a new possibility arises, namely, that the fine eye movements are an experimentally predictable derivative of both the stimulus and the percept. This is not to say that other explanations are not possible (*9*).

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References and Notes

1. G. H. Mead, *The Philosophy of the Act* (University of Chicago Press, Chicago, Ill., 1938).
2. Especially as elaborated by H. Cantril, A. H. Hastorf, and W. H. Ittelson, *Science* **110**, 461 (1949); W. H. Ittelson and H. Cantril, *Perception, A Transactional Approach* (Doubleday, New York, 1954).
3. R. B. Livingston, *Handbook of Physiology*, J. Field, Ed. (American Physiological Soc., Washington, D.C., 1959), sect. 1, chap. 31, p. 741.
4. Dr. T. N. Cornsweet was of invaluable aid in designing the apparatus.
5. J. Nachmias, *J. Opt. Soc. Am.* **49**, 901 (1959).
6. T. N. Cornsweet, *ibid.* **46**, 987 (1956).
7. R. W. Ditchburn and B. L. Ginsborg, *Nature* **170**, 36 (1952); R. W. Ditchburn, D. H. Fender, S. Mayne, *J. Physiol. (London)* **145**, 98 (1959).
8. L. A. Riggs, F. Ratliff, J. C. Cornsweet, T. N. Cornsweet, *J. Opt. Soc. Am.* **43**, 495 (1953).
9. This work was supported by a National Institute of Mental Health Research Training Fellowship. I am indebted to many people for ideas and assistance.
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Transfer of Maternal Calcium to the Offspring via the Milk

Abstract. By measuring the specific activities of milk and of maternal and filial long bones 3 months after calcium-45 had been given to the then nonpregnant mothers, it was found that the magnitude of the contribution of the maternal calcium stores to milk formation is similar to the contribution to the bone calcium of the offspring, that is, 10 to 15 percent of either milk calcium or filial calcium is maternal in origin.

Several groups of authors (*1–3*) have investigated the movement of maternal calcium to the developing fetus and have generally concluded that only a small portion of the maternal skeletal calcium stores is transferred to the embryo, most of the latter's bone calcium deriving from the maternal diet. The actual fraction of fetal calcium that is maternal in origin is imprecisely known, the figures reported varying from 28.6 percent (*1*) to 12 percent (*3*).

Hevesy, studying the conservation of maternal calcium in the offspring, concluded from data on calcium content and turnover in mice and their offspring that "The Ca^{45} taken in by the mother has thus only an opportunity of interchanging in the average with about 1/5 to 1/6 of the body calcium before being utilized in the building up of the embryo" (*4*, p. 15). This estimate thus agrees with the estimates cited.

Table 1. Comparison of specific activities of milk and of maternal and filial long bones. Percent dose Ca^{45}/gm Ca. (Dose given to mother 3 months earlier.)

Litter age (Days)	Mother		Milk	Litter		
	Ends	Shafts		Total	Ends	Shafts
1	13.6	20.0	1.75	2.57		
				0.99		
				0.96		
				1.29		
				Av. 1.45		
1	48.0	62.5	7.59	8.63		
				7.96		
				4.25		
				6.74		
				Av. 6.90		
10	43.2	50.0	4.61		7.00	4.97
					6.80	5.44
					5.66	5.23
					1.94*	4.97
					Avs. 6.49	5.15
16	21.6	29.4	3.12		4.67	4.84
					4.50	4.70
					6.45	5.63
					6.28	5.33
					Avs. 5.48	5.13

* This figure was not included in calculating the average.

The implication of these findings is that when the demand for calcium is high, as in pregnancy, the body can divert most of the incoming calcium atoms from the skeleton, their normal target organ, to the rapidly calcifying fetus (*5*). It seemed of interest to determine to what extent the pool of maternal calcium participates in milk formation and to estimate whether the endogenous calcium contributed by the mother to the milk calcium derives from the same source as the calcium supplied to the fetus.

To this end, 1-month-old female rats were given Ca^{45} (*6*) by intraperitoneal injection and caged together with males of the same age. Six to eight weeks later, when the females were found to be pregnant, they were separated and allowed to deliver and then to suckle their young. At predetermined times, the mothers and their litters were killed (ether anesthesia), their right humeri were dissected out, and, in the case of the mothers and older offspring, the proximal ends were separated from the shafts and analyzed for Ca and Ca^{45} (*7*). In the case of newborn rats, the entire humerus was analyzed; in the older suckling rats, all of the shaft, but in the mothers only a portion of the shaft (proximal metaphysis and part of diaphysis), was analyzed. In addition, the stomach of the offspring was excised, cut open, and the curdled milk was squeezed out and analyzed for Ca and Ca^{45}.

Table 1 shows the results of the analyses. It can be seen that the specific activities of the shafts of the mothers are higher than those of the ends; this indicates that much of the isotope, originally deposited at the epiphysial plates, is now in the shafts, in accordance with the pattern of growth of long bones worked out by Leblond *et al.* (*8*). The specific activities of the bones of the offspring were appreciably lower than those of the maternal bones, approximately 1/8 of the specific activity of the ends and 1/10 of the specific activity of the shafts of the maternal bones. These figures are qualitatively similar to, though perhaps lower than, the corresponding figures reported by others (*1, 3, 4*).

The specific activity of the milk recovered from the offspring is similar to that of their bones. This suggests that the magnitude of the contribution of maternal skeletal calcium stores to milk formation is similar to the magnitude of contribution toward fetal bone formation. In other words, about 10 to 15 percent of the calcium in milk or the filial skeleton is maternal in origin. This estimate represents a minimum figure, however, as some nonlabeled dietary calcium may enter the maternal skeleton and then be transferred to the fetus or the milk (*3*). Furthermore, the maternal bones were not labeled uniformly by the single injection of tracer (*7*). Consequently, some unlabeled maternal calcium may also have been transferred to the milk or the offspring.

A comparison of the fraction of the dose of Ca^{45} appearing in the milk (0.06 to 0.34, depending apparently on the relation of milk calcium secreted

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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References and Notes

1. C. L. Comar, I. B. Whitney, F. W. Lengemann, *Proc. Soc. Exptl. Biol. Med.* **88**, 232 (1955).
2. J. P. Feaster, S. L. Hansard, J. C. Outler, G. K. Davis, *J. Nutrition* **58**, 399 (1956); C. L. Comar, *Ann. N.Y. Acad. Sci.* **64**, 281 (1956); C. L. Comar and R. H. Wasserman in vol. 1 of series vi (Biological Sciences) of *Progress in Nuclear Energy* (Pergamon Press, London, 1956), p. 153.
3. R. H. Wasserman, C. L. Comar, M. M. Nold, F. W. Lengemann, *Am. J. Physiol.* **189**, 91 (1957).
4. G. Hevesy, *Kgl. Danske Videnskab. Selskab, Biol. Medd.* **22**, No. 9 (1955).
5. The as yet untested corollary of this statement is that when calcium intake is low, a larger fraction of the skeletal calcium is mobilized to meet the demands of the developing fetus.
6. Obtained on allocation from the Oak Ridge installation of the U.S. Atomic Energy Commission.
7. F. Bronner, *J. Gen. Physiol.* **41**, 767 (1958).
8. C. P. Leblond, L. F. Bélanger, R. C. Greulich, *Ann. N.Y. Acad. Sci.* **60**, 631 (1955). As the bone ends represent regions of greater metabolic activity (or turnover) than the bone shafts, one would expect on that ground alone that, as time goes on, a proportionately greater fraction of an isotopic label would be found in the shafts; for, once lodged there, it is less likely to be removed than if lodged in the epiphysial or metaphysial region. [See also J. S. Arnold and W. S. Jee, *Am. J. Anat.* **101**, 367 (1957)].
9. R. H. Wasserman, F. W. Lengemann, C. L. Comar, *J. Dairy Sci.* **41**, 812 (1958); W. J. Visek, R. A. Monroe, E. W. Swanson, C. L. Comar, *ibid.* **36**, 373 (1953).
10. The technical assistance of Mary F. Dallman is gratefully acknowledged.

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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.

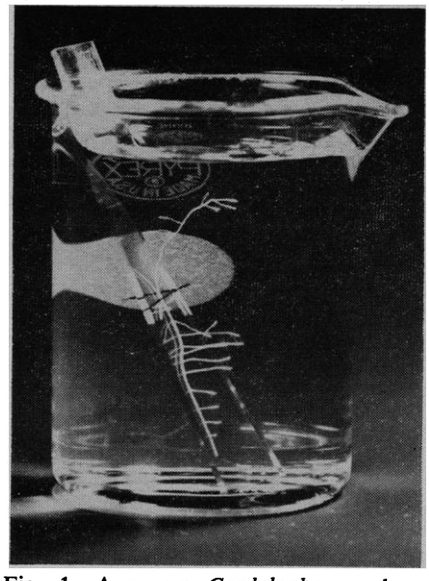

Fig. 1. A young *Cordylophora* colony growing on a slide in a 100 ml beaker of CCS5. The portion above the thread is unattached. There are 19 hydranths.

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

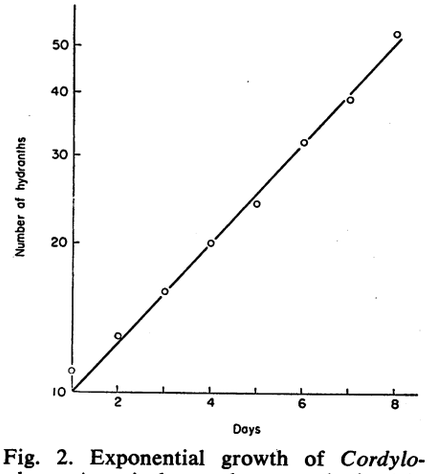

Fig. 2. Exponential growth of *Cordylophora*. A typical growth curve obtained in CCS5 at 22°C with one daily feeding and a second culture solution change, plotted on semilogarithmic paper. The interpolated doubling time is 3.0 days.