similarly produce new bone by autoinduction. Thus, RNA and degradation products of various intracellular proteinaceous materials, previously claimed to act as inductors, would enter only into the preinduction process. In the bone-induction process involving, as noted in Table 2, the interaction of living cells under normal conditions in the living animal, the inductor would not need to diffuse beyond subultramicroscopic distances. Cell interaction could take place immediately before mitosis, and the inductor could flow along membranes from one cell to another.

The evidence for osteogenesis by autoinduction is entirely morphological but, nevertheless, substantial. Bone formation occurs in extraskeletal implants of decalcified bone matrix in the interior of excavation cavities, and the new osteoblasts are derived, not from elements of the donor tissue, but from proliferating pleuripotent, ingrowing cells of the host. Comparable implants of *undecalcified* dead bone also produce osteogenesis, but only very rarely, only in scanty amounts, and then only after a latent period of several months (*15*). Ray and his associates (*16*) and Sherrard and Collins (*17*) describe rapid replacement of decalcified bone in bone defects, but as new bone arises from surrounding bone tissue (*18*), the orthotopic system does not offer convincing evidence of induction. In an extraskeletal or heterotopic implant of decalcified dead matrix, cell-induction sequences produce an entirely new ossicle with a marrow cavity as the end-product, and not merely histiotypic osteogenesis. Because differentiation of new-bone marrow occurs concomitantly with the process of new-bone formation by induction, the possibility of implanting decalcified bone matrix in subcutaneous soft parts in individuals with severe anemia, to produce a new site of active hematopoiesis, is important and interesting to contemplate. Uncertainty exists, however, about whether the period of survival of an ossicle in soft parts would be determined by the physiological demands for bone marrow, or mechanical stimuli for bone tissue. In a normal animal, extraskeletal ossicles decrease in size and may be entirely resorbed after a few years in the anterior abdominal wall.

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12 NOVEMBER 1965

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9 July 1965

Electrophysiological Studies of Chilean Squid Axons under Internal Perfusion with Sodium-Rich Media

Abstract. *Electrophysiological properties of giant axons of Chilean squid were examined by intracellular perfusion combined with various recording techniques. The amplitude of the action potential of the axons perfused with equal sodium concentrations (0.35M) on both sides of the membrane was approximately 75 millivolts (glutamate salts being used internally); the resting potential recorded with a calomel electrode was roughly 45 mv. These observations confirm and extend previous data, obtained from Chilean squid axons, which show that the amplitude of the action-potential overshoot does not agree with the Nernst equation applied to sodium ion. The discrepancy between these results and those obtained by others is attributed to differences in the procedures used to determine the resting potential.*

Giant axons in the stellate nerve of the Chilean squid, *Dosidicus gigas*, are very large (0.6 to 1.5 mm in diameter) and have no branches in the portion near the ganglion. Therefore, it is relatively easy to apply the method of intracellular perfusion with multiple cannulae to electrophysiological studies of these axons (*1*). This method gives important information concerning the physicochemical nature of the process of nerve excitation by directly controlling the chemical composition of the fluid media on both sides of the axon membrane.

Among several results obtained by this method (*1*), was the finding that *Dosidicus* axons could maintain relatively large action potentials under perfusion with a sodium-rich solution. With the same sodium concentrations across the membrane the resting potential of these axons was close to normal, and smaller than the amplitude of the action potential. The significant conclusions from these experiments are (i) that, at the inner surface of the axon membrane, a wide variation in the ratios of Na to K concentrations does not affect the resting potential significantly, and (ii) that combination of a high external with a low internal concentration of Na is not an indispensable condition for the maintenance of excitability. Furthermore, these experiments revealed a significant difference in physiological behavior between the inner and outer surfaces of the axon membrane.

Hodgkin and Chandler (*2*) have suggested that the technique of action-potential recording in the aforementioned study might have resulted in large, spurious electric responses because of the high resistance of the electrode. Therefore the electrophysiological properties of intracellularly perfused Chilean squid axons were examined with other recording techniques (*3*).

For intracellular perfusion the tech-

nique was substantially the same as that used before (*1*). A glass cannula, approximately 500 μ in diameter, was inserted into an axon, 60 mm long, held horizontally in a fluid medium on a Lucite chamber. During the process of insertion, mild negative pressure was applied in order to remove the axoplasm in the perfusion zone. A small cannula, approximately 200 μ in diameter, was inserted into the axon at the other end; this cannula was connected to a reservoir of perfusion fluid and was used as the inlet for perfusion. The large cannula was used for drainage of the perfusion fluid. The length of the perfused zone (that is, the distance between the inlet and outlet cannulae) was approximately 18 mm; on several occasions, the entire axon was perfused with artificial solutions.

In the first series of experiments (Fig. 1), a platinum wire, 50 μ in diameter and insulated with glass tubing, was used as the recording electrode. The electrode was inserted into the axon, through the outlet cannula, in such a manner that the bare recording tip (200 μ long) was in the middle of the perfusion zone. The potential difference between the ground electrode in the surrounding fluid medium and the internal recording electrode was measured with a unity-gain cathode follower (without positive feedback for neutralizing electrode capacity).

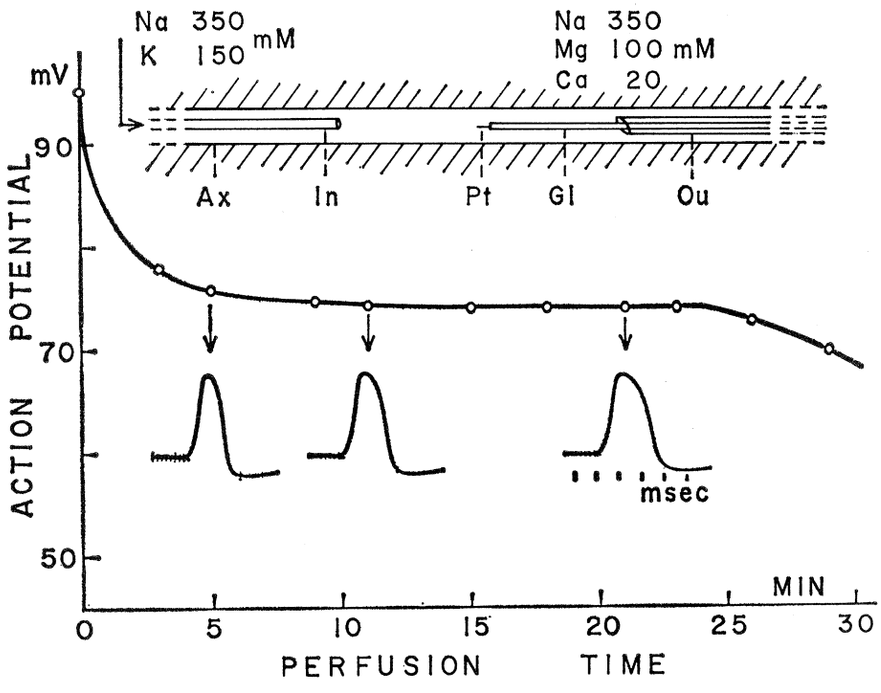

Fig. 1. (Top) Experimental arrangement (not to scale) for intracellular perfusion of a *Dosidicus* axon with sodium-rich solution. *Ax*, axon; *In*, inlet cannula; *Pt*, exposed surface of a platinum electrode; *Gl*, glass capillary covering the platinum wire; and *Ou*, outlet cannula. (Bottom) An example of the results obtained with the above arrangement. Three action-potential records are given. The axon diameter was 1.05 mm. Composition of perfusing fluid and external medium are given in the diagram.

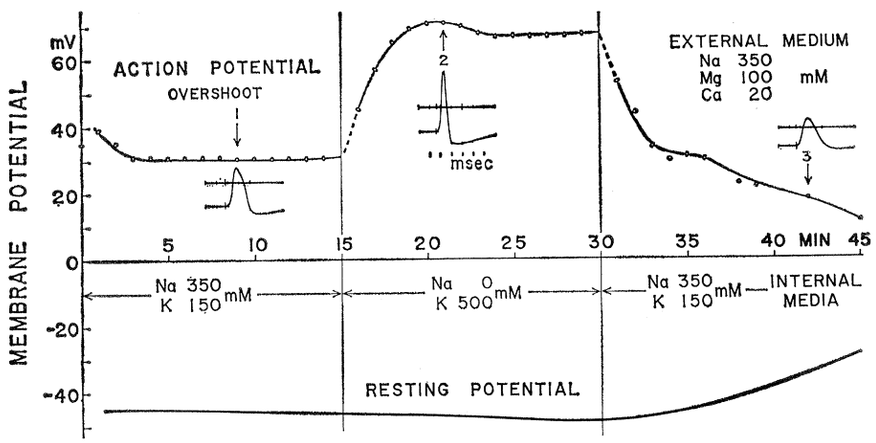

Fig. 2. Resting and action potentials of an axon intracellularly perfused first with a solution high in sodium, then with a solution high in potassium, and finally with a sodium-rich medium. A calomel electrode (0.6 megohm resistance) was used for recording. The diameter of the axon was 0.94 mm.

The external fluid medium was prepared by mixing 0.6*M* NaCl, 0.4*M* $MgCl_2$, 0.4*M* $CaCl_2$, and usually 12 percent glycerol solution by volume (no potassium salt was added); the *p*H of the solution was adjusted to 8.0 with tris(hydroxymethyl)aminomethane. The intracellular perfusion fluid was a mixture of 0.6*M* sodium glutamate, 0.6*M* potassium glutamate, and 12 percent glycerol; a phosphate buffer was used to adjust the *p*H to 7.3. The flow rate of the perfusion fluid was 30 to 100 μl/min. All experiments were carried out at room temperature (17° to 20°C).

Under continuous intracellular perfusion with a mixture of 0.35*M* Na^+ and 0.15*M* K^+, excitability of the axon was maintained for 20 minutes to 2 hours. However, under these conditions it was necessary to increase the concentration of the divalent cations in the external medium to maintain excitability. In most of our experiments, the external medium contained 0.1*M* $MgCl_2$ and 0.02*M* $CaCl_2$ (which was approximately twice as much as in normal sea water). With the Na^+ concentration on both sides of the axon membrane maintained at 0.35*M*, the amplitude of the action potential was roughly 75 mv (range: 70 to 80 mv). After onset of perfusion, there was a short period (about 4 minutes) of gradual decline; this period can be attributed to the time required for the sodium salt to diffuse through a layer (0.1 to 0.3 mm in thickness) of axoplasm remaining in the axon. The subsequent period of approximately constant action-potential amplitude often lasted more than 60 minutes, even when the entire 35-mm-long portion of the axon (distal to the orifice of the inlet cannula) was exposed to the perfusion fluid. The terminal phase of gradual decline of the action potential can be attributed to alteration of the macromolecular constituents of the membrane (*4*).

The resting potential of the axons intracellularly perfused with solution rich in sodium was measured as follows. A saturated KCl-calomel electrode (Beckman) was connected to a long glass capillary (0.13 to 0.18 mm in diameter) filled with 0.6*M* KCl solution. (The electrical resistance of the electrode was 0.6 to 1 megohm.) First, the recording tip of the electrode was immersed in the surrounding fluid medium; then, it was introduced into the interior of the axon in place of the platinum electrode (Fig. 1). The d-c potential difference across the axon membrane, as determined between the same electrode in the two positions, was about 45 mv (range: 42 to 50 mv).

The resting potential is insensitive (*4*) to a large change in the sodium-potassium ratio in the perfusion fluid (Fig. 2). Within about 4 minutes after the onset of perfusion with a sodium-rich solution, both the resting and action potentials became stationary. When the axon was then perfused with a sodium-free, potassium glutamate solution, there was an immediate increase in the amplitude of the action potential, a reflection of the effect of the internal sodium ion. There was no detectable change in the resting potential during the period of rapid increase in the action potential. When the perfusion fluid was switched back to the original sodium-rich solution, the resting potential remained practically unchanged (within approximately 3 mv). The gradual loss of excitability in the terminal period of perfusion was accompanied by a decline in the resting potential. Since sodium is less favorable than potassium for maintenance of the normal macromolecular state of the membrane (*5*), both the action and resting potentials tend to deteriorate sooner during perfusion with sodium-rich media.

The amplitudes of the action potentials obtained in our present experiments agree well with those reported earlier. The value stated by Tasaki and Takenaka (fig. 2 in reference *6*) in axons with 0.3*M* sodium on both sides of the membrane is about 80 mv. The value reported by Tasaki and Luxoro (*1*) on *Dosidicus* axons under similar conditions is about 70 mv (or slightly more). The value obtained by Hodgkin and Chandler (*2*) under slightly different experimental conditions is about 65 mv. We believe that the differences among these values are very small in view of the differences in the experimental procedures and in the external divalent cation concentrations. We conclude, therefore, that there is no significant error in our previous measurements of action potentials in axons perfused with sodium-rich media with recording electrodes having a relatively high resistance.

In the previous experiments on *Dosidicus* axons, precautions were taken to avoid errors in recording, either by (i) anesthetizing the unperfused portions of the axon with isotonic $MgSO_4$ solution, or (ii) eliminating the outlet cannula completely. A precaution taken by Tasaki and Takenaka (*6*) was to record the potential variation (v) in the outlet cannula together with the potential (V) in the middle of the perfusion zone; at the moment when $d(v-V)/dt$ vanishes, the capacitative flow of current through the wall of recording electrodes is close to zero and hence the error arising from the current is negligible.

The resting potentials recorded by Hodgkin and Chandler (*2*) vary between 80 and 110 mv and are very different from the values obtained by our technique (about 50 mv). Since the overshoot is defined as the difference between the action-potential amplitude and the resting potential, our value of the overshoot is much greater than the values reported elsewhere (*2*), and does not agree with the Nernst equation applied to sodium ion. We believe that the origin of the discrepancy in the overshoot values is not in the measurement of the action-potential amplitude but in the procedure for determining the resting potential.

The potential difference (electric) across the resting axon membrane cannot be rigorously defined from the standpoint of thermodynamics (*7*). For example, it is extremely difficult to evaluate the liquid-junction potentials between the internal and external solutions and the recording electrode, especially in the presence of divalent cations. In electrophysiology, the "resting potential" (or resting emf) can only be defined operationally; hence, the observed value of the resting potential may vary with the procedure used for the measurement. The procedure adopted elsewhere (*2*) (agar bridges and electrodes with unplatinized platinum) is very different from that in the present observations. In addition to the problems arising from salt bridges, it is well known that the potential of a platinum electrode changes drastically with slight variations in the electric current, in the O_2 tension, and in the concentration of trace polyvalent cations (*8*, p. 110). This latter factor may account for wide variation in the values of resting potential with such irreversible electrodes. Therefore, it is not very surprising that the results reported are very different.

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29 June 1965

Effect of Gamma Radiation on Dietary and Hormonal Induction of Enzymes in Rat Liver

Abstract. *The dietary induction of serine dehydrase, produced by the oral intubation of hydrolyzed casein to protein-depleted rats, is markedly inhibited by doses of γ-radiation of 400 roentgens or higher provided the irradiation is given within an hour after the initial dose of casein. If the irradiation is delayed until 7 hours after the initial dose of casein, induction is not inhibited. In contrast, the cortisone induction of tyrosine α-ketoglutarate transaminase is not inhibited by doses of γ-radiation up to 3200 roentgens; in some instances hormonal induction of this enzyme appears to be enhanced by irradiation.*

Previous studies (*1*) showed the effects of the antibiotic actinomycin D, as well as of the antimetabolite fluoroorotic acid, on the dietary induction of serine dehydrase. When actinomycin was administered at the beginning of