HISTOLOGICAL CHANGES IN REGENERATING PIECES OF DUGESIA DOROTOCEPHALA TREATED WITH COLCHICINE

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It has been shown that colchicine inhibits or completely abolishes regenerative changes in pieces of *Dugesia dorotocephala* (McWhinnie, 1955). Because of its selective stathmokinetic action it can be assumed that cellular studies, of colchicine-treated planarian pieces, should give evidence as to the source of blastema and regenerate cells. As early as 1902 Stevens reported numerous parenchymal cells in stages of mitosis, when untreated short transverse pieces of *Planaria lugubris* were regenerating. He suggested the embryonic nature of these cells which through multiplication and differentiation replaced the elements lost in section. With the use of x-rays Bardeen and Baetjer (1904) showed marked inhibition of regeneration in *P. maculata* and *P. lugubris*. Histological study showed no change in the cells of muscle, nerve, endoderm or gonad and an absence of mitosis in parenchymal cells. Control pieces showed mitotic cells in the parenchyma. Wiegand (1930) also demonstrated this point with several planarian species. On an indirect basis Curtis and Schultze (1934) emphasized the role of parenchymal cells in regeneration by comparing their number in species known to have high regenerative capacities (*P. maculata; P. agilis*) with one limited in regenerative ability (*P. fluviatilis*). Subsequent studies (Curtis, 1936) with x-rays showed a reduction of free parenchymal cells in proportion to the reduction in regeneration.

Colchicine inhibition of development has been shown by Beams and Evans (1940). Fertilized eggs of *Arbacia punctulata* were unable to divide if exposed to colchicine during the pre-metaphase interval and also showed a considerable decrease in viscosity.

Despite the evidence for the role of parenchymal cells in planarian regeneration, several workers have reported the absence of mitosis in normal planarian regeneration (Steinmann, 1926; Bandier, 1936; Clement, 1944). In an effort to demonstrate a specific source of cells which contribute to planarian regeneration, histological studies were made at selected intervals after colchicine treatment.

Materials and Methods

A stock of *Dugesia dorotocephala* was collected and maintained in the manner previously described (McWhinnie, 1955). Animals were sectioned into two halves at the level of the mouth. After sectioning, the pieces were separated into two groups. In one group, anterior and posterior halves were placed into $M/5000$ colchicine at the time of section. These were prepared for study at the end of exposure periods 3, 6 and 10 days. Pieces in the second group were placed into aerated tap water and were allowed to reconstitute for 24, 48 and 72 hours. At the end of each time interval these pieces were transferred to $M/5000$ colchicine where they were

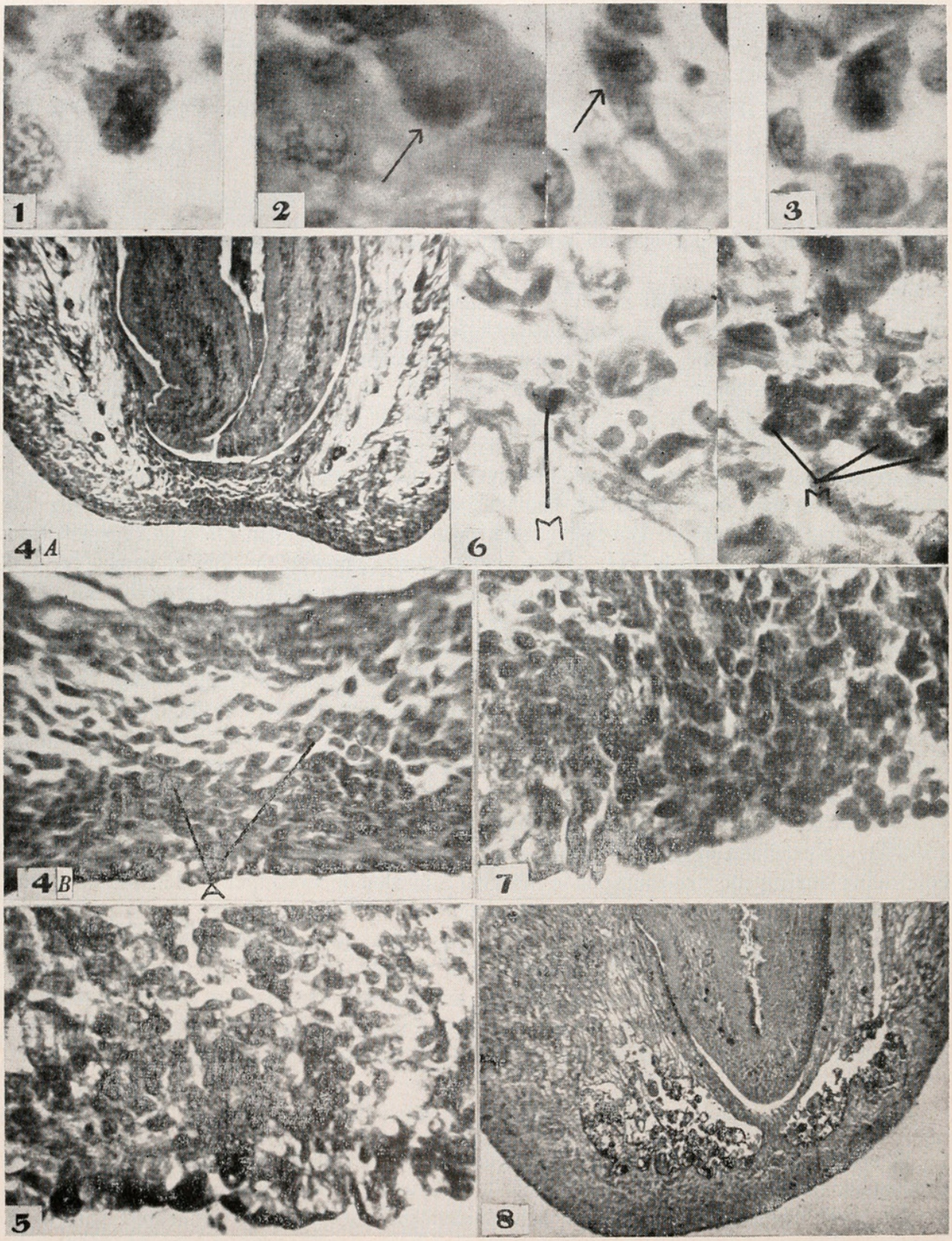

PLATE I

Explanation of Figures

FIGURE 1. Mitotic figure in a free amoebocyte in a three-day regenerating planarian piece, exposed to $M/5000$ colchicine from the time of section. $\times$ 1425.

permitted to remain for 12 and 24 hours. All pieces to be prepared for cell study were narcotized in 0.05–0.1% chloretone, killed and fixed in Bouin's fluid and embedded using the standard paraffin technique. These were sectioned at 6 micra in such a manner that both frontal and sagittal sections were obtained. Delafield's hematoxylin was used without a counterstain. Study was with 1000 and 1500 diameters magnification.

Results

The history of parenchymal cells in regenerating planarian pieces treated with colchicine demonstrates the paramount role of these cells in this developmental process. Planarian pieces treated with $M/5000$ colchicine for three days following section show many free amoebocytes in mitosis. These are uniformly distributed with extremely few in the area of the cut surface. Many of these cells appear elongate and in strands oriented to the cut surface, indicating migration. Mitotic figures were normal (Fig. 1). However, after 6 days' exposure to colchicine from the time of section most of the mitotic amoebocytes had abnormal chromosomal configurations and considerable pycnosis (Fig. 2). At 6 days these cells were still generally distributed throughout the parenchyma with never more than one to two in the region of the cut surface. Also, the large free amoebocytes were oriented into longitudinal strands. At the end of 10 days' exposure to colchicine a larger number of cells were at metaphase or beyond. However, at this time there was extensive cellular degeneration as evidenced by granulation of the nucleus, mitotic abberations and cytoplasmic vacuolation. Of the free amoebocytes undergoing degeneration many were oriented into migration strands.

Despite the apparent increase in number of parenchymal cells in mitosis from three to ten days after section in treated regenerating planarian pieces, the untreated pieces do not show this same progressive increase in number of cells undergoing division in time. Three days after section, untreated planarian pieces show some free amoebocytes in mitosis (Fig. 3) but none were observed in the region of section. On a comparative basis the number of mitotic figures was less than in the three-day colchicinized pieces. A marked decrease in the proliferation of these cells is apparent by six days after section when pieces regenerate in aerated tap water. However, at this time orientation of migratory strands to the area of the cut is

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Figure 2. Mitotic figures in free amoebocytes in a six-day regenerating planarian piece, exposed to $M/5000$ colchicine from the time of section. × 1425.

Figure 3. Mitotic figure in a free amoebocyte in an untreated three-day regenerating planarian piece. × 1425.

Figure 4. A. Long section through the cut surface of a planarian piece treated with $M/5000$ colchicine for 24 hours after 24 hours in water. × 150. B. Same as A. × 660. (A = amoebocyte.)

Figure 5. Long section of an untreated regenerating planarian piece 48 hours after section. × 660.

Figure 6. Parenchyma of a planarian piece treated with colchicine for 24 hours after 48 hours in water. Note free amoebocytes in metaphase and evidence of migration. × 1425.

Figure 7. Long section of an untreated regenerating planarian piece 72 hours after section. Note increased number of amoebocytes. × 660.

Figure 8. Long section of a planarian piece treated with $M/5000$ colchicine for 24 hours after 72 hours in water. An increase in the number of cells can be seen in the region of the cut surface. An increase in gut degeneration is apparent. × 150.

notable. By ten days after section, when the regeneration process is conventionally considered complete, there is no evidence of mitotic activity. Regenerating pieces of planaria had no mitotic activity in epidermis, muscle or endoderm tissue, whether the pieces had been treated with colchicine or permitted to regenerate in water.

When anterior and posterior halves of planarians were maintained in water for 24 hours and then transferred to *M*/5000 colchicine for 12 and 24 hours, large numbers of free parenchymal amoebocytes were in metaphase with considerably fewer in telophase. These cells had divided *in situ* as well as during migration to the area of the cut surface. Degenerative changes in the gut and parenchyma were not found after 12 hours and were minimal after 24 hours treatment. In this group some few mitotic cells showed degenerative changes as indicated by granulation of nuclear components and rupturing of the cytoplasm. Cells of the gut, fixed nuclei of the syncytium, muscle and epidermis were not in mitosis. Beneath the newly-formed epidermal covering there was a slightly larger number of free amoebocytes than in the rest of the parenchyma (Fig. 4 A and B). At this same time interval untreated pieces had extremely few mitotic cells but a considerably greater number of amoebocytes at the cut surface (Fig. 5).

A similar group of anterior and posterior halves was allowed to undergo regeneration for 48 hours before treatment with colchicine for 12 and 24 hours. These pieces showed a still greater accumulation of free amoebocytes and more metaphase figures beneath the epidermal covering than in the previous group, *i.e.*, colchicine treatment after 24 hours in water. Many amoebocytes *in situ* and in migration were at metaphase (Fig. 6).

In untreated 72-hour regenerates there were few mitotic figures, but numerous amoebocytes were densely packed at the region of the blastema (Fig. 7). Pieces treated for 24 hours showed fewer mitotic figures than those treated for 12 hours. Treatment with colchicine for 12 and 24 hours, after a reconstitution period of 72 hours in water, resulted in pieces with a greater number of amoebocytes in the region of the cut surface. At this time fewer mitotic cells were seen in all areas. Gut, as well as general parenchymal degeneration was more apparent than in the previous series (Fig. 8). On the other hand controls showed large numbers of amoebocytes in the blastema, little evidence of mitotic activity and no degenerative changes.

Discussion

Through the use of colchicine on regenerating planarian pieces it can be concluded that the cellular elements involved in restoration to wholeness are primarily the free amoebocytes of the parenchyma. In all cases observed, after a minimum of 24 hours of reconstitution, epidermis, co-extensive with the epidermis of the rest of the piece, covered the wound surface. In no case was mitosis observed in this tissue. Similar wound epithelial covering without cell proliferation has been demonstrated in amphibian limb regeneration (Lash, 1955). The cut surface is readily distinguishable from the rest of the piece by the lack of sub-epidermal pigment as well as by the localized density of the parenchymal cells. With the use of colchicine it would appear that the time course of mitotic activity during regeneration explains the short interval in which there is no apparent change after section, the time of onset of greatest cellular proliferation and the known difference in susceptibility to toxic influences through the regeneration period. Some studies made in this work show

that mitotic cells increase in number from the third to the tenth day after section when pieces are placed into colchicine at the time of section. However, observation of untreated pieces at the same time intervals strongly indicates a greater number of parenchymal cells in division at the third day after isolation than in pieces 6 or 10 days after isolation. The progressively larger number of metaphase cells found at longer time intervals after isolation and introduction into colchicine would simply emphasize the sustained inhibition in the presence of the alkaloid and consequently the accumulation of inhibited cells.

Evidence can be gained as to the time of greatest mitotic activity by permitting isolated pieces of planarians to initiate normal regeneration in water before placing them into colchicine. Halves of planarians placed into colchicine for 24 hours after initial regeneration in water for 24, 48, and 72 hours show a greater number of parenchymal cells in division at a final age of 72 hours (48 hours in water, 24 hours in colchicine). However, a substantial number of these cells were in division in pieces placed into colchicine for 24 hours after 72 hours' regeneration in water. While the number of mitotic figures found in this group was less than in the preceding it is also true that parenchymal degeneration was considerably greater than in that group. Under these conditions there is extensive gut degeneration, granular degeneration in patches of parenchyma as well as in the area of the cut surface. Mitotic cells at this time showed a range from normal metaphase to cells with chromosomes widely dispersed and granular in appearance. These modifications were not so apparent in pieces placed into colchicine after 48 hours in water.

The fixed 24-hour exposure to colchicine in these three groups with a greater susceptibility of pieces at the fourth day of reconstitution agrees with previous findings on the critical period in reconstitutional development. By studies of gross changes in planarian pieces and their susceptibility it was demonstrated that the fourth day in development is the most critical (McWhinnie, 1955).

While the highest mitotic activity appears at the third day after isolation and both gross and microscopic evidence show a high susceptibility at the fourth day, it would appear that the increased population of parenchymal cells and their migration to the cut surface constitute the most active and therefore the most susceptible period in planarian regeneration. This is visibly expressed by the toxic effects of colchicine on the fourth day.

It is suggested that the sequence of events in planarian regeneration includes an initial slow onset of division of parenchymal cells, rising to a peak at the third day after isolation. Associated with the rise in number of cells proliferating, oriented migrations of these cells to the cut surface follows. It would appear that the activity of migration is greatest through the fourth to the sixth day afer section. Some oriented strands found in 48-hour pieces indicate the onset of migration at this time. By the sixth day of regeneration, mitotic activity and cell migrations are subsiding and the remainder of the reconstitution period represents the time of morphogenetic changes to complete species organization, both internal and external.

It can be concluded also that the mechanism of colchicine inhibition of planarian regeneration is through its influence on parenchymal amoebocyte proliferation as well as reduced migration of those cells to the cut surface(s). It is entirely likely that colchicine-induced changes in viscosity (Beams and Evans, 1940) could account, in part, for the marked difference in cell density in the blastema of normal and treated regenerating pieces.

SUMMARY

1. Histological studies show that the mechanism of colchicine inhibition of regeneration in pieces of *Dugesia dorotocephala* is the stathmokinetic action it exerts on free parenchymal amoebocytes.

2. Parenchymal amoebocytes are the only cells exhibiting mitotic activity during the period of regeneration.

3. Mitotic activity reaches a peak at the third day of development while oriented migrations of amoebocytes appear to set in at the second day with marked movement through the fourth to the sixth day after section.

4. The free amoebocytes of the parenchyma constitute the exclusive source of cells participating in the replacement of parts lost when planarian worms are sectioned.

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