the pathways (Bungner's cords) preferentially followed by regenerating fibers. Here, the deficit was considerably greater than in experiment I, namely, at H: 42.2%; at E: 67.2%; at W: 67.5%.

Experiment IV. (6 cases.) Combining the operations of Exp. II and Exp. III. The differences were, at H: 39.4%; at E: 64.7%; at W: 68.8%. Comparing these figures with those of Exp. III, one notices again that the reduction of the proximal nerve source has failed to entail a corresponding reduction in the amount of fibers found within the limb. The absence of the peripheral sheaths, on the other hand, has caused in every case a material reduction in the peripheral supply (compare Exps. II and IV with I and III).

The data obtained seem to indicate that, (1) the branching of regenerating fibers is extensive enough to permit practically full reinnervation of a limb even from an undersized nerve source; (2) the degree to which a limb is repleted with regenerated fiber branches is controlled by factors residing within the limb and tending to limit the actual supply to approximately the normal amount; (3) the degenerating peripheral nerves may represent one major factor exerting numerical control over the admission of new fiber branches; but even in their absence the limb tissues seem to continue to exhibit a certain degree of control.

9341 P

Quantitative Studies on Nerve Regeneration in Amphibia. II. Innervation of Regenerated Limbs.

PAUL WEISS AND RAYMOND LITWILLER.

From the Department of Zoology, The University of Chicago.

When the developed limb of a urodele amphibian is amputated, masses of mesenchym accumulate at the wound and by subsequent growth and differentiation produce a new limb. Concomitantly, nerve fibers sprouting from the severed old nerve trunks invade the regenerating limb and provide its nerve supply. This process, studied in a preliminary manner by Weiss and Walker,[1] was given a more thorough examination in the present study.

Adult specimens of the Japanese newt, *Triturus pyrrhogaster* (Boie) and Mexican Axolotls were used, the latter in 2 different size (age) groups (75-85 mm. and 120-150 mm. in length). Fore

[1] Weiss, P., and Walker, R., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 810.

limbs were amputated at the body wall and allowed to regenerate. After varying periods of time, the regenerated limbs were preserved and sectioned. In the newt, Bielschowsky's silver stain for axons proved successful, while the data concerning the Axolotl are based mainly on myelin stains (Weigert-Pal). Tests in which 2 opposite limbs of the same animals were treated by the 2 different methods, staining on one side all fibers, on the other side the medullated fibers only, revealed that in regenerated fore limbs, 75-80% of the fibers are medullated. Every 20th cross section (cut at 10 micra) was measured, and the amount of nerve fibers contained therein was counted.

The number of nerve fibers present in the old limb base proximal to the level of amputation was rather constant: cca. 900 and 1200 fibers in the smaller and larger Axolotls, respectively. Distally to this level, in the regenerate, the number declines gradually in such regular manner that a definite relation between nerve supply and mass of innervated tissue suggested itself. The relation was revealed by the graphs in which the data were plotted, to be a remarkably simple one: If a regenerate is divided up into a series of slices of 200 micra each; if v_s is the mass of one of these slices; and if the number of nerve fibers terminating in this slice (determined as the difference between the nerve numbers counted in the 2 levels bounding the slice) is n_s; the ratio

$$k = \frac{n_s}{v_s}$$

was found to be nearly constant for all slices of a regenerate of a given age. In other words, the density (k) of nerve endings as well as, reciprocally, the mass of tissue innervated by a single fiber (1/k) are constants for a given limb. In a specific example, every slice of a regenerate of 50 days (Axolotl, 130 mm.) was calculated to contain 47 nerve terminations per one cmm. of volume. The factor k was found to decrease consistently with increasing length of regeneration. Furthermore, it is smaller in older than in younger animals.

The reported facts indicate that the tissues of a regenerating limb exert a definite control over the admission of new nerve fibers, establishing a "saturation" point for the number of possible terminations in a given peripheral volume. The fact that the saturation coefficient (k) decreases as regeneration proceeds, indicates that nerve fibers effect connections with the peripheral tissues at a very early stage of regeneration, and that the relations thus established are not essentially disturbed by the continued growth of the innervated tissue, largely muscle.