LETTERS TO THE EDITORS

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Longitudinal Migration of Phosphorus in a Sensory Nerve after Stimulation

THE difference between the longitudinal distribution of radioactive phosphorus in the sural nerve of rabbits at rest and in activity has been described in a previous communication[1]. It has now been shown that there is a similar redistribution of the total phosphorus in isolated nerves.

After cutting the nerve, the distribution of phosphorus first undergoes a series of changes, apparently because of stimulation by the injury discharges[2]. The sural nerves of rabbits anæsthetized with urethane 30 min. previously were ligated at the upper part of the thigh and middle of the leg and cut close to the ligature. The total phosphorus in the three sections, proximal, middle and distal, was estimated by the method of Berenblum and Chain[3]. The total phosphorus of resting nerve is between 300 and 400 μgm. per 100 mgm. wet weight, and if the three pieces of nerve were assayed immediately after it had been cut, the phosphorus content was approximately equal in all three pieces, the mean difference between the phosphorus content of the proximal and distal pieces being only 6 ± 4 μgm. per 100 mgm. (twenty-eight nerves). But a very different distribution was found if the nerves were either left in position in the rabbit's leg with the muscles and skin sutured over, or removed to a warm, moist chamber with a constant supply of carbon dioxide[4]. When the nerves were left for 10–50 min. after cutting the ends, the proximal phosphorus content was always higher than the distal, the differences amounting to 115 ± 10 μgm. per 100 mgm. (eleven nerves). The difference was greatest between 30 and 40 min. after cutting. However, in nerves left for 50–120 min. before assay, the distribution of the phosphorus between the three pieces was similar to that after immediate estimation as described above, amounting only to 10 ± 8 μgm. per 100 mgm. (eleven nerves). Everything points to the above sequence of events being due to excitation by injury discharges.

If the shift is due to activity, one would expect that electrical stimulation, after the nerve had gone through the above sequence, would again cause redistribution throughout the length of the nerve. Eleven sural nerves of rabbits anæsthetized with urethane were ligated and cut, and left for 50–110 min. These nerves were then stimulated electrically at their distal ends in the moist chamber for 20 min. The nerves were laid on wicks moistened with saline, in contact with silver/silver chloride electrodes, and the action potential was recorded throughout the period of stimulation by two similar electrodes supporting the other end of the nerve. The rate of stimulation was 30–60 per min. and the stimulus was maximal. The resulting difference between the phosphorus content of the proximal and distal pieces was marked, the content of the proximal piece being greater than the distal in all cases, and the difference amounting in the mean to 134 ± 19 μgm. per 100 mgm. (eleven nerves).

When the proximal end (that is, the end which was nearer the cord when the nerve was in the rabbit) was stimulated instead of the distal end, under the same conditions as just described, the phosphorus content of the *proximal* section again became greater than that of the distal section. The difference between proximal and distal was, in the mean, 123 ± 20 μgm. per 100 mgm. (ten nerves).

These results show that the distribution of phosphorus in the sural nerve of rabbits is radically changed by stimulation *in vitro*, and that the direction of the change is set, not by the direction of the propagation of the impulse, but by some inherent organisation of the fine structure of the nerve.

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Cytological Demonstration of Alkaline Phosphatase

Jacoby and Martin[1] have recently pointed out that diffusion of phosphatase may occur during the process of incubation which forms part of the technique of Gomori and Takamatsu for the cytological demonstration of alkaline phosphatase. I can confirm that artefacts may arise in this way. But there is no difficulty in deciding to what extent the results obtained by this method are valid, provided the technique is supplemented by some additional techniques published in 1946[2].

The manner in which the Gomori technique should be used is as follows: to ascertain the regions of highest phosphatase activity, sections should be incubated for a logarithmic series of times, the actual times being chosen according to the concentration of phosphatase in the tissue—for example, 5, 10, 20, 40, etc., minutes for normal rat kidney. The sites of highest activity appear positive for phosphatase before other sites, and no supplementary techniques are necessary for the correct localization of these sites. Sites of lower activity become evident after more protracted incubation times. Since these longer incubation times introduce the possibility of artefacts due to diffusion, supplementary techniques, such as those published in 1946, are necessary.

Most of the published work on the cytochemistry of phosphatase does not appear to have been controlled in this way, and is therefore open to question. Judging from this and other published work in cytochemistry, it appears that the technique of Gomori, like many other cytochemical techniques, suffers from a deceptive simplicity. It appears to be essential to have a good working knowledge of physics and chemistry for the proper use of these methods, even in a qualitative manner.

It should also be emphasized that while a number of other cytochemical techniques have been proposed for the demonstration of enzymes, such as acid phosphatase, phosphamidase, lipase, etc., no critical studies of these methods are yet available. Con-

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