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REVIEW

NEUROTROPHIC FACTORS AND REGENERATION IN THE PERIPHERAL NERVOUS SYSTEM*

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INTERRUPTION of axonal continuity is followed by a series of changes in the distal and proximal parts of the involved nerve fibers and in their cell bodies. These changes may lead to axonal regeneration and reinnervation or alternatively may end in neuronal death. Cajal (1928) was the first to suggest that regeneration of nerve fibers might be influenced by neurotrophic factors. The discovery of nerve growth factor (NGF) and the results of subsequent studies on this substance gave support to this concept. The research in this field developed along two entirely different lines. Firstly, the existence of factors, released *in vitro* or *in vivo* by muscle, Schwann or glial cells, that promote neuronal survival and neurite extension, was investigated. The existence of these factors (NGF and others) was confirmed, and NGF was purified. Secondly, other investigators examined proteins and polypeptides of endocrine origin and a number of other biochemically well-defined substances for possible neurotrophic effects.

The purpose of this paper is to review recently acquired knowledge about some of these neurotrophic factors. We shall confine ourselves mainly to effects on axonal regeneration in the peripheral nervous system. By way of introduction, a short outline of the histological and biochemical aspects of the events caused by axonal injury will be presented.

DEGENERATION AND REGENERATION

Degeneration

Waller (1850) was the first to study, by light microscopy, the changes in the nerve fibers distal to a nerve cut. Since then, the so-called Wallerian degeneration has been one of the most intensively studied experimental models of nerve lesions. It is generally accepted that the degenerative changes take place simultaneously along the length of the fibers, although a centrifugal course of events has been suggested (Lubinska, 1977; Sunderland, 1978).

The retrograde degenerative effects after crushing peripheral nerves are usually limited to the immediate neighbourhood of the lesion. The lesioned internode survives if injured

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in its distal half, but perishes if injured proximal to the nucleated region of the Schwann cell, which is essential for the survival of the myelin of the internode. The histological changes (chromatolysis) in nerve cell bodies were first described by Nissl (1892); this subject has been reviewed by Lieberman (1971), Sunderland (1978), Grafstein and McQuarrie (1978) and Selzer (1980). The rate and degree of the retrograde axon reaction depends on (1) neuronal type, (2) the distance between the lesion and the cell body, (3) the animal species, and (4) the age of the animal. Changes in the cell body occur within 24 hours following nerve injury. During chromatolysis there is a loosening of synaptic contacts and a widening of synaptic gaps. A reduction in the number of synaptic contacts on chromatolytic neurons has been reported for sympathetic neurons and motor neurons (Sunderland, 1978).

Regeneration

Experimental investigation of nerve fiber regeneration originated many decades ago. Current knowledge of the morphology of regeneration was summarized in reviews by Sunderland (1978) and Selzer (1980). Ramon y Cajal (1928) was a pioneer in this field, using silver-stained sections from peripheral nerves of several species. Following axon injury, sprouting occurs from the cut axon and from the nodes of Ranvier up to several segments proximal. The earliest sprouts develop after about six hours. The tips of the sprouts swell into growth cones. Forward growth occurs by advancement of the tip. The rate of regeneration decreases as the distance from the cell body increases. Regeneration is slower in old animals than in young. The age differences have been suggested to be related to a decrease in the rate of slow axonal transport.

Ramon y Cajal (1928) also stated that when the lesion is less disruptive the rate of regeneration is greater. This has been confirmed in later studies. Young (1942), for instance, showed that regeneration is facilitated by the presence of an intact endoneurial tube to guide the advancing axon. When the nerve is crushed, the endoneurial tube may be spared, and regeneration of each axon occurs within its original endoneurial tube. Generally, only one of the sprouts formed at the axon tip survives and regenerates within the endoneurial tube (Denny-Brown & Brenner, 1944). If the endoneurial tube is disrupted, as in nerve section, numerous sprouts grow out from each severed axon, but only a few successfully traverse the scar region and enter an endoneurial tube in the distal stump and regenerate. Therefore, in case of nerve section, the chances of effective nerve regeneration will be greatly enhanced if the regeneration fibers are able to find their way into the vacated endoneurial tubes of the distal stumps. In clinical practice it often proves necessary to minimize the gap by surgical repair techniques, such as end-to-end anastomosis or, when there is a large gap between the two sites, by end-to-end sutures using an autogenous nerve graft (Sunderland, 1978). In experimental studies a silicone tube has been applied between proximal and distal nerve parts, with favourable results (Lundborg *et al.,* 1982). It is clear that the most rapid and complete regeneration occurs after nerve crush, that is, when the endoneurium is left intact. The regenerating axons will contact the Schwann cells in the bands of Büngner. Whether or not the axons become myelinated depends on the type of signal produced by the axon, since the still undifferentiated Schwann cells are bipotential (Bray *et al.,* 1981). Effective regeneration

of the interrupted axons implies axon elongation, axon ensheathment, increase of axon caliber and re-establishment of terminal contacts. The diameters of the regenerating axons increase only slowly and seldom reach the original diameter, even two years after nerve injury. The myelin sheath also remains relatively thin (Schröder, 1972).

Though initially interpreted as wholly degenerative, it is now clear that in many ways the retrograde axonal reaction serves to prepare the cell body for the metabolic requirements of axonal regeneration. The subject has been reviewed by Grafstein & McQuarrie (1978).

In view of the increased requirements for structural materials that may be assumed to accompany axonal replacement and the increase in RNA metabolism, an increase in protein synthesis following nerve injury would be expected. Indeed, many studies have shown an increased incorporation of radioactive amino acids into protein during axonal regeneration (summarized by Grafstein & McQuarrie, 1978). Hall *et al.* (1978) studied amino acid incorporation in sympathetic ganglia, of which the postganglionic nerves had been cut, and detected changes in incorporation in only about 10% of the 300–400 different proteins that could be recognized after two-dimensional gel electrophoresis. Most of those that had changed showed increases in incorporation, but others showed decreases. The authors proposed a shift in protein synthetic activity following nerve injury from functional proteins (associated with synaptic transmitter metabolism) to structural proteins (cytoskeletal related proteins, like tubulin, actin and neurofilament proteins). Recently, more evidence has been presented to substantiate this hypothesis (Heacock & Agranoff, 1976; Giulian *et al.,* 1980; Benowitz *et al.,* 1981; Skene & Willard, 1981).

The protein-synthesizing machinery is located in the soma of the neurons, since the neurites are devoid of rough-surfaced endoplasmic reticulum and free polyribosomes. Survival of axons in general, as well as axons during regeneration, requires an intact transport system. Whether changes in axonal transport contribute to the regenerating processes is not clear. At least in goldfish optic nerve, there is a marked increase in the rate of axonal transport following nerve section (Grafstein & Murray, 1969; Grafstein & McQuarrie, 1978). However, in mammalian peripheral nerve, the rate of fast transport seems unchanged by axotomy (Ochs, 1976; Bisby, 1978) and it is not clear whether differences in the volume of fast transport occur (Griffin *et al.,* 1976; Ochs, 1976; Bisby, 1978). The situation regarding slow anterograde transport (Lasek & Hoffman, 1976), as well as the effects of nerve injury on retrograde transport, are equally unclear (Halperin & LaVail, 1975; Frizell *et al.,* 1976).

Conditioning lesion

The changes in molecular mechanisms involved in nerve regeneration appear within a few days following injury. Therefore it was suggested that "priming" in a distal part of the neurons would shorten the delay period following a more proximal lesion and enhance the outgrowth rate of the regenerating neurons. Indeed, several investigators demonstrated beneficial effects of a distal, preceding lesion (priming or conditioning lesion) on the rate of regeneration studied after a subsequent, more proximal lesion. McQuarrie *et al.* (1977) used the pinch test to locate the fastest growing sensory axons in the rat sciatic nerve and showed an increase of 23% in the outgrowth rate. In later studies

a stimulatory influence of a conditioning lesion 14 days earlier on the outgrowth of motor axons was measured by axonal transport methods, employing micro-injection of tritiated proline into the spinal cord ventral horns (McQuarrie, 1978). However, histofluorescence studies of norepinephrine-containing axons revealed that a conditioning lesion 14 days previously slowed the outgrowth rate of the adrenergic axons by 50%, though the initial delay was shortened (McQuarrie *et al.*, 1978). Using the toe-spreading test, Sebille & Bondoux-Jahan (1980a) were not able to show an enhanced recovery rate 14 days following a conditioning lesion in the sciatic nerve. The time course of the conditioning lesion effect on axonal regeneration was studied by Forman *et al.* (1980), using the pinch test. Conditioning intervals as short as two days or as long as 28 days resulted in accelerated outgrowth.

On the basis of these results, it might be suggested that some of the more prominent morphological and metabolic changes occurring in the cell body response to axotomy, which take more than a week to develop maximally (Grafstein & McQuarrie, 1978), do not underly the conditioning lesion effect. The beneficial effect could be the result of a change in the metabolism of the nerve cell body, e.g. increased RNA metabolism and changes in protein synthetic activity. In addition, control experiments showed that the effect was not part of a systematic response to surgery or Schwann cell proliferation (McQuarrie *et al.*, 1977).

It is clear that the main aim of the studies on the conditioning lesions has been a better understanding of both the retrograde reaction and the regenerative reaction. The question of whether the conditioning lesion stimulates regeneration has not been answered unambiguously. More advanced techniques than, for example, the classical pinch test, will be necessary to elucidate this problem.

Nerve growth factor

Nerve growth factor (NGF) was discovered when mouse sarcoma tissue was transplanted into chick embryos. The transplants caused a marked increase in the size of spinal sensory and sympathetic ganglia (reviewed by Levi-Montalcini & Angeletti, 1968). It appeared that the sarcoma produced a humoral factor (NGF) that consisted of some closely related proteins. NGF's biological activity resides in the so-called ß-subunit, which at physiological pH is part of a large zinc-containing complex with a molecular weight of about 140,000 daltons and sedimentation coefficient of 7S. This 7S complex also contains the α- and γ-subunits. The activity of the β-subunit (consisting of two identical 118 residue chains with a MW of 13,250 daltons) is inhibited in this complex (Harper & Thoenen, 1980).

NGF is a naturally occurring protein in all vertebrates, and NGF is required for the survival of its target neurons. Two particularly rich sources of NGF have been discovered: snake venom and the mouse salivary gland. Rat submandibular gland also provides a supply of NGF to the sympathetic neurons that innervate it (Hendry, 1975). It is suggested that Schwann cells and glia may act as sources of NGF and possible other neurotrophic factors (Varon & Bunge, 1978).

The generally recognized target tissues for NGF are the sympathetic ganglia and the embryonic dorsal root ganglia. For example, rats and guinea pigs, when immunized with

mouse nerve growth factor, produce antibodies that cross-react with their own nerve growth factor. The antibodies reach developing offspring of these animals both prenatally (rats and guinea pigs) and postnatally (rats). Depriving the fetus of nerve growth factor in this way results in the destruction of up to 85 percent of dorsal root ganglion neurons as well as destruction of sympathetic neurons (Johnson *et al.,* 1980).

NGF influences the development, maturation and maintenance of the sympathetic neurons at all stages of development and of the embryonic sensory ganglion cells. Other NGF targets have been proposed, but so far the action of NGF seems to be limited, at least in mammals, to the originally described target areas (Varon & Bunge, 1978). NGF is probably taken up at the nerve endings and transported in retrograde fashion. An impetus for investigations of the effect of NGF on regeneration was the observation that axonal outgrowth of post-ganglionic sympathetic neurons and immature dorsal root ganglion cells can be accelerated by NGF treatment (Levi-Montalcini & Angeletti, 1968). This effect of NGF can be attributed at least partly to an enhancement of protein synthesis by the nerve cell body. For example, NGF treatment caused increased cell body incorporation of labelled precursors into RNA and proteins (Angeletti *et al.,* 1965). Levi-Montalcini *et al.* (1968) described a dramatic accumulation of neurofibrillar material in dorsal root ganglion cells incubated *in vitro* with NGF. If one considers the role of microtubules in axoplasmic transport, and the importance of axoplasmic transport in sustaining nerve growth, this action of NGF would seem to be quite relevant to its role in promoting axon outgrowth. Changes in axonal transport by NGF were reported by Almon & McClure (1974), suggesting a selective effect of NGF, namely the rate of the faster phase of the transport was increased, whereas the rate of the slower phase was decreased by NGF. However, the role of NGF is not limited to a stimulation of protein synthesis and the induction of neurite outgrowth. NGF has been shown to induce an increase in the activities of some enzymes in the catecholamine synthetic pathways: tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) (Thoenen *et al.,* 1971). The requirement of NGF for the survival of its target neurons was clearly demonstrated by the observation that interruption of peripheral signals to a sympathetic neuron, either by axotomy or colchicine application, resulted in chromatolysis (Purves, 1975, 1976). The accompanying loss of pre-ganglionic synaptic contacts could be partially prevented by topical administration of NGF to the axotomized ganglion. These changes have been interpreted as indicating that a trophic influence from the periphery is mediated by an axonally transported supply of a NGF-like substance (Varon & Bunge, 1978).

Although NGF has been reported to partially block chromatolysis and synaptic disconnection of sympathetic ganglia after nerve damage, local NGF treatment did not reverse the changes in relative rates of synthesis of several proteins observed after nerve damage (Hall & Wilson, 1982). According to these authors, the absence of NGF in retrograde transport from target tissues cannot be the only signal for chromatolysis.

The possibility that the effects of NGF are mediated by a surface membrane effect, e.g. the stimulation of adenosine-3,5′-monophosphate (cAMP), has been investigated. Frazier *et al.* (1973) showed that the responses of chick embryonic sensory ganglia to NGF could not be mimicked by dibutyryl-cAMP, a cAMP analog that permeates the membrane, ruling out the possibility that cAMP acts as a second messenger in these

morphological responses. Also, Harper & Thoenen (1980) stated that the action of NGF on neurite extension and TH and DBH was not mediated by cyclic nucleotides. However, Garrels & Schubert (1979) showed that NGF caused quantitative modulation of protein synthesis in rat pheochromocytoma cells (PC12), and the responses were mediated by cAMP.

The observation that NGF is selectively transported by axons of sympathetic neurons (Hendry *et al.*, 1974) and dorsal root ganglia cells (Stöckel *et al.*, 1975), but not by motoneurons (Stöckel & Thoenen, 1975), makes it possible that many of the effects of NGF are presumably mediated by this selective retrograde transport system and subsequent intracellular actions. From these data it may be concluded that NGF will not play an important role in the clinical problems of regenerating peripheral motor nerve fibers.

Vitamin B

Vitamins are indispensable to the normal metabolic activity of the nervous system. Deficiency of the vitamin B complex gives rise to a series of neurological disorders. The important role of the vitamin B complex in neuronal metabolism inspired two groups of investigators to study the effect of vitamin B treatment on peripheral nerve regeneration.

In an electromyographical study, Yamatsu *et al.* (1976) were able to show that daily treatment with methylcobalamine (vitamin B12) during regeneration inhibited Wallerian degeneration and facilitated neural regeneration. Hasegawa *et al.* (1978) observed a significant stimulation of functional recovery by vitamin B treatment, as measured by the return of toe-spreading. Administration of the whole vitamin B complex gave the best results, though treatment with vitamin B1 or B12 alone also was able to stimulate the functional recovery.

The classical treatment of neurological disorders with the vitamin B complex is based on the important role of the complex in normal neurological functioning. Though sufficient amounts of vitamin B are necessary for successful regeneration processes, there is insufficient evidence to indicate vitamin B is a stimulatory agent on nerve regeneration.

Adenosine-3-5'-monophosphate

cAMP is viewed as a "second messenger" that translates extracellular messages into an intracellular response. Recent evidence suggests that cAMP also may regulate certain other neuronal functions, such as neurotransmitter synthesis and release, intracellular movements, carbohydrate metabolism and trophic and developmental processes. db-cAMP is a cAMP analog which is able to enter the cell and produce the same effects intracellularly as the second messenger cAMP, which is stimulated after hormone – receptor interaction. The trophic influence of cAMP on nerve development prompted several investigators in the last decade to study the influence of db-cAMP on regeneration processes.

There have been conflicting reports on the effect of db-cAMP on peripheral nerve regeneration *in vivo*. Pichichero *et al.* (1973) showed that the return of sensorimotor function was stimulated in crushed or hemisected rat sciatic nerves following intramuscular injections of db-cAMP. In contrast, McQuarrie *et al.* (1977) used the pinch test and were not able to detect changes in the rate of axonal growth in the sciatic nerve of

rats treated with db-cAMP. Also, no enhanced axonal elongation was observed by Black & Lasek (1979) following db-cAMP treatment, as studied by axonal transport. Gershenbaum & Roisen (1980) resolved these apparent discrepancies by showing that the results obtained depended on the test system used and that under appropriate conditions, for example the foot-flick test, db-cAMP did indeed enhance regeneration. Furthermore, they showed in a scanning electron microscopic study that the nucleotide stimulated degenerative processes, thereby removing obstructive myelin debris more rapidly and facilitating axonal regrowth.

The mechanisms through which the nucleotide acts to enhance the process of nerve degeneration and regeneration are still unclear. It is believed that cAMP stimulates the proliferation of Schwann cells (Appenzeller & Palmer, 1972). Additionally, Roisen *et al.* (1972) reported an important role of cAMP in microtubule assembly, thereby enhancing neurite elongation *in vitro*. Another site of action has been suggested by Carlsen (1982a), who proposed a local influence on nerve growth *in vivo* by cAMP, since adenylate cyclase activity is axonally transported and accumulates proximal to a nerve crush or transection. Moreover, a two-fold increase in enzyme activity in the proximal nerve stump occurs as regeneration begins and is sustained as nerve growth appears. The resulting increase in cAMP concentration in the regenerating nerve stump would stimulate the regeneration. In a further study, using a conditioning lesion seven days before the testing lesion in the rat sciatic nerve, Carlsen (1982b) suggested that adenylate cyclase activity turns around at the site of the conditioning lesion and accumulates distal to the test lesion, where it may enhance the development of regenerative sprouts.

Whether or not cAMP exerts its effects in neuronal cells by facilitation of microtubule assembly or by an action as a second messenger remains unclear at the moment. It already has been suggested that the actions of NGF and triiodothyronine are mediated by cAMP (Dumont *et al.*, 1971; Roisen *et al.*, 1972). It is conceivable that other humoral factors with receptors on neurons are able to stimulate regeneration processes via cAMP.

Gangliosides

Gangliosides are defined as sphingoglycolipids and are present in neuronal cell membranes. There is an increasing body of evidence to support the idea that CNS and PNS gangliosides may play an important functional role in various nervous tissues. There is good biochemical evidence that gangliosides are highly concentrated in the nerve terminals, mostly in association with the nerve terminal plasma membrane (Burton, 1976; DeRobertis *et al.*, 1976; Morgan *et al.*, 1976); exogenous gangliosides can be integrated into surface membranes after intravenous injection in the rat (Gorio *et al.*, 1980).

Ceccarelli *et al.* (1976) studied the effect of gangliosides on regenerating cholinergic and adrenergic nerve fibers. In an electrophysiological study they showed that treatment with brain gangliosides stimulated the regeneration and reinnervation process in both types of fibers. On the other hand, gangliosides did not influence the rate of regeneration of the fastest growing sensory axons, as measured by the pinch test (Sparrow & Graftstein, 1982; Verghese *et al.*, 1982). Sparrow & Grafstein (1982) were not able to detect changes in axonal transport or in the mean outgrowth distances, but they observed a significant increase in the number of regenerating axons following ganglioside treatment. In

addition, gangliosides have been shown to stimulate axonal sprouting in cultured dorsal root ganglia (Roisen *et al.*, 1981).

Neurophysiological experiments performed by Caccia *et al.* (1979) showed little effect of ganglioside treatment on the recovery of motor conduction velocity following nerve crush, but pointed to a synaptic effect. This was expressed by facilitating transduction of information at the neuromuscular junctions, produced by different stimulus intensities. Facilitation of excitability by ganglioside treatment during the process of reinnervation also was shown by Norido *et al.* (1981). Ganglioside treatment following sciatic nerve transection resulted in an earlier reoccurrence of polyphasic potentials in the gastrocnemius muscle than was seen following placebo treatment (Kleinebeckel, 1982).

It is clear from these data that ganglioside treatment accelerates regeneration processes. Evidence has been presented clearly indicating a beneficial effect of ganglioside treatment on the functioning of neuromuscular junctions (Ceccarelli *et al.*, 1976; Caccia *et al.*, 1979). In view of the fact that gangliosides must be considered as structural components of neural membranes and nerve terminals, the most probable mechanism of action of the gangliosides is on the growth cone, resulting in more sprouts and/or the formation and/or "maturation" of the neuromuscular junction. Axonal elongation does not seem to be influenced by ganglioside treatment.

Thyroid hormones

The thyroid hormones thyroxine (T4) and triiodothyronine (T3) are the natural secretory products of the thyroid gland. These hormones regulate numerous metabolic processes during development and post-natal life. The activity of the thyroid gland is greatly influenced by the thyrotropic hormone (TSH) of the anterior lobe of the pituitary, as part of a negative feedback mechanism. A proper functioning of the thyroid gland is necessary for the normal development of the nervous system in fetal and neonatal mammals (Sokoloff, 1971). It has been reported that in adult animals axonal regeneration is retarded in hypothyroidism (Marinesco & Minea, 1910; Talman, 1979), but can be restored to its normal rate by replacement therapy with T4 (Isenschmid, 1932). These observations formed the basis for further studies on the role of thyroid hormones in regenerative processes.

Experimental hyperthyroidism results in accelerated functional recovery from peripheral nerve injuries (McIsaac & Kiernan, 1975a; McQuarrie, 1975). Cockett & Kiernan (1973) studied longitudinal sections of silver-stained peripheral nerves and reported that exogenous T3 significantly increased the rate of axonal elongation distal to a crush lesion of the sciatic nerve of the rat, whereas McIsaac & Kiernan (1975b) showed in histological experiments that T3 accelerated neuromuscular reinnervation. Also, Yu & Srinivasan (1981) demonstrated enhanced axonal growth by low doses of T4 in rat hypoglossal nerve (motor nerve) following nerve section.

On the other hand, other experiments have failed to demonstrate accelerated axonal regeneration in T3-treated animals (Stelmack & Kiernan, 1977; Forman & Berenberg, 1978; Cotrufo *et al.*, 1979). According to Berenberg *et al.* (1977) treatment with exogenous T3 did not stimulate the regeneration rate of all types of axons. They showed that the recovery of motor function, as indicated by the return of toe-spreading, was

stimulated by T3 treatment; on the other hand no beneficial effect could be observed on the most rapidly growing sensory axons, as measured by the pinch test. The authors suggested that the accelerated recovery of motor function was mediated by an effect on the "maturation" of the regenerated axon rather than on axonal regrowth. Several possible mechanisms of action of the thyroid hormones have been proposed. They could stimulate the intracellular receptors or the intracellular concentration of cAMP by membrane–receptor interaction. The activated intracellular receptors or the increased level of cAMP then could stimulate metabolic processes including protein synthesis in the neurons. Cook & Kiernan (1976) suggested that the injured neurons in adult animals revert to an immature metabolic state during regeneration and become again responsive to thyroid hormones, resulting in an increased protein synthesis. Stelmack & Kiernan (1977) concluded from their study on regeneration of the rat facial nerve that more axolemmal components than axoplasmic proteins were synthesized following T3 treatment. Furthermore, Schwann cells were activated by the thyroid hormone, so that myelination was stimulated in both normal and regenerating fibers.

Improvement of neurologic recovery by thyrotropin releasing hormone (TRH) has been reported after spinal trauma in cats (Faden *et al.*, 1981). The authors did not mention the above described effects of T3 or T4 on axonal regeneration, but suggested a completely different mechanism of action. Following spinal injury, endogenous opioids (endorphins) would be released, which would cause a reduction in spinal cord blood flow, and thereby post-traumatic ischemia. Treatment with an opiate antagonist, naloxone, improves functional neurologic recovery. However, naloxone also blocks the analgesic action of the endogenous opioids, making the post-traumic pain even more severe. Holaday *et al.* (1978) proposed TRH to act *in vivo* as a partial physiologic opiate antagonist. However, TRH does not act at the opiate receptor and does not antagonize the analgesic effects of endogenous opioids or exogeneous opiates (Holaday *et al.*, 1978). Thus, the improved neurologic recovery by TRH after spinal trauma in cats, mediated by prevention of post-traumatic ischemia and its lack of effect on nociception, would indicate an unique therapeutic potential in spinal trauma in human beings (Faden *et al.*, 1981). In sum, the different proposed mechanisms of action of thyroid hormones on regeneration processes and the several negative findings make it difficult to judge their beneficial effect on regeneration processes. More experiments are needed to elucidate this issue.

Testosterone

It is known that steroid hormones act on their target tissues through a series of steps, resulting in increased RNA and protein synthesis. Motor neurons of cranial nerves and the spinal cord appear to be able to respond to androgens in a manner similar to other steroid target tissues (Sar & Stumpf, 1977). Therefore, the anabolic effect of steroid hormones has been studied on axonal outgrowth during regeneration of the hypoglossal nerve in rats. It was demonstrated by the HRP technique that administration of testosterone after nerve transection accelerated axonal outgrowth two weeks after surgery in adult and female rats (Yu & Srinivasan, 1981; Yu, 1982). Although testosterone might accelerate protein synthesis in the hypoglossal neurons (Yu, 1982), the site of action of testosterone may not be restricted to the neuronal cell body, but also may be at the site of injury. The literature concerning the influence of sex hormones on wound healing is large.

Testosterone treatment appeared to affect an alignment of cells and fibers in a more or less parallel fashion that reduced the amount of obstacle confronting the regeneration axons (Yu, 1982). This facilitation of passage across the site of injury enhances the process of regeneration.

Miscellaneous factors

A newly synthesized drug, isaxonine (N-isopropyl-amino-2-pyrimidine orthophosphate), has been shown to promote neurite outgrowth in cultured spinal ganglion of the mouse (Hugelin *et al.*, 1977). This drug appeared to be effective in nerve regeneration (Hugelin *et al.*, 1979). The authors reported that i.p. injection of isaxonine in the rat significantly increased the length of the most rapidly regenerating fibers. The effect was dose-dependent, and both sensory and motor functions returned earlier in treated animals. The cellular mechanisms responsible for the effect of isaxonine have not yet been elucidated.

A single injection of cyclophosphamide (24 hours after axotomy of the sciatic nerve in rats) was stated to accelerate the recovery rate of motor function by 62%, as tested by the return of toe-spreading reflex. Cyclophosphamide could exert its effect by a decrease in protein synthesis in cells involved in the immune response. Although the timing of the cyclophosphamide injection is compatible with the idea that the drug works within its period of immuno-suppressive activity (Paterson & Hanson, 1969), it is not possible to confirm that the motor recovery rate increase is due to this action (Sebille & Bondoux-Jahan, 1980b). Another possible mechanism of action of cyclophosphamide is the enhancement of cAMP content of the cell bodies, by inhibition of phosphodiesterase (Tisdale & Philips, 1975). The supposed increase in protein synthesis might result in an enhancement of axonal outgrowth. The acceleration of motor function return rate also could be due to such an activity (Sebille & Bondoux-Jahan, 1980b).

Polyamines, e.g. spermine, exert an essential regulation of cell growth, and their possible activity on peripheral axotomized neurons has been investigated. Daily treatment with spermine increased motor function by 137% (Sebille & Bondoux-Jahan, 1980b), as a result of an intensive increase in protein synthesis in the axomized neuron (Tabor & Tabor, 1964). On the other hand, polyamines also increase the uptake of extra materials by the axon (Itaya *et al.*, 1978) and enhance the axoplasmic flow of proteins (May & Oh, 1977). Such an activity could accelerate the uptake of trophic factors which trigger or enhance elongation of the severed axon and could increase the supply of newly synthesized proteins to the distal part of the regenerating axon.

Adrenocorticotropic hormone (ACTH)

ACTH is a polypeptide of 39 amino acids and is released from the distal part of the adenohypophysis. The amino acid sequence of the first 24 residues is similar in several species, whereas the composition of the C-terminal part of the molecule is slightly different. The classical endocrine effect of ACTH is the stimulation of the adrenal cortex to produce steroids. ACTH enhances the synthesis of glucocorticoids, probably by an interaction with cAMP (Li & Oelofsen, 1967). Furthermore, ACTH has a lipid mobilizing effect (adipokinesis) *in vivo* as well as *in vitro* (Li & Oelofsen, 1967). A third target for circulating or locally produced ACTH is the central and peripheral nervous system.

ACTH and small ACTH fragments have been shown to play an important role in the adaptation of the organism to environmental stimuli (De Wied, 1969). Since no endocrine or peripheral metabolic activities of the small ACTH analogs have been found, it is obvious that the influence of ACTH on behavior is due to an extra-adrenal effect. ACTH could exert a direct effect on central and peripheral nervous tissue (Gispen, 1980). The observed effects of ACTH-like peptides on the acquisition of conditioned avoidance behavior were paralleled by changes in RNA metabolism (Schotman *et al.*, 1972). In later studies, numerous effects of ACTH-like peptides on RNA metabolism and protein synthesis have been described (reviewed by Dunn & Schotman, 1981).

Long before nervous tissue was regarded as a target for ACTH-like peptides, the stimulatory effect of ACTH on the adrenal cortex, i.e. the production of glucocorticoids, was used in regeneration studies. ACTH acts as a useful immuno-suppressive and anti-inflammatory drug, effective in reducing both cellular and humoral immune responses and in retarding the formation of scar tissue (Weissman & Thomas, 1964; Scothorne, 1966).

The early studies on the effect of ACTH on CNS lesions have been reviewed by Berry *et al.* (1979). It appeared that, although the first results were promising, in later quantitative studies scar formation was indeed depressed in ACTH-treated animals, but no increase in CNS axon growth was observed. It also was demonstrated that the systemic humoral and cellular immune responses were significantly depressed by ACTH treatment, without an increase in CNS axon growth following brain damage (Berry *et al.*, 1979). Therefore, it must be concluded that the "barrier" and "immuno" hypothesis should be rejected.

ACTH also has been studied in regeneration processes because of its beneficial effects on immature rat skeletal muscle, on adaptation and on neural protein synthesis (Strand & Kung, 1980). These investigators showed that ACTH treatment following sciatic nerve crush in rats resulted in a more rapid recovery of functional movement and sensitivity, as measured in the foot-flick and toe-spreading tests. ACTH treatment also resulted in a more rapid growth of regenerating axons, an increased number of large end plates and an enhanced frequency of preterminal branching in the end plates. Since no effect of ACTH treatment could be observed on muscle protein synthesis, Strand & Kung suggested an action of the neuropeptide on the central motoneuron pool; ACTH could modulate the electrophysiological parameters of the perikarya and the synthesis and transport of neurotrophic and neurotransmitter substances to muscle.

The beneficial influence of ACTH on nerve regeneration also has been shown in terms of its effects on improving the functional efficiency of the regenerating motor unit. Electrophysiological studies indicate that ACTH (1 – 39) (in intact and adrenalectomized rats) has a specific effect on the reorganization of motor units during peripheral nerve regeneration. The development of small, low threshold motor units under fine control is encouraged by peptide administration. This contrasts with the formation of large, high threshold, coarsely controlled motor units that are found in the regenerating saline-treated controls, indicating that ACTH may organize the pattern of neuronal regeneration and the synaptic contacts formed by the new nerve terminals (Saint-Come *et al.*, 1982).

Furthermore, it has been shown that the active moiety of the ACTH-peptides responsible for the stimulation of peripheral nerve regeneration resides in a non-

steroidogenic part of the molecule (Bijlsma *et al.*, 1981). Detailed structure – activity studies in the rat, using the foot-flick test, revealed that ACTH sequences as small as ACTH(4 – 10), ACTH(4 – 9), a synthetic ACTH(4 – 9) analog (Org. 2766) and ACTH(6 – 10) enhanced functional recovery to a similar degree. This points to an extra-adrenal, presumably melanotropic effect of ACTH on the regenerating axons (Bijlsma *et al.*, 1983d).

Light microscopic analysis of the sciatic and tibial nerve at different intervals following sciatic nerve crush showed that the number of regenerating myelinated nerve fibers was increased by treatment with ACTH(4 – 10). Though the number of regenerating myelinated fibers was greater at all stages of regeneration, the stimulatory effect of the ACTH(4 – 10) treatment was most pronounced in the early stage of regeneration (Bijlsma *et al.*, 1983a). In a subsequent electron microscopic study, it was demonstrated that the ACTH(4 – 10) treatment stimulated the number of regenerated and unmyelinated fibers to a similar degree. However, the increased number of the regenerating fibers was accompanied by a decrease in axon diameter. There was no effect of the peptide on myelination *per se* (Bijlsma *et al.*, 1983b). These observations are in favor of an increased number of regenerating fibers rather than an enhanced outgrowth rate. From a different series of experiments it was shown by Verghese *et al.* (1982) that ACTH treatment of rats bearing a sciatic nerve crush did not enhance the rate of outgrowth of the fastest growing sensory axons. The results obtained by the pinch test are not representative for the average rate of nerve regeneration, and Verghese *et al.* suggested that ACTH exerts its effect on a specific phase of the regeneration or reinnervation process.

Though accelerated functional recovery can be related more easily to an enhanced outgrowth rate than to the observed increased number of outgrowing fibers, one could assume that functional recovery is related to the amount of reinnervation. Another possibility is that the ACTH treatment enhances the synaptogenesis. Indeed, there is some evidence that ACTH may affect neuromuscular functioning by a direct presynaptic action (Strand & Kung, 1980).

In addition to these observations, no changes in cell-free protein synthesis could be detected in the ventral horns of the lumbar spinal cord as a consequence of the lesioning and/or peptide treatment (Bijlsma *et al.*, 1983c). However, the amounts of proteins comigrating with purified actin and α- and β-tubulin were changed following sciatic nerve crush and/or ACTH (4 – 10) treatment. The peptide increased the relative amounts of actin and α- and β-tubulin in the ventral horns of the lumbar spinal cord of rats bearing crush lesions in their sciatic nerves by 26 and 40% respectively (Bijlsma, 1983).

The following working hypothesis for the action of ACTH and related neuropeptides on peripheral nerve regeneration is therefore formulated: ACTH and ACTH-fragments affect the availability of structural elements in regenerating neurons. Increased amounts of cytoskeletal proteins allow for outgrowth of larger numbers of sprouts. This ACTH effect is expressed in all the axons together constituting a mixed peripheral nerve. The diameters of the sprouts are smaller than those in untreated animals, as the proteins responsible for increase in thickness are not affected equally as are cytoskeletal and other structural proteins. The facilitation of functional recovery by ACTH and ACTH fragments can be the result of other aspects of the regeneration process as well, e.g. synaptogenesis.

In conclusion, the beneficial effects of ACTH fragments on peripheral nerve regeneration are well documented. Treatment of patients suffering from nerve damage with ACTH-like peptides may be of clinical importance. Since the neurotrophic effects can be exerted by small peptide sequences devoid of corticotrophic and toxic activity, treatment with these small sequences could be sustained for long periods. Furthermore, the higher numbers of regenerating nerve fibers make it more likely that, in the case of a more severe lesion than the above described crush lesion, the beneficial effects of ACTH might be even more pronounced, since more regenerating fibers could cross the site of injury.

It is clear that axonal regeneration in the peripheral nervous system can be regulated by a number of agents, some of them of endocrine origin. Beneficial effects on regeneration processes have been demonstrated by cAMP, gangliosides, NGF, testosterone, thyroid hormones and especially ACTH. Stimulatory effects of other substances such as isaxonine, cyclophosphamide and polyamines have been reported; however, these need further confirmation.

The effects of these agents are not always exerted on all types of axons. There is a certain degree of selectivity. Axonal regeneration is a complex pattern of events. It is likely that the reported agents manipulate only one of the processes involved; several of the trophic substances appear to exert their stimulatory effect via cAMP as a second messenger.

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REFERENCES

ALMON, R. R. & MCCLURE, W. O. (1974) The effect of nerve growth factor upon axoplasmic transport in sympathetic neurons of the mouse. *Brain Res.* **74**, 255 – 267.

ANGELETTI, P. V., GANDINI-ATTARDI, D., TOSCHI, G., SALVI, M. L. & LEVI-MONTALCINI, R. (1965) Metabolic aspects of the effect of nerve growth factor on sympathetic and sensory ganglia: protein and ribonucleic acid synthesis. *Biochim. biophys. Acta* **95**, 111 – 120.

APPENZELLER, O. & PALMER, G. (1972) The cyclic AMP content of sciatic nerve; changes after crush. *Brain Res.* **42**, 521 – 524.

BENOWITZ, L., SHASHOUA, V. & YOON, M. G. (1981) Specific changes in rapidly transported proteins during regeneration of the goldfish optic nerve. *J. Neurosci.* **1**, 300 – 307.

BERENBERG, R. A., FORMAN, D. S., WOOD, D. K., DESILVA, A. & DEMAREE, J. (1977) Recovery of peripheral nerve function after axotomy: effect of triiodothyronine. *Exp. Neurol.* **57**, 349 – 363.

BERRY, M., KNOWLES, J., WILLES, P., RICHES, A. C., MORGANS, G. P. & STEERS, D. (1979) A reappraisal of the effects of ACTH on the response of the CNS to injury. *J. Anat.* **128**, 859 – 871.

BISBY, M. A. (1978) Fast axonal transport of labeled protein in sensory axons during regeneration. *Exp. Neurol.* **61**, 281 – 300.

BLACK, M. M. & LASEK, R. J. (1979) Slowing of the rate of axonal regeneration during growth and maturation. *Exp. Neurol.* **63**, 108 – 119.

BRAY, G. M., RASMINSKY, M. & AGAYO, A. J. (1981) Interactions between axons and their sheath cells. *Ann. Rev. Neurosci.* **4**, 127 – 162.

BURTON, R. M. (1976) Gangliosides and proteins of brain synaptic components. *Adv. exp. Med. Biol.* **71**, 123 – 136.

BIJLSMA, W. A., JENNEKENS, F. G. I., SCHOTMAN, P. & GISPEN, W. H. (1981) Effects of corticotrophin (ACTH) on recovery of sensorimotor function in the rat: structure-activity study. *Eur. J. Pharmac.* **76**, 73 – 79.

BIJLSMA, W. A. (1983) The neurotrophic action of ACTH in peripheral nerve regeneration. Ph.D. Thesis, University of Utrecht, Utrecht, The Netherlands.

BIJLSMA, W. A., JENNEKENS, F. G. I., SCHOTMAN, P. & GISPEN, W. H. (1983a) Stimulation by $ACTH_{4-10}$ of nerve fiber regeneration following sciatic nerve crush. *Muscle Nerve* **6**, 104 – 112.

BIJLSMA, W. A., VAN ASSELT, E., VELDMAN, H., JENNEKENS, F. G. I., SCHOTMAN, P. & GISPEN, W. H. (1983b) Ultrastructural study of the effect of $ACTH_{4-10}$ on nerve regeneration: outgrowing axons become larger in number and smaller in diameter *Acta Neuropath.* (Berl). **62**, 24 – 30.

BIJLSMA, W. A., SCHOTMAN, P., JENNEKENS, F. G. I. & GISPEN, W. H. (1983c) Regeneration and protein synthesis in the spinal cord: the influence of ACTH-like peptides on cell-free protein synthesis. *Neurosci. Lett.* **38**, 297 – 302.

BIJLSMA, W. A., SCHOTMAN, P., JENNEKENS, F. G. I., GISPEN, W. H. & DE WIED, D. (1983d) Enhanced recovery of sensorimotor function in rats is related to the melanotropic moiety of ACTH/MSH neuropeptides. *Eur. J. Pharmac* **92**, 231 – 236.

CACCIA, M. R., MEOLA, G., CERRI, C., FRATTOLA, G., SCARLATO, G. & APORTI, F. (1979) Treatment of denervated muscle by gangliosides. *Muscle Nerve* **2**, 382 – 389.

CAJAL, Y. R. (1928) *Degeneration and Regeneration of the Nervous System,* Vol. 1. Hafner, New York.

CARLSEN, R. C. (1982a) Axonal transport of adenylate cyclase activity in normal and axotomized frog sciatic nerve. *Brain Res.* **232**, 413 – 424.

CARLSEN, R. C., (1982b) Comparison of adenylate cyclase activity in segments of rat sciatic nerve with a condition, test or test lesion. *Exp. Neurol.* **77**, 254 – 265.

CECCARELLI, B., APORTI, F. & FINESSO, M. (1976) Effects of brain gangliosides on functional recovery in experimental regeneration and reinnervation. *Adv. exp. Med. Biol.* **71**, 275 – 293.

COCKETT, S. & KIERNAN, J. A. (1973) Acceleration of peripheral nervous regeneration in the rat by exogenous triiodothyronin. *Exp Neurol.* **39**, 389 – 394.

COOK, R. A. & KIERNAN, J. A. (1976) Effects of triiodothyronin on protein synthesis in regenerating peripheral neurons. *Exp Neurol.* **52**, 515 – 524.

COTRUFO, R., DATTOLA, R., DEODATO, M., PISARNI, F. & MESSINA, C. (1979) Experimental hyperthyroidism fails to expedite reinnervation of muscles denervated by crushing sciatic nerves in rabbits. *Exp. Neurol.* **65**, 271 – 277.

DENNY-BROWN, E. & BRENNER, C. (1944) The effect of percussion of nerve. *J. Neurol. Neurosurg. Psychiat.* **7**, 76 – 85.

DEROBERTIS, E., LAPETINO, E. G. & FISZER DEPLOZA, S. (1976) Subcellular distribution and possible role of gangliosides in the CNS. *Adv. exp. Med. Biol.* **71**, 105 – 122.

DE WIED, D. (1969) Effects of peptide hormones on behavior. In: *Frontiers in Neuroendocrinology 1969,* Ganong, W. F. & Martini, L. (Eds.), pp. 97 – 140. Oxford University Press, New York.

DUMONT, J. E., WILLEMS, C., VAN SANDE, J. & NEVE, P. (1971) Regulation of the release of thyroid hormones: role of cyclic AMP. *Ann. N. Y. Acad. Sci.* **185**, 291 – 316.

DUNN, A. J. & SCHOTMAN, P. (1981) Effects of ACTH and related peptides on cerebral RNA and protein synthesis. *Pharmac. Ther.* **12**, 353 – 372.

FADEN, A. J., JACOBS, T. P. & HOLADAY, J. W. (1981) Thyrotropin-releasing hormone improves neurologic recovery after spinal trauma in cats. *New Engl. J. Med.* **305**, 1063 – 1067.

FORMAN, D. S. & BERENBERG, R. A. (1978) Regeneration of motor axons in the rat sciatic nerve studied by labelling with axonally transported radio-active proteins. *Brain Res.* **156**, 213 – 225.

FORMAN, D. S., MCQUARRIE, I. G., LABORE, F. W., WOOD, D. K., STONE, L. S., BRADDOCK, C. H. & FUCHS, D. A. (1980). Time course of the conditioning lesion effect on axonal regeneration. *Brain Res.* **182**, 180 – 185.

FRAZIER, W. A., OHLENDORF, C. E., BOYD, L. F., ALOE, L., JOHNSON, E. M., FERRENDELLI, J. A. & BRADSHAW, R. A. (1973) Mechanisms of action of NFG and c-AMP on neurite outgrowth in embryonic chick sensory ganglia: demonstration of independent pathways of stimulation. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2448 – 2452.

FRIZELL, M., MCLEAN, W. G. & SJÖRSTRAND, J. (1976) Retrograde axonal transport of rapidly migrating labelled proteins and glycoproteins in regenerating peripheral nerves. *J. Neurochem.* **27**, 191 – 196.

GARRELS, J. I. & SCHUBERT, D. (1979) Modulation of protein sythesis by NFG. *J. biol. Chem.* **254**, 7978 – 7985.

GERSHENBAUM, M. R. & ROISEN, F. J. (1980) The effects of dibutyryl cyclic adenosine monophosphate on the degeneration and regeneration of crush lesioned rat sciatic nerve. *Neuroscience* **5**, 1565 – 1580.

GISPEN, W. H. (1980) On the neurochemical mechanism of action of ACTH. *Progr. Brain Res.* **53**, 193 – 206.

GIULIAN, D., DESRUISSEAUX, H. & COWBURN, D. (1980) Biosynthesis and intraaxonal transport of proteins during neuronal regeneration. *J. biol. Chem.* **255**, 6494 – 6501.

GORIO, A., CARMIGNOTO, G., FACCI, L. & FINESSO, M. (1980) Motor nerve sprouting induced by ganglioside treatment. Possible implications of gangliosides on neuronal growth. *Brain Res.* **197**, 236 – 241.

GRAFSTEIN, B. & MURRAY, M. (1969) Transport of protein in goldfish optic nerve during degeneration. *Exp Neurol.* **25**, 494 – 508.

GRAFSTEIN, B. & MCQUARRIE, I. G. (1978) Role of the nerve cell body in axonal regeneration. In *Neuronal Plasticity,* Cotman, C. W. (Ed.), pp. 155 – 195. Raven Press, New York.

GRIFFIN, J. W., DRACHMAN, D. B. & PRICE, D. L. (1976) Fast axonal transport in motor nerve regeneration. *J. Neurobiol.* **7**, 355 – 370.

HALL, M. E. & WILSON, D. L. (1982) Nerve growth factor effects on protein synthesis after nerve damage. *Exp Neurol.* **77**, 625 – 633.

HALL, M. E., WILSON, D. L. & STONE, G. C. (1978) Changes in synthesis of specific proteins following axotomy: detection with two dimensional gel electrophoresis. *J. Neurobiol.* **9**, 353 – 366.

HALPERIN, J. J. & LAVAIL, J. H. (1975) A study of the dynamics of retrograde transport and accumulation of horseradish-peroxidase in injured neurons. *Brain Res.* **100**, 253 – 269.

HARPER, G. P. & THOENEN, H. (1980) Nerve growth factor: biological significance, measurement and distribution. *J. Neurochem.* **34**, 5 – 16.

HASEGAWA, K., MIKUNI, N. & SAKAI, Y. (1978) Effects of a Vitamin B complex on functional recovery after nerve injury. *Folia Pharmac.* **74**, 721 – 734.

HEACOCK, A. M. & AGRANOFF, B. (1976) Enhanced labeling of a retinal protein during regeneration of optic nerve in goldfish. *Proc. natn. Acad. Sci. U.S.A.* **73**, 828 – 832.

HENDRY, I. A. (1975) The response of adrenergic neurons to axotomy and NGF. *Brain Res.* **94**, 87 – 97.

HENDRY, I. A., STÖCKEL, K., THOENEN, H. & IVERSEN, L. L. (1974) The retrograde axonal transport of nerve growth factor. *Brain Res.* **68**, 103 – 121.

HOLADAY, J. W., TSENG, L. F., LOH, H. H. & LI, C. H. (1978) Thyrotropin releasing hormone antagonizes β-endorphin hypothermia and catalepsy. *Life Sci.* **22**, 1537 – 1543.

HUGELIN, A., TARRADE, T., ISTIN, M. & COELHO, R. (1977) Accéleration de la vitesse de croissance du neurone par une nouvelle substance neurotrope: le N-isopropyl-amino-2-pyrimidine. *C.r. Acad. Sci.* **285** (Serie D.), 1339 – 1341.

HUGELIN, A., LEGRAIN, Y. & BONDOUX-JAHAN, M. (1979) Nerve growth promoting action of isaxonine in rat. *Experientia* **35**, 626.

ISENSCHMID, R. (1932) Ueber den Einfluss von Thymus und Schilddrüse auf die Nerven-Regeneration. Versuche mit Thymokressin und Thyroxin. *Schweiz. Med. Wochenschr.* **62**, 785 – 789.

ITAYA, S. K., WILLIAMS, T. H. & ENGEL, E. L. (1978) Anterograde transport of horseradish peroxidase enhanced by poly-L-ornithine. *Brain Res.* **150**, 170 – 176.

JOHNSON JR, E. M., GORIN, P. D., BRANDEIS, L. D. & PEARSON, J. (1980) Dorsal root ganglion neurons are destroyed by exposure *in utero* to maternal antibody to nerve growth factor. *Science* **210**, 916 – 918.

KLEINEBECKEL, D. (1982) Acceleration of muscle reinnervation in rats by ganglioside treatment: an electromyographic study. *Eur. J. Pharmac.* **80**, 243 – 246.

LASEK, R. J. & HOFFMAN, P. N. (1976) The neuronal cytoskeleton, axonal transport and growth. In *Cell Motility, Book C: Microtubules and Related Proteins,* Goldman, R., Pollard, T. & Rosenbaum, J. (Eds.), pp. 1021 – 1051. Cold Spring Harbor, New York.

LEVI-MONTALCINI, R., CARAMIA, F., LUSE, S. A. & ANGELETTI, P. V. (1968) *In vitro* effects of the nerve growth factor on the fine structure of the sensory nerve cells. *Brain Res.* **8**, 347 – 362.

LEVI-MONTALCINI, R. & ANGELETTI, P. V. (1968) Nerve growth factor. *Physiol. Rev.* **48**, 534 – 569.

LI, C. H. & OELOFSEN, W. (1967) *The Adrenal Cortex,* Eisenstein, A. B. (Ed.), p. 185. Churchill, London.

LIEBERMAN, A. R. (1971) The axon reaction. *Int. Rev. Neurobiol.* **14**, 49 – 124.

LUBINSKA, L. (1977) Early course of Wallerian degeneration in myelinated fibers of the rat phrenic nerve. *Brain Res.* **130**, 47 – 63.

LUNDBORG, G., GELBERMAN, R. H., LONGO, F. M., POWELL, H. C. & VARON, S. (1982) *In vivo* regeneration of cut nerves encased in silicone tubes: growth across a six millimeter gap. *J. Neuropath. exp. Neurol.* **41**, 412 – 422.

MARINESCO, G. & MINES, J. (1910) Nouvelles recherches sur l'influence qu'exerce l'ablation du corps troïde sur la dégénérescence des nerfs. *C.r. Soc. Biol.* **68**, 188 – 190.

MAY, S. T. & OH, T. H. (1977) Possible role of polyamines in the neurotrophic regulation of muscle acetylcholesterase. *Exp. Neurol.* **55**, 493 – 497.

MCISAAC, G. & KIERNAN, J. A. (1975a) Accelerated recovery from peripheral nerve injury in experimental hyperthyroidism. *Exp. Neurol.* **48**, 88 – 94.

MCISAAC, G. & KIERNAN, J. A. (1975b) Acceleration of neuromuscular re-innervation by triiodothyronin. *J. Anat.* **120**, 551 – 560.

MCQUARRIE, I. G. (1975) Nerve regeneration and thyroid hormone. *J. Neurol. Sci.* **26**, 499 – 502.

McQUARRIE, I. G. (1978) The effect of conditioning lesion on the regeneration of motor axons. *Brain Res.* **152,** 597 – 602.

McQUARRIE, I. G., GRAFSTEIN, B. & GERSHON, M. D. (1977) Axonal regeneration in rat sciatic nerve: effect of a conditioning lesion and of dbc-AMP. *Brain Res.* **132,** 443 – 453.

McQUARRIE, I. G., GRAFSTEIN, B., DREYFUSS, C. & GERSHON, M. (1978) Regeneration of adrenergic axons in rat sciatic nerve: effects of conditioning lesion. *Brain Res.* **141,** 21 – 34.

MORGAN, J. C., TETTAMANTI, G. & GOMBOS, G. (1976) Biochemical evidence on the role of gangliosides in nerve endings. *Adv. exp. Med. Biol.* **71,** 137 – 150.

NISSL, F. (1892) Ueber die Veranderungen der Ganglienzellen am Facialiskern des Kaninchens nach Ausreissung der Nerven. Alg., *Z. Psychiat.* **48,** 197 – 198.

NORIDO, F., CANELLA, R. & APORTI, F. (1981) Acceleration of nerve regeneration by gangliosides estimated by somatosensory evoked potentials (SEP). *Experientia* **37,** 301 – 302.

OCHS, S. (1976) Fast axoplasmic transport in the fibers of chromatolyzed neurons. *J. Physiol.* **255,** 249 – 261.

PATERSON, P. Y. & HANSON, M. A. (1969) Studies of cyclophosphamide suppression of experimental allergic encephalomyelitis in Wistar rats. *J. Immunol.* **103,** 795 – 803.

PICHICHERO, M., BEER, B. & CLODY, D. E. (1973) Effects of dibutyryl cyclic AMP on restoration of function of damaged sciatic nerves in rats. *Science* **182,** 724 – 725.

PURVES, D. (1975) Functional and structural changes in mammalian sympathetic neurons following interruption of their axons. *J. Physiol.* **252,** 429.

PURVES, D. (1976) Competitive and non-competitive reinnervation of mammalian sympathetic neurones by native and foreign fibers. *J. Physiol.* **261,** 453.

ROISEN, F., MURPHY, R., PICHICHERO, M. & BRADEN, W. (1972) Cyclic adenosine monophosphate stimulation of axonal elongation. *Science* **175,** 73 – 76.

ROISEN, F. J., BARTFIELD, H., NAGELE, R. & YORKE, G. (1981) Ganglioside stimulation of axonal sprouting *in vitro*. *Science* **214,** 577 – 578.

SAINT-COME, C., ACKER, G. R. & STRAND, F. (1982) Peptide influences on the development and degeneration of motor performances. *Peptides* **3,** 439 – 449.

SAR, M. & STUMPF, W. E. (1977) Androgen concentration in motor neurons of cranial nerves and spinal cord. *Science* **197,** 77 – 79.

SCHOTMAN, P., GISPEN, W. H., JANSZ, H. S. & DE WIED, D. (1972) Effects of ACTH-analogues on macromolecule metabolism in the brain of hypophysectomized rats. *Brain Res.* **46,** 347 – 362.

SCHRÖDER, J. M. (1972) Altered ratio between axon diameter and myelin thickness in regenerated nerve fibers. *Brain Res.* **45,** 49 – 65.

SCOTHORNE, R. J. (1966) The effects of cortisone acetate on the response of the regional lymph node to a skin homograft. *J. Anat.* **90,** 417 – 427.

SEBILLE, A. & BONDOUX-JAHAN, M. 1980a) Effects of electric stimulation and previous nerve injury on motor function recovery in rats. *Brain Res.* **193,** 562 – 565.

SEBILLE, A. & BONDOUX-JAHAN, M. (1980b) Motor function recovery after axotomy: enhancement by cyclophosphamide and spermine in rat. *Exp. Neurol.* **70,** 507 – 515.

SELZER, M. E. (1980) Regeneration of peripheral nerve. In *Physiology of Periral Nerve Disease,* Summer, A. J. (Ed.), pp. 358 – 431. Saunders, Philadelphia.

SKENE, J. H. P. & WILLARD, M. (1981) Changes in axonally transported proteins during axon regeneration in toad ganglion cells. *J. Cell. Biol.* **89,** 86 – 95.

SOKOLOFF, L. (1971) The action of thyroid hormones. In *Handbook of Neurochemistry,* Lajtha, A. (Ed.). Vol. 5B, pp. 525 – 549. Plenum Press, New York.

SPARROW, J. R. & GRAFSTEIN, B. (1982) Sciatic nerve regeneration in ganglioside treated rats. *Exp. Neurol.* **77,** 230 – 235.

STELMACK, B. M. & KIERNAN, J. A. (1977) Effects of triiodothyronin on the normal and regenerating facial nerve of the rat. *Acta Neuropath.* **40,** 151 – 155.

STÖCKEL, K. & THOENEN, H. (1975) Retrograde axonal transport of nerve growth factor: specificity and biological importance. *Brain Res.* **85,** 337 – 341.

STÖCKEL, K., SCHWAB, M. & THOENEN, H. (1975) Specificity of retrograde transport of nerve growth factor in sensory neurons: a biochemical and morphological study. *Brain Res.* **89,** 1 – 14.

STRAND, F. L. & KUNG, T. T. (1980) ACTH accelerates recovery of neuromuscular function following crushing of peripheral nerve. *Peptides* **1,** 135 – 138.

SUNDERLAND, S. (1978) (Ed). *Nerves and Nerve Injuries,* 2nd ed. Churchill Livingstone, London.

TABOR, H. & TABOR, C. W. (1964) Spermidine, spermine and related amines. *Pharmac. Rev.* **16,** 245 – 300.

TALMAN, P. (1979) Measurement of peripheral nerve regeneration in the rat and the effects of pyronin or hypothyroidism. *Exp. Neurol.* **65,** 535 – 541.

THOENEN, H., ANGELETTI, P. V., LEVI-MONTALCINI, R. & KETTLER, R. (1971) Selective induction by nerve growth factor of tyrosine hydroxylase and dopamine beta-hydroxylase in the rat superior cervical ganglia. *Proc. natn. Acad. Sci. U.S.A.* **68**, 1598.

TISDALE, M. J. & PHILIPS, B. J. (1975) Inhibition of cyclic 3′, 5′-nucleotide phosphodiesterase; a possible mechanism of action of bifunctional alkylating agents. *Biochem. Pharmac.* **24**, 211 – 217.

VARON, S. & BUNGE, R. P. (1978) Trophic mechanisms in the peripheral nervous system. *Ann. Rev. Neurosci.* **1**, 327 – 361.

VERGHESE, J. P., BRADLEY, W. G., MITSUMOTO, H. & CHAD, D. (1982) A blind controlled trial of adrenocorticotropin and cerebral gangliosides in nerve regeneration in the rat. *Exp. Neurol.* **77**, 455 – 458.

WALLER, A. V. (1850) Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog, and observation on the alterations produced thereby in the structure of their primitive fibers. *Phil. Trans. R. Soc. Lond. (Biol.)* **140**, 432.

WEISSMAN, G. & THOMAS, L. (1964) The effects of corticosteroids on connective tissue and lysosomes. *Rec. Prog. Horm. Res.* **20**, 215 – 247.

YAMATSU, K., KANEKO, T., KITAHARA, A. & OHKAWA, J. (1976) Pharmacological studies on degeneration and regeneration of peripheral nerves. I. Effects of methylcobalamine and cobamine on EMG patterns and weight loss of denervated muscles following crush of the sciatic nerves in rats. *Folia Pharmac. Japan.* **72**, 259 – 268.

YOUNG, J. Z. (1942) The functional repair of nervous tissue. *Physiol. Rev.* **22**, 318.

YU, W. H. A. (1982) Effect of testosterone on the regeneration of the hypoglossal nerve in rats. *Exp. Neurol.* **77**, 129 – 141.

YU, W. H. A. & SRINIVASAN, R. (1981) Effect of testosterone and 5α-dihydrotesterone on regeneration of the hypoglossal nerve in rats. *Expl Neurol.* **71**, 431 – 435.