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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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

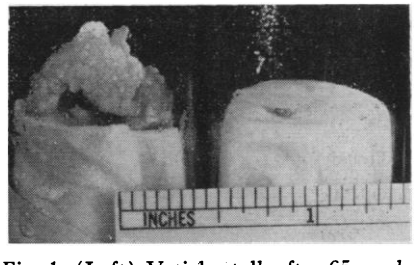

Fig. 1. (Left) Vesicle stalk after 65 weeks of in vitro growth. (Right) Dead vesicle stalk after 6 weeks of in vitro growth.

the locular membrane permitted entire vesicles (sac plus stalk) to be individually obtained by plucking the stalk at the base with a sharp pointed forceps. The vesicles thus removed were soaked in a buffer solution (0.10*M* phosphate, *p*H 7.0) for 15 minutes and placed on liquid nutrient media in screw-cap vials. The tissues were supported by reagent grade sea sand (Merck) or Du Pont cellulose sponge (yellow color) topped with Whatman No. 42 ashless filter paper discs.

The nutrient medium was entirely inorganic in composition except for sucrose as a carbon source (Table 1) and a small quantity of organic material supplied as ferric citrate or iron chelate-138 (Geigy Chemical Corp.). The medium was buffered by phosphate salts ($K_2HPO_4 + KH_2PO_4$) which also served as the sole phosphate source. The buffer system used was that of Colowick and Kaplan (*3*).

The vesicles started proliferating approximately 2 to 3 weeks after they were planted (May 1957) and are still producing new cells (13 January, 1959) (Fig. 1, left). Subcultures also carry on mitotic activity for long periods (10 months) and have gained in fresh weight as much as 2600 percent. The vesicle stalk appears to be the primary seat of growth activity. This tissue is capable of proliferating when it is attached to or removed from the juice sac. Occasionally the juice sac will proliferate, but only when the stalk is attached. Growth is most vigorous in the *p*H range 7.0 to 7.7 and occurs to a lesser extent at *p*H 6.0. The growth is visibly the same at all the above *p*H levels. None occurs at *p*H levels below 6.0 under the conditions employed.

Nutrient media which had supported the growth of the excised tissue for 81 days yielded entirely negative results when they were tested for aerobic and anaerobic microorganisms. The results suggest that mature lemon juice vesicles possess the inherent capacity to carry on cell proliferation for indefinite periods under relatively simple *in vitro* conditions.

Table 1. Composition of nutrient solution. Phosphate (0.01*M* total) was supplied by the buffer salts.

Compound	Amt. (g/lit.)	Ion or element (ppm)
$CaCl_2 \cdot 2H_2O$	0.1470	Ca^{++}, 40.0 Cl^-, 71.0
KNO_3	0.5090	K^+, 200.0 NO_3^-, 312.0
$MgSO_4 \cdot 7H_2O$	0.1080	Mg^{++}, 10.0 SO_4^{--}, 42.3
$MnSO_4 \cdot 4H_2O$	0.0050	Mn^{++}, 1.25 SO_4^{--}, 2.16
$ZnSO_4 \cdot 7H_2O$	0.0022	Zn^{++}, 0.50 SO_4^{--}, 0.738
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.0037	Mo, 2.0 NH_4^+, 0.0003
H_3BO_3	0.0028	B, 0.50
Fe-138*	0.0467	Fe^{+++}, 5.0 chelate, 41.0
Fe-Citrate†	0.0273	Fe^{+++}, 5.0 citrate, 16.86
$CuSO_4 \cdot 5H_2O$	0.0012	Cu^{++}, 0.30 SO_4^{--}, 4.50
Sucrose	34.250	

* Chelate-138 was used in all nutrient solutions buffered above *p*H 6.0.
† Ferric citrate was used in all nutrient solutions buffered at *p*H 6.0 and lower.

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18 August 1958

Requirements for Floral Initiation of Los Angeles Xanthium

Abstract. Cocklebur from the Los Angeles area was found to require more extensive short-day treatment for floral initiation than plants of the same species from the Chicago region. Data obtained by grafting the two regional types of cocklebur indicate that the leaves of the Los Angeles *Xanthium* produce a comparatively low amount of the flowering stimulus.

The cocklebur, *Xanthium strumarium* L. var. *canadense* (Mill.) T. and G. (*1*), which has been most commonly referred to in the past as *Xanthium pennsylvanicum* Wallr., is generally recognized as the most sensitive short-day plant now known. Although it can be maintained indefinitely in the vegetative state under long-day conditions, a single dark period of 8¾ hours or more will initiate floral development (*2*). The acknowledged esthetic deficiencies of the plant are more than compensated for by its usefulness as a test organism for assessing the effectiveness of a given treatment in terms of the short-day flowering response.

Most investigations employing *Xanthium* have used plant material derived from the fruit of wild plants collected in the vicinity of Chicago, Ill. The species range, however, extends in a wide belt from Florida through California, and from Quebec through North Dakota (*3*). Although it is taxonomically identical to the Chicago stock, *X. strumarium* from the Los Angeles area has been recognized (*4*) as being generally less sensitive to dark treatment than its Chicago counterpart.

The experiments described in this report constitute an evaluation of the critical day length for the Los Angeles *Xanthium* and, in addition, represent an attempt to determine a basis for the difference in response between the Los Angeles and the Chicago types.

Burs from wild Los Angeles stock were soaked in water for 12 hours before they were planted in flats of soil. Two weeks after emergence, 15 seedlings from each flat were selected for uniformity; all other plants were removed. All plants were maintained in cold frames throughout their growth period. Before and after the actual interval of photoinductive treatment the plants were maintained under continuous illumination, the natural day length being supplemented at night with incandescent light of intensity approximately 50 ft-ca at the leaf surface.

Two weeks after the last dark treatment the terminal buds were examined under a low-powered dissecting microscope and assessed with respect to the relative stage of floral development that had been attained. Results were evaluated in accordance with a numerical scale based on the diameter and morphological stage of development of the terminal staminate inflorescence. Vegetative plants were rated as zero on the scale. The first morphological change in the stem apex that could be clearly recognized as flowering was assigned a value of 1.0. A flowering apex measuring 0.25 mm in diameter was evaluated as 2.0. An additional increment of 1.0 was allowed for each 0.25-mm increase in the diameter of the developing inflorescence (*5*).

The treatments employed in the determination of the critical day length are listed in Table 1. From these data it is apparent that no flowering occurs in Los Angeles plants subjected to dark periods of 11 hours' duration or less. A single dark treatment of 20 hours was ineffective. The critical day length of the Los Angeles stock is, therefore, between 11 and 12 hours of darkness, and more than one photoinductive treatment is required for minimal floral initiation. Three photoinductive cycles with dark periods of 12-hour duration resulted in a low flowering response. An increase in

Table 1. Effect of dark treatment on the flowering response of Los Angeles *Xanthium*.

Darkness (hr)	Av. flowering response, with short-day treatments				
	No. of treatments				Continuous treatment
	1	3	5	5*	
9		0.0	0.0	0.0	0.0
10			0.0	0.0	0.0
11		0.0	0.0	0.0	0.0
12		0.2	1.9	0.7	10.9
13		0.9	4.0	2.9	
16	0.0	3.2			
20	0.0				

* Noninductive day length alternated with short-day treatment.