to the total dietary and body calcium pool available for milk production [9]), with the fraction of maternal calcium recovered in the offspring (*1–3*) confirms the conclusion drawn here, but this is the first report in which both parameters have been measured (*10*).

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10. The technical assistance of Mary F. Dallman is gratefully acknowledged.

12 April 1960

Culture of a Colonial Hydroid under Controlled Conditions

Abstract. A simple method has been developed for the cultivation of colonies of *Cordylophora lacustris*. The colonies, attached to microscope slides slanted in beakers, are grown in a culture solution containing five required ions. *Artemia* larvae are supplied as food. Increase in hydranth number is exponential with a doubling time of about 3 days.

Among the aquatic invertebrate metazoa, the colonial hydroids are particularly rich in unexploited potentialities for the study of growth and development at the tissue level. The exploitation of these potentialities cannot begin, however, until the organisms can be cultivated under controlled laboratory conditions. The accomplishments of Crowell, Hauenschild, and Kinne are important in this regard, for they have succeeded in growing three colonial hydroids in the laboratory [*Campanularia, Hydractinia,* and *Cordylophora,* see (*1*)]. Their methods, however, are rather elaborate and uncontrolled, involve the use of ocean water, and lack sufficient versatility to permit extensive variation of conditions. The present report describes a simple method for the controlled cultivation of a colonial hydroid, similar to the method for hydra developed so successfully by Loomis (*2*).

The organism is a brackish-water hydroid, *Cordylophora lacustris* Allman (*3*), which is unusually hardy, has great regenerative capacity, and forms a colony which is simpler in structure than that of many of the marine hydroids. Colonies of this sessile organism are grown attached to 1 by 3 inch microscope slides slanted in 100-ml beakers of culture solution (Fig. 1). Such cultures may be grown to a considerable density, whereas cultures grown in the bottoms of dishes quickly become necrotic. Many separate beaker-slide cultures may be maintained and observed with a minimum of effort.

A defined aqueous solution replaces brackish water. *Cordylophora* culture solution (CCS5) contains 0.05*M* NaCl, 0.001*M* $KHCO_3$, 0.005*M* $CaCl_2$, and 0.005*M* $MgCl_2$, and is made up in demineralized water (*4*). The sodium, potassium, calcium, and chloride ions are absolute requirements for growth, while in the absence of magnesium ions growth continues, but at a reduced rate. Bicarbonate ions are not required, but serve to buffer the solution. The proportions given are approximately optimal for growth. In contrast, *Hydra littoralis* requires only calcium (*2*) and traces of sodium (*5*) for optimal growth.

Hydroids are carnivores, and must be fed a completely undefined nutrient: living prey. The use of larvae of the brine shrimp, *Artemia*, for this purpose (*1, 2*) represents a giant step in the direction of controlled conditions, since *Artemia* larvae provide an unlimited supply of easily raised and highly uniform food. The dried eggs are hatched on a daily schedule (*6*), and each day the larvae are collected and washed, and the *Cordylophora* colonies are fed to repletion for about an hour. The culture solution is changed after feeding and again several hours later (*7*). Colonies are maintained at 22°C.

Asexual *Cordylophora* colonies are composed of three repeating units: hydranths, stems, and stolons. Tubular stolons grow out attached to the substratum, and perpendicular to the stolons uprights rise at regular intervals, each upright bearing a hydranth at its apex. The stems of the uprights lengthen and at intervals develop side branches which bear additional hydranths. Branches develop secondary and tertiary branches, and stolons also branch. While such a colony gives the general impression of a rambling bush, the pattern is highly regular and results from the relative rates of growth and spacing of the repeating units.

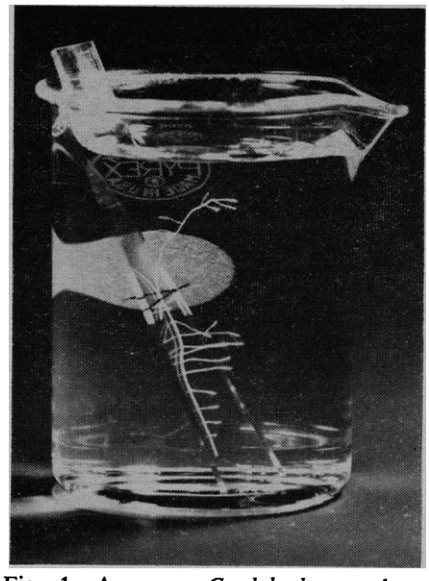

Fig. 1. A young *Cordylophora* colony growing on a slide in a 100 ml beaker of CCS5. The portion above the thread is unattached. There are 19 hydranths.

Secondary asexual colonies are started by removing single uprights from a well-developed colony and tying them to microscope slides with thread (*8*). A new stolon develops at the cut base of the upright, attaches to the slide, and begins the developmental sequence described above. Simultaneously, the original upright continues to elongate (without attaching to the slide) and branches (Fig. 1). Secondary colonies are allowed to develop for about a week and then are ready for use in experiments.

A measure is needed of what, in a general sense, constitutes increase with time in a growing colony. One unit

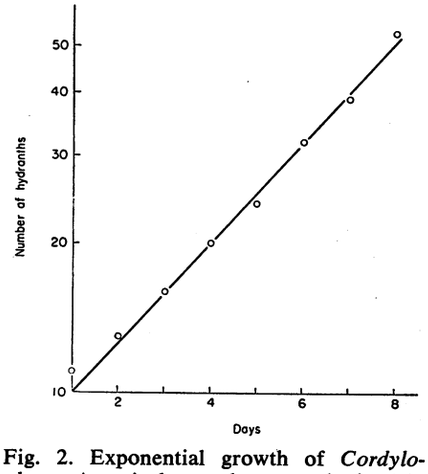

Fig. 2. Exponential growth of *Cordylophora*. A typical growth curve obtained in CCS5 at 22°C with one daily feeding and a second culture solution change, plotted on semilogarithmic paper. The interpolated doubling time is 3.0 days.

which correlates with the size of the colonies and can be measured with time (that is, without sacrificing the colonies) is the hydranth. A count of hydranth number with time under the conditions described reveals that the number increases exponentially for as long as hydranths can be counted accurately (Fig. 2). This is also true of *Hydra*, where the new hydranths quickly separate from the parents, and the count permits the determination of growth rate by the standard equations for exponential growth (*2*). Whereas Loomis found the average doubling time of *Hydra* to be slightly less than 2 days, the average for *Cordylophora* is 3 days.

By using growth rate as a measure of conditions, the influence of environmental variables on the growth of *Cordylophora* has been studied (*9*). Except for the composition of the aqueous environment and the feeding rate, the growth rate of colonies is relatively insensitive to variation of many parameters, including light, temperature, *p*H, and oxygen tension.

The method described has permitted the continual asexual growth of a clone of *Cordylophora* for over 2 years, providing vigorous and uniform material for experimental studies (*10*).

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4. Addition of 1.5×10^{-4} molar disodium diethylenediamine tetraacetate ("versenate") chelates heavy metals and makes it possible to prepare CCS5 in tap or distilled water.
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7. With daily feeding, a second daily CCS5 change is essential for vigorous growth. Colonies can be maintained without feeding for several months if the culture solution is kept clean and evaporation is avoided.
8. Silk thread is wound once around a slide, about an inch above the bottom, and sealed to one edge with a drop of molten wax. A single upright is slipped between the thread and slide.
9. A report of these studies is in preparation.
10. I wish to thank Dr. Norton Zinder for his helpful suggestions during the course of this work, and Dr. W. F. Loomis for a critical reading of the manuscript.

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Long-Range Detection of French Nuclear Tests of 1960

Abstract. With a relatively small number of strategically located ground-level air-filter stations, it has been possible to detect the presence of radioactive debris from the French nuclear test of February 1960 at a great distance from the test site, and to obtain data on the time of arrival of this debris and the extent of its north-south spread.

An atomic device of 60 to 80 kilotons' yield was fired by the French Government in the Sahara Desert near lat. 26° N., long. 0° at 0700 hours on 13 February 1960 when, according to news reports, weather conditions were such that all radioactive debris would be transported in an easterly direction. This debris would thus have to travel about three-quarters of the distance around the earth before it would intersect the 80th meridian network of air-filter stations operated by the U.S. Naval Research Laboratory (*1*, *2*).

As is shown in Fig. 1, the station at Miraflores, Panama Canal Zone, received the first indication of fission products from this test in the 2-day collection of 24–26 February, 12 or 13 days after shot time. The time of arrival at San Juan, Puerto Rico; Miami, Fla.; and Guayaquil, Ecuador, was a day or two later. Fission product activity reached its highest level at Miraflores in the collection of 26–29 February, when it was about 100 times the previous background of residual activity from past tests. Maxima occurred at the other sites at later times: Miami, 2–4 March; San Juan, 4–6 March; Guayaquil, 3–5 March. The second rise shown, during early April, is also probably related to the February test, since debris from the French test of 1 April would not be expected to arrive so soon. Debris from the much smaller second test could be hidden by residual activity from the first test; in any event its presence was not confirmed at any of these sites.

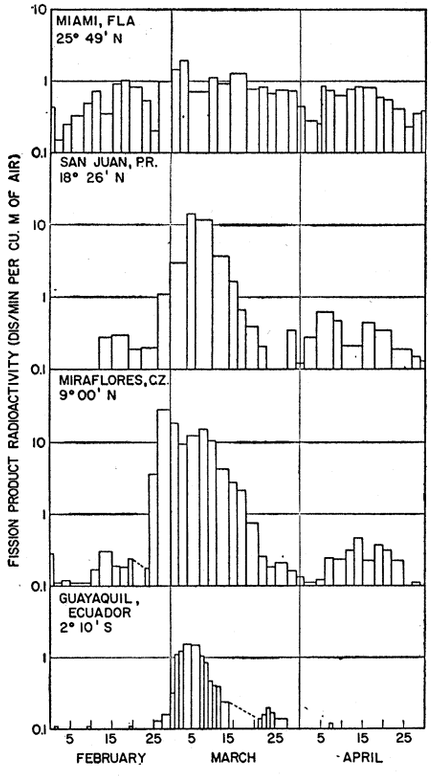

Fig. 1. Changes in the concentration of fission product radioactivity in the air along the 80th meridian (west) following the French nuclear tests of 1960.

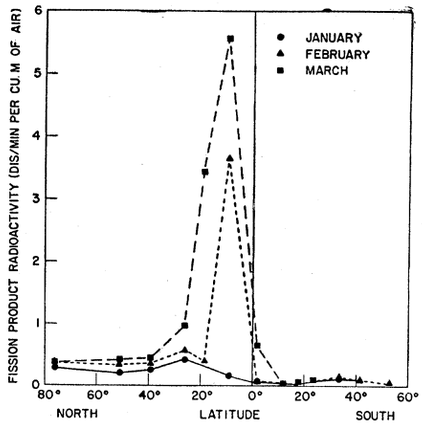

Fig. 2. Gross fission product β-activity in the air at ground level along the 80th meridian (west) during early 1960.

The extreme spread of this activity at the 80th meridian is shown in Fig. 2 to range from a few degrees south of the equator to just above lat. 25° N. No fresh activity was detected at Lima, Peru, or Washington, D.C., or at any stations north or south of these points. Conclusive proof of the absence of fresh debris at these other sites has been obtained through radiochemical analyses which showed the absence of the shorter-lived fission products. The progressive increase in the level of activity during the period January to March 1960 at the three northernmost stations is due to the predicted spring rise in activity in the Northern Hemisphere and is not associated with the French shot.

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