MECHANISMS OF NEURAL REGENERATION

PROGRESS
IN BRAIN RESEARCH
Volume 13

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MECHANISMS OF NEURAL REGENERATION

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Preface

The reviews of this volume are not meant to exhaust all topics within the subject of degeneration and regeneration but rather to probe deeply into a few selected aspects of the subject. The unique morphology of the neuron with its long cytoplasmic processes and its tremendous volume of distally situated cytoplasm transfers the problem of injury and regeneration to a different setting from the case in other tissues. Destruction of nervous tissue is significant of processes of a cell and less that of the entire cell, the problem of recovery and regeneration being mainly that of the processes of the individual neuron. Unlike other tissues when damaged, there is no neuronal multiplication, accumulation of cells and differentiation of cells. Therefore, our study of degeneration and regeneration of the nervous system is primarily a study of the neuron itself, in its reaction to injury, its physiology, its structure and the nature and growth of its cytoplasmic processes. This does not mean that there are no other consequences of nerve injury. Indeed, there are profound changes but they reflect the primary injury of the cytoplasmic processes: for example, changes both in the sheaths that surround the axonal threads and in some of the organs upon which the neuron ends. All these changes will be touched upon in the volume, but it is primarily the living threads of cytoplasm that we will deal with: their morphology, function and chemistry.

The Editors

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Axoplasmic Streaming in Regenerating and in Normal Nerve Fibres

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INTRODUCTION

'I put forward the hypothesis that in the body of the nerve cell a substance is formed from the nucleus and Nissl bodies which gradually passes into the nerve fibres; and also that stimulation of other cells by a nerve fibre is brought about by the passage of some of this substance into the cells on which the fibre acts . . . The nerve cells secrete a substance the passage of which from the nerve endings is necessary to stimulation'. 'The recovery of effect after transient fatigue I attribute to the passage of a portion of this substance down the nerve fibre to the nerve ending. The absence of recovery after prolonged stimulation I attribute to the whole of the substance in the nerve

fibres being used up, and to their being incapable of making more when severed from their nerve cells'.

These sentences were written by F. H. Scott in 1906. Audacious as they appear on the basis of the scant experimental evidence available at that early date, they formulate, in general terms, hypotheses of the chemical mediation of synaptic transmission, of perikaryal synthesis of some substances and of their transport along the axons, as well as interpretation of the disappearance of synaptic function after nerve section by the exhaustion of relevant materials and the impossibility of renewed supply.

All these hypotheses are still valid although their further elaboration and replacement of general by concrete terms has been unequal. Whereas immense developments have taken place during the intervening 60 years concerning the mechanism of synaptic transmission and of protein synthesis in the perikarya, our ideas concerning the mode of transport along axons remain uncertain.

This is due partly to inherent difficulties of detection and partly to the fact that relatively few investigations were undertaken with the explicit purpose of studying the movements of axonal contents. The data concerning these movements were obtained mostly in experiments in which other problems such as the site of synthesis or the physiological role of various materials were investigated together with the question of transport. The proposed interpretations dealt with all these interconnected problems and, so far as the axonal flow is concerned, often led to conflicting inferences. Thus, whereas the existence of some kind of transport of materials along axons is assumed, on various grounds, by most workers, opinions differ as to the mode of migration and the parameters of the postulated flow.

The purpose of this paper is to review and analyze some of the experimental results either suggesting or directly demonstrating the existence of axoplasmic movements (for embryological data see Levi-Montalcini and Angeletti, 1962), and to try to resolve, where possible, the apparent contradictions. A comment may be useful to clarify the relations between the content of the Tables and the text in chapters concerning indirect indications of the existence of axoplasmic flow. Some of the tabulated data were obtained with other purposes in mind and the problem of axoplasmic flow was touched upon by the authors only parenthetically or not at all. The interpretation of the authors where available is noted in the Tables. The interpretation proposed in the text is mine and is sometimes in conflict with the original proposals. In some instances unpublished details of experiments or of interpretation were kindly supplied by the authors and have been acknowledged in the footnotes.

I. MOVEMENTS OF AXOPLASM IN REGENERATING NERVE FIBRES

The most conspicuous shifts of axoplasm occur during the elongation of regenerating nerve fibres. The study of this phase of regeneration provided many quantitative data which greatly influenced the present concepts concerning the flow of axoplasm. It seems useful therefore to review briefly the behaviour and main features of elongating nerve fibres, although the various stages of regeneration, the formation of growth cones, the elongation of regenerating sprouts, their subsequent maturation and

establishment of peripheral connections, the relationship between morphological and physiological features of these processes, as well as the functional recovery of innervated organs have been subjects of extensive reviews (Cajal, 1928; Young, 1942; Seddon, 1954; Guth, 1956; Gutmann, 1958).

(a) Fibres in nerve trunks

Out of the end of the central part of a cut or crushed fibre, after a latent period of the order of days, one or several thin sprouts emerge. If the local conditions at the lesion are such that the growing sprouts may enter the nerve tubes of the peripheral stump, they grow there.

The presence of Schwann cells influences the rate of elongation (Williams, 1930) but regeneration is possible also without Schwann cells, as in cornea (Rexed and Rexed, 1951; Zander and Weddell, 1951).

From the very beginning the regenerating tip of the fibre is able to generate impulses on mechanical stimulation. In contrast to their poor electrical excitability the tips of regenerating fibres have a very low threshold to mechanical stimulation. It increases progressively with the ageing of regenerated parts (Konorski and Lubińska, 1946). Electrophysiological characteristics also change with progressive maturation of fibres (Berry *et al.*, 1944; Hursh, 1939; Erlanger and Schoepfle, 1946). Both Tinel's (1915) sign and Young and Medawar's (1940) 'pinch' method are based on mechanical excitability of regenerating sprouts. They permit the measurement of the length of regenerated fibres in the early stages, before functional completion, and the estimation of the rate of elongation of fibres uncomplicated by delays introduced by reinnervation of end organs.

The rate of regeneration is a few mm a day and varies little in peripheral nerves of various mammals (Table I). The only exception described so far is the trigeminal branch to the antlers of deer (Wislocki and Singer, 1946) which regenerates during their regrowth at a rate about ten times faster than that of other sensory fibres. The rate of regeneration of motor fibres, determined indirectly (Gutmann *et al.*, 1942) seems to be somewhat slower. An indication of slower regeneration of motor fibres is also suggested by Diamond's (1959) experiments, in which impulses in ventral and dorsal roots generated by pinching the regenerating nerve were recorded.

Regeneration may be induced repeatedly in the same group of axons without apparent impairment. Thus Duncan and Jarvis (1943) crushed 9 times a branch of the facial nerve in cat, obtaining after each lesion a complete recovery at similar periods of time.

The rate of regeneration is very temperature-dependent (Tables II and III). No elongation occurs below a certain critical value: 8° for the sciatic of frogs (Lubińska, 1952a) 26° for tissue cultures of chick embryos (Mossa, 1927) and an environmental temperature of below 2° for the caudal nerve of rats (Gamble, 1957). Above these values the rate of regeneration increases with temperature within the physiological range (Mossa, 1927; Lubińska and Olekiewicz, 1950). Subsequent maturation of regenerating fibres is also accelerated by temperature (Gamble, 1958; Jha *et al.*, 1959).

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TABLE I

RATES OF ELONGATION OF REGENERATING MAMMALIAN NERVE FIBRES*

Animal	*Nerve*	*Lesion*	*Rate in mm/24 h*	*Remarks*	*References*
Mouse	Sciatic	Crush	2–3		Wyrwicka, 1950
Rat	Tibial	Crush	3.3		Konorski and Lubińska, 1945
	Peroneal	Crush	2.4		
Rabbit	Dorsal auricular	Crush	2.5		Weddell, 1942
	Peroneal	Cut and suture	3.5		Gutmann *et al.*, 1942
		Crush	4.4		
	Peroneal	Cut and suture, at hip	2.6	In cross-unions tibial–peroneal and peroneal–tibial the growth rate is that of the central stump	Haftek, 1963
		Cut and suture, at knee	1.9		
	Tibial	Cut and suture, at hip	3.3		
		Cut and suture, at knee	2.6		
	Tibial	Crush	3.4		Konorski and Lubińska, 1945
		Cut and suture	3.3		
	Median	Crush	4.5		
	Ulnar	Crush	3.2		
	Radial	Crush	2.3		
Cat	Sciatic	Cut and suture	3–4		Berry *et al.*, 1944
	Peroneal	Crush	4.8		Konorski and Lubińska, 1945
	Median	Crush	4.7		
	Ulnar	Crush	4.5		
	Radial	Crush	4.9		
Dog	Phrenic	Crush	4.8	Action potentials	Erlanger and Schoepfle, 1946
	Tibial	Crush	3.7		Konorski and Lubińska, 1945
	Peroneal	Crush	3.4		
Monkey	Tibial	Crush	3.5		Konorski and Lubińska, 1945
Baboon	Tibial	Crush	3.9		Konorski and Lubińska, 1945
	Peroneal	Crush	2.5		

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Deer	Trigeminal	Shedding of antlers	15–20	Sensory fibres during growth of new antlers	Wislocki and Singer, 1946
Man	Radial	Cut and suture	1.6	Motor recovery — Rate fast initially then slowing down	Seddon *et al.*, 1943
			1.7	Tinel's sign — Rate fast initially then slowing down	
	Median, Ulnar		3 Forearm	Tinel's sign	Sunderland, 1947
			0.5 Wrist	Tinel's sign	
	Sciatic		2 Leg	Tinel's sign — Initial rate depends on the level of the lesion	
			0.5 Ankle	Tinel's sign — Initial rate depends on the level of the lesion	
	Radial	Axonotmesis	1.9 Elbow	Motor recovery — Initial rate depends on the level of the lesion	
	Radial	Axonotmesis	0.8 Mid-forearm	Motor recovery — Initial rate depends on the level of the lesion	
	Radial	Suture	1.2 Elbow	Motor recovery — Initial rate depends on the level of the lesion	
	Median	Suture	5.8 → 1.4	Tinel's sign	Sunderland and Bradley, 1952
	Ulnar	Suture	2.8 → 0.1	Tinel's sign	
	Musculo–cutaneous	Suture	2.7 → 0.8	Tinel's sign	
In developing animals:					
Cats, dogs and rabbits several weeks old	Sciatic		2–3	Regeneration. Histological methods	Cajal, 1928
Rat 1–3 days old	Caudal cutaneous nerves		2	Growth	Lubińska (unpublished)
4–26 days old	Caudal cutaneous nerves		2.8	Growth	
Embryo of guinea-pig	Pyramidal tract		6–7	Growth. Histological methods	Kimel and Kavaler, cited by Flexner, 1950
Chick embryo 3 days	Not stated		0.24	Growth. Histological methods	Cajal, 1928

* The data collected in this Table were obtained directly on the regenerating fibres. Transsynaptic tests were used only for human motor fibres. For other rates of regeneration, estimated from times of functional recovery, see Gutmann *et al.* (1942). The character of regenerating fibres is undetermined in the data obtained by histological methods or by testing the action potentials. All other data concern sensory fibres.

TABLE II

RATES OF ELONGATION OF REGENERATING AMPHIBIAN NERVE FIBRES

Animal	*Nerve*	*Lesion*	*Temperature* °C	*Rate in mm/24 h*	*Remarks*	*References*
Toad, *Bufo bufo*	Sciatic	Crush	9.1 12.1 15.7 21.8 25.8	0.6 0.6 0.6 1.0 1.4		Lubińska and Olekiewicz, 1950
Frog, *Rana esculenta*	Sciatic	Crush	8.9 12.5 17.1 21.9 25.9	0.6 0.6 0.8 1.2 2.2		Lubińska and Olekiewicz, 1950
Salamander	Cutaneous branches in the tail	Cut	—	0.48	Observed for 1 h. Average over longer periods is lower	Speidel, 1935b
Hyla crucifer, tadpole	Cutaneous branches in the fin	— Cut	— —	0.4–1.4 0.2–2.0	Outgrowth of new fibres Regeneration	Speidel, 1933, 1950 Speidel, 1935a
Rana sylvatica, tadpole	Cutaneous branches	Cut	20	0.3 In Schwann sheaths 0.12 Without sheaths	Figures corrected for latent period Figures corrected for latent period	Williams, 1930
Hyla crucifer, tadpole	Lateral line nerve	Cut	20	0.4 In Schwann sheaths 0.12 Without sheaths	Figures corrected for latent period Figures corrected for latent period	Williams, 1930

It is not clear, as yet, whether the rate of regeneration changes with the distance of fibre tips from cell bodies. Whereas both in the rabbit (Gutmann *et al.*, 1942) and in frogs and toads (Lubińska and Olekiewicz, 1950), the rate of advance of regenerating fibres appears to be constant throughout the length of the nerve, in man a declining rate of regeneration along the limbs and a marked fall (to about 1/6) in the wrist and the foot were observed (Seddon *et al.*, 1943; Sunderland, 1947; Sunderland and Bradley, 1952). Since the rate of regeneration is greatly influenced by temperature, the strong decrease of the rate in the distal parts of human nerves is probably at least partly due to temperature gradients along the limbs.

However even when precautions were taken to maintain a constant temperature along the regenerating nerve (Haftek, 1963) a difference of rates appeared (in the rabbit) according to the distance of the lesion from cell bodies, faster growth being observed with more proximal lesions. The initial rate was in each case maintained throughout later regeneration. These experiments seem to indicate that only the level of lesion and not the distance travelled by axon tips influences the rate of regeneration.

(b) Individual fibres in vivo *and in culture*

Whereas the study of regenerating nerve trunks at daily intervals indicates a steady advance of regenerating fibres, direct microscopical observations of individual fibres reveal a more complex behaviour. The main source of information concerning the behaviour of individual growing and regenerating fibres *in vivo* is Speidel's (1932, 1933, 1935a, b, 1950) remarkable series of experiments in which cutaneous nerves in the dorsal fin of tadpoles were observed over long periods in intact animals. He describes the emission of new nerve sprouts, the proliferation of primitive Schwann cells, their migration between and along fibres, their orderly application to successive stretches of axons, and formation of myelinated internodes in normal development and during regeneration. Speidel also studied the influence of various factors on the behaviour and appearance of axons and myelin and the sequence of changes in fibres undergoing Wallerian degeneration.

The growing axons present terminal enlargements, growth cones, with many branching pseudopods. The growth cones advance through the tissues by typical amoeboid motion, pseudopods being incessantly extended and retracted. The encountered obstacles may block the progress, causing enlargement of the growth cone and further branching at or near the tip. The branching may be temporary or permanent. Rarely the terminal portion of the axon is pinched off and degenerates. Sometimes the fibre recedes. A growth cone about to retract draws in its filamentous processes and becomes smoothly rounded. After a time the advance is resumed. Thus the elongation of fibres is intermittent, periods of advance alternating with those of arrest or retraction.

The back and forth movements of axoplasm are not confined to the growth cones. Slow protoplasmic streaming of both granular materials and clear neuroplasm may be discerned along the axons. It occurs in both directions but the flow towards

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TABLE III

RATES OF ELONGATION OF GROWING AND REGENERATING FIBRES IN TISSUE CULTURES*

Animal	*Tissue*	*Temperature in °C*	*Rate in mm/24 h*	*References*
Rana palustris, embryo	Walls of the neural tube, primordia of cranial ganglia		0.37–1.34	Harrison, 1910
Chick embryo	Various parts of the nervous system	38	0.56	Levi, 1934
Chick embryo, 7 days incubation	Mesencephalon	26	0.06	Mossa, 1927
	Mesencephalon	37	0.20	
	Mesencephalon	38	0.43	
	Mesencephalon	39	0.79	
	Mesencephalon	41	0.34	
Chick embryo, 8–12 days incubation	Spinal ganglia	37	0.41	Nakai, 1956
	Dissociated neurons from spinal ganglia	37	0.16	
Chick embryo, 7–12 days incubation	Lumbar ganglion	—	1.2	Hughes, 1953
	Midbrain	—	0.62–1.01	

* Most of these observations were made on regenerating fibres or on mixtures of growing and regenerating fibres. The initial outgrowth from neuroblasts is seen only in Harrison's experiments, where parts of the neural tube were explanted before differentiation. All observers stress the discontinuous character of elongation, intervening periods of rest and variable velocities at various moments.

growth cones predominates. Speidel stresses the close resemblance of behaviour of nerve fibres in living tadpoles and in tissue cultures.

In cultures *in vitro* the amoeboid activity of the ends of growing nerve fibres, involving incessant changes of shape and formation and disappearance of branches was

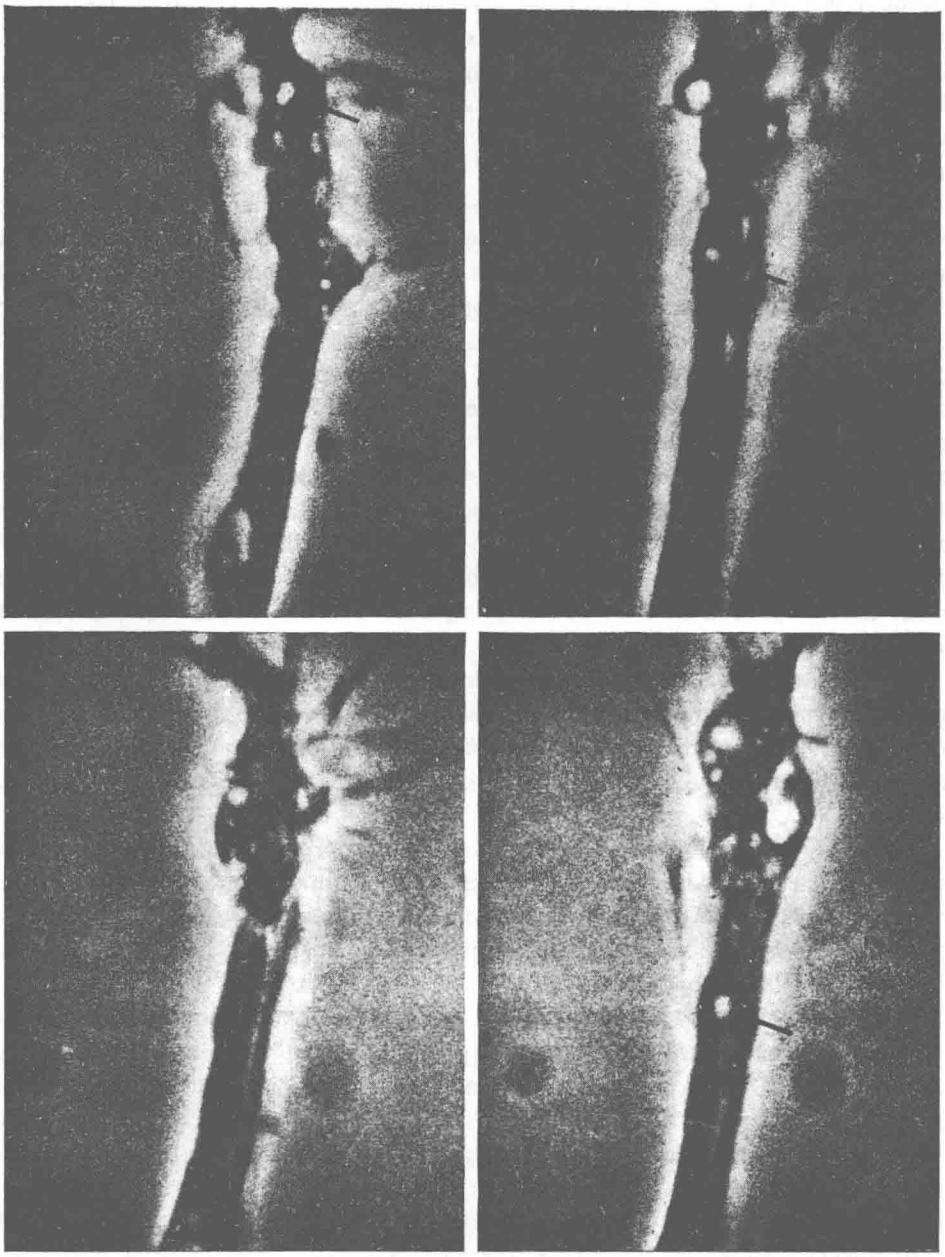

Fig. 1. Terminal part of an axon in tissue culture (from the dorsal root ganglion of an 8-day-old chick embryo). Vacuole taken in at the tip migrates in the axon in cellulipetal direction. Time intervals indicated in min. (From a film of C. M. Pomerat kindly supplied by the author.)

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first described by Harrison (1910) and was seen by all later workers. The advance of growing tips in culture is also variable and intermittent. Average rates are shown in Table III.

Bidirectional migration of mitochondria and other granules along the axons and ascent of droplets (Fig. 1) pinocytozed at the endings was seen in cultures from various parts of the nervous system of various animals (Matsumoto, 1920; Hild, 1954; Hughes, 1953; Hayden *et al.*, 1954; Nakai, 1956; Geiger, 1958; Rhines, 1959, and many others). This incessant movement of particles up and down the axons in tissue cultures is conspicuous in the beautiful films of Pomerat (1960) and Nakai (1956), unfortunately irreproducible here.

The bidirectional pattern of streaming is not a particular characteristic of growing axons. It was seen in many types of elongated cells or cell processes in plants, protozoa and cultures *in vitro* of metazoan tissues. For example, Jahn and Rinaldi (1959) describe cytoplasmic streaming in reticulopods of a Foraminiferan, *Allogromia*. The configuration and dimensions of these thin processes, a few microns in diameter and up to 15 mm in length, resemble those of axons. In every pseudopod the streaming occurs always simultaneously in both directions. In radial reticulopods one stream goes toward the body and the other away from the body. In reticulopods that form cross-connections of the reticulum, each stream goes in the direction opposite from the other. In thin pseudopods all of the visible granules are streaming. The granules can be traced individually as they move to the tip of the filament and then turn 180° around the tip and start back toward the base of the filament. In larger pseudopodia many narrow pathways of the stream lying side by side are observed. The rate of streaming under conditions of observation was about 8–15 μ/sec.

A very wide range of rates of streaming was observed in various materials. In plant cells the velocities are of several microns per second in root hair and pollen tubes, 40–80 μ/sec in the internodes of *Characeae* and over a millimetre per second in plasmodia (Kamiya, 1959). In animal cells the streaming is usually considerably slower.

The streaming is influenced by a great variety of external and metabolic factors. It may be arrested for a while by mechanical, electrical or optical stimulation and resumed shortly afterwards. In *Characeae* the cessation is correlated with the occurrence of action potentials. The vast experimental material obtained on plant cells is analyzed in Kamiya's (1959) monograph.

In regenerating nerve fibres in culture two types of axoplasmic motion may be detected: a bulk advance of axoplasm in the proximo-distal direction and a bidirectional streaming in the axons as visualized by movements of axoplasmic inclusions. What is the relation between these phenomena? The answer to this question is suggested by the study of velocities of both processes. In a few cases the rate of axoplasmic streaming and that of elongation of fibres, both very variable, were measured under the same experimental conditions. The velocity of streaming was found to be 8–40 times higher than that of elongation. These results are shown in Table IV which also includes data calculated from other types of experiments showing similar ratios of velocities of both processes.