References and Notes

1. A. W. Bernheimer and L. L. Schwartz, *J. Bacteriol.*, in press.
2. S. Razin, *Recent Progr. Microbiol.* **8**, 526 (1963).
3. R. M. Chanock. L. Hayflick, M. F. Barile, *Proc. Nat. Acad. Sci. U.S.* **48**, 41 (1962).
4. A. W. Bernheimer and L. L. Schwartz, *J. Gen. Microbiol.* **30**, 455 (1963).
5. A. W. Bernheimer, *J. Exp. Med.* **90**, 373 (1949).
6. G. Weissmann, H. Keiser, A. W. Bernheimer, *J. Exp. Med.* **118**, 205 (1963).
7. S. Razin, *J. Gen. Microbiol.* **36**, 451 (1964).
8. J. L. Kavanau, *Structure and Function in Biological Membranes, I* (Holden-Day, San Francisco, Calif., 1965), p. 132.
9. P. F. Smith, *Bacteriol. Rev.* **28**, 97 (1964).
10. M. M. Weber and S. C. Kinsky, *J. Bacteriol.* **89**, 306 (1965).

11. We thank Dr. H. E. Morton for supplying cultures of *M. laidlawii*, Dr. H. J. Morowitz for *M. gallisepticum*, and Dr. J. G. Tully for *M. neurolyticum*. Supported in part by grants from the Life Insurance Medical Research Fund and the National Institute of Allergy and Infectious Diseases (AI-02874-06) and by a PHS Research Career Program Award (5K6-AI-14, 198-03).

7 April 1965

Cell Proliferation in Hydra: An Autoradiographic Approach

Abstract. *Hydra cells take up substantial amounts of tritiated thymidine if the label is injected into an animal's gastric cavity shortly after feeding. Autoradiographic analysis shows that the isotope becomes incorporated into cell nuclei. The distribution of labeled nuclei probably represents the pattern of cell proliferation and is similar to the distribution of mitotic figures in histological preparations. The data show that cell proliferation is distributed along the body column and that there is no localized growth zone.*

Hydra is customarily used for studying morphological changes and regulation, yet little is known about the cellular growth patterns underlying these activities. Experimental studies on hydra have led Tripp (*1*) and, more recently, Brien and Reniers-Decoen (*2*) to postulate that growth and cell proliferation occur primarily in the subhypostomal (*3*) region. Such a growth pattern was largely deduced, however, and no direct analysis of the distribution of cell proliferation has been presented to support their thesis. A possible reason for this lack of analysis is the difficulty workers have experienced in finding mitotic figures in hydroid tissue (*4*). One approach would be to label dividing cells with tritiated thymidine and study their distribution by autoradiography, but previous attempts to label the nuclei of hydra (*5*) have been hindered by the relative impermeability of hydra to organic molecules in the external milieu (*6*).

In this report, I describe a successful method for administering tritiated thymidine to normal hydra so that the label becomes incorporated into dividing cell nuclei. To overcome the problem of permeability, thymidine solution is injected into the gastric cavity of hydra immediately after the animal has been fed *Artemia nauplii*. This presents the label directly to the digestive tissue. The colloidal nature of the mucus and partially digested food is essential in preventing the isotope from leaking out of the animal.

Hydra littoralis (*7*) were grown in the medium of Loomis and Lenhoff (*8*) with distilled water substituted for tap water. Culture density was maintained at one hydra per 15 ml of growth solution, the solution being renewed daily 1 hour after the animals were fed *Artemia nauplii*. The hydra were grown for 4 weeks before they were used in experiments, so that all animals had similar growth histories. A fine glass pipette was inserted through the mouth into the gastric cavity and 0.1 μl of tritiated thymidine (*9*) solution (0.1 μmole) was delivered by a 50-μl syringe (*10*). The average amount of isotope retained by the hydra 1 minute, 20 minutes, and 24 hours after injection was 89, 78, and 24 percent, respectively, as determined by monitoring the medium. This retention of substantial amounts of thymidine shows that the administration procedure was efficient.

Extended hydra were fixed in Lavdowsky's fluid (*11*) 24 hours after the thymidine was injected. Serial paraffin sections 5 μ thick were cut perpendicular to the long axis of the animals. The methods of Kopriwa and Leblond (*12*) were used for staining the sections and for the autoradiographic analysis. The sections were stained with Harris' hematoxylin, and the slides coated with Kodak KTB-3 liquid emulsion, exposed at 4°C, and developed in D-170. After exposure for 7 days, most of the labeled nuclei were associated with about 75 photographic grains each. The cytoplasm was relatively free of activity, indicating that little label was shunted into other synthetic metabolic pathways by either the hydra or its microbial flora.

The segment of radioautograph shown in Fig. 1 is taken from the middle stomach region. Incorporation

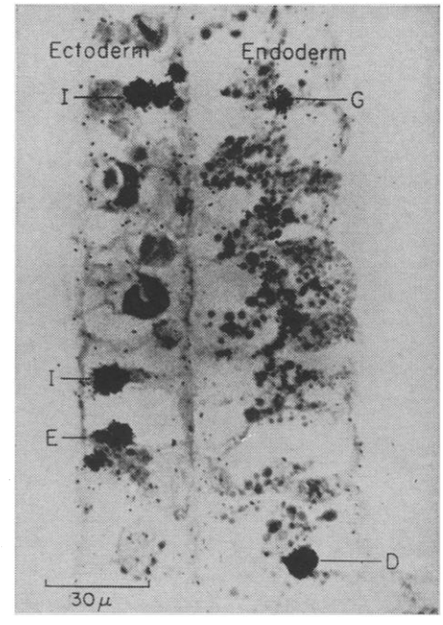

Fig. 1. Autoradiograph of hydra injected with triated thymidine. Endoderm (right) and ectoderm (left) are cut in cross section through the middle stomach region. Nuclei showing isotope incorporation in this photograph are: gland cell (*G*), digestive cell (*D*), interstitial cell (*I*), and epithelio-muscular cell (*E*).

of tritiated thymidine was observed in nuclei of all major types of cells distinguished in this study: digestive, interstitial, and gland cells in the endoderm; and cnidoblasts, interstitial, and epithelio-muscular cells in the ectoderm.

Labeled nuclei occurred at all levels

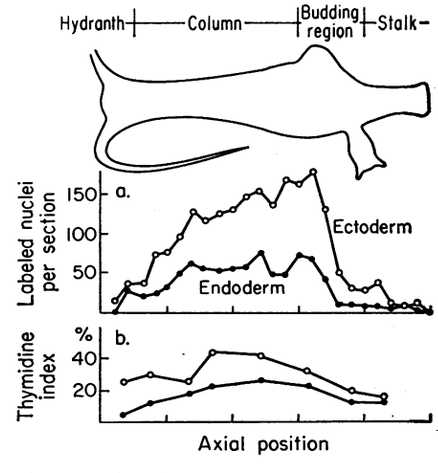

Fig. 2. Axial distribution of labeled nuclei in a hydra. Axial position (abcissa) is indicated by the drawing above the graph. Thymidine index is the percentage of nuclei which are labeled. Solid circles, endoderm; open circles, ectoderm. Counts of labeled nuclei were made on every 20th section. Thymidine indices were calculated for single sections at the positions indicated.

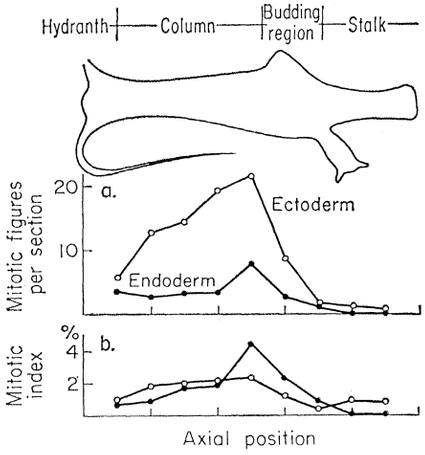

Fig. 3. Axial distribution of mitotic figures in a control hydra. Axial position (abcissa) is indicated by the drawing above the graph. Mitotic index is the percentage of cells in division. Solid circles, endoderm; open circles, ectoderm. Fixed in Lavdowsky's fluid and stained by Feulgen method. Each point represents the average value of counts on four histological sections. The peak in the endodermal mitotic index, a feature exhibited by some animals, probably represents an incipient bud.

along the axis in both the ectoderm and endoderm, but in a characteristically graded distribution (Fig. 2*a*). Thus, the numbers of labeled cells per histological section increased from the tentacular region to the budding zone. A similar increase in the abundance of cells, however, resulted in a more uniform distribution of the proportion of nuclei incorporating label (Fig. 2*b*). This proportion increased about twofold between the upper column and the budding region, and declined gradually in the stalk. Little or no activity was found in the distal hypostomal region or in the tentacles.

The majority of labeled nuclei in the endoderm were those of digestive cells. In the ectoderm the interstitial cells contained most of the label. The distribution of labeled epithelio-muscular cells was similar to that of digestive cells. No evidence was found for strong localization of activity in the subhypostomal region.

To confirm that this axial distribution of labeled nuclei actually reflected the pattern of cell proliferation in hydra, these data were compared with those obtained from mitotic counts on control animals. The similarities between the distributions of thymidine incorporation and of mitotic cells may be judged by comparing Figs. 2 and 3; these similarities show that preferential absorption of the label by certain cells or body regions does not affect the subsequent pattern of nuclear incorporation.

Mitotic counts also confirm that all the types of cells that were labeled, except the cnidoblasts forming nematocysts, undergo frequent mitosis: cnidoblasts presumably are labeled as a result of their origin from labeled interstitial cells. These results are of interest, since in the past there has been some uncertainty as to whether highly differentiated hydra cells divide at all (*13*).

The feature of growth along the entire body axis is consistent with the earlier qualitative observations on cell proliferation (*14*) but it is not in agreement with the subhypostomal growth theory of hydra (*15*). The experimental basis for this latter theory of localized growth is the demonstration of a proximal movement of cells down the column. This phenomenon has been reexamined quantitatively (*16*) and found to favor the new interpretation of broadly distributed growth. Thus, patterns of thymidine incorporation, distributions of mitotic figures, and experimental studies all indicate that proliferation in *Hydra littoralis* takes place over most of the body column.

RICHARD D. CAMPBELL
Rockefeller Institute, New York 10021

References and Notes

1. K. Tripp, *Z. Wiss. Zool.* **132**, 476 (1928).
2. P. Brien and M. Reniers-Decoen, *Bull. Biol. France Belg.* **83**, 293 (1949).
3. The term subhypostomal refers to the region just below the hydranth (see Fig. 2).
4. See discussion, in *The Biology of Hydra*, H. M. Lenhoff and W. F. Loomis, Eds. (Univ. of Miami Press, Coral Gables, Fla., 1961), pp. 312–316.
5. S. K. Brahma and A. Basu, *Exptl. Cell Res.* **27**, 178 (1962); A. L. Burnett, R. Baird, F. Diehl, *Science* **138**, 825 (1962).
6. H. M. Lenhoff, *Exptl. Cell Res.* **23**, 335 (1961); X. Kolenkine, *Bull. Biol. France Belg.* **89**, 169 (1955).
7. Carolina Biological Supply Co.
8. W. F. Loomis and H. M. Lenhoff, *J. Exptl. Zool.* **132**, 555 (1956).
9. Schwarz BioResearch, Inc.; specific activity, 6.6 curies per millimole.
10. D. L. Claybrook, in *The Biology of Hydra*, H. M. Lenhoff and W. F. Loomis, Eds. (Univ. of Miami Press, Coral Gables, Fla. 1961), p. 233.
11. E. Gurr, *Medical and Biological Staining Techniques* (Interscience, New York, ed. 2, 1956), p. 110.
12. B. Kopriwa and C. P. Leblond, *J. Histochem. Cytochem.* **10**, 269 (1962).
13. C. H. McConnell, *Biol. Bull.* **64**, 86 (1933).
14. ———, *Science* **72**, 170 (1930); *Biol. Bull.* **64**, 86 (1933); *ibid.*, p. 96; K. Schneider, *Arch. Mikroskop. Anat.* **35**, 321 (1890).
15. P. Brien and M. Reniers-Decoen, *Bull. Biol. France Belg.* **83**, 293 (1949); A. L. Burnett, *J. Exptl. Zool.* **146**, 21 (1961).
16. R. D. Campbell, *Am. Zool.* **4** (abstr.), 380 (1964).
17. These investigations form part of a program of study under the direction of Dr. Paul Weiss, aided in part by grant No. CA-06375 from the National Cancer Institute.

1 February 1965

Genetic Mosaicism in Adult Mice of Quadriparental Lineage

Abstract. *Genetic mosaic mice can be produced by aggregating, during cleavage stages, the blastomeres of two embryos of different genotype into a single cluster, and by transferring the developing aggregates to the uterus of a surrogate mother. Substantial numbers of such composite embryos survive past birth. Among the living adult mosaic mice are individuals within which cells of markedly different immunogenetic constitution coexist. Through the incorporation of appropriate genetic markers into mosaics, many new possibilities now present themselves for analysis of biological problems during embryonic as well as adult life.*

Five years ago, we began a series of technical explorations intended to culminate in the fairly routine production of genetic mosaic mice. The mosaic condition was to be established in the cleavage period of the egg, within a day or two after fertilization. This was expected to lead not only to a more precocious, but also to a more extensive, admixture of cells characterized by genetic differences than might be achieved either spontaneously through accidents, such as vascular anastomoses between cattle co-twins (*1*), or experimentally by means of limited cell inocula in fetal stages (*2*). The known, and in fact considerable, genetic armamentarium available in the mouse could, under the projected circumstances, be made to serve as an instrument of potentially great resolving power when brought to bear upon problems such as differentiation, within the context of the organism itself.

A suitable means of realizing this end seemed to be through reassemblage of early blastomeres from separate embryos into a single group which, if developmental potentialities were still quite labile, might be capable of normal ontogeny. Procedures were therefore required for removal of the egg envelope (zona pellucida) without injury, for facilitation of rapid adhesion among cells within the aggregate, and for cultivation of the conjoined members in vitro under conditions favorable for maintenance of viability during these rearrangements. All the techniques necessary for producing genetic mosaicism at any time during cleavage were developed in this laboratory and have already been described (*3, 4*). The in-