Communications

Electron microscopical study of Coats's disease

RAMESH TRIPATHI AND NORMAN ASHTON

Department of Pathology, Institute of Ophthalmology, University of London

At the present time there is only one report in the literature on the ultrastructural changes to be found in Coats's disease and this is limited to a description of subretinal and intraretinal "ghost cells" (Manschot and de Bruijn, 1967). We have recently had an opportunity to carry out an electron microscopical examination of an early, typical case and, although our findings add little to existing knowledge of the aetiology of the condition, they are of value in interpreting the morbid histological appearances of conventional microscopy and in understanding its pathogenesis.

Case Report

A female child was referred to hospital with a 6-month history of a white pupil and divergent squint in the right eye. The child was otherwise healthy and there was no relevant family history. Examination under general anaesthesia revealed extensive retinal detachment and a dense white plaque in the macular area. The left eye was normal. Retinoblastoma was diagnosed and the eye enucleated.

Pathology

MATERIAL AND METHODS

The globe was immediately bisected through the ora serrata and both the anterior and posterior segments were placed in 2.5 per cent. buffered glutaraldehyde solution.

Macroscopically, the anterior segment showed no abnormality and there was no evidence of a tumour within the posterior segment, where the white plaques seen clinically could now be identified as intraretinal exudates. There was widespread thickening and opacification of the retina and this was particularly evident at the posterior pole and at the temporal periphery, where dense yellowish-white intraretinal exudates could be seen in intimate relation to dilated, irregular, and aneurysmal vessels. The latter area, together with the underlying choroid and sclera, was selected for both light and electron microscopical examination (Fig. 1). Another section of the retina was removed for trypsin digestion, but this failed owing to the glutaraldehyde fixation.

Electron microscopy

Small blocks of tissue from the retina, choroid, ciliary body, iris, trabecular meshwork, and cornea were dissected and post-fixed in one per cent. osmium tetroxide solution for electron microscopy. After dehydrating in ascending grades of ethanol and clearing in epoxy-propane, the tissue blocks were embedded in Araldite and sections were cut on a Huxley ultramicrotome. Thick sections (1–2 microns) for light microscopy were stained with toluidine blue. Thin sections (500–800 Å) for electron microscopy were stained with uranyl acetate and lead citrate. Electron micrographs were taken with an AEI EM6 electron microscope.

FIG. 1 *Painting of segment of open eye, showing exudates at the temporal periphery intimately related to dilated, irregular, and aneurysmal vessels. This area was selected for light and electron microscopical examination*

FINDINGS

Histological

Sections showed a pronounced and irregular thickening of the retina, which, in the areas examined by light microscopy, showed no evidence of detachment. The thickening was attributable (a) to massive exudation with the formation of cystic cavities containing eosinophilic fluid in the inner retina; (b) to a heavy infiltration of the retina with foam cells and "ghost cells" particularly in the inner layers; (c) to a diffuse glial proliferation (Fig. 2). Intensely eosinophilic exudates were seen in some parts of the outer retina. These pathological changes had completely disrupted the retina so that its normal architecture was lost (except in some areas where the outer retina was well preserved), the visual cells appearing relatively normal with their outer segments lying directly upon a pigment epithelium showing no abnormality apart from occasional proliferative foci.

All sections showed gross pathological changes in the retinal vessels, many of which were dilated, thick-walled, and surrounded by proliferating glial cells, among which numerous pigment-bearing cells could be seen. Perls's stain for iron pigment gave negative results. Some of the larger vessels showed greatly thickened hyaline walls containing PAS-positive material, small aggregations of red cells, and pigment. Mononuclear and polymorphonuclear cells were present within the lumina of some vessels and were also seen perivascularly together with spindle cells and numerous eosinophils (Figs 2, 3, and 4). Mallory's phosphotungstic acid haematoxylin stain (PTAH) showed fibrin within the deep exudates

FIG. 2 Retina greatly swollen by exudate, foam cells, and glial proliferation, with disruption of normal structure. The vessels show gross hyaline thickening, narrowing of their lumina, and perivascular infiltration. The pigment epithelium shows mild proliferative changes. Paraffin section. Haematoxylin and eosin. $\times 100$

FIG. 3 Hyalinized vessel, showing a perivascular proliferation of spindle cells and surrounding gliosis. Paraffin section. Haematoxylin and eosin. $\times 210$

FIG. 4 Hyalinized vessel, showing inflammatory cells, including eosinophils, within the lumen and perivascularly. Paraffin section. Haematoxylin and eosin. $\times 145$

and within the cystic cavities. The choroid and sclera were normal. Light microscopy of Araldite sections (Figs 5, 6, 7, and 8, overleaf) confirmed these findings and showed in addition platelet thrombi in some vessels and the presence of large aneurysmal vessels which were devoid of endothelium and lined only with fibrous tissue; they were either empty or contained plasma clot (Figs 7 and 8).

Electron microscopy

Sections of cornea, trabecular meshwork, iris, and ciliary body showed no structural abnormality. Sections of the retina, which included the areas of the lesion, revealed the

FIG. 5 Hyalinized vessel, lined with endothelium and containing platelets and red blood cells in the lumen; red cells and pigmented cells are seen in the wall which is surrounded by dense gliosis. Araldite section. Toluidine blue. $\times 665$

FIG. 6 Hyalinized vessel similar to that shown in Fig. 5 but with extensive haemorrhage in its wall. Araldite section. Toluidine blue. $\times 665$

FIG. 7 Hyalinized vessel, showing a plasma thrombus, loss of endothelium, and pigmented cell infiltration in the wall. Araldite section. Toluidine blue. $\times 415$

FIG. 8 Dilated vessel, thin-walled and devoid of endothelium, forming a cyst-like space in the retina, which is densely infiltrated with "ghost cells". Araldite section. Toluidine blue. $\times 165$

underlying choroid to be essentially normal and Bruch's membrane to be intact. The pathology was confined entirely to the retinal components. The most conspicuous structural alterations were observed in the blood vessels located in the area of retinal thickening, where their walls were mostly greatly thickened and replaced by a laminated fibrous coating consisting mainly of basement membrane-like material infiltrated with

FIG. 9 Electron micrograph of a blood vessel, showing a lumen containing plasma (P), platelets (PL), and erythrocytes (E). The vessel wall is here represented by a laminated fibrous coat infiltrated with plasma, blood corpuscles (BC), and some cellular debris (arrowed). Note that the endothelium (EN) is still continuous, although collections of fibrin (F) in the subendothelial space are apparent. G = Perivascular glia. Approx. $\times 3,650$

serofibrinous exudates, blood corpuscles, and some cellular remains (Figs 9, 10, and 11). An intact endothelial lining was observed in some vessels (Figs 9 and 10), but the majority were completely devoid of endothelium and pericytes; blood corpuscles, plasma, and fibrin filled the lumina of these vessels (Figs 11, 12, and 13). The more dilated or aneurysmal and telangiectatic vessels showed an irregular wall invariably infiltrated with plasmoid and fibrinous material (Figs 12 and 13). In places this fibrous wall was extremely thin or even absent and the lumen extended up to the basement membrane of the surrounding glial cells (Fig. 12). The external limit of the vessel wall in most cases was demarcated from the surrounding retina solely by a thin basement membrane anchored to the sur-

FIG. 10 Electron micrograph of the wall of a blood vessel, showing a laminated arrangement of basement membrane-like material infiltrated with erythrocytes (E), platelets (PL), lipid droplets (L), and some cellular debris (arrowed). The endothelium (EN) is showing cystic changes in the mitochondria. The external limit of the vessel wall is demarcated by the basement membrane (BM) of the surrounding glia (G). Approx. $\times 6,650$

FIG. 11 Electron micrograph of a blood vessel in the retina containing erythrocytes (E), plasma (P), and fibrin (F), and showing a total absence of the vascular endothelium; the vessel wall is represented by a laminated fibrous coat only. Note the plasmoid (P) and fibrinous (F) transudates both within and outside the vessel coat and the perivascular mantle of glial cells (G). Approx. $\times 2,400$

FIG. 12 *Electron micrograph of a telangiectatic blood vessel, showing erythrocytes (E) and fibrin (F) in the lumen. Note the extension of the lumen in some places (arrow) up to the basement membrane of the surrounding glial mantle (G), while in other places fibrous remnants of the wall (FW) intervene between the two. Approx. $\times 6,650$*

FIG. 13 *Electron micrograph of the fibrous wall of an aneurysm, the extent of which is demarcated by an electron dense basement membrane (BM), presumably formed by the surrounding Müller cells (M). Note the presence of plasmoid material within the lumen (Lu) and also in the fibrinous wall (F), the latter consisting of basement membrane material and fine fibrils (approximately 100 Å in diameter and having a beaded pattern along the long axis). $\times 16,650$*

FIG. 14 Survey electron micrograph of the intraretinal exudates, showing plasmoid (P) and fibrinous (F) materials. Note also the lipoidal inclusions (L) in a Müller cell. $\times 4,000$

FIG. 15 Electron micrograph of intraretinal macrophages ("foam cells"), showing oval or circular electron optically empty spaces (L) which were originally occupied by lipid droplets. P = Plasmoid exudate. $\times 8,000$

FIG. 16 *Electron micrograph of a macrophage ("foam cell"), showing intracellular lipoidal inclusions (L). $\times 6,600$*

FIG. 17 *Electron micrograph of an intraretinal macrophage, showing intracellular lipoidal inclusions (L), residual bodies (R), and nucleus (N). Note also the surrounding plasmoid (P) and fibrinous (F) exudates and Müller cell (M). $\times 10,000$*

rounding glial cells (Fig. 13). In some instances, however, the existence of a blood vessel or aneurysm was recognized only by the presence of a space filled with plasma, fibrin, blood corpuscles, and macrophages, and incompletely surrounded by a basement membrane of the adjacent glial cells. No new vessels were found as described by Wise (1961).

Although the structural disorganization involved the entire area of the retinal lesions studied electron microscopically, it was most marked in the anterior layers (*i.e.* nerve fibre, ganglion cell, and inner nuclear layers), the elements of which were much reduced in number. In other areas the retina was greatly thickened because of the massive accumulation of plasmoid and fibrinous exudates (Fig. 14) and occasional blood corpuscles. The visual elements showed patchy degenerative and atrophic changes, which extended into the outer nuclear layer to a minor degree. The external limiting membrane appeared generally intact. The pigment epithelium showed occasional focal proliferative changes and in places was separated from the nervous components of the retina. The subretinal space appeared electron-optically empty, except for occasional cellular fragments of visual elements, pigment epithelium, a thinly distributed plasmoid material, and a few foreign cells.

The nervous layers of the retina were infiltrated by foam and "ghost" cells and macrophages (Figs 15, 16, and 17), although the maximum infiltration was seen in the middle and anterior layers. The majority of the macrophages contained lipoidal globules and a few also contained clumps of pigment granules, which in some sections appeared indistinctly membrane-bound and were probably secondary lysosomes (Fig. 17). The Müller cells in some areas appeared hypertrophic and generally showed an increase in intracellular fibrils and rough surface endoplasmic reticulum; in many places, however, the cellular architecture was destroyed. There was a striking perivascular proliferation of glial cells.

Discussion

The passage of large colloidal particles into the delicate tissues of the central nervous system, as occurs in plasmatic exudation, disrupts the organization of the nervous elements and severely impairs their function. It is, therefore, clearly essential that these tissues should be protected from their entry, and in the case of the retina this is normally achieved by two barrier systems, one located in the inner retina, apparently at the level of the vascular endothelium (Ashton, 1965; Cunha-Vaz, Shakib, and Ashton, 1966) and the other in the outer retina at the level of the pigment epithelium. Breaching of either of these two barriers results in subretinal and/or retinal exudation and, although the resulting clinical and pathological pictures vary according to the location, extent, severity, and chronicity of the exudative process, they are distinct pathogenetically only as regards the cause of the initial leakage. There is no doubt that Coats's disease is an example of such a barrier break-down, and, since the classical paper by Reese (1956), it has been generally accepted that this is due to retinal telangiectasis, but the exact cause of this anomaly is still obscure (Manschot and de Bruijn, 1967); a genetic basis has been suggested (Small, 1968). Although in the past there has been considerable controversy, admirably summarized by Woods and Duke (1963), few would disagree today that all the pathological changes – intraretinal and subretinal exudation, haemorrhages, lipid and fibrin deposition, phagocytic proliferation ("ghost cells"), and ultimate glial and fibrous tissue organization – most probably stem, directly or indirectly, from the abnormal vascular permeability. Since the exudates arise in the inner retina from a non-inflammatory vascular disease,

as in hypertensive and diabetic retinopathy, it is now apparent that as regards pathogenesis Coats's disease was inappropriately termed "external exudative retinitis."

This present examination of the ultrastructure of a single and very early case of Coats's disease (no subretinal organization or cholesterol depositions being present), while not revealing the cause of the condition, permits comment on a number of its important aspects and suggests a sequence of the pathological changes.

The prominent vascular abnormalities varied from a gross thickening of the walls of the smaller vessels with relatively normal or slightly dilated lumina and continuous endothelium, to a gross thinning of these walls with irregular and dilated lumina and a total absence of the endothelial lining. The lumina in both instances contained impacted red cells, plasma, thrombi, or platelet aggregations. By electron microscopy the mural thickening was found to be due to insudated lipid, plasma, and fibrin, together with abundant basement membrane-like material, cellular debris, and leucocyte and macrophage infiltration. Initially, the leakage of plasma into the vessel wall must occur through the endothelium but this, although showing degenerative changes as evidenced by cytoplasmic vacuolation, was frequently seen to be continuous with its junctions intact. At a later stage the endothelium was entirely absent and the vessel wall came to consist solely of a thick laminated coat of plasmoid and fibrillar elements, immediately surrounded by glial cells. It is not surprising that a vessel walled by such an acellular porous membrane should become aneurysmal or telangiectatic and permit a continual outpouring of its contents, including the occasional haemorrhage, into the adjacent tissues. Finally, the walls of these telangiectatic vessels may absorb leaving the basement membrane of the surrounding glial cells to form a channel for the blood; at this stage extensive haemorrhage may occur and organization follow. These pathological changes, therefore, suggest that an early event in Coats's disease is abnormal endothelial permeability; whether this is primarily due to a deficiency in structure or function, that is a failure to provide the normal blood-retinal barrier, is not at present clear, but it would seem that both the telangiectasis and leakage may be secondary to it. On such a basis there would be no difficulty in accepting Coats's Groups I and II, that is cases of exudative retinopathy with and without obvious vascular changes, as stages of the same condition.

The presence of basement membrane-like material and blood components in the thickened vascular walls explains the brilliant PAS-positive staining seen by light microscopy, which was originally noted by Reese (1956) and confirmed by Duke and Woods (1963), Manschot and de Bruijn (1967), and many others. The exudates in the retina also stain positively with PAS but less intensely.

Very similar – and sometimes identical – ultrastructural appearances of stratified or laminated mural thickening with fibrillar material, have been described in a number of other conditions in which increased vascular permeability is known to occur or might be supposed to be a factor. Examples are to be found in diabetic retinal microaneurysms (Toussaint and Dustin, 1963; Bloodworth, 1964), in diabetic vessels in the kidney (Bergstrand and Bucht, 1959) and toe (Banson and Lacy, 1964), and in the retinal vessels of diabetic dogs (Bloodworth and Molitor, 1965), in small vessels from a healed cornea (Szalay and Pappas, 1970), and in renal (McGee and Ashworth, 1963) and experimental hypertension (Ashworth and Grollman, 1959). These appearances have usually been described simply as "basement membrane thickening", but one wonders to what extent the mural thickening may be attributable, as in Coats's disease, to an abnormal endothelial permeability to protein.

The extension of exudate into the retina itself, causing disruption of its structure and wide

separation of the cellular membranes to form large extracellular spaces containing plasma, fibrin, erythrocytes, leucocytes, and macrophages, is completely non-specific in character. Such ultrastructural appearances have already been described in the brain (Lampert and Carpenter, 1965; Bubis and Luse, 1964) and in the retina in experimental ischaemia (Ashton, 1965; Shakib and Cunha-Vaz, 1966) and experimental hypertensive retinopathy (Ashton, Peltier, and Garner, 1968). The presence of leucocytes around the abnormal vessels and within their walls has also been noted previously, and the first case of Manschot and de Bruijn (1967) also showed eosinophils as in our case; this is probably no more than a mild low-grade inflammatory reaction to the exudation.

We agree with Manschot and de Bruijn (1967) that haemorrhages are not an essential feature as originally thought by Coats (1908) and subsequently doubted by Leber (1916), and would add that the deposition of cholesterol crystals, although highly characteristic, may also be absent, being rather a measure of the severity or chronicity of the exudative process than a specific feature.

As regards the "ghost cells" seen so typically in Coats's disease, there is controversy about their origin, there being two main schools of thought: (i) that they originate from retinal histiocytes (macrophages) and (ii) that they derive from pigment epithelium. Manschot and de Bruijn (1967) favour the latter origin and, although allowing that some subretinal macrophages may have migrated from the retina, do not refer to them as "ghost cells". These authors differentiate on an ultrastructural basis, macrophages containing ingested pigment from pigment epithelial cells which have become phagocytic, *i.e.* ingested pigment is contained in a vacuole whereas native pigment lies free in the cytoplasm. Unfortunately, in pathological material, we have not found this a valid distinction, for on the one hand vacuolar membranes around ingested pigment may be lost in the degenerating macrophage, and on the other hand native pigment may come to lie in pathological vacuoles in the proliferating pigment cell.

Another difficulty is that proliferating pigment epithelial cells may fail to form new pigment (Gloor, 1969) and, to add to the confusion, glial cells have some role in phagocytic activity and may also become laden with lipid, as already observed by Shakib and Ashton (1966) and further confirmed by this present study. In a previous communication from this department, we discussed the origin of retinal macrophages at some length and gave reasons for supposing that they originate both from the local proliferation of resting macrophages in the retina itself (cells which gained access to the retina at the time of vascularization), and from a contribution of similar cells from the blood stream, when the blood-retinal barrier is deficient (Shakib and Ashton, 1966), as in Coats's disease. We believe, in common with Marshall and Michaelson (1933), that the majority of "ghost cells" are lipid-laden macrophages, which have arisen in the above two ways, especially as in our present case proliferation of the pigment epithelium was minimal and in some areas, where "ghost cells" were prolific, this layer even appeared quite normal. There is no doubt, however, that pigment cells have phagocytic properties, as demonstrated by Young and Bok (1969) in the case of rod outer segments, and it is well known that they can proliferate and invade the retina to assume the appearance of macrophages, as recently shown in photocoagulation experiment on rabbits (Gloor, 1969). It may be concluded, therefore, that in cases where the pigment epithelium becomes involved in the exudative injury its cells may contribute to the macrophage reaction of the retina ("pigment epithelial macrophage" of Gloor, 1969), and so to the "ghost cell" population, but when this stage is reached they cannot be separately identified. It would also be generally agreed that pigment epithelial cells, together with fibroblasts from the retina, form the subretinal

fibrous tissue, but since this was absent in our present case it provides no further evidence in this direction.

Summary

An ultrastructural study of an early case of Coats's disease confirms the widely-held view that an abnormal permeability of the retinal vessels is fundamental in its pathogenesis. It is suggested that the pathological changes may initially derive not from telangiectasis, but from a functional or structural breakdown of the blood-retinal barrier (vascular endothelium), giving rise to plasmatic vasculosis and mural disorganization, and that these result in aneurysmal dilations and telangiectasis. Leakage of blood components then increases to form intraretinal and subretinal exudates, haemorrhages, lipid and fibrin deposits, with phagocytic proliferation, disorganization and destruction of the retinal elements, and eventually glial and fibrous tissue organization.

The characteristic profusion of macrophages and "ghost cells" within the affected areas is most likely due to the local proliferation of resting histiocytes in the retina itself, augmented by blood-borne macrophages and migrating pigment epithelial cells.

It is a pleasure to acknowledge the technical assistance of Mr. G. E. Knight and Mr. V. Elwood and the secretarial help of Miss. K. O'Malley.

References

- ASHTON, N. (1965) *Trans. ophthal. Soc. U.K.*, **85**, 199
 ———, PELTIER, S., and GARNER, A. (1968) *Ibid.*, **88**, 167
 ASHWORTH, C. T., and GROLLMAN, A. (1959) *Arch. Path.*, **68**, 148
 BANSON, B. B., and LACY, P. E. (1964) *Amer. J. Path.*, **45**, 41
 BERGSTRAND, A., and BUCHT, H. (1959) *J. Path. Bact.*, **77**, 231
 BLOODWORTH, J. M. B. (1964) In "Small Blood Vessel Involvement in Diabetes Mellitus", *Proc. Conf.*, 1963, ed. M. D. Siperstein, A. R. Colwell, and K. Meyer, p. 81. American Institute of Biological Sciences, Washington, D.C.
 ——— and MOLITOR, D. L. (1965) *Invest. Ophthalm.*, **4**, 1037
 BUBIS, J. J., and LUSE, S. A. (1964) *Amer. J. Path.*, **44**, 299
 COATS, G. (1908) *Roy. Lond. ophthal. Hosp. Rep.*, **17**, 440
 CUNHA-VAZ, J. G., SHAKIB, M., and ASHTON, N. (1966) *Brit. J. Ophthalm.*, **50**, 441
 DUKE, J. R., and WOODS, A. C. (1963) *Brit. J. Ophthalm.*, **47**, 413
 GLOOR, B. P. (1969) *v. Graefes Arch. Ophthalm.*, **179**, 105
 LAMPERTT, P., and CARPENTER, S. (1965) *J. Neuropath. exp. Neurol.*, **24**, 11
 LEBER, T. (1916) In "Graefe-Saemisch-Hess Handbuch der gesamten Augenheilkunde", 2nd. ed., band 7, teil 2, kap XA, 11 halfte. Engelmann, Leipzig.
 MANSCHOT, W. A., and de BRUIJN, W. C. (1967) *Brit. J. Ophthalm.*, **51**, 145
 MARSHALL, J., and MICHAELSON, I. C. (1933) *Trans. ophthal. Soc. U.K.*, **53**, 102
 MCGEE, W. G., and ASHWORTH, C. T. (1963) *Amer. J. Path.*, **43**, 273
 REESE, A. B. (1956) *Amer. J. Ophthalm.*, **42**, 1
 SHAKIB, M., and ASHTON, N. (1966) *Brit. J. Ophthalm.*, **50**, 325
 ——— and CUNHA-VAZ, J. G. (1966) *Exp. Eye Res.*, **5**, 229
 SMALL, R. G. (1968) *Trans. Amer. Acad. Ophthalm. Otolaryng.*, **72**, 225
 SZALAY, J., and PAPPAS, G. D. (1970) *Invest. Ophthalm.*, **9**, 354
 TOUSSAINT, D., and DUSTIN, P. (1863) *Arch. Ophthalm. (Chicago)*, **70**, 96
 WISE, G. N. (1961) *Amer. J. Ophthalm.*, **52**, 637
 WOODS, A. C., and DUKE, J. R. (1963) *Brit. J. Ophthalm.*, **47**, 385
 YOUNG, R. W., and BOK, D. (1969) *J. Cell. Biol.*, **42**, 392