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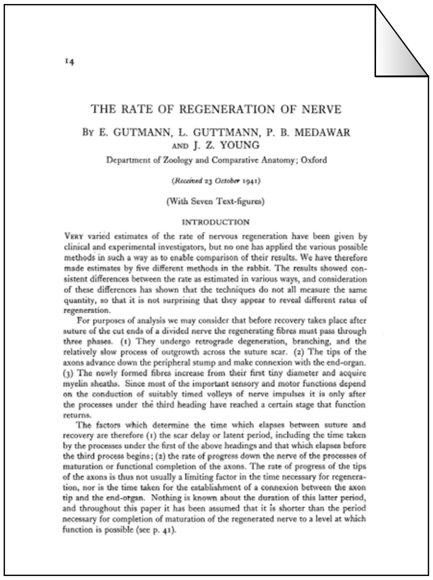
TRANSLATIONAL NEUROSCIENCE DURING THE SECOND WORLD WAR



14

THE RATE OF REGENERATION OF NERVE

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(With Seven Text-figures)

INTRODUCTION

VERY varied estimates of the rate of nervous regeneration have been given by clinical and experimental investigators, but no one has applied the various possible methods in such a way as to enable comparison of their results. We have therefore made estimates by five different methods in the rabbit. The results showed consistent differences between the rate as estimated in various ways, and consideration of these differences has shown that the techniques do not all measure the same quantity, so that it is not surprising that they appear to reveal different rates of regeneration.

For purposes of analysis we may consider that before recovery takes place after suture of the cut ends of a divided nerve the regenerating fibres must pass through three phases. (1) They undergo retrograde degeneration, branching, and the relatively slow process of outgrowth across the suture scar. (2) The tips of the axons advance down the peripheral stump and make connexion with the end-organ. (3) The newly formed fibres increase from their first tiny diameter and acquire myelin sheaths. Since most of the important sensory and motor functions depend on the conduction of suitably timed volleys of nerve impulses it is only after the processes under the third heading have reached a certain stage that function returns.

The factors which determine the time which elapses between suture and recovery are therefore (1) the scar delay or latent period, including the time taken by the processes under the first of the above headings and that which elapses before the third process begins; (2) the rate of progress down the nerve of the processes of maturation or functional completion of the axons. The rate of progress of the tips of the axons is thus not usually a limiting factor in the time necessary for regeneration, nor is the time taken for the establishment of a connexion between the axon tip and the end-organ. Nothing is known about the duration of this latter period, and throughout this paper it has been assumed that it is shorter than the period necessary for completion of maturation of the regenerated nerve to a level at which function is possible (see p. 41).

A copy of Gutmann et al.'s 1942 classic paper 'The Rate of Regeneration of Nerve' can be accessed from http://jeb.biologists.org/cgi/content/abstract/19/1/14

A peculiar concomitant of war is to stimulate highly focused, collaborative scientific research endeavors that lead to extraordinary accomplishments. The developments of radar and of nuclear weapons (The Manhattan Project), are particularly well-known examples. Likewise, groups of biomedical researchers have been assembled in wartime to aid in the efforts to treat the injuries that inevitably occur. The paper discussed here (Gutmann et al., 1942) is a result of one such effort in Britain, during the Second World War.

Of all the non-fatal injuries that occur in battle, the most disabling are neural, because of the limited ability of the human nervous system to regenerate. With this in mind, the British Army and the British Medical Research Council convened a group of neurologists and neurosurgeons in 1939 to confront the special challenges of neurological injury. The group was led by Hugh Cairns and Herbert Seddon, Professors of Surgery at Oxford University; Seddon was also an expert in nerve injuries and their repair. They recruited J. Z. Young (1903–1997), a brilliant cellular neuroscientist, to establish a unit devoted to peripheral nerve repair. Young had already made an unforgettable contribution to neurobiology by discovering and characterizing the giant fiber system of the squid (Young, 1939), whose large caliber axon and huge synapse made possible the Nobel Prize-winning work of Alan Hodgkin and A. F. Huxley on axonal conduction (Hodgkin, 1964; Huxley, 1964) and of Bernard Katz on synaptic transmission (Katz, 1971).

Young, in turn, assembled a team that was, in his words 'a curious mixture' (Young, 1993). His three co-authors on the paper featured here were Ernst Gutmann, Ludwig Guttmann and Peter Medawar. Gutmann (1910–1977) was a Czech physician who, according to Young (1993), had arrived in Britain as a prisoner of war. He was sent to the group in response to their request for 'someone to clean out the animal house' (Young, 1993), but it soon became apparent that he was a knowledgeable and technically gifted researcher. Guttmann (1899–1980) had been a leading neurologist in Germany, who had fled to England in 1939. His refugee status prevented him from resuming his practice, so he was eager to join a group that not only allowed him to gain a livelihood but also to use his skills. Medawar (1915–1987) was a Fellow at Oxford who had studied under Young, then gone on to do pioneering work on the analysis of cell growth in tissue culture. Others in Young's group, but not authors of this paper, included M. Abercrombie, D. Barker, W. Holmes and F. K. Sanders.

Together, the group undertook a series of investigations aimed at understanding why the regeneration of peripheral sensory and motor axons was often imperfect and how it could be improved. '*The Rate of Regeneration of Nerve*' published in *The Journal of Experimental Biology* in 1942, was their first major work. It was written, as the title suggests, to re-examine a simple question. The rate of regeneration had been studied previously by several neuroscientists, beginning with Ramon y Cajal (1928), but there was little consensus on what the rate was, and little clarity on why estimates varied so widely. Gutmann and colleagues were aware that the time of recovery from a nerve lesion was affected by several factors including a latent period after nerve damage and the speed at which nerves regrew. But the distinction between the rate of growth and the separate, significant interval before functional recovery was not well appreciated when these experiments were planned.

The basic protocol that the team used was to damage a peripheral nerve so that its axons would be severed, leading to degeneration of the distal part. They then followed the progress of regrowth from the cut stump over time. The experimental data in the paper consist of graphs plotting millimeters of regeneration *versus* days following nerve injury. Experimental subjects included young and adult rabbits and a few dogs; both sensory and motor nerve regeneration were monitored. Injuries included cuts, crushes near the spinal cord, crushes near the end organ, cuts followed by suture, and nerve cross-anastomoses, in which the proximal stump of one nerve was joined to the distal stump of another. Regeneration was assessed in several different ways. By directly pinching the nerve at various positions along its length, they could find the sensitive growing tip from which reflexes could most easily be elicited. Importantly, however, they also monitored recovery of sensory or motor function after muscle or skin were reinnervated. In most cases, no regeneration was seen for 3–7 days. Axons then appeared to grow at a constant rate of 3–5 mm per day. Surprisingly, however, functional recovery was not detectable for 2–3 weeks after the 'pinch test' indicated that the first axons had reached the end-organ.

It was this difference between the results obtained by the pinch test and by monitoring functional recovery that particularly caught the authors' attention. The failure of previous workers to clearly distinguish regeneration from recovery almost certainly led to the general view that axons grew much more slowly (~1 mm day^{-1}) than Gutmann and co-workers found. The authors noted that direct histological measures of axonal length, such as those made by Ramon y Cajal (1928), might have provided further insight; they also noted that Cajal's estimates of rate were fully consistent with their own. On the other hand, the direct behavioral assessment of functional recovery by Gutmann and the team added a new dimension. In any event, by making the distinction, and by rigorously quantifying their results, Gutmann et al. (1942) provided data on which other scientists could rely.

The differences between the rate measurements of Gutmann et al. and those of some predecessors also raised new questions. In the 1942 paper's Discussion, the authors considered possible explanations for the unexpected delay between axon arrival and functional recovery; they were unable at the time to decide amongst them, but the clear demonstration that regeneration and recovery were very different was a powerful impetus for further work. Indeed, perhaps the most important feature of the paper by Gutmann et al. (1942) is that it laid a solid foundation for much of the work the group went on to do. One main set of studies was aimed at elucidating the factors that limited the efficacy of regeneration. For example, Holmes and Young (1942) asked whether delaying the onset of regrowth had a deleterious effect. To this end, they compared regeneration under two different conditions: after the two ends of the nerve were cut and sutured together immediately or after the two ends were kept apart for protracted periods before being sutured to initiate regrowth. They found that delay decreased the number of axons that regenerated, but not the rate of regeneration, thus providing a novel explanation for the deleterious effects of delay on recovery of function. Gutmann and Sanders (1943) pointed to some aspects of recovery that were incomplete even under optimal conditions, such as the restoration of fiber diameter, and Sanders and Young (1944) then performed experiments suggesting that physical constrictions in the Schwann cell guides through which regeneration occurred were important determinants of the limitation. Knowing that these constrictions increased with time after denervation, they stressed the importance of performing nerve repair surgery as soon as possible following injury.

A second set of follow-up studies was strictly practical (see Young, 1942, and Guth, 1956 for summary and citations). For example, Gutmann showed that fine white silk was a superior suture material to catgut or colored silk; woman's hair, he thought, was better still. Young and Medawar recommended the use of fibrin clots for holding nerve ends together when suturing was infeasible. Gutmann, Sanders and Young tested various materials for their ability to serve as grafts that could support regeneration when nerves were so damaged that suturing was impossible. The work on sutures and grafts presaged the current interest in 'biomaterials' to aid nerve repair and regeneration by a half century (Schmidt and Leach, 2003). Likewise, the surgeon in the group, Seddon, rapidly applied some of the conclusions derived from work on experimental animals to humans (Seddon et al., 1943), in an early example of minimizing the 'bench to bedside' lag.

For neuroscientists, perhaps the most impressive of the studies building on that of Gutmann and colleagues (1942) was a definitive analysis of what happens when regenerating motor axons have finally completed their journey to the muscle, and are faced with the problem of re-establishing a synapse. Gutmann and Young (1944) asked '*what is the delay between the arrival of the tips of nerve fibres at a muscle and the onset of the power of transmission of impulses from one to the other*'? Also, following from the differences in efficacy between prompt and delayed regeneration, they asked whether '*this delay* [is] *the same after short and long periods of muscle atrophy*'. Emphasizing the practical implications of their work, they pointed to several ways in which delaying repair degraded regenerative ability. Among many influential observations, they showed that the Schwann cell guides critical for regeneration through the distal nerve also play critical roles in accurate reinnervation of muscle fibres. In this regard, as with so many others, they expanded on the seminal observations of Ramon y Cajal (1928) and his students. Some of our own recent work has extended this theme still further, by using live imaging in transgenic mice to document the guidance that Schwann cell tubes provide to regenerating motor axons and the factors that regulate its efficacy (Sanes and Lichtman, 1999; Nguyen et al., 2002; Pan et al., 2003). Our 'high-tech' measurement of regeneration rate, 2.7 mm day^{-1} (Pan et al., 2003), is remarkably similar to that obtained by Gutmann et al. (1942) six decades earlier.

As the war came to an end, the members of the group went their own ways. Of the four authors, Gutmann was the only one to continue working on the peripheral nervous system. He received a PhD from Oxford University, UK for his work with Young, then returned to Czechoslovakia, where he eventually became Head of the Department of Physiology and Deputy Director of the Czechoslovakian Academy of Sciences (Tucek, 1978). His work on regeneration, neurotrophic relations and aging in the neuromuscular system (Gutmann and Hanzlikova, 1973; Gutmann, 1976) was world-class at a time when little basic research of note was being done in Eastern Europe. Guttman, whose interests remained primarily clinical, turned to the practical applications of the work he had done with the group. He founded the National Spinal Injuries Centre at Stoke Mandeville near London, and remained as Director until 1966. A pioneer in organized physical activities for the disabled (Guttmann, 1973), he began 'Stoke Mandeville Games for the Paralyzed' in 1948, and oversaw its transformation into what is now the international Paralympic Movement. Medawar pursued another war-time project, on the factors that limit the success of skin grafts. His investigations on transplantation immunity won him a Nobel Prize in 1960

(Medawar, 1991). Young moved to University College London, where he was Chair of Anatomy from 1945 until 1974 (Boycott, 1998). He turned to cellular studies of memory in the octopus, an animal whose prodigious feats of learning he was among the first to appreciate (Young, 1965). His interests shifted gradually to more cognitive levels, and eventually beyond laboratory science to the philosophy of brain and mind (Young, 1988).

In conclusion, the Second World War stimulated intensive, focussed research on nerve regeneration. It is hard to imagine any other motivation for the painstaking experiments that Gutmann et al. (1942) undertook. These studies, which were more descriptive than experimental, provided a lasting, reliable data set upon which to compare the effects of various interventions on nerve regrowth. This paper has been cited more than 270 times over the years, with 10% of those in the 21st century, provide ample testimony to its value. The eminent individuals involved and the brilliant careers that each had are also a testament to the power of a pressing societal need that drove these independent-minded individuals to work together for the greater common good.

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