Wellcome Film Project

Peripheral Nerve Injury

The Scientific Basis of Medicine

With Dr PK Thomas, Royal Free Hospital, London.

Introduced by Dr Ian Gilliland

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<**Dr Ian Gilliland to camera**>

Dr Peter Thomas is Consultant Neurologist at the Royal Free Hospital and the Royal National Hospital, London. Prior to this he was Senior Lecturer in Neurology at the Institute of Neurology, Queens Square. His studies abroad took him as Assistant Professor to McGill University in Montreal. His particular contribution has been to nerve damage which is the subject of today's lecture. Dr Peter Thomas.

<**Dr Peter Thomas to camera**>

The exigencies of the second world war provided a powerful stimulus for research into nerve injury and the mechanisms of repair. This yielded much information of practical clinical importance as well as leading to observations of general biological interest. Over the past decade, the main advances have been in analysis of the ultrastructural changes by electron microscopy and it's largely these that I wish to

review in this talk. Before doing so it may be helpful to recall a few relevant points as to the normal structure and ultrastructure of peripheral nerve trunks.

<**Thomas, seated, refers to slide showing section through human** serial nerve >

This is a transverse section through the normal human serial nerve showing a number of fascicles each containing nerve fibres. The fascicles are bounded by the perineurium and held together by a connective tissue component, the epineurium. The epineurium also contains variable amounts of fat and the main blood vessels, the vasa nervorum. The blood supply is provided by the nutrient vessels which enter a longitudinal anastomotic network in the epineurium of arterioles and venules which communicate through the perineurium with another longitudinal anastomotic network of capillaries within the fascicles.

<**Thomas stands and walks over to wall display of multiple images. Refers briefly to an electron micrograph of the perineurium. Then returns to seated position and refers to series of slides showing the structure of peripheral nerves**>

If we look at the perineurium by electron microscopy, we have appearances which are shown in this preparation. It is seen to be composed of a number of layers of fat and cells with intervening zones containing collagen fibrils. The perineurium is an interesting structure which from physiological observations has been shown to provide a diffusion barrier between the inside and the outside of the fascicle.

This slide is a myelin stain of two fascicles showing the dense population of myelinated nerve fibres. The next slide is a diagram taken from one of Joseph Long's papers showing the organisation of a myelinated fibre as revealed by light microscopy. In transverse section, there's a central section surrounded by the myelin sheath, outside which is the Schwann cell and then a connective tissue layer derived from the endoneurium. In longitudinal section we see the axon with the surrounding myelin sheath, the Schwann cell with its nucleus and the endoneurial connective

tissue sheath. The Schwann cells, sorry the myelin, is interrupted at intervals by the nodes of Ranvier.

The next slide shows measurements made on the distance between the nodes of Ranvier, measurements of internodal length. If we plot the length of the internodes against their diameter, we find that the larger fibres possess longer internodes than the smaller. The reason for this relationship is that at the time of myelination, internodal length is short. The number of internodes doesn't change during development and as the nerve fibres increase in size they become stretched and internodal length increases. The reason for this relationship between internodal length and diameter is that the fibres that become myelinated first ultimately achieve the largest diameters and therefore come to have the longest internodal length. The ones down here with the short internodal length are the ones that are myelinated latest during development.

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<**Thomas stands and returns to wall of images, narrates over electron micrographs showing transverse sections of myelinated nerve fibre**>

Turning now to the ultrastructure of myelinated nerve fibres. Over here we have an electron micrograph of a transverse section through a small myelinated nerve fibre with the axon surrounded by the myelin sheath, and related to the satellite cell, the Schwann cell, with its nucleus, cytoplasm containing endoplasmic reticulum and mitochondria and surrounded by the basement membrane or basal lamina.

The next micrograph shows a small myelinated nerve fibre at higher magnification. The axon is seen to contain various organelles, neurofilaments, microtubules and mitochondria, surrounded by the lamellated myelin sheath, and this is the associated Schwann cell with its nucleus.

This section shows the way in which the myelin is formed during development. Here is the axon, this the Schwann cell and this, the nucleus. The Schwann cell surface

membrane becomes spiralled around the axon, opening out at this point – this is the so-called mesaxon.

In the next section, we have a thicker myelin sheath but again we can see the Schwann cell surface membrane turning in and then beginning to spiral around the axon. The myelin, the compact myelin is formed by obliteration of the intervening Schwann cell cytoplasm – these areas of Schwann cell cytoplasm, here, are obliterated to form the compact myelin. This is a partial Schmidt-Lanterman incisure, clefts that persist in the myelin with Schwann cell cytoplasm between the myelin lamellae.

<**Thomas, seated, refers to slides next to him detailing the ultrastructure of unmyelinated axons. Then back to camera**>

Turning now to the ultrastructure of unmyelinated axons. In this slide, which is a diagrammatic representation, taken from a paper by Elfin, in transverse section we have a Schwann cell with its nucleus and associated with this and invaginated into the cytoplasm on small mesaxons are a number of unmyelinated axons. The situation in human nerves, this is from an animal nerve, is slightly different in that each unmyelinated axon tends to be associated with a single Schwann cell process.

Well, so much for the background of normal structure. To turn now to nerve injury, in 1943 Seddon proposed a classification of nerve action which, although subsequently modified by some authors, still provides a useful subdivision of the main types of nerve injury.

<**Thomas over table listing classification of nerve injury, to camera between points**>

First of all neurapraxia refers to situations where there is a localised conduction block without distal degeneration of the axon. The muscles do not undergo denervation atrophy and nerve conduction is present if the nerve is stimulated below the lesion. Recovery is fairly rapid and is complete and when it occurs, takes place more or less

simultaneously throughout the territory of the nerve. A typical example is ‘Saturday-night’ paralysis of the radial nerve.

Secondly, axonotmesis refers to situations where the axons are interrupted but with preservation of the connective tissue framework of the nerve, Wallerian degeneration occurs below the level of the lesion so that recovery has to take place by axonal regeneration which is a slow process. The rate of axonal regeneration is of the order of a millimetre or so per day. Recovery begins in the most proximal part of the territory of innervation of the nerve, but the nerve fibres return to their former terminations so that the functional result is good.

Now, thirdly neurotmesis refers to injuries where, in addition to axonal interruption, the connective tissue framework of the nerve is also disrupted. It’s possible to divide this category depending upon the degree of disorganisation of the supporting structures. As with axonotmesis, Wallerian degeneration, of course, occurs below the level of the lesion and recovery has to be by axonal regeneration. However, even with good apposition between the cut ends of the nerves, recovery is always appreciably less good. This is because misrouting of the regenerating axons at the site of injury so that many fail to regain their former terminations.

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<**Thomas to camera**>

Now, to begin with the pathological basis for lesions of neurapraxic type, we have no direct information for man and our ideas are derived by extrapolation from animal experiments and from nerve conduction studies. A possible animal model was provided by Denny-Brown and Brenner in 1944 and later analysed in more detail by Mayer and Denny-Brown. This consisted of the application of a pneumatic pressure cuff around the hind limb of a cat, which gave rise to a local conduction block under the cuff, with features typical of neurapraxia. Denny-Brown demonstrated that the histological change in the nerve fibres consisted of a local demyelination with preservation of axonal continuity. The loss of myelin was mainly on either side of the

nodes of Ranvier. Recovery was accompanied by remyelination. Denny-Brown considered that the lesion was ischemic in origin, however, recent observations on the same experiment in the baboon by Professor Gilliat and his collaborators strongly suggests that the damage is mechanical in origin and produced by pressure from the tourniquet. It's not clear yet whether the results of this experimental model can be transferred to man. Nevertheless, it seems very likely that pressure pauses in man are associated with local demyelination since nerve conduction velocity is reduced at the site of the lesion.

Before discussing the changes at the site of injury with lesions resulting in axonotmesis or neurotmesis, I wish to discuss the features of Wallerian degeneration which take place, distal to the point of injury, in both these situations.

Within 24 hours after axonal interruption, the axons distal to the lesion shrink in size, the myelin retracts from the nodes of Ranvier and both the myelin and the axons develop an unduloid outline. The myelin breaks into ovoids which contain the interrupted portions of axon. This is complete on all myelinated nerve fibres after 4 to 5 days. The breaks in the myelin during ovoid formation occur at the Schmidt-Lanterman incisures. This was demonstrated by Webster, and this slide is from one of Webster's papers.

<**Thomas over slide illustrating Wallerian degeneration. Then back to camera**>

This shows the ovoids containing axonal debris, and it can be seen that the breaks in the myelin are occurring at the clefts of Schmidt-Lanterman.

The earliest ultrastructural changes during Wallerian degeneration have been observed in the exoplasm. The vacuoles of the endoplasmic reticulum disrupt and then the neurofilaments and neurotubules fragment and accumulate as clumps of granular debris. This begins at about 24 hours after nerve section. Dense lamellar bodies, which have been shown to be associated with acid phosphatase activity and therefore to be lysosomal in nature, appear in the axon and tend to accumulate adjacent to the nodes of Ranvier.

<**Thomas stands and refers briefly to an electron micrograph on wall of images. Then back to camera**>

These are shown in this electron micrograph over here. These are these dense lamellar bodies. This section also shows an accumulation of mitochondria which tends to take place adjacent to nodes of Ranvier at about 36 hours after nerve section. This node is widened because of retraction of the myelin, partly because of myelin breakdown; you can see myelin debris within the Schwann cell cytoplasm, and partly because of retraction of the myelin. The myelin has become separated from the axon by Schwann cell processes which intervene between the two.

The Schwann cell processes also grow into the incisures of Schmidt-Lanterman. The Schwann cells are therefore actively involved in the breakdown of the myelin.

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<**Thomas to camera, then over diagrams and electron micrographs on wall showing aspects of nerve cell degeneration**>

With interruption of the axon, the myelin ends come together to form the ovoids. Over here we have a diagram of a longitudinal section through a degenerating nerve fibre at about 7 days after nerve section. The basal lamina or basement membrane, which had originally surrounded the Schwann cell, has collapsed and surrounds ovoids containing myelin debris. Around this are Schwann cells which are beginning to proliferate. If we take a section through the fibre at about this point, we have the appearances shown in this micrograph. The collapsed basement membrane, which forms a tubular structure, contains Schwann cell processes together with myelin and axon debris.

Returning to the diagram, if we take a section at this level through the ovoid, the appearances seen in the electron microscope correspond to this picture. The ovoids containing axonal debris, surrounded by Schwann cell processes. A portion of the

ovoid is seen at higher magnification in this micrograph. Here is the ovoid surrounded by Schwann cell processes and contained within the basement membrane forming the basement membrane tube. The myelin ovoid is thus seen to be extracellular in position.

This myelin may then undergo breakdown as is seen in this micrograph. Here again we have the axonal debris, here the myelin ovoid, here are Schwann cell processes and the surrounding basement membrane. The myelin is breaking up into fascicular and tubular elements. Some of these breakdown products, which are probably the result of a purely physical breakdown, are shown in the next slide.

Here we have a number of Schwann cell processes, this is the basement membrane, and between the Schwann cell processes is this material consisting of hexagonally packed tubes derived from myelin breakdown.

Not all of the myelin breakdown takes place in an extracellular position. One can certainly find, at times, degenerating myelin within Schwann cell processes. This is illustrated by a section at this level. Here's the Schwann cell process with a small myelin ovoid. And here, a micrograph of this type of appearance – the basement membrane tube enclosing a number of Schwann cell processes, one of which contains a small myelin ovoid.

The myelin debris is then broken down within the confines of the basement membrane tubes within cells that have all the appearances of macrophages. This is a phagosome from one of these cells containing a myelin figure, some myelin debris. This is another of these phagosomes containing debris of varying types. And up here, another portion of a vacuole that is empty, presumably having contained lipid that has been dissolved out during the preparative procedures. These phagosomes can be shown to be associated with acid phosphatase activity and therefore are lysosomal in nature.

The next micrograph is an electron microscope preparation with a Gomori stain for acid phosphatase. The black reaction product of the stain is related to one of these

vacuoles containing myelin debris. This leads to the presence within the basement membrane tubes of macrophage-like cells containing multiple vacuoles, together with Schwann cells that have proliferated.

I've shown this diagrammatically in the next slide. Here, the basement membrane tube, the basal lamina tube, containing proliferated Schwann cells with their processes and here, this macrophage-like cell with numerous vacuoles.

These macrophages can be seen, as is shown in the next electron micrograph, leaving the basement membrane tubes – here is one of these large macrophages, this is basement membrane along here, and at that point, with the macrophage breaking through.

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Other basement membrane tubes can be seen containing Schwann cell processes but also spaces related to disrupted basement membrane, presumably where macrophages have left the tubes. These spaces become lined by new basement membrane as is shown in the next electron micrograph. Here we have the basement membrane that had surrounded the original nerve fibre, a bit more of the original basement membrane up there, and here a cavity that presumably contained a macrophage that has left the tube, that has become lined by new basement membrane and filled with collagen fibrils. The end product of this process is a basement membrane tube filled with proliferated Schwann cells, the so-called Büngner band.

In the next electron micrograph here is one of these Büngner bands, the basement membrane enclosing Schwann cells and proliferated Schwann cell processes. The macrophages that have left the basement membrane tubes can be seen within the endoneurium – a cell with a nucleus and multiple vacuoles containing myelin debris.

<**Thomas, seated, to camera**>

These cells then accumulate around the blood vessels and ultimately leave the nerve. The origin of these macrophages has been disputed. A number of early electron microscope studies suggested that they were transformed Schwann cells. However, more recent studies with the cell labelling techniques, by Asprey and by Olson, have indicated that they are ordinary macrophages and most probably have a mainly haematogenous origin.

To turn now to the changes at the site of injury. The experimental model that has been employed to examine the axonotmesis type of lesion is to crush the nerve with fine, smooth-tipped forceps. This will interrupt the axons but leave intact the connective tissue framework, so that the regenerating axons regenerate along their original pathways and thus reach their former terminations; this accounting for the good functional results after such injuries.

The details of the changes at the site of injury have been clarified by electron microscopy. Haftek and I examined nerves compressed with smooth-tipped forceps. In nerves fixed immediately after compression at the site of injury, the nerve fibres are represented merely by flattened basement membrane tubes containing small amounts of debris.

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<**Thomas stands and moves back to wall of images, narrates over micrographs showing aspects of changes at nerve injury sites**>

Over here we have the appearances seen immediately after nerve compression, we can see the flattened basement membrane tubes containing small amounts of myelin and cellular debris.

The next micrograph shows the appearances about an hour after the nerve compression when the material that has been squeezed to either side has flowed back into the compressed region. The basement membrane tube has expanded by

myelin and cellular debris. Although we found many considerably distorted basement membrane tubes, in no instances were the tubes ruptured.

When we came to look at the compressed region, 2 or 3 days after the injury, we found that the basement membrane tubes were filled with regenerating axons and Schwann cell processes. It's therefore these basement membrane tubes that define the pathways that guide the regenerating axons and their associated Schwann cells back across the compressed region.

<**Thomas, seated, narrates over slides showing a diagram and micrograph of nerve transection**>

Next, I wish to turn to a consideration of lesions of the neurotmesis type and will restrict myself to a discussion of the situation when the continuity of the nerve is completely interrupted. When a nerve is transected, the cut ends immediately separate from one another. The reason for this is uncertain although it has recently been suggested that the perineurial cells may possess contractile properties. Outgrowths then take place from both cut ends, with axon sprouts and Schwann cells growing out from the proximal stump to form the so-called neuroma. The outgrowth from the distal stump is at least as vigorous and may give rise to long processes termed 'ghost nerves'. This is illustrated in the diagram that is being displayed.

There has been some dispute as to the nature of the outgrowth from the distal stump. In this section we have the distal stump and here the outgrowth seen in longitudinal section. By light microscopy, it's extremely difficult to decide what is the nature of the cells that are appearing from the distal stump. But it's now clear from electron microscope studies, that the proliferated Schwann cells grow out from their basement membrane tubes in the distal stump and give rise to branching columns with a mass of collagenous connective tissue.

<**Thomas stands, returns to wall of images and narrates over electron micrographs showing various aspects of nerve transection and regeneration**>

Over here, we have a longitudinal section through the tip of the distal stump. The collapsed basement membrane tube that had surrounded the Schwann cell in the intact nerve contains Schwann cell processes. We know they are Schwann cells by their cytological features.

The next slide shows the region where the nerve was transected. Here is the collapsed basement membrane tube and this the precise point of transection with a cell process of uncertain nature extending through it.

In the next slide we have again the precise region of transection with the mouth of one of the basement membrane tubes and the cut end of the basement membrane, and extending out through the mouth of the tube a number of Schwann cell processes, surrounded by basement membrane, as is typical of these cells.

The micrograph over here is taken through the outgrowth itself and shows Schwann cell columns, cells closely related to each other and bounded by basement membrane. They form a branching network within the outgrowth.

<**Thomas, seated, to camera**>

When this outgrowth makes contact with the neuromatous outgrowth from the central stump, the regenerating axons are guided by the Schwann cell columns to the Büngner bands in the distal stump. Each axon in the central stump gives rise to numerous axon sprouts which interlace in the gap between the cut ends, increasing the chances of axons achieving their former terminations at the periphery. However, as I mentioned earlier, the functional recovery after nerve transection is always substantially less than perfect.

A further factor of importance in this direction arises where a damaged and fibrotic segment of the nerve is removed prior to approximation of the ends for surgical repair. The disposition of the fascicles in the nerve trunk constantly changes along the length of the nerve and it's therefore impossible to avoid mismatching between

the fascicles of the two cut ends, thus reducing the chances of axons reaching functional terminations.

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<**Thomas to camera, briefly refers back to previous diagrams intermittently**>

When the axons reach the distal stump, myelin takes place by formation of the spiral mesaxons by the Schwann cells in the way we've seen before – a regenerating axon being surrounded by early myelin formation.

The myelin then increases in thickness as axon diameter increases. The next slide shows a later stage of development: here we can see the basement membrane tube with Schwann cells, one of which has associated with it an axon which is becoming myelinated together with a number of unmyelinated axon sprouts.

The careful, quantitative studies by JZ Young and Paul Weiss and their collaborators show that proper maturation of fibre-diameter of these regenerating nerve fibres only occurs if the axons reach a functional termination at the periphery.

Another interesting feature of regenerating nerve fibres is that internodal length is more or less uniformly short, whatever their diameter.

<**Thomas over graph showing observations made on rabbit peroneal nerve**>

In the next slide are some observations made on the rabbit peroneal nerve some years ago by Cragg and myself, confirming earlier observations by Vizoso and JZ Young and others. We have here internodal length plotted against fibre-diameter. And on the unoperated side of the nerve we see the normal relationship between internodal length and diameter with the larger fibres having longer internodes. In the lower graph we have observations made on internodal length and fibre-diameter after the nerve has been crushed and allowed to regenerate fully. We will see that diameter has returned back pretty well to normal levels, but internodal length is much

the same whether the fibres are large or small. The reason for this is that during regeneration, the Schwann cells space themselves out along the regenerating axons, at much the same sort of spacing as during normal development, and then myelinate the axons. The fibres increase in size but as this experiment was performed in an adult animal, the nerve is not elongating and so the internodes remain uniformly short.

<**Thomas over diagrams and electron micrographs showing effects of nerve constriction, to camera in between**>

Now, a further interesting type of nerve injury is seen when nerves are constricted. This was originally studied some years ago by Paul Weiss and his collaborators. They found that if a nerve was crushed and the regenerating axons were allowed to grow through the constriction applied to the nerve more distally, the axon became greatly enlarged or ballooned, immediately above the constriction. Weiss interpreted this as an interruption in the proximo-distal flow of exoplasm but this explanation has not yet been entirely validated.

This experimental situation has recently been analysed in an electron microscope study by my colleague, Peter Spencer. He applied a constriction to the peroneal nerve in rats with a ligature and then crushed the nerve at a higher level using smooth-tipped forceps.

If we could have the next slide. Immediately above the constriction, he found that the regenerating axons were greatly enlarged. This is one of these greatly ballooned axons which hasn't had any myelin developed around it. The next slide shows the situation at the region of constriction where the axons are smaller but again have no myelin around them. And the next slide is taken from the region below the constriction where one again has rather small axons but these are now becoming myelinated.

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<**Thomas to camera**>

Now, finally I wish to mention briefly something about unmyelinated axons. The degeneration of unmyelinated axons resulting from nerve transection has not been studied extensively so far by electron microscopy. In general terms, the process seems to be similar as for myelinated fibres although the precise time course of events has not been established and there may be differences in detail. The manner of removal of the degeneration products has also not yet been established. Quantitative studies by Joseph and others using light microscopy, however, have clearly shown that the amount of accompanying Schwann cell proliferation is much less.

The regeneration of unmyelinated axons was studied some years ago in the rabbit vagus nerve by Evans and Murray. If we could have the next slide.

<**Thomas over diagrams and micrographs showing study of crushed rabbit vagus nerve**>

They studied the rabbit vagus nerve and found that if they crushed the nerve just below the diaphragm, regeneration of the unmyelinated axons took place, leading to a distribution pattern almost indistinguishable from normal. This is a useful experimental situation because the abdominal vagus contains almost entirely unmyelinated axons. Up in the cervical region, the vagus contains a mixed population of unmyelinated and myelinated axons and most of the myelinated axons leave in the recurrent laryngeal branch.

Evans and Murray found that if they crushed the vagus nerve in the neck, they found that very few of the unmyelinated axons regenerated down as they should have done into the abdominal vagus. Instead they became diverted into the recurrent laryngeal nerve where they were seen arrayed around the regenerating myelinated fibres.

If we could have the next slide. This is taken from their paper: the regenerating myelinated axons haven't taken up the silver stain very well, but they're surrounded by these large numbers of regenerating unmyelinated axons.

Dr King and I have recently investigated this experimental situation by electron microscopy. If we could have the next slide. We also crushed the vagus nerve in the neck and then had a look at the recurrent laryngeal nerve at intervals after injury. The next slide. We confirmed the observation made by Evans and Murray that the regenerating myelinated fibres had associated with them large numbers of unmyelinated axons and associated Schwann cells.

The next slide shows one of these regenerating myelinated fibres at higher magnification, in relation to which is a concentric arrangement of Schwann cell processes and numerous regenerating unmyelinated axons.

These results, therefore, indicate that the pattern of regeneration of unmyelinated axons is greatly influenced by the presence or absence of myelinated fibres, although the explanation for this has not yet been established. This observation may well have important consequences in relation to the regeneration of unmyelinated axons in man, in nerves with a mixed population of myelinated and unmyelinated fibres.

There are many other aspects of nerve injury that I could have considered, such as the important changes that take place central to the lesion, but I've now reached the end of my allotted time.

<**End credits**>