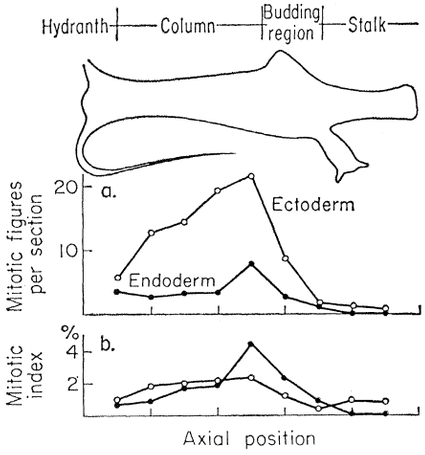

Fig. 3. Axial distribution of mitotic figures in a control hydra. Axial position (abcissa) is indicated by the drawing above the graph. Mitotic index is the percentage of cells in division. Solid circles, endoderm; open circles, ectoderm. Fixed in Lavdowsky's fluid and stained by Feulgen method. Each 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.

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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-

cidence of success which they yield is close to 100 percent, not only in promoting the reorganization of two separate embryos into a single one, but also in retaining participation of the respective components and in permitting the resultant embryos to progress to the blastocyst stage in vitro (*3, 4*). Other methods for combining eggs (*5*) have proved to be substantially more difficult and less effective. A detailed comparison of the two sets of procedures has been presented previously (*4*).

We have focused subsequent efforts upon promoting the possibility of implantation and sustained development of the embryos after transferring them from culture to a favorable recipient female. Many technical modifications have been progressively employed in the course of these studies, and the general trend has continued in the direction of increased viability. Survival of the mosaics past birth in appreciable numbers now seems assured. The current rate of survival is 34 percent, considering only the second half of the heterogeneous series of experiments to date. This represents a substantial increase over the rate of 28 percent obtained in the first half. Judging from the most recent data, a further improvement can be expected. At present over 100 such postnatal survivors are on hand, each having originated from two fertilized cleaving eggs. They comprise a variety of genotypic complements which have been brought into association in pairs within these individuals. The oldest animals are adults of relatively advanced age; some have produced numerous progeny.

The mosaic population includes hermaphrodites. This fact is consistent with expectation, in view of randomness of egg pairing with respect to sex chromosomal constitution.

One of the mosaic combinations can be seen in Fig. 1. The foster mother (albino) is shown with three young for which she served only as an "incubator." Each of the three is of quadriparental lineage and was derived in the following manner. One egg from a mating of inbred C57BL/6 female × C57BL/6 male, and another of pure C3Hf-strain parentage were obtained; both were stripped of their envelopes with pronase during mid-cleavage, and all of the blastomeres from each were aggregated under the conditions recognized as critical (*3, 4*). (Inclusion of all the cells, rather than half of them, from each contributor leads at first to formation of a double-size embryo, but normal size is restored before birth.) The unified cellular mass was allowed to develop to the blastocyst stage in culture and was then transferred surgically to the uterus of the surrogate mother in which pseudopregnancy had been induced by means of a sterile mating with a vasectomized albino male. The young were born at the normal time, and appeared healthy.

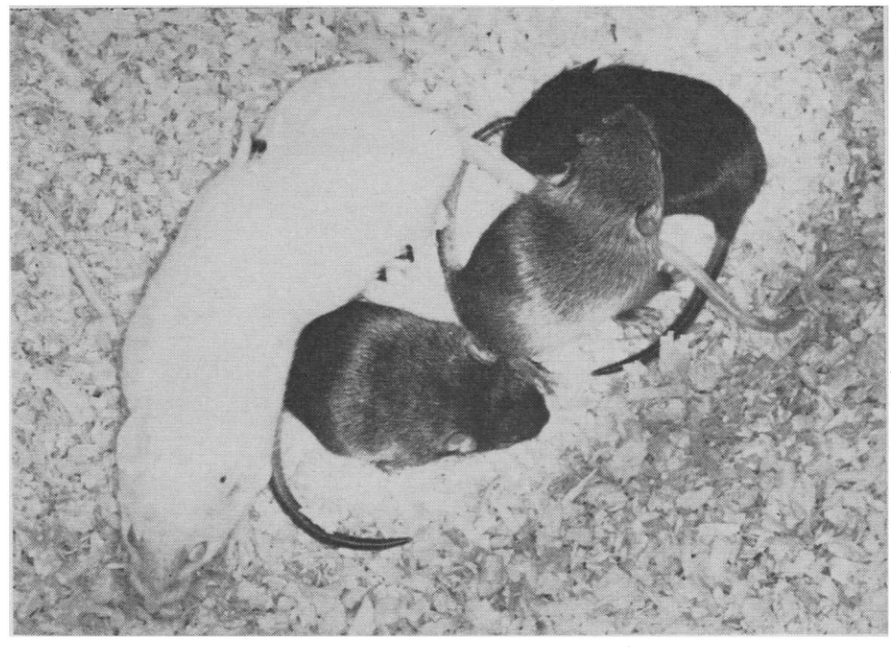

Fig. 1. Albino foster mother (with surgical wound clips visible) and her three "offspring" at 11 days of age. The coat colors are black (upper), agouti (middle), and mottled (lower). Each young mouse developed from an embryo in which blastomeres of independent C57BL/6 and of C3Hf origin were combined.

These remarkable animals, however, are mosaics for alleles at the *H-2*-locus, which exerts the strongest influence over graft recognition of any of the immunological histocompatibility factors identified in the mouse (*6*). The C57BL/6 strain carries the $H\text{-}2^b$-allele; C3Hf is $H\text{-}2^k$. Mice of either of these strains ordinarily reject grafts received from the other type.

Genetically controlled pigmentary differences in the strains of origin afford the possibility of external evidence of mosaicism. Both participants have black melanocytes. Mice of strain C3Hf, however, are *agouti*, while those of strain C57BL/6 are *non-agouti*. In the former, each hair has a subterminal yellow band which accounts for the characteristic coloration and which expresses a localized periodic influence of the hair follicle upon its melanocytes (*7*).

The C57BL/6 ↔ C3Hf mosaics have three classes of coat color: all or almost all black (non-agouti); all or almost all agouti; or mottled, with both colors (Fig. 1). Those which are mottled exhibit a striking pattern of markings. The pattern is one not previously observed in mice, and it appears to be repeatable from one mottled individual to another. Mosaicism in the monochrome as well as the mottled animals is under examination by means of a number of immunological and other markers.

Clearly, the incorporation of multiple, selected genotypes into single mice now affords a tool for the investigation of a great many biological questions in entirely new ways. Indeed, under such experimental conditions disclosure of hitherto unsuspected parameters might be anticipated, and these could lead to a useful reformulation of some of the questions.

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