OTHER PAPERS READ AT THE 1983 ANNUAL SCIENTIFIC MEETING OF THE INTERNATIONAL MEDICAL SOCIETY OF PARAPLEGIA

PERIPHERAL NERVE IMPLANTS IN THE SPINAL CORD IN EXPERIMENTAL ANIMALS

By DAVID H. WILSON, F.R.C.S.

Consultant Surgeon, Accident and Emergency Department, The General Infirmary, Leeds, U.K.

Summary. This paper challenges the concept of 'spinal cord autotomy' as an essential process which will prevent the penetration of spinal cord fibres into a peripheral nerve implant. It proposes that mechanical distraction between the graft and the cord is the main reason why spinal cord fibres fail to traverse a graft. Clinical, neurophysiological and histological evidence is presented to show that embedding an autogenous peripheral nerve implant into a trough in the dorsal half of the cord is effective in restoring continuity in some of the long fibre tracts. Reference is made to other recent work which supports these observations. Possible methods of enhancing the number of fibres which traverse the graft are discussed and an attempt is made to anticipate the possible results of the clinical application of this technique in spinal cord injury patients.

Key words: Experimental spinal cord implants; Pulsed electromagnetic field therapy (PEMF).

Introduction

THE first report of using a peripheral nerve implant to graft the spinal cord was published by Ramon y Cahal in 1928. Since then several researchers have attempted to repeat the experiment but, until ten years ago, only Sugar and Gerard (1940) claimed to have had any success.

Histological examination of these implants usually showed that there was an area of necrosis of the spinal cord adjacent to each end of the graft. Ramon y Cahal considered that this was an essential reaction, inherent in spinal cord tissue, and he described it as 'spinal cord autotomy.'

Spinal Cord Autotomy

In the 1970s Kao studied the mechanism of spinal cord cavitation. He postulated that if, after transection of the cord, the two halves were held in close proximity, then a single cyst would form between them. If, however, a gap was left between the two parts of the cord, connective tissue would invade the gap creating a central scar with cavitation on each side of it (Kao & Chang, 1977).

He then investigated the effect of resecting a portion of cord and using pieces of autogenous sciatic nerve to fill the gap. The results indicated that the process of 'spinal cord autotomy' occurred at each end of the graft.

In a further series of experiments he came to the conclusion that if there was a delay of a week between performing the cordotomy and introducing the graft, then the process of 'spinal cord autotomy' would have been completed within the seven days and no further reaction would occur between the cord and the graft. (Kao, Chang and Bloodworth, 1977).

FIG. 1

A trough has been cut into the dorsal half of the spinal cord. The ventral half remains to give stability to the cord.

Close scrutiny of this work lead me to think that there might be another possible explanation for this phenomenon. I considered that it might be due to mechanical distraction between the cut surfaces of the cord in the first experiment, and between the cord and the graft in the later experiments.

To test this hypothesis I performed a series of operations in which I created a trough in the dorsal half of the spinal cord, but preserved the ventral half to form a bed on which to place the graft, which would act as a splint for the graft and so minimise distraction between it and the cut surfaces of the cord (Fig. 1).

This concept of the importance of minimising any mechanical distraction between the cord and the graft has recently been strongly supported by Breig and his co-workers, who have shown that if, after performing a cordotomy in an experimental animal, the spine is fixed in extension so as to maintain intimate contact between the two halves of the cord, then neurites grow across the gap and so called 'spinal cord autotomy' does not occur (Breig *et al.*, 1982).

Spinal Cord Implants

The first tissue I used for graft material was the animal's own spinal cord.

FIG. 2

The piece of spinal cord which has been resected is rotated through 180° and reinserted to form an autogenous graft.

A portion of the dorsal half of the cord was resected, rotated through 180° and then reinserted. In three consecutive animals these autogenous spinal cord grafts all failed to survive (Fig. 2).

I then repeated the procedure on three more cats but this time, having resected the portion of the cord, I discarded it and used a piece of the sensory branch of the radial nerve from the forelimb as donor tissue. In all of these animals the grafts were fully incorporated into the spinal cord (Fig. 3).

FIG. 3

A portion of the sensory branch of the radial nerve from the forelimb of the animal has been resected to form a peripheral nervous system graft in the spinal cord.

Post-operatively the cats were observed clinically and their walking ability when traversing a trammel was recorded on cine-film. One year after grafting, evoked sensory potential studies showed that impulses from the sciatic nerve could be recorded from a micro-electrode inserted into the dorsal columns proximal to the graft (Fig. 4). The animals were then sacrificed and the spinal cord resected for histology. A longitudinal section through the junctional zone between the graft and the cord is shown in Figure 5; on the left is the darker stained fibrous tissue of the peripheral

FIG. 4

An Evoked Sensory Potential recorded one year after inserting the peripheral nervous system implant into the spinal cord. The stimulus has been applied to the sciatic nerve and the recording made from a micro-electrode inserted to a depth of 0.85 mm in the dorsal column rostral to the graft. The trace indicates that a second order neurone in the spinal cord has been triggered by the sciatic nerve stimulus.

FIG. 5

A longitudinal section cut through the junctional zone between the peripheral nerve graft (on the left) and the spinal cord (on the right). A Van Gieson stain has been used to show the fibrous tissue of the peripheral nerve blending with the glial tissue of the spinal cord.

nerve implant which blends into the lighter stained glial tissue of the cord on the right. There is only the slightest degree of cavitation at the interface between the two tissues.

In a transverse section through the region of the graft. A silver stain has been used to show the axons (Fig. 6). The peripheral nerve implant is in the centre of the figure with spinal cord to each side of it. The density of spinal cord fibres passing through the graft is approximately normal near the surface but falls to 15–20 per cent in the centre of the graft.

A transverse section through the dorsal columns a few millimetres rostral to the graft is shown in Figure 7. The central part of the figure shows large numbers of small regenerating axons which presumably traversed the graft and are now forming a reconstituted ascending tract.

Further evidence of the ability of spinal cord axons to traverse a peripheral nerve graft has been provided by Aguayo and his co-workers in Montreal (David and Aguayo, 1981). Using rats, they resected a piece of peripheral nerve 3.5 centimetres in length and inserted one end of it into the animal's medulla. The main length of the nerve was then placed in the subcutaneous tissue of the back of the animal and the distal end was inserted into the spinal cord in the lower thoracic region. Approximately six months later they divided the cord in the upper thoracic region and divided the peripheral nerve bridge at its mid-point. They then used horse radish peroxidase for axonal tracing and demonstrated that there was both ascending and descending spinal cord fibres in the bridge and that they penetrated the cord on re-entry to a distance of up to 3 mms, which is a

FIG. 6

A transverse section through the graft stained by a Palmgren silver stain to demonstrate the axons. Normal spinal cord is seen at the edges of the figure. The peripheral nerve implant is in the centre. Axons have penetrated the graft in large numbers near the surface. In the centre of the implant nerve fibre density is only 15–20 per cent of that seen in the normal spinal cord.

substantial distance in the rat. Whether or not these fibres then made meaningful synapses is yet to be determined.

Discussion

All these observations provoke further questions. Can it be demonstrated that the elongated axons, which have traversed the graft, eventually achieve a valuable and functional synapse? If only 20 per cent of fibres succeed in crossing the graft, will this proportion be sufficient to produce a worthwhile improvement in a patient's condition? Finally, are there techniques which can be used to improve the percentage of fibres which traverse the graft?

This last question has several possible solutions. De la Torre (1980) has recently reviewed various chemotherapeutic agents which might achieve a favourable manipulation of the neurochemical environment of the graft. Yeo's work on hyperbaric oxygen therapy is another likely contender (Yeo *et al.*, 1976). Procedures to improve the circulation in the region of the graft, such as Goldsmith's omental grafting technique, may also prove to be beneficial (Goldsmith *et al.*, 1982).

I have previously reported on the use of pulsed electromagnetic field therapy to accelerate the regeneration of peripheral nerves and to promote the healing of experimental cordotomies (Wilson & Jagadeesh 1976). The value of this treatment in stimulating peripheral nerve healing has recently

FIG. 7

A transverse section through the dorsal columns a few millimetres rostral to the graft. A Palmgren silver stain demonstrates many small, regenerating nerve fibres especially in the centre of the figure.

been confirmed in an independent study (Raji & Bowden 1982). Remarkable results from using this form of therapy to achieve healing in long-standing non-union of fractures have been reported by many workers, principally Bassett in America (Bassett *et al.*, 1981) and Sharrard and his group in Sheffield in England (Sutcliffe *et al.*, 1980). A study is now in progress to determine the efficacy of pulsed electromagnetic field therapy in enhancing the penetration of spinal cord fibres across peripheral nerve implants.

The reason for this investigation is the hope that the technique may eventually lead to the possibility of achieving some improvement for spinal cord injury patients. Consequently it is important to appreciate the limitations of what might be accomplished. If a peripheral nerve graft was successful in a patient there could be some restoration of function in the

long fibre tracts which formerly traversed the area of the spinal cord injury. The nerve cell bodies in that area have been permanently destroyed and cannot recover. Thus a patient with a lesion extending from C₆ to C₈ might regain some sensation in bladder or bowel or some awareness of the body below the level of the lesion. There would, however, be no return of motor function in the upper limbs, the nerve cell bodies which furnish the motor nerves of the brachial plexus having been permanently destroyed. To describe such an improvement as a 'cure' would be a gross misrepresentation of what had been achieved.

The partial restoration of transmission in some of the long fibre tracts of the spinal cord is all that could result from the use of this technique. Nevertheless, I consider it to be a goal worth pursuing.

Acknowledgements

I wish to thank Dr P. P. Newman for help with the neurophysiological assessment of the animals, and Dr D. G. F. Harriman for the preparation and interpretation of the histological material.

RÉSUMÉ

Cet article conteste l'idée de l'autotomie de la moëlle épinière comme étant un procédé qui empêche la pénétration des fibres nerveuses dans une greffe de nerf périphérique. On suggère qu'une rupture mécanique entre la greffe et la moëlle empêcherait les fibres de traverser la greffe.

L'évidence clinique, physioneurologique et histologique présentée démontre que l'introduction d'une greffe autogène de nerf périphérique dans un croux de la moitié dorsale de la moëlle aide à restaurer la continuité de certaines longues fibres. Ces observations sont appuyées par d'autres travaux récents auxquels on fait allusion.

On discute de différents moyens qui pourraient encourager l'augmentation de fibres nerveuses traversant la greffe et on essaie d'anticiper les résultats d'une application clinique de cette technique chez des malades atteints de blessure à la moëlle épinière.

ZUSAMMENFASSUNG

Dieses Referat bestreitet das Konzept der Rückenmarkautotomie als wesentlichen Vorgang, der die Rückenmarkfasern daran hindert, in ein peripherales Nervenimplantat hineinzuwachsen. Es schlägt vor, dass mechanische Störungen zwischen Lappen und Rückenmark die Hauptursache sind, dass die Rückenmarkfasern nicht imstande sind, durch einen Lappen zu durchwachsen. Klinische, neurophysiologische und histologische Beweise werden erbracht, um nachzuweisen, dass das Einbetten eines autogenen peripheralen Nervenimplantats in eine Vertiefung in der dorsalen Hälfte des Rückenmarks erfolgreich in der Wiederherstellung der Kontinuität in einigen von den langen Fasertrakten ist. Es wird auf weitere Arbeiten jüngerer Ursprungs hingewiesen, die diese Beobachtungen unterstützen. Mögliche Methoden davon, die Anzahl der durch den Lappen durchwachsenden Fasern zu erhöhen, werden diskutiert, und man versucht, die möglichen Ergebnisse einer klinischen Anwendung dieses Verfahrens bei Patienten mit Rückenmarkverletzungen vorauszusehen.

REFERENCES

- BASSETT, C. A. L., MITCHELL, S. N. & GASTON, S. R. (1981). Treatment of Ununited Diaphyseal Fractures by pulsing Electromagnetic Fields. *Journal of Bone and Joint Surgery*, **63-A**, 511-523.
- BREIG, A., RENARD, M., ŠTEFANKO, S. & RENARD, C. (1982). Healing of the severed spinal cord by biomechanical relaxation and surgical immobilisation. *Anat. Clin.*, **4**, 167-181.
- DAVID S. & AGUAYO, A. J. (1981). Elongation of central nervous system axons into peripheral nervous system "bridges" in the adult rat. *Science*, **214**, 931-933.

- DE LA TORRE J. C. (1980). Chemotherapy of Spinal Cord Trauma. *The Spinal Cord and its reaction to traumatic injury*. In: W. F. Windale (Ed.) pp. 291-310. Marcel Dekker Inc. New York.
- GOLDSMITH, H.S., STEWARD, E., CHEN, W. F., & DUCKETT, S. (1982). Application of intact omentum to the normal and traumatised spinal cord. In C. C. Kao (Ed.) *Spinal Cord Reconstruction*. Bunge, R. P. Raven Press, N.Y.
- KAO C. C. & CHANG L. W. (1977a). The mechanism of spinal cord cavitation following spinal cord transection. *J. Neurosurg.*, **46**, 197-209.
- KAO C. C., CHANG L. W., BLOODWORTH, J. M. B. (1977b). The mechanism of spinal cord cavitation following spinal cord transection. Delayed grafting with and without spinal cord retranssection. *J. Neurosurg.*, **46**, 757-766.
- RAJI, A. R. M. & BOWEN RUTH E. M. (1982). Effects of high peaked pulsed electromagnetic field on degeneration and regeneration of the common peroneal nerve in rats. *Lancet*, **2**, 444-445.
- RAMON Y CAHAL (1928). *Degeneration and Regeneration in the nervous system*. Oxford University Press, London.
- SUGAR O. & GERARD R. W. (1940). Spinal cord regeneration in the rat. *J. of Neurophysiology*, **3**, 1-19.
- SUTCLIFFE, M. L., SHARRARD, W. J. W. & MACEachern, A. G. (1980). The treatment of Fracture Non-union by electromagnetic induction. *Journal of Bone and Joint Surgery*, **63-B**, 123.
- WILSON, D. H. & JAGADEESH, P. (1976). Experimental regeneration in peripheral nerves and the spinal cord in laboratory animals exposed to a pulsed electromagnetic field. *Paraplegia*, **14**, 12-20.
- YEO, J. D., MCKENZIE, B., HARDWOOD, B. & KIDMAN, A. (1976). Treatment of paraplegic sheep with hyperbaric oxygen. *Med. J. Aust.*, **1**, 528-540.