der of the tubules than in the rest of the tissue) might be correlated with at least in part to difficulties of antibody penetration into the dense and packed collagenous dentinal matrix.

Type I dentinal collagen consists of large and well organized cross-banded fibrils with a pattern similar to the peroxidase deposits found in previous studies[9,11]. This might indicate that the antigenic sites of collagen molecules are not altered by the demineralization process. On the other hand, the type I trimer, biochemically detected in lathyritic rat dentine[12] or normal bovine dentine[5] cannot yet be immunologically and morphologically distinguished from type I fibrils, thus leaving this question open for further investigation.

Finally, it seems obvious that odontoblasts, which were shown to elaborate type I procollagen only[6,13] are the only cells responsible for the synthesis of dentinal collagen. Thus, the dentine probably does not represent a mineralized pulp collagen matrix as the latter contains large amounts of type III collagen[14].

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2 Carmichael, D.J., Dodd, C.M., and Nawrot, Calcif. Tissue Res. *14* (1974) 177.
3 Scott, P.G., and Veis, A., Conn. Tissue Res. *4* (1976) 117.
4 Munksgaard, C.M., Rhodes, M., Mayne, R., and Butler, W.T., Eur. J. Biochem. *82* (1978) 609.
5 Munksgaard, E.C., and Moe, D., Archs oral Biol. *25* (1980) 485.
6 Lesot, H., and Ruch, J.V., Biol. Cell *34* (1979) 23.
7 Thesleff, I., Stenman, S., Vaheri, A., and Timpl, R., Devl Biol. *70* (1979) 116.
8 Sauk, J.J., Gay, R., Miller, E.J., and Gay, S., J. oral Path. *9* (1980) 210.
9 Grimaud, J.A., Druguet, M., Peyrol, S., Chevalier, O., Herbage, D., and El Badrawy, N., J. Histochem. Cytochem. *28* (1980) 1145.
10 Graham, R.C., and Karnovsky, M.J., J. Histochem. Cytochem. *14* (1966) 291.
11 Wick, G., Bjorn, R.O., and Timpl, R., Lab. Invest. *39* (1978) 151.
12 Wohllebe, M., and Carmichael, D.J., Eur. J. Biochem. *92* (1978) 183.
13 Karim, A., Cournil, I., and Leblond, C.P., J. Histochem. Cytochem. *27* (1979) 1070.
14 Lechner, J.H., and Kalnitsky, G., Archs oral Biol. *26* (1981) 265.

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Restoration of full mass in nerve-intact muscle grafts after delayed reinnervation[1]

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Summary. A rat muscle freely grafted with the motor nerve intact becomes restored to full mass and contractile function, in contrast to the reduced weight of a standard free graft. By crushing the nerve to a nerve-intact graft and delaying reinnervation, full mass is still restored. One can conclude that earlier reinnervation is not the reason for the success of nerve-intact grafts, but that it is rather due to reinnervation along preserved Schwann cell channels.

Autogenous free grafts of entire muscles undergo a sequence of degeneration and regeneration of muscle fibers before becoming functionally reintegrated with the host[2]. Yet, typical standard free muscle grafts become stabilized at weights and contractile strengths only 35–50% of those of their normal counterparts[3].

In a recent experimental model, rat extensor digitorum longus (EDL) muscles were freely grafted as before, but with the motor nerve to the muscle left intact[4]. The early development of nerve-intact grafts was identical to that of standard free muscle grafts (with no preserved nerve connections), but the nerve-intact grafts were eventually restored to normal mass and normal or near-normal contractile properties. In searching for variables that might account for the difference between the 2 types of grafts, no difference was found in the number of muscle fibers between standard grafts, nerve-intact grafts or control muscles, but the muscle fibers in nerve-intact grafts were considerably larger than those of standard grafts. A major difference between standard and nerve-intact grafts was the time of formation of functional neuromuscular junctions. in standard grafts, neuromuscular junctions showed demonstrable function around the end of the 3rd postoperative week, whereas in nerve-intact grafts, after the initial degeneration of the ischemic terminal portions of the nerves, neuromuscular transmission was recorded 8 days after transplantation. On the basis of these results, it was suggested that the success of nerve-intact grafts might be due to the earlier restoration of functional neuromuscular junctions in these grafts. This idea was in accord with the earlier work of Hall-Craggs and Brand[5], who found improved muscle regeneration after they had previously crushed the motor nerve and allowed earlier access of the regenerating nerve fibers to the muscle.

We tested the timing hypothesis by designing the nerve-intact-crush model. In this, the EDL muscle was grafted with the motor nerve intact, but the sciatic nerve was also crushed so that regenerating nerve fibers innervated the graft at the same time after grafting (21–24 days) as reinnervation occurs in standard EDL grafts. If timing of reinnervation were the critical variable, one would expect the mass of nerve-intact-crush grafts to be less than that of nerve-intact grafts and similar to that of standard grafts.

Methods and results. This experiment was conducted on 59 male 175–200 g Sprague-Dawley and Wistar rats. All animals were anesthetized with ether. In experimental legs the EDL muscle was grafted with an intact nerve, and the sciatic nerve was crushed with forceps (fig. 1, left). Silver stained (Palmgren) preparations and confirmatory electromyographic studies, conducted as in our previous study[4],

revealed that the type of reinnervation in nerve-intact-crush grafts and standard grafts was comparable. 10 standard grafts and 10 nerve-intact-crush grafts in opposite legs of the same rats were studied, with no neuromuscular (NM) transmission in 3 18-day grafts, evidence of NM transmission in 2 27-day grafts of each type and evidence of NM transmission in 2 of 5 grafts of each type taken at 21 and 24 days.

A comparison between the weights of a paired series of nerve-intact and nerve-intact-crush grafts is illustrated in figure 2. During the 1st postoperative week, both kinds of grafts showed a rapid drop in weight due to the removal of damaged cytoplasm from the original muscle fibers. Then the weights of both types of grafts progressively increased until by 60 days there was no difference in the mean weights of both kinds of grafts and non-operated control muscles. The relative weights of the nerve-intact-crush grafts (fig. 2) are less than those of nerve-intact grafts because of the atrophy caused by the temporary denervation of the entire leg in the crush experiments. As seen in figure 2, non-grafted control muscles from legs subjected to the same type of nerve crush were also relatively lighter than normal EDL muscles.

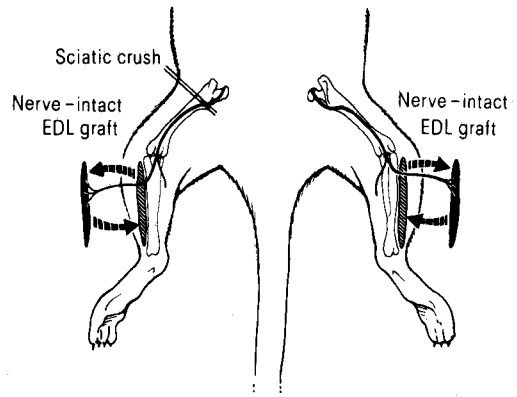

Figure 1. Diagram of operative procedure used in this experiment. On the right side an EDL muscle is removed from its bed with only its motor nerve left intact and is then grafted back into its original bed. On the left (nerve-intact-crush) the muscle is grafted as on the right, but the sciatic nerve is also crushed where indicated.

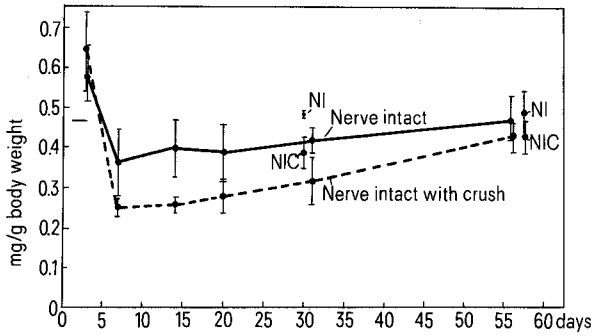

Figure 2. Weights of paired nerve-intact vs nerve-intact-crush rat EDL grafts expressed as mg/g b.wt. NI, normal EDL muscles to control for the weights of the nerve-intact grafts; NIC, ungrafted EDL muscles subjected to prior sciatic nerve crush to control for weights of mature nerve-intact-crush grafts. Each point in the curve is a mean ± SE of 7 grafts. Each control point (NI, NIC) is a mean of 8 muscles.

Discussion. These results show that both nerve-intact and nerve-intact-crush grafts ultimately become restored to the mass of non-transplanted control muscles (fig. 2). This is in contrast to the relatively poor restoration of mass in standard muscle grafts. One can conclude that the earlier restoration of neuromuscular transmission is not the variable that accounts for the full restoration of mass in nerve-intact grafts because full restoration was also accomplished in the nerve-intact-crush grafts in which functional reinnervation was delayed for as long as it is in standard grafts. Thus the hypothesis that the mechanism of success of nerve-intact grafts is the earlier return of a functional nerve supply can be rejected. We do not know, however, the time beyond which return of innervation to a muscle graft would result in a reduction in the mass of a graft.

On the basis of accumulated evidence, it now appears that the critical factor in nerve-intact grafts is the broader distribution of regenerating nerve fibers within the grafts through the mechanically undisturbed nerve pathways in the grafts. Because the nerve trunks in standard grafts are severed, it is less likely that nerve fibers growing into the grafts could find their way back to the sites of all the original motor endplates. The results of both McMahan's group[6] and Bader[7] have demonstrated the importance of the original synaptic areas in the reinnervation of regenerating muscle. Not only the direct counts of Bader[7], but also an earlier study, with choline acetyltransferase activity as an indicator[8], showed a reduced innervation in standard EDL grafts in the rat. From the practical standpoint of improving the quality of muscle transplants, it would appear that anastomosis of a proximal nerve segment with the nerve trunks leading into a freely grafted muscle would be the recommended procedure on the basis of our knowledge to date[9].

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2 Freilinger, G., Holle, J., and Carlson, B.M., Muscle transplantation. Ed. G. Freilinger. Springer, Wien 1981.
3 Carlson, B.M., and Gutmann, E., Pflügers Arch. *353* (1975) 215.
4 Carlson, B.M., Hník, P., Tuček, S., Vejsada, R., and Bader, D., Physiol. bohemoslov. *30* (1981) 505.
5 Hall-Craggs, E.C.B., and Brand, P., Exp. Neurol. *57* (1977) 275.
6 Marshall, L.M., Sanes, J.R., and McMahan, U.J., Proc. natl Acad. Sci. USA *74* (1977) 3073.
7 Bader, D., Devl Biol. *77* (1980) 315.
8 Carlson, B.M., Wagner, K.R., and Max, S.R., Muscle Nerve *2* (1979) 304.
9 Faulkner, J.A., Markley, J.M., and White, T.P., in: Muscle transplantation, p. 47. Ed. G. Freilinger. Springer, Wien 1981.

0014-4754/83/020171-02$1.50 + 0.20/0