digestive gland. In many snails of natural infection the digestive gland was found to be reduced to about one-fifth of its usual mass. In one specimen, which died shortly after being brought into the laboratory, 1647 daughter rediae, by actual count, were removed from the snail.

Recognizable cercarial embryos were first seen within the daughter rediae at 33 days. They are fully formed when they leave via the birth pore of the redia. After entering the lung cavity, the cercariae emerge from the snail via the respiratory aperture. The total time from exposure of the snails to the miracidium to the first emergence of the cercariae from the respiratory aperture was 40–46 days.

Details of the life cycle with systematic considerations will be published later.

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The Cultivation of Hydra Under Controlled Conditions

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Despite the classical studies that have been carried out on the regenerative capacities of hydra, as well as their extensive use in teaching courses, no reliable method has been available for their continued culture under controlled conditions, a fact that may explain their having been neglected to date as experimental animals.

Previous methods of culturing hydra have required the use of such variable and uncontrolled media as pond (*1–5*) and aquarium (*6*) water and, in addition, have needed subcultures of the water-flea *Daphnia* or other crustacean to provide the living food needed by all species of hydra. Unfortunately, present methods of culturing *Daphnia* are as unsatisfactory as those for culturing hydra; one recent publication has stated (*7*): "So far as we have been able to determine by experimentation, there has been no 'sure-fire' method discovered of keeping a culture of *Daphnia* permanently in the laboratory." The usual technique is to maintain four or five cultures in large wooden tubs, or barrels, so that there is a good chance that at least one culture will contain numerous *Daphnia* at any given time.

Even with an adequate supply of living food available, culturing hydra has been difficult. Hyman has stated (*4*): "The great difficulty in the continuous

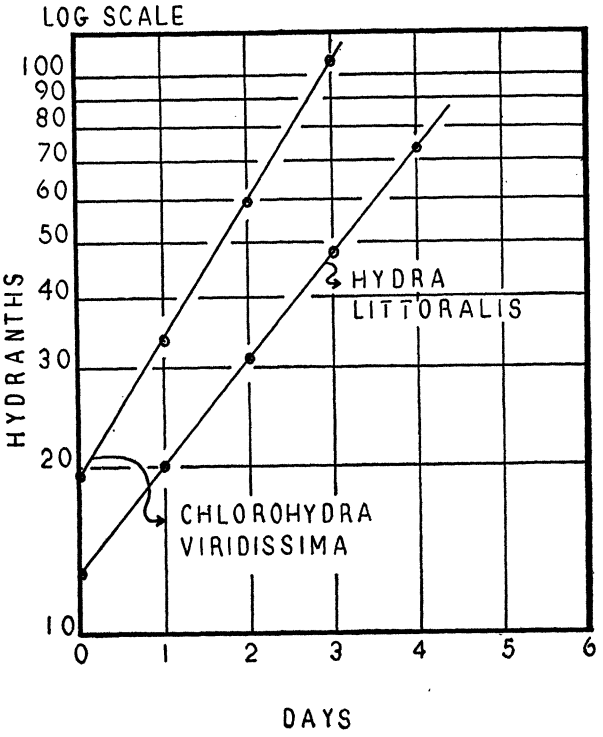

FIG. 1. Logarithmic increase of hydranths in two species of hydra grown under controlled conditions at 20° ± 0.5° C (provisional identification of hydra Littoralis).

culture of hydra is the occurrence of the phenomena of 'depression.' In spite of every care, hydra will pass into this state at intervals and, unless prompt measures are taken, will die out. In depression, column and tentacles fail to expand, the animal ceases to feed, shortens to a stumpy appearance, and finally disintegrates from the tips of the tentacles aborally."

The method of culturing hydra described here avoids depression by controlling both the medium and the food supply of the hydra. In place of pond water, a chemically defined solution is utilized. In place of *Daphnia* cultures, the dried and stable eggs of the brine shrimp *Artemia* are used as a source of living crustacea. As these dried eggs are viable for years, they may be hatched on schedule in reproducible batches of any desired size.

By utilizing the technique described below, rapid logarithmic reproduction (asexual) has been observed (Fig. 1), and thousands of hydra obtained daily with a minimum of effort. All the species studied to date have grown well under these conditions,[1] and depression has been entirely avoided.

1) Brine shrimp eggs[2] are hatched serially at room temperature on a 48-hr schedule, the daily routine

[1] Purchased hydra are often received in a state of depression. On receipt they should be placed singly in test tubes and fed and changed daily until actively budding. After a clone of 10–20 has been formed, they may be transferred to larger vessels.

[2] Brine shrimp eggs are available in quantity from aquarium stock companies.

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.

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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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