SOME EFFECTS OF TEMPERATURE UPON THE RATE AND PROGRESS OF WALLERIAN DEGENERATION IN MAMMALIAN NERVE FIBRES

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Diverse opinions have been held regarding the site of onset and the subsequent progress of degenerative changes in an injured peripheral nerve fibre. Recent investigations have supported two opposite concepts of this process. Most workers describe centrifugal or progressive degeneration in which degenerative changes begin proximally at the lesion and then spread distally from it (Parker, 1933; Parker & Paine, 1934; Rosenblueth & Dempsey, 1939; Rosenblueth & Del Pozo, 1943 and Causey & Palmer, 1953). Weddell & Glees (1941), however, reported the reverse of this, degenerative changes being more rapid in cutaneous nerve plexuses than in the nerve trunks which supplied them, and this was thought by Erlanger & Schoepfle (1946) to support their own findings obtained by physiological means.

It is well established that the rate of degenerative change in nerve fibres is affected by temperature (Merzbacher, 1903; Torrey, 1934; Gamble, Goldby & Smith, 1957) so that an apparent progression of the process proximo-distally (or vice versa) might indicate no more than a temperature gradient along the course of a degenerating nerve. Proximo-distal temperature gradients are present in nerves in appendages such as the limbs (see, for example, Dubois, 1951) or the tail. Such gradients can be varied in different ways, and the experiments to be described were made to investigate their effects upon the local rates of Wallerian degeneration in nerves.

METHOD

The large ventral caudal nerve in young adult rats was used. Proximally, for about 2·5 cm. of its course, it lies relatively deep among muscles, and the overlying skin is hairy. For the rest of its course it lies between tendons, and the overlying skin is scaly and covered only by short, sparse hairs. In this region the subcutaneous temperature is considerably lower than in the peritoneal cavity. It can be controlled by altering the environmental temperature, and is measurable by means of a thermocouple inserted through a hyperdermic needle.

All operations were carried out under ether anaesthesia and with full aseptic precautions. In four animals the nerve was cut as high as possible in the deep part of its course. After 5, 7, 10 and 25 days in a cold room at 2–3° C, the animals were killed and lengths of nerve removed for histological examination. In seven animals the nerve was cut about 2 cm. distal to the hairy part of the tail. In five of these the skin was then stripped from the distal 6–7 cm. of the tail, and this part inserted through an abdominal incision into the peritoneal cavity. It was secured there by sutures, abdominal muscle to tail tendons and abdominal to tail skin. In the two

remaining animals the skin was not stripped, but after careful shaving and washing in disinfectant the tail was sutured into the peritoneal cavity as with the first five. The 'tail grafts' were borne by the animals without any sign of distress or even discomfort and no sign of infection was found.

Six of the animals with tail grafts were kept in a cold room for periods ranging from 4 to 25 days. One animal (where the tail skin had been stripped) was kept in a heated cage at 35° C. for 7 days.

Immediately after death lengths of nerve were removed, stretched on cards and fixed in 1% osmium tetroxide.

(*a*) *Grafted tail.* Two lengths were removed; one from immediately below the lesion and therefore subject to environmental temperature, and the other from that part of the tail which lay in the peritoneal cavity.

(*b*) *Ungrafted tail.* Two lengths were removed; one immediately below the lesion, in the hairy part of the tail, the other from near the tip of the tail.

TEMPERATURE MEASUREMENT

In the cold room it was found that the environmental temperature inside the cages varied between 6° and 9° C. Intraperitoneal temperatures varied little; 36° C. was the lowest and 39° C. the highest recorded. Subcutaneous temperatures in the root of the tail (the hair-covered part) were almost equally constant and were never more than 1° C. below that of the peritoneal cavity. Temperatures in the distal part of the tail were always much lower, but varied considerably even in the same animal at different times. The higher temperatures (up to 26° C.) were recorded in animals which had been asleep with their tails tucked beneath them, the lower (down to 6° C.) in the same animals a few minutes later when they were moving actively about their cages. No record was kept of activity, but much time was spent asleep. Probably the tail temperature was more often and for longer periods nearer 20° than 10° C. It should be noted, however, that the measured subcutaneous temperature was never found to be less than 12° C. below peritoneal temperature at the end of the proximal one third, nor less than 18° C. below peritoneal temperature at the tip of the tail.

Under similar environmental conditions, animals with the tail grafted into the peritoneal cavity showed more constant subcutaneous temperatures in the exposed part of the tail. These always lay between 10° and 13° C., that is about 25° C. below the temperature in the peritoneal cavity and of the part of the tail within it.

RESULTS

The stages of degeneration in the sheaths of myelinated nerves which can be demonstrated by staining wth osmium tetroxide are well known and do not need extensive discussion. They are illustrated in Pl. 1, and are as follows (approximately in chronological order of their appearance): retraction of myelin from the nodes; fragmentation of the sheath into large oval droplets or 'digestion chambers'; the breaking of the large droplets into smaller ones; the gradual removal of all osmophilic material. In the present material the differences in the state of degeneration reached in the

cold and warm parts of the same injured nerve are such that they can be illustrated better in photographs than by verbal description.

Where the nerve had been sectioned in the hair-covered part of the tail 5 days previously, almost all the fibres in its warm proximal part were markedly degenerate, most of them as strings of digestion chambers (Pl. 1, fig. 1), although in a few fibres only retraction of myelin at the nodes had occurred. In the cold, distal part of the same nerve, no fragmentation had occurred, and apart from slight retraction of myelin from the nodes, the fibres were substantially normal in appearance (Pl. 1, fig. 2).

In a similar animal kept for 25 days, the whole process of degeneration was more advanced; in the proximal part of the nerve much of the altered myelin had disappeared (Pl. 1, fig. 3), while in the distal part fragmentation of myelin and its retraction from the nodes were the only signs of degenerative change (Pl. 1, fig. 4). The animals killed after 7 and 10 days showed similar differences between the proximal (warm) and distal (cold) parts of the nerve.

Where the distal part of the tail was kept warm within the peritoneal cavity, the opposite result was obtained. Four days after tail graft and nerve section the cold proximal part of the nerve stump showed no other sign of degeneration than retraction of myelin at the nodes in a small proportion of its fibres (Pl. 1, fig. 5), while in its warm distal part 'digestion chambers' had formed in almost all its fibres (Pl. 1, fig. 6). In a similar animal kept for 25 days the whole process of degeneration had again proceeded further. The proximal part showed myelin fragmentation or retraction of myelin at the nodes in many fibres (Pl. 1, fig. 7), other fibres being still normal in appearance. In the distal part, however, the breakdown of myelin was far advanced and much of the debris had disappeared (Pl. 1, fig. 8). Similar, though less obvious, differences between warm and cooled parts of the nerve were found in the 7- and 10-day specimens.

Removal of the skin from the distal, grafted part of the tail might, by damage to its blood supply or to its cutaneous branches, alter the rate of degenerative change within the grafted, warm part of the nerve. In two animals the uncut nerve from the opposite side of the tail was examined as a control; no sign of abnormality was found. In two other animals where grafting was performed without stripping the skin it was found as before that degenerative changes in the distal, warm part of the nerve were more advanced than in its proximal, cold part. It is clear that in these experiments local damage, whether through stripping off cutaneous branches or by interference with the cutaneous circulation had little, if any, effect upon the main trunk of the nerve.

One animal, where the tail was grafted and the nerve cut, was kept for 5 days in a cage warmed to 35° C. No difference was detectable between the stage of degeneration reached by the fibres in proximal and distal parts of the nerve. In this animal there could have been very little difference between the temperature of the exposed and the grafted parts of the tail.

One may conclude that it was the temperature difference which was the cause of the more rapid onset and progression of degeneration in the grafted part of the tail in the other animals and that insertion into the peritoneal cavity does not by itself have any effect.

DISCUSSION

These experiments have shown that the acceleration of the process of Wallerian degeneration which is caused by a rise in temperature can be produced locally in any part of the length of a nerve trunk, i.e. that the onset and course of the process is more rapid in the part of the nerve which is warm, whether this is near to or far from the lesion, than in the cooler parts. There is no reason to doubt that the changes which take place in the axon are similarly affected since other experiments (e.g. Gamble, Goldby & Smith, 1957) show that changes in the sheath greater than an incipient retraction of myelin at the nodes are accompanied by degeneration in the axon. It should be pointed out that the temperature differences in the present investigation were large, of the order of 15° C. and that they resulted in gross differences in the stage of degeneration reached which could be demonstrated easily in simple teased preparations.

On the basis of these experiments it would be possible to conclude that where no substantial temperature differences exist along a degenerating nerve, degeneration occurs simultaneously and at an equal rate along its whole length. This appears to be the case when the relatively gross changes are taken as the criterion of degeneration, as the experiment with one of our rats (the specimen kept at 35° C.) shows. When other histological or physiological criteria are used, different results have been obtained and these require discussion in relation to the present findings.

Physiological investigations have taken as the criterion of degeneration the breakdown of conduction in the nerve, or other changes in its electrical properties (Parker, 1933; Rosenblueth & Dempsey, 1939; Rosenblueth & Del Pozo, 1943; Erlanger & Schoepfle, 1946) and, except in the case of Parker's work, apply only to the first 4 days after section. The results have been somewhat equivocal. Rosenblueth & Dempsey (1939) and Rosenblueth & Del Pozo (1943), working on the cat's peroneal and popliteal nerves, concluded that degenerative changes began proximally near the lesion and progressed distally. Erlanger & Schoepfle (1946), working on the phrenic nerve of the dog, concluded that failures in conduction are abrupt and occur 'at random loci increasing in frequency peripheral-wards, or possibly with failure proceeding centripetally'.

The present experiments, using as evidence the comparatively gross histological changes which occur 4 days or more after nerve section, are not directly relevant to the physiological observations. They do suggest, however, that since large temperature differences can have so much effect in these later stages, smaller differences may well affect the physiological changes in the earlier stages. It was in limb nerves (peroneal and popliteal, Rosenblueth & Dempsey (1939); Rosenblueth & Del Pozo (1943)) that evidence for centrifugal progression was found, and centrifugal temperature gradients have been demonstrated in the limbs of mammals. Huggins, Blockson & Norman (1936) showed in the rat and the rabbit that this would account for a reduction in temperature of from 4 to 8° C. in the bone marrow in the distal segments of the limbs, and we have found similar differences in the subcutaneous temperature in rats' limbs in an environmental temperature of 22° C.

Parker (1933) worked on the sciatic nerve of the frog, a poikilothermic animal where no temperature gradient would be expected. However, his results do not

necessarily lead to the conclusions he drew from them. He cut the sciatic nerve close to its origin in the limb plexus, and found, between 12 and 15 days later, that identical stimuli at various points led to stronger muscular responses when those points were in the distal part of the nerve. He concluded that breakdown in conduction had occurred earlier in the proximal part of the nerve. If, however, the breakdown in conduction occurs first at particular loci on the course of individual nerve fibres (as the work of Erlanger & Schoepfle (1946) suggests) and if these loci occur randomly with no preference for the proximal rather than the distal part of a nerve fibre, then the same results would still be obtained. The longer the length of nerve tested, the better the chance that all its fibres would have lost the power of conduction at one point or another, and the shorter the length so tested the better the chance that failure in some of its fibres would have occurred above the level of the stimulus applied, leaving intact fibres below still capable of conduction. Since, therefore, in the investigations by Rosenblueth & Dempsey (1939) and Rosenblueth & Del Pozo (1943), the results might clearly have been affected by a temperature gradient along the nerve, one can conclude only that there is no satisfactory evidence for a progression of changes either distally from the lesion or proximally towards it. It is, indeed, probable that the failure of conduction does occur at loci distributed at random along the course of the nerve, and in any future investigation of this matter, the temperature conditions will require careful control.

Previous histological investigations have also given equivocal results and the earlier work was reviewed by Parker & Paine (1934). They themselves, working on the lateral line nerve of the catfish (in which no temperature gradient would be expected) considered that histological changes were first evident proximally and progressed distally. The histological differences, however, were small and their statements do not seem to be supported by the illustrations. The work of Weddell & Glees (1941), on nerves in the rabbit's ear, suggests an opposite conclusion. They found, at various times up to 96 hr. after nerve section, that histological evidence of degeneration was more marked in the peripheral cutaneous plexuses than in the same fibres more proximally situated in the parent nerve trunk. The most recent investigation is that of Causey & Palmer (1953), who used as their criterion of degeneration the lengthening of the nodes caused by retraction of myelin occurring up to 96 hr. after section of a nerve. They worked on the phrenic nerve and the nerve to the medial head of gastrocnemius in the rabbit, and found that lengthening of the nodes was much more marked proximally (near the lesion) than distally, particularly in the early part of the period studied. It is difficult to account for these results in terms of a temperature gradient, although the possibility that such a gradient exists cannot be completely excluded, particularly in the nerve to the gastrocnemius. The possibility that the original trauma of cutting or crushing the nerve may have interfered with the blood supply also cannot be excluded, although it does not appear a likely cause of the effect described.

Taking the present results (which deal only with fairly obvious histological changes produced by large temperature differences) into account, the only conclusions which can be drawn are as follows. In the first few days following injury to a nerve, there is some evidence that the changes which accompany the process of degeneration begin proximally and progress distally. This effect has not been evident in all the

relevant investigations, and could be due to causes other than inherent characteristics of the nerve fibre or of the degeneration process. Among these causes are temperature gradients along the course of the nerve; these undoubtedly become of great importance when they are large, and influence the appearances found in later stages (4 days or more after injury) very considerably. It is probable that smaller temperature gradients could produce similar effects in earlier stages, especially where electro-physiological or histological methods are used which are capable of demonstrating very small changes in the behaviour or structure of the nerve fibre. It is clear that for work of this kind, poikilothermic animals where temperature gradients are less likely to be a complicating factor, have considerable advantages.

SUMMARY

In the Wallerian degeneration of cut nerve fibres, local temperatures play an important part in determining the site of onset and the subsequent rate of degenerative change; cold retards and warmth accelerates degenerative change whether acting upon the proximal or distal part of the fibre.

The results have been discussed in relation to the findings of other investigations in this field.

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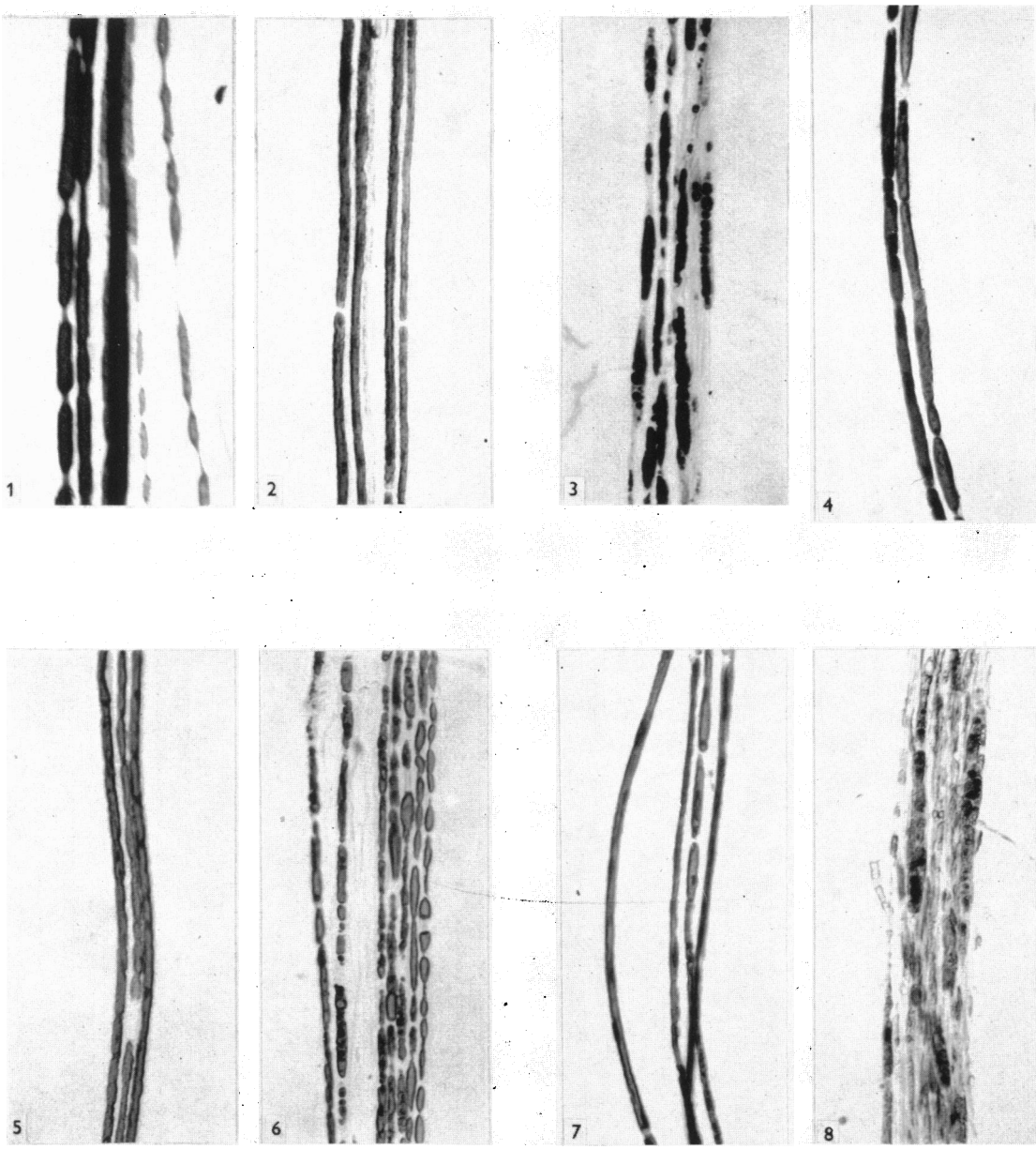

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EXPLANATION OF PLATE

All nerves were fixed in 1 % osmium tetroxide and teased in glycerine. The photographs were taken at 200 magnifications.

Fig. 1. Fibres of ventral caudal nerve, from its proximal warm part, 5 days after section in the 'root' of the tail.

Fig. 2. Fibres of the same nerve from its distal, cold part.

Fig. 3. Fibres of ventral caudal nerve, from its proximal warm part, 25 days after section in the 'root' of the tail.

Fig. 4. Fibres of the same nerve from its distal, cold part.

Fig. 5. Fibres from the ventral caudal nerve in the proximal, cold part 4 days after section in grafted tail.

Fig. 6. Fibres from the same nerve in its distal, warm part.

Fig. 7. Fibres from the ventral caudal nerve in its proximal, cold part 25 days after section in grafted tail.

Fig. 8. Fibres from the same nerve in its distal, warm part.