cal events leading to parthenogenesis in *D. mangabeirai* have also been reported (*9*). The evidence again points to an automictic type with fusion of haploid nuclei following the completion of maturation divisions.

Completion of maturation in the "unfertilized" eggs of other insect genera is not always associated with parthenogenesis. For instance, in the grasshopper, *Melanoplus,* maturation divisions proceed in essentially the same way as in eggs which receive sperm (*10*). Here the stimulus for eggs to develop beyond metaphase of the first division evidently acts during the process of egg laying. This also may be true of *D. melanogaster* (*11*).

W. W. DOANE
Osborn Zoological Laboratory, Yale University, New Haven, Connecticut

References and Notes

1. A. F. Huettner, *J. Morphol.* **39**, 249 (1924).
2. B. P. Sonnenblick, *Biology of Drosophila,* M. Demerec, Ed. (Wiley, New York, 1950), p. 62.
3. W. W. Doane, *Drosophila Inform. Service* **32**, 121 (1958).
4. M. Rabinowitz, *J. Morphol.* **69**, 1 (1941).
5. E. H. Strasburger and L. Körner, *Biol. Zentr.* **59**, 366 (1939).
6. E. Guyénot and A. Naville, *Cellule rec. cytol. histol.* **39**, 25 (1929).
7. H. D. Stalker, *Genetics* **39**, 4 (1954).
8. L. Sanger, thesis, Washington Univ. (1958).
9. W. H. Murdy and H. L. Carson, *Am. Naturalist* **93**, 355 (1959).
10. R. L. King and E. H. Slifer, *J. Morphol.* **56**, 603 (1934).
11. This work was supported by predoctoral fellowship No. 28404 from the National Science Foundation.

20 June 1960

Regeneration of Hydra from the Endoderm

Abstract. The endoderm of hydra, when cultured independently, can differentiate so that the total animal is reconstituted. Reconstitution or regeneration, or both, occurs without the demonstrable aid of the interstitial cells.

Two theories are held concerning the regeneration of hydra. One states that interstitial cells must be present for regeneration to take place and that the interstitial cells differentiate into the new cells of the regenerate (*1*). Papenfuss and Bokenham (*2*) claimed that the constituents of both cell layers were necessary. Brien and Reniers-Decoen (*3*) and Chapman and Tilney (*4*) have followed the differentiation of the interstitial cells and concluded that they function to form gametes and nematocysts.

However, Kanajew (*5*), employing histological techniques, traced vitally stained cells in the regenerates and concluded that endoderm may be formed solely from pre-existing endodermal cells without the aid of the interstitial cells. Tokin and Gorbunowa (*6*) and von Bertalanffy and Rella (*7*) demonstrated that total regeneration of hydra can take place from a reaggregated mass of cells obtained from the pedal disc. Histologically, only endodermal and ectodermal cells are present in the pedal disc. In addition, Brien and Reniers-Decoen (*8*) showed that regeneration could proceed after the destruction of the interstitial cells by x-radiation. The same authors, by means of vital staining and grafting experiments, observed that an individual cell is labile, and found that it goes through several histological changes during its life cycle. In 1958 Zwilling (*9*) reported that total reconstitution of *Cordylophora* can be obtained from the ectoderm.

This is a preliminary report on experiments to show that total regeneration or reconstitution, or both, of hydra can develop from the endoderm alone.

The animals, *Pelmatohydra oligactis,* were collected and then cultured under simulated natural conditions. The procedure consisted first of the removal of the anterior end of the animal; then the remainder of the animal was treated with a buffered mixture of saturated solutions of pancreatin and amylase at room temperature, to digest the mesogleal layer. When the mesoglea was sufficiently digested, the two cellular layers of the animal were mechanically separated. The isolated endoderm and ectoderm were cultured separately in a saturated solution of sulfadiazine in filtered "pond" water at approximately 15° to 20°C. Within a few hours the endoderm formed a smooth-surfaced solid ball. Under optimum conditions, between 18 and 24 hours later, the ball formed an elongated hollow mass. At the end of 2 days of culture the elongated mass developed the ability to contract both longitudinally and circumferentially when gently stimulated mechanically. By the end of 3 days at least one tentacular nub could be seen. Between days 5 and 6 three short tentacles, complete with batteries of nematocysts, formed around a definable hypostomial region. The tentacles had the ability to elongate and contract. On the 7th day microcrustacea were introduced into the culture medium as food. The capture and utilization of food by the newly reconstituted animals were used as the criteria of their complete reconstitution.

In no case did the separated ectodermal layer reconstitute, although the cells often remained together for a few hours. Then they would either separate and disintegrate, or the whole mass would flatten on the bottom of the culture dish and disintegrate.

Stained preparations of the separated ectodermal and endodermal cell masses have shown that no histologically recognizable interstitial cells were included with the endoderm in these regeneration experiments. However, interstitial cells and all stages of their development into nematocysts were recognized in the ectodermal preparations.

Work is presently in progress to refine the procedures used to obtain the endoderm and to describe cytologically the events that take place during the reconstitution or regeneration of hydra from the endodermal layer.

DIANE K. NORMANDIN
Department of Zoology, University of Illinois, Urbana

References

1. P. Tardent, *Wilhelm Roux' Arch. Entwicklungsmech. Organ.* **146**, 593 (1953); ———, "Some principles governing the process of regeneration in hydroids," paper presented at the 18th Annual Growth Symposium, June 1959, unpublished.
2. E. J. Papenfuss and N. A. H. Bokenham, *Biol. Bull.* **76**, 1 (1939).
3. P. Brien and M. Reniers-Decoen, *Bull. biol. France et Belg.* **83**, 293 (1949).
4. G. B. Chapman and L. G. Tilney, *J. Biophys. Biochem. Cytol.* **5**, 69 (1959).
5. J. Kanajew, *Wilhelm Roux' Arch. Entwicklungsmech. Organ.* **122**, 736 (1930).
6. B. P. Tokin and G. P. Gorbunowa, *Zhur. Obshchei Biol.* **3** (1934) summary in German, p. 306.
7. L. von Bertalanffy and M. Rella, *Wilhelm Roux' Arch. Entwicklungsmech. Organ.* **141**, 99 (1941).
8. P. Brien and M. Reniers-Decoen, *Bull. biol. France et Belg.* **89**, 258 (1955).
9. E. Zwilling, *Biol. Bull.* **115**, 319 (1958).

10 June 1960