OCULAR FINDINGS IN A CASE OF HEREDITARY OCHRONOSIS*

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THE literature apparently contains only four pathological examinations of the eye in hereditary ochronosis, and all of very recent date. The first two were carried out on biopsy material (Seitz, 1954; Rodenhäuser, 1957) and the other two on the whole eye (Rones, 1960; Allen, O'Malley, and Straatsma, 1961). An ochronotic eye enucleated because of a mistaken diagnosis of malignant melanoma was examined only macroscopically as the discolouration of the sclera was thought to be due to formalin fixation (Skinsnes, 1948). The present report deals with a further example of this condition, and comprises the first histological account of an ochronotic globe removed during the life of the patient.

Case History

A 60-year-old farm labourer complained of pain over his left eye for a period of 3 weeks, and was admitted to the Royal Victoria Eye and Ear Hospital, Dublin on April 27, 1962, under the care of one of us (F.S.L.). He was also suffering from increasing pain, stiffness, and deformity of the back, shoulders, hips, knees, and elbows. These general symptoms had started about 7 years previously, originally affecting the left knee, the other joints having been involved progressively over the intervening period. He believed his back had been somewhat stooped since boyhood. Disability was such that he walked slowly with the aid of a stick, and dressing was possible only with assistance. His gait was further impaired by a fracture of the neck of the femur sustained in 1960.

Over the past 3 to 4 years he had noticed some darkening and discolouration of his face and the appearance of small black nodules on his hands. Only in recent years had he become aware of any unusual appearance of his urine, and he maintained it was darker now than as a child. During the 3 months preceding admission he had developed breathlessness on exertion and some dependent oedema. There had been no exposure to phenol or hydroquinone. He had been in hospitals on two previous occasions; in 1940 with bronchitis and in 1960 for treatment of a fractured hip. There was no record of any comment on his urine at either time.

Family History.—The patient is unmarried and has only one sibling, a brother aged 59, also single, who is reported free from any evidence of ochronosis. The parents were not related and died at an advanced age; neither had any skin or urinary pigmentation, although both were arthritic, especially the father. There are numerous maternal cousins all living in America and not known to be affected. On the paternal side there are no cousins as the father had only one brother who died unmarried.

Physical Examination.—There was a severe dorsal kyphosis with loss of movement of spinal and hip joints. Knees and elbows were distorted in outline with pain, crepitus, and gross limitation of movement. There were no effusions and the hands, wrists, feet, and ankles were relatively normal. The face showed a fine punctate blackish-brown pigmentation mainly around the eyes, over the bridge of the nose, and on the upper lip. Mucous membranes were not involved. The external

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ears were thickened and, though of normal colour, showed marked loss of translucency, particularly in the upper parts. Sweat and cerumen were of normal colour as were teeth and nails. On the web between the left thumb and first finger were several black subcutaneous nodules a few millimeters in diameter and another was present on the palmar surface of the wrist.

There was moderate cardiac enlargement and normal heart sounds with no murmurs and regular rhythm. Rales were audible over both lungs, jugular venous pressure was raised, and there was oedema of both feet and ankles. The blood pressure was 180/90. An electrocardiogram showed left ventricular preponderance. Neurological examination showed no special features.

Urine.—This was brown on passing, darkening slightly on standing over 48 to 72 hours. On the addition of alkali the typical black discolouration appeared on the surface and ultimately spread throughout. Positive reactions for reducing substance were given on testing with Fehling's and Benedict's reagents, and also with "clinitest" tablets. Enzymatic testing for glucose was negative. A transient blue colour developed with the addition of ferric chloride, and exposed photographic film was promptly developed by a drop of alkalinized urine (Fishberg, 1942). Increased protein feeding resulted in the passage of considerably darker urine. Slight albuminuria was present, but no casts, cells, or organisms.

Radiological Examination.—The spine showed the typical appearances described by Pomeranz, Friedman, and Tunick (1941) and more recently by Simon and Zorab (1961), namely, dense calcification and narrowing of the intervertebral discs. There was also extensive osteophyte formation, tendon calcification, and loss of joint space in the knees, hips, shoulders, and elbows. Evidence of healed fracture of the femur was also seen. There was left ventricular enlargement, widening of the aorta, and some pulmonary congestion. Renal function tests showed no major impairment.

With rest and diuretics the signs of congestive cardiac failure cleared over a few weeks.

Ocular Findings.—The visual acuity was 6/6 in the right eye and nil in the left. Both eyes showed areas of brownish-black pigmentation in the sclera on the nasal and temporal sides of the cornea. Pigmentary spots were observed with the aid of the slit lamp in both corneae at the corneo-scleral margins in similar regions (Fig. 1).

FIG. 1.—Right eye, showing pigmentation in sclera on temporal side, and pigmentary spots at corneo-scleral margins.

RIGHT EYE: Apart from these pigmentary deposits, the only abnormality observed was nipping of the retinal veins at the arterio-venous crossings.

LEFT EYE: The bulbar conjunctiva was congested and ciliary injection was present. The cornea was hazy and oedematous. The pupil was irregular in shape because of posterior synechiae. The fundus could not be seen with the ophthalmoscope. Intra-ocular pressure (applanation tonometer) was 18 mm. Hg in the right eye and 78 mm. Hg in the left. Efforts to relieve the increased

intra-ocular pressure in the left eye by local therapy and Diamox were without avail. A radioactive phosphorus test showed an increased uptake in the left eye of 50 per cent. on the nasal side in the horizontal meridian.

As the general condition improved and the left eye was still blind and painful it was enucleated on June 13, 1962, and was sent to the Institute of Ophthalmology, London, for pathological investigation.

Pathological Findings

Macroscopic Examination.—Situated at 3 and 9 o'clock immediately posterior to the limbus and apparently extending backwards as far as the ora serrata there were two heavily pigmented brownish-black areas in the sclera. The congested conjunctiva was stripped away to show the scleral pigmentation more clearly, and pigment granules were then also seen at the corneo-scleral margins in the same regions (Fig. 2). The globe was opened horizontally and numerous haemorrhages were seen in the retina which was *in situ*; the optic disc was deeply cupped. To study the retinal vessels a portion of the retina was removed for trypsin digestion.

FIG. 2.—Enucleated left eye, showing heavy scleral pigmentation at 3 and 9 o'clock (the conjunctiva has been removed to demonstrate this more clearly). Note also discrete pigmentary spots at corneo-scleral margins.

Microscopic Examination.—The eye showed the pathological features of two distinct conditions.

(i) *Thrombotic Glaucoma.*—As is characteristic, there were dense vascular peripheral synechiae obstructing the filtration angles, retinal arteriolosclerosis, scattered retinal haemorrhages, and deep cupping of the optic disc. The digest preparation of retina showed numerous micro-aneurysms on the venous side of the capillary network, as frequently found in cases of retinal venous occlusion.

(ii) *Ocular Ochronosis.*—The pathological changes were confined to the sclera, episclera, and corneo-scleral junction. On both sides of the section, corresponding to 3 and 9 o'clock,

FIG. 3.—Horizontal section of whole globe showing extent of pigmentation at base of insertions of lateral recti. The optic disc is deeply cupped. Haematoxylin and eosin. $\times 3.75$

there was an intense golden-brown discolouration of equal intensity, sharply localized to the region of the pars plana at the base of the insertions of the lateral recti (Fig. 3). It was partly due to a fine granular deposit resembling melanin and partly to a diffuse staining of the scleral fibres. Pigmentation was most intense in the centre of these patches and gradually diminished at the periphery; it involved the full thickness of the sclera (Fig. 4A), but the adjacent and more anterior episclera showed a different type of change. Here the pigment was aggregated into irregular granulations varying in size from minute granules to large coalescent masses (Figs. 4B; 5). They were associated with parallel strands and clumps of lightly-pigmented swollen vermiform fibres, which stained positively for elastic tissue and were eosinophilic and fuchsinophilic (Table, overleaf, p. 410), thus closely resembling the characteristic changes of pingueculae (Figs. 4C; 6). Anteriorly there were a few structureless amber-coloured globules of varying size situated subepithelially in the

FIG. 4.—Higher-power view, showing four types of pigmentary change in affected areas.

- A—Diffuse staining of sclera with fine granular deposit;
- B—Irregular granular masses in episclera;
- C—Clumps of lightly pigmented swollen vermiform fibres;
- D—Structureless amber-coloured globules on outer layers of corneo-sclera.

Haematoxylin and eosin. $\times 52.5$.

FIG. 5.—High-power view of irregular granular masses in episclera, as shown in Fig. 4-B. Haematoxylin and eosin. $\times 110$.

FIG. 6.—High-power view of a clump of swollen vermiform fibres in episclera, as shown in Fig. 4-C. Safranin. $\times 440$.

FIG. 7.—High-power view of structureless amber-coloured globules of corneo-sclera, as shown in Fig. 4-D. Haematoxylin and eosin. $\times 110$.

TABLE
HISTOLOGICAL OBSERVATIONS

Stain	Site	Sections	
		Unbleached	Bleached
Haematoxylin and Eosin	Normal fibres Vermiform fibres Granular masses Pigmented sclera Corneal globules	Unstained Eosinophilic Obscured by pigment Obscured by pigment Obscured by pigment	Unstained Intensely eosinophilic Eosinophilic Faintly eosinophilic Eosinophilic
Periodic acid-Schiff	Normal fibres Vermiform fibres Granular masses Pigmented sclera Corneal globules	— — — — —	Negative Negative Negative Negative Negative
Periodic acid-Schiff and Haematoxylin	Normal fibres Vermiform fibres Granular masses Pigmented sclera Corneal globules	— — — — —	Negative Negative Negative Negative Negative
Orcein Elastic	Normal fibres Vermiform fibres Granular masses Pigmented sclera Corneal globules	Purple-brown Deep purple-brown Obscured by pigment Obscured by pigment Obscured by pigment	Purple-brown Deep purple-brown Light purple-brown Deep purple-brown Light purple-brown
Weigert Elastic	Normal fibres Vermiform fibres Granular masses Pigmented sclera Corneal globules	Purple Deep purple Obscured by pigment Obscured by pigment Obscured by pigment	Purple Deep purple Red Red Red
Verhoeff Elastic	Normal fibres Vermiform fibres Granular masses Pigmented sclera Corneal globules	Purple Purple-black Purple-black Obscured by pigment Purple-black	Purple Purple-black Purple-black Purple-black Purple-black
Phosphotungstic Acid Haematoxylin	Normal fibres Vermiform fibres Granular masses Pigmented sclera Corneal globules	Purple Purple-black Obscured by pigment Obscured by pigment Obscured by pigment	Purple Purple-black Purple-black Red Unstained
Toluidine Blue	Normal fibres Vermiform fibres Granular masses Pigmented sclera Corneal globules	Blue Blue Obscured by pigment Obscured by pigment Obscured by pigment	Blue Blue Dark blue Intense blue Dark blue
Safranin	Vermiform fibres Granular masses Corneal globules	Bright red Bright red Bright red	— — —

outer stromal layers of the corneo-sclera (Figs. 4D; 7). Posteriorly fine aggregations of granular pigment were lightly scattered throughout the sclera within and along the collagen bundles, particularly in the outer layers (Fig. 8, opposite). These diminished in concentration towards the disc region where only a few minute deposits were seen. The remainder

of the eye was entirely free from abnormal pigmentation. No intra-ocular, episcleral, or orbital vessels were involved.

FIG. 8.—Posterior sclera, showing scattered pigment granules within and along collagen bundles. Haematoxylin and eosin. $\times 440$.

The scleral pigment, together with the normal melanin in the retinal pigment layer and choroid, was completely removed by bleaching, and it could then be seen that the scleral tissue in the affected area was entirely acellular, in contrast to the adjacent sclera where the normal numbers of nuclei were present (Fig. 9*a, b*).

FIG. 9.—Bleached section, showing complete absence of nuclei in the area of sclera affected by ochronotic pigmentation (*a*) as compared with non-pigmented adjacent sclera which shows normal cellularity (*b*). Haematoxylin and eosin. $\times 150$.

In these preparations it could also be seen that the previously pigmented areas in the episclera, but not those in the sclera, were associated with an underlying hyaline degeneration (Fig. 10), of which the neighbouring lightly pigmented vermiform fibres were clearly a part. Nevertheless the latter fibres were a distinctly different type of change and were continuous with normal elastic fibres in the unaffected adjacent episclera, whereas the hyaline degeneration was more suggestive of a breakdown in collagen.

FIG. 10.—Bleached section, showing episcleral granular mass; depigmentation reveals underlying hyaline degeneration, which is orceinophilic. Orcein elastic stain. $\times 440$.

In summary, there were four pathological components in the ochronotic zone: *lightly pigmented swollen vermiform fibres* in the episclera of the affected area; *granular pigmented masses* lying immediately beneath; *deeply-pigmented sclera*; and *amber structureless globules* at the limbus. The details of their staining reactions, together with those of the normal elastic fibres in the sclera, before and after bleaching are shown in the Table.

Discussion

Alkaptonuria is a rare condition for, although it has been recognized for over 3 centuries and was accurately described 100 years ago, only 200 cases have been recorded, including a hundred with clinical ochronosis (Martin, Underdahl, Mathieson, and Pugh, 1955). The first case in Ireland was reported by Smith and Armstrong (1882), who quoted Stephenson as suggesting that the incidence of the disease in Northern Ireland is 3 to 4 per 1,000,000 of the population. Many others have reviewed the subject and of special value are the publications of Knox (1958) on the historical, genetic, and chemical aspects, Martin and others (1955) and Galdston, Steele, and Dobriner (1952) on the clinical features, and Lichtenstein and Kaplan (1954) on the pathological findings. The best review in the ophthalmic literature is that of Smith (1942), and valuable papers on the ocular findings have recently

been provided by Rones (1960) and Allen and others (1961). With so many able accounts of hereditary ochronosis in the literature, we need discuss here only the particular features of our own case in relation to previous reports.

From the clinical and radiological points of view, our case presented the classical picture of advanced ochronosis, although its first presentation at the age of 60 years is unusual, since the abnormality is characteristically present and detectable within 48 hours after birth (Garrod, 1902); Rose (1957), however, described a very similar case wherein the condition was first recognized in a woman aged 57 years. The ochronotic pigmentation of the sclera and corneal periphery were exactly as described in the first comprehensive ophthalmological publication by Sallmann (1926), who considered their symmetrical arrangement to be of diagnostic significance. The corneal involvement is particularly characteristic; indeed, according to Smith (1942) the presence in the superficial layers of the limbal tissue, on both the temporal and nasal side, of sharply-defined brown globules, which on retro-illumination resemble oil droplets on water, cannot be confused with any other slit-lamp appearance. Although in our case pigmentation was limited to the scleral and corneo-scleral regions, occasional involvement of the skin of the lid and of the conjunctiva has also been reported (Smith, 1942).

From the pathological point of view, the type and distribution of pigmentation in the sclera (most marked at the insertions of the lateral recti), the structureless amber globules in the corneo-sclera, the degenerate pigmented fibres in the episclera, the pigmented granular masses, and the absence of any associated inflammatory reaction are findings which closely correspond to those previously reported (Seitz, 1954; Rodenhäuser, 1957; Rones, 1960; Allen and others 1961). There appear to be three distinct processes at work, the pigment being deposited in oil-like globules at the limbus, as a fine granular deposit in the scleral collagen (which is also diffusely stained), and in a combined form in the episcleral fibres, which undergo swelling and convolution to produce the vermiform type of degeneration typically seen in pingueculae.

Whether these vermiform fibres are degenerate elastic tissue or not is difficult to determine. The fact that they are continuous with normal fibres and that both stain for elastic tissue (Table) would seem to favour a true degeneration of elastin, and this was assumed to be the case in the reports of Seitz (1954) and of Rones (1960). On the other hand, Gillman, Penn, Bronks, and Roux (1955) have shown that collagen fibres may undergo a form of degeneration in which the fibres assume the staining reactions of elastic tissue; this they called "elastotic degeneration". Unfortunately, it is not easy to distinguish the two types of degeneration as the tinctorial properties are so similar; moreover, it would seem possible that they could occur together. This may explain the results obtained in the Table, for while both orcein and Verhoeff's elastic tissue stains gave positive results for all the types of degeneration found in the ochronotic zone, differences were uncovered by the Weigert stain and phosphotungstic acid haematoxylin stain, which gave a purple reaction for normal and vermiform fibres, and a red reaction in the granular masses and areas of scleral pigmentation. It is impossible to be certain of the significance of these reactions at the present time, and they are recorded in detail here for future work, but we suppose that the vermiform fibres probably represent a true degeneration of elastic

tissue, whereas the granular masses and scleral changes were more in the nature of an "elastotic" degeneration of collagen. The nature of the globular structureless deposits is quite obscure.

Our findings that the pigment in the sclera was indistinguishable from melanin is also in agreement with other reports. It cannot, however, be chemically identical with melanin for the pigmentation of ochronosis is far from harmless and may give rise to destructive lesions in the affected tissues. In this way pigment deposition may lead to arteriosclerosis, aortic stenosis, nephrosis, and disabling arthritis. It was, therefore, particularly interesting in our case to find that, after the scleral pigment was removed by bleaching, the area involved was found to be entirely devoid of cells. This would appear to provide striking evidence of the toxic effects of ochronotic pigment and has not been previously reported. In view of these findings an inquiry was sent to Dr. B. Rones asking him to re-examine the bleached sections of the case he reported; he replied that he had also found the scleral zones of pigmentation to be completely acellular. Dr. R. Allen kindly loaned us the material from his reported case; here the injurious effect of ochronotic pigment was less striking, but in the main it was true that no surviving cells were to be found in the pigmented areas.

The reason for the peculiar distribution of pigment at certain selected sites is well discussed by Allen and others (1961). There seems to be no obvious relation between ochronotic pigmentation and the sites where melanin is normally present. Moreover, the pattern of deposition of melanin in pathological conditions bears little relation to that seen in ochronosis. Although the scleral distribution might suggest it, light is not known to influence ochronotic pigmentation, and the old view that ochronotic pigment is deposited in tissues with poor vascularity (Virchow, 1866) is only partially true, for, as pointed out by Allen and his co-workers (1961), avascular structures such as the cornea, lens, and vitreous are generally not involved. The pigment of ochronosis is, therefore, unique in its distribution and destructive effects, while its exact nature and the reason for its localization remain obscure.

Summary

The histopathological examination of an eye from a case of hereditary ochronosis is described. This is the fourth such account in the literature and the first examination of an ochronotic globe removed during the life of the patient.

The eye showed thrombotic glaucoma, which had led to enucleation, and ochronosis, characterized by intense golden-brown pigmentation in the anterior sclera in the horizontal plane at 3 and 9 o'clock, extending from the limbus to the region of the ora serrata. These areas showed swollen vermiform fibres in the episclera, granular masses lying immediately beneath, a deeply pigmented sclera, and amber structureless globules at the limbus.

The staining reactions of these various components are reported in detail and reasons are given for supposing that the vermiform fibres are probably degenerate elastic fibres, while the staining reactions of the granular masses and pigmented sclera may have been due to "elastotic degeneration" of collagen. The nature of the limbal globules is unknown.

Although the ochronotic pigment was indistinguishable from melanin, its peculiar destructive nature is emphasized, and is illustrated in this case by the new finding of total cellular destruction in the pigmented area of the sclera.

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