The respective roles of mitotic activity and of cell differentiation in Planarian regeneration

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Blastema formation, which is the first step of regeneration in adult Metazoa, is generally considered to be merely an accumulation of undifferentiated cells provided by mitotic activity, which occurs near the wound following amputation. Afterwards, these undifferentiated cells are thought to differentiate rapidly, thus affecting the organization of the regenerate.

Some authors have postulated that a close similarity exists between these undifferentiated cells and the blastomeres of a young embryo, and that the influence exercised by the stump tissues in blastema differentiation is a typical inductive process. This concept would imply two successive steps in regeneration: (1) blastema formation, exclusively dependent upon mitotic activity, even after the previous accumulation of undifferentiated cells resulting from migration (Dubois, 1949), (2) the transformation of the blastema into a regenerate as differentiation occurs.

Up to now, the first step seemed to be confirmed by experiment, since some authors have observed that some experimental factors which prevent regeneration (X-rays and mitoclastic poisons) inhibit mitoses. However, it has not yet been possible to produce any evidence of the occurrence of a completely undifferentiated state in the blastema. As soon as it has grown large enough to be surgically separated from the old tissues, it is already fully determined (Sengel, 1960).

Experiments carried out in our laboratory have shown repeatedly that mitotic activity is not the determinant factor in regeneration. Indeed, a high mitotic activity does not necessarily cause blastema formation, and blastema formation is sometimes possible when mitoses are inhibited. The first morphological manifestation of regeneration probably follows the onset of cell differentiation in the undifferentiated material.

MATERIAL AND METHODS

Experiments have been performed on the freshwater planarians Dugesia gonocephala (and its asexual form D. subtentaculata) D. tigrina, D. lugubris and Polycelis cornuta.

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Cytological observations have been made on smears. The whole fragment to be studied is gently drawn along a slide and stained by the Pappenheim panoptic technique (May-Grünwald, Giemsa). All fragments used for counting mitoses were of the same size. They had previously been treated with mitoclastic poison (colcemide, $10^{-4}$ M) for 24 h. Mitoses were counted over the whole surface of the smears.

X-irradiation was also performed under standard conditions. Worms generally received 10 250 r (95 kV, 8 mA, 25 min to the whole body (Chandebois, 1963a)).

For in vitro cultures, a special planarian arginine-free medium has been devised (Chandebois, 1963a). Fragments excised by two transverse sections were cultured in watch glasses on agar (0·5 % in the medium). They were immersed in a drop of fluid medium.

**CYTOLOGICAL EVENTS IN THE PARENCHYMA AT THE BEGINNING OF REGENERATION**

First, let us recall how the preliminary regeneration processes occur in planarians (Chandebois, 1962). The blastema cells are apparently fused into a syncytium. The same undifferentiated elements are also found in the fixed parenchyma of the intact worm. In normal conditions, their participation in regeneration is clearly shown by their mitotic activity near cut surfaces (Plate 1, fig. A). Free and strongly basophilic cells, the cells described as type I by Prenant, which accumulate near the wound and show a variety of mitotic figures, might seem to play a direct part in building the regenerate. They are in fact generally considered as 'neoblasts', i.e. totipotent cells. However, they cannot be found in the young blastema itself and they are probably formed by the syncytium elements (Plate 1, B, C). Indeed, many cytological observations have revealed that the number of chromosomes varies greatly in these cells of type I, from one chromosome to a number much higher than the diploid number (Plate 1, D1, E1, E2, E3, E4, F). These variations depend upon repeated

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**PLATE 1**

The origin and the various possible transformations of cells of type I in *Dugesia gonocephala* ($2n = 16$). Cells of type I originate from syncytium nuclei (A) around which a basophilic border of cytoplasm appears (B). This cytoplasm is finally delimited from the syncytium by a plasma membrane (C). These cells liberate cytoplasmic buds in the syncytium all through their life (D). The buds divide themselves (Db 1) and swell and disappear in the syncytium (Db2). Cells of type I show mitotic figures (D1 is a young cell with 16 anaphase chromosomes), they do not divide at the end of telophase (D2) and grow polyploid (F is a tetraploid cell). They also undergo unequal amitoses (E), so that during further mitotic events they exhibit various numbers of chromosomes (E1, E2, E3, E4 respectively show 1, 3, 4 and 8 chromosomes). The cytoplasm of cells of type I finally enlarges (G1) and is no longer clearly limited from the neighbouring syncytium. They form 'residual plasms' (G2) in which buds and chromosomes can often be observed (H). b, Cytolysing buds. The photographed cells are from cultured fragments since their cytology is clearer. All these structures have been observed in regenerating fragments (see Chandebois, 1962).
unequal amitoses (Plate 1, E) and upon the failure of the cytoplasm to divide after telophase (Plate 1, D2, F). It is clear that such cells have no histogenetic potencies. They finally swell, forming ‘residual plasms’ in which endomitoses and amitoses can always be observed, and then cytolysce (Plate 1, G1, G2, H). All through their life, these cells undergo a ‘pinching off’ process which, from in vivo observations, seems to be very active (one bud per cell each hour). These buds also enlarge and finally disappear in the cytoplasm of the syncytium (Plate 1, Db2). Many observations in hanging-drop cultures and on regeneration in irradiated worms suggests that these cells have a rapid turnover and are not reserve cells, as I previously believed. Their formation, division and final cytolysis can be completed within 48 h.

In brief, amputation is followed by an activation process in the syncytium—that is to say, the acceleration of the synthesis of nucleic acids. RNA synthesis is performed by cells of type I which then liberate the RNA by cytolysis. This explains why the activated syncytium does not show either nucleoli or cytoplasmic basophilia, but only increased mitotic activity. Differentiation of syncytium elements provided with RNA liberated by the cells of type I is the only factor which acts in blastema formation. Experimental evidence for this view will now be amplified.

THE ROLE OF MITOSES IN BLASTEMA FORMATION

The action of X-irradiation on the regeneration of Dugesia subtentaculata and D. tigrina.

Wolff & Dubois (1947) have observed that X-irradiation kills interphase neoblasts so that sectioned irradiated worms produce rapidly resorbed small blastemata. Dubois (1949) has shown that some accumulation of neoblasts is possible after an amputation, but they rapidly disappear. She therefore thought that the neoblasts must divide at least once to form the blastema, so that a mere accumulation of migrant neoblasts is not adequate for regeneration. When these small and rapidly resorbed blastemata appear, they are composed of healthy neoblasts, the progeny of those which were in mitosis during the irradiation.

Analogous experiments carried out on D. subtentaculata (Chandebois, 1963) furnished quite different results. When these worms were irradiated with 10250 r and were transected immediately afterwards, they produced normally differentiated regenerates after the same latent period as the controls. But growth eventually stops, no morphallaxis occurs, necroses appear in the regenerate and in the old tissues, and the worm dies after 2 weeks. A complete inhibition of regeneration is always obtained when the transection is made 48 h or more after the irradiation. Smears of irradiated regenerating planarians only show degenerating prophase nuclei. After colcemide treatment, no stathmokinesis can be observed even during the first hours following the irradiation. It therefore appears that in these particular conditions blastema formation occurs without any mitotic activity.
Irradiation of *D. lugubris* (Grégoire, unpublished results) has shown another aspect of the independence of mitotic activity and regeneration. In this species, a complete inhibition of regeneration is only possible when the amputation is done 7 days after the irradiation. When the transection is done earlier, regeneration is always possible, but the later the section, the smaller the regenerate obtained. From 15 to 20 days after irradiation, necroses appear in the ventral epidermis near the genital orifice in regenerating worms as in controls which have only been irradiated. The planarians die 20–30 days after amputation.

Table 1. *Average number of mitoses in each of the three cell types of undifferentiated parenchyma of irradiated and non-irradiated worms* (*Dugesia lugubris*) *undergoing regeneration*

<table>
<thead>
<tr>
<th>Delay of regeneration</th>
<th>Non-irradiated controls</th>
<th>Irradiated worms</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>48 h</td>
<td>37</td>
<td>7</td>
</tr>
<tr>
<td>72 h</td>
<td>66</td>
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<tr>
<td>10 days</td>
<td>—</td>
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<tr>
<td>14 days</td>
<td>6</td>
<td>—</td>
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<td>16 days</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>18 days</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>20 days</td>
<td>—</td>
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</tr>
</tbody>
</table>

C.I., Cells of type I; R.P., residual plasms; s., syncytium.

Mitotic activity has been measured in worms decapitated just after irradiation and in non-irradiated worms by the technique described above, but without previous block by the mitoclastic poison, which is very toxic to this species (Table 1). As in *D. subtentaculata*, mitotic activity is completely inhibited as early as the first day following amputation. But its restoration is possible and can be observed by 10 days after irradiation—that is to say, when the ability to form a blastema has just disappeared. Mitotic activity increases during the following days, when the first necroses are to be seen. In this species also, therefore, regeneration is possible in spite of a complete lack of mitoses and it can no longer be obtained when mitotic activity is restored.

*Variations in mitotic activity with body-level in Dugesia tigrina and in Polycelis cornuta* (Bermond, unpublished results)

In *P. cornuta*, as in all species of planarians, the rate of regeneration depends upon the level of transection. In the middle region of the body it is much poorer than at the ends. Head regeneration at this level is often completely inhibited. When it occurs, it is delayed and often teratomorphic. In other words, ‘head frequency’ is low. It was therefore interesting to try to discover if these phenomena are correlated with a depression of mitotic activity. For this purpose,
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Planarians have been cut into 7 fragments of equal length, namely: 1 head (A1), 2 prepharyngeal (B1 and B2), 2 pharyngeal (C1 and C2) and 2 post-pharyngeal fragments (D1 and D2) (see Text-fig. 1). We shall not consider the results concerning A1 and D2 fragments for they are bounded by only a single transection.

Table 2. Numbers of mitoses in the five specified portions of the body, of ten individuals of P. cornuta

<table>
<thead>
<tr>
<th>Cells of type I</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
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<tr>
<td>B1</td>
<td>73</td>
<td>41</td>
<td>175</td>
<td>162</td>
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<td>70</td>
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<tr>
<td>B2</td>
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<tr>
<td>C1</td>
<td>210</td>
<td>197</td>
<td>154</td>
<td>230</td>
<td>110</td>
<td>110</td>
<td>102</td>
<td>144</td>
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<td>106</td>
<td>18</td>
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<td>44</td>
<td>21</td>
<td>60</td>
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<td>50</td>
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Residual plasms

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<tr>
<td>B2</td>
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<td></td>
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<td>10</td>
<td>3</td>
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Syncytium

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<th>8</th>
<th>9</th>
<th>10</th>
<th>Average</th>
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<tr>
<td>B1</td>
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<td>26</td>
<td>48</td>
<td>27</td>
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</tr>
<tr>
<td>B2</td>
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<td></td>
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<td>30</td>
<td>17</td>
<td>34</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>22</td>
<td>41</td>
<td>5</td>
<td>29</td>
<td></td>
<td>18</td>
<td>36</td>
<td>16</td>
<td>26</td>
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<td></td>
<td></td>
<td>21</td>
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</table>

The number of mitoses was counted by the technique described above, in ten worms whose C1 fragment survived, between 1 and 6 days after their isolation. In nine worms (all but number 5) the highest number of mitoses in each of the three undifferentiated cell types was found in the anterior pharyngeal fragment (C1, Text-fig. 1, Table 2). The average numbers of mitoses in the B1 and D1 fragments were respectively 33 % and 29 % of the average number in C1 fragments. So we obtained just the converse of the expected results: mitotic activity is much higher at levels which exhibit a low head frequency than at levels which regenerate with a high rate. Similar results have been obtained for the other species D. tigrina.
Individual variations in mitotic activity in fragments from a particular level of *Dugesia subtentaculata*

When many anterior prepharyngeal fragments of *D. subtentaculata* are isolated and allowed to regenerate in tap water at the same temperature, healing, blastema formation, and eye and pharynx differentiation show exactly the same latent periods in all. One might expect that their mitotic activity would be equally uniform. Such fragments, isolated from worms of the same batch, were killed after 1–15 days of regeneration. Mitoses were counted in cells of type I, in residual plasms and in the syncytium. Table 3, in which the results are summarized, clearly shows the great variability in mitotic activity, which contrasts with the uniformity of the regenerative processes. These variations might depend upon virus infections. In fact, in numerous specimens which exhibited a low mitotic activity, serious damage to cell structures could be observed which often affected the nuclei and resembled densonucleoses described by Vago, Meynadier & Duthoit (1964) in insects. However, this had no influence on the rate of blastema formation.

In conclusion, it can be affirmed that blastema formation does not depend
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upon cell proliferation. A high mitotic rate does not always ensure regeneration, and the absence of mitoses does not necessarily result in a failure of regeneration. As the syncytium is always present in adult worms, even after heavy irradiation, it is clear that an inhibition of regeneration, whatever its origin may be, cannot be the result of a lack of undifferentiated material, but a process which inhibits its conversion into blastema tissues. We shall see below that the inhibited mechanism is probably cell differentiation.

Table 3. Variations in mitotic activity among prepharyngeal fragments of Dugesia subtentaculata

<table>
<thead>
<tr>
<th>Days after isolation</th>
<th>Maximum nos. of mitoses</th>
<th>Minimum nos. of mitoses</th>
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<tr>
<td>1</td>
<td>425</td>
<td>97</td>
</tr>
<tr>
<td>3</td>
<td>623</td>
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<td>5</td>
<td>1610</td>
<td>435</td>
</tr>
<tr>
<td>7</td>
<td>1323</td>
<td>336</td>
</tr>
</tbody>
</table>

C.I., Cells of type I; R.P., residual plasms; S., syncytium.

ANALYSIS OF THE VARIOUS FACTORS WHICH INHIBIT BLASTEMA FORMATION

I have made an approach to the study of blastema formation by means of an analysis of the action of factors which inhibit regeneration: (1) irradiation, (2) the failure of, or abnormalities in, initial healing. A large number of observations have led me to the conclusion that in each of these conditions cell differentiation cannot be realized, either because the syncytium is not provided with RNA or because the autoregulative systems cannot impose their differentiation upon those elements of syncytium normally prepared to respond appropriately.

Irradiation

In irradiated worms, syncytium can always be observed until death occurs. As a blastema forms even when mitoses are blocked, the inhibition of regeneration can but be explained by its inability to produce differentiated tissues.

The ability of *D. subtentaculata* to regenerate for about 10 days when it has been sectioned immediately after irradiation, together with the complete lack of regeneration in worms cut 48 h after irradiation, clearly shows that it is not cell differentiation itself which is inhibited, but a preliminary mechanism which becomes quite impossible 48 h after irradiation and which until then is sufficient to allow differentiation for about 10 days. After this interval of 48 h in *D. subtentaculata*, no cells of type I can be found, perhaps not because they are destroyed by X-rays, but rather because the syncytium can no longer produce
them, and because their numbers are rapidly exhausted, for they are short-lived cells.

In *D. lugubris* (Grégoire, unpublished results), cells of type I can always be seen in the parenchyma of irradiated worms. This suggests that in this species the inhibition of the preliminary processes of differentiation is related to the using up of cells of type I rather than to the inhibition of their formation.

Irradiation therefore demonstrates that blastema formation remains possible as long as a phenomenon which is supposed to promote cell differentiation is not inhibited, which is dependent on the cytolysis of cells of type I at the very beginning of regeneration. It is probable that RNA necessary to protein synthesis in the syncytium is liberated in this step.

*The failure of healing*

The first step in regeneration is always wound healing. When the continuity of the epidermis cannot be restored, no blastema can be formed. When wound healing is delayed, blastema formation is also always delayed. In water, as in toxic solutions, the failure of healing exposes the parenchyma to unfavourable conditions which could be considered the crucial factor for the inhibition of the regenerative processes.

*In vitro* cultures enabled Chandebois, (1968) to study the true role of healing. Planarian fragments have been placed in a medium convenient for histiotypic cultures, in which undifferentiated cells can multiply in an anarchic manner. Generally, wound healing does not occur in such a medium. The undifferentiated syncytium shows numerous mitoses and produces a great number of cells of type I. These cells migrate towards the medium, into which they are liberated. Their cytolysis occurs in the medium, so that syncytium does not retrieve the RNA. Finally, the elements of the syncytium are gradually converted into cells of type I, so that, in spite of its mitotic activity, the fragment is finally reduced to its irreversibly differentiated cells (Text-fig. 2). The exhaustion of the fragment can be complete in 2 or 3 days, but it is generally much delayed by the swelling of gastrodermal cells (Plate 2, fig. A), which hinders the migration of cells of type I. In such fragments, a high mitotic activity can still be observed after 2 weeks of culture (Plate 3, fig. A, B). Thus in spite of a complete lack of cicatrization, the parenchyma remains in good physiological condition and undifferentiated elements are able to proliferate without showing any sign of differentiation.

In some cases, however, fragments are able to heal, even after 10 days of anarchic proliferation and then can reform normal worms. Thus these cultures

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**PLATE 2**

Two features of the behaviour of fragments of the planarian *Dugesia gonocephala* cultured in vitro. g.c. Gastrodermal cells.

Fig. A. Fragment exhibiting a strong swelling of gastrodermal cells.

Fig. B. Fragment showing a cephalic regenerate formed after the healing of the anterior cut.
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show two alternatives in the behaviour of the syncytium. As long as the wound remains open, it cannot produce any differentiation. As soon as the ventral and dorsal epidermis close the wound, proliferation is limited. Differentiation begins and always leads immediately to normal regeneration.

![Diagram of Planarian regeneration]

Text-fig. 2. Scheme of the disturbance in the activity of cells of type I in fragments cultured in a nutritive medium. Above: the activity of the undifferentiated tissue in a regenerating fragment. Syncytial elements, enriched by dedifferentiated cells, produce the differentiated cells of the regenerate, while cells of type I are formed continuously and rapidly cytolysed. Below: the activity of the undifferentiated tissue in a cultured fragment. Cells of type I migrate towards the medium where they are cytolysed so that the syncytium does not retrieve the elaborated RNA.

Plate 3

Photographs of sections of cultivated fragments (Dugesia subtentaculata). m, Mitosis; g.c., Gastrodermal cells. Fixation with Bouin–Holland’s fluid; P. Masson’s stain.

Fig. A. Gastrodermal cells and parenchyma of a cultured fragment (5 days).
Fig. B. As A (7 days).
Fig. C. Tumour-like excrescence in a cultured fragment of 8 days.
Generally, the anterior wound alone is closed, while the posterior wound liberates cells and shows a high mitotic activity; only a head is produced (Plate 2, fig. B). This observation clearly demonstrates that the determinant factor in the option is not the composition of the medium or the physiological condition of the fragment, but only the restoration of epidermal continuity.

Some cultured fragments taken from a zone of morphallaxis (i.e. tissues lying behind a head regenerate), and whose dorsal epidermis had been injured, have shown tumour-like excrescences formed only of syncytium (Plate 3, fig. C). However, at the periphery the cells had a ciliated epidermal layer exhibiting a low degree of differentiation (neither basal membrane nor cell membranes and no rhabdites). The restoration of epidermal continuity had not been followed by the differentiation of the syncytium.

Other observations have shown that the determinant role of healing is not in the mere closure of the wound. In marine planarians I have shown (Chandebois, 1957) that the regeneration cannot occur when, because of muscular contractions, the dorsal epidermis of the right side fuses with that of the left, and similarly with the ventral epidermis. Under these conditions, the original ventral edge remains quite separated from the original dorsal edge of the section. Thus it is the drawing together of ventral and dorsal edges, previously isolated, which appears as the starting-point for regeneration, by evoking differentiation in the available elements of the syncytium.

**DISCUSSION**

The ability of planarians to regenerate without mitotic activity shows that their parenchyma keeps a permanent stock of undifferentiated elements, namely the syncytium, which can be directly converted into differentiated elements. On the contrary, extensive mitotic activity in the syncytium does not necessarily result in regeneration. This allows us to assert that the rudiment of a regenerate, which is called a blastema, is not due to a mere protrusion of the undifferentiated cellular material previously accumulated near the wound, which later undergoes determination under the influence of the old tissues. On the contrary, the formation of the blastema requires changes in the undifferentiated material, which are impossible when no wound healing occurs or when cells of type I are lacking and which are the first step of cell differentiation. The blastema is built up by cells which look undifferentiated but are already engaged in differentiation, i.e. are determined cells.

This new conclusion, combined with previous experimental results and observations, calls for a re-examination of regenerative processes of planarians.

(1) The autoregulatory properties of a morphogenetic field can be attributed to the ability of any of its component regions to be transformed into any other (Abeloos, 1965). Consequently we have first to find out how these transformations occur at the cellular level. The heterogeneity of a morphogenetic field is
due to its differentiated cells and depends upon their nature, distribution, and life-span. However, differentiated cells alone cannot bear field properties, since the transformation of any region into any other would imply cell de-differentiation and multiplication, which evidently is impossible for a great number of cell types. On the contrary, one might think that the field properties are only located in undifferentiated cells whose mitotic activity and powers of subsequent differentiation vary according to the intrinsic properties of the various regions, themselves depending upon their position in the field (Abeloos, 1965). This hypothesis is not confirmed by our experiments. We have seen above that when no wound healing occurs in cultured pieces of planarians, the parenchyma is maintained in good physiological condition. It proliferates without any differentiation or, being converted into cells of type I, gradually disappears from the fragment. However, healing sometimes becomes possible at the anterior transection, even after 10 days of anarchic proliferation. In every case, the subsequent formation of a normal head is obtained. This means that normal patterns of differentiation can be restored in the syncytium. It is therefore probable that undifferentiated tissue is a passive material whose proliferation and differentiation are controlled by differentiated cells. Both differentiated cells and undifferentiated cells are necessary to realize autoregulation, so that field properties are shown by cell populations whose differentiated elements are produced from one undifferentiated cell type. I have called such a population a cell transformation system (Chandebois, 1963a, 1965).

In the literature one finds many experimental results which show that the mitotic activity of undifferentiated cells and the choice they make between many potentialities are controlled by differentiated cells (for example, the proliferation and differentiation of epithelia: intestine, Grobstein, 1954; epidermis, Seilern Aspang & Kratochwil, 1965; hair follicles, Wolbach, 1950). The metabolic activity of differentiated cells also depends upon extracellular conditions which themselves are generally modified by the metabolic activity of neighbouring differentiated cells, or distant ones in the special case of endocrine cells. Such interactions can be the origin of modifications in differentiated cells: dedifferentiation, modulation, accelerated senescence. We can suppose that similar controls always exist between the various cell types of a given system.

As every type of differentiated cell originates from the syncytium (Chandebois, 1962), planarians have a single cell transformation system in which numerical equilibrium is realized between the irreversibly (for instance epidermis) and the reversibly (for instance muscle) differentiated cells. If irreversibly differentiated cells are lost from the system, activation increases in the undifferentiated elements and eventually the surplus of reversibly differentiated cells can dedifferentiate until a new equilibrium is reached (Chandebois, 1963b, 1965).

But it is evident that equilibria are not of the same nature everywhere in the system. They present qualitative modifications (nature of cells, spatial distribution) and quantitative ones (proportions of the various cell types, renewal rates).
The specific properties of a given region cannot be free from any influence emanating from differentiated cells of an adjacent region, and this creates a close co-ordination between all the parts of the system. When the pool of differentiated cells is modified in one part of the field, the activity of undifferentiated elements is modified from place to place. Cell production is necessarily modified qualitatively and quantitatively. In consequence, reversibly differentiated cells can undergo modulation or dedifferentiation so that new patterns are produced.

(2) When one performs an amputation on a planarian, cells which lie near the wound are deprived of the influence normally exercised by the differentiated cells which have been eliminated. The main result is an activation of undifferentiated elements; that is to say, an increased synthesis of nucleic acids. Histologically, this activation is displayed by an increase in mitotic activity and by a large production of cells of type I. The larger the number of differentiated cells removed, the stronger the activation may be. This will be the reason why the highest mitotic activity is exhibited by pharyngeal fragments, which are farthest from both ends of the body. Activation is probably not the only result of an amputation. Some cells cannot maintain their specific activity and undergo dedifferentiation. If no healing occurs and if the parenchyma is cultured in favourable conditions, as in a culture medium, activation occurs and no differentiation can be seen. In these conditions, the maintenance of activation cannot be explained as a specific action of the culture medium since, in the same fragment, a head can regenerate on the healed anterior surface, while the parenchyma near the posterior, unhealed wound always shows a high degree of activation.

Thus, normal healing is the sine qua non of regeneration; it is the only condition which is able to cause undifferentiated elements to undergo differentiation so as to reform the lost parts. It is very important to emphasize that a normal healing does not mean any closure process which restores epidermal continuity, but the true union of ventral and dorsal edges of the wound. When this condition is not realized, no regenerate can be formed. This is the case in strongly contracted pharyngeal sections where the two halves of the dorsal edge of the wound fuse together, as do the two halves of the ventral edge.

In planarians, blastema formation begins with the determination of the distal part (Chandebois, 1957). This determination seems to occur as soon as wound healing is over and we may suppose that these two phenomena are closely linked. Indeed, wound healing causes a juxtaposition of dorsal and ventral tissues, which exhibit different patterns. Ventral epidermal cells are very different from the dorsal epidermis and do not show the same properties (especially after irradiation; Chandebois, 1963b). The ventral side is also characterized by the presence of nerve cords and by the thickness of the muscular layers. At the ends of the body, these two types of organization contact each other. Cicatrization, by a simple mechanical process, establishes a similar confrontation so that new relations between the various types of differentiated cells now resemble those
of a distal end. These new cell patterns necessarily influence the differentiation of the syncytium, whose elements have previously multiplied, and cause them to produce cells which restore an equilibrium in the next level, and so on until the intermediary parts are reformed.

In short, regeneration is the progressive restoration of equilibria twice disturbed, first by the transection which evokes activation and dedifferentiation and next by normal wound healing which institutes new patterns near the level of the wound.

**SUMMARY**

It is generally suggested that regeneration consists of two successive steps: (a) blastema formation by undifferentiated cells which undergo mitoses; (b) the differentiation of those cells by an inductive process proceeding from the stump.

1. Numerous experiments and observations on various freshwater planarians suggest that blastema formation is not dependent upon the mitotic activity of the parenchyma. In planarians transected immediately after irradiation regeneration is possible in spite of the complete lack of mitoses. When planarians are cut some days later, however, regeneration always fails, even in fragments whose mitotic activity is already restored (*D. lugubris*). Fragments of the anterior pharyngeal region, which exhibits the lowest head regeneration frequency, show a mitotic activity higher than fragments from other parts of the body. In *D. subtentaculata*, infection by viruses considerably lowers mitotic activity in some worms, which regenerate, however, with the same latent period as healthy worms.

2. Blastema formation is effected by elements already engaged in differentiation. It is always inhibited when differentiation becomes impossible; for instance, when the formation of cells of type I is stopped (by irradiation), when no wound healing occurs (histiotypic cultures) or when fusion of the dorsal and ventral edges of the cut does not occur.

3. The role of wound healing in the determination of the cells of the regenerate is discussed.

**RÉSUMÉ**

*Les rôles respectifs de l'activité mitotique et de la différenciation cellulaire dans la régénération des Planaires*

Les auteurs admettent généralement deux étapes dans la régénération des Planaires; (a) l'édification du blastème due à l'accumulation de cellules indifférenciées que se multiplient par mitose; (b) la différenciation de ces cellules grâce à une action inductrice exercée par la souche.

1. De nombreuses expériences et observations réalisées chez diverses espèces de Planaires d'eau douce ont permis d'établir que la formation du blastème est indépendante de l'activité mitotique observée dans le parenchyme du fragment. Chez des Planaires sectionnées immédiatement après une irradiation, la régénération est toujours impossible, même si l'activité mitotique est déjà restaurée (*D. lugubris*). Des fragments prélevés dans la région pharyngienne
antérieure — où la fréquence de la tête est la plus basse — montre une activité mitotique nettement plus élevée que celle des autres fragments. Chez *D. subtentaculata*, des infections virales abaissent considérablement l’activité mitotique de certains individus qui régénèrent cependant à la même vitesse que les individus sains.

2. La formation du blastème est réalisée par des éléments déjà engagés dans la voie de la différenciation; elle est inhibée chaque fois que cette différenciation est bloquée, par exemple si la formation des cellules de type I est stoppée (irradiations), si la cicatrisation ne se produit pas (cultures histiotypiques) ou si elle ne conduit pas à l’affrontement des bords dorsal et ventral de la section.

3. Le rôle de la cicatrisation dans la détermination des cellules du régénéré est discutée.

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REféRENCES


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