ENDINGS PRODUCED BY SOMATIC NERVE FIBRES GROWING INTO THE ADRENAL GLAND

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New light has been shed on the mechanism of peripheral nerve regeneration by the experimental work of the past few years. It has been found that the re-establishment of a peripheral connexion has a profound influence on the diameter of regenerating fibres in the case of both sensory and motor somatic nerves. The nerve fibres which grow out from the central stump of an interrupted nerve are numerous and small. If these fibres are not allowed to reach their end-organs they fail to mature, so that the regenerated stretch of nerve contains numerous small fibres (Weiss, Edds & Cavanaugh, 1945; Sanders & Young, 1945, 1946). Those fibres which reach their end-organs increase to their normal diameter and in some way bring about the disappearance of the excess of small fibres.

However, this influence of the end-organ is not the only factor controlling the diameter reached by regenerating fibres. Young (1942) and Simpson & Young (1945) demonstrated that when a somatic nerve is united to the non-medullated anterior mesenteric nerve in rabbits medullated fibres are formed in the latter, though they are of smaller diameter than those produced by union of somatic to somatic nerve. A similar result was obtained following anastomosis of somatic nerve to the finely medullated greater splanchnic nerve. Hammond & Hinsey (1945) have shown similar effects with other nerves. These results show that the size reached by regenerating fibres depends on the diameter of the central stump fibres and on the size of the peripheral tubes into which they grow, as well as on their terminal connexions.

The present investigation deals with the distribution and nature of the nerve fibres which reach the adrenal following anastomosis of a somatic nerve as central stump to the greater splanchnic nerve. It is well established that in a normal animal preganglionic fibres run direct from the splanchnic nerves to end in the adrenal medulla (Elliott, 1913; Hollinshead, 1936; Young, 1939). The aim was to see whether the large somatic axons would grow to large size in the adrenal, and whether they would follow the pathways of the normal fibres to the gland, remaking the normal nerve plexus in the medulla. This constitutes a new test of the effect of central and peripheral influence on nerve regeneration. Should it prove that somatic fibres make large endings in autonomic plexuses this might provide a new method of investigating the more obscure points of peripheral autonomic nerve distribution, by using the readily identified somatic myelinated fibres as 'tracers'.

OPERATIVE METHOD

Adult rabbits were used. The abdomen was opened under nembutal and ether anaesthesia; the left greater splanchnic nerve was then identified and cut 1·5 cm. proximal to the anterior mesenteric ganglion, the central end being evulsed. The central ramus of one of the upper two lumbar nerves was then dissected free from the underlying muscle and cut 4 cm. from the lateral border of psoas. The proximal end was brought into apposition with the distal cut end of the greater splanchnic and joined by the application of a solution of human fibrinogen freshly made up from dried material and mixed with thrombin. The dried fibrinogen and thrombin were kindly supplied by the Lister Institute and proved very convenient for the purpose.

The animals were allowed to survive the operation for periods ranging from 100 to 200 days. Biopsy was then carried out under nembutal anaesthesia, both adrenals, and the site of anastomosis, being removed for fixation.

HISTOLOGICAL METHODS

Normal glands were stained by the Bielschowsky-Gros, Cajal, and Bodian methods, but the latter only was used in staining the experimental glands. Certain modifications have been introduced into the Bodian method and the exact technique used was as follows:

(1) Fixation of the glands in Carnoy's solution for 2 hr. (absolute alcohol 60 parts, chloroform 1 part, glacial acetic acid 1 part).

(2) Dehydration and paraffin embedding.

(3) Sections were cut at 15μ thickness and stained with 1 % Protargol in distilled water (Protargol supplied by Winthrop Chemical Co., New York). Sections were left in this solution for 24 hr. at 37° C.

(4) Distilled water—10 min.

(5) 1 % oxalic acid for periods varying from 10 sec. to 1 min.

(6) Reduction. A solution of hydroquinone 1 %, sodium sulphite 5 % was used. Reduction is complete in about 1½ min.

(7) Wash in running tap water for 1 min. followed by distilled water for 1 min.

(8) Toning, 0·1 % gold chloride 4 min.

(9) Oxalic acid 2 % for 2–3 min. This stage was observed under the microscope and the process stopped when the fibres appeared black on a grey background.

(10) Wash in distilled water for 1 min.

(11) Fix in hypo, wash, dehydrate and mount in balsam.

If the sections appear purple after stage 9 the period in oxalic acid in stage 5 should be increased.

NORMAL INNERVATION OF THE ADRENAL

To obtain a standard of comparison the innervation of the normal adrenal gland was reinvestigated using Bielschowsky, Cajal and a modified Bodian method. The results confirmed the work of Hollinshead (1936), Willard (1936) and Young (1939). The subcapsular plexus gives off a number of nerve bundles which pass through the cortex towards the medulla in a radial direction. These bundles occasionally give off branches while passing through the cortex, but in spite of careful search no nerve endings were found in the cortex adjacent to the nerve bundles, so presumably the few branches found in this region eventually reach the medulla. Alpert (1931) and others have claimed that nerve fibres end around cortical cells, but it is now generally held that this tissue has no direct innervation, all nerves within it passing either to blood vessels or to the medulla. At the cortico-medullary junction the nerve bundles can often be seen to bend, becoming oriented parallel with the margin of the medulla before they finally enter it.

In marked contrast to the poverty of fibres in the cortex, the medulla is very richly innervated. Nerve bundles can be traced inwards from the trunks at the cortico-medullary junction; usually they accompany the blood vessels. They soon branch profusely to form a nerve plexus around the lobules of chromophil tissue cells (Pl. 1, fig. 1). From this plexus fine nerve fibres are given off which ramify among the cells of the lobule, thus ensuring that all the chromophil cells are in contact with at least one nerve fibre.

It is probable that stimulation of the medullary cells is effected by direct contact with the fine fibres of the nerve network. Bulbs of various sizes have been described (Hollinshead, Willard), some situated in the course of the fibre and some at its termination, but they are not sufficiently numerous to be the main factor in the transmission of impulses. Thus Willard (1936) found that there were only 1·4 boutons per fifty chromophil cells in adult guinea-pigs.

No nerve cells were found in the medulla of the rabbit, although several preparations show well-stained ganglia in the extracapsular tissue.

EXPERIMENTAL FINDINGS

(1) *In the cortex*

In the glands innervated by somatic nerve fibres marked abnormalities are very clearly seen. First there are fibres far thicker than any normally present, derived from the axons of the donor nerve. A proportion of these pass radially in the nerve trunks, as in the normal arrangement, through the cortex to the medulla. In the cortex considerable lengths of thick fibre can be seen in even a single section (Pl. 2, fig. 5). Very many of the new fibres, however, have escaped from the nerve trunks into the neighbouring cortex, where they wander for some distance from the parent trunk (Pl. 2, figs. 4, 5). They are thus found in groups scattered throughout the cortex, each group having in its centre

a nerve trunk (Pl. 2, fig. 4). An example of a fibre actually leaving a trunk is shown in Pl. 2, fig. 5.

Only short lengths of these escaped fibres were seen in any one section, and in general they tended to be disposed tangentially, which facts indicate a tortuous course. Indeed, it proved very difficult to trace them in continuity even in serial sections.

It must be stressed again that the fibres found in the cortex adjacent to the nerve trunks in a normal gland are few in number and remain close to the parent trunk. These thick fibres in the hetero-innervated glands were present in far greater number and had wandered much farther from the trunk than is the case in the normal arrangement (Pl. 2, fig. 4). It is clear therefore that these fibres had not grown down pre-existing sheaths but had escaped from their tubes in the trunk.

These thick fibres showed an irregular undulating or varicose outline in their whole course, with frequent branching. The great majority did not reach the medulla but ended in the cortex. Their endings had the form of a club-shaped expansion with numerous short bulbous outgrowths, giving an irregular coarse stellate appearance (Pl. 2, fig. 7). Here and there a much finer filament was seen springing either from the main club-shaped expansion or from one of its excrescences (Pl. 2, figs. 6, 7). These filaments ran for a short distance amongst the cortical cells and terminated in a fine or coarse bouton type of ending.

In addition to these thick fibres, fine fibres, regular in outline and histologically similar to those found in the medulla of a normal gland, were seen in large numbers around the nerve trunks, and spread out for a short distance into the surrounding cortex (Pl. 2, fig. 5). In a few cases these fine filaments could be seen to arise from one of the thick nerve fibres in the trunk but the majority appeared to have no relation to them. Furthermore, they differed from the thick fibres in their mode of distribution, they ran tangentially and formed a plexus amongst the cortical cells. The result was a most striking increase in the overall richness of nerve fibres in the cortex. However, it must be emphasized that these nerve fibres were all close to the bundles running through the cortex, large areas of cortical tissue situated at a distance from such bundles were quite devoid of nerve fibres, as in the normal gland.

(2) *Pattern in the hetero-innervated medulla*

Here again large and small fibres were present. They entered the medulla as components either of a nerve bundle or of the cortical plexuses. In contrast to the appearance in the normal medulla, where the intercellular plexus is found developed in a uniformly rich manner in all areas (Pl. 1, fig. 1), it is not so developed in the medulla of the experimental gland, especially in those surviving 100 days (see below). A network similar to the normal was found only in discrete areas comprising a small portion of the whole, in most cases

less than one-third. These scattered, richly innervated areas presumably contain fibres derived from the lesser splanchnic and lumbar sympathetic chain and not interrupted by the operation. So the areas of medulla showing a relative poverty in their fine fibre innervation (and comprising at least two-thirds of the whole) can be regarded as those to which the lesser splanchnic and lumbar sympathetic make no contribution (see Young, 1939). Whereas at 100 days the poverty of the medullary plexus in these areas was striking, in one rabbit killed at 200 days there was a definite advance towards the normal density of innervation.

The thick fibres in the medulla occurred singly or in small groups, in the form of either straight or curved lengths, showing a slightly undulating outline and branching infrequently (Pl. 1, figs. 2, 3). These fibres were nowhere seen to develop a plexus arrangement. They appeared to end abruptly with no more than slightly bulbous expansion at their tips. They did not display any of the elaborate behaviour seen in the cortex. The fine fibres were found in small numbers amongst the chromophil cells. They branched frequently, but after 100 days the striking feature was that these fibres had not reproduced the normal rich medullary plexus. Even at 200 days there were considerable areas of medulla still poorly innervated.

DISCUSSION

Behaviour in the cortex

It is clear that the innervation pattern described above has been produced by the interaction of at least two abnormal factors. In the first place the regenerating somatic fibres often escape from the sheaths to invade the cortical tissue. Secondly these escaped fibres were subjected to grossly abnormal conditions; the adrenal cortex is densely cellular and produces a considerable mechanical obstruction to growth.

Many observations have been carried out to determine the effect of obstruction on the growing axon. Cajal (1928) investigated the effect of ligatures tied round regenerating nerve trunks and describes how the fibres proximal to the constriction formed large balls, with very fine exploratory filaments sent out from them in an attempt to force a path or find an alternative route. Speidel (1933), while observing the growth cones of regenerating nerves *in vivo*, described the formation of giant cones when formidable obstructions were reached. It has also been observed that during the early stages of normal regeneration, even in the absence of any artificial obstruction, the central end of the axon attached to the cell often swells. This expansion of the axon at the tip of the central stump is then a normal phenomenon in regeneration, but it is greatly exaggerated where there is obstruction (Weiss *et al.* 1945). This has led to the view that the nerve cell exerts a turgor pressure on the whole unit. The fine filaments arising from the club-shaped masses presumably represent an outflow into such small cracks as are available.

The thick fibres

The most likely explanation of the large club-shaped masses of axoplasm found in the cortex of the experimental animals is, then, that they are the result of obstruction to the longitudinal growth of the thick somatic axons at these points. After an axon became held up by the cortical cells the turgor pressure from the nerve cell continued to pour axoplasm into the tip.

The fine fibres

There are two possible sources of these fibres: (*a*) they may be derived from the autonomic and pain fibres in the somatic nerve, (*b*) some are definitely branches of the thick fibres. One example was found of a fine plexus being formed in the cortex from the fibre taking origin from a thick axon. It must be stressed, however, that most of the fine filaments arising from the thick fibres terminated in boutons a short distance from their source. It is probable that the fine fibres were derived to some extent from both these sources.

Behaviour in the medulla

The adrenal medulla consists of groups of chromophil cells surrounded by the sinusoidal venules. It is a far less compact tissue than is the cortex and hence obstruction phenomena would be expected to be less. As a result the thick fibres do not produce the club-shaped terminations found in the cortex, but rather form straight or curved rods with only a slight swelling at the tip.

Another striking feature is the absence of any formation of a plexus by these thick fibres. It would be interesting to investigate whether any adrenalin is produced when these somatic nerves are stimulated. Theoretically adrenalin could be produced, for both the somatic fibres and the preganglionic sympathetic fibres which normally innervate the adrenal medulla secrete acetylcholine (acetylcholine production was demonstrated in the adrenal by Feldberg, Minz & Tsudzimura (1934). Experiments to investigate this possibility are now being undertaken.

The fact that somatic fibres growing into the adrenal form fibres which are much larger than those normally present confirms the finding of Simpson & Young (1945) and Hammond & Hinsey (1945) that the size of a parent fibre has an influence on that of fibres regenerated from it, irrespective of their termination.

CONCLUSION

The conclusions so far are, then, that the influence of a central somatic fibre persists in a foreign autonomic tissue and determines that the regenerating axons shall be thick, but that this influence is not sufficiently strong to produce the formation of somatic end organs. It can also be concluded that the adrenal is unable to impose its own nerve pattern on the ingrowing fibres.

We have seen that nerve regeneration is not purely a non-specific process of downgrowth at random, but is markedly influenced by both central and

peripheral factors. It is only when an axon grows down to its own periphery and when the periphery is innervated by its own central axon that the normal pattern is reproduced. The part that function plays in the regeneration process is at present little understood, it may be that when an axon reaches a suitable periphery it begins to function (at first inefficiently) and that this acts as a stimulus which brings about the modelling of the terminal ramifications necessary for the more efficient carrying on of these functions. Further experiments are necessary to elucidate the part function plays in nerve regeneration.

SUMMARY

1. Somatic nerve fibres made to grow into the great splanchnic nerve and adrenal gland of rabbits produced the following abnormalities: (*a*) Many fibres escaped from the nerve bundles running through the cortex and proceeded for some distance among the cortical cells. They end as large bulbs, presumably when obstructed. (*b*) Fibres reaching the medulla end as large rods and do not form typical plexuses.

2. It is therefore demonstrated that the type of nerve termination produced in this gland during regeneration is affected by the nature of the central stump from which the fibre proceeds.

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REFERENCES

ALPERT, L. K. (1931). The innervation of the suprarenal glands. *Anat. Rec.* **50**, 221–233.

CAJAL, S. R. (1928). *Degeneration and Regeneration of the Nervous System.* Oxford.

ELLIOTT, T. R. (1913). The innervation of the adrenal glands. *J. Physiol.* **46**, 285–290.

FELDBERG, W., MINZ, B., & TSUDZIMURA, H. (1934). Mechanism of nervous discharge of adrenaline. *J. Physiol.* **81**, 286–304.

HAMMOND, W. S. & HINSEY, J. C. (1945). Diameters of nerve fibres in normal and regenerating nerves. *J. comp. Neurol.* **83**, 79–91.

HOLLINSHEAD, W. H. (1936). The innervation of the adrenal glands. *J. comp. Neurol.* **64**, 449–467.

SANDERS, F. K. & YOUNG, J. Z. (1945). Effect of peripheral connexion on the diameter of nerve fibres. *Nature, Lond.*, **155**, 237–238.

SANDERS, F. K. & YOUNG, J. Z. (1946). The influence of peripheral connexion on the diameter of regenerating nerve fibres. *J. exp. Biol.* **22**, 203–212.

SIMPSON, S. A. & YOUNG, J. Z. (1945). Regeneration of fibre diameter after cross-unions of visceral and somatic nerves. *J. Anat., Lond.*, **79**, 48–65.

SPEIDEL, C. C. (1933). Studies of living nerves. *Amer. J. Anat.* **52**, 1–79.

WEISS, P., EDDS, M. V. JR., & CAVANAUGH, M. (1945). The effect of terminal connexions on the caliber of nerve fibres. *Anat. Rec.* **92**, 215–233.

WILLARD, D. M. (1936). The innervation of the adrenal glands of mammals. *Quart. J. micr. Sci.* **78**, 475–486.

YOUNG, J. Z. (1939). Partial degeneration of the nerve supply of the adrenal. A study in autonomic innervation. *J. Anat., Lond.*, **73**, 540–550.

YOUNG, J. Z. (1942). Functional repair of nervous tissue. *Physiol. Rev.* **22**, 319–347.

EXPLANATION OF PLATES

All figures are of adrenal glands of rabbits stained with Bodian's method.

Plate 1

Fig. 1. Normal medulla. The rich plexus is found amongst the chromophil cells.

Fig. 2. Hetero-innervated medulla, 100 days. The thick and fine fibres are seen running together inside the nerve bundle.

Fig. 3. Cortico-medullary junction of hetero-innervated gland, 100 days. A thick fibre is seen leaving a bundle to branch in the surrounding tissue. *b*, bundle cut transversely.

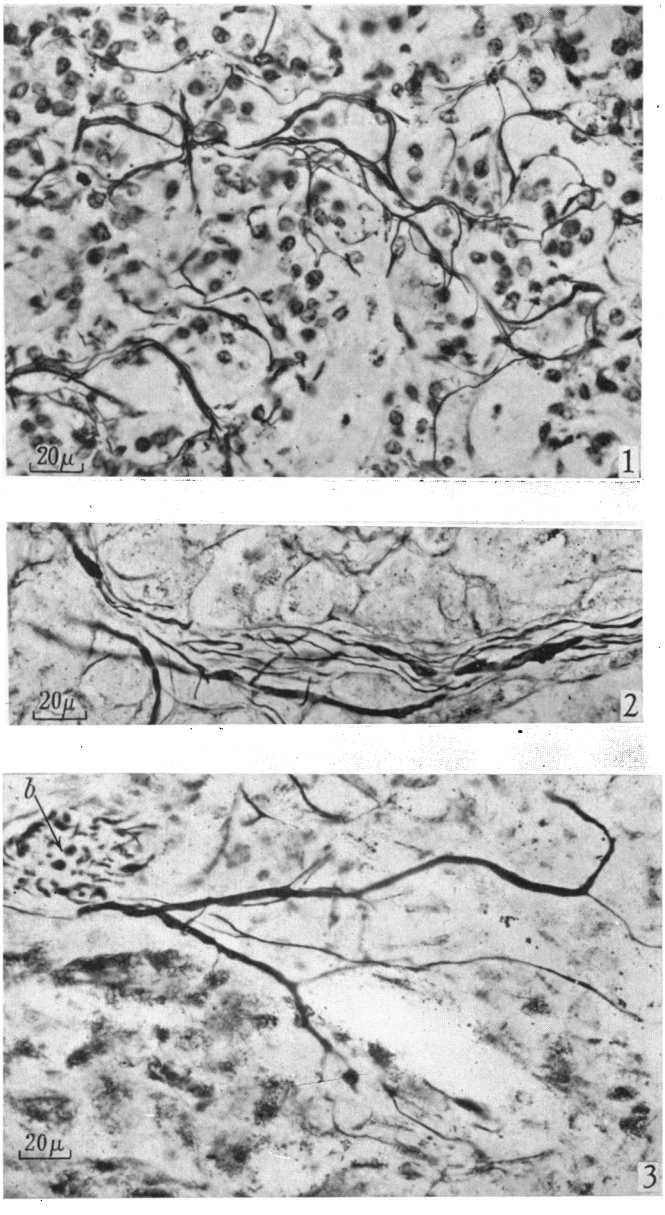
Plate 2

Fig. 4. Hetero-innervated cortex, 100 days. A nerve bundle (*b*) is cut transversely. A number of thick somatic fibres which have escaped into the neighbouring cortex are seen.

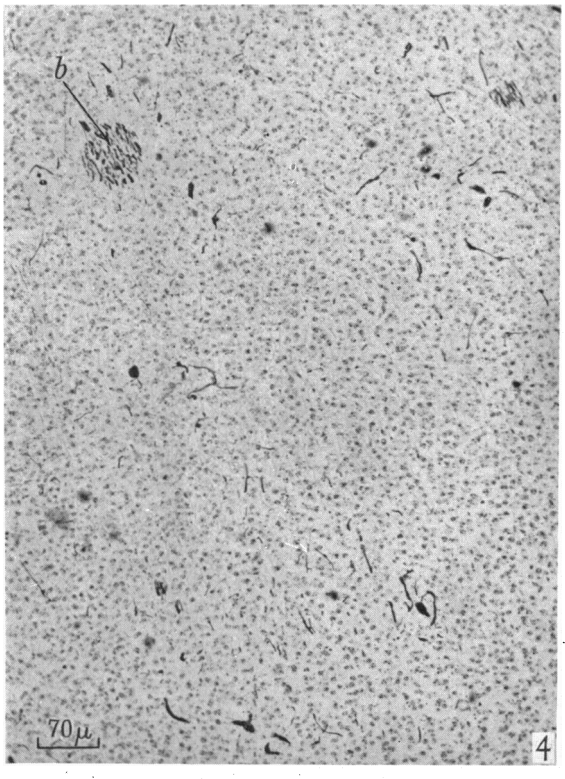
Fig. 5. Hetero-innervated cortex, 100 days. A nerve bundle is seen passing through the cortex. Most of the fibres are fine, but a thick one can be seen escaping into the cortex. Numerous fine fibres are also seen running between the cell columns.

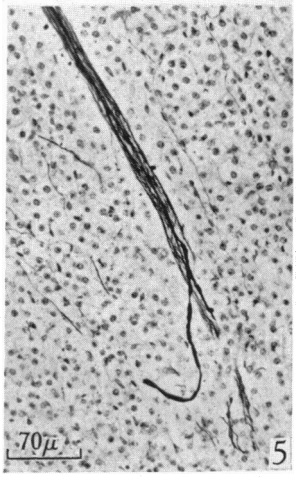
Fig. 6. Hetero-innervated cortex, 100 days. A higher power view of the cortex around a nerve bundle (*b*). The varicose appearance of the thick fibres can be seen.

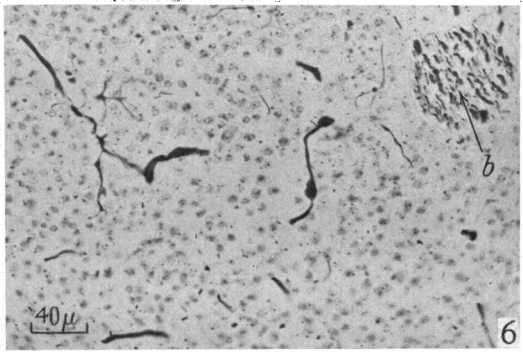
Fig. 7. Hetero-innervated cortex, 100, days showing the club-shaped terminations of the somatic fibres. Fine processes (*p*) ending in boutons can be seen arising from these masses.

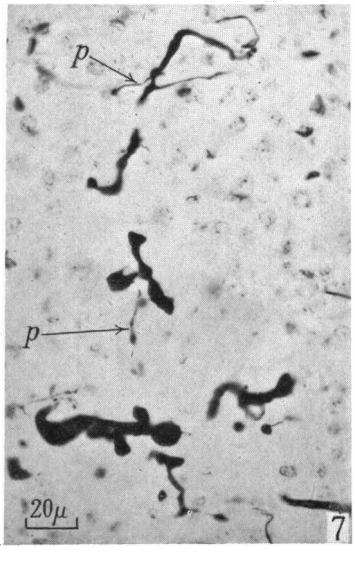


Figs. 1—3









Figs. 4—7