Communications

Amyloidosis of the cornea

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The observed incidence of ocular amyloid is to some extent a function of the diligence with which it is sought, recent reports having shown that it is by no means so rare as was once believed. In the last decade there have been several reports of conjunctival amyloidosis, associated in most instances with trichomatous pannus formation (Mathur and Mathur, 1959; Madangopal, 1962; Richlin and Kuwabara, 1962; Norn, 1964; Halasa, 1965; Stansbury, 1965; and Smith and Zimmerman, 1966). Vitreous opacities, perivascular deposition, and ocular nerve involvement are prominent in one category of systemic familial amyloidosis (Falls, Jackson, Carey, Rukavina, and Block, 1955; Kaufman, 1958; Kaufman and Thomas, 1959; and Paton and Duke, 1966).

Involvement of the limbus in predominantly conjunctival lesions has been reported by Coats (1915) and Renard, Dhermy, and Nguyen Van Ba (1965), but descriptions of amyloidosis in the cornea proper have until recently been exceptionally rare. The first case appears to have been that of Lewkojewski (1930) in which amyloid was deposited immediately beneath the epithelium, in the absence of any obvious predisposing cause, in an 8-year-old boy. No further cases were reported until Stafford and Fine (1966) described an 11-year-old girl with similar corneal deposits in an eye which showed several long-term complications of retrolental fibroplasia. Shortly afterwards McPherson, Kiffney, and Freed (1966), as a result of a retrospective study of 200 eyes, reported six more cases, all of which occurred in conjunction with chronic ocular disease of various types, and in one of which there was evidence of stromal deposition. More recently Collyer (1968) has described an example of corneal amyloidosis in an eye which had sustained a penetrating limbal wound some 41 years earlier. Another example of stromal amyloidosis, linked in this instance with hypergammaglobulinaemia, was presented by König and Pur (1966). In addition it has been shown by Seitelberger and Nemetz (1961) and Klintworth (1967) that the genetically determined lattice dystrophy of the corneal stroma is almost certainly yet a further type of familial amyloidosis.

Two instances of corneal disease due to subepithelial amyloid deposition have recently been seen at the Institute of Ophthalmology and a retrospective study of eleven cases originally diagnosed as nodular corneal dystrophy (including Salzmann's dystrophy) has revealed a further three. An analysis of these five cases is the purpose of this report.

Material and methods

Of the five cases to be described, one was an enucleation specimen, two were full-thickness corneal discs, and two were partial-thickness discs removed during keratoplasty. The tissues were fixed in formalin and, in the case of the corneal discs, embedded in paraffin wax and sectioned at a thickness

of 5 μ . The whole eye specimen was sectioned at 14 μ after embedding in celloidin after the method described by Ashton (1967).

The following histological staining techniques were employed:

Haematoxylin and eosin; congo red combined with examination in polarized light for birefringence and dichroism; methyl violet for metachromasia; thioflavine T on paraffin sections as described by Hobbs and Morgan (1963) for fluorescence microscopy (using an HBO 200 mercury vapour ultra-violet source and BG 12 (exciter) and Zeiss 53 and 44 (barrier) filters); Van Gieson; Masson trichrome; Wilder's silver stain for reticulin; periodic acid-Schiff; Hale's colloidal iron technique; toluidine blue.

Electron microscopy The celloidin-embedded whole eye preparation was taken back to absolute alcohol and selected portions of the cornea were post-fixed in osmium tetroxide. Thin sections were cut using a Huxley microtome and stained with uranyl acetate followed by lead citrate and then viewed with an AEI-EM6 electron microscope.

Results

Case 1

A woman aged 52 years presented with severe pain of 3 weeks' duration in an eye which had been blind for at least 15 years. There had been intermittent episodes of pain throughout this period and she gave a history of injury to the eye in childhood. Enucleation, which had been advised previously but refused by the patient, was considered imperative on this occasion. The contralateral eye was apparently healthy and there was no family history of ocular disease.

Histopathology The cornea, which macroscopically was opaque and ulcerated in the centre, showed widespread deposits of amorphous eosinophilic material between the epithelium and Bowman's membrane. The epithelium was largely atrophic, although in places it had proliferated down into the amorphous material, and the central ulcerated zone was accompanied by mild chronic inflammatory cell infiltration and scarring of the superficial stromal lamellae. While chiefly superficial to Bowman's membrane, there was also a little deposition of eosinophilic material in the superficial stroma but no abnormality was seen in the deeper layers. This material was subsequently shown to have the staining properties of amyloid (Figs 1 and 2, opposite). Descemet's membrane was moderately thickened and lamellated with a paracentral spur, considered to be related to the initial injury in childhood, projecting into the anterior chamber. No abnormality could be detected in the remaining ocular structures.

Electron microscopy Because the tissue was not fixed in conventional fixative for electron microscopy there was considerable cellular artefact present. Fortunately, however, amyloid appears to tolerate formalin fixation without significant distortion (Cohen and Calkins, 1959) so that comparison of individual fibril measurements in this specimen with those reported by other workers was considered to be justified. The material identified histologically as amyloid was composed of an haphazard feltwork of interlacing fibrils 80–100 Å in diameter many of which showed a beading effect at 40–70 Å intervals (Fig. 3, see p. 75). Moreover, there was occasionally a suggestion of a double fibril such as has been described by Boéré, Ruinen, and Scholten (1965) in both primary and secondary forms of amyloidosis. Since the material examined meets all the ultrastructural criteria of amyloid there can be no reasonable doubt as to the nature of the fibrils in this instance.

Tonofilaments in the basal epithelial cells showed some resemblance to the extra cellular amyloid fibrils but, as there was quite marked fixation artefact, precise measurement of these structures was impracticable.

Case 2

A 37-year-old man gave a history of a football injury to his left eye 14 years previously followed by progressive loss of vision. Clinical examination revealed a deep corneal opacity and prominent endothelial folds which were thought to indicate a rupture of Descemet's membrane. A lamellar keratoplasty was performed.

FIG. 1 Case 1. Section of cornea, showing subendothelial accumulation of congophilic material. Congo red and haematoxylin. $\times 48$

FIG. 2 Case 1. Identical field to that shown in the previous figure viewed between crossed polarizing screens to demonstrate birefringence and green dichroism of the subendothelial deposits. Congo red and haematoxylin. $\times 48$

FIG. 4 Case 3. Lamellar disc of cornea showing amyloid deposition between strands of proliferated squamous cell epithelium. Congo red and haematoxylin. $\times 120$

FIG. 3 Case 1. Electron micrograph, showing the fibrillar structure of the amyloid material. $\times 48,000$

Insert: Higher magnification shows beading effect on individual fibrils and occasional suggestion of a double fibril (arrow). $\times 150,000$

Histopathology The partial-thickness corneal disc showed marked atrophy of the epithelium with oedematous swelling of most of the basal cell layer. Immediately beneath the epithelium and chiefly superficial to Bowman's membrane was a wide layer of amorphous eosinophilic material which stained positively for amyloid. These deposits which extended across the full diameter of the disc were almost completely acellular apart from a very few lymphocytes and distorted spindle cells at the periphery. Parts of Bowman's membrane showed a little spotty calcification but no abnormality was seen in the superficial stroma.

Case 3

A 74-year-old man presented with a unilateral corneal opacity and ingrowing eye lashes which had been a source of irritation for many years. The cornea on the other side was apparently healthy despite a similar degree of trichiasis and there was no family history of ocular disease. A lamellar graft operation was carried out.

Histopathology The corneal epithelium, which showed excessive prickle cell development and a slight tendency to keratinization of the surface layers, had proliferated down into the underlying tissue to form an irregular network of branching epithelial strands. The spaces between the epithelial strands were filled by an homogeneous eosinophilic material which stained positively for amyloid (Fig. 4, see col. pl.). Lying within this matrix were scanty distorted and pyknotic spindle cells. Bowman's membrane had been totally replaced and on the deep surface of the specimen there was a thin layer of collagenous stroma infiltrated by small numbers of plasma cells and lymphocytes. There was no evidence of neovascularization.

Case 4

A 42-year-old woman first developed defective vision at the age of 24 years. She showed bilateral corneal opacities most severe on the right side and a full-thickness keratoplasty was performed on this eye. There was a history of visual impairment due to corneal disease in other members of the patient's family showing a dominant genetic transmission pattern (see Family tree).

Histopathology The corneal epithelium was irregular and many of the basal cells were oedematous. Between the epithelium and Bowman's membrane was a plaque of dense intensely eosinophilic hyalinized fibrous tissue and it seemed likely that this had been the chief cause of the patient's disability. In addition there were within the substantia propria many tiny foci of amyloid-staining material apparently formed in intimate association with the collagen fibres, although silver staining showed that such areas also contained branching argyrophilic fibres. The amyloid lesions were present at both deep and superficial levels and were generally ill-defined (Fig. 5). At one edge of the specimen Bowman's membrane and the superficial stromal lamellae showed spotty calcification, the former structure being partially deficient in this region. No obvious abnormality was seen in Descemet's membrane.

FIG. 5 Case 4. Cornea viewed in ultra-violet light shows strongly fluorescent amyloid deposits in the substantia propria. Thioflavine T. $\times 540$

Case 5

A 19-year-old girl presented with bilateral corneal opacities, which were diagnosed clinically as Salzmann's nodular dystrophy, and bilateral cataracts. She first developed corneal lesions at the age of 7 years, and lens changes appeared 2 years later. She had been a premature baby and was microcephalic with a slightly subnormal intelligence. There was no family history of eye disease. A full-thickness keratoplasty was performed on the right eye, this being the one most severely affected.

Histopathology The corneal epithelium was markedly atrophic, being reduced in places to the thickness of a single cell. Bowman's membrane was overgrown throughout almost the whole of the specimen by dense pannus and in the axial portion of the disc there was some hyalinization of the substantia propria. Near to the periphery on one side there was a little focal chronic inflammatory cell infiltration of the stroma associated with a minor degree of neovascularization, while some of the collagen fibres in this region showed a patchy increased eosinophilia. Such foci gave a positive staining reaction for amyloid (Figs 6 and 7).

FIG. 6 Case 5. Amyloid formed in the superficial stroma at the periphery of the cornea. Congo red and haematoxylin. $\times 270$

FIG. 7 Case 5. Similar field viewed in ultra-violet light shows multiple fluorescent deposits. Thioflavine T. $\times 640$

Details of the various staining reactions in these cases are given in the Table.

Table *Histological staining reactions of the corneal deposits*

Case no.	Age (yrs)	Sex	Congo red	Dichroism	Birefringence	Methyl violet metachromasia	Thioflavine T fluorescence	Van Gieson	Masson	PAS	Colloidal iron	Toluidine blue metachromasia	Wilder silver impregnation
1	52	F	+	+	+	—	+	Khaki	Blue	±	—	—	+
2	37	M	+	+	+	+	+	Yellow	Blue	+	—	—	+
3	74	M	+	+	+	—	+	Pink	Blue	±	+	—	+
4	42	F	±	+	+	+	+	Pink	Red	—	—	—	+
5	19	F	+	+	+	+	+	Pink	Blue	±	—	—	—

Discussion

Despite the fact that none of the histological staining reactions currently used in the recognition of amyloid is absolutely specific, there can be little cause to doubt the validity of the diagnoses in the cases under review, particularly in the instance where the diagnosis was confirmed electromicroscopically. Thus, while positive staining with Congo red is also shared by eosinophil granules, enterochromaffin granules, and elastic tissue (Pearse, 1960), such substances present no diagnostic problem in the cornea. Moreover the green dichroism of Congo red stained amyloid when viewed between crossed polarizing screens is regarded by Cohen (1967) as the single most useful diagnostic procedure, while Missmahl (1957) claimed that it is specific. Metachromasia with methyl violet is a reliable indicator when present but it is some times negative in established cases of amyloidosis especially those of the primary type (Symmers, 1956). Fluorescence after thioflavine T staining is a sensitive method for demonstrating amyloid and, though it has recently been shown that several other tissue components also react (Lehner, 1965; McKinney and Grubb, 1965), the technique is useful when combined with other methods. The Van Gieson staining reaction often imparts a khaki colour to amyloid (Symmers, 1956) but its chief value in the present context was to exclude hyaline material of collagenous origin.

As others have found, staining reactions for mucopolysaccharide substances were inconstant. The PAS reaction was positive to a variable degree in four of the five cases which accords with the findings of Cohen (1966) that purified amyloid fibrils contain 4.6 per cent. glycoprotein. The colloidal iron and toluidine blue staining reactions were even more capricious but, since according to Adams (1967) the acid mucopolysaccharide component of amyloid is largely a reflection of the ground substance in which the fibrils are embedded, these findings do not materially affect the reliability of the diagnosis in these cases.

As has already been observed, very few cases of corneal amyloidosis have been reported but that this is probably not representative of the true incidence is indicated by the studies of McPherson and others (1966), who found amyloid in 3 per cent. of an unselected group of 200 retrospectively studied cases. Recently non-systemic deposits of amyloid were encountered with age such that it was 25.4 per cent. in people over the age of 50 (Ravid, Gafni, Sohar, and Missmahl, 1967). These authors also found that it tended to occur at a younger age in those with some underlying lesion such as a fibrosed heart valve. They further commented that, whereas systemic amyloidosis is frequently associated with widespread vascular involvement, the focal non-systemic form is distributed predominantly outside the vessels in the pericapillary connective tissue. Though many organs were

shown to develop these deposits their studies did not include examination of the eye. It would seem entirely possible, though for different reasons, that Cases 1, 2, 3, and 5 of the present series might belong to this category in which amyloid deposition is apparently related to connective tissue senescence. Thus, in Cases 1 and 2, there was clinical evidence of previous trauma, with some supporting histological evidence of this in the first and, while there was no scarring apparent at the time the corneae were removed, this does not preclude the possibility of earlier pannus formation which had subsequently been replaced by amyloid. Case 3 was an elderly subject and this conceivably was of greater significance that the clinical history of trichiasis. Inclusion of Case 5 in this group is probably justified on histological grounds, since amyloid deposits formed in the connective tissue surrounding peripheral capillary blood vessels and in association with a subepithelial fibrous plaque.

The patient with stromal amyloidosis and a familial background of corneal disease (Case 4) presents all the clinical, genetic, and pathological features of lattice dystrophy. The lesions in the cornea showed the morphological and histochemical staining features shown by Jones and Zimmerman (1961) to be characteristic of this disease, while Klintworth (1967), in an extremely thorough study of three such cases, showed that lattice dystrophy is almost certainly a localized form of heredo-familial amyloidosis.

There is another form of hereditary amyloidosis which affects the eye: this has a systemic distribution and is associated with peripheral nerve involvement (Falls and others, 1955; Kaufman, 1958). Transmitted by a dominant gene as in familial corneal amyloidosis, the predominant ocular manifestations are perivascular deposits in the retina and choroid with vitreous opacities, but corneal involvement has not been reported and it would seem therefore to be a quite separate entity.

A principal source of amyloid in the systemic forms is cells of the reticulo-endothelial system (Teilum, 1966) but, except possibly in the one patient (Case 5) who showed mild plasma cell infiltration in the vicinity of the deposits, the source of the amyloid in these corneal lesions is obscure. In Case 3 there was prominent epithelial cell hyperplasia and Stafford and Fine (1966), discussing a very similar case, drew attention to the morphological similarities between amyloid fibrils and the tonofilaments of the squamous epithelium. But in their case, as in the case examined electron microscopically in the present series, precise comparisons could not be made because of fixation artefacts. There is, however, no good reason to suppose that such epithelial structures should be the precursors of amyloid, it being generally believed that they are the forerunners of keratin (Robertis, Nowinski, and Saez, 1960; Pedler, 1962). Epithelial cell hyperplasia in such corneas is more likely a non-specific secondary response.

McPherson and others (1966) observed that the subepithelial location of the amyloid material in most of their cases was suggestive of origin from previous pannus. A similar distribution was also observed in three of the present cases. There is no evidence, however, that amyloid is ever derived from degenerate fibrous tissue, the amyloid fibril and collagen differing widely in both their ultrastructure and their amino-acid composition (Cohen, 1966; Adams, 1967).

If the association with fibrous tissue is meaningful, and in an avascular structure like the cornea it is tempting to believe that it might be, it is more likely to be related to altered metabolic activity in the fibrocytes (or keratocytes in the case of stromal lesions). While the idea that fibrocytes might in certain circumstances be stimulated to form amyloid protein is not new (Warren, 1930) as yet there is no direct evidence for it. It is noteworthy, however, that Klintworth (1967) found that some of the stromal fibroblasts in cases of lattice dystrophy showed evidence of synthetic activity and were surrounded by an electron

dense material which he considered might represent a component of amyloid. Moreover, the current belief that reticulo-endothelial cells, which are a known source of amyloid, and fibroblasts are phylogenetically closely related (Curran, 1964; Symmers, 1966) suggests that the hypothesis is not inherently unreasonable.

Summary

An investigation of thirteen cases presenting with a nodular form of corneal dystrophy revealed five instances of local amyloidosis. In three the amyloid was deposited directly beneath the surface epithelium and in two of these the condition was preceded by trauma. In one amyloid was formed in relation to a chronically inflamed and vascularized focus in the superficial stroma, while the fifth case presented all the features of lattice dystrophy.

The diagnosis, made on the basis of histological staining reactions, was confirmed ultra-structurally in one instance.

Apart from the case of heredofamilial corneal amyloidosis (lattice dystrophy) there seemed some reason to include these cases with the recently defined group of non-systemic amyloidosis in which senescence and previous local pathology appear to play a major role.

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