Corneal dystrophy in Cockayne's syndrome

J. D. BRODRICK AND A. J. DARK

Sheffield

Cockayne (1936) described two progeria-like dwarfs who were brother and sister. Both had defective vision associated with atypical pigmentary degeneration of the retina and optic atrophy presumably of consecutive type. Their general development had been somewhat retarded from early childhood, so that by the age of 6 years cachectic dwarfism together with mental retardation had become manifest. It was also evident that they were deaf to some degree. Progressive atrophy of the subcutaneous and orbital fat and a scaly erythematous dermatitis, the latter aggravated by exposure to wind and sunlight, had contributed to an appearance of premature senility. No biochemical abnormalities were detected. Both patients showed maxillary prognathism and x rays revealed thickening of the skull bones and unusually small pituitary fossae.

Cockayne (1946) reviewed these patients 10 years later and found evidence of even further mental and physical deterioration. The girl was blind with occluded pupils filled with lens remnants, the result of bilateral dissection operations. The boy had a visual acuity estimated to be between 3/60 and 6/60. This was due mainly to consecutive optic atrophy although lens opacities may have contributed to his visual impairment.

Cockayne's syndrome thus includes dwarfism with prognathism, mental deficiency, deafness, pigmentary degeneration of the retina with consecutive optic atrophy, cataracts, thickening of the skull bones, and small pituitary fossa.

The syndrome has been described in a further 27 instances, subsequent authors contributing a wide variety of additional features with variable incidence. These include characteristic nephropathy leading to hypertension as well as hepatosplenomegaly, progressive upper motor neurone and cerebellar dysfunction, kyphosis, joint contractures, disproportionately large extremities, and intracranial calcification in the region of the basal ganglia, lateral ventricles, and frontal lobes (Neill and Dingwall, 1950; McDonald, Fitch, and Lewis, 1960; Paddison, Moossy, Derbes, and Kloepper, 1963; Windmiller, Whalley, and Fink, 1963; Spark, 1965; Wilkins, 1965; Ohno and Hirooka, 1966; Rowlatt, 1969; Coles, 1970; Moosa and Dubowitz, 1970). Defective tear secretion and absence of sweating have frequently been noted and a demyelinating peripheral neuropathy has recently been described (Moosa and Dubowitz, 1970). A comprehensive review of the pathological changes can be obtained by referring to the paper by Rowlatt (1969) in which detailed necropsy findings are described.

The ophthalmoscopic appearances have been those of fine, granular pigment spots scattered more or less evenly over the whole fundus. No tendency to bone corpuscular formation or perivascular pigment sheathing has been noted, but the retinal arteries have invariably been constricted and the optic discs consecutively atrophic. The foveal reflex has frequently been reported absent. Other occasional ocular findings include lens opacities and nystagmus. Poor dilatation of the pupils with a variety of mydriatics has been a consistent observation.

Coles (1970) recently described the presence of somewhat unusual corneal opacities associated with photophobia in two brothers with Cockayne's syndrome. Although he gave a clinical description, histological studies were lacking, but it seemed probable to him that the corneal lesions might be yet another manifestation of this disease, even though they had not been noted by any previous author. Corneal ulceration associated with photophobia was, however, mentioned in one of the cases described by Paddison and others (1963), but no details were given.

A child with Cockayne's syndrome and intense photophobia has recently been examined and found to have corneal opacities similar, if not identical, to those described by Coles (1970). In view of the unknown aetiology of the disease, it was naturally of interest to study the microscopic changes in the cornea in the hope that they might throw additional light on the underlying pathology.

Case report

A boy born in 1963 was examined at the age of 7 years. He was born at full term, after a normal pregnancy and normal delivery, and with a normal birth weight of 2.8 kg. There was no relevant family history, and no consanguinity. Failure to thrive was noted between 7 and 12 months. His weight at 1 year was 7.7 kg. (normal 10.3 kg.).

Physical and mental development in early childhood was normal but subsequent progress was grossly retarded. He could sit at 14 months but has never learned to walk without assistance. Progressive kyphosis was first noted at 1½ years. The joints became stiff with limitation of movements. Dryness of skin had been noted since birth with erythema and vesiculation on exposure to even mild sunlight (photodermatitis). Sweating and tearing have not been noted. Two major convulsions occurred at 2 and 3 years of age.

Ocular examination

In 1967 he had fine nystagmus, poor fixation, hypermetropic astigmatism, and a delicate pigmentary stippling of both fundi. Lid closure was normal but pupillary dilatation with a variety of mydriatics was poor.

In 1969 iris stromal hypoplasia was observed, but there was no other anterior segment pathology. The pigmentary deposits in the fundi were then noted to predominate in the mid and far periphery. There was no bone corpuscular formation or perivascular pigmentary sheathing, and the vessels, discs, and maculae were said to be normal.

In September, 1969, a protracted period of photophobia and redness, involving both eyes, commenced. This persisted in spite of local treatment with a variety of antibiotics and steroids up to the date of our examination in January, 1971.

Examination January, 1971

GENERAL

The appearance was that of a wrinkled little old man with a bird-like nose and deeply sunken eyes (Fig. 1). The skin was dry and there was atrophy of the subcutaneous fat. The weight and height were grossly abnormal (weight 9.0 kg., normal 23.8 kg.; height 88.75 cm., normal 122 cm.). He was pigeon-chested and had kyphosis. There was marked limitation of movement of elbows, hips, and knees; the fingers and toes were short with partial syndactyly of the second and third toes. There was no cardiovascular anomaly. Tendon reflexes were sluggish and the plantar response withdrawal. The testes were undescended. Careful audiometric study revealed no defect. Mental development was assessed as that of a 2-year-old child (Stanford Binet).

A detailed study on this patient was carried out by Moosa and Dubowitz (1970) and we owe the following data to them:

FIG. 1 Photograph of patient at age 7 with normal control (reproduced by courtesy of Dr. V. Dubowitz)

Blood: no abnormalities.

Urine: mucopolysaccharide screening test normal.

Radiology: calcification observed in the region of the walls of the lateral ventricles and the posterior fossa. There was an overall increase in density of the skull bones; the pituitary fossa appeared normal.

Electroencephalogram: no definite abnormality seen although the record was low voltage.

Nerve biopsy: active and regenerating segmental demyelination.

Muscle biopsy: overall decrease in fibre diameter with retention of normal differentiation into the two histochemical types.

Electromyogram: normal.

Nerve conduction velocities: significantly reduced in the ulnar nerve and posterior tibial nerve.

Chromosome analysis (leucocytes): normal.

Moosa and Dubowitz (1970) concluded that these observations were evidence of a demyelinating peripheral neuropathy.

OCULAR EXAMINATION

Schirmer's test without local anaesthetic showed wetting beyond 10 mm. in 5 minutes. Under light general anaesthesia, an attempt was made to estimate the corneal sensation by eliciting a blink reflex with 4-0 nylon threads (Zander and Weddell, 1951 a, b). The validity of the test under these circumstances is questionable because of the difficulty in controlling the depth of anaesthesia, but even so there did appear to be a marked difference in response between the left cornea which produced a normal blink reflex and the right cornea in which the reflex was grossly diminished.

Naked eye appearance

Both eyes were white. The transverse corneal diameter of each eye was 11 mm. The lower three-fifths of the right cornea was semi-opaque and no anterior chamber detail could be made out through it.

A juxta-limbal opacity was present in the infero-nasal quadrant of the left cornea, and transillumination of this eye revealed a moderate but definite defect in the pigment epithelium of the iris.

Biomicroscopy (Fig. 2)

RIGHT EYE

The superficial corneal opacity measured 10×5 mm., extended to the limbus, and reached just above the mid-pupil. It was slightly elevated and the overall appearance was that of an aggregate

FIG. 2 Drawing of corneal opacities, naked eye appearance, and optical section of cornea. $\times 10$

of whitish granules. Superficial vessels ran in from the limbus for a distance of about 5 mm. No specific stain with fluorescein or rose bengal was noted. Examination of all but the upper two-fifths of the anterior chamber was precluded by the corneal opacity. The area that could be examined appeared normal apart from iris stromal hypoplasia and defective pigment epithelium.

LEFT EYE

The white epithelial opacity in the lower nasal quadrant measured 4×3 mm., the inner border being irregularly scalloped. The opacity showed some roughening but was not elevated. There was superficial vascularization. The anterior chamber was quiet and normal in depth. The lower half of the iris was hypoplastic, and transillumination showed pigment epithelial defect in this region. The pigment ruff was absent. The lens was clear.

Fundoscopy

No view was obtained of the right fundus. The left fundus showed distinct pallor of the optic disc which was surrounded by an atrophic halo. There was regular attenuation of the arteriolar tree, the venules being normal in size, and a glial sheath round the inferior temporal vein extended to the mid-periphery. There was fine pigmentary stippling of the posterior pole, increasing in density in the mid and far periphery. A foveal reflex was present. No bone corpuscular formation or perivascular pigmentary sheathing was noted.

Electroretinogram

The electroretinogram showed a generalized low voltage response, a maximum photopic spike of 25 μ v being obtained. The flicker electroretinogram was absent. A keratectomy biopsy was taken from the right cornea.

Microscopy of keratectomy specimen

Material and methods

Four pieces of greyish tissue $2 \times 2 \times 1$ mm. were obtained. Two pieces were fixed in calcium formol, embedded in paraffin, and serial sectioned.

The other two fragments were fixed in osmified glutaraldehyde and embedded in Araldite.

The paraffin sections were stained with routine methods including toluidine blue, periodic acid-Schiff, Alcian blue, methyl violet, and Sudan black. The araldite-embedded material was cut both vertically and tangentially, and sections appropriate for both light and electron microscopy were obtained. The thicker sections were stained with toluidine blue and examined by light microscopy.

(1) LIGHT MICROSCOPY

Considerable epithelial dysplasia is evident in the deeper layers of the prickly cells which are acanthotic, nine layers replacing the normal four to five. The epithelial cells show moderate pleomorphism, especially the basal cells, which are rounded up and have lost their normal columnar palisade arrangement (Fig. 3). Bowman's layer is replaced by moderately basophilic amorphous masses which are approximately oblong in shape and are orientated vertically so that in places a regular filiform appearance is observed. A variable deposit of blue-black granules is present immediately adjacent to this layer and embedded in the underlying stroma (Fig. 4). These granules give a positive von Kossa reaction which is eliminated by pre-treatment with dilute mineral acid and are therefore presumably calcium. They show no affinity for periodic acid-Schiff, Alcian blue, methyl violet, or Sudan black.

FIG. 3 *Thin section, showing marked epithelial dysplasia with acanthosis and pleomorphism of the basal cells. Blocks of dense amorphous tissue replace Bowman's layer (arrowed) and deep to this are the dark granules (von Kossa). Toluidine blue. $\times 420$*

FIG. 4 *Higher power view of Bowman's region stained as in Fig. 3. $\times 1,275$*

Tangential sections complement the above findings with one exception. A lightly-stained, filigree-like reticulum can be seen to separate the amorphous blocks in Bowman's layer. It is less lightly stained than the blocks and could possibly be the remnant of the original Bowman's layer (Fig. 5).

FIG. 5 Tangential section at the level of Bowman's stromal interface, showing filigree-like reticulum surrounding amorphous blocks. Toluidine blue.

× 1,275

Most sections incorporate a few stromal lamellae attached to the epithelium. These look normal with no evidence of neovascularization, inflammation, or other pathological processes, and the few keratocytes that are observed appear to be normal.

FIG. 6a Electron micrograph, showing almost complete absence of the basement membrane—hemidesmosome system. Bowman's layer (BL) is replaced by blocks of amorphous tissue (A). Adjacent to this is the von Kossa-positive material, seen in this picture as black granules (B). Lead citrate. × 18,500

E Basal layer of epithelium s Stroma

FIG. 6b Electron micrograph, showing remnant of basement membrane (arrowed) separated from amorphous blocks (A) by fluid filled with debris (B). Lead citrate. $\times 18,500$

FIG. 7 Electron micrograph, showing amorphous blocks in Bowman's region. They are composed of fibrillar material which is densely felted at the periphery, and more loosely centrally. The von Kossa-positive granules appear as electron dense, crystal-like structures. Lead citrate. $\times 27,500$

(2) ELECTRON MICROSCOPY

The main features described above are confirmed and somewhat amplified. The basement membrane—hemidesmosome system is for the most part absent; where present it is separated from the underlying amorphous masses (A) in Bowman's region by fluid filled with amorphous debris (B) (Fig. 6 a, b).

The amorphous block-like areas replacing Bowman's layer are seen to have a denser (darker) periphery and a lighter core at $\times 40,000$. The light central area is filled with fine fibrillae about 80 Å wide with occasional beading (in the underlying stromal collagen the corresponding measurement is 200 Å). Fewer fibrils are present in the denser outer rim which is composed of electron dense material beyond the resolution of our microscope. The von Kossa-positive deposits are seen as intensely electron dense crystal-like structures (Fig. 7).

Discussion

Clinical aspects

This child shows the characteristic features of Cockayne's syndrome, with typical physical appearance, cachectic dwarfism, mental retardation, photodermatitis, intracranial calcification, joint contractures, and peripheral neuropathy. The ophthalmoscopic and ocular findings are consistent with the diagnosis and the newly described corneal changes have been discussed in detail. The electroretinogram has been recorded and this showed a grossly subnormal response indicative of some extensive degenerative process affecting the retinal layers.

Cockayne's syndrome is currently being considered as a form of leucodystrophy (McDonald and others, 1960; Norman and Tingey, 1966; Ohno and Hirooka, 1966; Moossy, 1967; Rowlatt, 1969; Moosa and Dubowitz, 1970), a concept supported by the delayed onset of clinical features, the remorseless progression and ultimate pattern, and the necropsy findings of demyelination, patchy necrosis, and calcification in the subcortical white matter, brain stem, and cord.

Recurrent photophobia and redness were seen in at least five of the 29 recorded cases of Cockayne's syndrome. In four of these (8 eyes), including the present case, naked eye changes of the anterior corneal layers were observed (Paddison and others, 1963; Coles, 1970). It thus seems reasonable to include corneal dystrophy with recurrent epithelial erosions as a further manifestation of the syndrome, a concept supported by the histological changes described above.

In this microscopical study the most striking changes are found in the region of Bowman's layer, which has been replaced by irregular masses of vertically-orientated fibrillar material. This appearance, together with the almost total absence of the true basement membrane system, is reminiscent of the ultrastructural changes found in Reis-Bücklers' dystrophy (Rice, Ashton, Jay, and Blach, 1968). In both conditions it is difficult to establish whether one is observing the end-point of a degenerative process affecting Bowman's layer or fibrillogenesis replacing Bowman's layer which has been destroyed by recurrent ulceration or some other process.

Rice and others (1968), describing Reis-Bücklers' dystrophy, have noted that similar, if not indistinguishable, changes occur in a number of dystrophies which are clearly unrelated, *e.g.* the lattice, granular, and macular types. Moreover, such changes have been described in the regenerative stages after herpetic and traumatic ulceration (Rice and others, 1968).

It is not intended to imply that the corneal changes we have found in Cockayne's syndrome are identical to those found in Reis-Bücklers' dystrophy, for there are clearly

distinctive differences in topography and microscopic appearances. Thus the epithelium does not show much change in Reis-Bücklers' dystrophy apart from epitheliolysis of the basal cells and an unusual accumulation of 'dark' cells. This is in contrast to the marked dysplastic features found in our case and calcification of the superficial stroma is not a feature of Reis-Bücklers' dystrophy. In addition, the finger-like amorphous blocks in Bowman's layer are not found in Reis-Bücklers' dystrophy.

If indeed the changes observed here are those of fibrillogenesis rather than degeneration, it may be that the prime defect resides in the epithelial cells. It is possible that epitheliolysis results in a breakdown of lysosomes releasing collagenase or other cathepsins which cause dissolution of the basement membrane and Bowman's layer. An alternative suggestion is that the granular accumulations in Bowman's layer are indicative of a primary degenerative condition in this region which results in the changes described in the overlying basement membrane and epithelium. In either case the primary defect may be related to the underlying metabolic defect which is responsible for the general manifestations of the syndrome.

Summary

- (1) A patient with Cockayne's syndrome has been described and the general and ocular features have been discussed.
- (2) The electroretinogram has been recorded and the response was found to be grossly subnormal.
- (3) A detailed description has been given of the corneal opacities found in our patient and the light and electron microscopical and histochemical findings have been described.
- (4) It would appear that a characteristic corneal dystrophy is an inconstant feature of Cockayne's syndrome which may be due to either a primary degenerative change in Bowman's layer or to a primary defect in the epithelial cells.

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