Reappraisal of the retinal cotton-wool spot: a discussion paper[1]

David McLeod BSC FRCS
Moorfields Eye Hospital, London EC1V 2PD

In 1969, Professor Norman Ashton discussed the then current views on retinal cotton-wool spots at the Section of Ophthalmology of the Royal Society of Medicine. It is appropriate now to reappraise the nature of cotton-wool spots since our thoughts on this subject have changed significantly in the intervening years.

Cotton-wool spots are localized areas of dense white swelling of the retinal nerve fibre layer. They often have a zigzag internal structure, a feathered edge but an otherwise well-delineated form and an approximately 1 mm dimension; they project slightly into the vitreous and sometimes deflect retinal vessels. For many years it had been widely held that these lesions represented microinfarcts of the inner retina. There were, however, several objections to this 'microinfarction' hypothesis. Firstly, although the relationship between arteriolar occlusion and cotton-wool spots was well established for hypertensive retinopathy, local obstruction of the feeding vessel was not a universal accompaniment of cotton-wool spots, e.g. in anaemic retinopathy and carotid artery occlusion (Ashton 1970). Secondly, a fundamental difference was observed between both the clinical and the histopathological appearances of cotton-wool spots and retinal infarction. Cotton-wool spots essentially involve just the nerve fibre layer of the retina, and their dense whiteness appears to result from an accumulation of organelles in the distended axon terminals (cytoid bodies) contained within the lesions (Ashton 1970). On the other hand, retinal infarction produces a grey translucent swelling with ischaemic necrosis and vacuolation of the whole of the inner half of the retina. Ashton emphasized that the essential determinant of the cotton-wool spot – organelle aggregation in axon terminals – was not a feature of necrosis but was predominantly a 'living reaction' in the axon.

How, then, could this aggregation of organelles in axon terminals be explained both in terms of axonal physiology and retinal circulatory pathology? The experiments of Ashton and coworkers (1966) went some way towards providing an answer. The retinal circulation of pigs was focally occluded by injection of glass microspheres into the external carotid artery with resulting embolism of small retinal arterioles. Areas of grey retinal swelling up to 6 mm in diameter appeared within a few minutes and corresponded to zones of vascular non-perfusion on fluorescein angiography. Ischaemic damage was identified histologically throughout the inner half of the retina and, even at only one hour following occlusion, nerve fibres at the borders of the lesions showed increased granularity. In the following days, the ischaemic areas became more densely white, smaller lesions becoming whiter than larger areas. Furthermore, an enormous accumulation of organelles (often showing degeneration) appeared in grossly distended axon terminals especially at the periphery of the lesions. Two possible mechanisms of intra-axonal organelle aggregation were considered: (1) proliferation of organelles *in situ;* (2) migration of preformed organelles by axoplasmic flow. For reasons which have been critically reviewed elsewhere (McLeod 1976), Shakib & Ashton (1966) came down in favour of the former mechanism. They suggested that a nonspecific reactive proliferation of organelles occurred in axons arising from ganglion cells which had survived at the 'hypoxic' periphery of ischaemic ('anoxic') areas.

Stimulated by informal discussions with Professor Ashton, I re-evaluated retinal cotton-wool spots by reviewing hundreds of fundus photographs of patients with ischaemic retinopathies. It was immediately evident that cotton-wool spots and retinal infarcts

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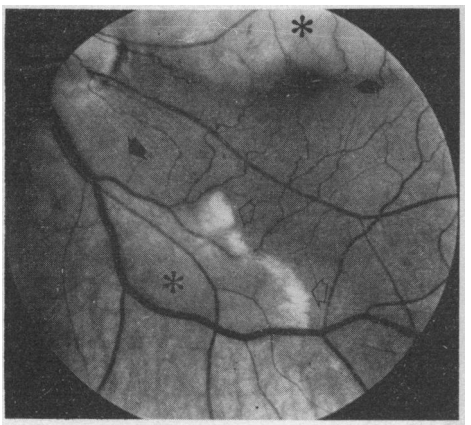

Figure 1. Fundus photograph of case of central retinal artery occlusion (left eye). Retinal infarction (asterisks) except in inferior macular area supplied by a cilioretinal arteriole. White axoplasmic debris has accumulated where nerve fibres cross boundary between viable and necrotic retina (open arrows). No axoplasm along borders with no axonal crossings (closed arrows)

frequently coexist and do so in a very specific fashion (McLeod 1975). White lesions identical in all respects to cotton-wool spots were to be found wherever nerve fibres crossed the boundary between ischaemic and viable retina after retinal arteriolar occlusion (Figure 1) or branch retinal vein occlusion with capillary closure. In some cases, a progressive accumulation of white material at the edge of an infarct could be observed over a period of days (Figure 2). No white material was found, however, where nerve fibres ran parallel to the edge of an ischaemic area or where this boundary corresponded to the temporal horizontal 'raphe' (the inappropriately designated line temporal to the macula where nerve fibres pass superonasally or inferonasally as arcuate bundles above or below the fovea – Figure 1).

The nature and significance of this relationship had not previously been appreciated, though it had been illustrated in numerous publications. It appeared that here was compelling evidence against the hypothesis of '*in situ* hypoxic proliferation' (since a zone of white swelling would be expected completely to surround any infarct). On the other hand, the clinical findings were entirely compatible with the hypothesis that the cotton wool-like material accumulated as a result of obstruction of orthograde or retrograde axoplasmic transport (on the peripheral side or disc side of ischaemic lesions respectively or both). In general, the

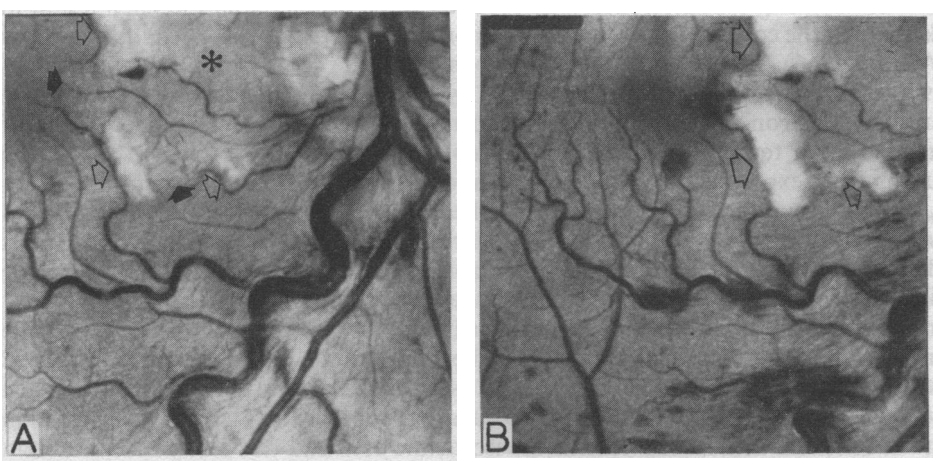

Figure 2. Fundus photographs of case of central retinal vein obstruction with cilioretinal infarction (right eye) (courtesy of Dr J F Cullen). A, Parapapillary retinal infarction (asterisk). Early accumulation of axoplasmic debris along borders of infarct crossed by nerve fibres (open arrows – obstructed orthograde transport). No axoplasm along border with no axonal crossings or where parafoveal ganglion cell bodies are involved in infarct (closed arrows). B, Same eye a few days later. Minor increase in haemorrhages outside area of infarction, but gross increase in axoplasmic debris along borders of infarct crossed by nerve fibres (open arrows)

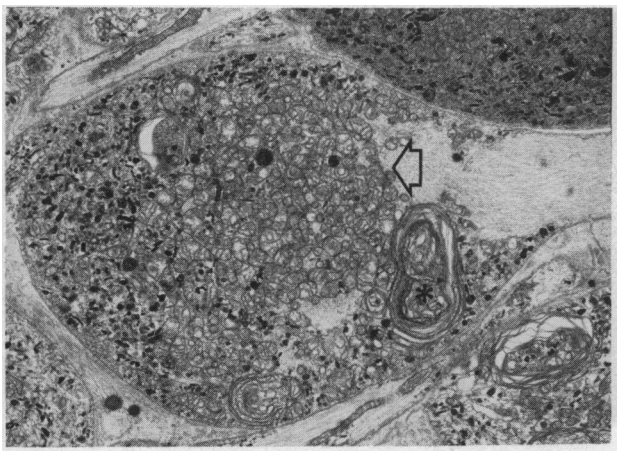

Figure 3. Electron micrograph of spheroidal axon terminal. Mitochondria have accumulated as a result of obstruction of retrograde axoplasmic transport (arrow), with membranous whorl formation (asterisk). (Reproduced from McLeod *et al.* 1977, with kind permission)

accumulation of such 'axoplasmic debris' on the peripheral side of an ischaemic lesion exceeded that on the disc side of the lesion; this correlated with the known prolonged continuance of orthograde axoplasmic transport following axonal injury compared with the relatively brief period of continuing retrograde transport (Lubinska 1964). The absence of a white border-zone along those edges of infarcts without axonal crossings was similarly explicable.

The experiments of Ashton and others were therefore repeated, small retinal arterioles in pigs being occluded by argon laser photocoagulation (McLeod *et al.* 1977). After two days, areas of pale swelling of the inner retina were noted corresponding to angiographically delineated zones of capillary non-perfusion. Disappointingly, the edges of the ischaemic zones were only marginally whiter than the central regions (unlike the clear distinction observed in most human retinae). Nevertheless, histology revealed three distinct zones in each lesion in sections taken along the course of the retinal nerve fibres (as had previously been illustrated by Shakib & Ashton 1966). The central zone of necrosis involved the whole of the inner half of the retina, while zones of swelling of the nerve fibre layer alone could be identified on each side of the infarct. By electron microscopy, these border zones were confirmed as consisting of hugely distended axon terminals packed with cytoplasmic organelles (Figure 3). Thus, the lesions resulting from experimental retinal arteriolar occlusion were not equivalent to cotton-wool spots, as had been inferred by Dollery and colleagues (1966), but were infarcts bordered by distended axon terminals in the retinal nerve fibre layer; only these border zones in fact corresponded to cotton-wool spots. No border zone of axonal distension and organelle accumulation was found where nerve fibres ran parallel to the edge of an ischaemic area.

We then attempted to distinguish by autoradiography the cause of the organelle aggregation at the borders of retinal infarcts. A radioactive amino acid (tritiated leucine) was injected into the vitreous cavity at the same time as the laser arteriolar occlusion, and the retinal distribution of label was studied after two days (experiment A). The label was found in viable ganglion cells and their axons outside the ischaemic area and was also concentrated in axon terminals at the peripheral edge of ischaemic lesions (Figure 4). However, there was no excess of grains over the axon terminals on the disc side of the lesions. Assuming that the mechanism of accumulation of organelles was the same on each side of the lesions, we felt this proved conclusively that the organelle aggregation resulted from obstruction of (orthograde) axoplasmic transport rather than *in situ* reactive proliferation. Otherwise, why were there no

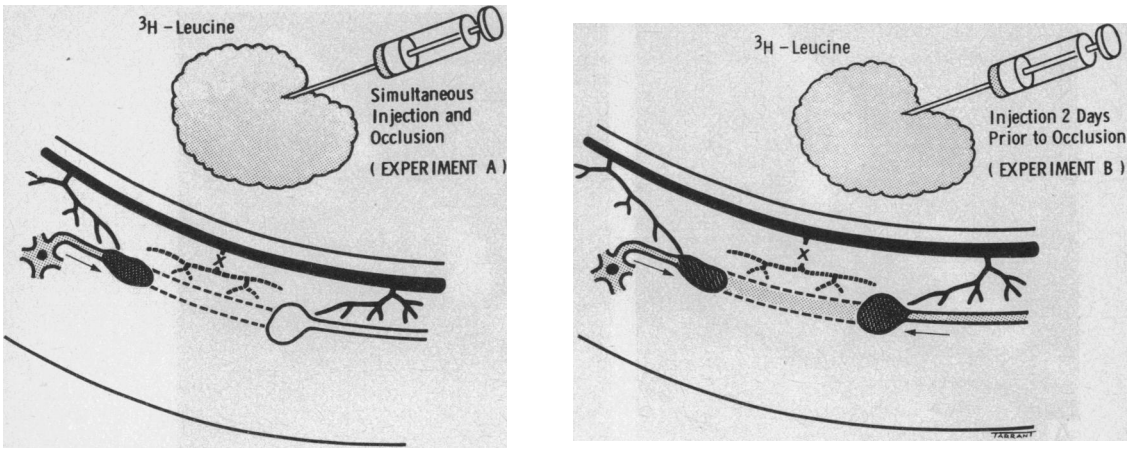

Figure 4. Summary diagram of autoradiographic findings in retinal ganglion cell axons after experimental branch retinal arteriolar occlusion (x) and intravitreal injection of tritiated leucine. Experiment A: Accumulation of radioleucine in axon terminals proximal (peripheral) to inner retinal infarct, but no accumulation on distal border of infarct. Obstruction of orthograde axoplasmic transport demonstrated (arrow). Experiment B: Accumulation of radioleucine in axon terminals both proximal and distal to infarct. Obstruction of both orthograde and retrograde axoplasmic transport demonstrated (arrows). (Reproduced from Mcleod *et al.* 1977, with kind permission)

grains over axon terminals on the disc side of the lesions? The tritiated leucine had clearly been taken up by ganglion cells peripheral to ischaemic lesions, incorporated into proteins and transported towards the optic disc, being obstructed in its course by destruction of axon segments within the areas of ischaemia.

In some experiments, leucine was injected into the vitreous cavity some time prior to arteriolar occlusion in order to 'prime' the entire ganglion-cell system with label (experiment B). Label then became concentrated on each side of the retinal infarcts, thus demonstrating obstruction of both orthograde and retrograde axoplasmic transport, and showing that there was no constraint to leucine-uptake on the disc side of ischaemic lesions (Figure 4). We were also able to show exactly the same processes on either side of axonal interruptions induced by laser burns heavy enough to disrupt the retinal nerve fibre layer. This confirmed the nonspecific nature of the changes developing in relation to axonal injuries.

These findings were important in two respects. Firstly, the particular circumstances of our experiment provided a means of finally proving that organelle aggregation following axonal injury results purely from 'passive' interruption of normal axonal physiology, and is not an 'active' proliferation at a site in the neurone where such proliferation is otherwise thought not to occur. Secondly, our findings indicated that the nature of cotton-wool spots should be

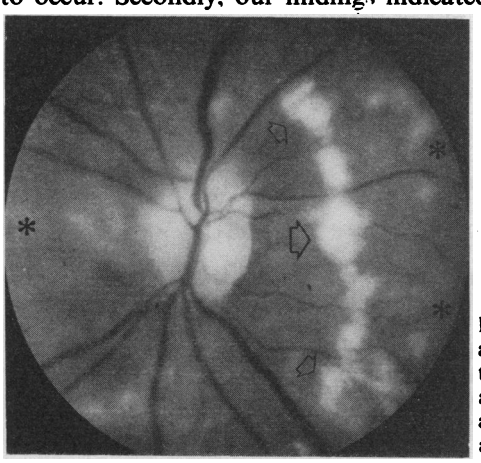

Figure 5. Fundus photograph of case of partial central retinal artery occlusion (left eye). Retinal infarction (asterisk) except in temporal parapapillary area. Axoplasmic debris has accumulated where nerve fibres cross boundary between viable and necrotic retina (open arrows – obstructed retrograde axoplasmic transport)

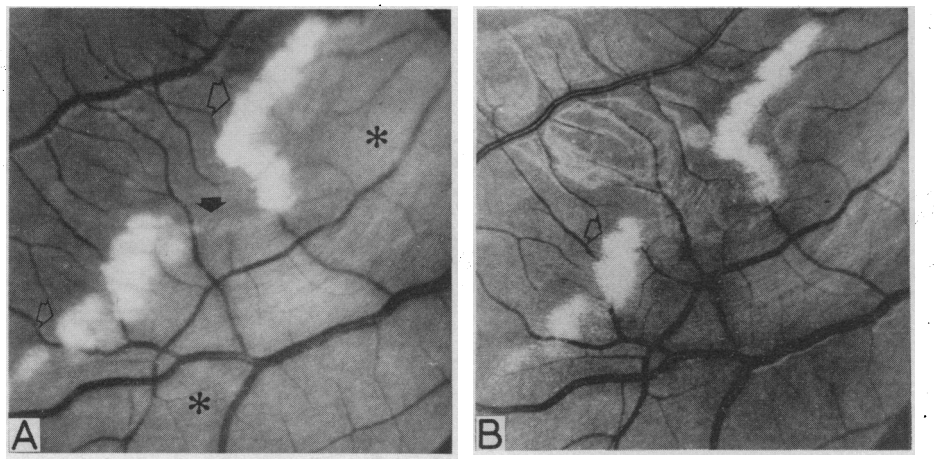

Figure 6. Fundus photographs of case of branch retinal artery occlusion (right eye) (courtesy of Mr M D Sanders). A, Retinal infarction (asterisks) inferior to disc and macula. Axoplasmic debris has accumulated where nerve fibres cross boundary of infarct (open arrows – obstructed orthograde axoplasmic transport). No white accumulation where nerve fibres course parallel to border of infarct (closed arrow). B, Same eye two weeks later. Signs of retinal infarction have virtually disappeared, leaving apparently 'isolated' cotton-wool spots (open arrow) which show a granular appearance during phagocytosis

reappraised. Cotton-wool spots should not be regarded as microinfarcts of the inner retina and 'must be evaluated on a broader basis than focal ischaemia' (Ashton 1970). They are accumulations of axoplasmic debris in the nerve fibre layer of the retina resulting from obstruction of axoplasmic transport. In most instances, cotton-wool spots do not represent the whole area of ischaemic inner retina but merely reflect obstruction of axoplasmic flow in axons crossing into much larger ischaemic areas (Figures 1 & 2). This is particularly well demonstrated in cases of partial central retinal artery occlusion where multiple peripapillary cotton-wool spots develop at the border of an area of ischaemia involving most of the inner retina (Oji & McLeod 1978) (Figure 5).

In many instances, apparently isolated cotton-wool spots represent axoplasmic debris at the edge of an infarct, the clinical signs of which have disappeared (or become much less evident) in the days or weeks following the vascular occlusion (Figure 6). It is true that, in small ischaemic lesions, the two border zones of axoplasmic debris may coalesce and in some instances one might speculate that there is no complete axonal disruption but only a temporary and possibly incomplete hold up of axoplasmic transport (as seen at the optic disc in papilloedema). The subsequent disappearance of the white swelling may then reflect re-establishment of axoplasmic transport rather than phagocytosis of axonal material.

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