STUDY OF PERIPHERAL NERVE REGENERATION IN THE SILICONE CHAMBER MODEL USING HUMAN AMNIOTIC MEMBRANE MATRIX AS SUBSTRATUM

BY

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GUIDE

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CERTIFICATE

This is to certify that Dr. Asha Vijayaraghavan carried out the work entitled **'STUDY OF PERIPHERAL NERVE REGENERATION IN THE SILICONE CHAMBER MODEL USING HUMAN AMNIOTIC MEMBRANE MATRIX AS SUBSTRATUM'** under my guidance and personal supervision and that it is her original work. This project has been done as part of her postdoctoral research fellowship in the department of Neurology, SCTIMST, Trivandrum, 1991-1992.

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CONTENTS

INTRODUCTION

Peripheral nerve regeneration has been the subject of extensive experimental work ever since Ramon y Cajal described this phenomenon in 1928 (1). A lot of clinical research is focussed on this area even today because of the formidable challenge posed by the repair of traumatic nerve injuries. Interest in this field has been renewed by the discovery of various growth factors which have trophic (nutritional) and tropic (directional) functions on the axon. With the help of these novel biological approaches it may be possible to achieve the ultimate goal of establishing perfect anatomical continuity and functional recovery of severed nerves. The search for the ideal teachniques and biological means to achieve this target, if fruitful, will have enormous potential not only in the repair of traumatic nerve injuries and nerve injury related to surgery like cancer surgery, but also in experiments of CNS regeneration.

Evolution of various nerve repair techniques:

The traditional method of anastomosing the severed nerve ends to bridge the gap is fraught with problems of foreign body reactions to suture materials and tension at the repair sites, resulting in injury to the regenerating nerve fibres and misrouting of axons.

Meticulous microsurgical techniques and direct end to end repair of funicles have minimized the problems of misrouting and random growth of axons into incorrect Schwann cell tubes in the distal segment. But there remained problems like endoneural necrosis, microbleeding, oedema and fibrosis interfering with the growth of axons and the critical factor of gap length in choosing cases for surgical repair (2).

Autologous nerve grafting is another repair technique of bridging nerve gaps of variable lengths. These grafts serve as a passive conduit for axonal regeneration but the major limitation to its clinical application is the requirement of donor nerves especially in multiple nerve injuries. A number of homografts and heterografts have been evaluated but all were found to be immunologically unacceptable (2).

Sutureless nerve repair using tubulization is an attractive alternative to avoid these complications. This technique involves the enclosure of the ends of a severed nerve by a preformed tube which holds the stumps in alignment, guides the regenerating axons to the distal stumps and collects the products from the reactive Schwann cells.

A wide range of biological and non biological materials have been tried for tubulization (3-7). Tubular prosthesis of permeable, semipermeable and bioresorbable materials are being tried by experimentalists though their clinical use is yet to be established.

The silicone chamber model for nerve regeneration was developed in 1986 by Lundborg and Varon (8-10). It is an entubation model in which the rat sciatic nerve is transected and cut ends enclosed within a silicone tube leaving a 10 mm gap between the nerve stumps. This model has helped to study the sequential events occuring during successful regeneration and the effect of various substances on nerve regeneration.

The important factors which have been identified as necessary for functional return of activity of a severed nerve are (i) gap length (ii) matrix components on which the nerve grows (iii) accuracy of neurite terminal reconnection (iv) neuronotrophic environmental factors.

In vitro studies have revealed that growth factors alone like Nerve Growth Factor (NGF) offer little advantage as they only stimulate the growth of neurites but have no specific directional influence (11, 12). Hence no useful functional recovery can be achieved with them alone. But

extracellular matrices have been found to provide an organized directional growth (11). Evaluation of the purified extracellular matrix components like collagens, proteoglycans, fibronectin and laminin have shown that laminin possesses the highest neurite promoting effect (13-18). This effect of laminin is seen more pronounced with the use of intact extracellular matrices.

The human amniotic membrane (HAMM) is a naturally occuring, rich source of laminin, in addition to its other components like type IV collagen, heparan sulfate and proteoglycans. The neurite promoting influence of the basement membrane surface of HAMM for neurons is well established in vitro (19,20). It has also been tested in adult CNS rat neurons in vivo (19,21). Human amniotic membrane was rolled and implanted into a cavity created between rat septum and hippocampus (19,21). The cholinergic neurons grew axons through the implant into the hippocampus. Although no immunosuppressive drugs were administered, there was no significant immunologic reaction to HAMM. The HAMM is easily available from hospitals, has a consistency which allows folding, coiling, stretching and suturing. Its activity is stable in vitro for more than 6 months at 4^{o}C.(19). In addition it has been proposed that it may have additional growth advantages by the presence of, or release

of other neuronotrohic factors contained in the membrane or released as a result of the interplay between the membrane and the neurite growth cone and its receptors(12). Hence this membrane is a valuable material which can be tried as prosthetic bridge in experimental regeneration studies of CNS and PNS. Although the rat amniotic membrane has been tried in PNS regeneration studies in the silicone chamber model (22) as well as to restore erectile functions after ablation of cavernous nerves in rats (12), the role of human amniotic membrane has not been established in the study of peripheral nerve regeneration.

AIMS OF THE PRESENT STUDY:

1. To construct a silicone chamber model for rat sciatic nerve regeneration.

2. To study the chamber regenerate at varying intervals and observe the sequential changes in the migration of the cellular components, Schwann cells, axons, myelin, perineurium and vasculature and formation of nerve fascicles.

3. To assess the effect of human amniotic membrane matrix (HAMM) as a substratum for nerve regeneration in the rat. If effective, to compare the regeneration on the test side using HAMM, with controls constituted by the empty silicone chamber anchored to the severed nerve ends.

4. To study the usefulness of immunosuppressive agents, if there is any immunological reaction to HAMM.

5. To develop a pilot study to guide future systematic investigations using biodegradable tubes and test other growth promoting and guiding factors.

MATERIALS AND METHODS:

Human fetal membranes were obtained from full term placentas within 24 hours of normal delivery. The amnion was separated and rinsed in phosphate buffered saline containing penicillin, streptomycin and fungizone. It was then incubated in 0.1% ammonium hydroxide for 10 to 15 minutes. The epithelial cells were removed by gentle brushing and repeated rinsing. The membrane was then put in the PBS solution containing pencillin, streptomycin and fungizone at $4^{o}C$.

The silicone chambers were constructed from silicone tubing stock. 15 mm long pieces of sterile silicone tubes with an internal diameter of 1.5 mm were used.

Adult Wister male and female rats (n = 30) each weighing 200-250 gms served as the experimental animals (Photo No.1).

Surgical Procedure:

The animals were deeply anaesthetised using intraperitoneal pentobarbital (40mg/kg). Under strict aseptic conditions the sciatic nerve on each side was exposed and freed from the surrounding tissues and the epineurium was removed (Photo No.2). The nerve was then

transected at midthigh level proximal to the tibial and peroneal bifurcation.

A sterile silicone tube which is longitudinally slit on one side was placed in the thigh and the proximal and distal nerve stumps were pulled for a distance of 2.5 mm into each end of the slit tube. The stumps were then anchored with a single perineural 8-0 suture to the silicone tube at each end. On the test side, the rolled piece of sterile HAMM was introduced into the silicone tube and placed longitudinally along the interstump gap with the ends of the membrane touching the cut ends of the nerve (Photo No.3 and 4).

The slit in the silicone tube was then sealed off with silicone adhesive. On the control side the same procedure was performed except that the tube was left empty (Photo No.5). The tubes were buried under muscles and the wound sutured with silk.

The rats were given cotrimoxazole (Trimethoprim 5mg/kg) for a week and kept isolated for 48 hours in the post operative period. The rats were sacrificed at 3,5,8 and 12 weeks after the surgical procedure. 6 animals received immunosuppressive therapy with Azathioprine (2.5 mg/kg) starting 1 week pre-op and continued till 5 weeks post op, when they were sacrificed.

At autopsy the test and control sides were re-exposed through the same incision. The regenerate including 5 mm of the proximal and distal stumps were removed from each side. The silicone tube was carefully slit and separated from the regenerate.

The proximal and distal stumps and the regenerate were examined for infection, inflammation, degeneration and the presence of Schwann cells, axons, myelin and vasculature by H and E staining and E.M. examination.

Electron microscopic examinations were done in 6 specimens. The specimens were fixed in phosphate buffered 3% glutaraldehyde followed by 1% osmium tetroxide. The specimens were then embedded in resin and 1 micrometer sections were cut on an ultramicrotome using a glass knife and stained with toluidene blue.

RESULTS:

In all, 27 animals survived the post operative period.

Gross examination in situ showed a thin encapsulation around the tubes on both test and control sides (Photo Nos. 6 and 7). On separating the silicone tubes, good anatomical continuity was seen between the proximal and distal stumps by a regenerate on both test and control sides in all the specimens(Photo No.8).

Macroscopically, the regenerate on the control side was thin, (less than 1 mm), whereas the test side regenerate appeared thicker and had a uniform thickness of 1-1.5 mm. (Photo No.8). Histopathological examination (H & E) of the control side showed the following features:

At 3 weeks: (n=2) The regenerate showed occasional nerve fibres, few Schwann cells and perineural cells (Photo No.9..).

At 5 weeks (n=10). The regenerate showed numerous Schwann cells, myelinated axons and formation of nerve fasicles with few perineural cells. The mean endoneural diameter was 0.5 mm. (Photo No.10a,b.)

At 8 weeks (n= 5) The regenerate showed numerous nerve fibres including myelinated fibres forming well circumscribed nerve fascicles, numerous proliferating

Schwann cells and few perineural cells. The endoneural diameter ranged from 0.5 mm to 1 mm (Photo No. 11).

At 12 weeks (n=4). There were well formed nerve fascicles with numerous myelinated nerve fibres and a well defined perineurium. Numerous Schwann cells and small blood vessels were also seen (Photo No. 12a,b).

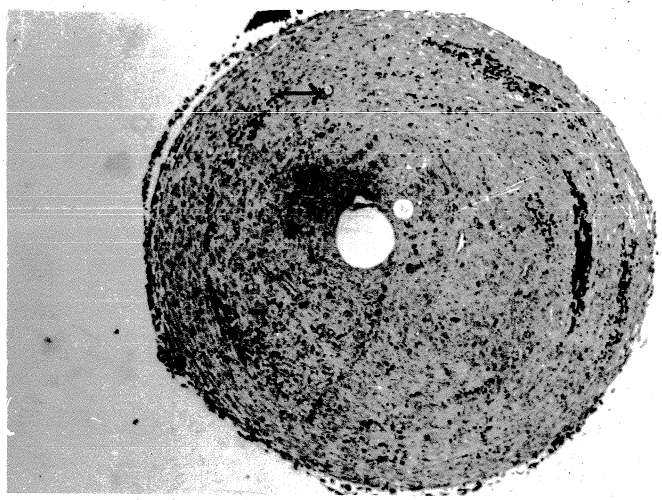
Test side: In all the specimens from 3rd to 8th weeks there was evidence of chronic inflammatory reaction in the regenerate with plenty of lymphocytes, macrophages, increased vascularity and occasional giant cells. The human amniotic membrane was seen as a homogenous eosinophilic material occupying the central area of the regenerate with spindle cells infiltrating from the periphery. Numerous circumferential fibrocytes were also seen. There was no evidence of Schwann cell proliferation or nerve fibres (Photo No 13,14a,b).

The samples from the proximal and distal nerve stumps showed nerve fascicles undergoing degeneration.

At 12 weeks, the proximal portions of regenerate of the test side showed evidence of Schwann cell proliferation with few nerve fibres in the periphery. There was a striking appearance of lymphocytes at the junction between the

advancing nerve fibres, Schwann cells and the amniotic membrane (Photo No 15, 16, 17)

Azathioprine treated group (n=6) at 5 wks: The test side of this group showed evidence of chronic inflammation as seen in the group without Azathioprine (Photo No. 18.). The regeneration on the control side was identical to that seen without Azathioprine. (Photo No 19)



hoto 9: Control 3 wks, cross section (C.S) 50x, showing few Schwann cells, few nerve fibres(->).

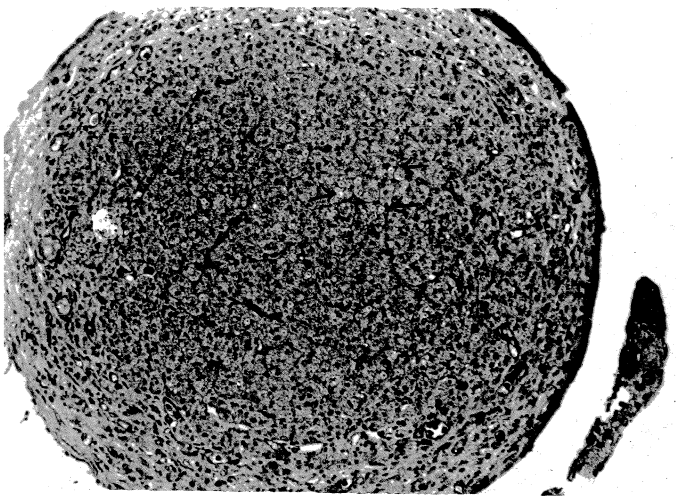
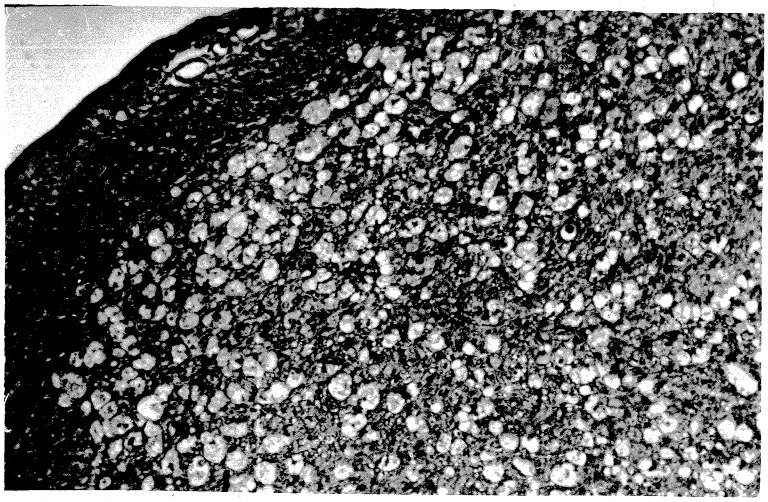


Photo 10a:Control 5 wks, C.S 50 x, showing numerous myelinated fibers (->), few Schwann cells.



hoto 10b:Control 5 wks, C.S 50 x, showing same as 10a.

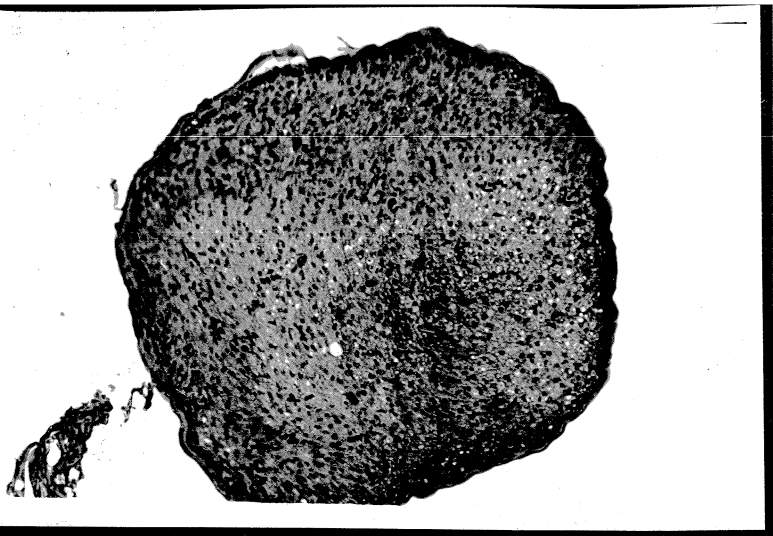


Photo 11:Control 8 wks, C.S 50 x, showing myelinated fibers (->), Schwann cells and perineural cells (x).

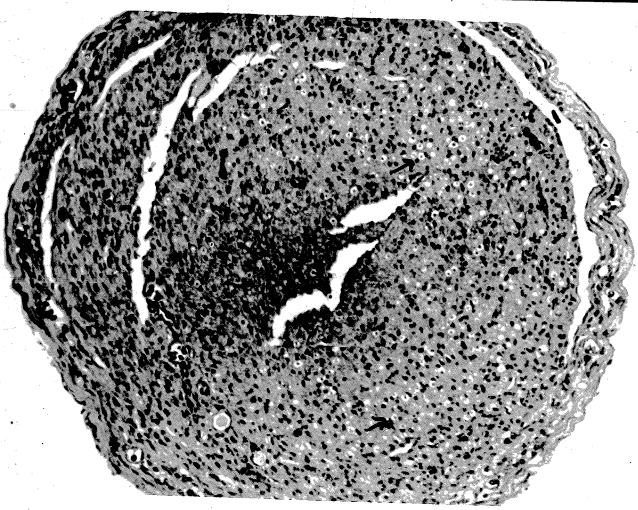
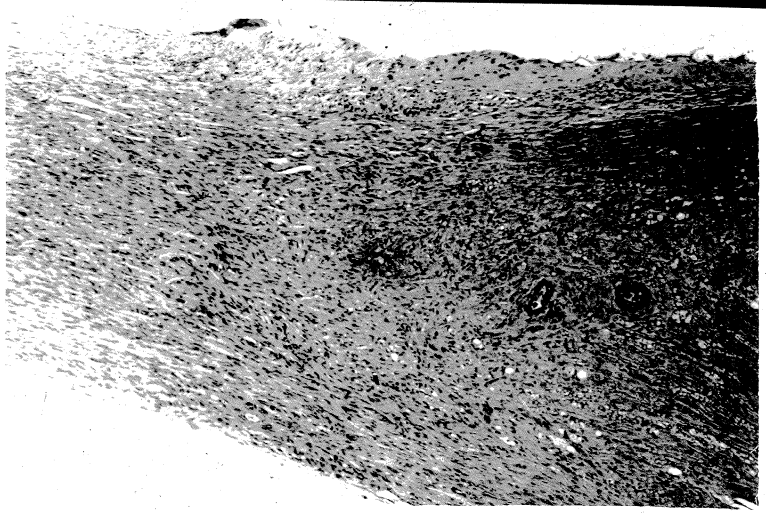
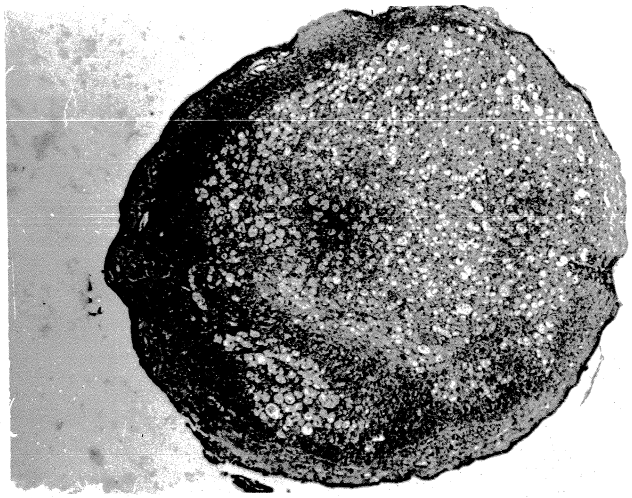


Photo 12a:Control 12 wks, C.S distal end of regenerate 50 x, showing numerous myelinated fibers (->) and perineural cells (x).



oto 12b:Control 12 wks, longitudinal section (L.S) 20 x, showing regeneration.



oto 14b:Test 8 wks, C.S 20 x, showing same changes as Photo 13.

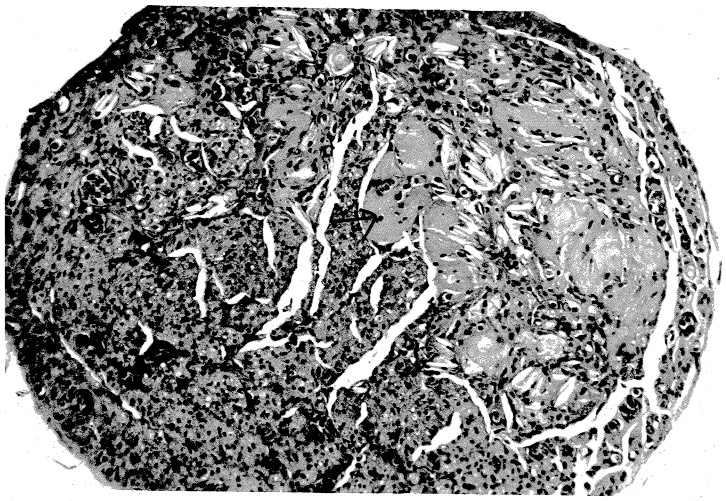
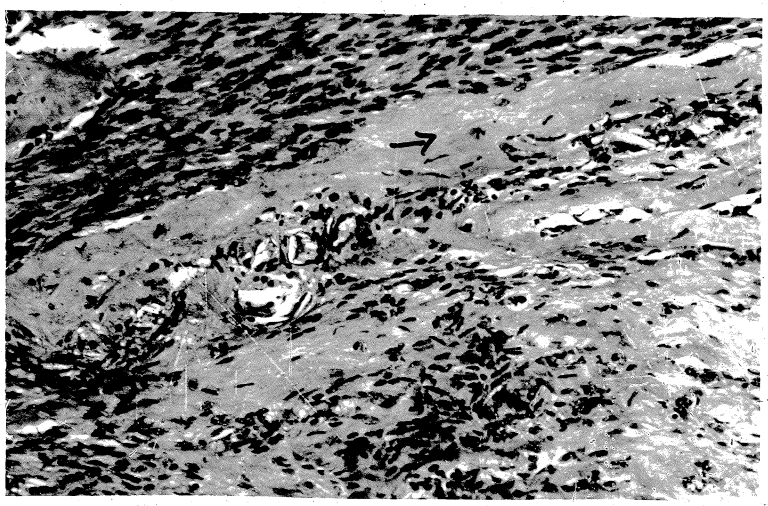
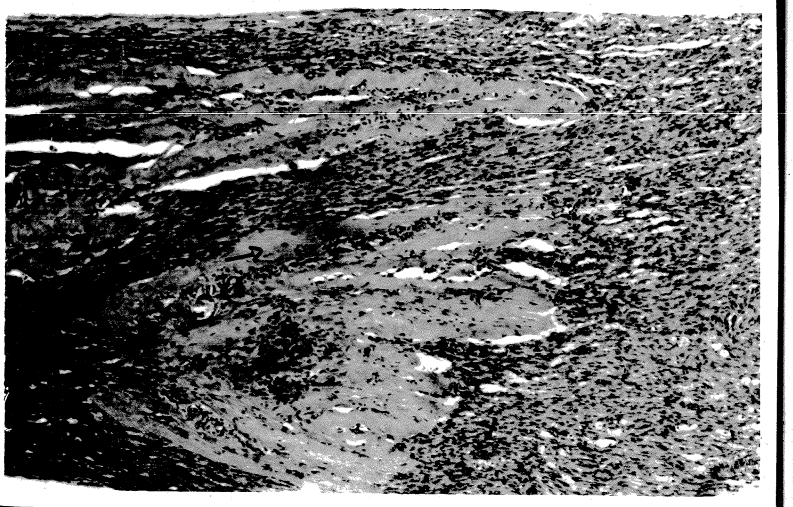


Photo 15:Test 12 wks, C.S 50 x, few myelinated fibers (x) at periphery and central coil of amniotic membrane (->)



hoto 16:Test 12 wks, C.S 50 x, same changes as photo 17.



to 17:Test 12 wks, L.S 20 x, showing inflammatory reaction (x) between membrane (->) and Schwann cells (.)

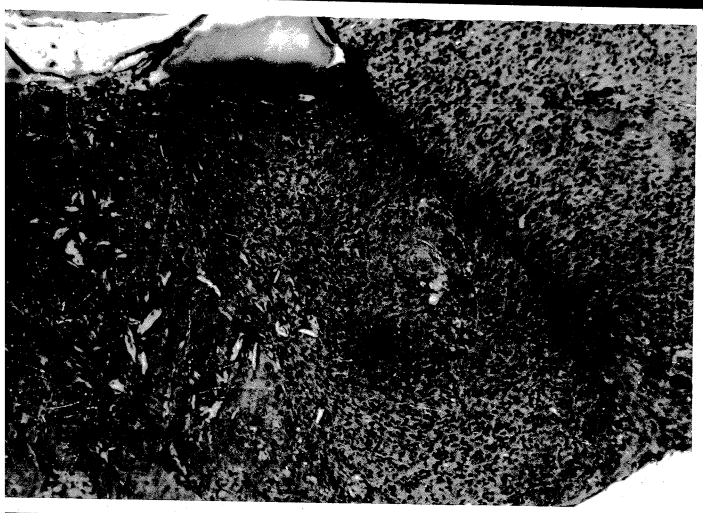
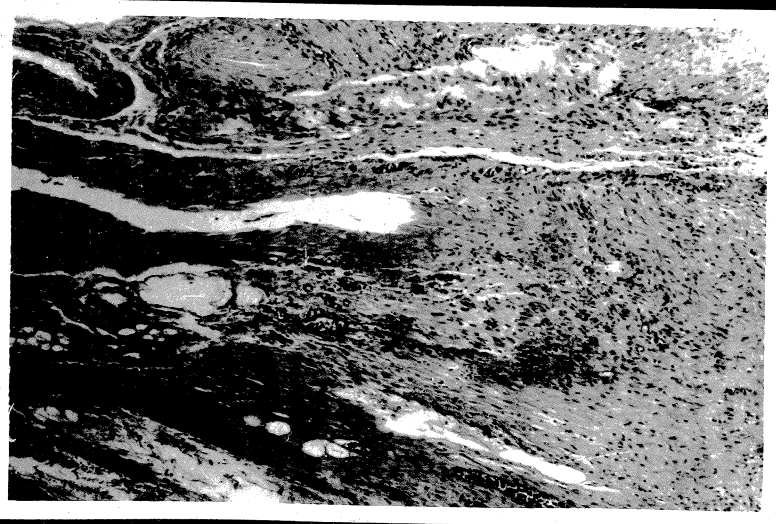


Photo 18:Test 5 wks, Imuran; (L.S) 20 x showing membrane (->) with adjacent chronic inflammatory cells (x) and numerous Schwann cells (.) in the periphery.



to 19:Control 5 wks, Imuran; L.S 20 x showing same as 10 a.

DISCUSSION

The silicone chamber model for nerve regeneration, developed by the Lundborg and Varon research groups has been used as an entubation model to study the spatial and temporal sequence of nerve regeneration(23-25). It is also the standard entubation technique to study other entubation materials as well as the effect of various growth and neurite promoting factors in vivo (26-29). Currently there is a resurgence of interest in the entubation technique, with the failure of the most meticulous microsurgical techniques to achieve adequate functional recovery after nerve severance (30). It is now well accepted that further refinement in surgical techniques is not possible and a more biological approach to nerve injuries using various growth and neurite promoting factors may turn out to be a superior mode of treatment. It has to be tested whether modifying the environment at the site of nerve injury using these biological methods will help to overcome the major clinical problems of misdirection of axons at the repair site and slow outgrowth rate of axons which allow fibroblasts and collagen to proliferate and create a barrier.

In this study the silicone chamber model was constructed and implanted in the rat sciatic nerve. The temporal progression of cellular components migrating into

the chamber was found to be identical to the well established results of earlier reports (22-25). Evaluation upto 3 months after implantation showed well formed nerve fascicles containing increased number of myelinated axons.

In this study human amniotic membrane (HAMM) coil implanted in the peripheral nervous system was found to elicit an immunological reaction preventing successful regeneration. The chamber regenerate, though grossly well formed and continuous with the proximal and distal stumps, was constituted by remnants of the membrane, fibrocytes, lymphocytes, macrophages and a few giant cells. Test specimens of regenerate examined 12 weeks after the implantation showed migration of proliferating Schwann cells and regenerating axons into the proximal part of the regenerate. However in the distal segment, the chronic inflammatory cell reaction formed a barrier to their advancement at the junction between the Schwann cells and the membrane.

Earlier experiments in vivo using HAMM in the CNS have shown good neurite promoting activity and growth of axon on the HAMM and into the denervated target tissue (19,21). The membrane caused no greater inflammatory reaction in the brain beyond that seen in response to the aspirative lesioning in the brain.

Though in the present study implants were done under sterile conditions and using different batches of HAMM, there was an identical type of inflammatory reaction, which reflects that it is an immunological response to heterospecific tissue rather than an exogenous infection. The presence of such a reaction seen in the PNS to heterologous tissue, but not in the CNS, is probably due to the immunologically privileged status of the brain or some unknown factors operating in the PNS.

Immunosuppressive therapy using Azathioprine in a dose of 2.5 mg/kg starting one week prior to surgery and continued till autopsy was not found to be beneficial in suppressing the inflammatory reaction. All the rats which received immunosuppression were autopsied at 5 weeks. It is not known whether continued immunosuppression would have helped to augment the attempts at regeneration seen with HAMM at 12 weeks on the test sides.

Rat amniotic membrane (RAMM) has been successfully used in the silicone chamber model (22). Recently, experiments using RAMM grafts sutured over the resected ends of cavernous nerves without entubation showed enhancement of electrically stimulated penile erection and mating behaviour in rats rendered impotent by surgery (12). In both these

experiments there was no immunological reaction to homologus tissue in the PNS.

Histological and physiological evidence of efficacy of HAMM in peripheral nerve regeneration and its superiority over existing repair techniques have to be established in animal experiments before it can be tried for clinical use. It would be worthwhile to repeat the study in rats using greater immunosuppression with higher doses of Azathioprine (5mg/kg) or a combination of drugs (Azathioprine, prednisolone and cyclosporin A). If the immunological reaction is prevented, HAMM may prove to be as efficacious as RAMM in peripheral nerve regeneration in rats and probably in humans too. In that case bioimplants made of biodegradable material containing HAMM may prove useful in the treatment of nerve injuries. It is possible that homologous transplants with HAMM in humans may not elicit an immunological reaction just as RAMM did not in the PNS or CNS of rats.

CONCLUSION

1. The silicone chamber model of nerve regeneration was created in rat sciatic nerve. The results of nerve regeneration were comparable with well established existing results and thus standardized.

2. In the peripheral nervous system HAMM was found to elicit an immunological reaction which inhibited Schwann cell proliferation and axon growth.

3. At longer intervals (12 weeks) an attempt at nerve regeneration was seen on the test side with HAMM though its complete advancement into the distal segment was arrested by inflammatory reaction.

4. Treatment with Azathioprine in a dose of 2.5 mg/kg did not suppress the immunological reaction.

5. A trial with stronger immunosuppression using higher doses of Azathioprine or combination of immunosuppressant drugs might hep in preventing this immunological reaction.

REFERENCES

1. Cajal S Ramón Y. Degeneration and Regeneration of the Nervous system. Oxford University Press, London. 1928. Quoted in Sutherland S. Nerves and Nerve injuries, Edinburgh: Churchil Livingstone, 1978.

2. Lundborg G. Nerve Injury and Repair, Edinburgh, Churchill Livingstone, 1988.

3. Seckel BR, Chiu TH, Nyilas E, Sidman RL. Nerve regeneration through synthetic biodegradable nerve guides: Regulation by target organ. Plast reconstr Surg 1984; 74: 173-81.

4. Uzman BG, Villegas GM. Mouse sciatic nerve regeneration through semipermeable tubes. A quantitiative model. J Neurosci Res 1983; 9: 325-38.

5. Bernard Knoops BS, Hernan Hurtado BS, Phillipe Vanagular. Rat sciatic nerve regeneration within an acrylc semipermeable tube and comparison with a silicone impermeable matter. J Neuropath 1990; 49: 438-48.

6. Russell Griffiths, Kenneth Horch, Larry Stensas. A collagen and fibrin tube for nerve repair. Restorative Neurol and Neurosci 1990; 1: 339-46.

7. Joseph M. Rosen, Huun N Pham, Gorden Abraham, Lou Harold, Vincent R Hentz. Artificial nerve graft compared to autograft in a rat model. J of Rehab Res & Dev. 1989; 26: 1-14.

8. Varon S, Williams LR. Peripheral nerve regeneration in a silicone model chamber: cellular and molecular aspects. Periph Nerve Repair Regen. 1986; 1:9-25.

9. Lundborg G, Longo FM, Varon S. Nerve regeneration model and trophic factors in vivo. Brain Res. 1982; 232: 157-61.

10. Lundborg G, Hanson HA. Regeneration of peripheral nerve through a preformed space. Preliminary observations on the reorganisation of regenerating nerve fibers and perineurium. Brain Res 1979; 179: 573-76.

11. Varon S, Hagg T, Manthorpe M. Neuronal growth factors. In: Seil FJ, Ed. Neural Regeneration and transplantation. New York: Alan R. Liss. Inc., 1989: 101-21.

12. Burgers JK, Nelson RJ, Quinlan DM, Walsh PC. Nerve growth factor, nerve grafts and amniotic membrane grafts restore erectile function in rats. J Urol 1991; 146: 463-8.

13. Marston Manthorpe, Eva Engvall, Erkki Ruoslahti et al. Laminin promotes neurite regeneration from cultured peripheral and central neurons. J of cell biol. 1983; 97: 1882-90.

14. Rogers Sherry L, Letournean Paul C, Palm Sally L, McCarthy James, Furch Leo T. Neurite extension by peripheral and central nervous system neurons in response to substratum - bound fibronectin and laminin. Dev. Biol. 1983; 98: 212-20.

15. Bunge Richard P, Bunge MB. Evidence that contact with cnnective tissue matrix is required for normal interaction between Schwann cells and nerve fibers. J Cell Biol. 1978; 78: 943-50.

16. Davis GE, Varon S, Engvall E, Manthorpe M. Substratum binding neurite promoting factors: relationships to laminin. Trends Neurosci 1985; 8: 528-32.

17. Baron-Van Evercooren A, Kleinman HK, Ohno S., Marangos P., Schwartz JP, Dubois-Dalcq ME. Nerve growth factor, laminin and fibronectin promote neurite growth in human fetal sensory ganglia cultures. J. Neurosci Res 1982;8: 179-94.

18. Joshua Sanes R. Laminin for axonal guidance. Nature 1985; 315: 714-5.

19. George DE, Scott BN, Engvall E, Varon S, Manthorpe M, Gage FH. Human amniotic membrane serves as a substratum for growing axons in vitro and in vivo. Science 1987; 236: 1106-9.

20. George DE, Engvall E, Varon S, Manthorpe M. Human amnion membrane as a substratum for cultured peripheral and central nervous system neurons. Dev Brain Res 1987; 33: 1-10.

21. Gage FH, Blaker SN, Davis GE, Engvalle E, Manthorpe M. Human amnion membrane matrix as a substratum for axonal regeneration in the central nervous system. Exp Brain Res. 1988; 72: 371-80.

22. Danielson N, Muller H, Pettman B et al. Rat amnion membrane matrix as a substratum for regenerating axons from peripheral and central neurons: effects in a silicone chamber model. Dev Brain Res 1988; 39: 39-50.

23. Williams LR, Longo FM, Powell HC. Spatial-temporal progress of peripheral nerve regeneration within a silicone chamber: Parameters for a bioassay. J Comp Neurol 1983; 218: 460-70.

24. Longo FM, Skaper SD, Manthorpe M, Wiliams LR, Lundborg G, Varon S. Temporal changes in neuronotrophic activities accumulating in vivo within nerve regeneration chambers. Exp Neurol 1983; 81: 756-69.

25. Danielson N. Regeneration of rat sciatic nerve in the silicone chamber model. Restorative Neurol & Neurosci 1990; 1: 253-9.

26. Politis MJ, Ederle K, Spencer PS. Tropism in nerve regeneration in vivo. Attraction in regenerating axons by diffusible factors derived from cells in distal nerve stumps of transected peripheral nerves. Brain Res 1982; 253: 1-12.

27. Kanje M, Skottner A, Lundborg G. Effects of growth hormone treatment on the regeneration of rat sciatic nerve. Brain Res 1988; 475: 254-8.

28. Kanje M, Skottner A, Sjoberg J, Lundborg G. Insulin like growth factor - 1. (IGF-1) stimulates regeneration of the rat sciatic nerve. Brain Res 1989; 486:396-8.

29. Longo FM, Manthorpe M, Skaper SD, Lundborg G, Varon S. Neuronotrophic activities accumulate in vivo within silicon nerve regeneration chambers. Brain Res 1983; 261: 109-17.

30. Lundborg G. Nerve regeneration probelems in a clinical perspective. Restorative Neurol & Neurosci 1990; 1: 297-302.