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Non-cellular Regeneration Processes in the Integument of the Flatworm *Geoplana abundans*¹

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Abstract. Some terrestrial Tricladids, like *Geoplana abundans*, develop unusual regeneration processes in the course of replacing lost parts of their integument. Specimens fixed in 5 consecutive stages of generation show the following phenomena: first formation of a membrane over the wound; then establishment of a plasmodial covering layer under the membrane through re-utilization of various tissue fragments; then migration of nuclei into this layer and their adjustment to the new position; finally formation of a complete integument without cellular structure but with clear delimitation from the other tissues by a basal membrane.

Key Words

Non-cellular regeneration
Integument-regeneration
Regeneration of Flatworm
Geoplana regeneration

No neoblasts participate in the formation of this integument but later an immigration and vigorous multiplication of neoblasts take place, always below the basal membrane. Still later the plasmodial integument itself also takes up a definite cellular structure. The possible role of similar phenomena in the pathological histology of higher organisms is pointed out.

Introduction

Processes of regeneration have been studied very thoroughly for many years as several good surveys of recent years indicate [HAY, 1966; Goss, 1969] and yet, there are still some aspects of this enigmatic developmental phenomenon which need clarification and do not fit in the general framework of existing knowledge. The regeneration of lost parts in flatworms is generally considered to be the work of neoblasts [WOLFF, 1962]. Although their significance is slightly different under different experimental conditions their role as the main source of regenerated structures is undeniable [BRONSTED, 1955; LENDER, 1960; PEDERSEN, 1959; WOLFF, 1962]. (No attempt will be made here to review the extensive literature.) However, exper-

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iments to be described below indicate that terrestrial flatworms—generally considered to be poor regenerators—have regeneration processes which are, in their first phase, independent from the function of neoblasts. In fact, this primary process is entirely non-cellular, i.e. new structures are formed without multiplication or dedifferentiation of cells. The existing structural elements are partly demolished, partly reconstructed and thus provide the first rudiments of new structures.

Material and Methods

The experiments were carried out with *Geoplana abundans* (Froelich) which has on the dorsal side seven black longitudinal stripes on a bright yellow background. These stripes play a role in the observation of the regeneration process because their bending over the cut surface indicates the progress of wound healing with great precision.

In the first series of experiments 21 specimens were used. Because of the somewhat unexpected results the experiments were then repeated in a second series with 14 specimens. The flatworms were collected in nature and were kept for 2 weeks in culture bowls where they were abundantly fed with terrestrial isopoda. Afterwards they were starved for one week before the experiments were started. One specimen or fragment was used only once to follow the regeneration.

The amputations were made in Petri dishes on wet filter paper with a flame-sterilized scalpel. The line of section was halfway between the anterior and posterior end of the body axis. After the immediate injury contractions have ceased the fragments to be observed were transferred to another Petri dish with wet filter paper and kept in dark. The regeneration process was observed on the cut surface of the posterior pieces, the anterior halves were discarded. This eliminated any variability due to the gradient factor and the data could be pooled for evaluation.

Bouin's solution was used as fixative (washed out with 70% alcohol), and the pieces were routinely embedded and sectioned. Sections of 3.5 and 7 μ thickness in groups of 16 were mounted on slides. For orientation Hauser's triple stain technique was used but for specific purposes Azan, Masson-Goldner and iron hematoxylin (Heidenhain) staining was also carried out. Observations were made with a Zeiss-Lumipan microscope and Zeiss phase contrast equipment, photographs were made with a Seibert (Wetzlar) Photoautomat.

Results

The process of regeneration was studied in 5 consecutive stages. The first was immediately after amputation when the postoperative convulsions have ceased. The second was 1 h after amputation, the third 5 h later (i.e. 6 h after amputation), the fifth 24 h after amputation and the sixth 72 h (3 days) after it.

Stage 1. Immediately after amputation. The region of the wounds shows a very characteristic appearance (fig. 1). The open wound is reduced to a minimum by strong contraction of the circular muscle fibers. Macroscopically this manifests itself in the curvature of the black dorsal stripes. The lateral portion of the integument is also contracted toward the midline. It is strongly bent inward and ends abruptly at the edge of the wound. These curvatures of the integument are also clearly noticeable on the microscopic sections. Nevertheless, the wound is still open at this time in the center. All the tissues which were cut through by the amputation are freely exposed. Within 10 min one can notice a tendency of these tissues to be pushed outward which indicates a considerable inner pressure. On many sections fixed at this stage one can find on the surface solid structures which have been eliminated, such as intestinal lining, muscle fibers, accumulation of nuclei of unknown cell types, etc. This indicates that the organism provides an almost instantaneous wound closure. In this first stage neither new structures nor changes in existing tissues can be noticed. The only change which can be observed is a swelling of the cut ends of muscle

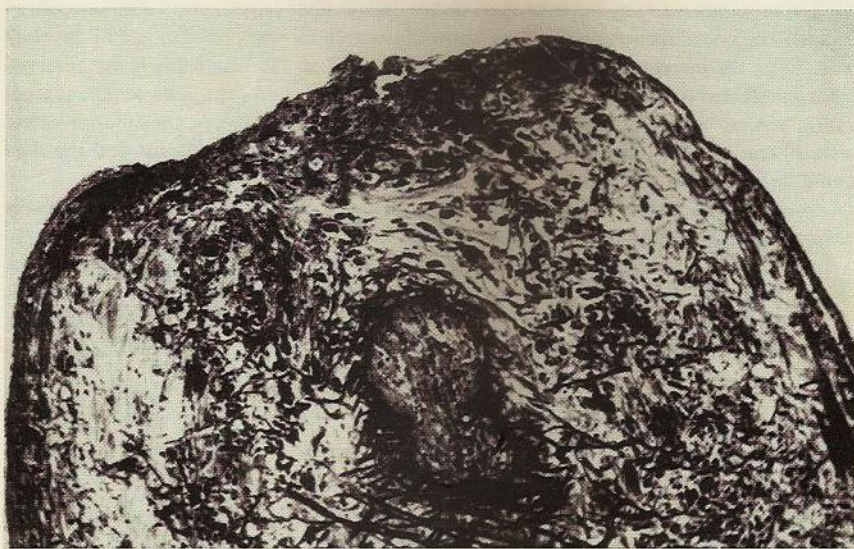


Fig. 1. General view of the transected region immediately after the amputation. The curvature of the integument is due to muscular contraction in the cutaneous muscle system. 100 \times .

fibers. But even this can be considered as an overcontraction of injured fibers, i.e. a physiological phenomenon rather than a morphological change in the tissue.

Stage 2. One hour after amputation. Some of the structures which appear in the microscopic picture at this stage represent the most distinctive characteristics of this whole regeneration process. The integument gradually relaxes its contracted condition over the wound. But the marginal cells are different in their appearance from the cells of the intact epidermis. Instead of being columnar they are flattened. The position of the nuclei is also changed. Their long axis is now parallel to the surface, not perpendicular to it as in the intact epidermis. Sometimes these marginal cells appear shaped like a wedge whose edge begins secreting a membrane.

One can distinguish 3 different kinds of such newly produced membranes. The first seems to grow out of the integument (fig. 2), but is not a simple continuation of the basal membrane or the cuticle. These original membranes disappear with the last intact epidermal cells. Moreover, the new membrane shows quite different staining properties. Whether it has a fibrillar structure or not only electron microscopic studies could decide. In the light microscope it appears, even highly magnified, homogeneous. It has the same staining properties as the cytoplasm.

The second kind of new membranes appears not in the edge of the wound but in the center (fig. 3). This type is similar to the first but is not connected to cells. Sometimes nuclei of mesenchymal origin are included in these membranes giving the impression of a pseudo-cellular organization. (It would be difficult to produce objective evidence against the true cellular nature of these structures but the author is satisfied that they are not cells in the strict sense.) Neither mitotic nor amitotic cell divisions were ever observed in the vicinity of these membranes, nor even structures which could be interpreted as indicative of cell divisions. The membranes have another characteristic feature: they stain very strongly with Heidenhain's iron hematoxylin.

The third type is actually a double structure (fig. 4). It is a layer which is composed of formed cellular elements. These have the tendency to melt into a homogeneous tissue limited by a distinct outer membrane which differs sharply.

All these membrane types may be encountered side by side in the same section but their appearance is irregular. Sometimes the one type, on other occasions the other is prevalent and no explanation was found so far for this variation. There is no time sequence in their appearance.

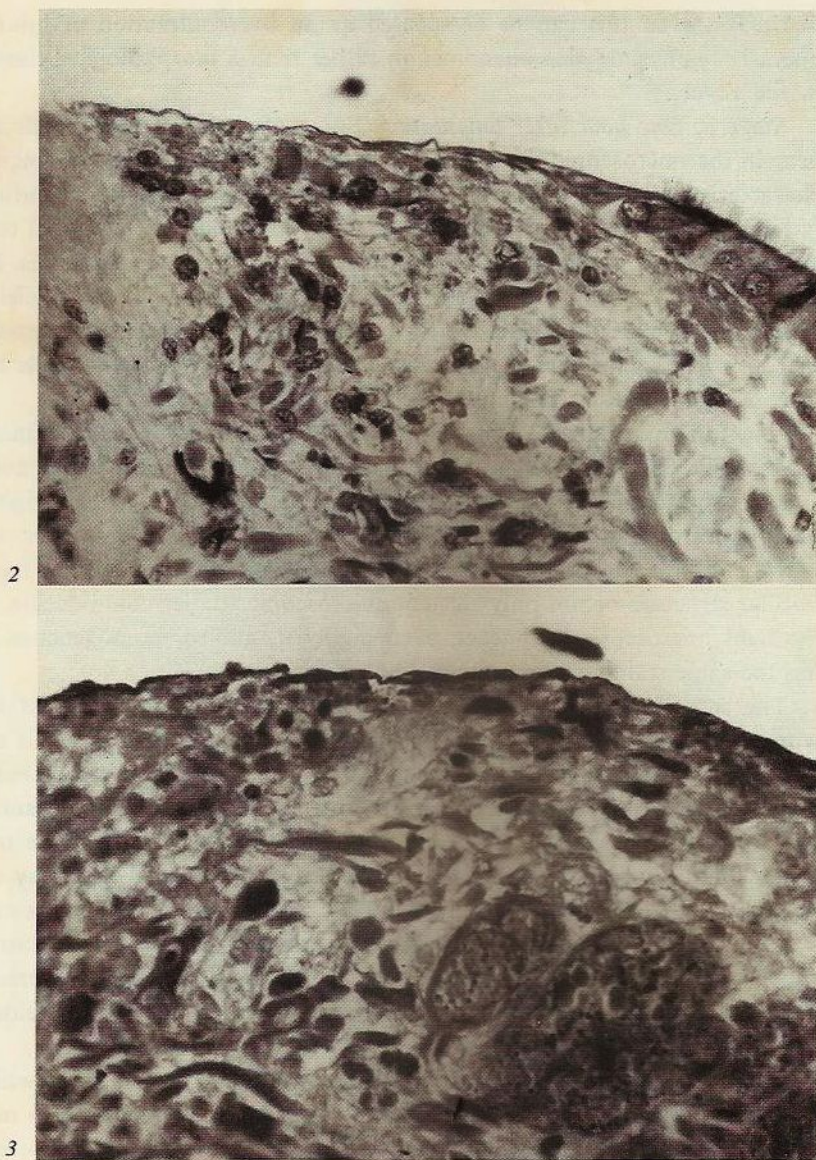


Fig. 2. The formation of a fine membrane arising from the marginal cells of the epithel. 400 \times .

Fig. 3. Fully formed surface membrane in the central portion of the wound. 550 \times .

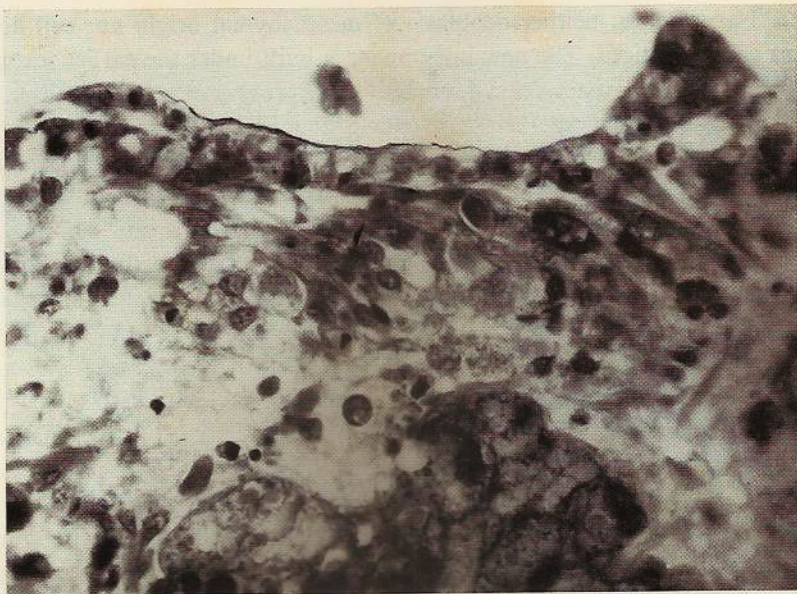


Fig. 4. Thick sheath derived from the debris of the intestinal epithel and other tissue fragments. Note a fine, clearly defined cuticle on the surface. 550 \times .

Stage 3. Six hours after amputation. The time span of 6 h brings no further structures. However, one can notice the beginning of a phenomenon which appears fully developed only in the next stage, i.e. 24 h after amputation. This is a thickening of the muscle fibers, which is distinctly different from that swelling mentioned in the description of stage 1. This latter is due to contractions of the injured muscles while in stage 3 the enlarged portions show at the edge signs of dissolution (fig. 5).

By this time in most cases the entire wound surface is covered with a continuous membrane and the re-building of a new integument has started. In one preparation of this stage an interesting case of reconstruction was observed. The new covering layer has closed out a number of testicular tubules. Obviously, the highly specialized structures of spermatogonia and spermatocytes are not suitable as raw material for reconstruction, while sperm is immediately decomposed and its substances incorporated in new structures.

Stage 4. Twenty-four hours after amputation. At this stage the main characteristics of the regeneration process are even more clearly apparent (fig. 6). The formation of the new integument is in full progress by this

time. Nevertheless, neither neoblasts of mesenchymal origin nor cell divisions can be noticed. The new integument is built under the earlier formed membrane, from existing tissue material by a process of reorganization. It is of no importance whether these tissue components come from endoderm or mesoderm; they will be in any case transformed into integumental epithel—which was of ectodermal origin in the first place! The 2 theoretically separate processes of decomposition of tissue debris and building of a new integument go here hand in hand and are parallel rather than consecutive processes. Thus one can spot in the newly formed integument segments which betray by their staining properties that their origin is different from that of the rest. Nevertheless, they seem to be completely incorporated in the new structure. Soon new migrant nuclei start to appear in the integumental layer. These nuclei are in the beginning of diverse structure and size. Some are small, compact nuclei, originating from secretory cells, others medium-sized mesenchymal nuclei but there also large vesicular nuclei of intestinal epithel cells. It should be emphasized that the appearance of these nuclei is not a process of cell migration as no cytoplasmic sheath can be seen around the nuclei.

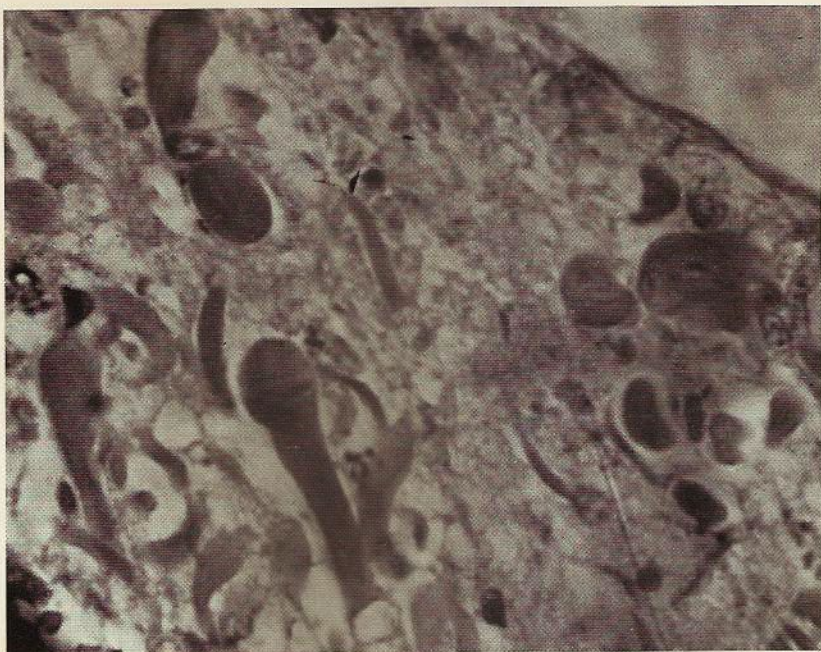
The cytoplasmic ground substance of the new integumental layer slowly becomes uniform and shows a fine granular structure. The nuclei which migrated into it also take up a uniform average size (however, not within 24 h). The new integument is at this time not yet separated from the tissues below it. It touches directly on the mesenchyme. On the outer surface of the integument cilia are formed but this process was not followed closely enough by the author and will not be described here.

Muscle fibers begin to grow into the regenerating area from all directions. Whether new fibers can be formed independently from the existing cutaneous muscle system is questionable. Simultaneously with the appearance of muscle fibers a new basal membrane also appears (fig. 6, arrow). It is formed by the mesenchyme which indicates that in the case of these Turbellarians the basal membrane has nothing to do with the 'epithel' i.e. the integument of our terminology.

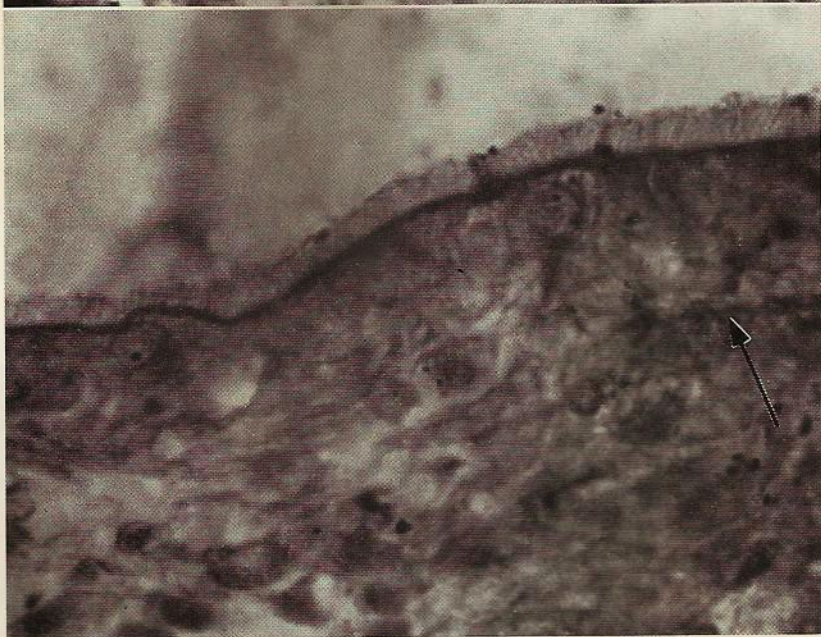
The new integument is in the beginning purely plasmodial in nature: no membranes divide it into cellular compartments. One cannot call it a synci-

Fig. 5. Partial destruction of muscle fibers. Swelling and lysis occurs at the cut ends. 1,000 \times .

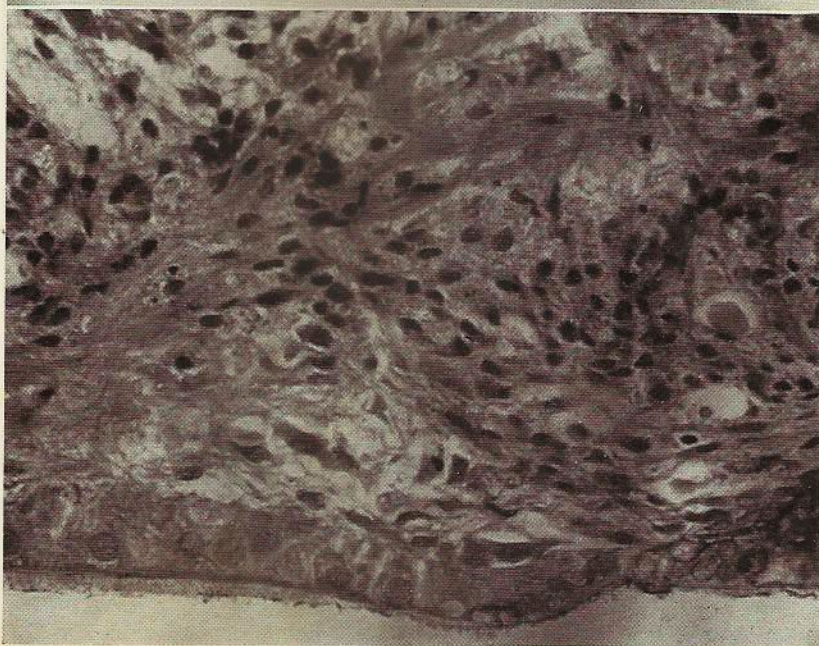
Fig. 6. The new integument, continuous with the mesenchyme. Note the beginning of the formation of a new basal membrane at right (arrow). 1,000 \times .



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6



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8

tium as it does not have a cellular organization in the first place. But the plasmodial integument gradually changes into the regular structure, made up by individual cell units as in the normal epithel of intact individuals. However, this gradual transformation takes place only in later stages. Cell membranes were not noticed at 24 h, not even at 72 h with the techniques employed in this study.

Stage 5. Seventy-two hours after amputation. In 3 days the formation of the plasmodial integument is completed. Various inclusions, such as rhabdites, secretion granules and fibrous secretions may now appear in it. The cutaneous muscle tube and the basal membrane are also completed. In the mesenchyme below the muscle tube for the first time secretory cells and cell groups appear. A lively migration of neoblasts starts (fig. 7) but it has no role in the formation of the integument as by this time the basal membrane completely separates the integument from other tissues. The neoblasts participate in the formation of the regeneration blastema and exhibit strong mitotic activity. But all these processes take place now below the integument (fig. 8). The really large scale reorganization processes, aimed at replacing lost organs, are just starting at this stage. But these are not the subject of this paper.

Discussion

On the basis of the observations described above two fundamental questions can be raised. First, is the regeneration a single process, taking place always and everywhere in the same manner, by the same mechanism or are there essentially two different kinds of regeneration, a cellular and a non-cellular one. If so, can both of them be called 'a replacement of lost parts'? The second question is a continuation of the first one: are the processes described in this paper more than just a wound healing?

To the author it seems that both questions can be answered in the affirmative. As regards the first question, the positive answer has been given already in the relevant literature on regeneration. This is obviously too bulky to be reviewed here. The positive answer to the second question is based on the consideration that a complete reconstruction of a part of an organ system—in this case the integument—comes surely under the head-

Fig. 7. Regenerating 'epithel' (integument) and agglomeration of neoblasts in the mesenchyme. 550 \times .

Fig. 8. Fully regenerated epithel with cellular structure restored. 150 \times .

ing of 'replacement of lost parts'. This is the more so because in the reconstruction of the integument alien material is utilized from other tissues and organs. Through the reconstruction process a new functioning organ is established.

Is this type of regeneration a phenomenon restricted only to lower invertebrates? Or can a similar plasmodial growth process occur in higher organisms too where it might give rise to abnormal increase, hyperplasia or hypertrophy, let alone malignant growth of parts? Perhaps some of the morphological findings in tumors and other pathological conditions can be explained with the help of the results described here. Extracellular matrices play an imcreasing role in the thinking of developmental biologists [HAY, 1968, HAY and REVER, 1969] and phenomena like the ones encountered in lower invertebrates may give further impetus to research in this direction.

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