THE EFFECT OF PREGANGLIONIC SECTION ON THE NEURONS OF THE SUPERIOR CERVICAL GANGLION IN RABBITS

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INTRODUCTION

There is evidence that at least in some cases if the afferent connexions of a neuron are allowed to degenerate the perikaryon undergoes morphological changes. These changes, known collectively as transneuronal degeneration, are especially marked in the cells of the lateral geniculate body following section of the optic nerve (Minkowski, 1920; Clark, 1932; Clark & Penman, 1934). In this situation the process is rapid and more complete in primates and slower and less severe in other mammals such as the rabbit and cat (Minkowski, 1920, 1922; Cook, Walker & Barr, 1951).

Transneuronal degeneration has been described elsewhere in the central nervous system. Warrington (1898, 1899) found chromatolysis in the spinal cord of the cat and the monkey following dorsal root section or transection of the cord. Barron (1933) described chromatolysis in anterior horn cells after section of the pyramidal tract. Foerster, Gagel & Sheehan (1933) have described transneuronal effects in the spinal cord following posterior root section, and Foerster & Gagel (1934) found degenerative changes in the cuneate nucleus after section of the posterior columns.

Other writers (Schimert, 1939; Hare & Hinsey, 1940) have failed to confirm the occurrence of transneuronal degeneration in spinal cord neurons. Cook *et al.* (1951), in a study involving section of cortico-spinal tracts or dorsal root fibres, found that '...no cell changes were observed in experimental animals which could not be found in control animals or explained by factors other than partial isolation'.

From these conflicting results it would appear either that the transneuronal effect is not so pronounced in the case of spinal cord neurons, or that the effect depends on total isolation of the neuron.

It is possible that neurons in different parts of the nervous system react to a varying extent to deafferentation. Clark (1943) considers that the severe reaction found in the cells of the lateral geniculate body after optic nerve section is '...indicative of the extreme specificity of function of the cells in this nucleus'.

Complete isolation of neurons is also possible in the autonomic nervous system, e.g. in the superior cervical ganglion by cutting the preganglionic trunk. The reaction of the cells of this ganglion is not so severe as that of the cells of the lateral geniculate body. The changes which have been described following preganglionic section are shrinkage of the neuron with some crenation of its margin and a tendency of the Nissl substance to become aggregated at the periphery of the cell (Sternschein, 1920; de Castro, 1923). However, Gibson (1940) failed to find any changes in the ganglion cell bodies of the superior cervical ganglion following preganglionic section.

The present study is a reinvestigation of the transneuronal effect in the superior cervical ganglion. An attempt has been made to estimate any change in cell size by quantitative methods.

MATERIAL AND METHODS

Adult rabbits of various breeds and both sexes were used. Anaesthesia was begun with pento-barbital sodium (Nembutal), the dose given being 30 mg./kg. body weight; this was supplemented where necessary by open ether. Using full aseptic precautions the cervical sympathetic trunk was exposed at the level of the cricoid cartilage and then stimulated by means of a faradic current. The resulting dilation of the pupil was taken as confirmation of the identity of the isolated nerve.

The trunk was then divided as low down as possible and the cut ends sutured into muscle so as to prevent regeneration. The trunk of the opposite side was exposed and stimulated but not divided.

Survival times were allowed up to 150 days, the animals being biopsied under Nembutal-ether anaesthesia. The superior cervical ganglia and the part of the cervical sympathetic trunks immediately caudal to them were removed. The ganglia were allowed to adhere to small cards and then fixed in Bouin's fluid (picric acid 75 ml.; 40% formaldehyde 25 ml.; glacial acetic acid 5 ml.), while the nerves, stretched on cards in which a window had been cut, were put into Bodian's fixative (40% formaldehyde 15 ml.; glacial acetic acid 5 ml.; absolute alcohol 80 ml.).

After embedding in paraffin the ganglia were cut longitudinally at 5μ thickness and the majority of the mounted sections stained with Heidenhain's azo-carmine and aniline blue. Some sections, however, were stained with buffered thionin or Borrel's blue in order to observe changes in the Nissl substance. In this respect it may be noted that the azo-carmine stain, in autonomic ganglia also gives a good picture of the Nissl bodies.

As the ganglion of the unoperated side was used as a control, the operated and unoperated ganglia from a particular animal were put through the staining and photographic processes together, and as far as possible under identical conditions.

The problem of making a quantitative estimate of cell size in the superior cervical ganglion is rendered difficult by the fact that the density of the connective tissue stroma does not permit the use of the method of optical section for measuring the diameter of the neuron.

The following procedure was therefore used. Photographs were taken at 750 diameters of random fields. In each field the eight largest cells showing at least one nucleus and nucleolus were traced on to good-quality tracing paper. The resulting outlines were cut out and weighed using a micro-balance, the weights being then converted to square micra. One hundred cell areas were measured for each ganglion and the mean of these results calculated. The figure from the operated side was then compared statistically with that of the control ganglion.

The method of tracing, cutting out, and weighing for the estimation of small irregular areas has been previously discussed by Scammon & Scott (1927) and Mainland (1929). The latter finds that celluloid of uniform thickness gave the greatest accuracy, but this is laborious to work with.

The use of tracing paper has the advantage that the cell outline is easily traced and then cut out with scissors. As it was thought possible that the thickness of the paper would not be sufficiently uniform to give the required degree of accuracy, a survey was carried out taking samples from the beginning, the middle and the end

of a roll of paper. Standard areas were punched out and on weighing these it was found that the variation was in fact very small (Table 1). The accuracy of the tracing and cutting out was tested by making a number of estimates of the same cell. These were found to be in close agreement (Table 2).

Table 1. *Weight in grams of standard discs* 1 *cm. in diameter, punched from a roll of tracing paper* 30 *in. wide and containing* 60 *ft. of paper. Samples of twelve discs each were taken at the beginning and end of the roll and at* 20 *ft. intervals between the two*

0 ft.	20 ft.	40 ft.	60 ft.
0·0036	0·0040	0·0038	0·0040
0·0040	0·0038	0·0040	0·0041
0·0042	0·0039	0·0039	0·0040
0·0040	0·0040	0·0038	0·0040
0·0038	0·0039	0·0040	0·0042
0·0038	0·0040	0·0040	0·0040
0·0038	0·0039	0·0040	0·0040
0·0040	0·0040	0·0040	0·0039
0·0040	0·0040	0·0039	0·0040
0·0040	0·0039	0·0039	0·0040
0·0039	0·0038	0·0040	0·0040
0·0040	0·0040	0·0040	0·0042
Mean weight (g.) 0·0039	0·0039	0·0039	0·0040

Table 2. *Weight in grams of the outlines of two neurons cut out of tracing paper. Fifteen outlines were weighed from each neuron*

	1st neuron	2nd neuron
	0·0183	0·0217
	0·0176	0·0222
	0·0174	0·0220
	0·0174	0·0219
	0·0174	0·0220
	0·0178	0·0220
	0·0182	0·0212
	0·0176	0·0218
	0·0176	0·0222
	0·0178	0·0228
	0·0176	0·0226
	0·0180	0·0226
	0·0176	0·0230
	0·0176	0·0220
	0·0182	0·0223
Mean	0·0177	0·0222
S.D. of the mean	$<0·0001$	$<0·0001$

RESULTS

The changes which have been described in the neurons of the superior cervical ganglion following preganglionic section are mild chromatolysis, crenation of the cell margin, aggregation of Nissl bodies at the periphery and displacement of the nucleus to the side of the cell (Sternschein, 1920).

In the present investigation chromatolysis was found to be difficult to detect. The Nissl substance in the neurons of the superior cervical ganglion is powdery in appearance, so that evidence such as the fragmentation of coarse granules, characteristic of chromatolysis in spinal motoneurones, is not found. However, the Nissl substance stains less easily after preganglionic section, the cells appearing paler.

This change is first seen at about 40 days after operation and persists at 160 days (Pl. 1, figs. 2, 4 and 6).

The other changes mentioned were not found to be characteristic of the deafferented ganglion. Crenation of the cell margin, peripheral aggregation of Nissl bodies and eccentric position of the nucleus were all noted in normal ganglia (Pl. 1, figs. 3, 5). A noticeable feature of the ganglion of the operated side is the greater amount of stroma (Pl. 2, figs. 2, 4 and 6), which shows an increase in the number of non-neuronal nuclei, as might be expected from the increase in Schwann cell population in the presence of degenerating preganglionic myelinated axons. Joseph (1950) has shown that there is an increase in the nuclear count of nerves containing small myelinated fibres during degeneration, but not in the case of nerves containing non-myelinated axons only.

It is difficult to estimate qualitatively any change in cell size in the operated compared with the normal ganglion. This difficulty is partly due to the fact that few neurons are cut equatorially, and partly to the increase in connective tissue stroma.

On considering the results of the measurement of cell areas (Table 3), it is seen that although the mean cross-sectional area of the neurons of the deafferented side is smaller than that of the control for the 20- and 30-day survival times, the difference only becomes statistically significant at 40 days.

Table 3. *Changes in neuronal area following isolation*

Survival time in days	Mean area of 100 neurons (μ^2)		Difference (μ^2)	Difference (%)	Significant ? (t test)
	Normal ganglion	Deafferented ganglion			
20	750	720	30	4·0	No
30	827	790	37	4·4	No
40	713	649	64	8·9	Yes
80	817	734	83	10·1	Yes
100	839	694	145	17·2	Yes
160	682	559	123	18·0	Yes

This reduction in size progresses from 8·9 % at 40 days to 17·2 % at 100 days and is still of about the same order (18 %) at 160 days.

DISCUSSION

The time course and degree of the neuronal shrinkage observed in these experiments is of about the same order as that described by Cook *et al.* (1951) in the neurons of the lateral geniculate body of the cat following eye enucleation. They found that reduction in cell size appeared at 63 days and reached a level of 25 % of the cross-sectional area of the cell body, where it remained constant up to a survival period of 10 months. The interpretation of the morphological changes which occur in isolated neurons is difficult and involves consideration of the cytochemistry of the Nissl bodies as well as alterations in the neuron and its environment.

The significance of the changes which occur in chromatolysis of neurons is not clear. It has been shown by Caspersson (1940) and Landström, Caspersson & Wohlfart (1941) that the Nissl substance consists essentially of nucleoproteins, the

nucleotide components being possibly of the ribose type. Cook *et al.* (1951) found that the reduction in neuronal volume in the deafferented neurons of the lateral geniculate body was accompanied by depletion of the Nissl substance and diminution in size of the nucleus and nucleolus. They suggested that these findings may imply a depression of nucleic acid and nucleoprotein metabolism, since the nucleus and especially the nucleolus is intimately concerned with the synthesis of Nissl bodies (see Hydén, 1943). Furthermore, these authors point out that the inactivity resulting from deafferentation may be partly responsible for this depression, as Hydén has also reported that the metabolism of ribose nucleoprotein is influenced by electrical stimulation of the neuron. On the other hand, Liu, Bailey & Windle (1950), in a critical investigation of the effect of electrical stimulation on neurons, were unable to confirm Hydén's findings.

Gersh & Bodian (1943) investigated the chemistry of chromatolysis in spinal motoneurons following dorsal and ventral root section. They used ultra-violet absorption methods similar to those of the Stockholm school, and found insufficient evidence to determine the nucleus or the nucleolus as the site of replacement of the Nissl bodies. Their work suggests that although the nuclear changes in nucleotide and protein components during chromatolysis may be similar to those taking place in the cytoplasm, they occur to a much smaller extent. As a result of their studies and those of Schoenheimer (1942), these authors consider that 'the enzyme mechanisms disturbed in chromatolysis are present both in the nucleus and the cytoplasm'.

There is a tendency to refer to all changes in the appearance of the Nissl substance of neurons as chromatolysis, with the implication that the physico-chemical mechanism underlying such changes is similar in all cases. Alteration in the Nissl bodies occurs in widely differing circumstances; as a result of disease, the action of toxins, axonal section or isolation of neurons. It seems unlikely that the chemical changes undergone by the Nissl material are the same in all instances. That such changes may not be always of the same kind, even as judged by microscopical observation is seen from the attempt to classify chromatolysis into stages by Campbell & Novick (1946). These authors investigated the spinal cord of cats following section of the sacral roots and observed that the affected neurons varied in two directions from normal. Three groups of neurons showed the progressive disintegration of the Nissl bodies usually described as chromatolysis, whereas in two other groups the Nissl particles were larger and more intensely staining than normal. The time course of the changes occurring in transneuronal degeneration is different from that of chromatolysis resulting from axonal section. The earliest onset of transneuronal degeneration is seen in the lateral geniculate body of primates 7 days after isolation (Clark, 1943), whereas the chromatolytic change following axon section is apparent 43 hr. following operation (Gersh & Bodian, 1943).

Chromatolysis is associated with changes in cell size. Gersh & Bodian (1943) noted in their experiments that the chromatolytic neurons appeared more spherical in outline, and that they had increased in volume.

In the case of transneuronal degeneration, as seen in the lateral geniculate body or the superior cervical ganglion, the neurons undergo a progressive reduction in size, no phase having been recorded in which the neuronal volume is increased,

except that swelling of neurons which have undergone deafferentation has been described in the sensory nucleus of the fifth nerve about 100 days after alcohol injection of the Gasserian ganglion (Penman & Smith, 1950).

The reaction of the neurons of the superior cervical ganglion to deafferentation is not as severe as that which occurs in the lateral geniculate body, either in the amount of neuronal shrinkage, or in the degree of severity of the chromatolytic reaction. It may be that sympathetic ganglion cells are less sensitive to changes in their environment than the neurons of the lateral geniculate body. Murray & Stout (1947) observed migration and division of sympathetic ganglion cells from adult animals when cultured *in vitro*. This does not appear to have been demonstrated in the case of neurons from the central nervous system and may indicate greater powers of autonomy in the case of the ganglion cells.

Little is known about the functional condition of neurons which have been subjected to deafferentation. No investigation of the physiological state of the neurons of the lateral geniculate body following isolation appears to have been attempted.

According to Gibson (1940) the soma potentials of isolated neurons of the superior cervical ganglion appear normal. This was judged by firing antidromic impulses down the postganglionic trunk after preganglionic section. However, antidromic invasion of the neuronal soma does not occur physiologically, and the interpretation of such results is difficult (Eccles, 1950).

Also in the case of the superior cervical ganglion, Cannon & Rosenblueth (1936) observed that as indicated by contractions of the nictitating membrane, its neurons were more sensitive to circulating acetylcholine after deafferentation.

In the peripheral autonomic nervous system it seems that denervated structures generally show an increased irritability to circulating chemical agents, as stated in Cannon's 'Law of Denervation' (1939), and the 'Law' has since been extended to the central nervous system, as seen from the work of Drake & Stravraky (1948). So far, this increased sensitivity of denervated structures has not been explained.

Complete deafferentation of neurons is practicable only in certain situations such as the lateral geniculate body or the superior cervical ganglion. Such complete isolation is always followed by the morphological changes known as transneuronal degeneration; there is no definite evidence of change in the case of partially isolated neurons. It is likely that changes in the immediate environment of the neuron, such as the disintegration of afferent axons and the disappearance of synaptic terminals, together with the reaction of the connective tissue, play an important part in establishing transneuronal degeneration. The absence of normal stimulation may affect the neuronal metabolism, but there is no certain evidence of this.

SUMMARY

1. A qualitative and quantitative study has been made of the changes which occur in the neurons of the superior cervical sympathetic ganglion of the rabbit following preganglionic section.

2. The changes observed were less severe than those which have been described for some neurons of the central nervous system during transneuronal degeneration. In the isolated ganglion the Nissl substance did not stain as strongly as in the

normal, and the mean cross-sectional area of the neurons was reduced. There was an increase in the connective tissue of the ganglion.

3. The interpretation of the morphological changes which take place in deafferented neurons is difficult. The absence of the normal stimuli may cause alterations in the neuronal metabolism, but it is likely that the change in the immediate environment of the cell caused by the disintegration of the afferent terminals is an important factor.

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REFERENCES

BARRON, D. H. (1933). Structural changes in anterior horn cells following central lesions. *Proc. Soc. exp. Biol., N.Y.*, **30**, 1327–1329.

CAMPBELL, B. & NOVICK, ROSALIND (1946). A quantitative method for the study of chromatolysis. *Proc. Soc. exp. Biol., N.Y.*, **61**, 425–427.

CANNON, W. B. (1939). A law of denervation. *Amer. J. med. Sci.* **198**, 737–750.

CANNON, W. B. & ROSENBLUETH, A. (1936). The sensitisation of a sympathetic ganglion by preganglionic denervation. *Amer. J. Physiol.* **116**, 408–413.

CASPERSSON, T. (1940). Methods for the determination of the absorption spectra of cell structures. *J.R. Micr. Soc.* **60**, 8–25.

CASTRO, F. DE (1923). Evolución de los ganglios simpáticos vertebrales y prevertebrales. Conexiones y citoarquitectonia de algunos grupos de ganglios en el niño y hombre adulto. *Trab. Lab. Invest. biol. Univ. Madr.* **20**, 113–208.

CLARK, W. E. LE GROS. (1932). A morphological study of the lateral geniculate body. *Brit. J. Ophthal.* **16**, 264–284.

CLARK, W. E. LE GROS (1943). The Doyne Memorial Lecture: The anatomy of cortical vision. *Trans. ophthal. Soc. U.K.* **62**, 229–245.

CLARK, W. E. LE GROS & PENMAN, G. G. (1934). The projection of the retina in the lateral geniculate body. *Proc. Roy. Soc.* B, **114**, 291–313.

COOK, W. H., WALKER, J. H. & BARR, M. L. (1951). A cytological study of transneuronal atrophy in the cat and rabbit. *J. comp. Neurol.* **94**, 267–291.

DRAKE, C. G. & STRAVRAKY, G. W. (1948). An extension of the 'Law of denervation' to afferent neurones. *J. Neurophysiol.* **11**, 229–238.

ECCLES, J. C. (1950). The responses of motoneurones. *Brit. med. Bull.* **6**, 304–311.

EMMELIN, N. (1952). 'Paralytic secretion' of saliva. An example of supersensitivity after denervation. *Physiol. Rev.* **32**, 21–46.

FOERSTER, O. & GAGEL, O. (1934). Die tigrolytische Reaktion der Ganglienzelle. *Z. mikr.-anat. Forsch.* **36**, 567–575.

FOERSTER, O., GAGEL, O. & SHEEHAN, D. (1933). Veränderungen an den Edösen im Rückenmark des Affen nach Hinterwurzeldurchschneidung. *Z. ges. Anat.* 1. *Z. Anat. Entwbesch.* **101**, 553–565.

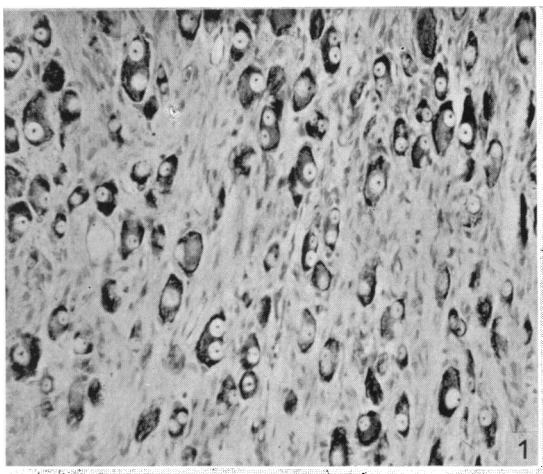
GERSH, I. & BODIAN, D. (1943). Some chemical mechanisms in chromatolysis. *J. cell. comp. Physiol.* **21**, 253–279.

GIBSON, W. C. (1940). Degeneration and regeneration of sympathetic synapses. *J. Neurophysiol.* **3**, 237–247.

HARE, W. K. & HINSEY, J. C. (1940). Reactions of dorsal root ganglion cells to section of peripheral and central processes. *J. comp. Neurol.* **73**, 489–502.

HYDÉN, H. (1943). Protein metabolism in the nerve cell during growth and function. *Acta physiol. scand.* **6**, Suppl. 17.

JOSEPH, J. (1950). Further studies in changes of nuclear population in degenerating non-myelinated and finely myelinated nerves. *Acta anat.* **9**, 279–288.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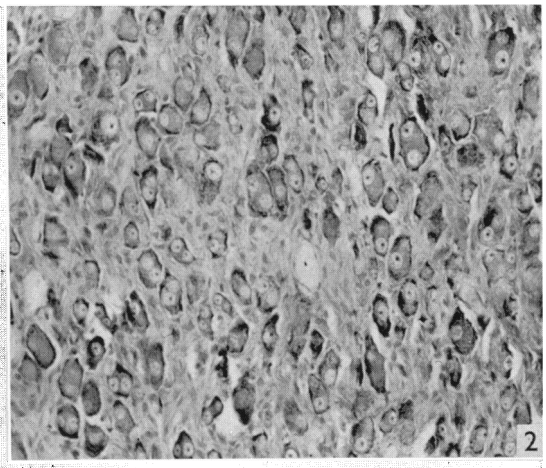
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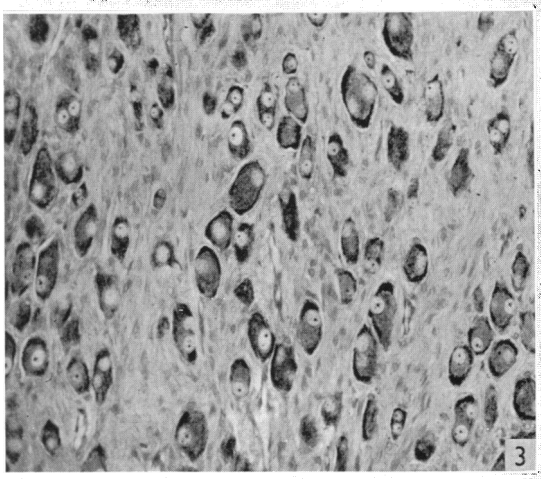
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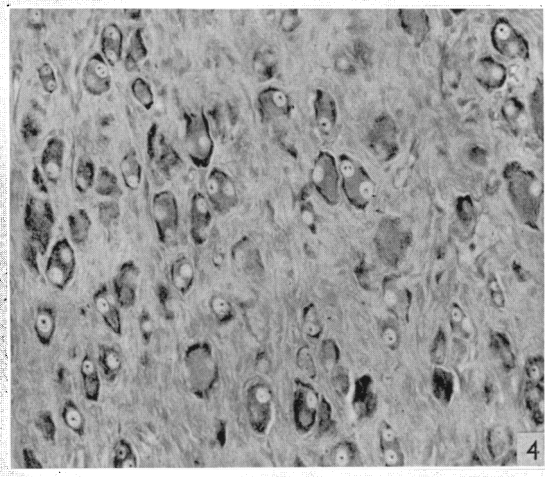
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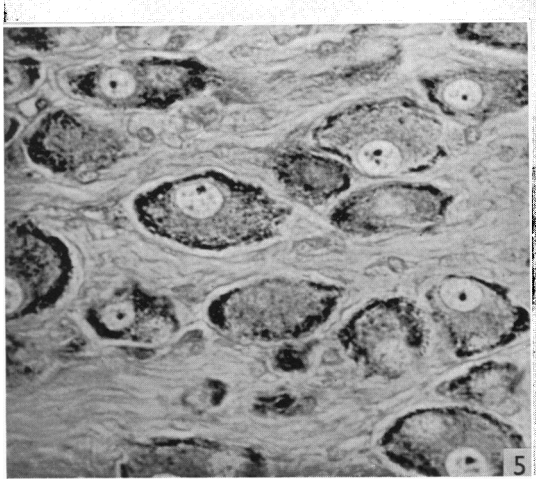
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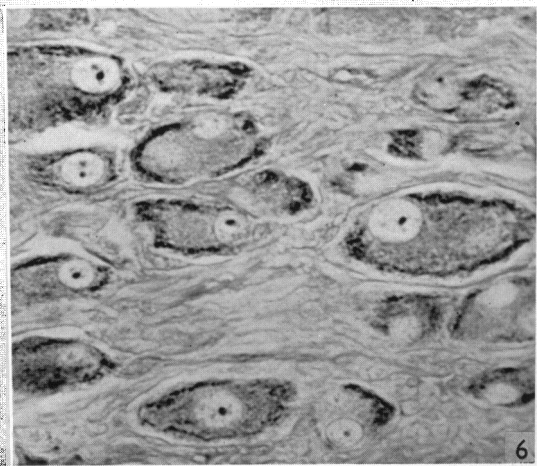
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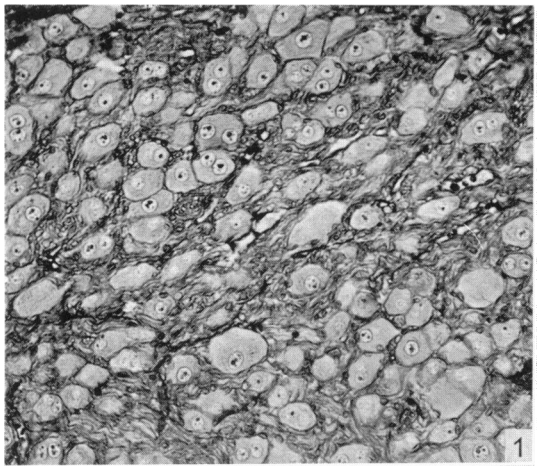
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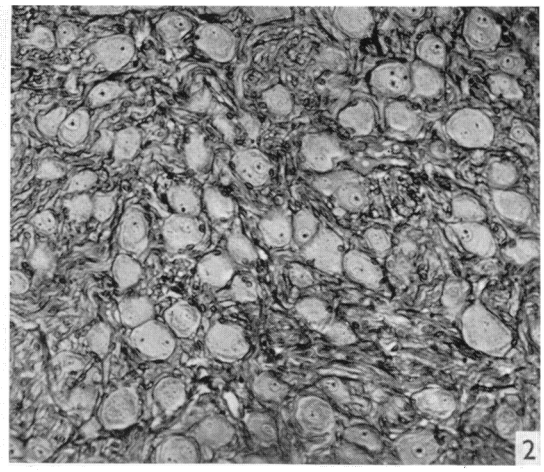
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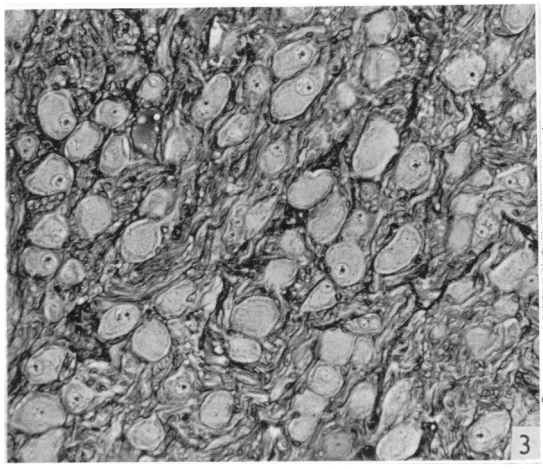
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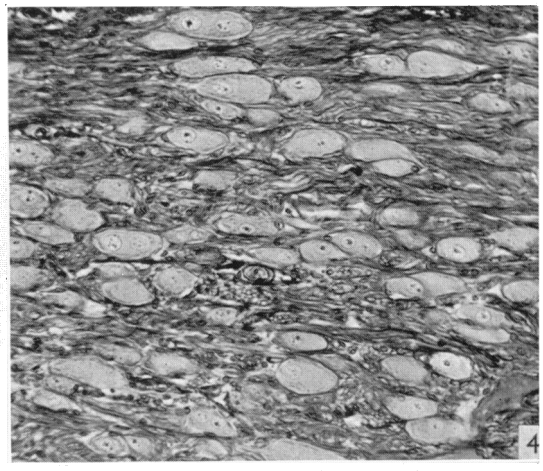
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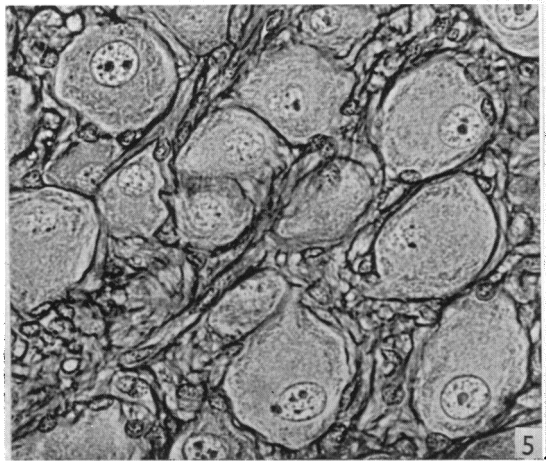
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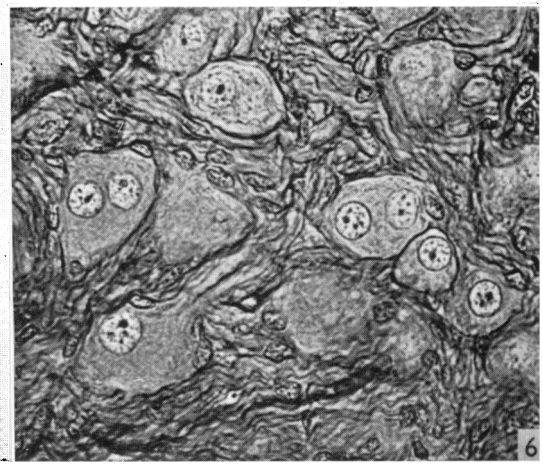
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LANDSTRÖM, H., CASPERSSON, T. & WOHLFART, G. (1941). Über den Nucleotidumsatz der Nervenzelle. *Z. mikr.-anat. Forsch.* **49**, 534–548.

LIU, C., BAILEY, H. L. & WINDLE, W. F. (1950). An attempt to produce structural changes in nerve cells by intense functional excitation induced electrically. *J. comp. Neurol.* **92**, 169–181.

MAINLAND, D. (1929). The technique of estimating small irregular areas in biological research with notes on the tests of accuracy. *J. Anat., Lond.*, **63**, 345–351.

MINKOWSKI, M. (1920). Über den Verlauf, die Endigung und die zentrale Repräsentation von gekreuzten und ungekreuzten Sehnervenfasern bei einigen Säugetieren und beim Menschen. *Schweiz. Arch. Neurol. Psychiat.* **6**, 201–252.

MINKOWSKI, M. (1922). Sur les conditions anatomiques de la vision binoculaire dans les voies optique centrales. *Encéphale*, **17**, 65–96.

MURRAY, MARGARET R. & STOUT, P. S. (1947). Adult human sympathetic ganglion cells cultivated *in vitro*. *Amer. J. Anat.* **80**, 225–273.

PENMAN, J. & SMITH, MARION C. (1950). Degeneration of the primary and secondary sensory neurones after trigeminal injection. *J. Neurol. Psychiat.* **13**, 36–46.

SCAMMON, R. E. & SCOTT, G. H. (1927). The technique of determining irregular areas in morphological studies. *Anat. Rec.* **35**, 269–277.

SCHIMERT, J. (1939). Das Verhalten der Hinterwurzelkollateralen im Rückenmark. *Z. ges. Anat.* **1**. *Z. Anat. EntwGesch.* **109**, 665–687.

SCHOENHEIMER, R. (1942). *The Dynamic State of Body Constituents*. Cambridge, Mass.: Harvard University Press.

STERNSCHEIN, E. (1920). Das Ganglion cervicale supremum nach proe- und postganglionäre Durchschneidung. *Arb. neurol Inst. (Inst. Anat. Physiol. ZentNerv.) Univ. Wien*, **23**, Heft II, 155–176.

WARRINGTON, W. B. (1898). On the structural alterations observed in nerve cells. *J. Physiol.* **23**, 112–129.

WARRINGTON, W. B. (1899). Further observations on the structural alterations observed in nerve cells. *J. Physiol.* **24**, 464–478.

EXPLANATION OF PLATES

PLATE 1

Fig. 1. (Rabbit 219.) Nissl preparation of the superior cervical ganglion of the control side. × 160.

Fig. 2. (Rabbit 219.) Nissl preparation of the superior cervical ganglion of the operated side 40 days after isolation. × 160. The Nissl substance is paler than that seen in the control.

Fig. 3. (Rabbit 221.) Nissl preparation of the superior cervical ganglion of the control side. × 160.

Fig. 4. (Rabbit 221.) Nissl preparation of the superior cervical ganglion of the operated side 80 days after isolation. × 160. There is increased pallor of the Nissl substance.

Fig. 5. (Rabbit 218.) Nissl preparation of the superior cervical ganglion of the control side. × 440.

Fig. 6. (Rabbit 218.) Nissl preparation of the superior cervical ganglion of the operated side 40 days after isolation. × 440. The Nissl substance does not stain so strongly as that of the control ganglion.

PLATE 2

Fig. 1. (Rabbit 220.) Azo-carmine and aniline-blue preparation of the superior cervical ganglion of the control side. × 160.

Fig. 2. (Rabbit 220.) Azo-carmine and aniline-blue preparation of the superior cervical ganglion of the operated side 40 days after isolation. × 160. There is a slight increase in the amount of connective tissue.

Fig. 3. (Rabbit 222.) Azo-carmine and aniline-blue preparation of the superior cervical ganglion of the control side. × 160.

Fig. 4. (Rabbit 222.) Azo-carmine and aniline-blue preparation of the superior cervical ganglion of the operated side 80 days after isolation. Some increase of connective tissue may be seen.

Fig. 5. (Rabbit 223.) Azo-carmine and aniline-blue preparation of the superior cervical ganglion of the control side. × 440.

Fig. 6. (Rabbit 223.) Azo-carmine and aniline-blue preparation of the superior cervical ganglion of the operated side 80 days after isolation. × 440. An increase of the connective tissue may be seen.