Kayser-Fleischer ring

A pathological study

J. HARRY AND R. TRIPATHI

Department of Pathology, Institute of Ophthalmology, University of London.

Although the brownish area at the corneal periphery in the region of Descemet's membrane was first described by Kayser (1902), and Fleischer (1912) recognized it as a part of Wilson's disease (hepato-lenticular degeneration), there have been no previous reports in the ophthalmic literature of the United Kingdom of a pathological study of the lesion which is now recognized as a pathognomonic finding in this disease.

Case Report

CLINICAL HISTORY

The patient, a young man who was the only child of unrelated parents was involved, at the age of 19, in a road accident in which he was knocked unconscious. On recovering consciousness he was found to be suffering from a loss of balance, together with deafness in the left ear; tremor of the head and hands developed later and this spread to involve the lower limbs. It was, however, several years before a diagnosis of Wilson's disease was made and treatment with British anti-Lewisite begun. There was an initial improvement, but this was not maintained, and when the dose was increased visual disturbance and muscular cramps developed. A course of penicillamine treatment was commenced and this resulted in considerable improvement. The treatment was, however, rather sporadic and after a few days the patient had the first of several haematemeses; these were shown to be associated with oesophageal varices, and a splenectomy and spleno-renal shunt was performed. His condition subsequently deteriorated dramatically and in spite of intensive treatment with penicillamine he lapsed into a comatose state in which he remained for a year before he finally died at the age of 34, some 15 years after the initial onset of symptoms.

OPHTHALMIC EXAMINATION

The cornea of each eye showed a broad complete Kayser-Fleischer ring.

BIOCHEMICAL STUDIES

These showed a very low caeruloplasmin and serum copper typical of Wilson's disease.

PATHOLOGICAL EXAMINATION

(a) General

The most striking features of the *post mortem* examination were seen in the brain and liver. The former showed conspicuous cortical atrophy, mainly over the frontal and parietal areas, wasting of the putamen, and a small thalamus and mid-brain. The liver showed a nodular coarse cirrhosis. Other findings included portal vein thrombosis and areas of bronchopneumonia.

This communication is dedicated to Prof. Dr. Josef Böck, Chief of the Second Eye Clinic of the University of Vienna, on the occasion of his 70th birthday.

(b) Ophthalmic

One eye, which was removed 2 hours after death, was fixed in 2.5 per cent. glutaraldehyde in Tyrode's buffer and sent to the Department of Pathology at the Institute of Ophthalmology for examination.

A small portion of cornea was removed for electron microscopical study and the remainder of the eye was embedded in celloidin. Sections 12–14 μ were cut for light microscopy, and stained with haematoxylin and eosin, and rubeanic acid. Typical staining reactions for copper were not obtained but phase-contrast microscopy revealed the presence of fine granular deposits in Descemet's membrane at the periphery extending inwards from Schwalbe's line as a band approximately 1 to 1.5 mm. broad.

The small portion of cornea was post-fixed in 1 per cent. osmium tetroxide, dehydrated through ascending grades of alcohol, and embedded in araldite. Thick sections (1–2 μ) for light microscopy and thin sections (500–700 Å) for electron microscopy were cut on a Huxley ultramicrotome using a glass knife. Electron micrographs were taken with an A.E.I. EM6 electron microscope.

The thick sections stained with toluidine blue demonstrated very clearly granules in Descemet's membrane (Fig. 1) similar to those which had been observed by phase-contrast microscopy.

FIG. 1 *Light photomicrograph of peripheral cornea, showing granular deposits in Descemet's membrane (DM) arranged essentially in two linear zones. Araldite embedded, 1 μ thick section, stained with toluidine blue. $\times$ 600*

Electron microscopy showed the presence of electron dense deposits of varying size lying mainly in Descemet's membrane, where they were arranged essentially in the middle third and in two linear zones (Fig. 2, overleaf); posteriorly, in relation to the endothelial surface, the deposits were fine and dust-like, while more anteriorly, towards the stromal surface, they were fewer in number but larger in size and showed what appeared to be a central nidus with a somewhat irregular peripheral zone (Fig. 3, overleaf).

An occasional small deposit was seen in the deep stroma and a few particles were present both within the endothelial cells and on the posterior corneal surface outside the endothelial cell membrane.

Analytical electron microscopy (A.E.I. EMMA 4, having combined features of transmission electron microscopy and electron probe x-ray microanalysis) confirmed that the deposits were, in fact, copper.

Discussion

Wilson's disease is an inborn error of copper metabolism in which the synthesis of caeruloplasmin, with which copper forms a stable compound in the blood, is diminished and there is increased absorption of copper from the gastrointestinal tract together with increased output in the urine. The bulk of circulating copper is normally bound to caeruloplasmin, but in Wilson's disease, as a result of the reduced serum caeruloplasmin, there is an increase in the amount of copper loosely attached to albumin and in this form it is readily deposited in the tissues, particularly in the liver and the brain. The consequence of this process is

FIG. 2 Survey electron micrograph of peripheral cornea, showing distribution of electron dense deposits. AC = anterior chamber; arrows point to fine deposits on posterior surface of endothelium (E), which shows cystic swelling of mitochondria (post mortem change). Note essentially linear distribution of electron dense deposits in Descemet's membrane (DM) and occasional deposits in adjacent stroma (S). Stained with uranyl acetate. $\times 10,000$

FIG. 3 At higher magnification, the larger deposits often show a relatively less electron dense central zone or nidus (N) with a denser peripheral zone (P). Electron micrograph. Uranyl acetate. $\times 80,000$

the development of nodular cirrhosis of the liver and degeneration of the basal ganglia and other areas of the brain, features which were seen in the case described and which accounted for the clinical condition.

The classical ophthalmological finding is the presence of the Kayser-Fleischer ring, which is considered pathognomonic of Wilson's disease. This ring appears as a dark band of pigmentation inside the limbus and varies in colour from greenish-yellow to golden-brown (Figs 4 and 5, opposite). Early workers, in the belief that Wilson's disease and kernicterus might have a common pathogenesis, had considered the possibility that the pigment was related in some way to the breakdown of haemoglobin, and Kubik (1922) claimed that he had obtained spectroscopical evidence to support this view. The idea that the corneal colouration might be due to the deposition of some heavy metal had also been entertained and the findings of large amounts of both silver and copper in the viscera, as reported by Rumpel (1913), added weight to this hypothesis. Jess (1922) suggested that the Kayser-Fleischer ring might be due to endogenously-deposited copper, although it was not until 8 years later that Haurowitz (1930) demonstrated an increased copper content in both brain and liver in cases of Wilson's disease. The question of either silver or copper deposition remained a controversial issue until Policard, Bonnet, and Bonamour (1936) demonstrated by histospectroscopical examination, the absence of silver and the presence of copper in the region of the ring. More recent work by Liebergall (1963) and Coleman, Sanders, and Field (1963) also supports this view.

FIG. 4 *Kayser-Fleischer ring as seen on clinical examination. Front view*

Many of the difficulties in the histochemical demonstration of the Kayser-Fleischer ring had arisen from the fact that the copper was apparently lost either in alcohol fixation or during the ether-ethanol treatment in celloidin-embedding (Kubik, 1922; Metzger, 1924; Brand and Takáts, 1951). In the case under discussion, however, the fact that in celloidin-embedded sections faint deposits were observed by phase-contrast microscopy proved that the ring was not entirely lost. This may have been due to prior fixation with glutaraldehyde, a factor which was also possibly responsible for the failure to demonstrate the deposits by histochemical means. Our own observations concerning the size, nature, and distribution of the electron dense deposits seen by electron microscopy largely confirm the findings previously reported by Uzman and Jakus (1957), and our analytical electron

FIG. 5 Kayser-Fleischer ring as seen in the slit-lamp

microscopical findings substantiate the electron probe microanalytical study of Tousimis and Adler (1963).

The nature of the copper deposit in the ring was discussed in some detail by Uzman and Jakus (1957) who, having produced evidence that a salt linkage between copper and an organic or inorganic ion is highly unlikely, suggested that the copper is, in fact, present in the form of a copper-chelate.

The mode of formation of the Kayser-Fleischer ring is also the subject of speculation. As pointed out by Uzman and Jakus (1957), the pattern of the copper deposits is highly suggestive of a process similar to that leading to the formation of Liesegang rings, and it can be postulated that the diffusing substance, probably ionic copper loosely bound to albumin, has its origin in the anterior chamber. The general pattern of the deposits suggests that the copper particles infiltrate into Descemet's membrane through the endothelial cells from the aqueous humour while the larger deposits with a central nidus (Fig. 3) probably result from coalescence of the smaller particles over a period of time. The question why the copper should react with some normal or abnormal constituent of Descemet's membrane to produce the structural pattern of the Kayser-Fleischer ring remains unanswered. Nor is it clear why this ring should be deposited at the periphery. It seems, however, likely that this may be related to the direction of aqueous flow and/or to some functional peculiarity of the peripheral corneal endothelium. An alternative hypothesis that the copper deposits originate from the limbal circulation seems to be less likely.

Summary

A case of Wilson's disease is described and the pathological and ultrastructural features of the Kayser-Fleischer ring are reported. That the deposits contain copper was demonstrated by analytical electron microscopy; their nature is discussed and deductions are made with regard to their mode of origin.

We are indebted to Dr. J. M. Walshe of the Department of Investigative Medicine, University of Cambridge, for providing the material and the clinical information, and to Dr. G. A. Gresham of the United Cambridge Hospitals for the general *post mortem* findings. We are grateful to A.E.I. Scientific Apparatus Ltd., of Harlow, Essex, for the analytical electron microscopical results, to Mr. V. J. Elwood and Miss Susan Legg for technical assistance, and to Mrs Evelyn Burr for secretarial help. Our thanks are also due to Prof. Norman Ashton for valuable guidance and criticism in the preparation of this paper.

References

- BRAND, I., and TAKÁTS, I. (1951) *v. Graefes Arch. Ophthal.*, **151**, 391
 COLEMAN, S. L., SANDERS, T. E., and FIELD, B. D. (1963) *Amer. J. Ophthal.*, **56**, 297
 FLEISCHER, B. (1912) *Dtsch. Z. Nervenheilk.*, **44**, 179
 HAUROWITZ, F. (1930) *Hoppe-Seyler's Z. physiol. Chem.*, **190**, 72
 JESS, A. (1922) *Klin. Mbl. Augenheilk.*, **69**, 218
 KAYSER, B. (1902) *Ibid.*, **40**(2), 22
 KUBIK, J. (1922) *Ibid.*, **69**, 214
 LIEBERGALL, G. S. (1963) *Amer. J. Ophthal.*, **55**, 1260
 METZGER, K. (1924) *Klin. Mbl. Augenheilk.*, **72**, 803
 POLICARD, A., BONNET, P., and BONAMOUR, G. (1936) *C.R. Soc. Biol. (Paris)*, **122**, 1120
 RUMPEL, A. (1913) *Dtsch. Z. Nervenheilk.*, **49**, 54
 TOUSIMIS, A. J., and ADLER, I. (1963) *J. Histochem. Cytochem.*, **2**, 40
 UZMAN, L. L., and JAKUS, M. A. (1957) *Neurology (Minneap.)*, **7**, 341