EXPERIMENTAL NEUROLOGY 95, 639–651 (1987)

# Immunocomplexes in Rat and Rabbit Spinal Cord after Injury

G. PALLADINI, M. GROSSI, A. MALECI, G. M. LAURO, AND B. GUIDETTI[1]

*Departments of Neurological Sciences and of Cellular and Developmental Biology, Università "La Sapienza," Rome, 00185 Italy*

*Received May 28, 1986; revision received October 21, 1986*

The possibility that, following a major lesion of the central nervous system, a humoral immune response could be evoked with formation of immune complexes "*in situ*" was investigated. For this purpose, an immunohistochemical study on rabbit and rat spinal cord at different times after surgical transection was carried out. The peroxidase–antiperoxidase method showed IgG decoration of the myelin sheaths starting a short time after surgery. The sera of intact and injured animals were then tested both by immunohistochemical methods on intact spinal cord sections and by immunoelectrophoresis on a protein extract of homologous spinal cord. The results showed in the rabbit the absence of antibodies to neural antigens before surgical injury and its appearance within a few days after surgery. On the other hand, in the rat, even before the injury, we found antibodies to neural tissue which decreased in the first few hours after injury, and returned to control values during successive days. The same experiments were conducted after a peripheral nerve lesion (sciatic nerve crush), but no immune response could be detected. The possible role of this immune response in the failure of axonal regeneration in mammalian spinal cord is briefly discussed. 

## INTRODUCTION

In higher vertebrates and particularly in mammals, the central nervous system is considered as an "immunologically privileged" organ (28) and therefore sequestered from the immune system; thus its antigens are considered "non self" (41) and a traumatic exposure of the central nervous system

Abbreviations: CNS—central nervous system, IgG—class G immunoglobulins, PAP—peroxidase–antiperoxidase, DDT—dithiothreitol, PMSF—phenylmethylsulfonyl, GAR—goat anti-rabbit, RAR—rabbit anti-rat.

[1] This work was supported by a grant of the Italian Ministry of Public Instruction. We thank Professor R. Guerrisi and Dr. E. Palatinsky for their neurosurgical assistance. Please address correspondence to Professor G. Palladini, Dipartimento Di Scienze Neurologiche.



0014-4886/87 $3.00

(CNS) is expected therefore to cause an immune response. Some authors have in fact observed the appearance of antibodies to neural antigens in this condition, both in experimental lesions (8, 26, 38) and in patients with spinal or head injuries (29, 32). In an experimental model, Gannushkina *et al.* (14) also observed IgG deposits at the lesion site, which were increased by previous immunization with whole brain homogenates.

However, the immunological nature of the phenomena has been disputed (2, 43) and their possible implications in the posttraumatic evolution of CNS lesions are still unknown. Therefore a reinvestigation of the problem appeared to be appropriate.

## METHODS

*Animal Subjects.* Ten New Zealand rabbits weighing 3 kg each (16 weeks old) and 30 Wistar rats weighing 150 g each (8 weeks old) were used.

*Surgery.* The lesion was made in eight rabbits and 25 rats under general barbiturate anesthesia. The spinal cord was transected at the lower thoracic level after laminectomy of the last dorsal vertebrae. The lesion of the peripheral nervous system was carried out in two rabbits and five rats, under general barbiturate anesthesia by crushing the sciatic nerve in the thigh 30 s with jewelry forceps. During the postoperative period the animals were housed in sterilized cages. The animals were killed at different times after surgery by an intraperitoneal barbiturate overdose.

*Sera.* Blood was collected from the animals, before surgery, 1 h after surgery, and on the following 3, 7, and 21 days. The sera were stored at −70°C after overnight absorption with homogenized homologous liver at 4°C.

*Histology.* The spinal cord and sciatic nerve from operated and intact animals were removed immediately after death, frozen in liquid nitrogen, and sectioned by a cryostat or embedded into paraffin using the freeze-drying method (Pearse Edward EPD3 tissue dryer) to ensure the best preservation of the tissue antigens (39). Longitudinal sections of spinal cord and sciatic nerve were processed by conventional histology, including Bodian's silver stain, and by immunohistochemical tests.

*Immunohistochemistry.* Longitudinal paraffin-embedded sections of the spinal cord and sciatic nerve were tested for the presence of class G immunoglobulins (IgG) by the peroxidase–antiperoxidase method (PAP). For this purpose, the rabbit specimens were incubated 30 min with normal goat serum diluted 1:25, to prevent the nonspecific binding of the goat immunoglobulin through its Fc fragments (1) and after 1 h at room temperature with a goat anti-rabbit (GAR) IgG (Miles Laboratories, Elkhart, Indiana), diluted 1:25 in phosphate-buffered saline (PBS). The sections were treated with a rabbit PAP complex (Miles) diluted 1:200 and then stained 5 min with a

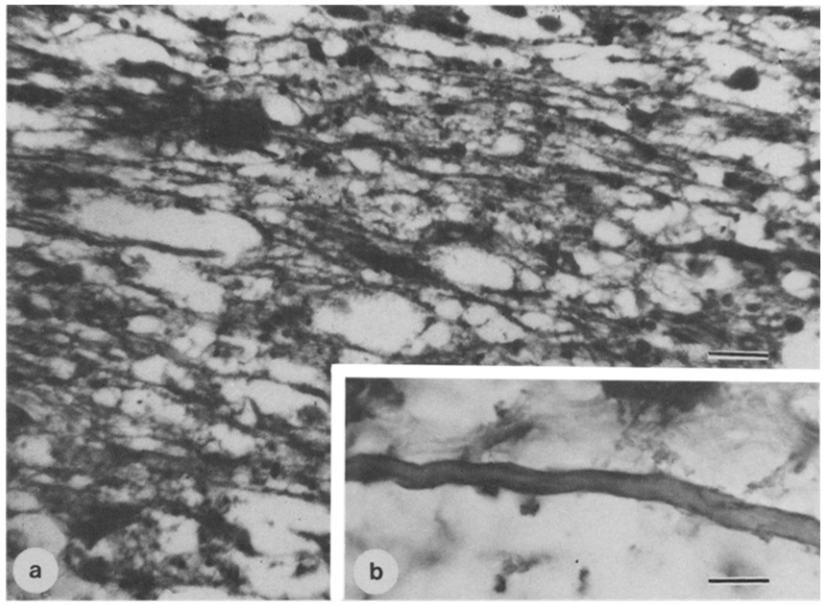


FIG. 1. a—Rabbit spinal cord longitudinally sectioned 7 days after surgical transection and tested by the PAP method for the presence of IgGs. Scale bar 83 $\mu$m. b—Higher magnification shows the localization of IgGs on the surface of the myelin sheath. Scale bar 6.2 $\mu$m.

fresh solution of 0.5% 3,3-diaminobenzidine–tetrahydrochloride (DAB) and 0.15% $H_2O_2$ in 0.05 *M* Tris–HCl, *p*H 7.6.

The rat specimens were treated similarly after incubation 1 h with a rabbit anti-rat (RAR) IgG (Miles) diluted 1:80, preceded by normal goat serum incubation.

To detect antibodies to CNS antigens in the sera of intact and operated animals, the indirect immunofluorescence technique was used on transverse and longitudinal cryostatic sections of intact spinal cord and sciatic nerve.

The rabbit spinal cord and sciatic nerve were incubated 30 min with normal goat serum and later for 30 min at room temperature with homologous sera collected before and after surgery. Then, the specimens were stained with rhodamine-conjugated F(ab')2 fragments-GAR IgG (Cappel, W. Chester, Pennsylvania), diluted in PBS 1:200, and the rat spinal cord and sciatic nerve were stained with rhodamine-conjugated F(ab')2 fragment-RAR IgG (Cappel) diluted in PBS 1:200 after incubation with normal goat serum and homologous sera collected before and after surgery. The sections were then observed with a Leitz Ploem-Opak fluorescence microscope with M2 filters combination.

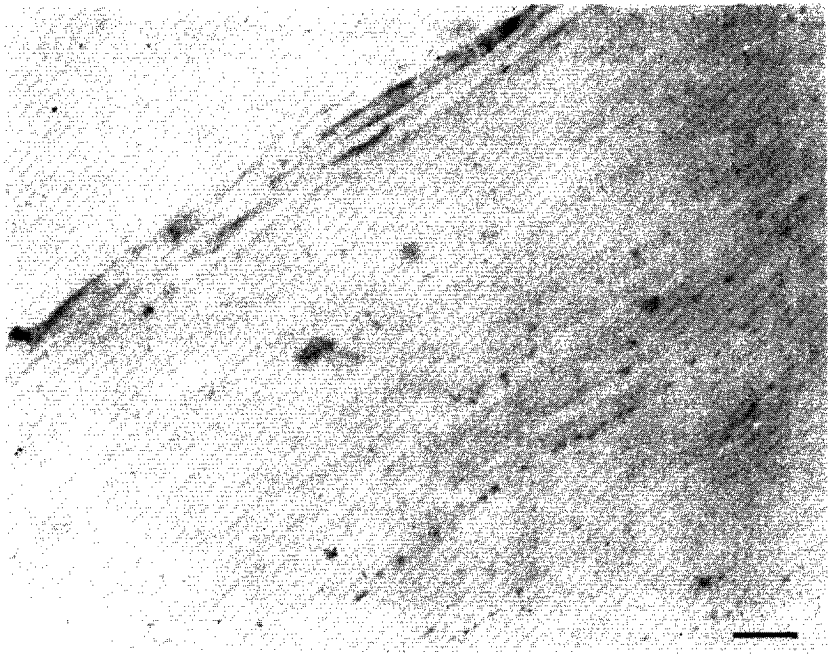

FIG. 2. Normal rabbit spinal cord longitudinally sectioned and tested by PAP method for the presence of IgGs counterstaining by hemalum. Scale bar 83 $\mu$m.

Immunohistochemical control assays were carried out eliminating the first antiserum in the PAP method and substituting the homologous serum with a heterologous (goat) nonimmune serum when the indirect immunofluorescence was used. The dissociation of immune complexes bound to the tissue was carried out by incubating sections with acetic acid, *p*H 2.5, 1 h at room temperature (16).

*Protein Extract of Spinal Cord.* The extract was obtained according to the method described by Willard and Simon (42) carried out at 4°C. Briefly, approximately 5 g rabbit or rat spinal cord, fresh or frozen at −70°C, were homogenized in 30 ml buffer A (5 m*M* EDTA, 2 m*M* dithiothreitol (DTT), 1 m*M* phenylmethylsulfonyl fluoride (PMSF), 10 m*M* Tris, *p*H 8.0) and then diluted to 360 ml with the same buffer. This homogenate was stirred 1 h then centrifuged 1 h at 100,000*g*. Proteins were precipitated from the supernatant by the addition of NaCl (final concentration 0.3 *M*), followed by stirring for 25 min, then centrifuging 1 h at 100,000*g*.

The pellet was resuspended at room temperature in 4 ml buffer A, containing 10 m*M* DTT, 3% sodium dodecyl sulfate without PMSF. The suspension was heated 5 min at 90°C and centrifuged 45 min at 100,000*g* at 20°C. The pellet was discarded and the supernatant stored at −70°C.

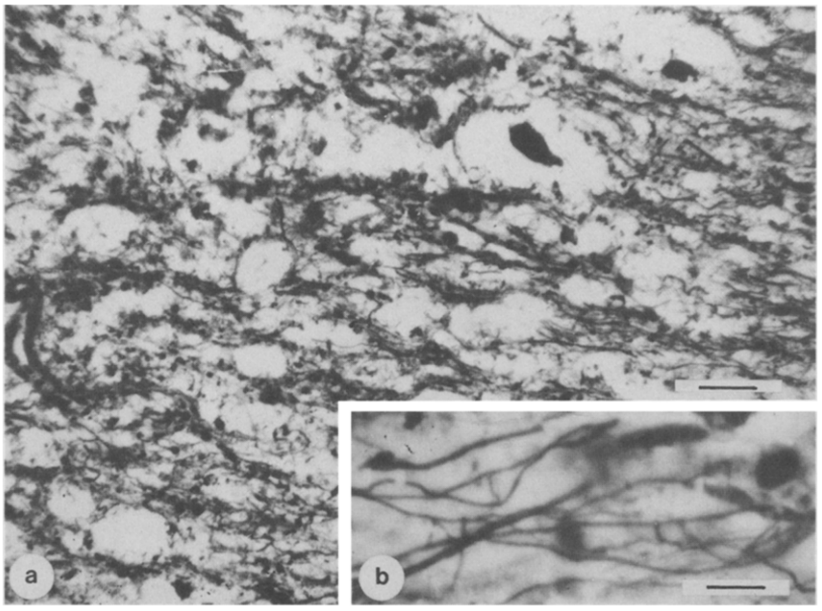


FIG. 3. a—Rabbit spinal cord longitudinally sectioned 7 days after surgery and stained by the Bodian method. Scale bar 83 $\mu$m. b—Same picture at high magnification showing growth cones. Scale bar 13 $\mu$m.

*Immunoelectrophoresis.* Five microliters of the protein extract, obtained as described above, were subjected to agarose gel electrophoresis (1% agarose in Veronal buffer, *p*H 8.2), applying a current of about 3 mA per slide with a voltage of about 6 V/cm.

After electrophoresis, the sera of injured and uninjured animals were placed in the central well overnight. The precipitin arcs were stained using Coomassie brilliant blue R-250.

## RESULTS

*Spinal Cord Lesion.* Starting 3 days after surgical transection in both rat and rabbit, the myelin sheaths appeared to be uniformly decorated by IgG antibodies along the full length of the spinal cord by the PAP procedure.

The immunohistochemical picture appeared grossly unchanged on the 7th and 12th day after the injury. The controls prepared either omitting the IgG antiserum or after pretreatment with acetic acid, *p*H 2.5, appeared negative. Also, sections of intact spinal cord, tested for the presence of IgGs adherent to the myelin sheaths, appeared negative (Figs. 1 and 2). Injured spinal cord sections made negative by the low *p*H treatment, became PAP positive with the same pattern if reincubated with homologous injured animal serum.

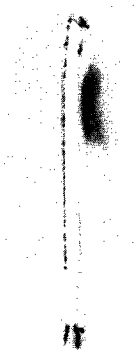

FIG. 4. Immunoelectrophoresis against spinal cord extract of rabbit sera collected 7 days after surgery.

Classical histology confirmed the morphological picture already described by Cajal (34, 35). During the first 7 days following the spinal lesion, adjacent sections stained with Bodian's method showed a strong development of growth cones at the tip of the injured fibers (Fig. 3) whereas the majority of the growth cones had disappeared after 21 days.

When the animal sera, collected before and after the surgical lesion, were tested by immunoelectrophoresis on a protein extract of homologous spinal cord, the results showed differences between rabbits and rats. The rabbit sera collected before surgery did not show any precipitin arc, and antibodies were detectable only 2 to 3 days after the injury; such reactivity was still present after 21 days, the longest survival time in our study (Fig. 4).

On the other hand, antibodies directed against CNS antigens could be detected in the sera of uninjured rats as well as of those that were operated. However, we found that the antibodies in rats were not detectable 1 h after the surgical injury, and then returned to the values of intact animals after 3 days (Table 1, Fig. 5).

The reactivity of the sera collected before and after injury was not affected by absorption on homogenized homologous liver.

The sera collected from the operated animals were tested on cryostat sections of intact spinal cord by indirect immunofluorescence. Serum antibodies stained the uninjured fibers and transverse sections clearly indicated the myelin sheaths, and not the axons, as the target of the antibodies (Figs. 6, 7). The pattern of immunostaining was similar to that observed in sections of transected spinal cord stained with IgG antiserum.

Sera from uninjured rats but not from uninjured rabbits contained antibodies, which decorated fibers of the intact spinal cords, confirming the re-

TABLE 1

Immunoelectrophoretic Analysis of the Animal Sera on a Proteic Extract of Homologous Spinal Cord

| Serum | Spinal cord |
|---|---|
| Intact rabbit | – |
| Operated rabbit | |
| 3 h after cordotomy | – |
| 3 days after cordotomy | + |
| 7 days after cordotomy | + |
| 23 days after cordotomy | + |
| 3 h after sciatic crush | – |
| 8 days after sciatic crush | – |
| 23 days after sciatic crush | – |
| Intact rat | + |
| Operated rat | |
| 1 h after cordotomy | – |
| 3 days after cordotomy | + |
| 7 days after cordotomy | + |
| 23 days after cordotomy | + |
| 1 h after sciatic crush | + |
| 8 days after sciatic crush | + |

sults obtained with immunoelectrophoresis (Table 2). Such reactions were inhibited by treatment with acetic acid *p*H 2.5 before incubating the sections with the labeled anti-IgG F(ab')2 antiserum.

*Sciatic Nerve Lesion.* The longitudinal sections of rabbit and rat sciatic nerves, removed 8 days after crushing, did not show any IgG adherent to the myelinated fibers when tested by the PAP method. At the same time, adjacent sections stained with Bodian's silver stain showed vigorous regeneration of the axons, bridging the lesion site.

The rabbit sera collected before and after the crushing did not show any precipitin arc with the spinal cord protein extract. The rat sera collected 1 h, 8 days, and 24 days after the surgical injury, reacted as the sera of the intact animals (Table 1).

Rabbit and rat sera collected before and after crushing the sciatic nerve were tested on cryostat sections of the intact peripheral nerve by the indirect immunofluorescence method. These sera did not recognize any antigenic structure associated with the myelin of the peripheral nervous system. The same sera tested on intact cord showed no variations compared with uninjured animal sera (Table 2).

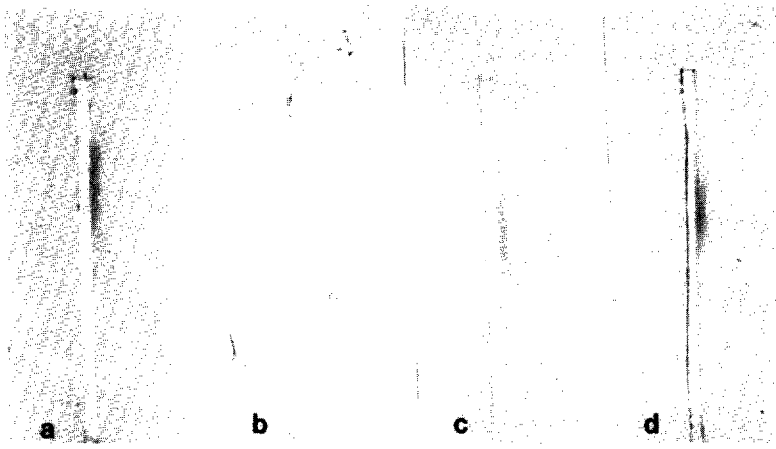


FIG. 5. Immunoelectrophoresis against spinal cord extract of rat sera collected at different times before and after surgery. The precipitin arc was present in a (serum of the intact animal), almost disappeared in b (1 h after transection), and was again evident in c and d (respectively, 3 and 7 days after transection).

## DISCUSSION

Our data show that within a short time after spinal cord injury, immune complexes appear at the myelin level in the transected spinal cord as evidenced by staining with IgG antibodies. Furthermore, our data show the rapid appearance of antibodies to spinal cord antigens in the sera of rabbits after spinal cord transection and the presence of such antibodies in nonoperated rats. The sudden appearance of these antibodies in the rabbit and their rapid decrease in the rat after injury followed by a striking increase within a short time, could be explained as a secondary (anamnestic) immunological response. The correspondence of the immunohistochemical pattern in the injured spinal cord stained with IgG antibodies and in the intact spinal cord immunostained with sera of operated animals suggests that the involved antigens were similar and probably related to the spinal cord antigens revealed by immunoelectrophoresis.

We believe that our immunohistologic findings are due to antigen-antibody reactions and not to nonspecific protein–protein interactions (2, 31). Sections were constantly negative when the first IgG antiserum was omitted in the PAP method and when the homologous serum was substituted with a heterologous serum in indirect immunofluorescence. The reaction was inhibited by preincubating the sections with low *p*H buffer, which is generally considered a method to release antibodies bound to antigens (16). The use of a specific anti-IgG serum excluded a specific IgM reactivity versus lipids

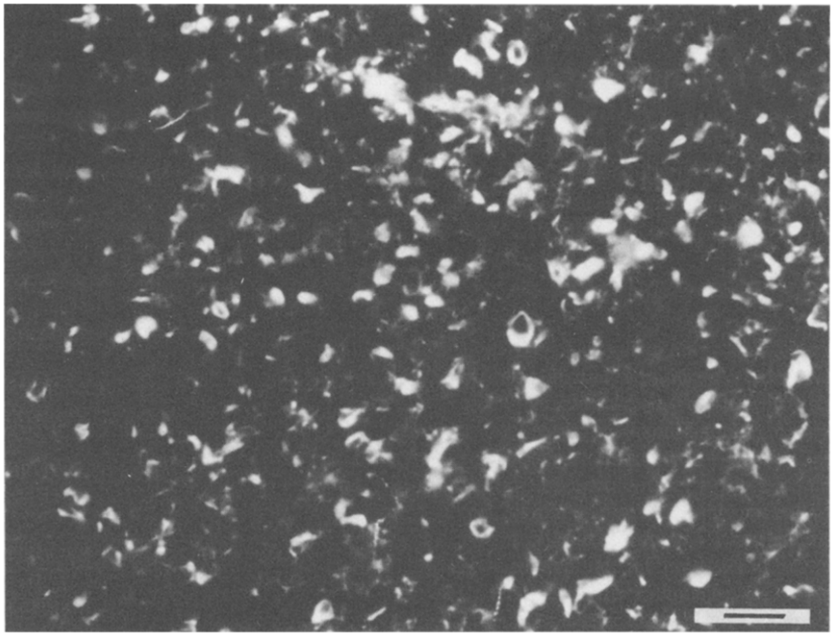

FIG. 6. Immunofluorescence of cross section of rat spinal cord, using homologous sera. Myelinated axons cut in cross section showed a positive reaction in the myelin sheath. Scale bar 35 $\mu$m.

(21). The pretreatment with nonimmune goat serum, that renders unavailable the binding sites for the Fc fragment of the IgG (12), showed that the reaction was not caused by a nonspecific binding of Fc, as proposed by other authors to occur in the CNS (1, 31). To exclude the presence of the receptors for heavy chains common to Fc and F(ab')2 fragments (1), sections of intact spinal cord were treated with IgG antibodies, without preincubation with normal goat serum; the results were completely negative.

Recent studies suggest that axonal growth is related to interactions between the growth cone and the substrate, and that it can be stopped by antibodies directed against the substrate (25, 37, 40) or against growth factors coming from the target organ (18, 19).

We believe therefore that the "abortive regeneration" (34, 35) of the central axons in mammals is not due to a real inability of the neurons or to scarring as proposed in the past (6, 26) but it could be related to the presence of a nonpermissive substrate for the CNS (3, 17). It is possible that the "nonpermissivity" of the CNS substrate could be related to the presence of the immune complexes observed on the myelin sheaths. Several data in the liter-

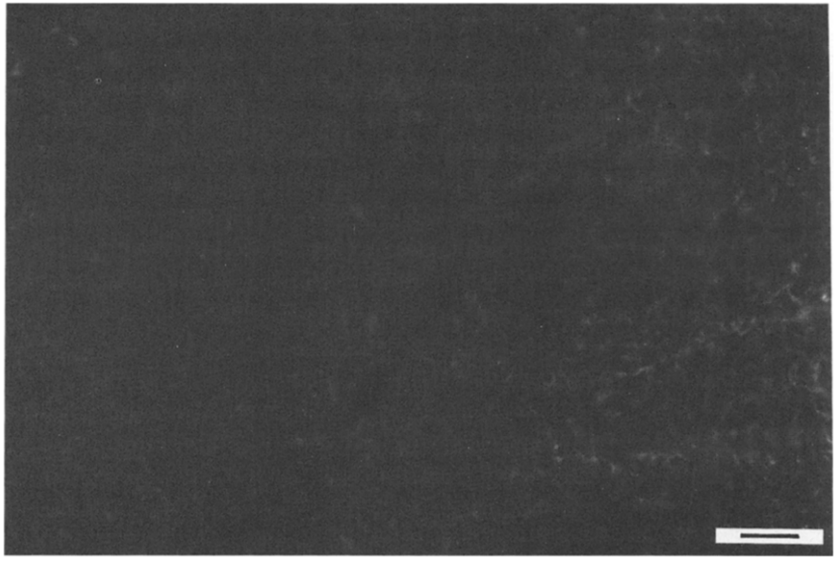

FIG. 7. The same as Fig. 6 substituting the homologous serum with an heterologous serum. Scale bar 35 $\mu$m.

ature are consistent with our hypothesis. The absence of CNS antigens and consequently of the immune complex formation may explain the easy growth of the central axons in peripheral nerve grafts (7, 9–11). Conversely, the presence of CNS antigens and therefore of the immune complexes could explain the inability of peripheral axons to grow in optical nerve grafts (27).

TABLE 2

Indirect Immunofluorescence of Intact Spinal Cord and Sciatic Nerve by Sera from Intact and Operated Animals

| Serum | Spinal cord | Sciatic nerve |
|---|---|---|
| Intact rabbit | – | – |
| Operated rabbit 7 days after cordotomy | + | – |
| Operated rabbit 21 days after cordotomy | + | – |
| Operated rabbit 7 days after sciatic crushing | – | – |
| Intact rat | + | – |
| Operated rat 7 days after cordotomy | + | – |
| Operated rat 21 days after cordotomy | + | – |
| Operated rat 7 days after sciatic crushing | + | – |

Furthermore, the lack of CNS antigens also explains, in our opinion, why, in some cases, the CNS fibers are able to invade fragments of nonnervous tissues (5, 23). The functional, although moderate, regeneration observed in newborn hamsters (22, 36), in hibernating animals (20), and in rat fetuses (15) may be related to partial immunodeficiency phenomena, while the ineffective regenerative enhancement observed after antimitotic (30), corticosteroid, or pyromen therapy (24) could be explained with the low activity of the immunosuppressive therapies in the anamnestic immune reaction (13). Moreover, our unpublished observations indicate that in *Lacerta sicula,* in which corticospinal regeneration is functionally complete (33), no *in situ* immune complexes are seen, probably in relation to the extreme slowness of antibody synthesis and to the rapidity of spinal regeneration in these animals.

Our hypothesis must be considered different from the immunologic primitive theory (4) which connects the abortive regeneration with an autoimmune response to neuron components that can stop axon growth. In our mind, on the contrary, autoimmune reactions directed against the environmental substrate render it nonpermissive for axonal growth.

## REFERENCES


1. AARLI, J. A., S. R. APARICIO, C. E. LUMSDEN, AND O. TONDER. 1975. Binding of normal human IgG to myelin sheaths, glia and neurons. *Immunology* **28:** 171–135.
2. AARLI, J. A. 1983. The immune system and the nervous system. *J. Neurol.* **229:** 137–154.
3. BARNES, D. M. 1985. What PNS nerves regenerate? *Science* **230:** 1024–1025.
4. BERRY, M., AND A. C. RICHES. 1974. An immunological approach to regeneration in the central nervous system. *Br. Med. Bull.* **30:** 135–140.
5. BERRY, M. 1979. Regeneration in the central nervous system. Pages 67–111 in W. T. THOMAS SMITH AND J. B. CAVANAGH, Eds. *Recent Advances in Neuropathology.* Churchill-Livingstone, Edinburgh.
6. BERRY, M. 1982. Post-injury myelin-breakdown products inhibit axonal growth: an hypothesis to explain the failure of axonal regeneration in the mammalian CNS. *Bibl. Anat.* **23:** 1–11.
7. BENFEI, M., AND A. J. AGUYAO. 1982. Extensive elongation of axon from rat brain into peripheral nerve graft. *Nature* **296:** 150–152.
8. BOWEN, F. P. 1986. Immunologic reaction after cortical lesions in rabbits. *Arch. Neurol.* **19:** 398–402.
9. CHI, N. H., A. BIGNAMI, N. T. BICH, AND D. DAHL. 1980. Autologous sciatic nerve graft to the rat spinal cord immunofluorescence studies. *Exp. Neurol.* **68:** 568–580.
10. CHI, N. H., AND D. DAHL. 1983. Autologous peripheral nerve grafting into murine brain as a model for studies of regeneration in the central nervous system. *Exp. Neurol.* **79:** 245–264.
11. DAVID, S., AND A. J. AGUAYO. 1981. Axonal elongation into peripheral nervous system "bridges" after central nervous system injury in adult rats. *Science* **214:** 931–933.
12. DONNENFELD, H., R. J. KASCSAK, AND H. BARTFELD. 1984. Deposits of IgG and C3 in the spinal cord and motor cortex of ALS patients. *J. Neuroimmunol.* **6:** 51–57.

13. EISEN, H. N. 1969. Immunology. Pages 491–496 in B. D. DAVIS, R. DULBECCO, H. N. EISEN, H. S. GINSBERG, AND W. B. WOOD, Eds., *Microbiology.* Harper, New York/Evanston/London/Tokyo.
14. GANNUSHKINA, I. V., B. B. JOHANSSON, AND L. I. PERSON. 1982. Increased blood–brain barrier dysfunction around cerebral stab wounds in rat immunized to brain antigen. *Acta Neurol. Scand.* **66:** 482–487.
15. GERARD, R. W., AND T. KOPPANY. 1962. Studies on spinal cord regeneration in rat. *Am. J. Physiol.* **76:** 211–212.
16. GILDEN, D., M. DELVIN, AND Z. WROMBLEWSKA. 1978. A technique for the elution of cell-surface antibody from human brain tissue. *Ann. Neurol.* **3:** 403–405.
17. GRAFSTEIN, B. 1980. Cellular mechanism for recovery from nervous system injury. *Surg. Neurol.* **13:** 363–365.
18. GURNEY, M. E. 1984. Suppression of sprouting at the neuromuscular junction by immune sera. *Nature* **307:** 546–548.
19. GURNEY, M. E., A. C. BELTON, I. V. CASHMAN, AND S. P. ANTEL. 1984. Inhibition of terminal axonal sprouting by serum from patients with amyotrophic lateral sclerosis. *N. Engl. J. Med.* **311:** 933–939.
20. GUTH, L., C. P. BARRET, E. J. DONATI, S. S. DESHPANDE, AND E. X. ALBUQUERQUE. 1981. Histopathological reactions and axonal regeneration in the transected spinal cord of hibernating squirrels. *J. Comp. Neurol.* **203:** 297–298.
21. HOFSTETTER, W., C. H. HEUSSER, AND K. BLASER. 1985. Nonspecific binding of mouse IgM antibodies to lipid antigen. *J. Neuroimmunol.* **7:** 207–214.
22. KALIL, K., AND T. REH. 1982. A light and electron microscopic study of regrowing pyramidal tract fibers. *J. Comp. Neurol.* **211:** 265–275.
23. KIERNAN, J. A. 1978. An explication of axonal regeneration in peripheral nerves and its failure in the central nervous system. *Med. Hypotheses* **4:** 15–26.
24. KIERNAN, J. A. 1979. Hypothesis concerned with axonal regeneration in the mammalian nervous system. *Biol. Rev.* **54:** 155–197.
25. LIESI, P., D. DAHL, AND A. VAHERI. 1984. Neurons cultured from developing rat brain attach and spread preferentially to laminin. *J. Neurosci. Res.* **11:** 241–251.
26. MASADA, S. 1965. Immunological studies of brain injuries. *Clin. Neurol.* **5:** 547–555.
27. MARX, J. L. 1980. Regeneration in the central nervous system. *Science* **209,** 379–380.
28. MEDAWAR, P. B. 1948. Immunity to homologous grafted skin. III. *Br. J. Exp. Pathol.* **29:** 58–69.
29. MIZRACHI, Y., AND A. OHRY. 1983. Systemic humoral factors participating in the course of spinal cord injury. *Paraplegia* **21:** 287–293.
30. PALLADINI, G., AND L. ALFEI. 1965. Observations concerning the regeneration of the spinal cord of the adult rat during treatment with antiblastic substances. Pages 515–519 in V. KIORTSIS AND H. A. L. TRAMPUSCH Eds., *Regeneration in Animals and Related Problems.* North-Holland, Amsterdam.
31. PEARSE, A. G. E. 1968. *Histochemistry Theoretical and Applied.* Vol. I, pp. 185–187. Churchill-Livingstone, Edinburgh.
32. PROCHAZKA, M., M. VOLTHEROVA, AND J. STEPHAN. 1971. Studies on immunological reaction after brain injury. II. *Int. Surg.* **55:** 322–326.
33. RAFFAELLI, E., AND G. PALLADINI. 1969. Rigenerazione delle cellule e degli assoni del midollo spinale dorsale di *Lacerta sicula. Boll. Zool.* **36:** 105–110.
34. RAMÓN Y CAJAL, S. 1907. Note sur la dégénérescence traumatique des fibres nerveuses du cervelet et du cerveau. *Trab. Lab. Rec. Biol. (Madrid)* **5:** 105–151.
35. RAMÓN Y CAJAL, S. 1914. *Estudios sobra la degeneracion y la regeneracion del sistema nervioso.* Moya, Madrid.

36. REH, T., AND K. KALIL. 1982. Functional role of regrowing pyramidal tract fibers. *J. Comp. Neurol.* **211:** 276–283.
37. SANES, J. R. 1985. Laminin for axonal guidance? *Nature* **315:** 714–715.
38. STEFAN, J., M. PROCHAZKA, AND M. VOLTNEROVA. 1971. Studies on immunological reactions after brain injury. I. *Int. Surg.* **55:** 316–321.
39. STEIN, H., A. HERYET, K. C. GATTER, AND D. Y. MASON. 1984. Freeze-dried paraffin-embedded human tissue for antigen labelling with monoclonal antibodies. *Lancet* **2:** 71–73.
40. THOENEN, H., AND D. EDGAR. 1985. Neurotrophic factors. *Science* **229:** 238–242.
41. WAKSMAN, B. H. 1983. Immunity and the nervous system: basic trends. *Ann. Neurol.* **13:** 587–591.
42. WILLARD, M., AND C. SIMON. 1981. Antibody decoration of neurofilaments. *J. Cell Biol.* **89:** 198–205.
43. WILLENBORG, D. O., E. A. STATEN, AND E. EIDELBERG. 1977. Studies on cell-mediated hypersensitivity to neuronal antigens after experimental spinal injury. *Exp. Neurol.* **54:** 383–392.