LETTERS TO THE EDITORS

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Mode of Blocking of Axonal Activity by Curare and Inhibitors of Acetylcholinesterase

Micro-injection of certain strategic substances into the axon of the squid (*Loligo pealii*) has been carried out in order to study further the correlations between observed electrical activity of the nerve and possible neuro-chemical events. A micro-pipette with a long shaftlet having a lumen at the tip not more than 5 μ in diameter can be readily inserted into the giant axon between a pair of external electrodes. At will, it can be placed at the site of one electrode. These manœuvres can be accomplished without damage to excitation of, or propagation in, the fibre. The present communication is confined to reporting the blocking action of eserine and prostigmine, stilbamidine and curare, injected in aqueous solutions (usually containing 0·2 per cent phenol red, which colours the injection site an orange-yellow). Total fluid injected varied from about 10^{-4} mm.^{3} to 10 or 12 mm.^{3}, usually, however, being of the order of 0·5–5 mm.^{3}.

Although eserine and prostigmine are both powerful inhibitors of acetylcholinesterase, it is well known that the first, applied externally, blocks axonal activity whereas the second does not[1]. Prostigmine is ineffective because it does not penetrate the permeability barrier of the fibre. The present experiments, however, show that local injection of either substance directly into the axon blocks excitation under the stimulating cathode or propagation of the spike beyond the injection site. 5–10 μgm. of either substance acts within 1–2 sec. About 0·1 μgm. of prostigmine blocks within 10–30 sec. About 1 μgm. of eserine is required to produce a block in the same length of time.

In the group of curare-like compounds, stilbamidine when externally applied blocks axonal activity[2], whereas *d*-tubocurarine does not, the difference again being ascribable to a permeability barrier for the latter. However, injection of either substance into the axon in extremely small amounts causes block. The minimal quantities to act within 10–20 sec. are about 10^{-4} μgm. for stilbamidine and 10^{-5} μgm. for *d*-tubocurarine.

With all four substances, injection of small droplets in the minimal concentrations results in a brief block and recovery, which is to be ascribed to diffusion of the substance along the axon and its consequent dilution at the injection site. Injection of a few cubic millimetres of the carrier fluid alone causes no block, and even when the entire axon is injected with the fluid, axonal activity is maintained for 15 min. or more. The blocking which then takes place is at no time reversible.

Acetylcholine chloride injected in relatively high amounts (1 μgm. and 0·01 μgm.) in a few experiments blocked activity. With these concentrations there was no initial excitatory phase, nor was there observed any alteration in the form of the terminal spike.

The above series of experiments demonstrates that certain substances ordinarily ineffective when applied externally do act in the interior of the axon once the permeability barrier is overcome. Both acetylcholine and curare, furthermore, exert a much more powerful action upon axonal conduction than upon synaptic transmission. It is to be noted that stilbamidine and *d*-tubocurarine, which are relatively weak inhibitors of acetylcholinesterase, exert their action in much smaller concentrations than do the powerful inhibitors eserine and prostigmine. The mode of action of the two groups of substances is therefore probably different. Considerable evidence has recently accumulated to implicate a 'receptor protein' in the axonal membrane upon which acetylcholine liberated in the course of activity exerts its effects. It seems likely that the curare group of substances owe their blocking action to competitive combination with this 'receptor protein'. The present experiments also indicate that acetylcholinesterase cannot itself be the 'receptor protein', as suggested by one of us[3]. A co-ordinated functioning of all three chemical components, acetylcholine, the esterase and the 'receptor protein', is required to effect the propagated electrical activity of the axon.

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[1] Bullock, T. H., Nachmansohn, D., and Rothenberg, M. A., *J. Neurophysiol.*, **9**, 9 (1946).
[2] Bergmann, F., Wilson, I. B., and Nachmansohn, D., *Biochim. et Biophys. Acta*, **6**, 217 (1950).
[3] Grundfest, H., First Conference on the Nerve Impulse, 39 (Josiah Macy, Jr., Foundation, N.Y., 1951).

Lethal Retardation of Growth by Electric Shock

It has been shown that electric shock retards regeneration of the corneal epithelium of rabbits[1] in a manner similar to the depression of mitotic activity of the skin epithelium by other forms of shock[2]. Between the cells of various tissues, there seem to be some differences in susceptibility to antimitotic action. This may depend, among other possible causes, on the speed of multiplication of the cells. In order to obtain a first impression of what is going on, it was considered very important to investigate the effect of repeated electric shocks on the weight of growing animals and their organs. The animals (rabbits) were divided into two groups each consisting of six animals of both sexes. Experiments with the first group were started on the eleventh day after birth, with the second group on the eighteenth. In each group three animals served as controls. The animals were treated by electric shock once a day with the exception of Sunday in the same manner as described earlier[1].

With regard to endurance there was a great difference between the two groups. The first group tolerated 25–30 treatments, the second group 44–52 treatments before death occurred. Twice a week the growth of all animals was checked by weighing. The accompanying graph shows the increase of weight as a percentage of the initial body-weight in the