all uterine fluid 24 hours before infusion with phenol red did, however, effect bromination.

Further studies are in progress to determine the site and mechanism of the bromination. Perhaps there exists, in certain marine animals, an enzyme system which catalyzes the bromination described above. The biological halogenation of phenols may be analogous to the biogenesis of thyroxin where tyrosine residues undergo iodination (*8*).

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3. The biological phase of this work was aided by a grant from the New York Heart Association.
4. Bromophenol blue is prepared in the laboratory by reacting bromine with phenol red in glacial acetic acid. See E. C. White and S. F. Acree, *J. Am. Chem. Soc.* 41, 1190 (1919).
5. I. M. Kolthoff and V. A. Stenger, *Volumetric Analysis* (Interscience, New York, ed. 2, 1947), vol. 2, p. 53. It should be noted that phenol red itself changes color from yellow to red at *p*H 6.8 to 8.0.
6. A small amount (1.6 mg) of the crystalline material was nevertheless submitted for analysis for bromine; 37.06 percent was found. This seems to fit for bromophenol blue decahydrate; bromium content calculated for $C_{19}H_{10}Br_4O_5S \cdot 10\ H_2O$, is 37.60 percent. The microanalysis was performed by William C. Alford of the National Institute of Arthritis and Metabolic Diseases.
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6 October 1958

## Ion Adsorption and Excitation

*Abstract.* A simple calculation shows that the K ions about to leave the nerve during excitation form a monolayer at the surface of the resting nerve and are not hydrated. The same applies to the Na ions which replace the K ions. This may be taken as an indication of the existence of a reversible ad- and desorption phenomenon.

An important ionic relationship associated with the excitation process becomes apparent in the following way. Let it be assumed that there is a specific volume $V_K$ of the nerve substance in which the original concentration $K_i$ of potassium ions changes to that of the bathing fluid $K_o$ as a result of the net K ion loss incurred during a single discharge. This takes the form

$$V_K = K_{flux}/(K_i - K_o)$$

Knowing $V_K$ and taking the flux quantity as distributed uniformly over the area of 1 cm², one can arrive at the thickness $d_K$ of the nerve layer immediately involved in the exchange of K ions.

This simplified system is considered to be a valid one because of the steplike character of the K ion concentration gradient existing in the resting state between the two phases (that is, axoplasm and bathing solution). An additional advantage is the fact that the thickness thus obtained corresponds to that of a monoionic layer, obviating the necessity of considering a possible diffusion process which would tend to restore the internal K ion concentration to a uniform level. Similar reasoning holds with regard to $d_{Na}$.

The actual calculations were performed on the basis of the net ion flux data (K = 4.3 pmole/cm² per impulse, Na = 3.7 pmole/cm² per impulse) given by Keynes (*1*). The inner and outer ion concentrations used are those given by Keynes (*1*) for the *Sepia* axon ($Na_i$ = 110 mmole/kg, $Na_o$ = 458 mmole/kg, $K_i$ = 272 mmole/kg, $K_o$ = 9.7 mmole/kg), and those given by Hodgkin (*2*) for the *Loligo* axon ($Na_i$ = 49 mmole/kg, $Na_o$ = 440 mmole/kg, $K_i$ = 410 mmole/kg, $K_o$ = 22 mmole/kg).

The results are as follows, the figures in parentheses being values obtained from the second set of data.

$$d_K = 1.6393\ A\ (1.109\ A)$$
$$d_{Na} = 1.063\ A\ (0.946\ A)$$
$$d_K/d_{Na} = 1.54\ (1.172)$$

Examination of these results shows that the thickness of the nerve layer involved in ionic fluxes lies in the range of the effective radii of the ions considered (K = 1.33 A; Na = 0.98 A). In addition, the average of the two $d_K/d_{Na}$ ratios obtained equals 1.367, which is very close to 1.357, the ratio of the effective radii of the K and Na ions.

It is thought that these results may justify the conclusion that the nerve layer immediately involved in ionic shifts allows for the presence of only one-half of a monolayer of nonhydrated cations. Accordingly, the original formulation is modified to take the form

$$f = A\ r\ (C_i - C_o)$$

where $f$ is the net flux in moles per impulse, the sign denoting its direction; $A$ is the area of the nerve in square centimeters; $r$ is the effective radius of the ion in centimeters; and $C_i$ and $C_o$ are the inner and outer ion concentrations in moles per cubic centimeter.

Conversion of the concentrations into osmotic pressures shows the flux to be inversely proportional to the absolute temperature, a relation which is in line with the experimental evidence presented by Shanes (*3*).

Taking Hodgkin's data for plasma concentrations (*2*) and the flux values as above, one obtains from the flux formula $K_i$ = 345.3 mmole/kg and $Na_i$ = 62.45 mmole/kg. These values agree with those given by Koechlin (*4*) for the squid giant axons ($K_i$ = 344 ± 15 mmole/kg, $Na_i$ = 65 ± 15 mmole/kg), thus indicating a possible confirmation of the validity of the derived expression.

The emergence of the effective ionic radii implies that electrostatic forces may be at work and that the excitation phenomenon may involve to a greater or lesser degree an adsorption phenomenon (*5*).

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5. I thank Dr. G. Ungar and Dr. B. W. Zweifach for their advice and helpful criticism.

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## Proliferation of Excised Juice Vesicles of Lemon in vitro

*Abstract.* Juice vesicles from mature lemon fruits will proliferate *in vitro* for indefinite periods. The comparatively simple tissue grows on a synthetic nutrient medium almost entirely inorganic in composition.

Many types of excised meristematic plant tissues or parts have been the subject of in vitro studies, but the successful culture of tissue from a mature fruit is mentioned only briefly in the literature. The first in vitro studies of fruit tissues were started by Schroeder (*1*) and are under way at present on several different fruit species (*2*).

In the present study mature lemon fruits (variety Eureka) were surface-sterilized by immersion for 20 minutes in a saturated calcium hypochlorite solution, rinsed with sterile water, and cut longitudinally into eighths. Removal of

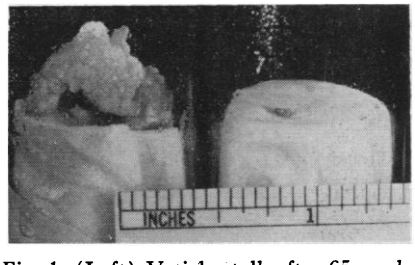


Fig. 1. (Left) Vesicle stalk after 65 weeks of in vitro growth. (Right) Dead vesicle stalk after 6 weeks of in vitro growth.