consisting of dusting a quarter of a teaspoonful (0.7 g) of *Artemia* eggs on the surface of 500 ml of 3.5 g/l NaCl solution contained in a large flat Pyrex dish (Corning 3-qt utility dish). It is convenient to prepare this salt solution by diluting a stock solution of saturated NaCl solution a hundredfold with tap water.

2) Forty-eight hours later, the hatched larvae are collected in a fine-mesh net, washed with L solution (see below), and added to the cultures of hydra. Living larvae can be separated from unhatched eggs by phototropic migration.

3) The cultures of hydra are grown at room temperature in similar shallow dishes in "Littoralis (or L) solution" of the following composition: 0.35 g/l NaCl; 0.07 g/l $CaCl_2$; 0.01 g/l $NaHCO_3$. This solution should be made up in either distilled or deionized water, as tap water is toxic to hydra in most localities. In practice, it is convenient to make up this solution by filling a gallon jug with water from a laboratory demineralizer after having added 10 ml each of the following two stock solutions: (1) 133.0 g NaCl, 26.6 g $CaCl_2$, demineralized water to 1 l; (2) 3.8 g $NaHCO_3$, demineralized water to 1 l.

4) The L solution in which the hydra are grown should be changed within 24 hr of feeding them with brine shrimp larvae. For this, it is only necessary to decant the old and fouled solution, replacing it with new, as most of the hydra will be found to adhere tenaciously to the glass bottoms of the dishes. The few hydra that are decanted may be easily collected by swirling the old solution in round containers; they will be found to collect in the center.

5) When active increase is not desired, hydra may be left in clean L solution without feeding for several weeks at room temperature, or for several months in a refrigerator. Such stored specimens will begin to bud once again approximately 48 hr after being returned to room temperature and fed daily with an excess of brine shrimp larvae.

References

1. HYMAN, L. H. *Trans. Am. Microscop. Soc.*, **48**, 322 (1929).
2. EWER, R. F. *Proc. Zool. Soc.*, **117**, 207 (1947).
3. HADLEY, C. E., and FORREST, H. *Am Museum Novitates*, No. 1423 (1949).
4. NEEDHAM, J. G. *Culture Methods for Invertebrate Animals.* Ithaca, N. Y.: Comstock (1937).
5. BRIEN, P. *Bull. soc. zool. France*, T. LXXVI, 277 (1951).
6. TURNER, C. L. *Biol. Bull.*, **99**, 285 (1950).
7. *Turtox Service Leaflet No. 39.* Chicago: General Biological Supply House.

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An Ultrafilterable Growth Factor for Tissue Culture[1]

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The use of tissue cultures as a means of screening chemotherapeutic agents and in studying fundamental problems of growth has increased considerably in the past few years. Attempts have been made to standardize the technique and define the results in specific chemical terms (*1, 2*). A purely synthetic medium, comparable to those used in bacterial cultures, would provide an ideal solution. Attempts at synthesizing a medium empirically have been made (*3, 4*) but the results have not been completely satisfactory.

Fischer and his co-workers (*5*) made a start toward finding factors, both in chick embryo extract and in other natural sources, which could simplify the present day media. Their work resulted in the use of some partially purified, but chemically undefined, fractions which could be substituted for embryo extract. In this laboratory, it is believed that an analytical approach to the problem is more promising than a synthetic approach. We have been able to obtain a fraction from the ultrafilterable portion of chick embryo extract which has been partially analyzed by paper chromatography and ionophoresis. It showed the presence of about five components.

Embryo extract prepared as previously described (*6*) was ultrafiltered. The ultrafiltrate was lyophilized and the powder was extracted with 70% ethyl alcohol. The alcohol extract was then passed through a column of Amberlite IR-120 in the hydrogen form and was washed through with two volumes of alcohol. The effluent was neutralized to pH 7.0 and evaporated to a volume of 1–3 ml. The solution was made to the original volume with distilled water. This fraction contained about 3% of the original ultrafilterable nitrogen and was as active as untreated ultrafiltrate when added back to thoroughly dialyzed embryo extract proteins. The method of culturing and defining the results were those developed in this laboratory by Signorotti, Hull, and Kirk (*2, 6*). At present the three ninhydrin positive materials and two ultraviolet absorbing materials which comprise this fraction are being identified.

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References

1. DAVIDSON, J. N., and LESLIE, I. *Nature*, **165**, 49 (1950).
2. HULL, W., and KIRK, P. L. *J. Gen. Physiol.*, **34**, 81 (1950).
3. WHITE, P. R. *Growth*, **10**, 231 (1946).
4. MORTON, H. J., MORGAN, J. F., and PARKER, R. C. *J. Cellular Comp. Physiol.*, **38**, 389 (1951).
5. ASTRUP, T., FISCHER, C., and VOLKERT, M. *Acta Physiol. Scand.*, **9**, 134 (1945).
6. SIGNOROTTI, B., HULL, W., and KIRK, P. L. *J. Gen. Physiol.*, **33**, 315 (1950).

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