Ocular Neovascularization
The Scientific Basis of Medicine

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University of London Audio-Visual Centre, 1976.
Made for British Postgraduate Medical Federation.

Produced by Michael Clarke.

Black-and-white
Duration: 00:26:09:18

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<Opening titles>

<Garner to camera>

To have blood vessels in tissues which rely on them for their metabolic requirements is one thing, their presence in tissues which don't is quite another. While in the first case they provide an essential lifeline, in the second they are not only superfluous but can create serious problems. Parts of the eyes which are not normally vascularised, for instance, are particularly vulnerable in this respect. The cornea in health is not normally vascularised since it receives its metabolic requirements via a process of diffusion from the immediate surroundings.

<Garner narrates over a series of close-up photographs of eyes referring to lesions with an indicator stick>

But should the cornea be damaged for any reason, perhaps due to trauma or infection or chemical burns or indeed a variety of other causes, it can result in blood vessels budding out from the conjunctiva at the margin of the cornea and growing into the transparent tissue.

<*Next photograph*> As might be expected, this can interfere with vision directly as well as disposing to the formation of opaque scar tissue around the vessels. <*Next photograph*> Again, blood vessels are not normally to be found on the surface of the iris. If they do develop in this situation, they are liable to become adherent to the back of the cornea especially towards the filtration angle where the anterior chamber is shallowest. This obstructs the outflow of aqueous through the trabecular meshwork and, depending on how much of the iris is involved, can result in a rise in intraocular tension and be a cause of glaucoma.

<Garner narrates over photomicrograph, using indicator stick>

A third ocular situation in which unwanted new vessels may develop is on the surface of the retina. Initially, preretinal blood vessels proliferate in the interval between the inner limiting membrane of the retina and the posterior phase of the vitreous and usually arise from the venous side of the retinal circulation. Such vessels are sometimes associated with shrinkage or refraction of the vitreous body and since they are attached to the back of that structure, tend to be drawn forwards.

Alternatively and indeed commonly, they may grow directly into the vitreous. But in either event, they are liable to bleed and to be complicated by perivascular fibrosis. This in turn can dispose to the formation of traction bands which pull on the retina and ultimately may result in its detachment.

<Garner to camera>

The clinical problem created by the growth of new blood vessels in the retina is due, in part, to our inadequacy in being able to deal with them and, in part, to our lack of understanding of the processes involved in their creation. Measures for their

treatment such as corneal grafting and surgical drainage procedures for the problems created by the vessels on the iris surface can scarcely be regarded as ideal procedures. Although it must be said that the use of the argon laser in the management of vessels on the retinal surface has achieved some very remarkable results. Even so, on the basis that prevention is better than cure, it is of great practical importance as well as academic interest to ask how and why new vessels should develop within the eye.

<Table>

Retinal Neovascularization
(Retinitis proliferans)

Diabetic retinopathy
Central retinal vein occlusion
Retrolental fibroplasia
Eales' disease
Sickle cell retinopathy

<Garner narrates over table>

If we begin with the retina we can start by listing some of the conditions in which new vessels can be seen. Now what these, and any other conditions giving rise to preretinal blood vessels, all show is reduced perfusion of the underlying circulation.

<Garner narrates over slide using indicator stick>

The disorder is characterised by focal obliteration of the capillary bed of which diabetes, seen here, and sickle cell retinopathy are classical examples. The new vessels, seen as bizarre channels on the retinal surface, are intimately related to the areas of failed perfusion. And since focal ischaemia is present before the new vessels develop, there would seem to be a good reason to postulate a cause and effect relationship.

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<Garner to camera>

Perhaps some of the most persuasive evidence in support of this view is provided by the events which follow the exposure of premature babies to excessive amounts of oxygen. And this state of affairs can be reproduced in the experimental animal.

<Garner narrates over series of slides, indicating lesions with stick>

And here is a retina from a newborn kitten, the vessels of which have been outlined by the use of Indian ink. And we can discern a capillary network based on three main arteriole venous channels. Now if such a kitten is kept in an oxygen enriched atmosphere for about three days, the vessels undergo <*next slide*> widespread and irreversible closure. In this case, the closure has been such that only a few stubs remain at the disc margin. And it might be noted in passing that the closure attributable to oxygen is limited to the retinal circulation, hence a still patent hyaloid artery.

<*Next slide*> I'll return to normal air. New vessels gradually invade the retina from the disc in an attempt, as it were, to repair the damage. Now this vascular proliferation is not confined to the retina, for many vessels break through the inner limiting membrane and give rise to massive preretinal vascularisation, which is seen here as a second layer of capillaries. In a premature baby this chain of events can result in the condition which we know as retrolental fibroplasia, a subject which was considered in some detail in an earlier talk in the televised Scientific Basis of Medicine lectures by Norman Ashton.

<Garner to camera>

There is then a strong connection between preretinal neovascularization and inadequate perfusion of the underlying retina. Even so it must be stressed that new vessels will not develop unless the retina remains viable.

<Garner narrates over table>

Total or near total cessation of retinal blood flow such as occurs in the occlusion of the central retinal artery, where there is no compensation from a hyperoxygenated choroid as in retrolental fibroplasia, and in which consequently tissue necrosis occurs, is not followed by the formation of new vessels. From this it has been postulated that hypoxic but still viable retinal tissue liberates or activates a factor which is able to stimulate the proliferation of vascular endothelium. Furthermore, since the vessels grow on the surface of the retina, it is suggested that the factor is diffusible and able to pass out of the retina and into the vitreous where it will tend to accumulate. And this sequence of events is summarised in these next diagrams.

<Garner narrates over diagrams>

If we can imagine that here we have a normally perfused retina, we then envisage that there is a reduction in the perfusion of the retina so that the retina still remains viable but in an embarrassed metabolic state. In this situation, it is postulated that a vasoformative substance is released which diffuses into the vitreous and there it will tend to accumulate because of the stagnant nature of the vitreous body.

Having accumulated, it then stimulates the vessels on the surface of the retina *<cut to next diagram>* to grow forwards towards the source of the stimulus.

<Garner to camera>

Although it has to be stressed that the presence of a vasoformative factor, indeed its existence, within retinal tissues is as yet to be demonstrated, there is a possible parallel with some work done by Falkman and his colleagues in the United States on the growth of tumours.

<Garner narrates over series of diagrams, referring to details with indicator stick>

They found that, up to a certain size, growing cancer cells can satisfy their metabolic needs by a process of simple diffusion from and into the surrounding tissue fluids. <*Cut to next diagram*> But beyond this critical size, the process is no longer adequate to maintain the cells locked in the centre of a rapidly-growing tumour mass. And when this stage is reached, a vasoformative substance or as they term it 'a tumour angiogenetic factor' can be extracted from the tumour cells. <*Cut to next diagram*> This appears to be responsible for the subsequent infiltration of the cancer tissue by proliferating blood vessels. And it is only on the basis of such vascular activity that further growth of the tumour is possible.

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<Garner to camera>

The tumour angiogenetic factor has been partially characterised and shown to be a complex of protein, carbohydrate and ribonucleic acid with a molecular weight of about 100,000. The RNA content amounts to about 25% of the whole and since the activity of the molecule is destroyed by prior treatment with ribonuclease, it may be that this represents the active principle.

Now in the search for a retinal vasoformative factor, Patterson and his colleagues in Baltimore have experimented on neonate animals and found that in animals exposed to toxic amounts of oxygen that in the recovery phase, that is in the vaso-proliferative phase in the early stage, there is an increased amount of protein in the vitreous. As have some experiments conducted by ourselves also shown that animals similarly treated do have an abnormal protein component in their vitreous in the early recovery phase. Now whether this does indeed represent a vasoformative factor or is merely a reflection of the increased permeability of such vessels, this being a well known property of such vessels, remains to be seen.

<Garner narrates over series of diagrams>

In the attempt to account for the association of preretinal vascular growth with retinal hypoxia, some workers have paid particular attention to metabolic changes within the affected tissues. In the healthy state, of course, the retina metabolises carbohydrate through an anaerobic pathway. But where there is reduced vascular perfusion, <*cut to next diagram*> anaerobic processes take over.

Could it be then that products of anaerobic glycolysis such as lactic acid, the latter being especially relevant in the context of diabetic retinopathy, accumulate and stimulate vessel growth? There is some encouragement to think that this might be possible in the common experience that new vessels stem from the venous side of the circulation, where any build up of acid metabolites would be greatest. Furthermore, some years ago, Imrie injected lactic acid directly into the vitreous of neonate kittens and was able to demonstrate neovascularisation in about 50%. However these experiments do not appear to have been confirmed and an attempt made by Norman Ashton some years ago using a similar model failed completely to show any vessel growth. So for the moment this rather attractive hypothesis must remain sub judice.

In our own quest for what has so far proved an elusive vasoformative factor in the retina, Dr Kisen(?) and I in the Institute of Ophthalmology have conducted a series of experiments using the retinae from kittens exposed to vasotoxic amounts of oxygen, using the cornea as a substrate on which to test our extracts.

The kittens were exposed to oxygen and then in the early stages of recovery – that is 4 or 5 days after removal from the oxygen at a time when we postulated that a vasoformative factor ought to be present but before we could actually discern new vessels – we removed the retinae and vitreous, extracted them and then re-suspended the extract in a small amount of saline.

<Garner narrates over diagram with indicator stick>

Then we introduced it into the cornea. And the way we did it was to use a technique which had originally been described by Morris and his co-workers some years

before. It involved making a small incision at the limbus. And then through that incision, introducing a metal Irish repositor moulded to the curvature of the cornea, and introducing it between the lamellae of the cornea within a point 1 to 2mm from the limbus on the opposite side. We then withdrew the repositor and using the track that it had made, we introduced a fine polythene catheter, the reservoir of which was filled with our retinal extract.

We then observed the animals over the next 10 to 12 days. And at the end of that period, we were able to discern in at least ¾ of them, a proliferation on the vessels towards the open end of the tube.

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Now, unfortunately, from our point of view that is, using control extracts from normal animals or using plain saline also resulted in vascular proliferation opposite the open end of the tube in a significant number. And so the import of our experiments is so far not clear. But we are pursuing them and considering the use of alternative substrates.

<Garner narrates over photomicrograph with indicator stick>

Rather less attention has been given to the cause of new vessels on the surface of the iris but it may well be that a similar mechanism is involved.

<Table>

Iris Neovasucularization
(Rubeosis Iridis)

Central retinal vein occlusion
Chronic uveitis
Diabetic retinopathy
Sickle cell retinopathy
Retinal detachment (long standing)

Iris neovascularization is commonly referred to as Rubeosis Iridis and it can complicate a variety of disorders, some of which are listed here. It can be seen that to a considerable extent, new vessels on the iris and on the retina have a common aetiology. Consequently, it is reasonable to postulate the presence of a vasoformative factor in this situation also.

<Garner narrates over diagram>

In the case of the iris, it is suggested that the vasoformative factor drains in a forward direction, especially in central retinal vein occlusion where drainage from the disc region is likely to be compromised. And it ultimately passes over the surface of the iris as it leaves the eye through the filtration angle of the anterior chamber. And as a result, vessels near to the surface of the iris will be stimulated to proliferate. Conversely, it was shown quite a few years ago by Edmund Smith that should new vessels develop in the disc region, there is much less chance of Rubeosis Iridis occurring since presumably this would provide an alternative escape route for any vasoformative factor.

<Garner to camera>

When we come to consider vascularisation of the cornea due to naturally occurring disease, we have, once again, to admit to considerable ignorance about the precise cause.

<Table>

Corneal Neovascularization
e.g. herpetic keratitis
trachoma

Post-traumatic inflammation
Chemical burns
Graft rejection

Stevens-Johnson syndrome

Rosea kera

<Garner narrates over table>

It is nevertheless seen in a large number of diverse disorders only a few of which are listed here. But it will be noted that many of them have an inflammatory element, whether it is due to direct infection or response to trauma or a primary immunological reaction.

<Garner to camera>

Clintworth working at Duke University in North Carolina has recently drawn attention to the possibility that it might be the inflammatory exudate which is providing the vasoformative stimulus. He and his colleagues have recently done some experiments in which they induced vascularisation of the cornea in experimental animals by a variety of means, including alkaline burns, bathing the cornea in Alloxan[?], direct trauma, infection and so forth. And in every instance, in which they were able to observe new vessels, they found that there was a preceding phase of inflammatory cell infiltration. And in some more recent experiments, they conclude that it is the neutrophil polymorph which is providing the positive stimulus.

But, might it not be that it is the tissue damage responsible for the inflammatory reaction which is itself providing the vasoformative stimulus rather than the exudate? Even so, in support of their contention, are their later series of experiments. They found that if they subjected animals to massive doses of radiation such as to knock out the circulating leukocytes and then tried to induce corneal vascularisation, they were unsuccessful. This is fairly conclusive evidence and yet even that is open to some doubt for the animals were by this time in a very moribund state as a result of the massive irradiation, and might it not have been that it was a general depression of tissue responsiveness rather than any specific lack of stimulus that was responsible for the lack of vascularisation?

Now it has been observed that a feature common to most if not all situations in which there is interstitial corneal vascularisation […]

<Garner narrates over diagram>

[…] is stromal oedema which as is seen on the right of the diagram results in separation of the individual lamellae. And as a result it has been argued that the chief reason that the intact healthy cornea, shown on the left, is not vascularised is the impenetrable nature of its extremely compact architecture. There is considerable support for this concept to the extent that interstitial oedema is considered to be a prerequisite for stromal vascularisation, but it is unlikely to be the full explanation since the cornea can sometimes be oedematous, as in Fuchs' combined dystrophy, without becoming vascularised.

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<Garner to camera>

Corneal vessels can be stimulated in experimental animals in many ways, some of which I have already mentioned. But a variety of amino acid and other nutritional deficiencies have also been implicated, although it must be said that the cornea of the human appears to be far less sensitive to dietary deficiencies. A possible parallel with veterinary vascularisation is provided by the vessels which sometimes complicated the use of contact lenses before adequate provision was made for gaseous interchange at the corneal surface. It has been argued that in those cases, the vessels were a response to tissue hypoxia engendered by the contact lens.

Now in the face of so much inconclusive and diverse evidence, it is difficult to construct a unifying hypothesis for corneal vascularisation.

<Garner narrates over diagram>

But it appears that we need to have some functional change within the cornea, either in the form of inflammation, tissue damage, nutritional deficiency, hypoxia or toxicity, or indeed some other factor which remains to be yet discovered. And the functional change induced by these substances or these methods provides a positive stimulus. But that in itself doesn't appear to be enough; we need to have a structural change in the cornea in the form of tissue oedema, and it may well be that this tissue oedema is itself attributable to these changes of a functional nature. But given the functional change, a positive stimulus that is, together with the opening up of the lamellae of the cornea, we then have the stage set for vascularisation of the cornea.

<Garner to camera>

In this talk I've touched on some of the problems caused by the vascular proliferation in the eye. I've referred to some of the possible stimuli, but it is only proper to add that we may be dealing not so much with a positive stimulus as with the removal of an inhibitory substance or factor. Known as chalones, substances of this kind are being recognised in a number of tissues including the kidney, liver and skin – indeed that in the skin is thought to be a glycoprotein. And the control of lens growth in the eye again is thought to be under the control of a chalone. But so far as vascularisation in the eye is concerned, we have as yet no direct evidence for such a factor. Nevertheless some recent work carried out in Chicago on articular cartilage, suggests that this may be a profitable line of investigation.

<Garner narrates over diagram>

It was found that as opposed to most other tissues, cartilage grafted on to chick chorioallantoic membrane is resistant to invasion by the vascularised mesenchyme of the host. They also found that this inhibition can be destroyed by prior extraction with guanidine hydrochloride. Subsequent experiments show that a factor is present in the extract which will inhibit the growth of vascular endothelium in tissue culture, and it is suggested that it is this which is responsible for the absence of blood vessels in cartilaginous tissues.

<Garner to camera>

In the light of these discoveries, we are now planning to look at corneal tissue from a similar standpoint. And it will be clear from what I have said that we are only just beginning to come to grips with the problem of untoward vascularisation in the eye. To resolve the outstanding questions is going to need the combined efforts of the ophthalmologists, the experimental pathologist and the biochemist. There is nevertheless some encouragement in that specific growth factors have been described in a number of other tissues: an epidermal growth factor, a fibrous growth factor and a nerve growth factor have all been described, and to a large extent have been characterised and shown to be polypeptides. They appear to act by activating the adenyl cyclase cyclic AMP system at the membrane of the appropriate cells. Some of these factors, specifically the nerve growth factor, have been shown to be antigenic. And by preparing specific antisera, it has been possible to induce a state of sympathectomy in animals when the vaccine is given to them.

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Now much as I would like to end on an optimistic note, I have to say that attempts we have made to produce antibodies to vascularising tissue in the eye have so far not met with any success. But it would be a real bonus, of a comparable effect as that observed in nerve tissue, could be provided in vascularising tissues of the eye, so that unwanted ocular neovascularisation could be prevented or removed by the administration of a specific vaccine.

<End credits>