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Methods for Perfusing the Giant Axon of Loligo Pealii

By

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It was possible to perfuse the interior of the giant axon of *Loligo pealii* or *forbesi*, [C. S. Spyropoulos, I. Tasaki and T. Teorell, National Institutes of Health Annual Report, NINDB, 1959; Baker, P. F., A. L. Hodgkin and T. D. Shaw, Preprint of Proc. Physiol. Soc. (1961); also cited in preprint, Baker, P. F. and T. D. Shaw (1961), Plymouth Marine Biological Laboratory report for 1960—1961 (in press)] with an artificial solution and maintain the resting potential and the ability of the axon to produce normal action potentials. The technique employed (Diagram A) in the first series of experiments was as follows: Using an axon of *Loligo pealii* about 25—35 mm in length, a column of axoplasm was removed by introducing a capillary (300 μ in diameter). Subsequently each end of the axon was cannulated using glass pipettes (approx. 200—300 μ in diameter) and fine nylon thread. The perfusing fluid was introduced through one cannula and drained through the other. The rate of perfusion varied; in most experiments it was of the order of 0.01 ml/sec. The perfusion rate was usually but not always controlled with the Phipps and Bird Syringe-Driver. This rapid rate effected a rapid equilibration of the diffusible components of the intracellular space. A pair of silver wires was introduced through the cannula for stimulation and recording (under space clamp conditions). This electrode assembly facilitated drainage by guiding the perfusate from the ejecting to the withdrawing cannula. In another series of experiments, this was accomplished by means of a calomel micropipette which was also used as a potential recording electrode. In later experiments, simpler methods were used and longer axons (35—50 mm). In the method illustrated by diagram B a long glass capillary (350 μ in diameter) with a long slit (20 mm long and 100 μ in width) on one side was employed.

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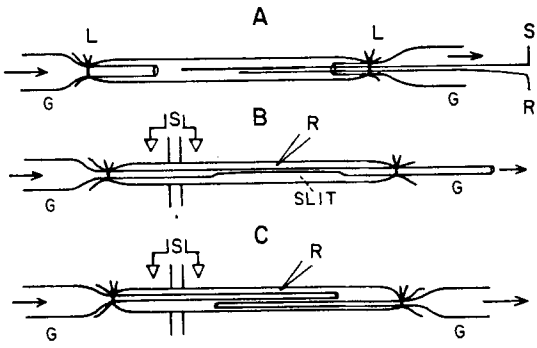

Fig. 1. *Illustration of various methods of perfusing isolated squid giant axons,* — A: Two separate glass cannulae (G) are tied with nylon thread (L) to the ends of the axon and a set of intracellular metal wire electrodes, for stimulation (S) and recording (R), are introduced through the distal cannula; when an accurate (D.C.) potential measurement is required, a calomel electrode is used. In this instance, external stimulation is employed. B: A single glass capillary provided with a long slit on one side is inserted to the extent that the slit is approximately half way through the axon and the tip protrudes through the end; external stimulation (S) across a vaseline gap and internal recording by means of a microelectrode pushed through the membrane are employed. C: Two separate cannulae, overlapping for about 20 mm in the middle portion of an axon, are used in conjunction with a microelectrode and external stimulating arrangements; the perfusate flows out of one cannula and is drained by the other. (Drawing not to scale.)

In C the perfusate was ejected through the opening of one of two overlapping (by about 20 mm) pipettes and collected through the opening of the second pipette. In another method, not illustrated in the figure, the perfusate flowed back toward the opening of the axon through which the perfusing glass capillary was introduced. In our technique there was always at the beginning a layer of axoplasm (50 μ or more) between the membrane and the zone of flowing fluid. Continuous perfusion usually resulted in erosion of the axoplasmic wall and in an appreciable reduction in its thickness.

Using isotonic KCl as a perfusate, the action potential remained normal for a period of approximately 30 min. This finding appeared to indicate that the internal sodium concentration could be reduced without changing the amplitude of the action potential. Conduction block was usually (but not always) preceded by prolongation of the duration of the action potential. Upon perfusing with sodium salt, instead of potassium, a rapid fall ensued in the resting potential and in the action potential amplitude. Using solutions of the same salt (Na or K) and fixing the concentration ratio across the membrane (with little adhering axoplasm), the membrane potential was found to depend on the level of the concentration ratios. Though the experiments were preliminary, this finding was interpreted as indicating the existence of fixed charge in the membrane (TEORELL, Progr. in Biophys. and Biophys. Chem., *3:* 305, 1953). The axons employed were 400—600 μ in diameter.

Quite recently, BAKER, HODGKIN and SHAW (cf. above reference) reported that they could remove nearly all of the axoplasm in their perfusion experiments. We cannot with certainty point to a particular difference in respective techniques that could account for their being more successful in maintaining the excitability for longer periods of time.