THE PHENOMENA OF REGENERATION IN EVERTED HYDRAS

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BY

JAMES EDWARD WASHINGTON

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CHAPTER I

INTRODUCTION

It has been known since the time of Trembley (1774) that hydras possess the ability to regenerate. The literature on regeneration of hydras is voluminous, but the data on the regeneration of everted hydras is less numerous. Roudabush ('33), after having everted hydras was able to get a migration of the cells of the ectodermal and endodermal layers through the mesoglea back to their respective position within twenty-four hours. The purpose of this work was to reinvestigate the findings of Roudabush and to observe the nerve cells during this process.

REVIEW OF LITERATURE

The first worker to observe that a hydra turned inside out might regenerate was the Swiss priest, Alexander Trembley (1774). He maintained erroneously that the ectoderm, which in the turned animals lined the enteric cavity, came to take over the function of the endoderm; while the endoderm which was the outside, assumed the function of the ectoderm.

In 1878 Engelman repeated a number of Trembley's experiments. He turned hydras inside out but could not keep them alive in this state. Soon after the operation the polyps turned themselves back with the ectoderm on the outside.

Nussbaum (1887) repeated Trembley's experiments but came to the conclusion that instead of reversal of functions, migration of the two layers took place. He described the sliding of the ectoderm over the endoderm which started at the mouth and a pore in the base, and continued until the entire ectoderm had reached its outside position. Ischikawa (1890) undertook the same problem and obtained the same results as Nussbaum.

Hadzi ('09) showed that previous to and during the process of bud formation certain of the interstitial cells wandered from the ectodermal region to the region of the newly forming endoderm where they gave rise to certain endodermal elements. Tannreuther ('09) found that the initial steps in the formation of buds in hydras was started in the interstitial cells. He found that the most active region of growth in the formation of the bud is found at the junction of the forming bud and parent. The cells in the region of the junction divided very rapidly and contributed almost entirely to its growth. McConnell ('32) showed that the sensory cells arose as modification of the interstitial cells when the bud began to form tentacles. When the tentacles had completed their growth, a network was formed in the ectoderm which gradually extended into the region of the basal disc. Sensory cells, found on the surface of the tentacles and mouth region, later developed processes which connected them with the nerve net.

Hadzi ('09) stated that the nematocysts migrated throughout the body of hydroids. Kepner and Baker ('24) have shown that nematocysts of hydras can migrate from the enteron to the epidermis of worms of the genus Microstoma, which have ingested the hydras as food. Jones ('41) showed that the nematocysts of Pelmatohydra oligactis arose in the ectoderm from differentiated interstitial cells. These young nematocysts passed through the mesoglea in the endodermal cells.

McConnell ('31) found that dissociated cells of hydras became amoeboid and were able to move about quite freely. These endodermal cells were seen to take up particles of food with pseudopods. Later, however, the cells were able to elaborate flagella.

Mattes ('25) stated that on wound healing in hydras the endodermal cells first came in contact and united. The ectodermal cells then

gradually grew over the endoderm until they came in contact. Kanajew ('29) stated that the process of wound healing was accomplished by the concentration of large endodermal cells which immediately became surrounded with large ectodermal cells. Mesoglea appeared later. Following this step the interstitial cells accumulated and surrounded the wounded region. Part of these cells were ectodermal in origin and part of them originated in the endoderm, and migrated through the mesoglea.

Tripp ('28) removed the foot of hydras and turned them inside out. When these hydras were grafted into the sides of other hydras two different results were obtained. The graft would partly revert the original anterior end and appear as a bud, or the ectoderm of the host and mouth rim of the graft would migrate over the endodermal surface of the inverted graft. In this case the graft absorbed its original ectoderm. Mutz ('30) demonstrated that small inverted pieces of the oral disc, transplanted either in the oral or aboral region of another hydra, acted as an organizer, if they were 0.75 mm. in size. It reversed the polarity of the tissue in the region to which it was grafted. The graft retained its individuality and induced a new formation.

Kanajew ('30) found that the interstitial cells played a subordinate role in regeneration and budding. The regenerated part, like the bud, at least as regard to its endoderm, is formed at the expense of the nearby differentiated tissues. This was demonstrated by histological examination and by transplantation of vitally stained parts. Papenfuss ('34) everted two hydras in order to have the endodermal layers on the outside. When these two endodermal layers were placed one upon the other, he noticed a fusion of the two layers. However, if one of the endodermal layers was removed, leaving the mesoglea exposed, it was impossible to get the endodermal layer fused with the mesoglea. By re-

moving the endoderm and mesoglea of hydras and placing the ectoderm of these hydras together, it was impossible to get fusion of the ectodermal layers. Ectoderm would neither fuse with endoderm nor with mesoglea. Papenfuss and Bakenham ('39) were unable to get a hydra to regenerate when the ectoderm and endoderm were cultured independently. They interpreted these results as an indication that the differentiated cells of one tissue layer were unable to transform into the cell types characteristic of the lacking tissue layer. The undifferentiated cells, the embryonic interstitial cells, did not become activated to regenerate this tissue.

CHAPTER II

MATERIALS AND METHODS

The animals used in these experiments were the brown hydras (Hydra oligactis). They were secured from Turtox Biological Supply House, Chicago, Illinois and Carolina Biological Supply Company, Elon College, North Carolina. These animals were fed daphnia. They were kept in the same glass container in which they were shipped and the debris and undigestible particles were removed daily from the container with a glass pipette.

The instruments used for everting the hydras were: a bristle from a nylon shaving brush inserted into the end of a match and held in place by means of clarite; watchmaker's forceps for holding the animal, and a depression slide. Paraffin was placed on the edges of the nylon bristle and the watchmaker's forceps in order to smooth them. The method used was similar to that of Roudabush ('33). A hydra was placed on a depression slide with a few drops of balanced aquarium water under a binocular microscope. It was stimulated to contract by carefully prodding it with the nylon bristle. When it had fully contracted its basal end was pushed with the bristle toward the anterior end which was held with the watchmaker's forceps. When the basal end had come in contact with the hypostome, the hydra was made to open its mouth by carefully manipulating the bristle and moving the forceps. The basal end was then pushed through the mouth until the animal was everted. After everting the hydra, the bristle was carefully withdrawn holding the animal everted with the forceps. They were observed to see if they reverted immediately. Those that remained everted were transferred to a glass container with some balanced water.

Animals to be fixed were allowed to remain in the inverted condition

for a predetermined length of time. They were anaesthetized with 0.1% chloretone solution; fixed in a solution of ten parts commercial formalin, fifty parts 95% alcohol, two parts glacial acetic acid and forty parts distilled water. The fixative was poured onto them in a stream from a glass pipette from the base anteriorly. No preliminary washing was needed for this fixative. The animals were passed through a series of graded alcohols, cleared in oil of bergamot and embedded in paraffin. They were sectioned at six micra and placed on slides. The sections were stained in Delafield's hematoxylin and counter stained in eosin. In order to show nervous elements the Protargol method of Bodian ('36) was used. The animals were fixed in a solution containing 5 ml. acetic acid, 5 ml. of formalin and 90 ml. of 80% alcohol for one hour. They were then washed in several changes of 70% alcohol. After passing them through the series of alcohols, they were cleared in oil of bergamot, embedded in paraffin, sectioned at six micra and placed on slides. The paraffin was removed with xylol. The sections were then passed through the series of alcohols to distilled water. After removing them from the distilled water, they were placed in a solution of 1% Protargol-S for twenty-four hours at 37°C., then washed in distilled water. They were placed in a reducing solution containing:

1 gm. hydroquinone
5 gm. sodium sulphite
100 cc. of distilled water,

for ten minutes. They were then washed thoroughly in distilled water. The sections were toned in a 1% gold chloride solution containing three drops of glacial acetic acid for ten minutes; then washed in distilled water. The sections were placed in a 2% oxalic acid solution for ten minutes to differentiate the nervous elements. The residual silver salts were removed by placing the sections in a 5% sodium thiosulphate solution

for ten minutes. The sections were washed thoroughly in distilled water, dehydrated and mounted in balsam.

Fig. 1 — A photograph of a normal hydra in the budding region. This is a longitudinal section under the high power of the microscope. (440X). Note:

(a) Cuboidal cells of the ectoderm

(b) Interstitial cells of the ectoderm

(c) Nematocysts of the ectoderm

(d) Columnar cells of the endoderm

(e) Interstitial cells of the endoderm

(f) Dark staining droplet cells of the endoderm

(g) Nutritive-muscular cells of the endoderm

Fig. 2 — A photograph of a hydra a few minutes after eversion in the basal region. This is a longitudinal section under the high power of the microscope. (440X).

Note:

(a) The columnar cells of the endoderm are external.

(b) The mucous gland cells of the ectoderm are internal.

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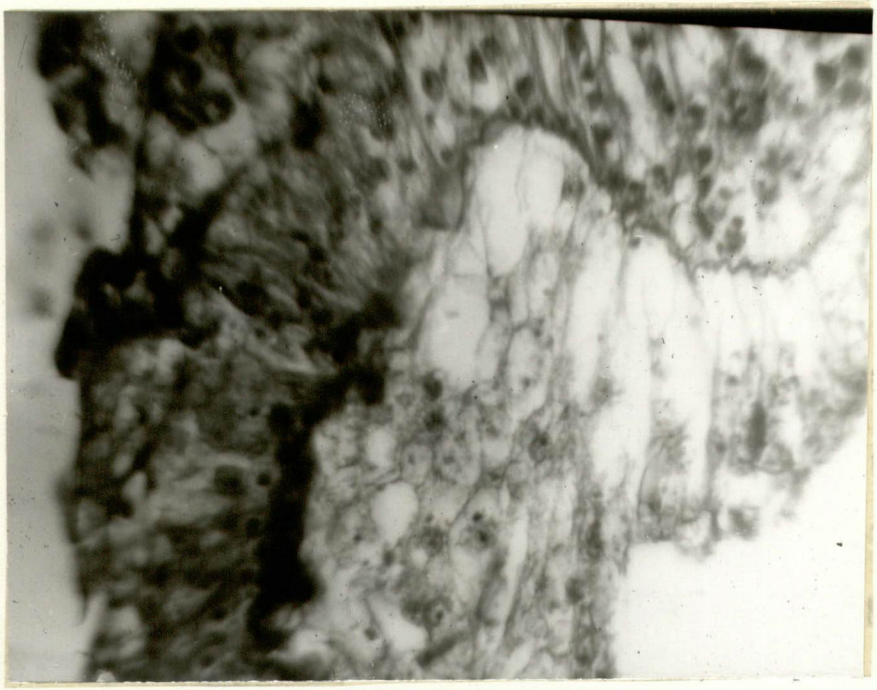

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Fig. 3 — A photograph of a three hour everted hydra in the budding region. This is a longitudinal section under the high power of the microscope. (440X).

Note:

(a) The columnar cells of the endoderm are external.

(b) Glandular cells in the inner layer.

Fig. 4 — A photograph of a hydra six hours after turning in the basal region. This was a longitudinal section under the high power of the microscope. (440X).

Note:

(a) Columnar cells of the outer layer

(b) Nematocysts of the outer layer

(c) Glandular cells of the inner layer

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Fig. 5 — A photograph of a hydra nine hours after turning in the budding region. This is a longitudinal section under the high power of the microscope. (440X).

Note:

(a) Nematocysts in the outer layer

Fig. 6 — A photograph of a hydra twelve hours after turning in the budding region. This is a longitudinal section under the high power of the microscope. (440X).

Note: — The cells of one layer cannot be distinguished from those of the other layer.

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Fig. 7 — A photograph of a hydra fifteen hours after eversion in the mouth region. This is a longitudinal section under the high power of the microscope. (440X).

Note:

(a) Nematocysts in the outer layer

(b) Gland cells of the mucous type in the inner layer

(c) Columnar cells of the inner layer

(d) Dark staining droplet cells in the inner layer

Fig. 8 — A photograph of a hydra eighteen hours after turning in the basal region. This is a longitudinal section under the high power of the microscope. (440X)

Note:

(a) The outer layer of cells was between cuboidal and columnar in shape.

(b) The inner layer of cells was of a simple columnar type.

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Fig. 9 — A photograph of a hydra twenty-one hours after eversion in the basal region. This is a longitudinal section under the high power of the microscope. (440X).

Note:

(a) Columnar cells of the inner layer

(b) Cuboidal cells of the outer layer

Fig. 10 — A photograph of a hydra twenty-four hours after eversion in the budding regions. This is a longitudinal section under the high power of the microscope. (440X).

Note:

(a) Cuboidal cells of the ectoderm

(b) Columnar cells of the endoderm

(c) Nutritive-muscular cells of the endoderm

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CHAPTER III

EXPERIMENTAL RESULTS

The data presented here is based upon experimental evidence collected from hydras fixed at the time of eversion, three, six, nine, twelve, fifteen, eighteen, twenty-one and twenty-four hours after eversion, and a normal hydra.

The hydras which were everted reacted in the following manner: First, some of them were not able to adjust themselves to the new situation, and almost immediately underwent "depression"; second, some of them returned themselves immediately; and third, some of them remained turned and regenerated. It was the third group of hydras from which the sections were made.

A normal hydra (Fig. 1) fixed has the following characteristics in a longitudinal section. The epithelial cells, which are the supporting cells, of the ectodermal layer are cuboidal in shape. The epidermis of the tentacles and oral region consists of an abundance of nematocysts, which with their attendant cnidoblasts are embedded in the supporting cells. Gland cells are frequent in the epidermis of the oral region and the pedal disc. Between or in the supporting cells are found small rounded cells, usually in clumps, which are the interstitial or indifferent cells. The endoderm consists mainly of large columnar epithelial cells called nutritive cells. These cells are highly vacuolated. Near the mouth gland cells of the mucous type are very abundant. They are elongated cells with conical free ends containing the nucleus and secretion granules. In the digestive region the endoderm is liberally sprinkled with gland cells of the granular type.[1]

Droplets secreted by the digestive cells which stained pink with eosin were found in the endodermal layer. The cells of the endoderm of

the epithelial disc are composed of columnar epitheliomuscular cells that contain food-vacuoles and which present no gland cells.[2] The mesoglea is a thin homogeneous plate bearing on its outer and inner surfaces the muscular processes of the ectodermic and the endodermic cells.[3] With these characteristics it was usually easy to identify the cells of the different layers.

A hydra (Fig. 2) fixed a few minutes after eversion had the glandular cells of the basal disc, which are the characteristic of the ectoderm on the inner layer. The tall columnar cells of the endoderm were external. The nematocysts, which are predominant in the ectoderm, appeared in the inner layer.

In sections made three hours after eversion (Fig. 3), the columnar cells on the outside were not as tall as they were when the animal was everted. Interstitial cells are found in the mesoglea. A few glandular cells appeared in the budding region of the inner layer.

In sections fixed six hours after turning (Fig. 4), there were a greater number of glandular cells in the inner region which once were the nutritive cells. Some of the nematocysts had migrated back to the outer layer. The cells of the outer layer were still columnar.

In sections made nine hours after eversion (Fig. 5) more nematocysts were found in the outer layer; however, the original endodermal cells were columnar.

In sections fixed twelve hours after eversion (Fig. 6) it was difficult to distinguish one layer of cells from the other. The cells were so arranged that those of the ectoderm could not be distinguished from those of the endoderm.

In sections made fifteen hours after eversion (Fig. 7) nematocysts were seen on the periphery of the outer layer. Mucous gland cells were

seen in the basal disc of the outer layer. Near the mouth, gland cells of the mucous type were present in the inner layer. Cells containing pink staining droplets were present in the inner layer also.

The sections fixed eighteen hours after turning (Fig. 8) had an outer layer of cells which were between cuboidal and columnar in shape. The inner layer of cells were of a simple columnar type. Cells containing more pink staining droplets were present in the inner layer.

The sections fixed twenty-one hours after turning (Fig. 9) had columnar cells on the inner layer which were larger than those of the eighteen hour everted animal. An abundance of nematocysts were seen in the outer layer. The outer layer of cells were cuboidal. Glandular cells were present in large numbers in the inner layer in the region of the nutritive-muscular cells. The latter cells showed vacuolated areas. Several cells containing pink staining droplets were present in the inner layer of cells.

The hydra fixed twenty-four after eversion (Fig. 10) had the cells of the endodermal and ectodermal layers in their respective positions.

The migration of the endodermal and ectodermal cells in all of these sections were rather uniform in the mouth, budding, and foot regions.

During the process of cells migration the number of interstitial cells, the undifferentiated embryonic interstitial cells, did not increase in number in the ectodermal and endodermal layers.

CHAPTER IV

DISCUSSION

The ability of cells to migrate in animals is not a recent phenomenon. Hadzi ('09) demonstrated that nematocysts migrated through the body of hydroids. Wilson and Penney ('30) have also shown that the amoebocytes of sponges moved from one area to another. Roudabush ('33) was able to obtain the migration of the cells in the endodermal and ectodermal layers through the mesoglea. In living turned Hydra viridissima he was able to get the clear ectodermal cells migrating to the outside through the mesoglea between the green cells of the endoderm. Nussbaum (1887) demonstrated that in everted hydras the cells returned to their respective position by the sliding of the ectoderm over the endoderm which started at the basal region. This work is not in agreement with the experimental findings of Nussbaum (1887), but it is in agreement with the experiments of Roudabush ('33). According to Nussbaum's (1887) findings, the ectodermal cells were predominant in the basal and mouth regions and fewer in number in the budding region during an early stage of cell migration. However, in this work the ectodermal cells were evenly distributed in the three regions during cell migration. In order for the ectodermal cells to be evenly distributed in the three regions they must have migrated through the mesoglea between the endodermal cells.

Trembley (1774) maintained that the ectoderm, which in the turned animals lined the enteric cavity, came to take over the function of the endoderm; while the endoderm which was outside, assumed the function of the ectoderm. In order for this to occur the ectodermal cells must transform into endodermal cells. It is impossible for ectodermal cells to take over the function of the endodermal cells because the endodermal

cells have a special function that cannot be performed by the ectoderm cells; namely, the secretion of digestive enzymes.

There was not a dedifferentiation of the interstitial cells of one layer and then a redifferentiation of these cells to the cells of the other layer. During the process of cells migration the number of interstitial cells, the undifferentiated embryonic cells, were not increased in number in the ectodermal and endodermal layers. Kanajew stated ('30) that the interstitial cells played a subordinate role in regeneration and budding. The regenerated part, like the bud in regard to its endoderm is formed at the expense of the nearby differentiated tissue.

Child stated ('19) that the anterior end of hydras had a higher rate of metabolic activity that that of the posterior end. In this experimental work the rate of metabolism did not seem to have any effect on the migration of cells in these areas. The cells in the posterior end migrated at approximately the same rate as those in the anterior and budding regions.

In the twelve hour evert the cells were in the process of migrating to their respective position, therefore, it was impossible to clearly delineate ectodermal from endodermal components.

According to Hyman ('40) the sensory cells, which are the ganglionic cells, of the epidermal and endodermal layer are similar in structure. Due to this factor it was hard to determine what happened to these sensory cells during the process of cells migration. However, the Protargol method of Bodian ('36) showed the ganglionic cells with their processes in the two layers from the time of eversion until complete reconstitution.

CHAPTER V

SUMMARY

1. Hydra oligactis can be everted and kept alive in this condition.

2. The hydras that remain turned regain normality by the migration of the cells constituting the two layers of the body back to their respective positions.

3. The migrating cells of each layer, individually or in groups, penetrate the mesoglea and filtrate between the cells of the opposite layer.

4. The process of cell migration takes approximately twenty-four hours.

5. The cells migrate at approximately the same rate of speed in the three regions: the head, budding and foot.

6. Protargol--S can be used to demonstrate the individual cells and nerve net of hydra.

FOOTNOTES

[1] Libbie H. Hyman, The Invertebrates: Protozoa through Ctenophora (New York, 1940), pp. 375-381.

[2] W. A. Kepner, "A New Histological Region in Hydra oligactis", Biol. Bull. (1928), vol. 54, p. 173.

[3] James Ritchie, Thomsons' Outlines of Zoology (New York, 1948), p. 187.

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