to that used by Miller (*9*). The effectiveness of the procedure was checked by histological verification of serial sections of the mediastinal contents. In early experiments control mice were subjected to a sham-thymectomy operation. As these animals proved to be indistinguishable from nonoperated controls the method was discontinued in later experiments.

All mice received a single supralethal radiation dose (912 r, 875 rad) from a 250 kv, 30 ma Maxitron x-ray apparatus (focus-mouse distance 58 cm, dose rate 65 rad/min, half value layer 2.1 mm Cu).

Suspensions of bone marrow cells were prepared as described previously (*8*). The donors of the bone marrow were male or female rats (100 to 125 g) of the inbred WAG strain or 8-week-old female mice of the CBA strain. Fetal liver cells were obtained from C57BL or CBA × C57BL F_1 fetuses on the 15th to 17th day of pregnancy. Each irradiated mouse was injected intravenously within 4 hours after irradiation with 2 to 5 million viable nucleated mouse bone marrow or fetal liver cells or with 17.5 to 26.0 million rat bone marrow cells. The mice were housed five to a cage and given unlimited access to a cubed ration and tap water. Suitable cleaning and sterilization procedures for cages and water bottles were strictly observed (*10*). The mice were observed daily. At least once between the 40th and the 90th day blood samples were taken from the nonisologous chimeras to ascertain whether the peripheral blood cells were donor or host type. Animals which showed signs either of incomplete chimerism or of the presence of thymic remnants were eliminated from the experiment (*11*). Since the results of the isologous experiments (with F_1 fetal liver or C57BL fetal liver) were closely comparable, the data were pooled. The results are presented in Table 1. From these data it is evident that in the isologous and in the parent-to-F_1 combinations secondary mortality is virtually absent in the controls, and late mortality is specific for the thymectomized mice. This late mortality is preceded by a wasting disease similar to that observed in neonatally thymectomized mice. The mortality in the isologous chimeras follows a similar pattern to that reported in a comparable study by Duplan and Monnot (*12*). In the heterologous chimeras there is a severe secondary mortality in the controls in the 2nd month after irradiation. In the thymectomized animals the mortality in the same period is much lower. The survival curve for each of four replicate experiments with rat bone marrow (combined in Table 1) shows this delayed mortality in the thymectomized mice.

In conclusion, under the conditions of these experiments thymectomy prior to the establishment of a radiation chimera is associated with a late mortality in those graft-host combinations where the controls show no secondary disease. In the combination in which there appears a severe early secondary disease without thymectomy, prior thymectomy is associated with a decrease and a delay in mortality. Since in the autologous combination a decreased recovery of immune activity in thymectomized chimeras has been described, it seems very likely that a similar disturbance in the immune reactivity of the donor cells will occur in homologous or heterologous chimeras. A decrease in mortality from secondary disease is in complete agreement with the supposition that this severe secondary disease is primarily caused by an immunological graft-versus-host reaction which, like other immune reactions, will be dependent on the presence of an intact thymus for the development of mature immunologically competent cells. Further study seems necessary to analyze the cause of the late mortality in the thymectomized isologous chimeras.

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Alkaline Phosphatase in Peripheral Nerves

Abstract. *By means of a simultaneous coupling azo dye technique, alkaline phosphatase at the node of Ranvier and the incisures of Schmidt-Lanterman has been identified. The presence of this enzyme at these sites may be of metabolic significance.*

Alkaline phosphatase in peripheral nerves has been studied (*1*). Although it has been described in the endothelial walls of the capillaries, its presence in neural tissue has been inconsistent and no mention has been made of its presence at the node of Ranvier nor at the incisures of Schmidt-Lanterman. We have now identified this phosphatase at the above-mentioned node and incisures.

Adult rats and cats were used. The sciatic nerve was removed under nembutal anesthesia. Care was taken to avoid crushing the nerves which upon excision were immediately immersed in cold (4°C) gum arabic for 10 to 20 minutes before the tissue was frozen rapidly in isopentane at −70°C. The period of immersion in gum arabic is varied in accordance with the diameter of the nerve, but it should not exceed 10 minutes for small nerves and 20 minutes for larger ones. The use of this cold hypertonic solution preserves the structure, although good results can be obtained by freezing directly. Next, sections (8 μ) are cu on the Cryostat and allowed to dry for 10 minutes; they are then fixed in cold formol-calcium solution at 4°C for 1 hour, washed for 30 minutes, and transferred to distilled water for 30 seconds. The sections are then placed in the naphthol AS-AN phosphate substrate with Fast red-violet LB (*2*) (*p*H 8.30) at room temperature for 1 hour (*3*). That the substrate have a *p*H of 8.30 is essential for demonstrating the activity at the incisures and nodes, as the capillaries stain over a *p*H range of 7 to 9. After incubation in the substrate, the

tissue is dehydrated in sucrose for 30 minutes before being mounted in water-soluble medium Plasdone C (*3*). The preceding technique is used in processing human specimens.

The use of AS phosphate gave reproducible definitive localization of alkaline phosphatase with light microscopy. These newer, substituted AS phosphates are distinctly superior to unsubstituted naphthol AS phosphate, and are intended to replace the previous simple naphthol phosphate substrate. The stability and permanency of the preparation are also greater than those obtained by other methods.

The results indicate where, in the nerve fiber, the alkaline phosphatase activity is localized, namely at the nodes of Ranvier and the incisures of Schmidt-Lanterman. There is no obvious difference between species except for relative size of fibers. The tissue of the rat, however, gave the appearance of having a more intense reaction than that of other species studied.

Under the microscope, a low-power view of the stained section presents a banding effect produced by the reaction at the incisures which can be seen to vary in size and shape (Fig. 1). The bands are irregularly spaced and of approximately the same intensity when stained. The capillaries, running longitudinally, are darker in staining (Fig. 1). The activity at the incisures is seen frequently throughout one internodal length. The number and shape of the incisures vary from fiber to fiber. This has been a consistent finding in the three species studied.

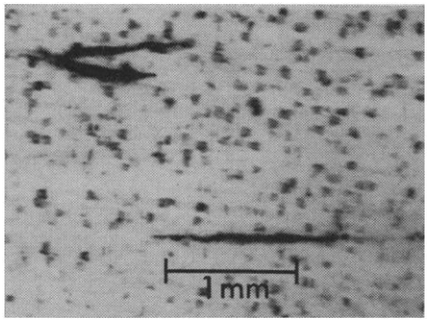

Fig. 1. Longitudinal section of normal rat sciatic nerve demonstrating activity of alkaline phosphatase. Note the irregular spacing of incisures and nodes. The dark-stained linear structures are capillaries (× 30).

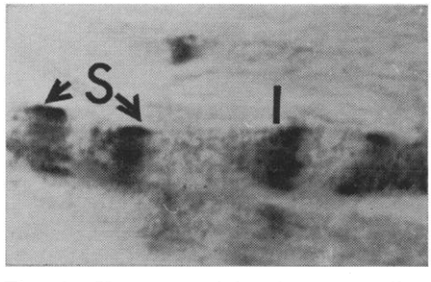

Fig. 2. Human peripheral nerve, alkaline phosphatase reaction appearing as small granules at the incisures of Schmidt-Lanterman (*I*). Note darker staining in outer portion of Schwann sheath (*S*) (× 200).

When studied at high magnification, the incisures frequently appear to be funnel-shaped (Fig. 2). At other times they appear as circumferential bands which stain more intensely in the outer region of the Schwann sheath (Fig. 3). Detailed observations of the incisures reveal the positive reaction as small granules in varying circumferential forms (Fig. 3) distributed along the incisures.

The appearance of alkaline phosphatase at the nodes resembles small rounded caps, concentrated around the axon (Figs. 3 and 4). The opposing ends of the nodes appear in close apposition at the internodal constriction (Figs. 3 and 4). The granular staining at the node is dense and gives the appearance of being more confined than at the incisures (Fig. 4).

Cylindroconic segments of variable lengths were described in myelin sheaths by Schmidt (*4*) in 1874 and Lanterman (*5*) in 1877. Cajal in 1928 discussed the controversy which arose with regard to their being functional structures or products of fixation and staining (*6*). Robertson (*7–9*) and Luxoro (*10*), using the electron microscope, described the fine structure of the incisures. Robertson (*9*) and Luxoro (*10*) show them to be shearing defects in the myelin in which the lamellae are separated widely at the major dense line and believe that they may play a part in membrane transport. If this is the case, the alkaline phosphatase in heavy concentrations at these points along the myelin sheath may aid in meeting the metabolic requirements of peripheral nerve.

The possibility of phosphatase activity in the "sodium pump" proposed by Tosteson (*11*), and the extension of Danielli's hypothesis (*12*) that the phosphatase is part of a protein in active membrane transport, might be considered. The final determination of the Schwann sheath molecular structure will not come until more is learned about the relationship between alkaline phosphatase and other enzymes which may be present in nerve myelin sheath.

Neither the reactants nor the products of the alkaline phosphatase reaction

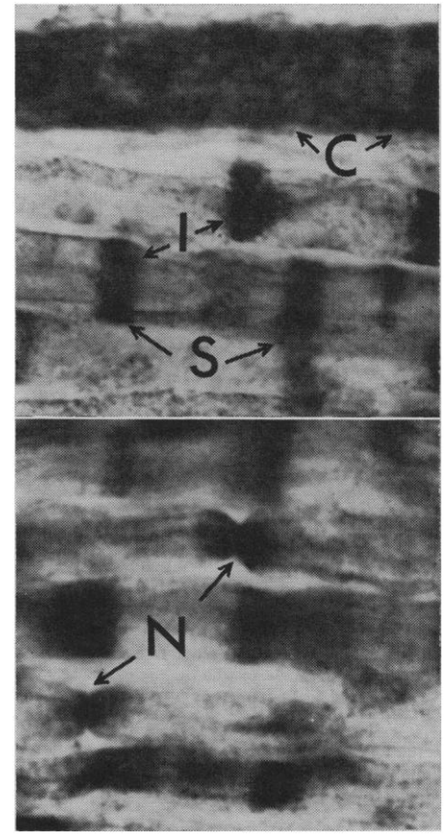

Fig. 3. Longitudinal sections of rat sciatic nerve demonstrating alkaline phosphatase activity. (Top) Positive areas of alkaline phosphatase activity distributed in endothelial wall of capillary (*C*), in incisures (*I*), and in Schwann cell sheath (*S*). (Bottom) Position of activity at nodes of Ranvier (*N*) (× 200).

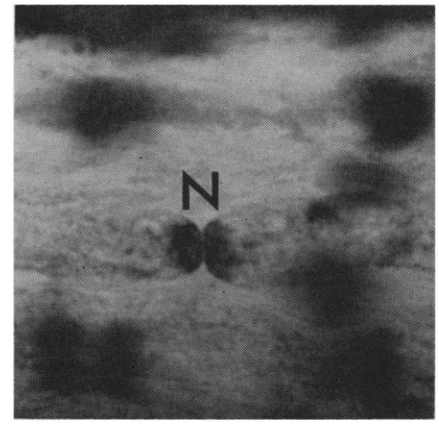

Fig. 4. Longitudinal section of rat sciatic nerve. Alkaline phosphatase activity appears more confined at node of Ranvier (*N*) (× 200).

have been identified. That this is a different type of alkaline phosphatase from that seen in capillary wall endothelium seems likely because of differing sensitivity to *p*H and environmental conditions.

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"Red" Skeletal Muscle Fibers: Relative Independence of Neural Control

Abstract. *Studies on skeletal muscle of rats and mice indicate significant differences between the behaviors of the two basic types of muscle fibers during the development of denervation atrophy. While the diameter of fibers possessing sarcoplasmic granularity ("red" fibers) is preserved for a long time after complete severance of motor and sensory nerves, fiber with agranular cytoplasm ("white" fibers) undergo rapid atrophy. It is concluded that, at least in this respect, the "white" fibers are more dependent on neural control than are the "red" fibers.*

It has been known for some time that, even in the same species, the rate of atrophy following severance of motor nerves is significantly different in the various skeletal muscles and that some fibers atrophy much more rapidly than do others within the same muscle (*1*). No satisfactory hypothesis has been offered to explain this peculiar phenomenon in denervated muscle and, in fact, the problems associated with the genesis and prevention of neural atrophies are still largely unsolved (*2*).

It is now well established that the terms "red" and "white," as applied to a muscle, do not imply homogeneity of the component fibers, but that most of the mammalian skeletal muscles are composed of two general categories of fibers—that is, "red" and "white" fibers, that differ from each other both in certain structural characteristics and biochemical activities (*3*). In this report it is shown that evaluation of the progression of denervation atrophy, from this point of view, not only offers an explanation for the difference in reactivity of the various muscle groups subsequent to nerve lesions, but also suggests that the "white" fibers are more dependent on neural control than are the "red" ones.

The object of this study was to analyze comparatively the behaviors of the two basic types of muscle fibers during the development of three forms of muscle atrophy: (i) denervation atrophy, (ii) disuse atrophy, and (iii) dystrophy that develops spontaneously in strain-129 mice suffering from a hereditary form of myopathy. Male and female rats and mice of various ages were used and the investigations were centered on groups of muscles in the hind limbs. Total denervation was effected through a suprapubic incision; the left obturator, femoral, and sciatic nerves were sectioned intrapelvically, and a portion of the axons, several millimeters in length, was then removed to prevent regeneration. Disuse atrophy was induced either by tenotomy or by application of a plaster cast (*4*); the tenotomized triceps surae was isolated in a Millipore tube to prevent adhesions or regeneration of the sectioned tendon. The strain-129 mice were selected from our breeder colony (*5*). The animals were killed by decapitation at various intervals ranging from 1 day to several months after surgical intervention, after the initiation of immobilization by a plaster cast, or, in the case of strain-129 mice, after the presence of dystrophy could be clinically diagnosed. At autopsy, the muscle specimens were frozen rapidly in liquid air, stored at −70°C, and sectioned in a cryostat in 10-μ thicknesses. Succinic dehydrogenase was demonstrated with 3-(4,5-dimethyl-thiazolyl-2)-2,5-diphenyl-tetrazolium bromide (MTT) (*6*); for routine morphologic studies the frozen tissue was fixed after sectioning with formalin and stained with hematoxylin-phloxine-saffron (HPS).

The distribution pattern of oxidative enzymes is different in the "red" and "white" fibers (*7*) and fibers showing significantly greater succinic dehydrogenase activity are usually smaller in diameter than those showing less activity (Fig. 1). The technique for detecting succinic dehydrogenase activity was not suitable for analyzing the two categories of fibers because soon after denervation a qualitative alteration in the normal distribution of succinic dehydrogenase ensued; gradually, all denervated fibers reacted uniformly in the test for this enzyme (Fig. 2).

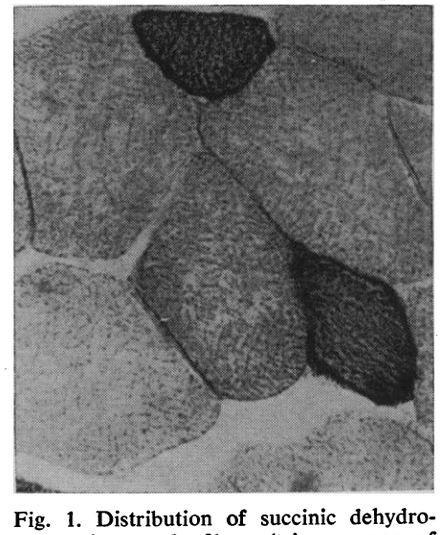

Fig. 1. Distribution of succinic dehydrogenase in muscle fibers (triceps surae of normal mouse).