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A. G. BROWN.

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CHANGES OCCURRING IN SENSORY STRUCTURES AFTER DENERVATION AND DURING REINNERVATION, AND IN INJURED AND REGENERATING AFFERENT NERVE FIBRES.

A. G. BROWN.

"There is still no published case in which the condition of the end organ in the skin has been thoroughly correlated with functional recovery. It is therefore not yet possible to say how far the excess of innervation and abnormal shapes of the endings are responsible for the aberrations of sensations which are observed. There is every reason to think that the correlations will be found to be close, though the imperfect maturation of the nerve trunks may be equally important in producing these aberrations.....In fact the whole process of normalisation of structure and function in the skin during recovery is well worth further study." (J. Z. Young 1942).

In spite of the widespread use of electrophysiological techniques for recording from peripheral nerve fibres, and the great increase in knowledge of the innervation of normal skin since the above was written, Guth was still able to say in 1956, "There are no major differences from the processes previously described." An attempt will be made in the present essay to correlate the well documented findings in the field with some recent experiments and to try to fill in, in part, the gap in knowledge referred to above. Needless to say more questions will be posed than answered.

The very nature of the work results in it being mainly descriptive, in answer to the question, "What happens to sense organs and sensory fibres after injury to the afferent nerve?" Until this question is answered fully it is perhaps unwise to ask the next one of "How do the changes/

changes observed come about?" Tentative answers will, however, be suggested, though this part of the essay, will,of necessity, be highly conjectural.

That structural changes occur in sensory receptors after denervation has been recognised since Vintschgau and Hönigshmeid cut the glossopharyngeal nerve of the rabbit, in 1877, The taste buds on the posterior part of the tongue on the same side as the cut nerve had disappeared by a month after the operation. Later Vintschgau (1880) described the histological changes concerned in greater detail and gave drawings of these changes. Since that time many workers have observed changes occuring in taste buds after denervation in various animal species, and a discussion has been carried out as to whether the denervated organs undergo atrophy, degeneration or dedifferentiation.

Structural changes in sense organs after denervation.

a) Fishes & Amphibia ; The work of Olmsted (1920 b) is the most convincing and provides objective evidence, as photomicrographs, of the changes occuring after denervation. The catfish, was used,and the barbels denervated by cutting the appropriate nerve. Seven days after operation, changes of degeneration were observed in the nerve coincident with an increase in the number of small leucocytes in the epidermis. By the twelfth to thirteenth day, that part of the sensory cell distal to its nucleus appeared to be attacked by leucocytes, and eventually there remained of the taste bud , a hollow shell, bounded by a single layer of cells, with scattered nuclei at the base and an irregular mass of smaller/

smaller granules and larger globules at the centre. The basement membrane of the epidermis, which normally lies deep to the taste buds, ~~disappeared~~. disappeared. These changes must have occured fairly rapidly since most of the specimens showed either little change or complete change, and in over 100 slides only five or six showed the intermediate stages in any detail. Mitoses were never seen in the sensory cells and it was concluded that the process was not one of dedifferentiation but one of degeneration. May (1925) confirmed these results and described the finer histological changes which occured, though no photo- micrographs were produced, only drawings. (Olmsted (1921) also cut the lignal nerve of the dog and after eight days in eighteen fungiform papillae no taste buds were seen, three had only the remains of one bud, and two had one apparently normal taste bud).

The lateral - line organs of various species of fish have also been useful preparations for the study of the changes occuring after denervation. Brockelbank (1925) cut the vagus nerve of one side in the catfish and described the ensuing changes, though she only reproduced camera lucida drawings to support her statements. After four days the receptor cells of the lateral-line organs began to lose their sharpness of outline and by thirteen days there was general degeneration, the few remaining sense organs being greatly reduced in size, the sensory cells had disappeared and only a few supporting cells remained. No evidence of attack on the denervated endings by leucocytes was observed, (cf. taste buds), and it was concluded that the organs had returned to an embryonic condition.

These/

These results were confirmed by Bailey (1937) for the same species of animal.

Speidel (1948) studied the lateral-line organs in the tadpole of the green frog, which is in the tadpole stage for one or two winters, and hence offers good opportunity for keeping the organs denervated for a considerable time by repeated cutting of the nerve. These studies were carried out on the living animal, anaesthetised with chloralose, observed in a transparent chamber. From one month to ten months after denervation there was a decrease in number of lateral-line organs from twenty-three to eight, whereas on the normal side there was an increase of from sixty-eight to one hundred and thirty seven. In the organs as remaining. the cells were smaller, as were the organs as a whole, and the hair processes of the organs were short and reduced in number.

b)Birds : The large sensory corpuscles of Grandry and Herbst in the bill of the duck have been studied by Tamura (1922)Boeke (1923) and Dijkstra (1933) all cited by Young (1942). After denervation there was some shrinkage of the cells but little degeneration or atrophy of the organs, at least up to four months.

c) Mammals: An important contribution to the problem of changes occuring in sense organs after denervation was that of Tower (1932) on the muscle spindles of the cat. Changes in these organs in the interrosseous muscles of the fore-paw were followed after cutting ventral and dorsal nerve roots separately. After cutting the ventral roots the degenerative changes which occured seemed confined to the poles of the spindles. Six months/

months after the operation the intrafusal fibres showed similar changes to the extrafusal fibres, namely, they were thinner, had less well marked striations and swolled nuclei; whereas the equatorial region was conspicuous, the nuclei appearing normal, being round, clear cut, never confluent, with small nucleoli and their arrangement in rows was undisturbed. The capsule reacted as a whole and was greatly thickened. After cutting the dorsal roots the polar regions were unaffected, but in the equatorial region after six to twelve months denervation, the nuclei were arranged irregularly, with cross striated sacroplasm between them, were smaller, polygonal or rectangular in outline, and sometimes confluent, their outlines being obscure. The capsule responded slightly or not at all. After cutting the sympathetic supply no changes were discernible.

An extensive study of changes in the end organs in the skin of the finger of the rhesus monkey was reported by Jalowy (1955). The Meissner and Vater Paccinian corpuscles showed no great changes after denervation to any extent. After three days of denervation the touch menisci were broken up and there was granulation in the vicinity of the touch cells. By twenty-five days many of the cells of the corpuscle were multipolar. Similar results were also reported for nerve endings in the skin of the snout of the Guinea pig(Jalowy 1934).

This survey of the literature establishes that many sense organs in several different animal species undergo histological changes after denervation. In general, the receptor cells themselves undergo the greatest/

greatest changes, the supporting and connective tissue components undergoing considerably less change. The presence of a nerve fibre is essential for the maintenance of the specific structure of a sense organ, as was demonstrated convincingly by Tower's (1932) elegant experiments. The influence of the nerve fibre may work over only a short range since within the muscle spindle the afferent nerves were responsible for the maintenance of the equatorial region, while the efferent fibres were responsible for that of the extrafusal fibres.

Structural changes in sense organs during reinnervation.

a) Fishes & Amphibia: The problem of what happens when nerve fibres are allowed to grow back into denervated organs has also been examined. Olmsted (1920 b) showed that, in the catfish, the appearance of the nerve fibres in the barbel was accompanied by the reappearance of taste buds which were capable of function as tested by appropriate stimuli in behavioural experiments. The regenerative process included an extension of the dermis into the epidermis and the proliferation of cells in the germinal layer at the tip of the papillae. The presence of the nerve determined regeneration of the whole barbel, and not only of the taste buds, since if regeneration of the nerve was prevented the barbel did not regenerate. These results were supported by photomicrographic evidence. Olmsted (1920 a) also showed that during regeneration of previously innervated barbels the growth of nerve into a region preceded the appearance of taste buds, and also of their forerunners, the dermal papillae, the formation of which was connected with the growth into them of a branch of/

of the nerve trunk. These results were confirmed by May (1925).

The problem was approached in a different way by Stone (1941), who used the salamander. In young embryos the tongue area was removed and transplanted to the area for the side of the body, and led to the development of the lower jaw and associated structures in the new position. When the operation was carried out at different stages of development; a) just before and during taste organ development the organs apparently developed normally, b) just after development of the taste organs they remained and developed apparently normally and c) at a stage when the taste organs were well developed and with the nerve innervating them, they remained until metamorphosis, when the graft was shed. Stone concludes ".....presumptive tongue forming tissue can develope taste organs in new positions on the body and these taste organs can arise independent of the gustatory nerve fibres and maintain themselves for a long time." The arrival of a nerve supply and the appearance of taste organs in Olmsted's and May's work was taken to be ".....nothing more than a coincidence." These experiments do not demonstrate the independence of taste buds of a nerve supply, though they indicate that they may be independent of the gustatory nerve supply in an embryo.

There is also disagreement concerning the lateral - line organs of these species. Stone (1938) in a study of the formation of these endings in the salamander, in which the placodes which form the lateral-line system were stained with Nile blue sulphate and transplanted to other larvae, concluded that the formation of both the primary and accessory endings was/

wasindependent of the nerve supply. Speidel (1947), studying the regeneration of the tail-tip of conscious amphibian tadpoles with the nerve prevented from regenerating by repeated cutting, reported that the usual pattern of lateral - line organ regeneration occured, and it was concluded that the ~~area~~ nerve was unessential for regeneration and that there was no evidence of induction by the nerve. Both of these experiments were carried out on living animals using the transparent chamber technique. Speidel (1948), however, using the same experimental procedure and the same animal species, reported that if reinnervation of the tail was allowed to take place the number of lateral - line organs increased.

Experiments on the lateral - line organs of the catfish are more conclusive. Brockelbank (1925) observed that during reinnervation the organs regenerated, the supporting cells differentiating before the receptor cells, Bailey (1937) reported that when the lateral - line was removed, with the nerve left intact, within two weeks the normal epithelium had become thickened over a nerve fibre, and had become organised into sensory organs, and mitoses were seen. By three weeks lateral - line organs were observed and medullated nerve fibres could be traced into them. Five weeks after the operation the organs were apparently completely differentiated. When the lateral - line and the nerve were both removed no new organs were formed. Finally, deflection of the nerve to a new path resulted in the formation of many epidermal lateral - line organs along the new course of the nerve fibres. Both the above investigations are less convincing because they lack documentary evidence, no photomicrographs being/

being reproduced.

b) Mammals: As far as mammalian sense organs are concerned little is known. Jalowy (1934,1935) states that only Merkel's discs show great changes during regeneration and that the loops which bind the discs together appear late in the course of regeneration.

The induction of sense organs by the nerve fibre consists of two distinct problems, namely, the initial formation of the ending and its reformation during reinnervation. The two processes are not necessarily the same. For the former process Stone's (1940) experiments, in which the embryonic tongue region was transplanted to the side of the body, do not demonstrate the independence of taste buds of a nerve supply, (vide supra). In the salamander the formation of the lateral - line organs is independent of a nerve supply according to Stone (1938), as is their reformation according to Speidel (1947). Both of these workers used direct microscopic examination of the living animal, a method which is unlikely to reveal fine nerve fibres. The possibility remains, however, that this may be a species peculiarity. In the catfish both Brockelbank and Bailey observed the formation of lateral - line organs in response to ingrowth of nerve fibres, and also in Bailey's experiments their formation, de novo, from "undifferentiated" epithelium after diversion of the path of the nerve fibre. Confirmation of Bailey's work is required.

The majority of workers support the idea that induction of sense organs is caused by the ingrowing nerve fibres, for both the formation and reformation/

reformation. That the underlying mechanism may be chemical has been postulated by several workers (Olmsted 1920, May 1925, Torrey 1924, Bailey 1937, and Young 1942), but the nature of such a mechanism remains obscure. If precursors of epithelial cells can be induced to form receptor cells, (which from the above review would appear to be the case, and evidence for which will be presented below), it may be that such a chemical mechanism acts by releasing latent potentialities within the cell e.g. by uncovering certain parts of the genetic information of the cells carried in the chromosomes. If all cells of one animal are viewed as having come from one parent cell, i.e. the zygote, all cells, in theory, contain the same potentialities i.e. the same genetic information. The induction mechanism may work in a similar way to that of virus particles infecting a cell, the chemical substance concerned being ribonucleic acid.

From Bailey's studies, epithelial cells, in the catfish, appear to possess the capability of giving rise to sensory cells in the presence of a nerve fibre, and the problem of whether sense organs atrophy, degenerate, or dedifferentiate has little import. If the atrophy or degeneration were reversible, at least during the early stages after denervation, it would be impossible to separate these processes from dedifferentiation.

Sensory changes after nerve injury: The problem of the changes occurring in the nerve fibres themselves after injury is best introduced by a short review of the sensory changes occurring in man after transection of cutaneous nerves and during recovery. The pioneering experiments were carried/

carried out by Henry Head and his collaborators (Head, Rivers and Sherren 1905, Head and Sherren 1905, Rivers and Head 1908), but the results of Trotter and Davies (1909) have been confirmed for the most part by Boring, (1916), Sharpey-Schafer (1927) andLanier, Carney and Wilson (1935). Trotter and Davies cut seven cutaneous nerves in themselves and in each case there was produced in the skin 1) a central area of complete loss of sensation, surrounded by 2) an area of partial loss to all cutaneous stimuli, which was in turn surrounded by 3) a large zone in which qualitative changes could be detected. The last two zones will be together called the intermediate zone.

Before regeneration of the cut nerves could have begun the intermediate zone began to shrink in size towards the area of complete anaesthesia. This shrinkage occurred a matter of days following transection of the nerve. (In the rabbit, as tested by the response to pin - prick, Weddell, Guttman and Gutmann (1941) found that the zone had shrunk as early as the day following division of the sural nerve). As the cut nerve regenerated, about ten to fourteen weeks following axonectomy and suture, all modalities of cutaneous sensation tested reappeared at about the same time, although the rate at which they progressed towards normal acuity varied e.g. cold sensibility was often more fully developed than heatsensibility.

During the period of recovery the sensations showed remarkable qualitative peculiarities, the thresholds for all types of stimuli were raised, but once reached the sensations were of an explosive nature and more/

more intense than normal. Schafer observed that within a few hours of either cutting or crushing cutaneous nerves, there was a continuous burning pain lasting for a few hours and two to three days later a similar pain developed of a very unpleasant nature which lasted for some three weeks.

Electrophysiological studies of injured and regenerating nerve fibres:

That the initial burning pain perceived soon after cutting or crushing the nerve may be due to spontaneous discharges from the damaged region of the fibre was demonstrated by Adrian (1932), who recorded, in acute experiments, three types of discharge from the cut ends of nerves of cats and rabbits; namely 1) aregular discharge with a frequency of from 150 to 200 impulses per second, 2) an irregular discharge with a frequency of less than 50/second, and 3) grouped discharges. The first two types merged into each other. Mechanical interference of the cut end of the nerve sometimes produced an abrupt change in frequency of the spontaneous discharge, or stopped it or even started one in a fibre which had previously been silent. Grouped discharges had an impulse interval of not greater than 10msecs. and usually about 5msecs., as did the continuous regular discharge. The irregular type, on the other hand had impulse intervals always greater than 7.5 msecs., and it was concluded that the detailed structure of the discharge was dependent on the period of recovery in the nerve fibre. In the phrenic nerve, synchronised discharges with a spike height of 10 to 30 times that of single fibres, were seen, and it was concluded that "an active fibre can cause a slight momentary/

momentary increase in the stimulus to other fibres.....owing to the action current which it produces." The fibres which were most likely to give rise to a persistent discharge were the afferent fibres of low diameter.

Skoglund (1942) in an extensive study of accommodation to a linearly rising current in motor and sensory nerve fibres in the cat, found that it was necessary to compensate a decreased rate of rise of the stimulus by increasing current strength. For sensory nerves, as compared with motor nerves, the rate of rise of the stimulus was of much less significance and the absolute threshold for sensory nerve fibres was lower than for motor fibres. It was also observed that afferent fibres, when cut, were much more prone than efferent fibres to show spontaneous activity. These results were confirmed for human nerve fibres by Kugelberg (1944) who used electrodes applied to the skin, the index of excitation in the appropriate fibre being taken when there was a palpable twitch of a muscle in response to stimulation of the skin over a motor nerve , and when such stimulation over a sensory nerve gave rise to a perceived sensation.

In an interesting series of investigations, Granit, Leksell and Skoglund (1944), showed that interaction between nerve fibres could take place at the cut or crushed end of a nerve. After cutting or crushing the sciatic and hamstring nerves in cats the ventral roots were stimulated, electrically, and recordings were taken from the dorsal roots. When the motor root was stimulated, activity was recorded in the sensory root, and vice versa, a smaller volley was recorded coming back in the motor root when/

when the sensory root was stimulated. This response was very sensitive to the general anaesthetic and was greatly increased for a period of five to ten minutes after freshly cutting or crushing the nerve. Since, according to Grundfest (1940), the fibres of the C group are practically lacking in accommodation, and it is highly probable that such fibres subserve painful sensations, it is to be expected that they will be easily excited by such fibre interaction, and some symptoms of causalgia, particularly the early burning pain perceived after nerve injury referred to above, could be explained on this basis.

That regenerating cutaneous nerves can be excited by appropriate stimulation over them can be assumed from the work of Head and co-workers, and Trotter and Davies, such a mechanism being the basis of Tinel's sign in neurology (Tinel 1915). Konorski and Lubinska (1946) reported that electrical activity could be recorded from regenerating nerve fibres in response to mechanical stimulation of them, and that freshly regenerated fibres are more sensitive to such stimulation than normal fibres, though no details of their experimental method were given.

Weddell et al. (1941) suggested that sensory recovery in denervated skin is due to several factors, the early recovery of sensibility in the intermediate zone being due to the recovery of function in remaining normal fibres. Why injury to a cutaneous nerve fibre should cause temporary suppression in adjacent normal nerves is difficult to understand. Such an explanation would require revision of the classical view of a nerve fibre being an autonomously functioning whole. The hypothesis could/

could be tested as the normal pattern of response from cutaneous sense endings is well documented, and it would be easy to observe if a lesion to one cutaneous nerve affected the responses in nerve fibres innervating the same and adjacent areas of skin. This experiment has apparently not been done. The early shrinkage of the intermediate zone was also correlated, (Weddell et al. 1941), with the ingrowth of fibres from the surrounding normal nerves, the growing tips of the axons being about 1mm. in front of the point where responses to a pin prick could be obtained in the unanaesthetised rabbit.

Many problems remain to be answered, particularly in regard to the provision of objective, electrophysiological, information on the changes taking place in sense organs during reinnervation. A suitable preparation for such investigation was recently discovered and experiments have been carried out in an attempt to clarify some of the points raised above. A preliminary report of the experiments has been published (Brown & Iggo 1963).

Experimental Work

The end organ chosen for study was originally described by Pinkus (1904) and more recently reported by Straile (1960) and by Jabonero and Casas (1961). It was independently observed by Iggo who has described its structure (Iggo & Muir 1963) and its response properties (Iggo 1963). Previous authors have undoubtedly recorded from afferent fibres innervating this organ in the rabbit and the cat, (Frankenhauser 1949, Maruhashi, Mizuguchi & Tasaki 1952, Hunt & McIntyre 1960). This organ, which will henceforth/

henceforth be called a touch corpuscle, occurs in the hairy skin of several species of animal, viz., cat, rat, rabbit, dog and monkey. In the cat, which animal was used exclusively in the present series of investigations, it is easily recognised, with the aid of a dissecting microscope in depilated skin, as an oval, dome - shaped structure, from 100μ to 300μ in its long diameter, containing a loop of capillary blood vessels and usually lying over a guard - hair. fig. 1.

The touch corpuscle has a characteristic histological appearance, fig. 2 . It consists of a modified epidermal layer, 4 to 5 cell nuclei in thickness, the adjacent unspecialised epidermis having only one layer of nuclei. This modified epidermis contains in its deepest layer, large cells, numbering 20 or more, which in van Giesen and silver stained preparations appear to contain a large sac - like region, on the dermal side of the cell, which is unstained. Immediately external to this layer the nuclei of the epidermis are arranged with their long axes at right angles to the surface of the corpuscles in a palisade arrangement. The largest cells (tactile cells) because of their clear cytoplasm are easily recognised, and enclose a disc of nervous tissue approximately 10μ in diameter and 1μ thick, densely packed with mitochondria, and which is the terminal expansion of a branch of a myelinated nerve fibre (Iggo & Muir). This disc lies deep to the nucleus of the cell. The tactile cells are enclosed within the basement membrane of the epidermis which is arranged, at the circumference of the touch corpuscle, in a complex, fimbriated manner. The dermis of the corpuscle contains a large amount of collagen and/

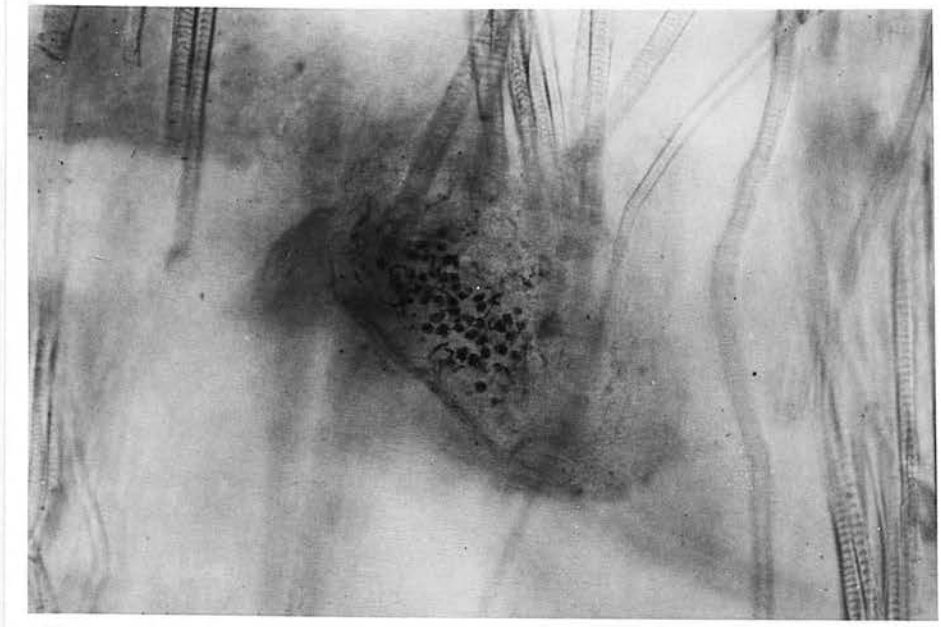


Fig. 1. A touch corpuscle in a methylene-blue stained preparation of normal cat's skin. The darkly stained discs of the tactile cells are arranged parallel to the surface of the corpuscle.

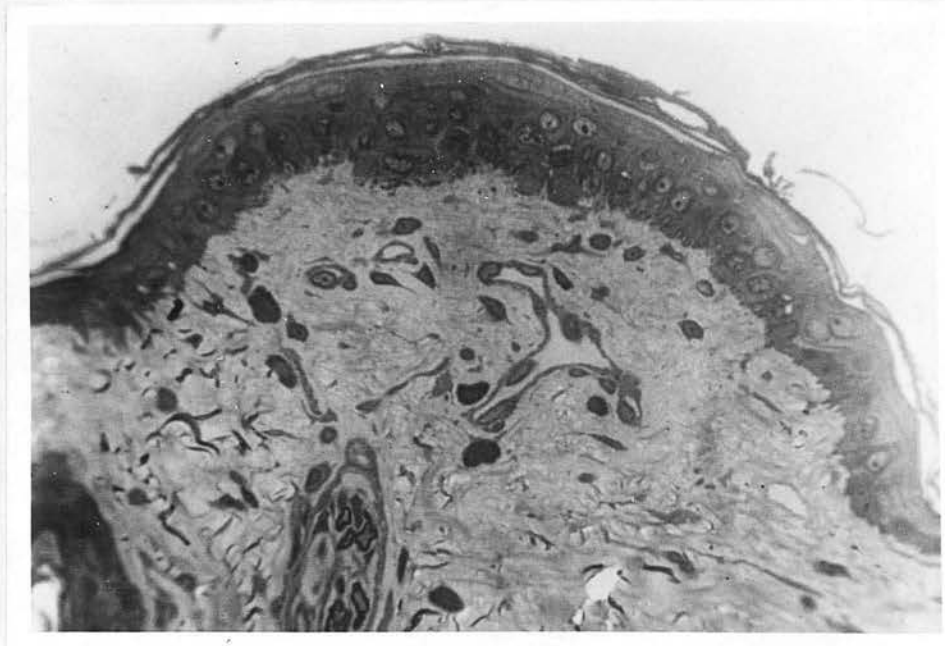
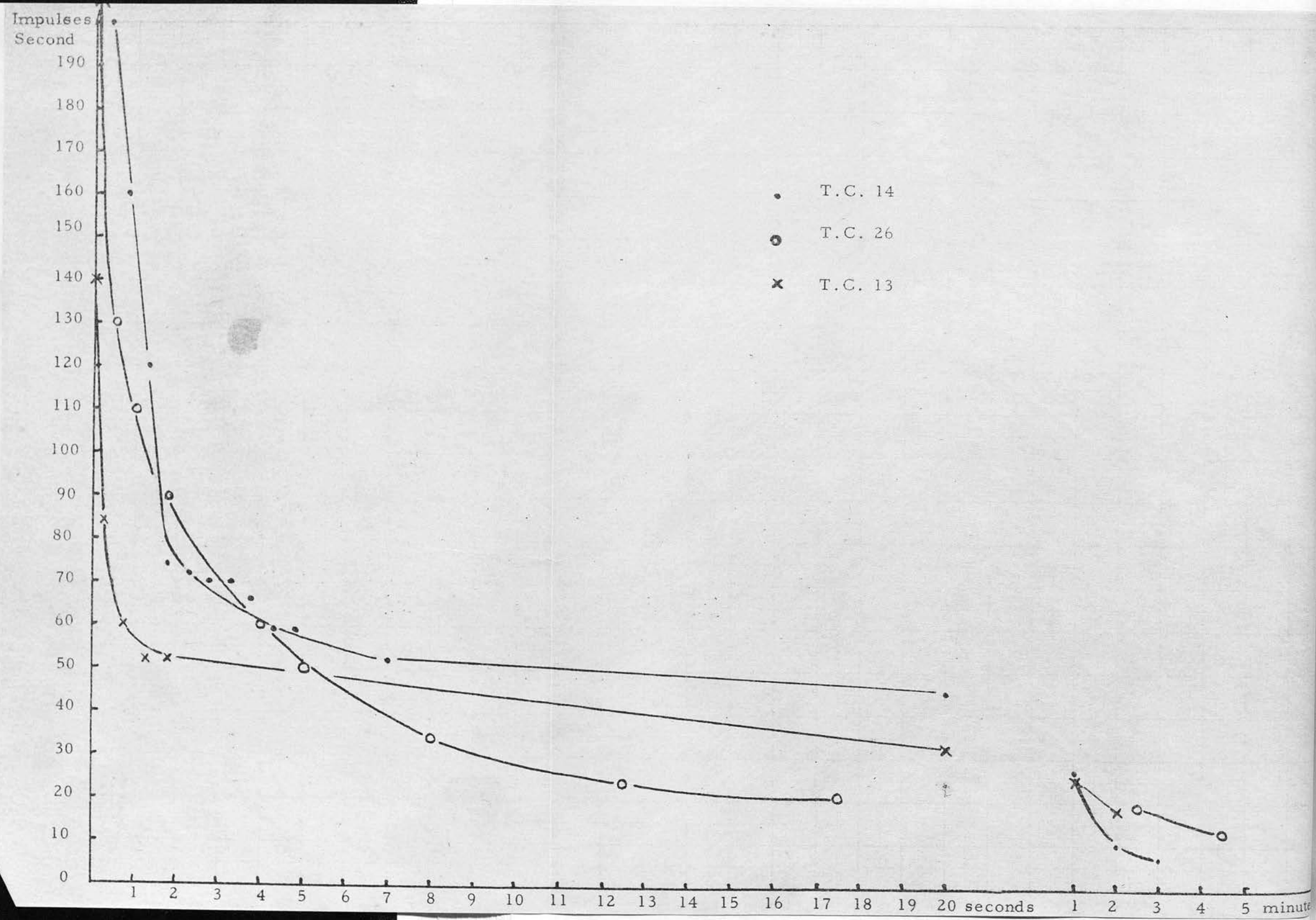


Fig. 2 Transverse section of touch corpuscle showing a particularly well developed fimbriated basement membrane structure at the dermo-epidermal junction. No tactile cells are seen in this section which is taken from the periphery of the corpuscle.


Impulses
Second
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
T.C. 14
T.C. 26
T.C. 13
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 seconds
1 2 3 4 5 minute

Fibre	C. V. m/sec. Nerve	Skin	Mech.Thresh.mg.	R.D.	No.of spots/fibre	Max.disch./sec. Mech.	Therm.
I	0	0	0	-	I	0	0
2	0	57	0	+	I	800	24
3	0	40	< 20	+	I	700	0
4	0	40	< I0	-	I	900	I8
5	45	0	<< 200	+	I	660	I7
6	95	0	2	+	2	760	I7
7	0	0	0	-	I	0	0
8	0	0	0	-	I	VHF	0
9	0	40	0	-	I	II00	45
I3	0	0	0	-	I	0	0
I4	0	0	0	-	I	0	0
I5	4I	36	0	-	2	0	0
I6	52	0	0	-	2	0	0
I7	0	0	< 5	-	2	0	0
2I	0	0	< 5	-	2	0	0
23	0	0	0	-	2	0	0
25	0	0	0	-	I	0	0
25b	0	0	0	-	2	VHF	0
30	72	0	> I0	-	I	0	0
3I	50	0	> 3	-	2	0	0
33	55	0	< 3	-	3	0	0
34	62	0	< 3	-	I	VHF	0
35	0	0	< 3	-	3	0	0

C.V.= conduction velocity Nerve= measured in the nerve trunk.
Skin measured from the skin.

RD resting discharge

\+ present. - absent. 0 not recorded.

Table I : Properties of 25 afferent fibres from touch corpuscles in normal skin.

and a loop of capillary blood vessels, as well as the branches of a single large myelinated nerve fibre, which run to the individual tactile cells.

A discharge of impulses in the afferent fibre can most easily be evoked by mechanical stimulation of the corpuscle, the threshold for mechanical stimulation being usually 3 to 5 mg. weight. The response shows a slow adaptation to maintained mechanical stimulation, lasting for 5 mins. or longer, and in response to sudden distortion of the ending a burst of impulses with a maximum frequency of 1,000 impulses per second, or more, can be elicited, fig 3 A discharge can also be evoked in the fibre by a fall in cutaneous temperature, e.g. a sudden cooling of from 40° to 20° C. causes a discharge of impulses in single fibres at frequencies not greater than 50 per second if the fibre is silent at the upper temperature. The discharge usually ceases before the skin has reached its lower temperature. If , however, there was a steady discharge in the fibre due to the weight of the thermal stimulator this discharge is affected by the cutaneous temperature (cf. Witt & Hensel 1959, Hunt & McIntyre 1960). A summary of the properties of these fibres appears in Table 1, these properties distinguishing the fibres from other myelinated axons innervating hair follicles in the same skin, and are always associated with a touch corpuscle at the sensitive spot in the skin. The conduction velocity of the fibres ranges from 40 to 95m./sec.(9 fibres). This preparation then, is useful for studying the changes occurring after denervation since it has a well defined structure, discharge properties which are known and easily recognised and it can be seen from the surface of/

of the skin.

Experimental Method.

Young adult cats were used throughout. Under ether anaesthesia, after induction with ethyl chloride, the saphenous nerve in the thigh was exposed and crushed with forceps for a length of 1 cm., or in some experiments resected for a length of 3 cm.

At intervals of 4 to 100 days after operation the animals were anaesthetised with ether and chloralose after ethyl chloride induction. The saphenous nerve was exposed and immersed in a pool of paraffin made by stitching the skin to a metal ring. Fine strands were dissected from the nerve 30 to 50 mm. proximal to the site of the lesion and set up for recording with the conventional electronic apparatus. The hairs on the area of skin innervated by the saphenous nerve were clipped short and, if required, removed with a commercial depilatory.

The skin was explored with a smooth ended glass probe and when the touch corpuscle was found, after confirming with the dissecting microscope, it was stimulated with a vertically mounted probe, the movements of which were fed to a second cathode-ray tube (Iggo 1960 a). The mechanical threshold of the ending was found using graduated nylon hairs, needing a known force to bend them. The temperature of the skin was changed using a thermode through which water at a known temperature was circulated, and the temperature changes of which were also fed to the second cathode-ray-oscilloscope. (Iggo 1950 b).

After identification with the dissecting microscope the position of the/touch corpuscle was noted as

the touch corpuscle was noted on a drawing of the leg of the animal, and the skin was marked with silver nitrate solution, applied by pinching the skin with fine watchmakers forceps dipped into the solution at a position 2 hairs distal to the touch corpuscle.

At the end of the experiment the skin was pinned onto a piece of balsa wood, removed and fixed in 10 % buffered formalin. It was then sectioned at 4 or 6 μ and stained with the van Giesen stain, or sectioned at 15 μ and stained by the Holmes silver method.

Results.

a) Histological: The earliest specimens for histological examination were taken 4 days after crushing the saphenous nerve, and by this time changes were already evident in the touch corpuscles. The epithelium appeared thinner than normal, the number of layers of nuclei in one case being down to a single layer and the filamentous arrangement of the basement membrane of the epidermis also appeared flattened. Tactile cells were few in number, scattered irregularly over the ~~capsule~~ corpuscle and showed certain characteristic changes, viz. shrinkage of the cell in an irregular manner producing a smaller clear space, the nucleus taking up a relatively larger part of the cell and appearing in a more central position. The nerve fibre had broken up into blebs of argentophyllic material, surrounding which were large numbers of Schwann cell nuclei. Many polymorphonuclear leucocytes were seen in the capillaries of the organs but none were seen outside the blood vessels.

In cases where the nerve had been resected the above changes were progressive/

progressive and by 20 to 30 days after operation the epidermis consisted of a single layer of nucleated cells similar in appearance to the adjacent normal epidermis. Tactile cells were absent or few in number, in which latter case they showed all the signs of denervation described above, and the basement membrane of the epidermis had lost its fimbriated appearance. The touch corpuscles were still recognisable by their tuft of capillaries and dense collagenous connective tissue, which remained for at least 30 days.

In those animals which had had the nerve crushed, changes accompanying regrowth of the nerve fibre back into the organ were seen from about the 20th day onwards in this preparation.

The first distinctive change which was observed with reinnervation was the great developement of the fimbriated basement membrane structure, which was particularly well seen in Holmes silver preparations, when the invaginations into the dermis extended in a regular sheet across the dermo-epidermal junction. At this stage, 16 to 20 days post-operatively, axonal twigs were making their appearance in the dermis of the corpuscle, and the epidermis was thickening, with the palisade layer becoming defined. By 25 to 30 days tactile cells had appeared in the basal layer of the epidermis, surrounded by basement membrane. In many instances a group of such cells ~~were~~ was present over a region where an axonal branch had grown, and the epithelium was considerably thicker than the adjacent epithelium of the touch corpuscle, where there were no tactile cells. By this time the axonal twigs in the dermis were numerous and some had grown to the tactile cells which now/

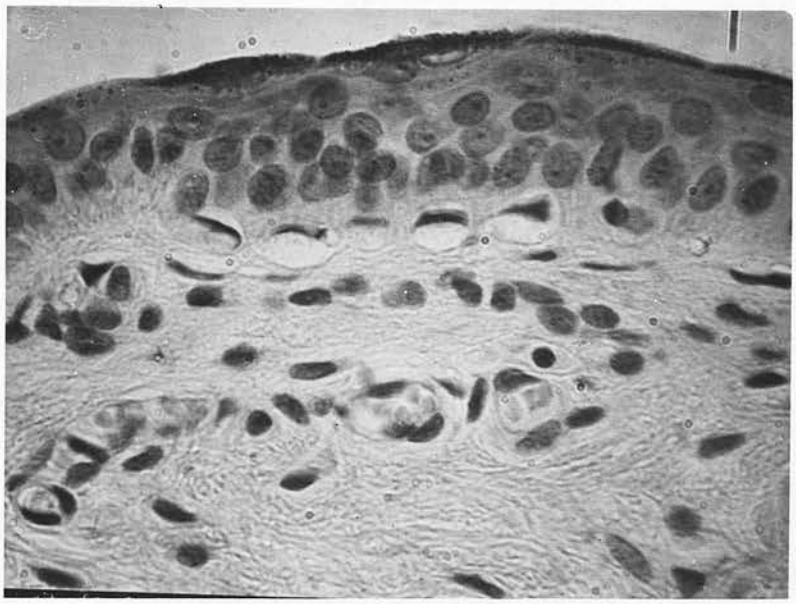
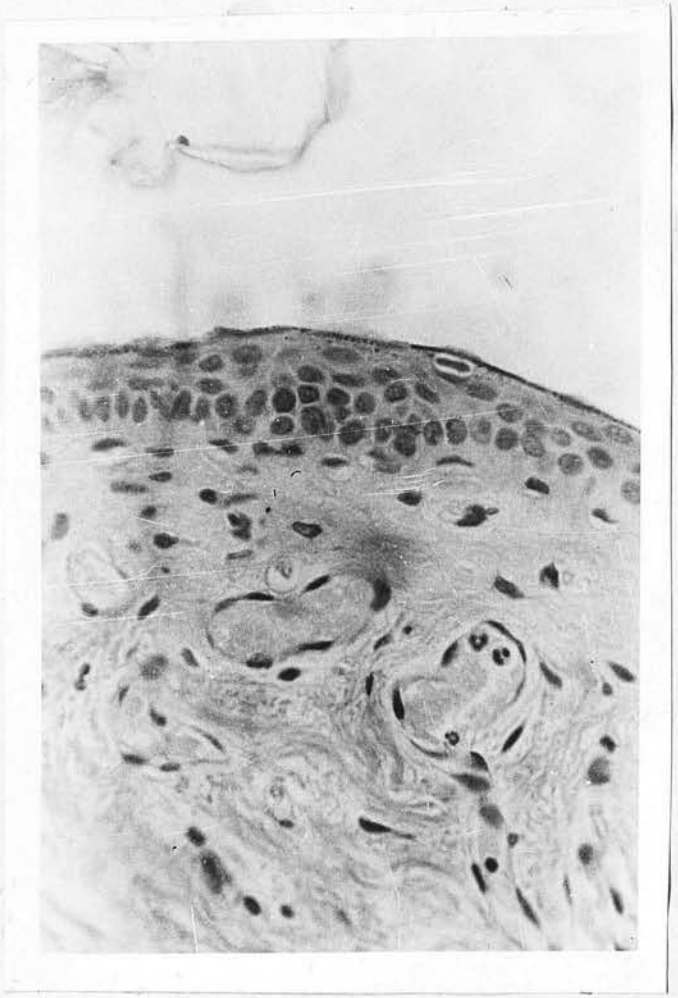


Fig.10 van Giesen preparation of transverse section of touch corpuscle 25 days after crushing the afferent nerve. The nerve fibres have grown back to the organ. Tactile cells have appeared over a row of Schwann cell nuclei and the epidermis has thickened over the line of tactile cells. The fimbriated basement membrane structure is visible.



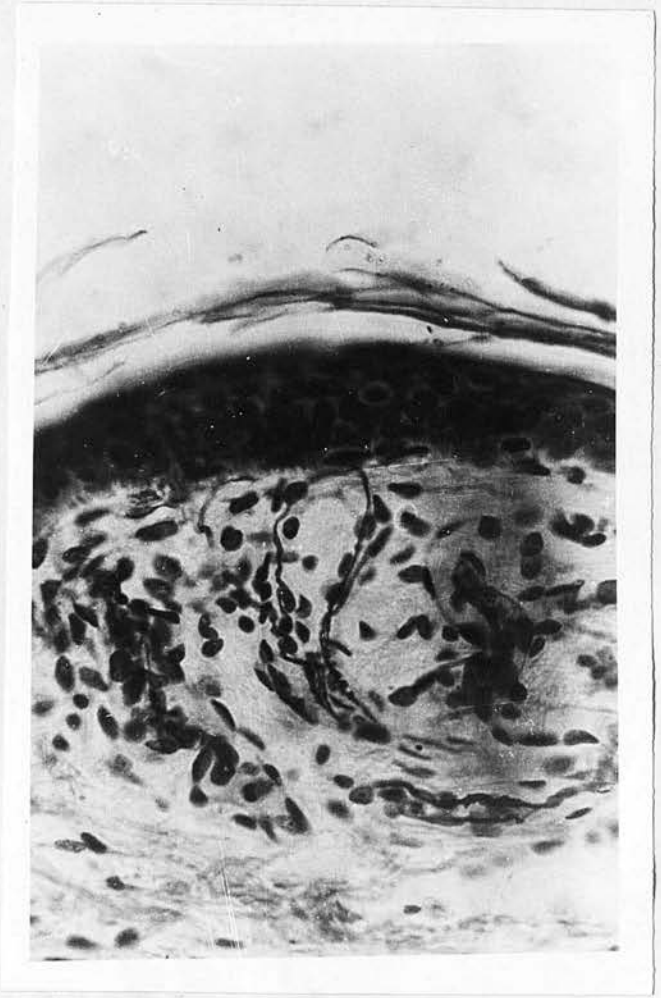
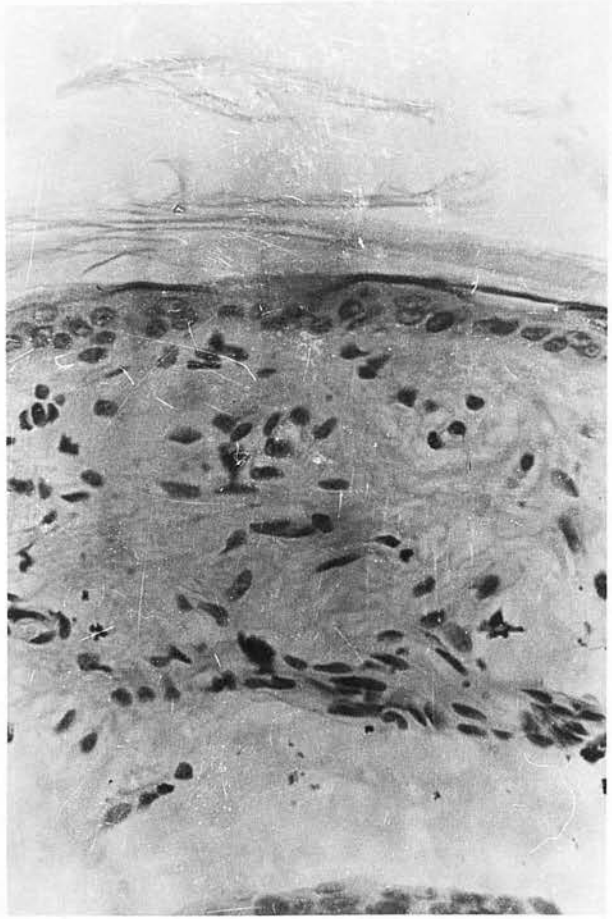


Fig.8 van Giesen & Fig.9 Holmes silver preparations of transverse sections of touch corpuscles 19 days after crushing the afferent nerve. The nerve fibres have grown back to the endings and the epidermis is thickerwith its basal layer of nuclei becoming arranged in the palisade manner. Tactile cells are present but the clear spaces are shrunken.



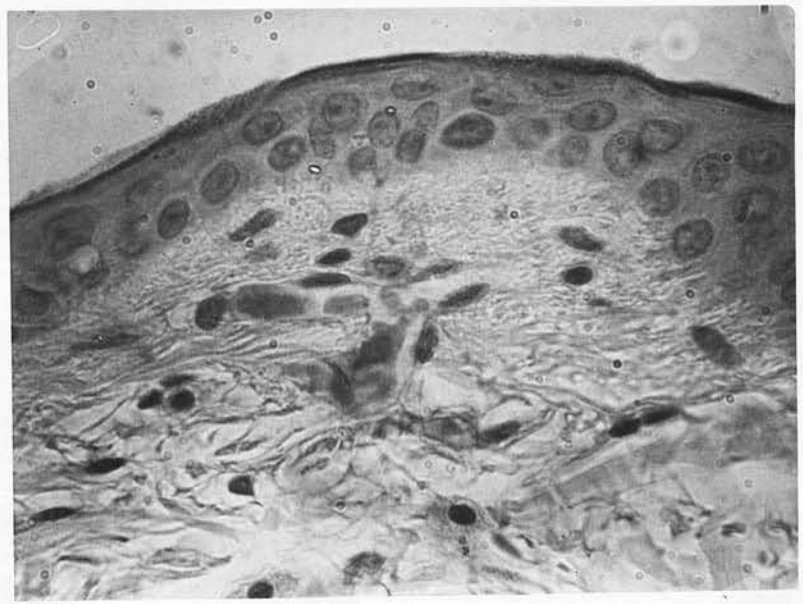


Fig.6 22days & Fig.769 days after cutting the afferent nerve, van Giesen preparations of transverse sections of touch corpuscles. Epidermis thin and similar to normal; tactile cells absent, collagen and blood vessels still present.

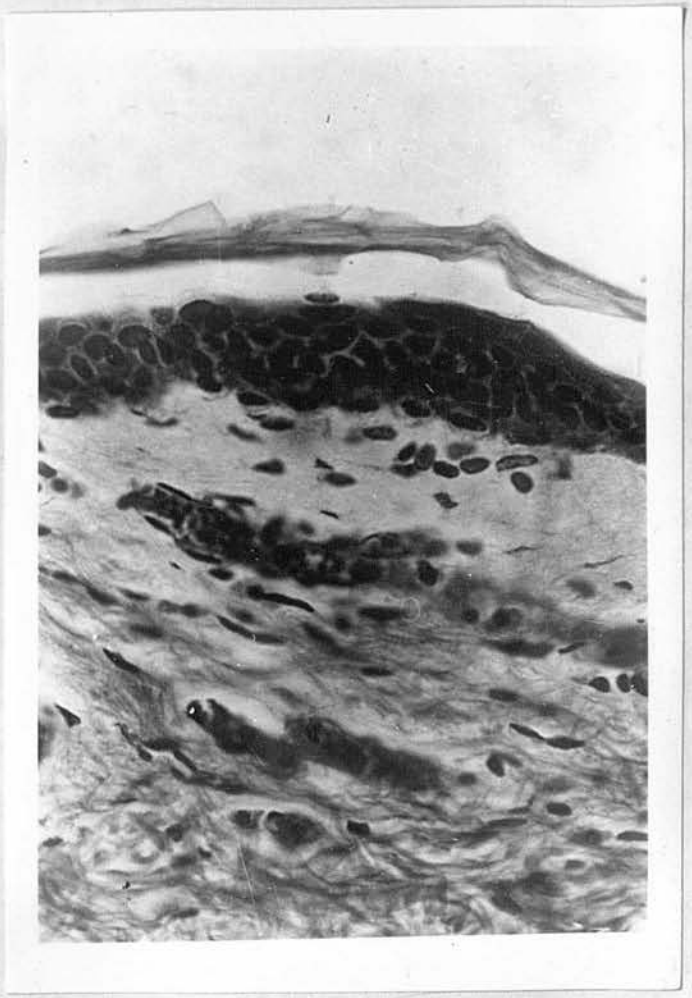


Fig.4 van Giesen, Fig.5 Holmes silver method; Transverse sections of touch corpuscles 4 days after crushing afferent nerve. Epidermis thinner than normal, tactile cells have shrunken clear spaces and are few in number.

now contained plates of nervous tissue.

With further developement the tactile cells increased in number and size, the epithelium developed, showing once again the 4 to 5 layers of nuclei with a palisade layer, until by 100 days the histological structure of the ending appeared normal.

Photomicrographs of the changes occurring in touch corpuscles following denervation and during reinnervation appear in figs 4 — 10.

b) Electrophysiological: 5 to 7 days after crushing the nerve, the majority of fibres had no resting discharge, but activity could be recorded by stimulating the skin over the scar. In response to firm stroking with a glass probe a short burst of impulses lasting 0.1 to 0.2 sec. could be elicited in both myelinated and nonmyelinated fibres. The mechanical threshold for such a response was of the order of 200 to500mg. weight. This sensitivity to mechanical stimulation was restricted to the area of skin overlying the axontip, and moved distally with time. fig 11

A minority of fibres in this group showed a persistent, spontaneous, fairly regular discharge of impulses, about 50/ sec. which could be inhibited by strong mechanical stimulation of the skin of the scar site, and by antidromic stimulation of the nerve. In some cases similar activity in other fibres could be evoked by brief mechanical stimulation of the skin, e.g. by drawing a glass probe quickly across the skin. The frequency of the steady discharge was accelerated by warming and slowed by cooling.

On one occasion reflected activity was observed, when an antidromic volley sent down the main nerve trunk resulted in a volley being recorded coming/

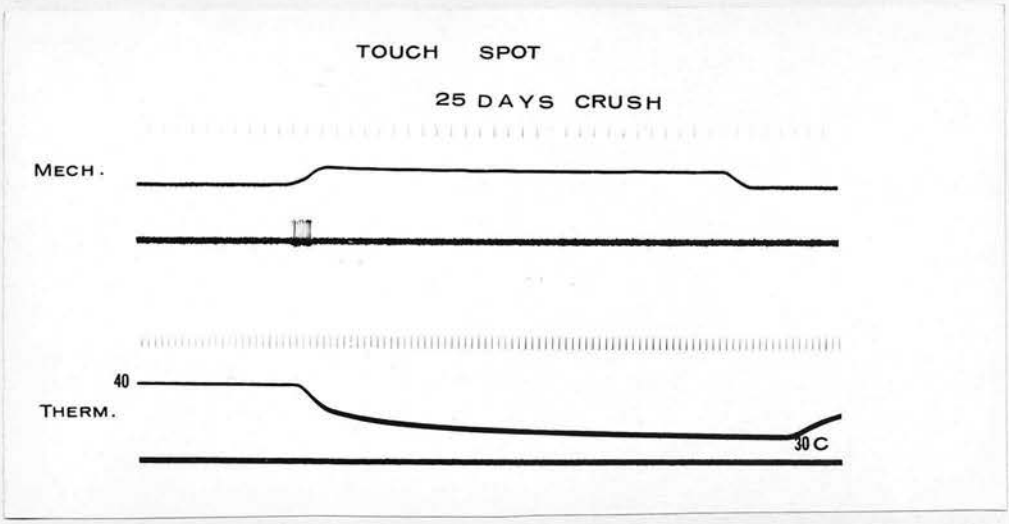


Fig.18 25 days after operation. Responses of a touch corpuscle to mechanical stimulation, there is a short burst of impulses with rapid adaptation. No respose to a fall in temperature offrom 40 to 30 C. Time 0.1 secs.

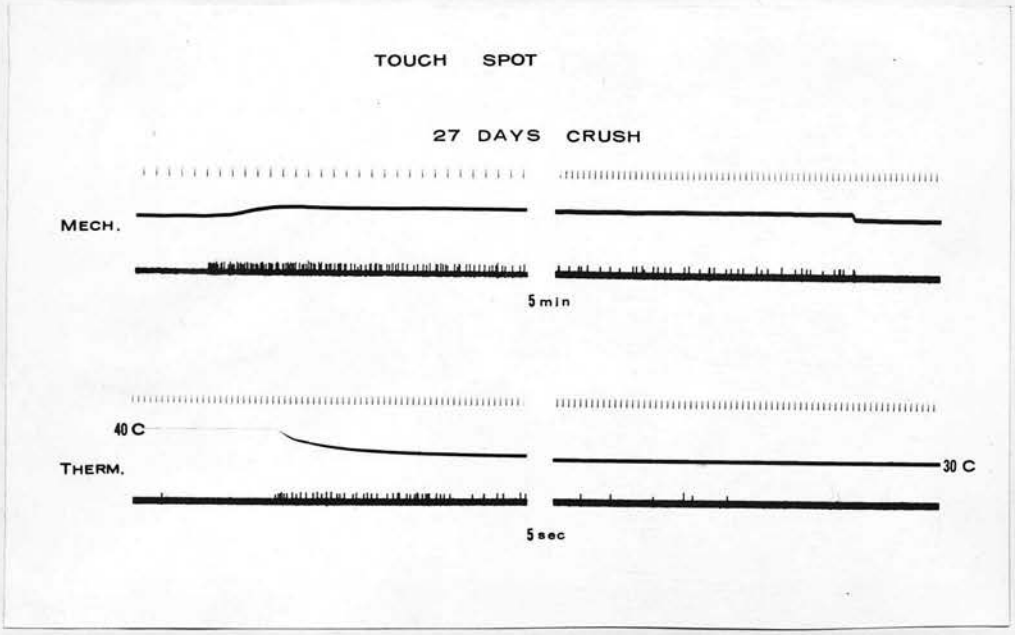


Fig.19 27 days afteroperation. Slowly adapting response to maintained mechanical stimulation. The touch corpuscle also responds to a fall in temperature of from 40 to 30 C. Time intervals 0.1 secs..

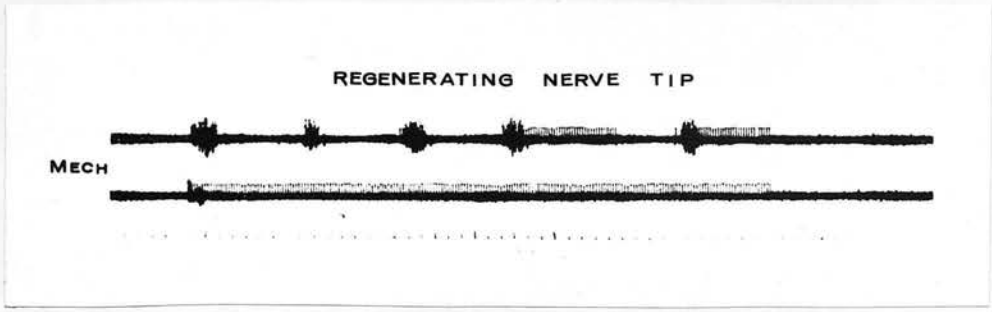


Fig.11 5 days after operation. Responses of regenerating nerve tip to mechanical stimulation. Prolonged discharge in one fibre, with a regular frequency. Time 0.1 secs..

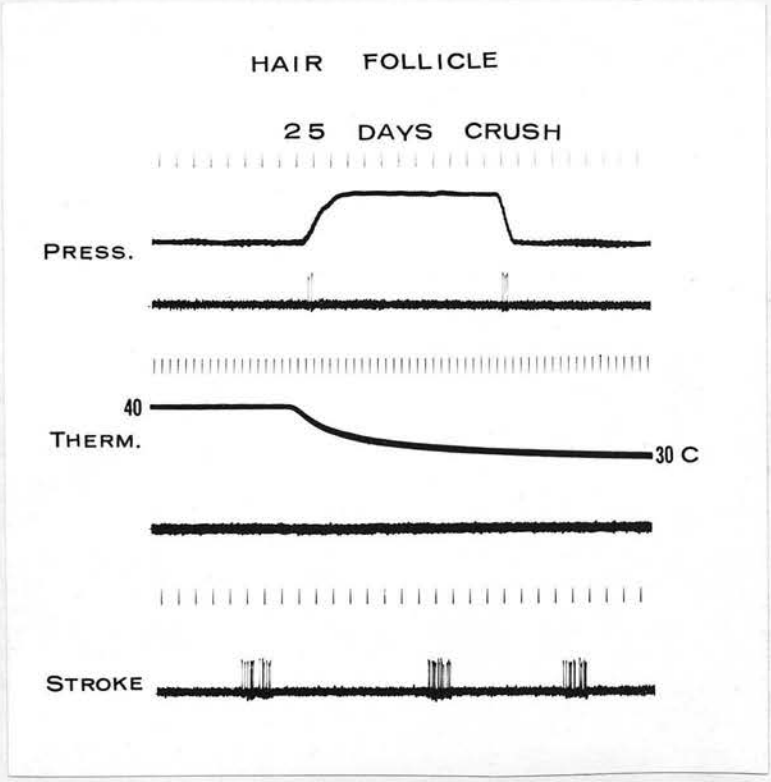


Fig 12 25 days after operation. Responses of hair follicle receptor, from above downwards; pressure with probe, thermal, stroking. Time 0.1 secs..

coming back in a strand of fibres dissected off from the main trunk.

16 to 19 days after operation no fibres observed had a resting discharge. Sensitive spots were widely dispersed in the skin innervated by the saphenous nerve, the responses obtained were essentially similar to the above, were not usually associated with a touch corpuscle, had receptive fields in the skin of the order of 1 sq. cm., responded to mechanical stimulation with a threshold of 200 to 500 mg. weight, showed rapid adaptation and were not stimulated by change in cutaneous temperature of 20°C. (40° to 20°C and vice versa).

20 to 23 days postoperatively two distinct types of receptive field were in evidence. 1) a highly localised, spot - like field, threshold greater than 200 gm. weight, sometimes associated with a touch corpuscle, and 2) a dispersed sensitivity of about 1 sq. cm., mechanical threshold greater than 200 mg. weight, and similar in size to that of hair follicle afferent fibres and which responded to pulling the hairs. fig. 12.

From this time to 30 days and coincident with the period of histological activity occurring with reinnervation of the corpuscles, many various responses were observed. In the early stages of this period responses similar to the spot - like ones of 16 to 23 days, were observed, generally at, or a few millimetres proximal to, to a touch corpuscle. As the period progressed the responses approached the normal. The mechanical threshold fell, in some cases to 3 mg. weight or less, the maximum impulse frequency rose, in some cases to over 1,000/ sec.; responses to temperature change at the skin surface were observed and the adaptation time to

mechanically/

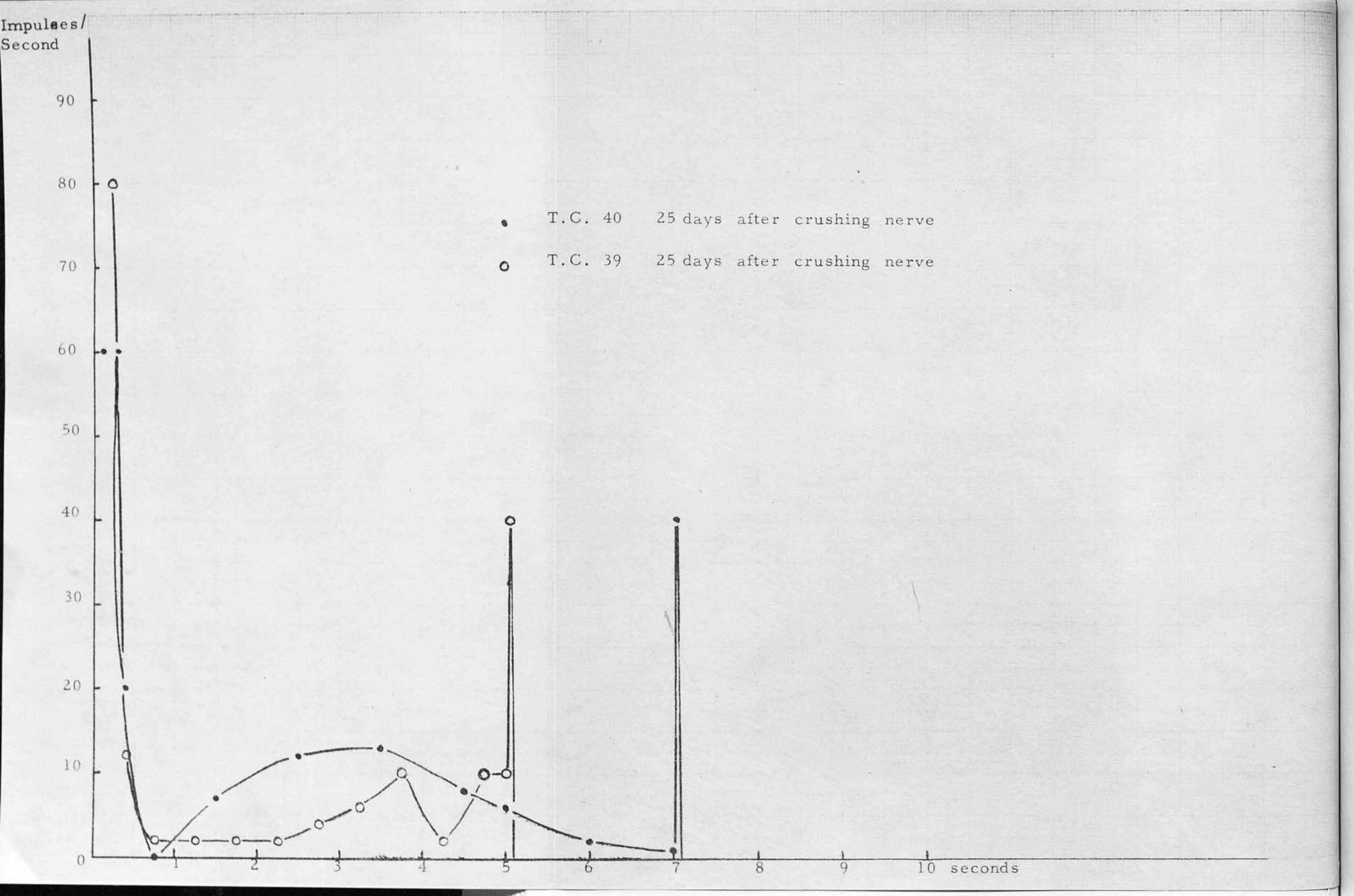

Impulses/
Second
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10 seconds
T.C. 40 25 days after crushing nerve
T.C. 39 25 days after crushing nerve

Fig. 17 Adaptation curves for 2 touch corpuscles 25 days after operation at an early stage of reinnervation, showing unusual responses to mechanical stimulation.
(There was no detecable change in the pressure applied during stimulation. See text.)

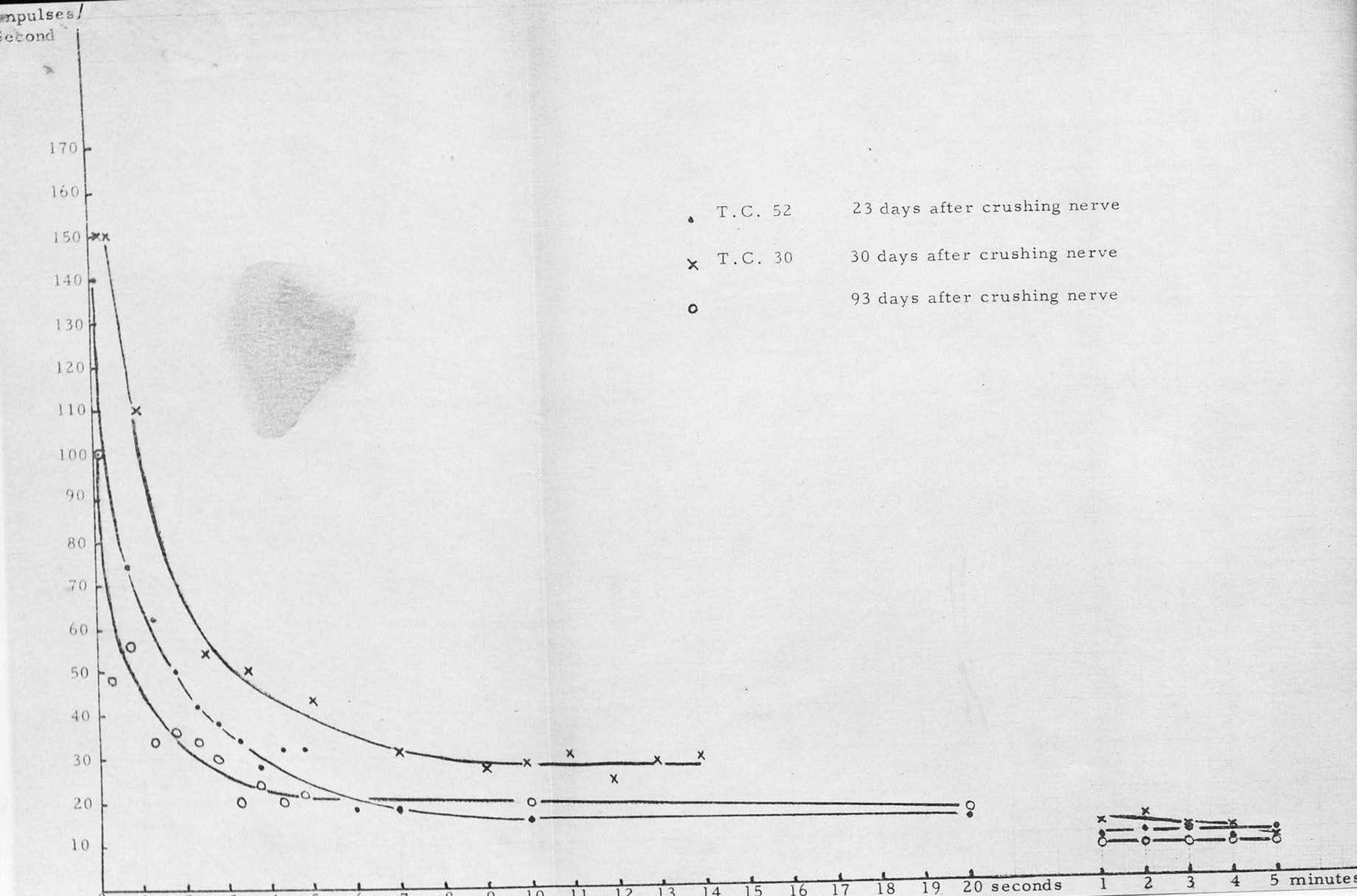
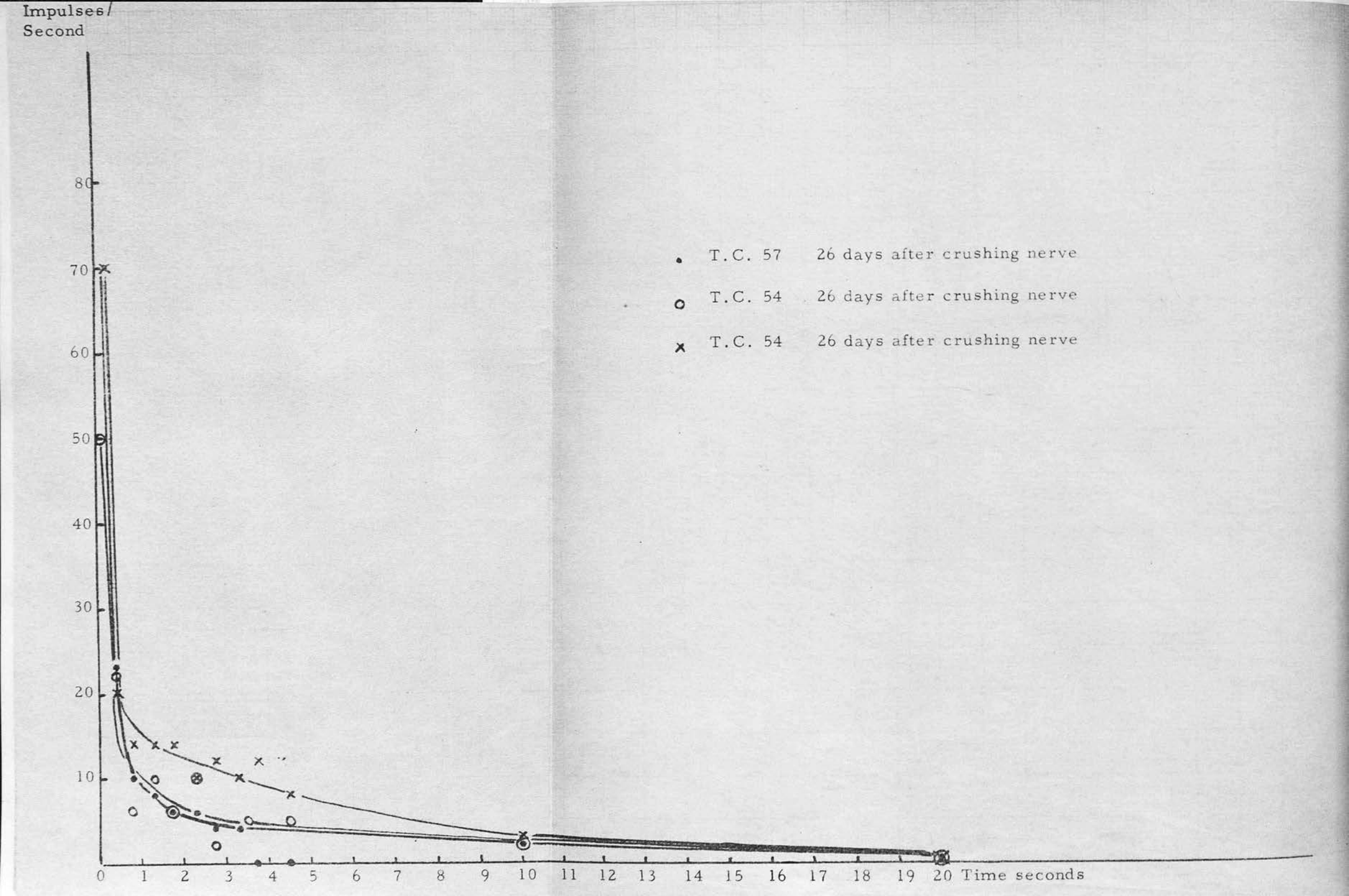

mpulses/
econd
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
T.C. 52 23 days after crushing nerve
T.C. 30 30 days after crushing nerve
93 days after crushing nerve
20 seconds
1
2
3
4
5 minutes

Fig. 16 Further stage in the reinnervation of touch corpuscles. The response to mechanical stimulation now shows a higher rate of firing at the initial response, the adaptaion rate is slower, and the endings respond for 5 mins. or more.


Impulses/
Second
80
70
60
50
40
30
20
10
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 Time seconds
T.C. 57 26 days after crushing nerve
T.C. 54 26 days after crushing nerve
T.C. 54 26 days after crushing nerve

	T.C.	C.V. m/sec.	Thresholds. Mech.	Thermal.	No.of spots /fibre.	Histology.
Cat S16 27 days	43	26.5	>3<10mg.	10°c.	1	Cellular epidermis, well developed basement membrane tactile cells few, mostly with shrunken clear spaces,
	44	26.5	10mg.	10°c.	2 } 1 common	Epidermis thin except over tactile cells, palisade present, 8-9 tactilecells / section, spaces shrunken; 2 Schwann sheaths. nerves dividing & running to tactile cells.
	45	o	>3<10mg.	+	2	Epid. well developed with palisade & basement memb. fimbriated.; many tactile cells, some normal.
	46a		>10mg.			Well developed epidermis,
	46b	o	<10mg.	+	3	few tactile cells with
	46c		≃10mg.			shrunken clear sacs; basement membrane developed. Nerve
	47a	33	<10mg.	+	2	fibres ending about tactile
	47b		>10mg.			cells.
	48	o	>10mg.	+	2	Epid. wellsdeveloped with palisade & fimbriated basement membrane. Tactile cells present, some of normal appearance. No nerve fibres in vicinity of epidermis.
Cat S14 28 days	33	55	o	o	1	
	34	66	<3mg.	o	1	
	35a		<3mg.	o		
	35b	60	<3mg.	o	3	
	35c		<3mg.	o		
	36	o	<3mg.	o	2	
Cat S8 30 days	27a					
	27b	90	200-500mg.	o	2	
	28	65	+	+	3	
	29	47.5	<5mg.	20	1(+)	

+ present -absent onot recorded.

Table 4: Properties of afferent fibres 27-30 days after nerve and of the touch corpuscles they innervated.

	T.C.	C.V. m/sec.	Thresholds Mech.	Thermal.	R.D.	No.spots /fibre.	Histology.
Cat SI7 23 days.	48a	o	>5<10			I	a) Small T.C., epith. thin; few small tactile cells.
	b		≃300mg.	o	-	Imm.prox. to T.C.	
	~~c~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	
	49	o	o	o	-	prox. to 2 T.C.	Epith. thin, nopalisade, no fimbriated basement membrane Nerve running to epidermis.
	50	o	o	o	-	I	Slightly thickened epidermis, no palisade, no tactile cells no fimbriated basement membrane.
	5I	o	o	o	-	I	Some thickening of epidermis, fimb. basement membrane present, no palisade, no tactile cells.
	52	o	< 3mg.	o	-	I	Epith. 3nuclei thick, basement membrane well developed, tactile cells scattered(about 5/ section.
	37	33	>I0	I0	-	2	Epid. 2-3 nuclei thick, no palisade ,basement membrane fimbriated,few atypical tactile cells.Nerve fibres in dermis.
	38	o	50-200mg.	20	-	2	Epid. thin, basement membrane developed, no typicaltactile cells; nerve fibresin dermis.
Cat SI5 25 days.	39	o	> 50mg.	-	-	2	Epid. 4-5 nuclei thick, well define d basement memb., few tactile cells.
	40	o	> 50mg.	-	-	2	Epid. 2-3 nuclei thick, palisade & b. memb. not well defined: moderate no. oftactile cells, some of normal appearance.
	42	o	o	-	-	I distal to spot.	
	4I	o	>300mg.	-	-	"	
	54	o	3mg.	o	-	2(3)	Epid. 2 nuclei thick; few typical tactile cells.
Cat SI8 26 days.	55	o	3mg.	o	-	3	Epid. 2-3 nuclei thick,few atypical tactile cells.
	56	o	o	o	-	I	
	57	o	3mg.	o	-	2	

R.D.: resting discharge. + present - absent o not recorded.

Table 3; Properties of afferent fibres 23-26 days after crushing the nerve, and of the touch corpuscles they innervated.

	C.V. m./sec.	Thresholds. Mech.	Therm.	R.D.	Remarks.
Cat S9 5 days	o	o	o	- +	Maj. of fibres silent, some with resting discharge. Stroking over scar with firm strokes stopped spontaneous discharge. Reflected activity. Sensitivity of all fibres to mech. stim. rapidly depressed by mech. stimulation.
Cat S52 7 days.	o	o	o	-	No electrical activity in nerve when skin stimulated mechanically or thermally.
Cat SI2 8 days.	o	very high	o	-	Burst of impulses lasting approx. 100- 200 msec. when skin over nerve tip stroked. Steady burst lasting 4-10 sec. in one fibre.
Cat S6 15 days	o	> 200-500 mg.	-	-	Discharge to mech. stim. > 5 impulses lasting 200 msec., except in one fibre in which there was a very steady discharge at 50/sec. after stroking the skin firmly, lasting 0.2-4.5 sec., which cut off abruptly.
Cat SI3 22 days.	o	> 200-500mg.			Burst of impulses when skin stroked.

C.V.: conduction velocity.
R.D.: resting discharge.
+ present. - absent. o not recorded.

Table 2: Properties of afferent fibres in the saphenous nerve 5-22 days after nerve crush, which had not reinnerva ted a touch corpuscle.

mechanically applied stimulation increased. In some cases impulses could be recorded from the fibre as long as 5 min. after the application of a maintained mechanical stimulus. fig. 13 - 14 .

Tables 2 to 4 summarise the above results and correlate them with the histological appearance of the touch corpuscles, and adaptation curves to mechanical stimulation are shown in figs. 15 - 17.

At 25 to 26 days several unexpected observations were made e. g. at the moment of applying the mechanical stimulus there was a short burst of impulses 60 to 80/sec. lasting for about 5/10 sec., which then fell away to, in one case zero and in another to 2/sec., the discharge then increasing in frequency again over the next 2 to 3 seconds and finally giving a burst of impulses at 40/sec. at the removal of the stimulus, there being no detectable alteration in the pressure exerted by the probe during the application of the maintained stimulus. (fig. 17.).

Individual touch corpuscles are normally supplied by a single myelinated axon but in one case a touch corpuscle, after reinnervation, received 2 afferent fibres, which was demonstrated both electrophysiologically and histologically, this has not been observed in the normal animal.

Discussion.

The experiments reported show convincingly another sense ending which undergoes histological changes after denervation. The maintenance of the specific histological structure of cutaneous touch corpuscles in the cat is dependent on a nerve supply; not only are the receptor cells themselves dependent/

dependent, but other elements of the organ, namely the epithelium and basement membrane, are too. The whole structure is a functional unit. By comparison with earlier work it can be assumed that, for the maintenance of such structure, sensory nerve fibres are required and that motor nerve fibres would be incapable of such maintenance.

Thus Gutmann (1945) joined the central end of the sural nerve to the peripheral end of the peroneal nerve in rabbits. The sensory fibres, when they reached the muscle, ran for long distances along or across the muscle fibres, branching and giving the appearance of a network. Empty end plates were rarely entered and when entered were passed through, no structures being formed in them. No annulospiral endings were formed in the muscle spindles. The sural nerve fibres also grew into the autonomous zone of the peroneal, of the skin of the dorsum of the foot, and there was recovery of sensibility to mechanical stimulation. Similar results were reported by Weiss and Edds (1945). It can be concluded that sensory nerve fibres will not innervate motor end plates (the earlier papers in this field e.g. Boeke had inadequate controls), and indeed the above work indicated that cutaneous afferent fibres are unable to innervate muscle spindles. If this is the case the degree of specificity of cutaneous nerve fibres is greater than previously thought. It is curious that Gutmann did not comment on this observation and it obviously needs confirmation, since it is of fundamental importance. That the sural nerve grew to the autonomous zone of the peroneal nerve and could be stimulated there, does not help in the matter of specificity, since it could have either reinnervated previously/

previously denervated sense organs, or else it was the ends of the nerve fibres themselves that were responding to stimulation. Weiss (Weiss and Edds) regards such specificity as due to the arrangements of molecules at the boundary membranes between cells, ".....intimacy of contact between a nerve fibre and its surroundings is determined by specific affinities between the molecular population on either side of the boundary membranes." This will be referred to later.

When the nerve fibres grow back into the touch corpuscles the specific structure of the organs is reconstituted. In nerve crush experiments it can be assumed (Young 1942) that the nerve fibres grow back along their original axon sheaths, and thus the problem of specificity does not arise. Cellular activity does, however take place in the ending when the nerve fibre grows back into it, and it might appear surprising that the basement membrane's fimbriation is the first change to appear , though epithelial cell proliferation follows it immediately. When a nerve fibre grows back to innervate a touch corpuscle it will reach the basement membrane at an earlier time than it reaches the epithelium. Thus if a chemical process is concerned in the reformation of the ending, as is probably the case, such a process would be expected to affect the basement membrane before the epithelium. That the tactile cells make their appearance only over those parts of the ending where a nerve fibre is growing indicates the short distance over which the induction process operates (cf. Tower).

Turning to the development of the specificity of nerve fibres and of their associated endings, some apparently conflicting observations have been/

been reported. Hogg (1941) in a study of human foetuses, observed that at 8.5 weeks of menstrual age the epidermis thickened and the basement membrane appeared. By ten weeks of menstrual age nerve fibres were visible and there was a rearrangement of cellular elements and the epidermis became thicker over the nerve fibres than elsewhere. These observations are reminiscent of the changes occurring in touch corpuscles during reinnervation. Miner (1956), however, showed that if, in frog tadpoles, a strip of skin from mid-dorsal to mid-ventral line was removed, rotated through 180° and replaced, it continued to develope in a way appropriate to the place from whence it came. The adult frog, when stimulated on its ventral surface responded by wiping its dorsal surface, and vice versa, and it was shown that the cutaneous nerves had not grown round to find their appropriate skin.

It was suggested, (Miner), that the integument undergoes a field - like differentiation, each locus having an unique array of chemical properties, which become stamped on the nerve fibre. This specificity then spreads proximally and appropriate synaptic connections are laid down in the central nervous system. This mechanism could be explained on the basis of the distribution of molecular populations at cell boundaries (Weiss), brought about by some such mechanism as proposed in the first part of this work; but it does appear, that the arrival of nerve fibres at the skin affects cutaneous cellular components in the human foetus, presumably at a time when the nerve fibres are unspecified, according to Miners hypothesis, unless of course the changes in the skin are coincidental, which seems unlikely. It could be argued that the ingrowing nerve fibres firstly affect the/

the epidermal cells which then, in turn, act back on the nerve fibre in a reciprocal way, though this is using the results of observations on two animal species. In the adult animal the specificity is presumably immutable and only the appropriate fibre can reinnervate the ending, and in so doing causes a considerable amount of cellular rearrangement.

Weiss (1939) distinguishes between modulation and true differentiation, the former process being the changes of cell properties which persist only as long as their initiating external influence, and reverse when it is withdrawn. The ability to respond appropriately to a known differentiating environment, has been called competence by Waddington (1940). In the touch corpuscle the basal layer of the epidermis may be capable of giving rise to either an epithelial cell or to a tactile cell, there being presumably no conversion of fully differentiated epithelial cells to tactile cells.

A further unsolved problem is that of the origin of the tactile cells of the touch corpuscle. Are they of ectodermal (neuroectodermal), or of other origin? If they are epidermal in origin a hypothesis such as that mentioned above may be called for, whereby latent potentialities in the epidermal cell are released by an approaching or contacting nerve fibre. If, however, the origin is from totipotent cells of the mesenchyme, although the mechanism involved might be similar, the specificity need not perhaps be so narrow. In this connection the observation that one touch corpuscle was observed to be innerveted by two nerve fibres, after reinnervation, from both of which electrophysiological recordings were taken, indicates that the relation is not a point-to-point one, as it were , but one concerned/

concerned with small areas and small numbers of fibres, or even a graded relation, one specificity merging into another.

When the regenerating fibres reach the skin, according to Gutmann and Guttman (1942) there is a delay of about 5 days from their arrival to the recovery of sensory function , as tested, in rabbits, using behaviour experiments. It was asked whether this "peripheral delay" was due to slower growth of fibres in the skin, maturation of the fibres, or to the fact that the arrival of a sufficient number of fibres is necessary for functional recovery. Obviously the latter two factors may play some part, the former being difficult to examine, but the experiments reported here do throw some light on the problem. Thus 25 days postoperatively responses were obtained by stimulating a touch corpuscle, mechanical threshold greater than 100 mg. weight, with a rapid adaptation, which showed histologically, a nerve fibre entering the dermis but not reaching the epidermis, which latter was thin, the fimbriated basement membrane structure being present but ill-defined and the few tactile cells present showing signs seen after denervation. In a 27 day animal many touch corpuscles had mechanical thresholds of the order of 10 mg. or less and here the epidermis was thicker, the palisade layer and basement membrane well defined and a large number of tactile cells of normal appearance. Thus in the case of this ending part of the time taken at the periphery is due to the reconstitution of the organ, both structurally and functionally.

Weddell, Sinclair and Feindell (1948), on the basis that single sensory spots in the skin are innervated by more than one nerve fibre, concluded that/

that if the density of innervation were reduced, the sensations perceived from such an area of skin would have an unpleasant quality. It cannot be denied that such reduction will alter the pattern of impulses passing up an afferent nerve trunk, and presumably alter the sensation perceived, both quantitatively and qualitatively. The sense organ under study, however, is normally innervated by only one afferent fibre, which fibre innervates several (1 to 4) endings. Though the direct application of animal experiments to human beings is to be deprecated it is suggested that part of the sensory experience perceived during reinnervation is due to the abnormal discharge pattern observed in such fibres.

That the basis for some of the unpleasant sensations perceived when afferent nerve fibres are damaged is the spontaneous discharges and fibre interaction reviewed above seems established. It is believed that the work reported here, when spontaneous discharges and fibre interaction were observed 4 to 5 days after crushing the nerve fibres, shows such discharges the longest time after nerve injury yet observed, and helps to remove the objection that such discharges do not last long enough to account for the burning pain perceived after nerve injuries. A neuroma was present at the injured region of the nerve and here presumably the normal insulation of the fibres was lost.

This study also emphasises that it is the end organ which determines the characteristic discharge properties of the afferent fibre to physiological stimulation and that when separated from their end organs all afferent nerve fibres respond in a similar way. This again is an explanation of/

of the unpleasant sensations perceived after nerve injury.

It is obvious that many problems remain unsolved and for the future the following types of experiments will be considered:

a) What occurs after cutting the nerve and preventing regeneration altogether? Do the touch corpuscles disappear completely with time, and if so by what mechanism?

b) What differences occur after cutting the afferent nerve and allowing regeneration to take place? Are all afferent fibres in a cutaneous nerve able to reinnervate a touch corpuscle and if so with a functional result? Are any aberrent endings formed?

c) Are motor fibres able to innervate touch corpuscles and cause them to recover their structure and function?

d) Are there any receptor potential changes during reinnervation of the touch corpuscles, since this preparation would appear to be useful for microelectrode studies?

e) Autoradiography experiments to determine the origin of the tactile cells.

The experiments reported here were carried out in collaboration with Professor A. Iggo of the Department of Veterinary Physiology, Royal (Dick) School of Veterinary Studies, whom I wish to thank for his constant advice and permission to use the material. I should like to thank, also, Mrs.W. Shanks and Mrs. M. Davidson for their technical assistance, and Mr. G. Renwick for preparing the photographs used.

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