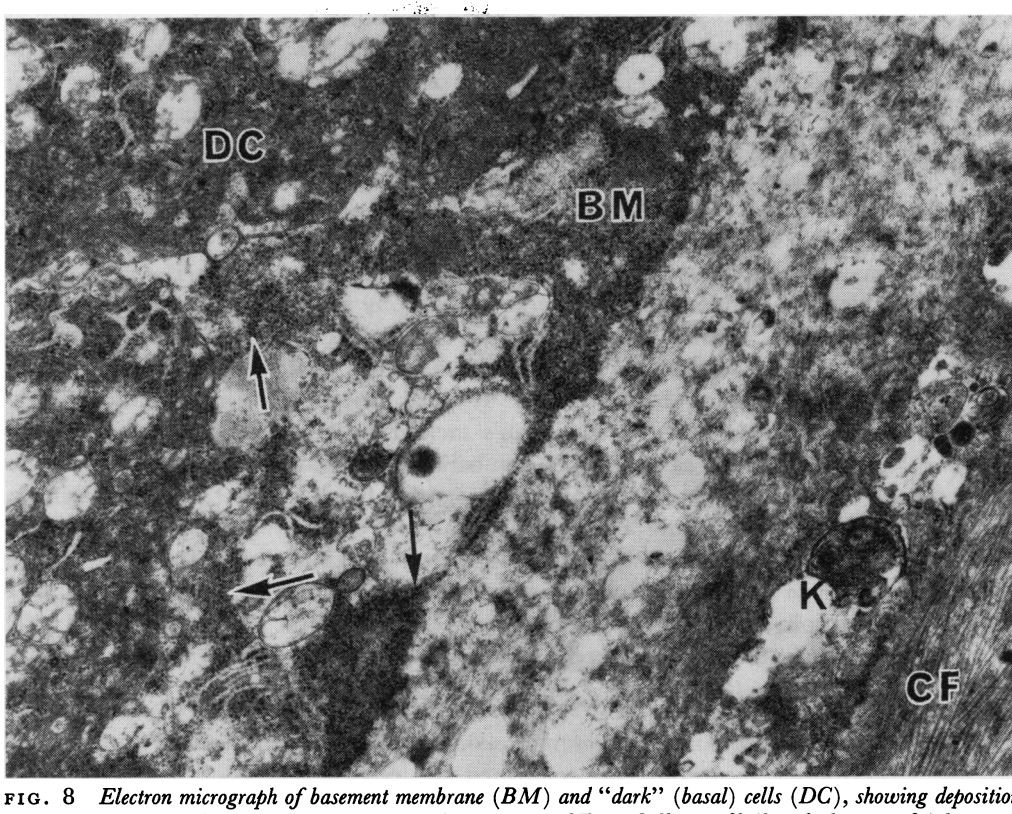

FIG. 8 *Electron micrograph of basement membrane (BM) and "dark" (basal) cells (DC), showing depositions of glycogen (arrowed). K = Degenerating keratocytes. CF = Collagen fibrils of the superficial stroma.* ×21,000

Discussion

The profiles of the crystalline deposits taken together with their histochemical properties leave no doubt that the material in question is cholesterol, thus confirming the report of Delleman and Winkelman (1968). The nature of the small round spaces seen in electron micrographs of the stroma is less certain, though there would seem to be some reason to believe that they were initially filled with a neutral fat which was subsequently removed during the embedding process, since frozen sections showed a heavy fine particulate impregnation with oil red O staining material. Should this be the case, its failure to react with osmium tetroxide suggests that it is a saturated triglyceride. The presence of such a material would account for the opalescence of the stromal lamellae not apparently infiltrated with cholesterol crystals that was noted on slit-lamp examination and which persisted in the deep layers after lamellar keratoplasty. Round or ovoid electron-lucent structures within the stroma were also described by Offret and others (1966), but are not really comparable to those seen in the present case in that they were membrane-bound and sometimes included osmiophilic material: in their case it is probable, as the authors suggest, that the vesicles were cytoplasmic organelles extruded from degenerate cells.

Impregnation of the Bowman's membrane present at the edge of the resected disc with cholesterol and neutral fat almost certainly corresponds to the corneal arcus noted clinically and as such is indistinguishable from that seen in association with increasing age.

In discussing the clinical and biochemical aspects not only of this man but also of his family, Bron and others (1972) have come to the conclusion that the corneal lesion was

the result of a localized genetic defect in cholesterol metabolism, complicated and intensified by a separately determined hereditary disturbance of plasma lipoprotein. Since accumulation of cholesterol in a crystalline form in the central cornea is not described in the systemic disorders of lipid metabolism (Fredrickson, Levy, and Lees, 1967), and as the diffuse lipid deposition in other parts of the cornea reflects the elevated levels of cholesterol and triglyceride in the circulating plasma, the findings in the present case are not incompatible with such an interpretation.

There is no ready explanation for the increased amounts of glycogen within and around many of the basal cells of the epithelium. It is improbable, however, that this finding is a specific component of Schnyder's dystrophy for two reasons: first, it does not appear to have been described in other reports of this condition, and secondly, comparable appearances have been reported in other disorders such as Reis-Bücklers' dystrophy (Rice, Ashton, Jay, and Blach, 1968). It seems more likely that the increase in dark cells is linked to destruction of Bowman's membrane and a degree of epithelial oedema, with extracellular liberation of glycogen being due to associated epitheliolysis in the basal layer.

Equally, the fine granular and sometimes fibrillar material seen within the collagenous lamellae of the substantia propria in electron micrographs is probably a non-specific response. Similar material, which may represent unaggregated collagen filaments, has been described in granular dystrophy (Tripathi and Garner, 1970), in Reis-Bücklers dystrophy, and in experimental scar tissue in rabbit cornea (Rice and others, 1968), as well as in the case of alleged Schnyder's dystrophy reported by Offret and others (1966).

Summary

Histochemical and electron microscopical study of corneal tissue from a case of Schnyder's crystalline dystrophy has confirmed the reports of earlier workers that the crystalline material is cholesterol. Stromal deposition of neutral fat and non-crystalline cholesterol was also demonstrated and it would seem probable that the corneal condition was due to a localized defect in cholesterol metabolism aggravated in this instance by a systemic abnormality of lipid transport. Non-specific changes, represented by destruction of Bowman's membrane and some disorganization of the superficial stroma, together with increased numbers of dark cells in the basal layer of the corneal epithelium, were also observed.

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References

BONNET, P., PAUFIQUE, L., and BONAMOUR, G. (1934) *Bull. Soc. Ophtal. Paris*, p. 225

BRON, A. J., WILLIAMS, H. P., and CARRUTHERS, M. E. (1972) *Brit. J. Ophthal.*, **56**, 383

DELLEMAN, J. W., and WINKELMAN, J. E. (1968) *Ophthalmologica (Basel)*, **155**, 409

FREDRICKSON, D. S., LEVY, R. I., and LEES, R. S. (1967) *New Engl. J. Med.*, **276**, 34, 94, 148, 215, 273

MALBRÁN, J. L., PAUNESSA, J. M., and VIDAL, F. (1953) *Ophthalmologica (Basel)*, **126**, 369

OFFRET, G., PAYRAU, P., POULIQUEN, Y., FAURE, J. P., and BISSON, J. (1966) *Arch. Ophtal. Paris*, **26**, 171

PAUFIQUE, L., RAVAULT, M. P., BONNET, M., and LAURENT, C. (1964) *Bull. Soc. Ophtal. Fr.*, p. 104

PEARSE, A. G. E. (1968) "Histochemistry, Theoretical and Applied", 3rd. ed., vol. 1, pp. 687–704. Churchill, London

RICE, N. S. C., ASHTON, N., JAY, B., and BLACH, R. K. (1968) *Brit. J. Ophthal.*, **52**, 577

SCHNYDER, W. F. (1929) *Schweiz. med. Wschr.*, **59**, 559

SÉDAN, J., and VALLÈS, A. (1946) *Bull. Soc. franç. Ophtal.*, **59**, 127

TRIPATHI, R. C., and GARNER, A. (1970) *Brit. J. Ophthal.*, **54**, 361

WENT, J. van, and WIBAUT, F. (1924) *Ned. T. Geneesk.*, **68**, 2996