PERSPECTIVE

Why cotton wool spots should *not* be regarded as retinal nerve fibre layer infarcts

D McLeod

Br J Ophthalmol 2005;89:229–237. doi: 10.1136/bjo.2004.058347

Cotton wool spots (CWSs) comprise localised accumulations of axoplasmic debris within adjacent bundles of unmyelinated ganglion cell axons. Their formation is widely held to reflect focal ischaemia from terminal arteriolar occlusion, but credible evidence supporting this view is lacking. CWSs are here purported to be nothing more than sentinels of retinal nerve fibre layer pathology, hence their recommended redesignation "cotton wool sentinels." After branch arteriolar occlusion, CWSs evolve as boundary sentinels of infarction, their uniform width suggesting a glial constraint to axonal expansion. In pre-proliferative diabetic retinopathy, CWSs form a C-shaped chain nasal to the disc and around the macula where they constitute sentinels of ischaemia affecting the entire retinal mid-periphery. The polymorphous CWSs evolving during acute panretinal hypoperfusion represent sentinels of an ischaemic penumbra. Those surrounding the disc in Purtscher's traumatic angiopathy are sentinels of neuronal damage from transient venous hyperdistension that overwhelms the protection afforded by peripapillary axonal decompartmentalisation.

its axon. For several days after localised axonal damage, both orthograde and retrograde axoplasmic transport will continue unabated in undamaged axon segments causing axon end bulbs to appear on each side of the point of injury. When large clusters of cytooid bodies arise in this way, they will expand the retinal nerve fibre layer (RNFL) and may protrude into the vitreous (fig 2).

THE PREVAILING VIEWPOINT: "THE FOCAL ISCHAEMIA HYPOTHESIS"

Look at most textbooks, periodicals, or websites and you will find that CWSs are perceived to be synonymous with focal retinal ischaemia, a view promulgated since the middle of the last century.⁴ Thus, CWSs are often construed as microinfarcts occupying the downstream territory of occluded retinal arterioles and, since the average size of a CWS is approximately 10% of the area of the optic disc,⁵ the arterioles in question are generally thought to be the terminal (or pre-capillary) arterioles. These vessels tend to branch from higher order arterioles at right angles and are at, or below, the limit of ophthalmoscopic resolution.⁶

An alternative iteration of the "focal ischaemia hypothesis" invokes localised infarction in the RNFL in the absence of significant damage to the inner half of the retina deep to this layer.^{4 7 8} In the context of hypertensive retinopathy, Friedenwald (1949) speculated that the deeper vessels might be less susceptible to spasm, or the inner retina deep to the RNFL may be less vulnerable to ischaemia, or the deep capillary bed might have more collateral connections.⁴ Subsequently, Henkind (1967) postulated that CWS formation reflects selective impairment of perfusion through the capillaries that supply the thickest part of the stratum opticum where CWSs are for the most part located.^{6 9} Accordingly, the radial peripapillary capillary plexus (RPCP) was portrayed as a distinct superficial vascular layer arising independently from the larger retinal arterioles and with only a limited potential for capillary collaterals. Other investigators, however, have emphasised the multi-layering of the capillary meshwork in the RNFL and the rich interconnections of the RPCP with the deeper capillary bed with which it shares a common (rather than an independent) arteriolar origin.^{10 11} Any exaggerated vulnerability of the

Cotton wool spots (CWSs) are conspicuous lesions of the innermost retina that were first observed in hypertensive retinopathy soon after the invention of the ophthalmoscope. They are potential components of the fundus picture in a wide variety of systemic diseases, or they may accompany signs of retinal vascular occlusion. As such, CWSs often coexist with other retinopathic features like haemorrhages, lipid exudates or oedema, or they may be "isolated." They may be discovered singly (fig 1) or in groups of similar, or not so similar, appearance.

"Cytooid bodies" have long been recognised as the histological hallmark of a CWS (fig 2). A cytooid body (or end bulb of Cajal)^{1 2} represents the terminal swelling of a disrupted ganglion cell axon that has expanded up to 10-fold (to some 5–25 µm diameter) while becoming crammed with mitochondria and other subcellular material as a result of obstruction of axoplasmic transport.³ Otherwise called axonal flow, this is the bidirectional trafficking of cargoes of organelles and molecules between the cell body (or soma) of a neuron and the synapses formed by

Professor David McLeod,
Research Group in Eye &
Vision Science, School of
Medicine, University of
Manchester, UK

Correspondence to:
Professor David McLeod,
Academic Department of
Ophthalmology,
Manchester Royal Eye
Hospital, Oxford Road,
Manchester M13 9WH,
UK; david.mcleod@
manchester.ac.uk

Accepted for publication
12 November 2004

Abbreviations: CRA, central retinal artery; CRV, central retinal vein; CWS, cotton wool spot; FFA, fundus fluorescein angiography; RNFL, retinal nerve fibre layer; RPCP, radial peripapillary capillary plexus

Figure 1 Retinal cotton wool spot. The unusually large white lesion (left) appears to be “isolated” at first glance. It masks the fluorescence of the underlying choroid on fundus fluorescein angiography (right), as well as showing venular dye leakage. (As explained later, this CWS is a “boundary sentinel” of parapapillary infarction of one disc area following occlusion of an arteriole an order higher than a terminal arteriole arising near the origin of the main inferior branch of the central retinal artery. Orthograde transport blockade is demonstrated.)

RNFL to ischaemia is then thought to mirror the high metabolic demands of ionic pumping in the axolemma of the unmyelinated ganglion cell axons.¹¹

Modern descriptions of CWSs concede that “RNFL infarcts” don’t simply represent localised areas of ischaemic necrosis. They embrace the concept that, by one means or

another, the intra-axonal flow of organelles must be interrupted (in order to generate Cajal’s end bulbs) or, at the very least, it must be seriously impeded. Indeed, Tso and Jampol’s definition of a CWS—“a disturbance of both retrograde and orthograde axoplasmic transport...due to focal retinal ischaemia”—incorporates this notion.¹² So, focal ischaemia causes focal axonal damage and obstruction of axoplasmic transport, thus generating a CWS (fig 3). Put another way, terminal axonal swellings (which constitute a CWS) derive from terminal retinal arteriolar occlusion. But do they? Although evidence has been adduced from a variety of sources to reinforce the intuitive appeal of the focal ischaemia hypothesis, a pervasive illusion of certainty about the mechanism of CWS formation has arguably clouded the interpretation of much of these data.

Clinical observations

Fundus fluorescein angiography (FFA) appears to strengthen the case for equating CWSs with focal retinal ischaemia by revealing patches of hypofluorescence corresponding to each white lesion. This is often taken to indicate that capillary flow within the affected area of inner retina has terminated as a result of a microvascular occlusion. Because of its colour and reflectance, however, a CWS in the RNFL will mask the fluorescence in the underlying tissues (fig 1),¹³ so the apparent dye filling defect is, at least in part, consequential

Figure 2 Cytoid bodies in the human retina. Heavy laser photocoagulation has resulted in axotomy in the retinal nerve fibre layer (RNFL) and, several days later, grossly distended axon end bulbs as demonstrated by silver staining of a flat retinal preparation (above) and in light microscopic section at right angles to the course of the axons (below). The axon bundles are contained within meridional compartments whose lateral walls comprise linear arrays of Muller cell processes (arrows). The cytoid bodies protrude into the vitreous cavity as they become packed into the compartments between the glial partitions. a = cross section of a medium sized arteriole indenting the RNFL from below. (Courtesy of Professor John Marshall.) Note: The axon end bulbs have evolved on the disc-side aspect of the laser burns in these two examples and reflect obstructed retrograde axonal flow. The obverse (soma-side) aspect of the pathology has not been illustrated here.

Figure 3 The “focal ischaemia hypothesis” of cotton wool spot (CWS) generation. Occlusion of a terminal (or precapillary) branch of a retinal arteriole (red) is widely believed to result in a small area of infarction (grey) in the RNFL wherein both orthograde and retrograde axoplasmic transport in the ganglion cell axons is obstructed. Axon end bulbs (solid black circles), developing during the ensuing few days, will therefore “point in both directions” within the CWS while potentially elevating the internal limiting membrane. Note: within the ischaemic patch, soma-side axon end bulbs are located on the left and disc-side axon end bulbs are to the right. The arrows indicate the direction of continuing axonal flow after the ischaemic nerve injury (→, orthograde; ←, retrograde). The same convention has been adopted in figures 7, 9, and 12.

upon and not causally related to CWS formation. Furthermore, the area occupied by a CWS doesn't necessarily reflect the size of the occluded blood vessel(s) and the corresponding area of capillary non-perfusion. In diabetic retinopathy, for example, CWSs usually occupy only a fraction of the area of hypofluorescence.^{14 15}

Human histopathological studies

In malignant hypertension, Friedenwald (1949) reported that collections of cytooid bodies are often to be found "between the terminal bifurcation of a terminal arteriole" (a location that he thought was in keeping with retinal microinfarction),⁴ while Ashton and Harry (1963) noted that the small arterioles and associated capillaries in the locality of CWSs sometimes stain for lipid (which is also a major biochemical constituent of the cytooid bodies themselves).² However, fixed luminal narrowings or obstructions in these arterioles weren't a notable feature of flat mounts of the retina in hypertension.^{2 4 16–18} Indeed, in no retinopathy has there been convincing histopathological corroboration of focal ischaemia whereby CWSs have been shown to be coterminous with the territories supplied by demonstrably occluded precapillary arterioles.

On the contrary, Ashton postulated that, because the patches of capillary closure that co-locate with CWSs in hypertensive retinopathy bear no relation to the territories of individual retinal vessels, these localised areas of non-perfusion could just as well be a consequence, rather than the cause, of the massive axonal expansion.^{2 16 17} Clearly, the neuronal swelling must be constrained to some extent by Muller's radial glia whose basal processes align to form meridional septa that fasciculate the ganglion cell axons (fig 2).^{19–21} Elevation of the local tissue pressure and secondary capillary closure might then be regarded in a similar light to a "compartment syndrome," and a vicious cycle can also be envisaged whereby, once initiated, axon end bulbs packing within a fascicle might beget more end bulbs.

A further standard of proof has thus far failed to be satisfied. The focal ischaemia hypothesis requires that both orthograde and retrograde axonal transport are obstructed within each lesion, so a CWS should comprise collections of both soma-side and disc-side axon end bulbs (fig 3). Silver staining picks out (and thereby highlights the continuity of) a small proportion of the nerve fibres in flat retinal preparations (fig 2), and would be an effective method of demonstrating this co-localisation. To date, however, axon terminals "pointing in both directions" within a CWS haven't been reported using this technique.^{22 23}

Experimental retinal vasculopathies

Embolisation of the inner retinal circulation is often cited as providing compelling experimental evidence that CWSs derive from focal ischaemia, but this isn't borne out by the facts. Terminal arterioles are not "end arterioles" because the retinal capillary net has multiple precapillary arteriolar inputs.^{6 24} It is therefore unsurprising that ischaemic damage failed to ensue in the dog retina following embolic occlusion of individual terminal arterioles using latex microspheres of between 7 µm and 14 µm diameter.²⁵ For terminal arteriolar occlusion to give rise to microinfarction, the collateral circulation would have to be deficient owing to the inherently limited anatomical connections of the capillaries (as might obtain in the RPCP according to Henkind),^{6 9} or to simultaneous perturbation of capillary perfusion in adjacent met-arterioles (say from multiple embolisation),^{25 26} or to a background of generalised hypoperfusion (say from systemic hypotension or vasoconstriction) upon which a local occlusion was superimposed.⁸

Ashton and colleagues (1966) embolised the pig micro-circulation with larger glass microspheres (of 15–40 µm

diameter) and observed localised areas of pallor in the downstream territories of occluded vessels within an hour.²⁶ The translucency of the ischaemic patches then increased over the following 2–3 days before retinal transparency was restored a few days thereafter. The lesions were interpreted as being CWSs, but the self fulfilling prophecy—that, because CWSs (as seen clinically) are microinfarcts, then the retinal infarcts (induced experimentally by microembolisation) must be CWSs—was incorrect. The occluded arterioles were generally of a size at least an order higher than terminal arterioles. Moreover, the predominant inner retinal pathology was oncosis, a mode of cell death characterised morphologically by diffuse swelling of the neurons and their organelles and signifying a catastrophic loss of cellular homeostasis through failure of ionic pumping in the plasma membranes.²⁷ Organelle accumulation within expanded axons in the RNFL was restricted to zones at the margins of the inner retinal infarcts.²⁶

Equivalent ischaemic patches were subsequently induced by laser end arteriolar occlusion and afforded the opportunity to secure unequivocal proof of obstructed orthograde or retrograde axoplasmic transport along ischaemic boundaries crossed by ganglion cell axons.³ These experiments also showed that vitreous oxygenation can spare from oncosis and axoplasmic hold-up those axons that are located within the innermost reaches of the RNFL. The ischaemic lesions thus comprised inner retinal infarcts "bracketed" by collections of soma-side and disc-side axon end bulbs respectively. Unfortunately, however, the RNFL of the pig retina is so thin that these border zones of accumulated axoplasm couldn't be differentiated ophthalmoscopically from the intervening area of translucent oncotomic infarction (fig 4), whence the misapprehension had arisen 10 years earlier as to the nature of the ischaemic patches induced by embolisation.

CWSs of various shapes and sizes develop in the posterior retina of primates with experimental renovascular hypertension.^{28–30} They have been attributed to occlusive sequelae of autoregulatory vasoconstriction in superficial arterioles of varying diameters, ostensibly to protect the RPCP against hyperperfusion. Focal hypofluorescence on FFA was thought to provide strong supplementary evidence for the focal ischaemia hypothesis of CWS generation, this despite an acknowledgement of axoplasmic masking.^{29 30} Furthermore, while ultrastructural examination confirmed that the white lesions were indeed collections of cytooid bodies, no information was forthcoming as to the direction(s) in which the axon end bulbs were pointing.^{18 28}

A DIFFERENT VIEWPOINT: COTTON WOOL SPOTS AS "SENTINEL" LESIONS

The continuing viability of (and axoplasmic transport within) axon segments contiguous with the site of axonal interruption determines the build up of organelles within a cytooid body.^{3 31} By shifting one's focus to the dynamic pathology of axonal flow obstruction, a variety of mechanisms can be revealed whereby disturbed neurovascular inter-relations in the RNFL will result in CWS formation.

Cotton wool spots as boundary sentinels of inner retinal ischaemia

After acute occlusion of a branch retinal end arteriole (which, by definition, exclusively supplies a circumscribed area of inner retina), one or more opaque lesions will evolve in the RNFL at the margin of grey inner retinal oncosis that marks the territory of the occluded vessel in such patients (figs 5, 6). These lesions result from obstruction of *either* orthograde *or* retrograde axoplasmic transport,^{3 31–34} and gaps will be seen where no ganglion cell axons cross the ischaemic boundary. This might be where the axons run parallel to the boundary

Figure 4 Comparative effects of occlusion of a small branch retinal end arteriole. Immediately after laser coagulation of a small arteriole in the pig retina, the capillary bed downstream from the occlusion shows non-filling with dye during the early venous phase of FFA (left). Two days later, a relatively homogeneous patch of retinal translucency has developed (middle). This pale lesion (of an area twice that of the optic disc) is not a single CWS but is a composite of inner retinal oncosis sandwiched between two zones of axoplasmic debris accumulation in the RNFL, as verified by histology and autoradiography.³ An equivalent optic disc-sized ischaemic patch in the human macula (right) comprises a central grey ischaemic strip, partially masked by haemorrhage, that is sandwiched between two white CWSs (arrowheads). The two boundary sentinels of this “bracketed infarct” comprise collections of soma-side (peripheral) and disc-side axon end bulbs respectively.

(figs 5, 6), or at the fovea (fig 5), or along the temporal horizontal raphe.^{3 31} Whereas clinical signs of oncosis appear within an hour of vascular occlusion, the accumulating axoplasm doesn’t become clearly evident until 6–18 hours later (fig 5). The amount of axoplasmic debris that finally builds up will depend primarily on the number of axons still actively transporting axoplasm and, therefore, on the thickness of the RNFL at the point of injury (fig 6).³ The uniform width of the white border (of the order of 200–300 µm) implicates a structural constraint to tissue expansion, presumably packing of axon end bulbs into the compartments formed by the radial glia (figs 1, 2, 5, 6).

Inner retinal transparency takes 7–14 days to be restored after oncotic infarction whereas any related axoplasmic debris takes 3–6 weeks to be phagocytosed. The signs of

Figure 6 Spatial and temporal features of inner retinal infarction. The oblique boundary of oncotic infarction inferonasal to the fovea has a shiny white margin (reflecting obstruction of orthograde axoplasmic transport) except for a gap where the ganglion cell axons run parallel to the ischaemic interface (left). The extent of opacification along this boundary reflects the relative thickness of the RNFL, the least accumulation of axoplasmic debris occurring where the RNFL is thinnest. Two weeks later (right) the CWSs appear to be “isolated” as the signs of oncosis have faded. The upper boundary sentinel is of uniform width but is unusually long. (Courtesy of Mr Michael Sanders.)

Figure 5 Temporal and spatial features of inner retinal infarction. A few hours after the onset of cilioretinal infarction from non-ischaemic central retinal vein (CRV) occlusion,³⁴ axoplasmic debris is just beginning to accumulate along those borders of a grey parapapillary oncotic infarct that are crossed by ganglion cell axons (top left). Two days later (top right), CWS formation from obstructed orthograde axoplasmic transport in the papillomacular bundle is fully established. Three weeks later the signs of oncosis have disappeared and the fading boundary sentinels appear to be “isolated” at this stage (bottom left). In bottom right (the same study as top right), “axoplasmic cuffing” (arrow) has evolved as a result of dilation and angulation of a major branch of the central retinal vein. (Courtesy of Dr Barry Cullen.)

oncotic necrosis having earlier disappeared, the CWSs will then appear to be “isolated” for the remainder (and indeed the majority) of the period during which there is fundusoscopic expression of inner retinal swelling after branch end arteriolar occlusion (figs 1, 5, 6). Thus, when a CWS of uniform width, and of length >1 mm, is discovered, it is likely to be a “boundary sentinel” (fig 7),³—that is to say, the CWS will be standing sentinel over an area of ischaemia that is larger (figs 1, 5), and potentially far larger (fig 6), than the CWS itself. Nevertheless, the appearance of the sentinel gives little indication as to the size of the infarct.

Contemporaneous obstruction of both orthograde and retrograde axonal flow is seldom observed. This is because the larger blood vessels and axon bundles in the RNFL follow a similar retinal course and limited numbers of neurons cross ischaemic interfaces after most retinal vascular occlusions. One boundary of the infarct may be located in the retinal periphery or along the horizontal raphe, or it may be embedded within the optic disc as is evident, for example, after occlusions of cilioretinal arterioles (fig 5) or early branches of the CRA (fig 1). Where two ischaemic boundaries are located close together elsewhere within the posterior retina (for example, in relation to an infarct of the size of the optic disc), no major difference tends to be discernible in the

Figure 7 The cotton wool spot as a boundary sentinel of inner retinal infarction. Embolic or thrombotic occlusion of a branch retinal end arteriole has resulted in oncotic inner retinal infarction. The axon end bulbs here reflect obstruction of retrograde axoplasmic transport along the disc-side aspect of the infarct. The CWS will generally be strikingly conspicuous on funduscopy in comparison with the infarct. Vitreous oxygenation may spare axons running immediately beneath the internal limiting membrane from anoxic damage.

appearance of the axoplasmic debris that accumulates along the soma-side and disc-side margins of the bracketed infarct (fig 4).^{3 35}

A distinctive pattern of retinal ischaemia characterises pre-proliferative (or severe non-proliferative) diabetic retinopathy wherein an extensive area of capillary non-perfusion develops throughout the retinal mid-periphery and beyond.^{15 36 37} This process is accompanied by the formation of comparatively small CWSs that are unusually slow to resolve.^{5 14} By making a composite of their locations in several patients,⁵ or as may be observed in individual patients,³⁷ the CWSs are typically distributed in an annulate pattern just external to the major vascular arcades and nasal to the optic disc (fig 8). The circle tends to be deficient temporally, resulting in a C-shaped configuration of the lesions.^{5 37} The location of this chain of boundary sentinels approximates to the interface between the perfused microcirculation of the central retina and the non-perfused capillary bed more peripherally. These extramacular CWSs are accumulations of axoplasmic debris indicating retrograde axoplasmic transport obstruction at sites where the RNFL is relatively thin.

Gaps are seen in what might otherwise have become a ring of axoplasm wherever the ischaemic interface is crossed by

Figure 8 Extramacular cotton wool spots (CWSs) in pre-proliferative diabetic retinopathy. Part of a chain of CWSs is seen in the superonasal quadrant of the left eye. Each CWS has evolved through obstruction of retrograde axoplasmic transport. The boundary sentinels lie in between radially directed retinal vessels, and each is of similar vintage.

medium sized blood vessels that radiate out towards the equator, including those branching from the major vascular arcades. These gaps appear to reflect direct tissue oxygenation across the walls of the vessels, allowing retrograde axonal flow to penetrate beyond the circle along perivascular corridors. Supportive evidence derives from histopathological documentation of sparing of a mantle of inner retinal tissue immediately surrounding arterioles that traverse areas of diabetic capillary closure³⁸ and from the preservation of retinal light sensitivity around such patent vessels.³⁹ The broader temporal gap in the chain of CWSs reflects the thinness of the RNFL on either side of the horizontal raphe and the fact that, there, the arcuate course of the axons tends to bypass the ischaemic interface.

The simultaneous occurrence of several CWSs, each of similar vintage (fig 8), indicates rapid progression towards proliferative retinopathy and rubeosis iridis.¹⁴ These neovascular consequences don't arise unless or until a substantial proportion of the retinal capillaries are non-perfused.^{36 40} Outgrowths of new vessels then emerge from retinal venules where they cross the same ischaemic interface as that which determined the site of earlier axoplasmic transport obstruction.⁴¹

Cotton wool spots as sentinels of an ischaemic penumbra

Further insights into the genesis of CWSs derive from clinical study of instances where the ischaemic interface is less well delineated. A profound reduction in perfusion pressure in the central retinal artery (CRA), for example, will cause the peripheral retinal circulation to be reduced to a trickle, but autoregulatory vasodilation in the immediate environs of the optic disc may be sufficient to maintain a zone of peripapillary retinal viability.^{32 33 42-44} This pattern of hypoperfusion is an inevitable consequence of the progressive increase in inner retinal volume (and the associated expansion of the retinal vascular bed) that occurs with increasing distance from the optic disc.⁴⁵ The arteriovenous perfusion pressure is necessarily the greatest around the disc, which is also where the arteriovenous pathways are the shortest, so a meridional metabolic gradient will arise from increasing oligoemia giving way to peripheral retinal ischaemia.⁴² Axoplasmic debris will accumulate at some point along this gradient as a result of retrograde transport obstruction (fig 9).

The CWSs that develop during acute panretinal hypoperfusion are typically disseminated in an irregular circle or oval at a variable distance from, and centred just temporal to, the optic disc (fig 10).^{42 43} This annulate pattern is a consequence of deferral or displacement of transport obstruction into the peripapillary RNFL from the edge of the optic disc where retrograde flow obstruction occurs after complete CRA occlusion.^{33 35 46 47} More obvious temporally, the CWSs tend to be polymorphous and are sometimes >300 µm in width (fig 10), presumably reflecting the gradual change from RNFL viability around the disc to peripheral non-viability (or at least from an ability, to an inability, to sustain retrograde axonal flow).

Evidence of *generalised* inner retinal hypoperfusion can be drawn from the observation of a delayed and retarded dye transit on FFA^{42 48} associated with a reduction in the electroretinogram b-wave, an increase in retinal oxygen extraction (causing exaggerated cyanosis in the retinal veins), and a relative afferent pupillary defect unless, of course, the ischaemia is bilateral.^{42 43} The focal hypofluorescence reflects dye masking by accumulated axoplasm and, possibly, localised closure of already hypoperfused capillaries secondary to RNFL expansion. The mechanism of CWS generation thus proposed stands up well against the alternative explanation for the fundus signs based on the

Figure 9 The cotton wool spot as a penumbral sentinel in panretinal hypoperfusion. Acute hypoperfusion of the CRA, with slow flow along its branches, creates an ischaemic gradient affecting progressively more peripheral locations in the inner retina. Retrograde axoplasmic transport is obstructed in the penumbral zone abutting the disc-side aspect of neural infarction. Direct oxygenation of the RNFL across the wall of the arteriole permits axon end bulbs to become embedded within the ischaemic retina.

focal ischaemia hypothesis. This would require the simultaneous onset of occlusions of multiple, superficial, peripapillary, precapillary arterioles of varying sizes, each giving rise to ischaemic spots in the RNFL adjacent to (but independent of) diffuse peripheral retinal infarction. Moreover, this retinopathic pattern has been reported in giant cell arteritis,^{43–49} in which condition the intraocular branches of the CRA aren't directly affected by inflammatory infiltration.

The CRA may be partially occluded in its end arterial course or it may be occluded outside the globe where, even if the luminal obstruction is complete, its effects might be mitigated by a substantial collateral circulation. Putative mechanisms of CRA occlusion include embolism and hypertensive vasospasm as well as giant cell arteritis.⁴² The responsible pathology might otherwise be located in the ophthalmic artery or in the carotid artery.^{6,7,50–54} Interestingly, ligation of both carotid arteries in rat strains with limited collateral flow through the circle of Willis produced an equivalent fundus picture 2 days post-occlusion associated with a marked retardation in dye transit on FFA.⁵⁵ In most animals, retinal "whitening" (here presumed to be accumulated axoplasm) developed in a wide zone around the optic

discs bilaterally but, redolent of the pattern of clinical presentation, the axoplasmic debris was restricted to the immediate peripapillary retina if the ischaemia was particularly severe.

Clinically, CWSs sometimes develop at an even greater distance from the optic disc, especially in the vicinity of the major temporal vascular arcades. Indeed, some of these lesions may become embedded within the ischaemic retina instead of demarcating it (figs 9, 10). Once again, this phenomenon appears to be attributable to the efficient diffusion of oxygen into the neuroretina across the walls of retinal arterioles.^{6,42–56} Retrograde axonal flow alongside these vessels will continue thereby until the course of the axon bundles diverges from that of the vessels, say at the bifurcation of an arteriole. A CWS will then evolve just beyond the vascular fork (fig 10).⁴² A mantle of neural tissue sometimes survives alongside retinal arterioles in otherwise atrophic inner retina after CRA occlusion⁵⁷ or after carotid artery occlusion.⁵⁸ This provides histopathological support for the precepts underpinning the embedding phenomenon.

The fundus picture of acute panretinal hypoperfusion typically resolves in 4–6 weeks, for the greater part of which time the CWSs will appear to be "isolated." Visual acuity may also recover remarkably in a similar time frame despite the development of optic atrophy and RNFL thinning.^{6,42–43} This partial recovery of vision is in keeping with the notion that the peripapillary area of retained inner retinal viability is separated from unsalvageable ischaemic retina by a zone of oligaemia wherein the neuronal tissue maintains its structural integrity while losing its capacity to function (at least temporarily). This zone corresponds to the "penumbra" in clinical stroke or after experimental middle cerebral artery occlusion,^{59,60} albeit in the brain the functionally silent tissue surrounds the infarct at its core. In the inner retina, nutrients and oxygen from the vitreous and the choroid are also likely to contribute to the metabolic gradients that arise. In due course, neurons within the penumbra may become necrotic through oxygen dependent self destruct mechanisms ("apoptosis"). This will cause the area of infarction to expand but with none of the clinical morphological changes associated with oncosis. Alternatively, the penumbral tissue may recover (for example, through relatively prompt reperfusion) leading to a greater or lesser degree of visual restoration and avoidance of longer term sequelae such as preretinal neovascularisation and rubeosis iridis.

Figure 10 Penumbral sentinels from acute panretinal hypoperfusion. An annulate pattern of polymorphous CWSs, including embedded lesions (arrow), evolves after partial CRA occlusion (left); a small cilioretinal arteriole crossing the inferotemporal disc margin, has contributed to deferral of retrograde axoplasmic transport blockade into the macular retina. Some of the CWSs are over 1 mm in width, and the shape of these penumbral sentinels contrasts with those of the boundary sentinels in figures 4 and 5. The oncotic swelling of the inner retina peripheral to the CWSs is heterogeneous, with perivascular sparing and a poorly developed cherry red spot at the fovea. After ischaemic CRV occlusion (right), retrograde axoplasmic transport blockade produces a similar pattern of CWS formation.

Figure 11 Purtscher's traumatic retinal angiopathy. The left fundus in each case shows peripapillary CWSs and haemorrhages, but no signs of generalised inner retinal oedema, between 1 and 2 weeks after chest and head injury. (Courtesy of Mr Nicholas Jones.)

That these CWSs thus represent “penumbral sentinels” invites speculation that endogenous neuroprotection might influence ischaemic manifestations in the fundus. A previous period of sublethal ischaemia or hypoxia, for example, is known to be capable of modifying the response of neural tissue to a subsequent ischaemic challenge by inducing metabolic downregulation and upregulation of protective growth factors.^{59–61} Through this temporary adaptation (lasting several days), tissue that would otherwise have suffered oncotic infarction will follow the alternative apoptotic route to necrosis or may even survive. Thus, the volume of inner retinal infarction arising after prolonged ischaemia may be significantly reduced but retrograde axoplasmic transport blockade in the RNFL (with CWS formation) can still be predicted. By this means, the CWSs may be signalling that, in order to improve the chances of neuronal survival, the energy metabolism of the retinal tissue has diminished to complement the reduced level of perfusion, a process that is effectively the converse of circulatory autoregulation, whereby tissue perfusion matches metabolism.

An annulate pattern of retrograde axoplasmic transport blockade, similar to that following partial CRA occlusion, often develops after severe occlusion of the central retinal vein (CRV) although some of the signs of ischaemia may be obscured by intraretinal haemorrhage (fig 10),^{5 42 62} The CWSs associated with ischaemic CRV occlusion should therefore be regarded as penumbral sentinels and not as expressions of focal ischaemia. Even if the luminal blockage in the CRV is relieved or bypassed, however, widespread intracapillary thrombosis usually prevents reperfusion of the retinal capillary net,⁶³ and neovascular glaucoma is likely to follow as a result. Less severe (non-ischaemic) CRV occlusion has little or no effect on axoplasmic transportation in the territory of the CRA, enabling axoplasmic debris to accumulate at ischaemic interfaces with the cilioretinal circulation (fig 5)^{31 34} and/or around angulated retinal veins (see below). Thus, the CWSs that accompany CRV occlusions aren't necessarily (penumbral) sentinels of severe panretinal hypoperfusion.

Cotton wool spot generation from vasoneural compression

Where axons in the RNFL encounter retinal veins that have become acutely tortuous, focal accumulations of axoplasmic debris may sometimes be seen that can be attributed to a disturbance of normal neurovascular anatomy. As noted, the larger retinal blood vessels and bundles of tightly packed ganglion cell axons tend to run a parallel course, the vessels generally being located beneath the RNFL.^{6 10 64} However, if axon bundles cross the path of vessels that are indenting the RNFL from below (fig 2), the axon fascicles will splay open

before regrouping on the far side of the artery or vein.⁶⁴ This splaying is believed to confer a degree of protective deformability of RNFL structure that obviates vasoneural compression. Nevertheless, it appears that this deformability can indeed be overcome and axon bundles can be compromised when segments of retinal veins suddenly impinge on the RNFL, resulting in “axoplasmic cuffing” of the veins (fig 5).

Axonal splaying at neurovascular crossings and associated breaches in glial septation are a characteristic feature of the peripapillary retina. Here the major retinal vessels and their larger side branches plunge obliquely through the RNFL from their superficial location on the optic disc in order to assume their usual position beneath the RNFL elsewhere in the fundus.^{11 21 64} Again, this neurovascular interaction may be the anatomical basis for CWS generation. For example, a plethora of CWSs in an annulate distribution some 2–4 mm in radius around the disc sometimes evolves in the immediate aftermath of hyperacute elevation of central (intrathoracic) venous pressure such as might derive from severe chest compression (fig 11).^{65–69}

The CWSs in Purtscher's traumatic retinal angiopathy have generally been attributed to multifocal retinal arteriolar occlusion,^{68 69} but the long held suspicion—that reflux of venous blood through the valveless jugular veins and cavernous sinus somehow underpins these changes^{65–69}—may well be correct. Transient supraphysiological hypertension within, and passive hyperdistension of, the thin walled

Figure 12 Cotton wool spot generation in Purtscher's traumatic retinal angiopathy. A branch retinal venule (blue), traversing the peripapillary RNFL, is postulated to have become acutely hyperdistended from transmission of transient extreme elevation of the intrathoracic venous pressure to the eye. Compression of adjacent axon bundles will result in obstruction of both orthograde and retrograde axoplasmic transport, with subsequent formation of “duplex” CWSs on the soma-side and disc-side aspects of the oblique venular segment.

retinal veins in the peripapillary RNFL might cause compression damage to the axon bundles if their innate protective deformability was to be overwhelmed. CWSs of “duplex” composition, from obstruction of axoplasmic transport on each aspect of the relevant venous segment, would then evolve over the following 48 hours or so as a legacy of the incident (fig 12). Indeed, in one published instance, early manifestations of axoplasmic debris accumulation were photographed on the disc-side of retinal veins within 2–3 hours of an automobile accident.⁶⁷ The lesions then expanded into dumbbell-shaped cotton wool patches straddling the veins and giving every indication of having arisen through obstruction of bi-directional axoplasmic transport.

The precise distribution and degree of bilateral symmetry of the CWSs will depend upon postural and anatomical factors in the neck (governing the transmission of elevated central venous pressure to the eyes)⁶⁹ and microanatomical features in the RNFL (such as the number and sites of neurovascular crossings and the limits of glial decompartmentalisation at these locations). Uveal engorgement, raised intraocular pressure, reflex arteriolar constriction, and sub-membranous intraretinal haemorrhages are other potential accompaniments. However, generalised inner retinal oncosis with cherry red spot formation, as seen after retinal fat embolism or in other “Purtscher-like” retinopathies,^{51 70 71} isn’t a feature (fig 11).⁶⁶ Otherwise, short term continuance of orthograde axonal flow in the RNFL wouldn’t be possible and soma-side axon end bulbs wouldn’t evolve.

CONCLUSION

Axoplasmic transportation in the RNFL can be obstructed in a variety of circumstances and by various means, both vascular and mechanical. The sentinel lesions that arise may favour the arterioles (as in acute panretinal hypoperfusion) or the venules (as in Purtscher’s traumatic retinal angio-pathy) or they may occupy the spaces between the arterioles and venules (as in pre-proliferative diabetic retinopathy). CWSs of uniform width and relatively long length are usually sentinels of oncotic inner retinal infarction after occlusions of branch end arterioles, and two such boundary sentinels may bracket a small infarct of the size of the optic disc. While unproved, the possibility remains that CWSs sometimes reflect occlusions of the smallest (terminal) retinal arterioles. However, this mechanism has no more basis in theory than several other mechanisms, and then only in the context of a restricted collateral microcirculation. What is certain is that, in practice, CWSs are frequently described as “RNFL infarcts” in circumstances in which they plainly aren’t, and often because the associated hypofluorescence on FFA is mistakenly taken to signify focal ischaemia. High resolution optical coherence tomography⁷² and fundus oximetry⁷³ may help to clarify some of the issues surrounding CWS formation in due course.

But does it really matter that CWSs are misconstrued as RNFL infarcts when their presence should anyway alert the clinician to the probability that the patient has significant underlying systemic disease?^{44 74 75} Well, yes! But isn’t this just semantic quibbling? Well, no! Such an oversimplification of the mechanism of CWS generation denies any in-depth appreciation of the diversity of neurovascular interactions in the retina and the ongoing life and death struggles that are integral to the evolution of these distinctive fundus features. Appreciating that CWSs are sentinel lesions may well become important, for example, in planning novel pharmacological interventions such as the local delivery of neuroprotective therapies to the retina in the future. Thus, although the “focal ischaemia hypothesis” of CWS generation has become thoroughly entrenched during the past 50 years, a broader perspective is now called for. By virtue of their characteristic

reflectance and generic neuropathological basis (and setting aside their diverse aetiology and varied morphology), these lesions should be redesignated “cotton wool sentinels.” Freeing the CWS from the conceptual straightjacket of the “RNFL infarct” should also foster our establishing the pathophysiological basis of the many other retinopathies of which CWSs are a component part, not least those associated with human immunodeficiency virus infection and systemic hypertension.

ACKNOWLEDGEMENTS

I am grateful to the Ophthalmic Imaging Department, Manchester Royal Eye Hospital and to Anne Corless of Tembo Medical Graphics for the illustrations, and to Dr John Harry and to departmental colleagues for constructive criticism of this article. There are no competing interests to declare.

REFERENCES

- 1 Ramon Y Cajal S. *Degeneration and regeneration of the nervous system*. Translated by May RM. London: Oxford University Press, 1928.
- 2 Ashton N, Harry J. The pathology of cotton-wool spots and cytooid bodies in hypertensive retinopathy and other diseases. *Trans Ophthalmol Soc UK* 1963;**83**:91–114.
- 3 McLeod D, Marshall J, Kohner EM, et al. The role of axoplasmic transport in the pathogenesis of retinal cotton-wool spots. *Br J Ophthalmol* 1977;**61**:177–91.
- 4 Friedenwald JS. A new approach to some problems of retinal vascular disease. *Am J Ophthalmol* 1949;**32**:487–98.
- 5 Mansour AM, Jampol LM, Logani S, et al. Cotton-wool spots in acquired immunodeficiency syndrome compared with diabetes mellitus, systemic hypertension and central retinal vein occlusion. *Arch Ophthalmol* 1988;**106**:1074–7.
- 6 Wise GN, Dollery CT, Henkind P. *The retinal circulation*. New York: Harper and Row, 1971.
- 7 Hollenhorst RW. Ocular manifestations of insufficiency or thrombosis of the internal carotid artery. *Am J Ophthalmol* 1959;**47**:753–67.
- 8 Klein BA. Comments on the cotton-wool lesion of the retina. *Am J Ophthalmol* 1965;**59**:17–23.
- 9 Henkind P. Radial peripapillary capillaries of the retina: I. Anatomy: human and comparative. *Br J Ophthalmol* 1967;**51**:115–23.
- 10 Shimizu K, Ujiie K. *Structure of ocular vessels*. New York: Igaku-Shoin, 1978.
- 11 Snodderly DM, Weinhaus RS, Choi JC. Neural-vascular relationships in central retina of macaque monkeys (*Macaca fascicularis*). *J Neurosci* 1992;**12**:1169–93.
- 12 Tso MOM, Jampol LM. Pathophysiology of hypertensive retinopathy. *Ophthalmology* 1982;**89**:1132–45.
- 13 Dollery CT, Hodge JV. Hypertensive retinopathy studied with fluorescein. *Trans Ophthalmol Soc UK* 1963;**83**:115–26.
- 14 Kohner EM, Dollery CT, Bulpitt CJ. Cotton-wool spots in diabetic retinopathy. *Diabetes* 1969;**8**:691–704.
- 15 Bresnick GH, Engerman R, Davis MD, et al. Patterns of ischaemia in diabetic retinopathy. *Trans Am Acad Ophthalmol Otolaryngol* 1976;**81**:694–709.
- 16 Ashton N. Diabetic retinopathy: a new approach. *Lancet* 1959;**2**:625–30.
- 17 Ashton N. Pathophysiology of retinal cotton-wool spots. *Br Med Bull* 1970;**26**:143–50.
- 18 Ashton N. The eye in malignant hypertension. *Trans Am Acad Ophthalmol Otolaryngol* 1972;**76**:17–38.
- 19 Pedler C. The inner limiting membrane of the retina. *Br J Ophthalmol* 1961;**45**:423–38.
- 20 Radius RL, Anderson DR. The histology of retinal nerve fiber layer bundles and bundle defects. *Arch Ophthalmol* 1979;**97**:948–50.
- 21 Quigley HA, Addicks EM. Quantitative studies of retinal nerve fiber layer defects. *Arch Ophthalmol* 1982;**100**:807–14.
- 22 Wolter JR, Goldsmith RI, Phillips RL. Histopathology of the star-figure of the macular area in diabetic and angiospastic retinopathy. *Arch Ophthalmol* 1957;**57**:376–85.
- 23 Wolter JR. Pathology of a cotton-wool spot. *Am J Ophthalmol* 1959;**48**:473–85.
- 24 Henkind P, Dollery CT. Pathophysiology of the retinal vascular bed following acute embolisation. *Invest Ophthalmol* 1966;**5**:204–7.
- 25 Gay AJ, Goldor H, Smith M. Chorioretinal vascular occlusions with spheres. *Invest Ophthalmol* 1964;**3**:647–56.
- 26 Ashton N, Dollery CT, Henkind P, et al. Focal retinal ischaemia: ophthalmoscopic, circulatory, and ultrastructural changes. *Br J Ophthalmol* 1966;**50**:281–384.
- 27 Majno G, Joris I. Apoptosis, oncosis and necrosis; an overview of cell death. *Am J Pathol* 1995;**146**:3–15.
- 28 Garner A, Ashton N, Tripathi R, et al. Pathogenesis of hypertensive retinopathy; an experimental study in the monkey. *Br J Ophthalmol* 1975;**59**:3–44.
- 29 Hayreh SS, Servais GE, Virdi PS. Cotton-wool spots (inner retinal ischemic spots) in malignant arterial hypertension. *Ophthalmologica* 1989;**198**:197–215.
- 30 Hayreh SS. Hypertensive fundus changes. In: Guyer DR, et al, eds. *Retina-vitreous-macula*. Chapter 27. Philadelphia: WB Saunders, 1999:345–68.

- 31 McLeod D. Clinical sign of obstructed axoplasmic transport. *Lancet* 1975;**2**:954–6.
- 32 McLeod D. Retinal ischaemia, disc swelling, and axoplasmic transport. *Trans Ophthalmol Soc UK* 1976;**96**:313–18.
- 33 McLeod D. Ophthalmoscopic signs of obstructed axoplasmic transport after ocular vascular occlusions. *Br J Ophthalmol* 1976;**60**:551–6.
- 34 McLeod D, Ring CP. Cilio-retinal infarction after retinal vein occlusion. *Br J Ophthalmol* 1976;**60**:419–27.
- 35 Pieh C, Safran AB. Blockage of retrograde axonal flow after retinal artery occlusion. *Arch Ophthalmol* 2003;**121**:1508–9.
- 36 Shimizu K, Kobayashi Y, Muraoka K. Mid-peripheral fundus involvement in diabetic retinopathy. *Ophthalmology* 1981;**88**:601–12.
- 37 Verdaguer J, le Clercq N, Holuigue J, et al. Nonproliferative diabetic retinopathy with significant capillary nonperfusion. *Graefes Arch Clin Exp Ophthalmol* 1987;**225**:157–9.
- 38 Bek T. Transretinal histopathological changes in capillary-free areas of diabetic retinopathy. *Acta Ophthalmol* 1994;**72**:409–15.
- 39 Bek T. Localised retinal morphology and differential light sensitivity in diabetic retinopathy. Methods and clinical results. *Acta Ophthalmol* 1992;(Supp 207):1–36.
- 40 Patz A. Clinical and experimental studies on retinal neovascularisation. XXXIX Edward Jackson memorial lecture. *Am J Ophthalmol* 1982;**94**:715–43.
- 41 Wong HC, Sehmi KS, McLeod D. Abortive neovascular outgrowths discovered during vitrectomy for diabetic vitreous haemorrhage. *Graefes Arch Clin Exp Ophthalmol* 1989;**227**:237–40.
- 42 Oji EO, McLeod D. Partial central retinal artery occlusion. *Trans Ophthalmol Soc UK* 1978;**98**:156–9.
- 43 McLeod D, Oji EO, Kohner EM, et al. Fundus signs in temporal arteritis. *Br J Ophthalmol* 1978;**62**:591–4.
- 44 Brown GC, Brown MM, Hiller T, et al. Cotton-wool spots. *Retina* 1985;**5**:206–14.
- 45 Ernest JT, Krill AE. The effect of hypoxia on visual function: psychophysical studies. *Invest Ophthalmol* 1971;**10**:323–8.
- 46 Wolter JR. The centrifugal nerves in the human optic tract, chiasm, optic nerve, and retina. *Trans Am Ophthalmol Soc* 1965;**63**:680–707.
- 47 Radius RL, Anderson DR. Morphology of axonal transport abnormalities in primate eyes. *Br J Ophthalmol* 1981;**65**:767–77.
- 48 David NJ, Norton EWC, Gass JD, et al. Fluorescein retinal angiography in carotid occlusion. *Arch Neurol* 1966;**14**:281–7.
- 49 McLeod D, Kohner EM. Ophthalmoscopic signs in temporal arteritis. *N Engl J Med* 1977;**297**:1180.
- 50 Miller SJH. Carotid insufficiency. *Trans Ophthalmol Soc UK* 1960;**80**:287–99.
- 51 Hedges TR, Knapp P, Schenk H. Ophthalmoscopic findings in internal carotid artery occlusion. *Am J Ophthalmol* 1963;**55**:1007–12.
- 52 Mooney A, Mooney D. Cotton wool spots in retina following carotid ligations. *Arch Ophthalmol* 1965;**373**:746–7.
- 53 Worrall M, Atebara N, Meredith T, et al. Bilateral ocular ischemic syndrome in Takayasu disease. *Retina* 2001;**21**:75–6.
- 54 Ong TJ, Paine M, O'Day J. Retinal manifestations of ophthalmic artery hypoperfusion. *Clin Exp Ophthalmol* 2002;**30**:284–91.
- 55 Spertus AD, Slakter JS, Weissman SS, et al. Experimental carotid occlusion: funduscopy and fluorescein angiographic findings. *Br J Ophthalmol* 1984;**68**:47–57.
- 56 Buerk DG, Shonat RD, Riva CE, et al. O₂ gradients and countercurrent exchange in the cat vitreous humor near retinal arterioles and venules. *Microvasc Res* 1993;**45**:134–48.
- 57 Zimmerman LE. Embolism of the central retinal artery. *Arch Ophthalmol* 1965;**73**:822–6.
- 58 Font RL, Naumann G. Ocular histopathology in pulseless disease. *Arch Ophthalmol* 1969;**82**:784–8.
- 59 Chen J, Simon R. Ischaemic tolerance in the brain. *Neurology* 1997;**48**:306–11.
- 60 Stenzel-Poore MP, Stevens SL, Xiong Z, et al. Effect of ischaemic preconditioning on genomic response to cerebral ischaemia: similarity to neuroprotective strategies in hibernation and hypoxia-tolerant states. *Lancet* 2003;**362**:1028–37.
- 61 Roth S, Li B, Rosenbaum PS, et al. Preconditioning provides complete protection against retinal ischaemic injury in rats. *Invest Ophthalmol Vis Sci* 1998;**39**:777–85.
- 62 Wolter JR. Retinal pathology after central retinal vein occlusion. *Br J Ophthalmol* 1961;**45**:683–94.
- 63 Hockley DJ, Tripathi RC, Ashton N. Experimental retinal branch vein occlusion in rhesus monkeys. III. Histopathological and electron microscopical studies. *Br J Ophthalmol* 1979;**63**:393–411.
- 64 Zhang X, Mitchell C, Wen R, et al. Nerve fiber layer splaying at vascular crossings. *Invest Ophthalmol Vis Sci* 2002;**43**:2063–6.
- 65 Stokes WH. Unusual retinal vascular changes in traumatic injury of the chest. *Arch Ophthalmol* 1932;**7**:101–8.
- 66 Marr WG, Marr EG. Some observations on Purtscher's disease: traumatic retinal angiopathy. *Am J Ophthalmol* 1962;**54**:693–705.
- 67 Kelley JS. Purtscher's retinopathy related to chest compression by safety belts. *Am J Ophthalmol* 1972;**74**:278–83.
- 68 Orzalesi N, Coghe F. Obstructed axoplasmic transport in Purtscher's traumatic retinopathy. *Ophthalmologica* 1980;**180**:36–45.
- 69 Archer DB, Earley OE, Page AB, et al. Traumatic retinal angiopathy—associated with wearing of car seat belts. *Eye* 1988;**22**:650–9.
- 70 DeVoe AG. Ocular fat embolism: a clinical and pathological report. *Arch Ophthalmol* 1950;**43**:857–63.
- 71 Blodi BA, Johnson MW, Gass JDM, et al. Purtscher's-like retinopathy after childbirth. *Ophthalmology* 1990;**97**:1654–9.
- 72 Gloesmann M, Hermann B, Schubert C, et al. Histologic correlation of pig retina radial stratification with ultrahigh-resolution optical coherence tomography. *Invest Ophthalmol Vis Sci* 2003;**44**:1696–703.
- 73 Crittin M, Schmidt H, Riva CE. Hemoglobin oxygen saturation (So₂) in the human ocular fundus measured by reflectance oximetry: preliminary data in retinal veins. *Klin Monatsbl Augenheilkd* 2002;**219**:289–91.
- 74 Wong TY, Klein R, Couper DJ, et al. Retinal microvascular abnormalities and incident stroke: the Atherosclerosis Risk in Communities Study. *Lancet* 2001;**358**:1134–40.
- 75 Wong TY, Klein R, Sharrett AR, et al. Retinal microvascular abnormalities and cognitive impairment in middle-aged persons: the Atherosclerosis Risk in Communities Study. *Stroke* 2002;**33**:1487–92.