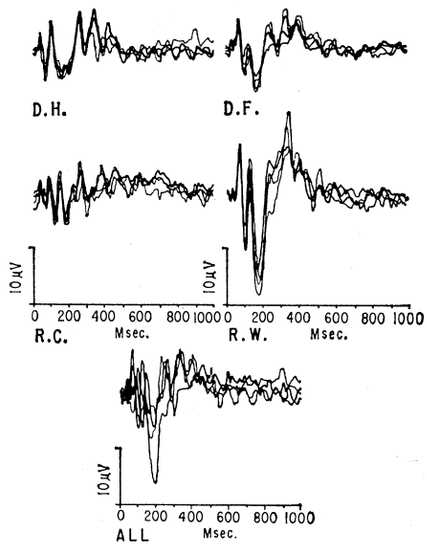

Fig. 1. Reliability and individuality of the evoked potentials. The first four illustrations show the superimposition of four averaged evoked responses recorded at weekly or longer intervals from subjects D.H., D.F., R.C., and R.W. The fifth shows the superimposition of the evoked responses of all four subjects.

brain-wave (electroencephalographic) patterns. Two had predominantly alpha patterns, two had predominantly beta patterns, and the remaining three had mixed alpha and beta patterns. Averaged evoked potentials were obtained from four subjects on four different occasions and from three subjects on three different occasions. Individual recordings were taken at weekly or longer intervals.

Inked plots of the resulting averaged evoked potentials yielded a complex wave consisting of eight distinct components in the first 300 msec of the response. These components were repeatedly observed with all subjects.

A base line for each plotted evoked response was established by drawing a horizontal line which touched the base of the largest positive deflection. Twenty-five ordinates erected from the base line, equally spaced over 300 msec, intersected the various components of the evoked response. A measure in millimeters was made of the distance from the base line to the point at which the vertical lines intersected the evoked response. Thus each of the ordinate values was expressed as a distance from the largest positive deflection of the evoked response.

Pearson product-moment correlations were then computed to determine the degree to which ordinate values of the evoked response obtained from one recording session were related to those obtained from other recording sessions.

To determine the degree to which inter-individual evoked responses were related, a mean for each of the 25 ordinates was computed for each subject from the values obtained from his repeated recordings. The mean ordinate values of each subject were then correlated with those of other subjects.

An analysis of the data indicated that those components of an individual's averaged evoked potential occurring in the first 300 msec were highly reliable over long periods of time, in this instance, intervals separated by a week or longer. Test-retest correlations of the averaged evoked response of each of the seven subjects ranged from .72 to .99, with a median correlation of .88. Inter-individual correlations proved to be much smaller, ranging from —.29 to .92, with the median correlation being .37.

These relationships between evoked responses can be portrayed graphically. Figure 1 illustrates that an individual's evoked response recorded at one time more closely resembles his own evoked response at other times than it does the evoked responses of other individuals. Each of the first four illustrations shows the superimposition of four evoked responses, recorded at weekly or longer intervals, from each of four subjects. An evoked response from each of the subjects is shown superimposed in the fifth illustration.

It should be noted that disparity among the responses occurs after about 300 msec in most cases. These later components are often characterized by rhythmic waves of an alpha frequency. An attempt was made in the present study to determine if the resting alpha frequency was related to the frequency of these rhythmic late components. A correlation between the average alpha and after-discharge frequencies of 17 additional subjects, whose evoked potential showed an after-discharge, was .58 ($P =< .02$). The correlation indicated that those with faster alpha frequencies tended to have faster after-discharge frequencies.

The variation in the response from different individuals deserves comment. Some reasonable possibilities are that it may be due to individual differences in scalp and skull thickness, the distance from excitatory areas of cortex to recording electrodes, or simply background frequency. This latter possibility was investigated. A rank order correlation was computed for frequencies of brain activity and amplitudes of the averaged evoked responses. It was not significant. However, the two "fastest" brains, predominantly beta rhythm background frequency, ranked first and second in amplitude of early components of the evoked response.

From these data it would appear that the earlier components of the averaged evoked response are reliable and stable over a period of weeks, as attested by the reliable intra-individual correlations. Although individuality is clearly reflected in the evoked response, its source is obscure and must await further investigation.

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References

1. G. D. Dawson, *Electroenceph. Clin. Neurophysiol.* **6**, 65 (1954).
2. R. G. Bickford, R. F. Galbraith, J. L. Jacobsen, *ibid.* **15**, 720 (1963).
3. C. Shagass and M. Schwartz, *J. Neuropsychiat.* **2**, 262 (1961).

8 November 1963

Dedifferentiation and Redifferentiation of Cells in *Hydra viridis*

Abstract. *The interstitial cell of the green hydra is formed by dedifferentiation of specialized gastrodermal cells. Similarly, the epidermal epitheliomuscular cells are probably formed by direct differentiation of algae-laden digestive cells that lose their algae and enclosed food droplets, migrate to the periphery of the animal, and begin the mucous secretion characteristic of epidermal cells.*

The origin of cells comprising a regenerate has been studied in all the major phylums. Invariably, such studies are complicated by the presence of so-called embryonic cells in the regenerating organism, which obscure the results. These cells, according to the animal in which they are found, have been designated interstitial cells, archeocytes, formative cells, neoblasts, mesenchyme cells, totipotent cells, plus a variety of less common names. They are usually characterized by a highly basophilic cytoplasm, lack of cytoplasmic organelles, and, except in vertebrates, the ability to migrate from dis-

Table 1. Histochemical and structural differences between the types of cells in *Hydra viridis*.

Cell type	Location	Cytoplasmic organelles	Alkaline phosphatase	SH (Gomori)	Hyaluronidase	Glycogen	Sudanophilia	Protein reserve
Interstitial	Epidermis	–	–	–	Partially labile	–	–	–
Gland	Gastrodermis	Zymogenic granules	+	–	Fast	–	–	–
Mucous	Gastrodermis	Mucous droplets	+	+	Labile	–	–	–
Digestive	Gastrodermis	Large vacuole	+	+ (Food droplet)	Fast	+	+	+
Epidermal epitheliomuscular	Epidermis	Mucous-secreting border	–	+ (Mucous droplet)	Fast	+	–	–
Cnidoblast cell	Epidermis	Nematocyst	–	+ (Nematocyst)	Fast	–	–	–

tant areas to the site of a wound. If these cells really are representatives of a persistent, embryonic stock, then they might be able to differentiate into any cell type found in the organism, so that a lost part could be regenerated without the direct participation of other cell types. However, it is also possible that during regeneration, specialized cells undergo a morphological and physiological dedifferentiation, assume the appearance of the neoblast, and then participate in the replacement of the missing structures.

It was reported recently that the isolated gastrodermis of a hydra can regenerate its epidermis (*1*). We decided that such a system would be useful for studying cell potencies during regeneration, provided we could identify precisely the cell types present in the isolated gastrodermis. We would have to study the regeneration process by histological methods during all stages of regeneration, and to know well the sequence of differentiation of neoblasts in the normal animal.

European strains of the green hydra, *Hydra viridis*, were used in the present study. This species lacks neoblasts in the gastrodermis, and the gastrodermal digestive cells are provided with an excellent marker in the form of algal symbionts. Unlike many other species of hydra, the gastrodermis does not contain mature cnidoblasts with functional nematocysts. It is, therefore, possible to isolate strips of gastrodermis containing only three cell types, namely gastrodermal digestive cells, zymogenic or gland cells, and mucous cells, all of which may be clearly distinguished by histochemical techniques (*2*) (Table 1). The digestive cells can also be observed easily in the living and fixed animals because of the algal cells within them. It should be stressed that cnidoblasts are not autoreproductive, but are formed exclusively by differentiation of interstitial cells (*3*). Also, in a normal animal which has no injury there is no evidence that dedifferentiation of gastrodermal elements occurs.

To separate the cell layers, animals were excised at the level of the subhypostomal growth region and through the proximal portion of the gastric region. This ring of tissue was then threaded on a glass capillary needle. The animal was transferred to a 0.5-percent solution of lyophilized trypsin (*4*) which was made up in a salt solution containing 0.1 percent NaCl, 0.2 percent $CaCl_2$, 0.01 percent $KHCO_3$, and 0.03 percent $MgSO_4$. After 4 minutes the animal was transferred to a dish containing 50 ml of the same salt solution without the enzyme.

The epidermis, readily identified by its lack of algae, was removed from the gastrodermis by teasing apart the layers with fine glass needles. Separate pieces of epidermis and gastrodermis were transferred individually in depression slides containing 0.5 ml of the salt solution and were incubated at 9°C. Such tissue fragments were often extremely small; in one instance, a hydra containing one tentacle, a hypostome, and a mouth opening regenerated from a piece measuring 270 μ by 40 μ.

Experiments were performed to establish that the gastrodermis so obtained was not contaminated with other types of cells. Tissue fragments from 25 hydras were fixed for 1 hour in 100 percent alcohol, then stained in aqueous toluidine blue, buffered at *p*H 7; they were then destained in 50 or 70 percent alcohol, dehydrated in absolute alcohol, and mounted in Permount. The fragments were examined histologically and none contained interstitial cells or cnidoblasts. Only gland cells, mucous cells and digestive cells were present (*5*).

Twenty strips of isolated gastrodermis were examined histologically, in groups of five, after 5 hours, 8 hours, 12 hours, and 24 hours of incubation. Some strips were also observed after longer periods, and in a few instances a hydra had regenerated, which had a well formed hypostome and mouth opening, a peduncle and a basal disc. However, the survival rate of fragments after 24 hours was small and later stages were not observed in detail histologically.

After 5 hours, the fragments were still lacking interstitial cells. Gastrodermal digestive cells had lined up around the periphery of the tissue mass with their basal nuclei pointing towards the center. Although symbiotic algae were still present within the cells, many of the algae had been voided to the central cavity. Thus, the gastrodermal cells at the periphery had much clearer cytoplasm than those in the center of

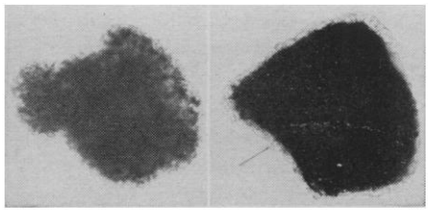

Fig. 1. (Left) A fragment of gastrodermis 4 to 5 hours after isolation. The cells which form the border still contain algal cells but are beginning to clear. (Right) The same fragment 24 hours after isolation. It is surrounded by a layer of large clear cells, some of which (arrow) still contain algae.

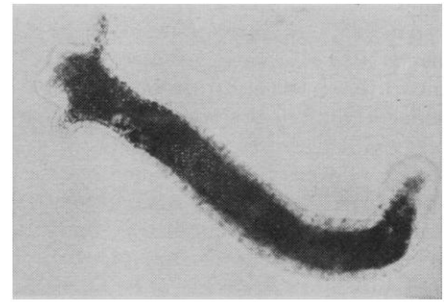

Fig. 2. A complete hydra regenerated from the fragment of gastrodermis shown in Fig. 1 (72 hours after isolation).

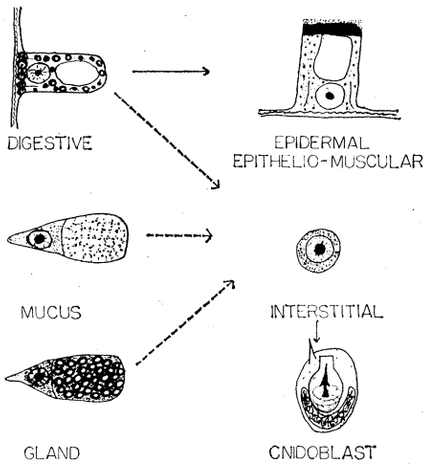

Fig. 3. Diagrammatic representation of the various routes by which interstitial cells might be formed from gastrodermal cells.

the mass which had not voided their algae. This process of algal voiding by digestive cells could be observed in the living masses of the tissue by examining them under the compound microscope at a magnification of 160.

Noticeable changes had also occurred in gland and mucous cells by this time. Their cytoplasm was more basophilic than usual, and gland cells located at the periphery of the mass had apparently voided their internal secretions to the surrounding medium. This would account for the highly basophilic nature of the cells, since the area surrounding their nuclei contained a considerable amount of RNA. However the RNA of the dedifferentiated gland cell was not necessarily similar to that of the interstitial cell.

No cnidoblasts were present in any of the preparations at this time. A well formed metachromatic border was observed around the periphery of the mass, probably representing the secretion of mucous cells, and the digestive cells in the periphery were more basophilic than those in normal animals.

After 8 hours, cells morphologically identical to interstitial cells were present in the peripheral border. After 12 hours, the number of small basophilic cells had greatly increased, but no mitoses could be found in the preparations. The gastrodermal digestive cells had become clearer due to the voiding of their algal bodies. No cnidoblasts were present at this stage.

After 24 hours, the number of small basophilic cells had decreased, but large numbers of cnidoblasts had formed. In each animal, about 24 cnidoblasts contained fully formed nematocysts, many of which discharged upon contact with the fixative. Other cells were in various stages of transition from interstitial cells to cnidoblasts. Polarity may have been already determined at this stage, since half the tissue mass (presumably the distal portion of the animal) was rich in cnidoblasts and interstitial cells, while the other half (the future peduncle and basal disc) was virtually devoid of these cells. The epidermis had formed and was almost free of algae (Fig. 2). In the few animals that were examined after 48 hours, the cells at the periphery of the mass had begun the mucous secretion typical of epidermal cells and were completely free of algae.

We also studied 25 fragments of epidermis, which were treated and incubated in the same way as the gastrodermis. The fragments all rounded up into small balls within several hours of isolation and remained in this form until they disintegrated 48 to 72 hours later. Prior to disintegrating, they showed no signs of differentiating into gastrodermal elements.

Our results thus show that epidermal, epitheliomuscular cells can arise directly from gastrodermal cells which lose their enclosed algae and food droplets and begin mucous secretion. Interstitial cells appearing in the mass do not arise from pre-existing interstitial cells, but from digestive cells, gland cells, or mucous cells which void their internal secretions. Therefore, the interstitial cells do not represent a "modulated" (*6*) form of a gastrodermal cell, since they are capable of differentiating into cnidoblasts containing mature nematocysts; neither do they represent a persistent embryonic stock which is maintained solely by the division of interstitial cells. Evidently, certain specialized cells in the hydra are not "end points" of development but can acquire new potency if properly stimulated (*7*).

Since this paper was accepted for publication, we succeeded in obtaining regeneration from gastrodermal fragments which contained only digestive cells and gland cells. Therefore, the mucous cells are not required for the formation of any epidermal cell type.

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References and Notes

1. D. K. Normandin, *Science* **132**, 678 (1960).
2. P. Brien and M. Reniers-Decoen, *Bull. Biol. France Belg.* **89**, 259 (1955).
3. A. L. Burnett, *J. Exptl. Zool.* **140**, 281 (1959); D. Slautterback and D. Fawcett, *J. Biophys. Biochem. Cytol.* **5**, 441 (1959); A. Hess, *The Biology of Hydra and Some Other Coelenterates*, H. M. Lenhoff and W. F. Loomis, Eds. (University of Miami, Coral Gables, 1961), p. 467; A. L. Burnett, *Ann. Soc. Zool. Belg.* **90**, 295 (1960).
4. Mann Research Laboratories, New York.
5. This whole mount technique is extremely sensitive for the detection of interstitial cells. For example, in *Hydra pirardi*, which loses its interstitial cells during sexuality, we have detected as few as two interstitial cells which had escaped the sexual process and had not differentiated into gametes. Digestive cells are readily recognized by their enclosed algal bodies whose nuclei stain intensely; mucous cells are detected by the presence of high metachromasia in the cytoplasm, and gland cells by their large zymogen granules. The stain might also be considered specific for nematocysts, because in whole animals, these structures stain blue-black and stand out vividly in the tissues. To test the ability of the whole mount method to detect small amounts of epidermis, several fragments of the gastrodermis were purposely contaminated with epidermis. Epidermal cells showed up quite distinctly. In addition, any contaminating epidermis that was present collected and formed a clear cap around a small portion of the border of the fragment. The epidermis did not spread out and form a uniform layer around the mass of gastrodermis.
6. P. Weiss, *Principles of Development* (Holt, Baltimore, 1939), p. 93.
7. Supported by the National Science Foundation and the U.S. Public Health Service.

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A Remarkably Reduced Vascular Plant in the United States

Abstract. *A clone-forming thallus lacking sex organs and propagating by gemmae occurs on rocks and tree trunks in the Appalachian region from Georgia to Virginia, Ohio, and Kentucky. Although bryophytic in appearance, the thallus is identified here for the first time as a greatly reduced variety of the vascular plant known as "shoestring fern,"* Vittaria lineata *(L.) J. E. Smith, or a closely related species. The normally dominant sporophytic phase of the life cycle has been eliminated and the plant exists only as a vegetative prothallus.*

Evolutionary reduction—the abbreviation or loss of organs and organ systems—has long been familiar to students of vascular plant morphology and phylogeny. The example to be reported here, however, is so extreme that the plant concerned, even though widespread and locally abundant in many localities in the Appalachian region, has been neglected in catalogues of the vascular floras of the majority of states where it occurs, with but rare