MACULAR CORNEAL DYSTROPHY

AN INHERITED ACID MUCOPOLYSACCHARIDE STORAGE DISEASE OF THE CORNEAL FIBROBLAST

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Macular corneal dystrophy, an uncommon, genetically determined human disease, is manifest clinically by slowly progressive visual impairment and is characterized morphologically by distinctive structural alterations in the cornea. Although many of the histologic features have been recognized for more than half a century, the precise nature of the condition has not been clearly established. Some have viewed the primary lesion as an intracellular process; others have regarded collagen degeneration as cardinal.

In the present investigation, light and electron microscopy, histochemical procedures, and biochemical analytical methods were employed to elucidate the pathogenesis of the lesions and the biochemical correlates.

MATERIAL AND METHODS

Sixteen corneal buttons were obtained by penetrating keratoplasty in 9 cases of macular corneal dystrophy. The clinical manifestations in each patient are summarized in Table I. Three of the patients investigated had had repeat transplants in an affected eye (cases 3, 6, and 8) after postoperative periods of 27, 46 and 60 months, respectively.

Representative portions of the cornea from each case were fixed in formalin, embedded in paraffin and sectioned at 7 μ perpendicularly to the epithelial surface. These and comparable sections of normal cornea procured from enucleated eyes and at necropsy were stained as follows: hematoxylin and eosin, Masson’s trichrome, Mowry’s modification of Hale’s colloidal iron reaction, periodic acid-Schiff (PAS), post-coupled benzylidene, performic acid-alcian blue, ferric ferricyanide, ninhydrin-Schiff, Millon, alcian blue, alcian blue-chromotrope 2R, luxol fast blue, osmic acid, toluidine blue, crystal violet, Verhoeff’s elastic tissue stain, Congo red, modified May-Grünwald-Giemsa, Wilder’s reticulum, and thioflavin T. Periodic acid-Schiff, Hale’s colloidal iron reaction, toluidine blue and crystal violet stains were also employed after hyaluronidase (bull testis), malt diastase, methylation, sulfation, methylation followed by sulfation, acetylation, and acetylation followed by saponification. Frozen sections of formalin-fixed tissue in 2 patients were stained with oil red O, luxol fast blue and osmic acid. Unstained corneal sections were examined for birefringence with polarized light.

A portion of the cornea from case 7 (with macular corneal dystrophy) and, for con-
### Table I
**Summary of Clinical Manifestations of Cases Studied**

| Case | Family | Sex | Race | Family history of corneal dystrophy | Age at onset of symptoms | Age at initial corneal transplant | Remarks |
|------|--------|-----|------|-------------------------------------|--------------------------|---------------------------------|---------|
| 1    | A      | M   | W    | -                                   | 19                       | 28                              |         |
| 2    | B      | M   | W    | +                                   | Early adulthood          | 47                              | Graft opaque within 1 year       |
| 3    | C      | M   | W    | +                                   | 14                       | 36                              | Bilateral corneal transplants. Repeat transplant, left eye |
| 4    | C      | M   | W    | +                                   | 14                       | 31                              | Graft opaque within 5 years      |
| 5    | C      | F   | W    | +                                   | 18                       | 41                              | Bilateral corneal transplants. Repeat transplant, left eye |
| 6    | C      | F   | W    | +                                   | 14                       | 39                              |         |
| 7    | D      | F   | W    | -                                   | 14                       | 39                              |         |
| 8    | E      | F   | W    | +                                   | Childhood                | 25                              | Repeat transplant, right eye     |
| 9    | E      | F   | W    | +                                   | 9-10                     | 18                              |         |
control purposes, representative sections of cornea from 2 enucleated eyes removed for glaucoma and melanoma, were sectioned into small cubes measuring less than 2 mm. These were fixed in cold Veronal-buffered 2 per cent osmium tetroxide for 45 minutes. Since the tissues from the case of corneal dystrophy had been inadvertently fixed for approximately 1 hour in 10 per cent formalin prior to osmication, the control tissues were treated in like manner. All were dehydrated in graded alcohols, infiltrated with styrene, and embedded in Epon. Thin sections were cut with the Servall Porter-Blum microtome, mounted on 300 mesh copper grids and stained for 45 minutes in 2 per cent aqueous uranyl acetate at 60°C. Electron microscopic examination was made with an RCA EMU-3G microscope at 50 kv. Companion 2 μ sections were stained with thionine-azure blue for purposes of orientation.13

Epon-embedded tissues did not manifest an affinity for colloidal iron. Small blocks of corneal tissue previously fixed in formalin and embedded in paraffin were therefore deparaffinized, sectioned into 2 mm. cubes and treated with colloidal iron as in the initial stages of Hale’s reaction.8 These were subsequently embedded in Epon and sectioned for electron microscopy.

A biopsy of the skin was performed on the forearm of a 62-year-old man (case 3) with clinically manifest macular corneal dystrophy since the age of 14 years. Diagnosis had been established in this patient by histologic examination of corneal tissue removed 8 years previously. Representative portions of the biopsy specimen were fixed in formalin, embedded in paraffin, and stained by the above techniques for light microscopy and histochemical evaluation. Additional portions were sectioned into small cubes, fixed in osmium tetroxide and embedded in Epon for electron microscopy. The acid mucopolysaccharide composition of a 24-hour specimen of urine from this patient was determined by the orcinol,14 carbazole,15 and naphthylresorcinol16 reactions, as well as by the method of DiFerrante and Rich.17

Results

With the exception of a variety of nonspecific epithelial changes, minimal abnormalities were evident on light microscopy of numerous hematoxylin and eosin stained sections of the corneal tissues in the 9 cases of macular corneal dystrophy. By contrast, when companion sections were stained with alcian blue or prepared by the PAS or Hale colloidal iron methods, conspicuous structural alterations were regularly visible (Fig. 1). Large quantities of abnormal material were present in the immediate vicinity of the spindle-shaped corneal fibroblasts (Fig. 2). The cells themselves were present in the expected concentrations and were positioned normally, with the long axis parallel to the corneal epithelium. Their cytoplasm, however, regularly contained numerous large and small particles measuring up to 3 μ across that stained intensely with alcian blue and by the PAS and Hale colloidal iron methods. Deposits with similar histochemical affinities were regularly present in the contiguous collagen. In some instances these measured up to 10 μ across, and when of this size were particularly numerous in the superficial portions of the cornea adjacent to Bowman’s membrane (Fig. 3). Most of the extracellular deposits, however, measured less than 1 μ across, were spherical and were scattered haphazardly in the collagenous stroma. Both the intra- and extracellular deposits were most prevalent
### Table II

**Staining Reactions of Accumulated Material in Macular Corneal Dystrophy**

| Method                        | Staining reaction | Remarks                                                                 |
|-------------------------------|-------------------|------------------------------------------------------------------------|
| **Protein**                   |                   |                                                                        |
| Post-coupled benzylidene      | Neg.              | Demonstrates tryptophan and indoles*                                   |
| Perforinic acid-alcian blue   | Neg.              | Demonstrates cystine*                                                   |
| Ferric ferricyanide           | Neg.              | Demonstrates SH groups*                                                 |
| Ninhydrin-Schiff              | Neg.              | Demonstrates \(\alpha\)-amino acids*                                   |
| Millon                        | Neg.              | Demonstrates tyrosine (hydroxy-phenyl groups)*                          |
| **Carbohydrates**             |                   |                                                                        |
| Periodic acid-Schiff          |                   |                                                                        |
| No pretreatment               | ++ + + + Pos.     | Demonstrates 1,2 glycols*                                               |
| Schiff control                | Neg.              | Demonstrates aldehydes*                                                 |
| After malt diastase           | ++ + + + Pos.     | Glycogen digested by malt diastase*                                    |
| After hyaluronidase (bull testis) | ++ + + Pos.     | Bull testis hyaluronidase digests hyaluronic acid, chondroitin sulfate A and B* |
| After acetylation             | Neg.              | Acetylation abolishes PAS positivity*                                   |
| After methylation             | ++ + Pos.         | Saponification restores acetylation-abolished PAS positivity*           |
| After sulfation               | ++ Pos.           |                                                                        |
| After acetylation followed by saponification | ++ + + Pos.     |                                                                        |
| After bisulphite              | ++ Pos.           |                                                                        |
| Hale's colloidal iron-binding method |                   |                                                                        |
| No pretreatment               | ++ + + + Pos.     | Acid mucopolysaccharides, esters of phosphoric acid, nucleoproteins and other proteins* |
| After malt diastase           | ++ + + + Pos.     |                                                                        |
| After hyaluronidase (bull testis) | ++ + Pos.     |                                                                        |
| After acetylation             | ++ + + Pos.       |                                                                        |
| After methylation             | ++ + Pos.         |                                                                        |
| After sulfation               | + Pos.            |                                                                        |
| After acetylation followed by saponification | ++ + + + Pos. |                                                                        |
| After methylation followed by sulfation | ++ + Pos. | An apparent difference between intra- and extracellular material |
| Alcian blue, pH 2.1           | ++ Pos.           |                                                                        |
| **Lipids**                    |                   |                                                                        |
| Oil red O                     | Neg.              | Stains acid mucopolysaccharides*                                        |
| Method                                      | After hyaluronidase (bull testis) | After acetylation and after acetylation followed by saponification | Masson's trichrome | Thioflavine T | Congo red | Polarized light | Notes |
|---------------------------------------------|-----------------------------------|-----------------------------------------------------------------|-------------------|---------------|------------|----------------|-------|
| Luxol fast blue                             | Neg.                              | ++Metachromasia (purple)                                      | Not stained       | Not stained   | Not stained | Not birefringent | Stains phospholipids * Demonstrates unsaturated fats and their derivatives, alcohols, ethers, SH and SS long chain aldehydes and group amines, ketones * |
| Osmic acid                                  | Neg.                              | Material not identifiable; some granules orthochromatic, others ++metachromatic (purple) |                   |               |            |                | Stains acid mucopolysaccharides, amyloid and other substances metachromatically * |
| Other methods                               |                                   | Most material not identifiable; some granules orthochromatic (blue) |                   |               |            |                | Sulfation enhances toluidine blue metachromasia * |
| Toluidine blue                              | ++Metachromasia                   | ++Metachromasia (mauve)                                       |                   |               |            |                | Stains acid mucopolysaccharides and other substances metachromatically * |
| No pretreatment                             |                                   | +Metachromasia                                               |                   |               |            |                | Sulfation enhances crystal violet metachromasia |
| After hyaluronidase (bull testis)           |                                   | ++Metachromasia (mauve)                                       |                   |               |            |                | |
| After malt diastase                         |                                   | ++Metachromasia                                              |                   |               |            |                | |
| After methylase                             |                                   | Material not identifiable                                    |                   |               |            |                | |
| After sulfaion                              |                                   | +++Metachromasia                                             |                   |               |            |                | |
| After methylation followed by sulfaion      |                                   | Material not identifiable                                    |                   |               |            |                | |
| After acetylation                           |                                   | ++Metachromasia                                              |                   |               |            |                | |
| After acetylation followed by saponification|                                   | ++Metachromasia                                              |                   |               |            |                | |

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in the axial portion of the cornea with a conspicuous tendency to lessen peripherally. Masson's trichrome preparations showed the corneal collagen to be intact, but staining of the corneal deposits was not manifest. Similarly, tissues prepared by Wilder's method demonstrated a generally intact reticulum pattern. The endothelial layer of the cornea occasionally contained minute amounts of colloidal iron-positive material, but in general these cells were unaltered. In corneal buttons removed after a previous transplant abundant intra- and extracellular deposits of accumulated material were noted in the peripheral portions of the corneal tissues, while the central sites of the previous corneal transplant were uninvolved. The results of the histochemical preparations are summarized in Table II.

The fine structure of the corneal fibroblasts was altered in a consistent manner although these changes varied notably in degree from cell to cell (Fig. 4). Most conspicuous was the presence of numerous large and small cytoplasmic vacuoles ostensibly originating as dilations of the endoplasmic reticular cisterna. These were recognized by the presence of a nearly continuous membranous layer of ribonuclear protein granules about their periphery (Figs. 5 to 9). The larger vacuoles were more uniformly spherical and were often confluent, thus imparting an engorged appearance and a honeycomb structure to the cytoplasmic compartments (Fig. 10). The organelles, characteristically sparse in normal corneal fibroblasts, were present in approximately normal numbers in macular corneal dystrophy. Mitochondria were displaced by the vacuoles. The rough endoplasmic reticulum encased the smaller vacuoles, as has been mentioned, and in fragmented form, littered the periphery of the larger lacunas. Generally, the content of both large and small intracellular vacuoles were electron-lucent, but in some regions had finely granular densities and rarely showed one or several osmiophilic particles or masses. The latter closely resembled those which with regularity occupied the extracellular vacuoles. The nuclei in normal corneal fibroblasts were slender and elongated and were rather densely filled with coarse granules, together with one or several nucleoli (Fig. 5). Prefixation in formalin caused the nuclear granules to marginate at the nuclear membranes. The nuclei of corneal fibroblasts in macular corneal dystrophy did not differ structurally from the normal. Rarely were they surrounded by a perinuclear cisterna which was always slender and not evidently associated with the cytoplasmic vacuoles; the latter characteristically occupied a bi-polar position in the cytoplasm. The surface plasma membranes were ostensibly intact though absolute assurance could not be obtained in the regions of small and numerous digital cytoplasmic processes that extended into the adjacent collagen. Cleavage
of the plasma membrane from the collagen bed was conspicuous in macular dystrophy but was present as well in control tissues and seemingly represented an artifact induced during tissue preparation by physical forces acting divergently upon contiguous structures with disparate consistencies (Figs. 5, 7 and 9).

Multitudes of small extracellular vacuoles measuring up to 2 μ were dispersed haphazardly and individually or in clusters throughout the collagen. Each was sharply demarcated from the collagenous fibers that were displaced and somewhat attenuated as they passed tangentially about the vacuoles. As was true of the intracellular vacuoles, the content of the extracellular ones was for the most part electron-lucent. In contrast, the extracellular vacuoles almost regularly contained one or several discrete and markedly osmiophilic masses. These were usually spheroid and measured up to 1 μ across. Their density often varied in central and peripheral areas and frequently exhibited a laminated pattern but not in a consistent manner (Figs. 11 and 12). Some extracellular vacuoles lay adjacent to the plasma membranes of fibroblasts but did not preferentially reside in this area.

The collagen fibers were everywhere well preserved and regularly retained the usual periodic cross-striational pattern. Even collagen fibers adjacent to the corneal fibroblasts or coursing about and between individual or clusters of extracellular vacuoles were uniformly intact (Figs. 11 and 12).

Tissues incubated in colloidal iron provided clear evidence of a selective affinity of this material for the content of cytoplasmic vacuoles, thus confirming information obtained by light microscopy (Fig. 13). The electron-dense iron-stained granules clustered, in great densities, about the margins of the cytoplasmic vacuoles and by contrast were rarely found, even as individual granules, elsewhere.

Companion electron micrographs of normal cutaneous fibroblasts and those in the skin of the patient with macular corneal dystrophy showed no notable structural differences; neither contained cytoplasmic vacuoles so conspicuous in the corneal fibroblasts of the same patient. Quantitative analysis of a 24-hour urine specimen from this patient disclosed the total amount of acid mucopolysaccharide excreted to be at the upper limit of normality (16.23 mg. per day). The carbazole/orcinol ratio was 0.669 (normal range 0.6 to 0.8) and the carbazole/naphthyl-resorcinol ratio was 8.38 (normal range 8 to 12).

COMMENT

Although light microscopy has clearly defined several of the cardinal histologic features of macular corneal dystrophy, largely because of
limitations in resolution divergent interpretations, have been placed upon the significance of these changes and their sequential relationships so that the fundamental nature of this condition remains uncertain. The presence of metachromatic staining material was recognized by Fuchs and was more precisely defined by Franceschetti and Babel as having weak affinities for hematoxylin and eosin and for the May-Grünwald-Giemsa, Gram and van Gieson stains, but manifesting a strong affinity for resorcin fuchsin. Wolter and Cutler directed attention to vacuoles of varying sizes in the cytoplasm of corneal fibroblasts, interpreting these cellular changes as primary and the hyalinization of the corneal lamellas as secondary. Contrariwise, however, Jones and Zimmerman emphasized the stromal aspects of the lesions, subdividing them as follows: (a) small focal areas of swelling within lamellas (intralamellar or "early" lesions); (b) large accumulations of amorphous granular material in areas where the lamellas were destroyed; and (c) small focal interlamellar deposits of amorphous granular material. In accord with the staining affinities of these materials for alcian blue and colloidal iron, these authors concluded that the deposits were composed, at least in part, of acid mucopolysaccharides. They postulated, however, that the disease process had its genesis primarily in mucoid degeneration of collagen, with subsequent phagocytosis of the mucoid material by the corneal corpuscles and endothelium.

Electron microscopy has provided additional information cogent to the pathogenesis of this disease. First, the collagen fibers, even as they circumvent large confluent lacunas or lie tangential to individual small extracellular vesicles, are themselves structurally intact. Second, the storage material, as it accumulates intracellularly, that is, within the corneal fibroblasts, appears initially not as pinocytotic or phagocytosed vacuoles, but as accumulations within the endoplasmic reticular canals and cisternae. Together these findings clearly define the fundamental nature of macular corneal dystrophy as an intracellular storage disease ostensibly caused by a genetically governed metabolic derangement.

Although numerous complementary histochemical procedures were employed to define the nature of the storage material, the cogency of the information does not exceed the specificity of these stains and this is not always of a high order. However, together they permit certain tentative conclusions. Of the 4 positive histochemical reactions, namely PAS, alcian blue, metachromasia and colloidal iron, the PAS stain is probably the least specific, as it demonstrates all substances with a 1,2 glycol

* Although Wolter and Cutler reported their case as having granular dystrophy of the cornea, Jones and Zimmerman drew attention to the fact that the clinical data of this case suggested macular dystrophy rather than granular dystrophy.
Ostensibly, alcian blue stains acid groups by salt linkage, while metachromasia, with dyes such as toluidine blue and crystal violet, derives a degree of specificity from dependency upon a significant density of free electron-negative groups which attract the polar cationic groups of the dye. Hale's colloidal iron technique is perhaps most specific since it depends upon the binding of ferric iron at low pH to acidic groups, a reaction made visible by staining of the bound iron. Diverse compounds such as hyaluronic acids, sulfated mucopolysaccharides, nucleoproteins, esters of phosphoric acid and like proteins are visibly demonstrated. This quartet of histochemical reactions by the intra- and extracellular storage material in macular corneal dystrophy characterizes it as an acidic molecule with an affinity for iron, having 1,2 glycol, carboxyl and possibly sulfate groups. Negative reactions for protein largely preclude this as a major constituent. The avidity of focal components of the extracellular material for osmic acid, in preparation for electron microscopy, suggests the presence of lipids. However, negative oil red O, luxol fast blue and osmic acid stains with light microscopy attest against significant concentrations. The storage material is evidently not glycogen as it was not soluble in water nor affected by diastase digestion. Thus, the corneal deposits stained readily by the fundamental histochemical techniques which are known to yield positive reactions with acid mucopolysaccharides, that is, PAS, alcian blue, metachromatic dyes and colloidal iron. As further ancillary evidence, the reactions of the corneal deposits to these histochemical procedures following sulfation, methylation, acetylation, and post-acetylation saponification were in accord with the reactivity of acidic polysaccharides. As bull testis hyaluronidase had no detectable effect on the PAS or iron-binding staining reactions, hyaluronic acid, chondroitin sulfate B, and chondroitin sulfate C probably did not constitute a significant component of the corneal deposits.

Although numerous workers have preferred to denominate the fixed connective tissue cells of the corneal substantia propria as corneal corpuscles, stromal cells or keratocytes, the term corneal fibroblast would appear preferable in view of their morphologic similarity to fibroblasts in other connective tissues. Furthermore, the stromal cells of the cornea have been shown to synthesize collagen. The striking centrifugal diminution in the amount of storage material within the corneal tissues and the absence of similar deposits in cutaneous fibroblasts therefore lends additional support to the concept that fibroblasts are not all metabolically alike. Although fibroblasts in various connective tissues are similar in structure, they exhibit apparent functional differences due to intrinsic or extrinsic factors and may form acid mucopolysac-
charides that differ notably quantitatively and qualitatively. It should be emphasized, however, that the precise extent and degree of the fibroblast storage phenomenon in macular corneal dystrophy remains in doubt pending detailed histologic, histochemical and biochemical studies at necropsy examination of affected patients.

**SUMMARY**

Macular corneal dystrophy has clearly manifested its genetic origin but has successfully concealed the nature and genesis of its lesion. Information concerning these was sought by the present investigation of 9 cases, employing light and electron microscopy, histochemistry and biochemical analytic procedures.

The lesions were regularly characterized by the presence of small and large vacuoles within the cytoplasm of corneal fibroblasts. Stored material, by its positive staining with PAS, alcian blue, colloidal iron, and metachromasic dyes was tentatively identified as an acid mucopolysaccharide. Electron microscopy showed the earliest intracellular accumulations to occur within the cisternae of the endoplasmic reticulum and larger accumulations formed confluent vacuoles that distended and distorted the cytoplasmic compartment. Small droplets and large lacunas with similar histochemical staining characteristics were present throughout the corneal stroma. Although the collagenous fibers were displaced by these accumulations, their fine structure was regularly well preserved.

The cutaneous fibroblasts of a patient with macular corneal dystrophy were not structurally altered and the urine did not contain an excess of acid mucopolysaccharide. Together these findings provide strong evidence that macular corneal dystrophy represents a further example of a genetically determined metabolic disorder manifestly restricted to the corneal fibroblasts and characterized by the intracellular storage and ostensibly by extracellular migration and accumulation of excessive quantities of acid mucopolysaccharide.

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**Legends for Figures**

**Fig. 1.** Appearance of formalin-fixed, paraffin-embedded section of cornea in macular corneal dystrophy stained by Mowry's modification of Hale's colloidal iron method. × 128.

**Fig. 2.** Deposits of colloidal iron-positive material appear in the vicinity of corneal fibroblasts. × 678.

**Fig. 3.** Prominent accumulations of colloidal iron-positive material are evident in the superficial portion of the cornea adjacent to Bowman's membrane. Macular corneal dystrophy. Stain as in Figures 1 and 2. × 678.
FIG. 4. Montage of tissues in macular corneal dystrophy. Fibroblasts are interspersed in normal numbers between the lamellar layers of collagen. They are greatly distended and distorted by the presence of vacuoles that honeycomb their cytoplasm. Lacunas often border the fibroblasts and small electron-lucent vesicles are present throughout the collagen. × 1,800.

FIG. 5. The normal corneal fibroblast is elongated, with scant cytoplasm, sparse cytoplasmic organelles and few cytoplasmic vesicles. × 9,600.
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Fig. 6. A corneal fibroblast in macular corneal dystrophy is conspicuously altered by large and small solitary and confluent cytoplasmic vacuoles. The nucleus is normal in appearance. Numerous discrete spheroid vesicles are scattered haphazardly in the collagen. \( \times 7,000 \).

Fig. 7. A fibroblast, less severely involved, shows a general distention of the endoplasmic cisternae which retain their peripherally arranged ribonuclear protein granules. \( \times 14,000 \).
Fig. 8. Similar distention of endoplasmic cisternae is accompanied by more irregular and often confluent cytoplasmic vacuoles. Small vacuoles lie within the surrounding collagen. \( \times 14,000 \).

Fig. 9. A higher magnification of the area shown in Figure 6 exhibits a complex pattern of distended endoplasmic cisternae and a predominance of irregular large vesicles honeycombing the entire cytoplasm. \( \times 21,000 \).
Fig. 10. Lakes of electron-lucent material greatly predominate in the cytoplasm of more severely involved cells. Smaller vesicles are present in the contiguous cytoplasm. × 19,000.

Fig. 11. An electron-lucent vacuole contains an irregular osmiophilic mass and is compressed between collagen fibers. The collagen is well preserved and shows normal cross-striational periodicity. × 64,000.
FIG. 12. An aggregate of solitary and confluent vesicles displaces the collagen but is not associated with evident collagenous degeneration. × 49,000.

FIG. 13. The cytoplasmic vesicles within corneal fibroblasts manifest a strong affinity for colloidal iron in conformity with this staining reaction by light microscopy. × 14,000.