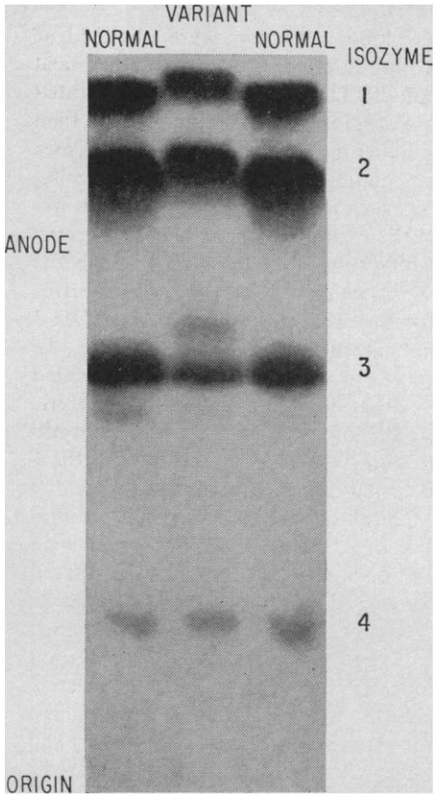

Fig. 3. Comparison of starch-gel electrophoretic patterns of the variant LDH erythrocyte isozymes presumably resulting from double affection at the *b* allele with two normal patterns.

served on the gel in LDH-3 and LDH-4 which contain progressively fewer B subunits and progressively more of the normal A subunits. Isolation and characterization of the variant B subunit are required for the elucidation of these problems. Unfortunately the normal and variant LDH-1 are so close in electrophoretic mobility that recombination experiments in which normal and variant isozymes were mixed and frozen according to the method described by Markert (*1*) failed to shed any light on the nature of the variant B subunit.

The frequency of 0.003 of LDH variants in this study of 1200 patients is of the same order of magnitude as the frequency of 0.009 obtained by Kraus and Neely (*4*) in an investigation of 940 apparently normal hospital employees from Memphis, Tennessee. In this study, in agreement with Kraus and Neely, a higher frequency of variants was observed among Negroes; also, a greater number of A than B variants occurred. Frequencies are best calculated individually for each electrophoretically distinguishable variant. Since the primary structures of isozymes with identical electrophoretic mobilities may differ, it may prove incorrect to assume that two unrelated individuals possess identical point mutations because their corresponding variant lactic dehydrogenase isozymes migrate similarly. Likewise, LDH variants may exist with normal electrophoretic mobility so that the total number of different LDH variants may far exceed that determined solely by electrophoretic screening.

Additional LDH variants have been observed by electrophoretic screening of erythrocytes from various populations (*8*). The populations screened so far have been too small and the number of LDH variants discovered have been too few to determine whether differences in the frequency of variants exist among populations. A greater frequency of variants among Negroes may eventually be established.

The nature of the LDH polymorphism, particularly whether it might confer a selective advantage, such as has been suggested in the case of the sickle-cell heterozygote, is not known. However, if the mutation were neutral, the current frequency of LDH variants in the order of 1 percent in some populations might result solely from the accumulation of mutations over many previous generations. Neutrality is becoming increasingly accepted with the growing number of inherited protein variations which, like the LDH polymorphism, appear to be unassociated with disease. Even if disadvantageous, the LDH polymorphism could still be explained as arising solely from mutation, were it only mildly disadvantageous, and selection against it consequently would be negligible. Alternatively, the observed frequency of variants is also compatible with the notion of transition from an environment in which the mutation had been adaptively advantageous to one in which it no longer was so.

ELLIOT S. VESELL

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

References and Notes

1. C. L. Markert, *Science* **140**, 1329 (1963); E. Appella and C. L. Markert, *Biochem. Biophys. Res. Commun.* **6**, 171 (1961).
2. C. R. Shaw and E. Barto, *Proc. Nat. Acad. Sci. U.S.* **50**, 211 (1963).
3. S. H. Boyer, D. C. Fainer, E. J. Watson-Williams, *Science* **141**, 642 (1963); W. E. Nace, A. Claflin, O. Smithies, *ibid.* **142**, 1075 (1963).
4. A. P. Kraus and C. L. Neely, Jr., *ibid.* **145**, 595 (1964).
5. E. S. Vesell and A. G. Bearn, *Proc. Soc. Exp. Biol. Med.* **94**, 96 (1957); E. S. Vesell, *Ann. N.Y. Acad. Sci.* **94**, 877 (1961); E. S. Vesell and A. G. Bearn, *J. Gen. Physiol.* **45**, 553 (1962).
6. A. P. Kraus compared the electrophoretic mobilities of two of these three variants with his Memphis variants.
7. P. J. Fritz and K. B. Jacobson, *Science* **140**, 64 (1963); L. A. Costello and N. O. Kaplan, *Biochim. Biophys. Acta* **73**, 658 (1963).
8. E. S. Vesell, in *Progress in Human Genetics*, A. G. Steinberg and A. B. Bearn, Eds. (Grune and Stratton, New York), vol. 4, in press.
9. I thank Dr. P. R. McCurdy of D.C. General Hospital, Dr. J. J. McCarthy and Dr. C. E. Luhrs of Georgetown University Medical Center, and Dr. J. S. Shaver of Suburban Hospital for making available clinical material.

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Cellular Segregation and Heterocytic Dominance in Hydra

Abstract. *Hydra heterocytes—that is, hydra containing cells from both normal and mutant animals, were used for studying some developmental properties of mixed cell populations. Cells which initially had originated from a nonbudding mutant, and which subsequently became part of a heterocyte of normal appearance, were able to proliferate, to segregate, to come off as buds, and to express their phenotype in those buds. Furthermore, the heterocytes underwent a transformation in which the mutant phenotype eventually became dominant in all instances.*

Somatic cells that spontaneously mutate may profoundly influence adjacent normal tissues. To investigate such influences, I am studying progeny originating from heterografts made between normal and mutant hydra of the same species. Hydra offer experimental advantages for this type of study in that, unlike most other metazoa, hydra can be made to grow continuously and to reproduce solely by asexual means. Consequently, the mutant somatic cells do not die with the host, but are distributed indefinitely among the progeny where interactions with normal cells may be allowed to express themselves.

Developmental mutants of hydra, which rarely have been reported, were obtained in the following manner. We induced mass cultures (*1*) of *Chlorohydra viridissima* (Florida strain, 1961) to form eggs and sperm by maintaining them in "M" culture solution (*2*) for a week without washing. Hatching from the resultant fertilized eggs were a number of mutant hydra having morphological aberrancies. One such animal was used in the experiments described in this report. This hydra was unusual

in that (i) it did not reproduce asexually by budding; (ii) when bisected transversely, both cut portions developed heads but did not regenerate bases; (iii) in comparison with normal hydra, its size (Fig. 1), dry weight, and protein content (Table 1) were about ten times as great; (iv) it often took on multipolar shapes (Fig. 1*B*); and (v) it could not be induced to differentiate gonads under conditions which induce gonads in the normal animal.

A clone of this mutant was obtained by repeatedly bisecting the animal transversely; the sectioned parts developed mouths and tentacles, ate, and grew. During the month following this operation, a single animal usually gave rise to approximately 10 heads along its body tube; from each head there developed a new body tube, and thus was formed a multipolar (many-headed) parent animal (see some multipolar forms in Fig. 1*B*). Eventually the multipolar animal separated into single monopolar individuals (*3*).

This mutant, like many *C. viridissima* hatched from eggs, was not infected with algae; hence all comparisons (Table 1, Fig. 1) are made against the non-mutant aposymbiotic (albino) animals. Infection of the aposymbiotic mutant hydra with algae did not change the characteristic developmental peculiarities of that mutant. Other properties of this mutant and its cytology will be described elsewhere.

Heterografts between the anterior portion of the mutant and the posterior part of a normal albino hydra commenced to bud progeny, most of which were phenotypically clearly either mutant or normal. Regardless of appearance, however, each offspring had some developmental properties of the normal animal and, at the same time, also had some properties peculiar to the mutant. Such offspring are most probably composed of cells originating from both parts of the graft. Therefore, to facilitate further discussion, these progeny henceforth will be called "heterocytes" according to the definition put forth by Filosa (*4*) for an organism containing cells of the same species but having different inheritable characteristics. Final validity for this deduction, however, will rest upon further cytological evidence.

Table 1. Protein content and dry weight of normal and mutant *C. viridissima*. All animals were without algae. Protein was measured on pooled groups of ten animals by the method of Lowry *et al.* (*8*). For dry weight measurements, individual lyophilized animals were weighed on a quartz fishpole balance (*9*); three animals were used for each determination.

Type of hydra	Protein content (μg/hydra)	Dry weight (μg/hydra)*
Normal	6.32	10.78 ± 2.9
Mutant	49.07	132.9 ± 16.7

* Plus or minus standard derivation.

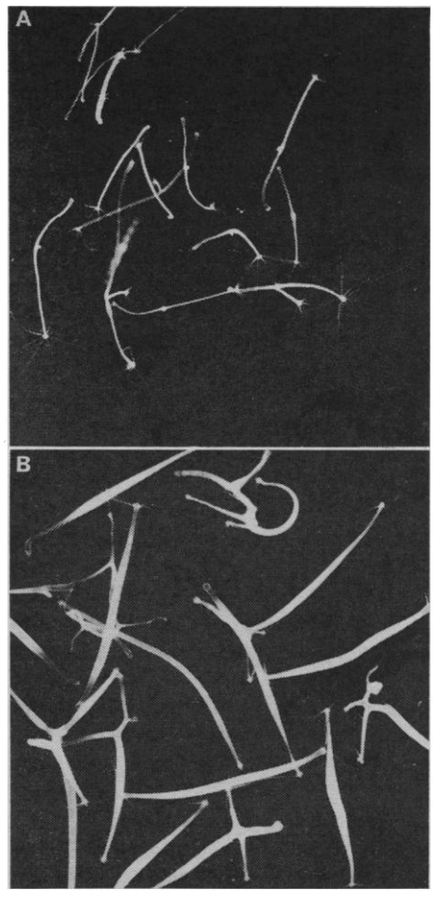

Fig. 1. (*A*) Normal aposymbiotic *Chlorohydra viridissima* (Florida strain, 1961) (× 3.2). (*B*) Mutant *C. viridissima* (× 3).

Heterocytes resembling normal hydra were placed singly in separate Petri dishes and each was allowed to reproduce, by budding, a group of about 100 hydranths (*5*). The number of hydranths in each colony increased at a logarithmic rate, and the doubling time (*6*) was measured (Table 2, column 2). Among the groups derived from each heterocyte there were a number of animals having the characteristic morphological and developmental properties of the mutant (Table 2).

This experiment shows, first, that it is possible to obtain through budding of a single heterocyte, progeny of two distinct phenotypes. Thus, mutant somatic cells appear to proliferate in a host individual, to segregate, to come off as a bud, and to express their phenotype in those buds. Secondly, the groups which had the largest number of mutants had the slowest doubling times. On the other hand, those colonies having doubling times close to those of normal symbiotic and aposymbiotic *C. viridissima* (approximately 1.5 days) gave rise to few mutant heterocytes. Perhaps both the slower growth rate and the large proportion of mutants among the progeny reflect a greater number of mutant cells in the parent organism.

A practical benefit ensued from this finding (Table 2). By growing large numbers of hydra derived from normal-appearing heterocytes, we were able to harvest "mutants" in vastly greater numbers than would be possible through increasing the clone by means of the tedious bisecting procedure. A culture of such "derived" mutants is shown in Fig. 2. It would appear as though the mutant cells, which normally are difficult to obtain in large numbers because of the inability of the mutant to bud, can be obtained in great numbers through the vehicle of the heterocyte. In essence, the "normal" hydra acts as a "tissue culture" for raising mutant cells. This procedure for "rearing" mutants has also been applied successfully in this laboratory with another kind of non-budding hydra mutant of the same species.

An unusual change in phenotype was observed among these normal-appearing heterocytes. Every such animal, reared for periods varying from 2 weeks to 5 months, invariably changed its morphological and reproductive characteristics and became gradually transformed into an animal of mutant phenotype. Never has a polyp of mutant phenotype been observed to transform into one of normal phenotype.

Experiments illustrating the transformation of a heterocyte of normal appearance into one of mutant phenotype

Table 2. Production of "normal" and "mutant" progeny by budding of five heterocytes of normal appearance. The clonal logarithmic growth rate k was obtained by using the equation for logarithmic growth (*6*): $k = \ln 2/T$. The term T refers to the time, expressed in days, in which the numbers of hydranths (*5*) doubled in number.

T	k	Progeny		Progeny as mutant (%)
		Normal	Mutant	
1.60	0.43	115	1	0.87
1.65	.42	126	8	5.97
1.80	.39	132	9	6.38
1.90	.36	57	9	15.8
1.95	.35	50	19	27.6

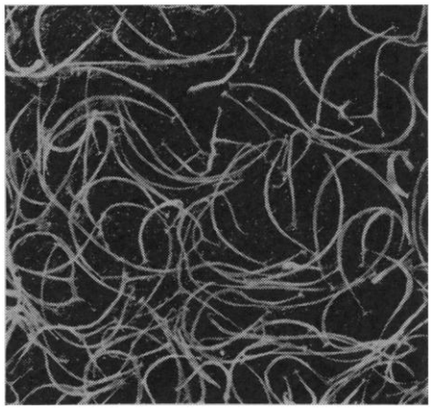

Fig. 2. Colony of "mutants" derived by budding of normal-appearing heterocytes (approximately same size).

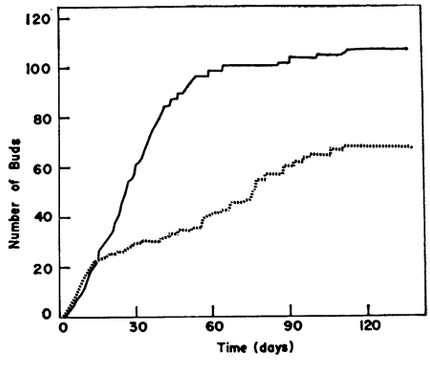

Fig. 3. Budding rate of two heterocytes resembling normal hydra.

were carried out as follows. As a daily procedure, two normal-appearing heterocytes were fed excess *Artemia* nauplii (*1*), their culture solution was changed twice, and the number of buds they produced was recorded. Both animals budded at a normal rate for at least 2 weeks (Fig. 3). At that time one animal (dotted line) became slightly larger, and started to take on the appearance of a mutant. This transforming animal continued to bud, but at a slower and irregular rate. After a month, budding ceased and the once normal-appearing heterocyte was gradually transformed, like the mutant, into a multipolar animal. This multipolar animal then slowly divided into six monopolar mutant-like animals (*3*), a few of which sporadically gave off a number of small bud-like individuals. After 100 days virtually all "bud" production ceased, with the mutant phenotype finally dominating the resultant monopolar animals.

The other heterocyte (solid line) continued to give off buds at a constant rate for about 40 days before it began undergoing a similar transformation. The monopolar mutant-like individuals derived from this normal-appearing heterocyte showed fewer latent bursts of budding activity than did the individuals derived from the other.

These experiments are but two of more than 25 conducted, in which the normal-appearing heterocytes always became mutant. The rate at which this transformation took place probably depended upon the number and location of mutant cells in the original heterocytes (*7*). The mechanism underlying this transformation remains unknown at this time.

The results of these experiments (Table 2, Fig. 4) demonstrate two properties of mixed populations of somatic cells: cellular segregation and heterocytic dominance. These two phenomena, as they occur in hydra, may have broad biological significance. For example, with respect to development, we have a system whereby cells of one set of inheritable characteristics can exert their developmental propensities over those of another, and can be expressed in the phenotype of the animal. With reference to evolution, the eventual dominance of mutant somatic cells may play a major role in the evolution of metazoan species reproducing solely or primarily through asexual reproduction. Lastly, the hydra heterocyte allows us to study a system in which mutant somatic cells can metastasize throughout an animal and eventually dominate the host tissue in a manner analogous to like processes of some tumors.

HOWARD M. LENHOFF
Laboratory for Quantitative Biology and Zoology Department, University of Miami, Coral Gables, Florida

References and Notes

1. W. F. Loomis and H. M. Lenhoff, *J. Exp. Zool.* **132**, 555 (1956).
2. L. Muscatine, in *The Biology of Hydra: 1961*, H. M. Lenhoff and W. F. Loomis, Eds. (Univ. of Miami, Coral Gables, Fla., 1961), p. 255.
3. This slow asexual division of a multipolar animal into monopolar animals is not "budding," which refers to a specific, rapid, developmental process of asexual reproduction in hydra.
4. M. Filosa, *Amer. Naturalist* **96**, 79 (1962).
5. A hydranth, as used in this context, is defined in H. M. Lenhoff and W. F. Loomis, *J. Exp. Zool.* **134**, 171 (1957).
6. W. F. Loomis, *ibid.* **126**, 223 (1954).
7. It should be kept in mind, however, that in cases of heterocytes made from other types of mutants, it may be possible for the normal phenotype to dominate.
8. O. Lowry, N. Rosebrough, A. Farr, R. Randall, *J. Biol. Chem.* **193**, 265 (1951).
9. O. Lowry, *ibid.* **140**, 183 (1941).

10. Supported by grants from the USPHS (GM 11226-02) and the Florida Division of the American Cancer Society, and by an investigator award of the Howard Hughes Medical Institute. This work was presented at a meeting of the American Society of Zoologists, Knoxville, Tenn., 1964.

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Sonar System of the Blind: Size Discrimination

Abstract. *Measurements were made of the ability of four blind subjects to use echoes to discriminate between objects of different sizes placed in front of them. Threshold estimates indicate that objects with area ratios as low as 1.07/1 could be discriminated.*

Kellogg (*1*) demonstrated that two blind subjects using information from echoes could detect differences in the sizes of standard objects placed in front of them. He did not, however, obtain measurements as to how fine a discrimination of this nature could be made. We have tested the ability of a group of blind subjects under uniform testing conditions to make size discriminations by using echoes. Measures were obtained as to how much of a difference in size must exist between two similarly shaped targets before one is judged as larger or smaller than the other. The echo-information was obtained from vocalizations produced by the subjects. The results obtained can be said to represent one measure of echo-acuity of the blind.

The subjects were four blind males, 20 to 30 years old. Each had been blind for at least 5 years and none had any spatial vision. Their hearing had been tested and found essentially normal. The subjects had received previous echo-detection training during earlier experiments and had developed their own echo-producing vocalization which they emitted when seeking or identifying targets suspended in front of them. The vocalizations ranged from sharp tongue clicks to elongated "S" sounds.

Testing was done in a specially constructed room. It was relatively isolated from noises outside the laboratory and the walls, ceiling, and floor were covered with sound absorbing materials. Though not anechoic, the room provided a relatively constant auditory environment in which to perform the tests. The average sound level in the room was 43 decibels on the C scale of a sound level meter with reference pressure 0.0002 dyne/cm^2. The apparatus used to present the circular sheet-metal targets to the subject extended down from a cupola above the room. These disc-shaped targets were raised and lowered on a metal rod by an experimenter in the room above. Subjects were seated in a testing chair