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Hydras, under constant laboratory culture conditions showed an annual recurrence of phase variation. From February to September, they behaved normally, following which they entered into a phase of depression lasting for a varied period of time each year. Just prior to the onset of depression, the hydras produced gonads (mainly testes), showed an occasional variation in their body contours and their sensitivity to chemicals increased. Some of the hydras became abnormally tall and thin (1 - 1.5 cm) and their contractility was significantly reduced. Once the depression had set in, hydras lived in such a phase with an altered behavioural pattern for a long period. In the present investigation the two phases of hydra - namely, depressed and dedepressed (normal) - would be taken into account.

1. Morphological Semblances

A tabulation of the morphological semblances between the normal and the depressed hydras is given in Table 1. The length of the body column in unstretched as well as in fully stretched state was found to be more in depressed hydras. The bud zone appeared to shift more towards the oral extremity. The distance between the basal disc and the bud zone increased from 0.9 mm in normal hydras to 1.5 mm during the depression phase. The relative tentacle length with respect to the body column decreased from 210% to 100% in depressed phase variants.
2. Behavioural Semblances

2.1. Locomotory Behaviour

The locomotory activity was found to be minimum just after feeding during the normal phase of hydrams. In 72 hr starved cultures, all hydrams exhibited vigorous locomotory activity in search of food. The hydrams either adhered to the bottom of the culture dish or remained afloat. Those sticking to the substratum kept up an exploratory activity in all the directions by contracting, bending, re-extending and sweeping the bottom with their tentacles. After repeated activity of this type, the hydrams detached and moved towards the surface of the medium by producing a gas bubble, clearly visible as a bright spot at their basal discs. The floating hydrams either remained vertically suspended from the surface with tentacles stretched downwards or moved horizontally on the surface in a circular manner. Table 2 compares the locomotory behaviour of the two phase variants. In normal phase, 89.5% hydrams were floating (80.5% vertically and 9% horizontally) and 10.5% were attached to the bottom of the culture dish, whereas in depressed condition, the percentage of the floating hydrams decreased to 36.9% (25.6% vertically and 11.3% horizontally) and that of the ones adhering to the base increased significantly (63.1%).
2.2. Feeding Behaviour

The normal mode of capturing the prey with tentacles and paralysing the same by nematocysts was observed in both the phase variants. The immobilised prey was pushed into the enteron with the help of tentacles within 2 min. By 30 min, the process of ingestion was complete. The Artemia caught, but not ingested were finally released back into the culture medium. The number of Artemia caught and ingested and the time taken for egestion in both the phases has been recorded in Table 3. The number of the prey captured was more in depressed compared to normal hydra, but the number finally ingested was almost equal. The time interval between ingestion and egestion was longer in depressed hydras compared to that of normal.

3. Budding and Growth Rate

Bud production was seen at regular intervals in a well fed culture. The bud initiated as a small conical protruberance which increased in length and developed a pair of tentacles and hypostome at its distal end. It started feeding and responding to stimuli independent of the parent after the attainment of tentacular length. It developed a basal disc at the proximal end by 40 hr and finally got detached from the parent. Table 4 represents the budding capacity of the two phase variants. The time taken from the bud initiation
to bud detachment was 46 hr in normal hydoras and 42 hr in depressed ones. The daughter bud took 51 hr for maturation, i.e., for producing its own bud in the normal, and 59 hr in the depressed phase. The time interval between the successive bud initiation was 24 hr in normal and 27 hr in depressed hydoras.

The population growth of normal and depressed hydoras is expressed in terms of linear increase in the number of polyps (starting with 5 hydoras) and the number of hypostomes (total number of polyps plus total number of buds) (Fig. 3). For the first 5 days, the growth (lag phase) in the normal hydoras was slightly slower than that of the depressed, but later it accelerated and by the end of 10 days the total number of polyps (128) and hypostomes (172) in normal was much higher as compared to that of the depressed (79 polyps and 132 hypostomes).

4. **Cellular Semblances**

The phase variation was analysed at tissue level. Following changes were observed in histological preparations of the hydoras from the initiation of depression generally seen to have begun from October till its recovery to normal state in February.
4.1. Initial Cellular Marking of Depression

The histological preparations of depressed hydram showed a varied degree of cellular changes from thinning of the ectoderm to the disarray and asymmetry in endodermal lining (Fig. 4A-I). The depressed hydram invariably displayed a distortion in the radial symmetry in the arrangement of different cell types. A longitudinal section of the hydra in the month of October (Fig. 4A) confirmed that ectoderm as well as endoderm of the two sides did not correspond in their thickness. The ectoderm often lacked full representation of all the cell types. The number of nematoblasts and stem cells was significantly reduced. This condition was seen in a greater magnitude in some cases (Fig. 4B). The body wall was reduced in thickness and many cell types disappeared rendering empty spaces (Fig. 4C) in the cell wall.

4.2. Persistence of Depression Leading to Disarray of Cell Types

Sections taken from depressed hydram in November showed continued asymmetry of the cell arrangements, reducing cell numbers and rendering many more empty spaces in the body wall (Fig. 4D). The longitudinal section demonstrated not only the cellular features mentioned earlier but also showed lack of gland cells. Another cellular disorganisation was seen in the form of expulsion of nucleus like bodies from
the cells belonging to one side of the hydra body wall (Fig. 4E). Such bodies in the space of the enteron showed many divisions. A magnified view of Fig. 4D has been presented in Fig. 5. In extreme cases, this might reduce the normal cell aggregation resulting in the occurrence of large spaces and often hypertrophied cells. Figure 4F presents such a situation of expulsion of nucleus like elements on one side of the body wall near the basal disc.

When the hydras returned to the normal state, the characteristic layering of the body wall returned to normal symmetry. That means the thinning out of the body wall was compensated by restituting more cells to make it thickened. No further elimination of free nucleus like elements and their free divisions (Fig. 4G) were seen. By this time, the normal structure of the body wall reappeared gradually (Fig. 4H, I).

5. Regenerative Semblances

5.1. Basal Disc Regeneration at Various Body Levels

The basal disc regeneration time in both normal and depressed hydras increased as the level of the cut was shifted more towards the distal end as is evident from Table 5. In the presence of hypostome, the basal disc restructuration took about 11, 16, 19 and 28 hr at level
1, 2, 3 and 4 respectively in normal hydra. Excision of hypostome in the similar experiments resulted in a delay in the regeneration time at all the levels. The restitution time was approximately 13, 16, 25 and 30 hr respectively at level 1, 2, 3 and 4. Though the time of basal disc regeneration (in presence of hypostome) at level 1 was less in depressed hydras than that of the normal, at all other levels the time taken was more than the normal (Table 5). In the presence of hypostome the basal disc regeneration was accomplished in about 10, 16, 21 and 30 hr at level 1, 2, 3 and 4 respectively. A different regeneration pattern was observed in the depressed hydras on removal of the hypostome. The time for restitution of basal disc in the absence of hypostome was about 18, 16, 19 and 22 hr at level 1, 2, 3 and 4 respectively.

A schematic diagram of the regeneration timings of basal disc is depicted in Fig. 6. In normal hydras, the time of basal disc regeneration in the absence of hypostome was more than that in the presence of hypostome at all the levels. But in the depressed hydras the removal of hypostome affected the regeneration time differently. At level 1, there was a delay; at level 2 almost no change; at level 3, a slight stimulation and at level 4, an appreciable stimulation of regeneration was observed.
5.2. **Isolate Regeneration at Various Body Levels**

The regeneration time of the isolates removed from different body levels along the column was studied. Table 6 shows the time required for the hypostome and the basal disc restitution. In the normal isolates, at level 1, the hypostome regenerated in approximately 20 hr and the basal disc in 24 hr. At level 2, the hypostome and the basal disc regeneration took about 21 and 26 hr respectively. At level 3, 25 hr and 30 hr was the approximate time required for the restitution of hypostome and basal disc respectively. At the level 4 isolate the hypostome regenerated in about 30 hr, whereas the basal disc regenerated in 27 hr. At level 5, the time for hypostome and basal disc regeneration was 35 and 22 hr respectively.

In the isolates excised from the depressed hydras the regeneration of hypostome and basal disc took about 23 and 27 hr at level 1; 24 and 31 hr at level 2; 28 and 32 hr at level 3; 35 and 25 hr at level 4 and 29 and 19 hr at level 5, respectively (Table 6).

The pattern of regeneration of the normal and depressed isolates together has been shown in Fig. 7. In the normal isolates the hypostome regeneration took more time as the level of the cut was shifted towards the proximal end. The time for basal disc regeneration was more than that of the hypostome at level 1, 2 and 3 and less than that of
hypostome at level 4 and 5. In the depressed isolates, the hypostome increased from level 1 to level 4, but decreased at level 5. The basal disc regenerated earlier than the hypostome at level 1, 2 and 3 and later at level 4 and 5.

6. Isolate Regeneration Semblances

6.1. Topographic Variations

The successive topographic changes in an isolate regeneration till its ultimate transformation into an axiated hydra body were followed by taking the time lapse photographs. In the normal isolate, the regeneration process was accomplished in 5 steps. The first step, wound healing, began immediately after the isolation. It was evident by the gradual spreading of the endoderm accomplished by the vigorous movement of body wall to fill up the central space. The wound was completely healed by the end of 2 hr (Fig. 8A), giving the isolate the appearance of a small compact ball. This stage continued till about 12 hr with gradual loss of cells. As a result a considerable shrinkage was observed at 12 hr (Fig. 8B).

The next step in regeneration was a permanent distortion of the round shape of the isolate giving way to a mechanism of an anteroposterior stretching of the cell mass (Fig. 8C). At the same time, the polarity of the future head and the basal disc regions was laid down.
The future hypostome area was recognised by the appearance of two small projections (Fig. 8C) and their elongation into tentacles. After the two ends - the proximal and the distal - were determined, a further stretching of the middle region was continued to set apart the two ends in opposing directions (Fig. 8D). The basal discs started appearing at 24 hr, and by 29 hr (Fig. 8E) all the isolates had a well developed hypostome and basal disc.

In the isolates taken from depressed hydramas, the process of regeneration began at the same pace as the normal, but it failed to gear through all the steps successfully. The wound healing was accomplished by the normal time (Fig. 9A). By 12 hr, the shrinkage of the cell mass was observed due to cell loss (Fig. 9B). The anteroposterior stretching was conspicuously absent. The regenerates remained more or less roundish in shape, but the tentacular rudiments made their appearance by 21 hr (Fig. 9C). The elongation of the tentacles took place by 24 hr along with a little stretching of the body column (Fig. 9D). Figures 9E and F showed a further failure of basal disc regeneration in the isolates that had failed to achieve an anteroposterior axis. Such isolates sometimes also showed presence of ectopic tentacles (Fig. 9G). This stage continued till 48 hr (Fig. 9H), where the basal disc still had not appeared in all the isolates.
6.2. **Cellular Variations**

The cellular variations were studied in terms of histology and relative density of various cell types during regeneration of the isolates.

**6.2.1. Histology**

The histology of the normal and depressed anterior gastric annuli was studied following routine procedures. The annuli isolated from depressed hydras showed a wide range of variation during their regeneration. Some underwent almost a normal process of regeneration, whereas others displayed extreme form of depression. Figure 10A shows the wound healing process being accomplished by coalescence of endoderm. There was also an initiation of the ectodermal delamination. The internal layering of the endoderm was undergoing reorganisation. The reorganisation of the endoderm continued (Fig. 10B) resulting in regrouping of the cells. The cells were laterally compressed to assume the future crescent shaped nature in the hypostomal area. Similarly, the basal disc formation also initiated at the other extremity. The endoderm was reorganised and the ectodermal cells transformed into oblong shape typical of the basal disc. Figure 10C represents a case of typical basal disc differentiation in normal isolate regeneration. The hypostome was also well differentiated in this isolate. The ectoderm had a poor representation at some places.
Figure 10D shows the case of impaired regeneration. The hypostome did not differentiate fully. The cells assumed a slight crescent shape. The ectoderm showed thinning at many points. The endoderm also showed empty spaces towards hypostome area. The mesoglea was well differentiated.

Another form of inhibited differentiation was seen in Figure 10E. At some places, the ectoderm as well as the endoderm showed empty spaces. Both the body layers lacked typical representation of the cells. Figure 10F shows four transverse sections exhibiting varying range of inhibition in the isolates. In the first section about half of the body wall demonstrated a complete failure to differentiate. Whereas the other half had a well developed ectoderm and endoderm separated by a clearly marked mesoglea. In the second section about \( \frac{1}{4} \)th of the body wall failed to differentiate, whereas in the 3rd and 4th sections, the cell layers were well differentiated. Figure 10G is a case of extreme inhibition, where both the layers are very poorly differentiated even after 40 hr of regeneration. Some cases showed a better differentiation of the cell layers but basal disc regeneration was impaired (Fig. 10H).

6.2.2. Cell Counts

Fluctuations in total number of cells in regenerating isolates and relative density of various constituent cell types have been enumerated by tissue maceration and
cell counting techniques (Tables 7, 8). The studies were performed from 20 hr onwards because the depressed and the dedepressed isolates could not be distinguished morphologically before this particular stage.

6.2.2.1. Variations in the Total Cell Quanta and Epithelial Cells in Regenerating Isolates

Figure 11 shows the variation in the total cell number and the number of epithelial cells in normal and depressed isolates during regeneration. The total cell number did not show much variation from 28 hr onwards during regeneration of the normal isolate. At 20 hr, the total cell number per isolate was 5459 and it reduced to 4808 cells at 24 hr. At 28 hr, the number rose remarkably to 6599 cells and remained almost same with a slight decrease at 32 and 44 hr. The number of epithelial cells also followed the same trend of fluctuation as shown by the total cell number, except at 20 to 24 hr, where it increased in contrast to the decrease in total cell number.

During the regeneration of depressed isolate, the total cell density was more in the initial stages, but decreased significantly at later stages (Fig. 11). The total cell number was 6941 at 20 hr which decreased to 5939 and 5090 at 24 hr and 28 hr respectively. The number rose to 5428 at 32 hr and reduced significantly to 3321 at 36 hr. There was a gradual increase in total cell density thereafter till 48 hr. The number of epithelial cells
showed variations similar to that shown by the total cell number as a function of time. The number ranged from a maximum of 1425 at 20 hr to a minimum of 662 at 40 hr.

6.2.2.2. Relative Cell Density of Various Cell Types During Isolate Regeneration

The relative cell density of a particular cell type was calculated as its number in relation to hundred epithelial cells present in the same isolate at the same stage. Figure 12 and Tables 7 and 8 show the variation in relative cell density with respect to time during regeneration of normal and depressed isolates.

The interstitial cells were categorised as big interstitial (big I) cells and the little interstitial (little I) cells, depending on their size. The relative cell density of both these types showed similar trend of fluctuations till 36 hr (Fig. 12A). The number was high at 20 hr, decreased till 28 hr and increased again till 32 hr. Then on there was no appreciable change till 36 hr. The relative density of little I cells maintained an increasing trend after 36 hr till 48 hr, whereas that of the big I cells outlined a decline till 40 hr, increased at 44 hr, and remained almost same till 48 hr.
The nematoblasts of all the three types, namely stenotele, desmoneme and isorhiza were counted together at regular intervals of time during 20-48 hr of isolate regeneration. Their number remained very low compared to that of I cells during the entire period of regeneration (Table 7). The relative density of big I-cells decreased during 20-24 hr, followed by no change during next 4 hr. It started increasing gradually during 28-48 hr with a dip at 44 hr (Fig. 12A).

The relative density of differentiated cell types was very low compared to that of the epithelial and the I cells (Fig. 12B). The nematocytes showed very minor fluctuations in their relative density during regeneration. Two small peaks of relative density of nematocytes were observed at 24 and 36 hr. During the rest of the regenerative period, it remained comparatively low. However, a gradual elevation was observed 40 hr onwards.

The relative density of sensory plus nerve cells showed a slightly different pattern (Fig. 12B). It increased from 20 to 24 hr followed by a decline till 32 hr. After this period, there was a gradual increase culminating into a peak at 44 hr, followed by a decline.

The gland plus mucus cells together constituted more to the bulk of total cell density than the nematocytes.
Their relative density decreased from 20 to 24 hr remained constant till 28 hr, and increased gradually thereafter till 48 hr, with a slight decline at 40 hr (Fig. 12B).

The relative density of various cell types was found to be different during regeneration of depressed isolates (Table 8; Fig. 12C). Unlike the normal, two peaks I-cells were obtained at 24 and 40 hr (Fig. 12C). The relative density increased from 20 to 24 hr forming the first peak and decreased thereafter. Minimum relative density of big-I cells was seen at 32 hr, and that of little-I cells at 28 hr. After this dip, the relative density of both the cell types rose gradually till 40 hr and declined till 48 hr.

The relative cell density of nematoblasts was more in depressed than in normal isolates. There were 2 sharp peaks of nematoblast differentiation at 28 and 40 hr. The number decreased from 20 to 24 hr and increased till 28 hr forming the first peak. It was followed by a decline till 36 hr. The second peak was observed at 40 hr and a decline followed it (Fig. 12C).

The number of nematocytes was very low compared to that of the nematoblasts, but their relative density followed essentially the same profile as that of the nematoblasts with peaks at 28 and 40 hr (Fig. 12D).
The sensory plus nerve and the gland plus mucus cells showed similar changes in their number. Their relative densities increased from 20 to 24 hr and remained almost same till 28 hr. There was a dip in the number of sensory plus nerve cells and a peak in gland plus mucus cells at 32 hr. From 36 hr onwards, again the changes in the relative densities of the two groups of cell types were similar, increasing gradually till 44 hr and decreasing thereafter (Fig. 12D).

6.3. Profiles of Macromolecular Synthesis During Isolate Regeneration

The successive changes in incorporation of the labelled precursors into DNA, RNA and proteins were studied at regular intervals during the entire period of isolate regeneration from the time of amputation to its complete restructuration. Although, the reappearance of the lost parts took place much earlier, the synthetic studies were continued till 48 hr to get a picture of complete matura­tion of the regenerate.

6.3.1. DNA Synthesis

A graphical representation of the radioactive precursor incorporation into newly synthesized DNA during regeneration of anterior gastric annuli of normal and depressed hydramas has been depicted in Fig. 13. The incorporation of labelled compound in regenerating depressed
isolates was very low compared to that in the normal isolates.

The overall synthetic profile of DNA during regeneration of normal isolates revealed 2 peaks of synthetic activity. The first peak was observed at 18 hr. The increase in activity started at 6 hr and continued till 18 hr. At 18th hr the rate of synthesis was nearly three times than that at 6 hr, and it remained almost same till 30 hr forming a plateau. Following this plateau, there was a sharp decline in synthetic rate till 33 hr. The rate of synthesis started increasing at 33 hr and reached a maximum at 45 hr. This peak of synthetic activity was much higher than the previous one. Following the second peak, there was a decline in the activity.

In the depressed regenerates, a very low synthetic activity of DNA was observed. Figure 13 revealed two peaks of incorporation during regeneration of isolates taken from depressed hydras. The peaks were noticed at 12 and 18 hr. There was a gradual increase in synthetic activity from 3 hr onwards, which reached the maximum at 12 hr, followed by a decline till 15 hr of regeneration. There was a further burst of fresh DNA synthesis at 18 hr, which was the highest point of activity in depressed isolates. This peak point almost corresponded with the lowest synthetic point in normal isolate. There was a sharp decline
after 18 hr and the activity remained very low 21 hr onwards. Though, there were minor peaks at 24, 30 and 39 hr, they were insignificant. The overall profile of DNA synthesis during regeneration of isolates from depressed hydrams was much lower than that of normal isolate.

6.3.2. RNA Synthesis

Figure 14 depicts the incorporation of $^3$H-uridine into RNA during the regeneration of the isolates from normal as well as depressed hydrams. The synthetic activity during regeneration of the depressed isolates was considerably lower than that of the normal ones.

There were 4 peaks of synthesis during regeneration of normal isolates. The first peak was constituted by a sharp increase in incorporation during 6 to 12 hr, followed by a decline till 15 hr. The second peak was recorded at 18 hr, followed by a fall in incorporation till 24 hr. Another peak was observed at 27 hr. The rate of incorporation declined till 30 hr, and elevated gradually till 39 hr and rapidly till 42 hr, resulting in the highest peak at 42 hr, which was followed by a sharp decline till 48 hr.

During the regeneration of isolates from depressed hydrams, an altogether different profile of RNA synthesis was observed (Fig. 14). There was only one peak at 12 hr. The
synthetic activity was found to be very low compared to that of the normal isolates. At 3 hr, the incorporation was almost \( \frac{1}{8} \) of the normal isolates. The major peak at 12 hr was constituted by a steep increase in incorporation from 9 to 12 hr, followed by a gradual decrease. The synthetic activity decreased till 21 hr and remained very low after that.

6.3.3. **Protein Synthesis**

The incorporation of \(^{14}\text{C}-\text{isoleucine into freshly synthesized proteins during normal and depressed isolate regenerations has been depicted in Fig. 15. As in the case of DNA and RNA, the synthesis of protein was also reduced during the regeneration of depressed isolates as compared to that of the normal ones, though the differences were not as extreme as in other macromolecules. Figure 15 shows that during the regeneration of the isolates excised from normal hydras, four peaks of synthetic activity of the proteins were observed at 9, 15, 30 and 42 hr. The rate of synthesis started increasing at 3 hr and remained high at 6 and 9 hr, following which it declined sharply till 12 hr. At 12 hr the synthetic activity increased again, reached a maximum at 15 hr and thereafter decreased gradually till 24 hr. The initiation of third peak, which was as high as the second one, started at 24 hr. The peak was seen at 30 hr, following which the rate of incorporation decreased and reached the
lowest point at 36 hr. The last minor peak was observed at 42 hr, which was followed by decline in the rate of synthesis.

During the regeneration of the isolates excised from the depressed hydras, an alteration in the profile was also observed along with reduction in the rate of protein synthesis. Though the height of peak points was more or less equal in depressed and normal isolates, the overall rate of synthesis remained low in depressed isolates. The initial rise in the synthetic activity was same as in normal isolates from 3 to 6 hr. The decline which was observed at 12 hr in normal isolates, appeared only at 18 hr in the depressed isolates. Following this, the synthetic pattern in depressed isolates followed that of the normal ones with a temporal shift of 3-6 hr till 36 hr. The last peak that was observed in normal isolates at 42 hr was missing in the depressed isolates.

6.4. Variation in Enzyme Activity During Isolate Regeneration

Three different enzymes, namely, alkaline phosphatase (ALP), acid phosphatase (ACP) and Na\(^+\),K\(^+\)-ATPase were assayed during the regeneration of the normal and depressed isolates.
6.4.1. Assay of Alkaline Phosphatase Activity

The changes in the specific activity of alkaline phosphatase during isolate regeneration are shown in Fig. 16. The activity is expressed as per cent change with respect to the control. The control is 24 hr starved hydas. There were 3 peaks of activity during regeneration of the normal isolates (Fig. 16). The first small peak was observed at 4 hr, when the activity reached 112% that of the control. It decreased thereafter till 12 hr, beyond which a gradual increase till 28 hr was monitored. At 28 hr, maximum activity was observed, which was 127% that of the control. During 32 to 36 hr, the specific activity decreased. The third peak was seen at 44 hr followed by declination. The magnitude of this peak was smaller than that of the second peak and higher than that of the first peak.

During the regeneration of isolates taken from the depressed hydas, 3 peaks of enzyme activity were marked (Fig. 16). The first two peaks did not coincide with those of the normal, whereas the third peak coincided. The first peak at 12 hr was constituted by a steep increase in the enzyme activity from 4 to 12 hr followed by a declination till 16 hr. The second peak at 24 hr showed an increasing phase of enzyme activity from 16 to 24 hr and decreasing phase from 24 to 36 hr. The rising phase showed a steep increase in the activity from 16-24 hr, whereas the declining
phase showed a steep fall in the activity till 28 hr, followed by a gradual decrease till 36 hr. The magnitude of the second peak was 166% that of the control value. The third and last peak was observed at 44 hr.

6.4.2. Assay of Acid Phosphatase Activity

The profile of acid phosphatase activity showed three peaks during the regeneration of normal isolates (Fig. 17). The first peak was observed at 3 hr, following which there was a gradual decline till 6 hr and a sharp decline till 15 hr. The acid phosphatase activity was minimum at 15 hr, where only 54% activity was observed, compared to the situation seen in control. From 18 hr onwards, the specific activity started increasing again till 27 hr, with a dip in the activity during 21-24 hr. The highest peak was observed at 27 hr where the activity was 199% of the control. It was followed by a sharp decline bringing down the activity to 66% of the control at 30 hr. The third peak at 39 hr was preceded by an increase in enzyme activity with a dip during 33-36 hr, and was followed by a steep fall in activity.

The activity of acid phosphatase showed a different pattern during the regeneration of depressed isolates. There were 3 major and one minor peak of the enzyme activity. Figure 17 shows that the specific activity decreased during initial 3 hr. The first minor peak was seen at 6 hr
having the enzyme activity only 94% of the control. It was followed by a decrease to 71% at 9 hr. The first major peak at 12 hr showed the enzyme activity to be 156% of the control. This was followed by a declination till 18 hr. The second peak, observed at 24 hr, was comparable to first peak in its magnitude. Following it, there was a decrease in specific activity till 33 hr, reaching a value of 74% of the control. The third peak observed at 45 hr was preceded by a gradual increase in the enzyme activity during 33 to 36 hr and 39 to 42 hr and a steep increase during 36 to 39 and 42 to 45 hr. Enzyme activity was highest at this hour following which it declined till 48 hr.

6.4.3. Assay of Na⁺,K⁺-ATPase Activity

The changes in the activity of Na⁺,K⁺-ATPase during regeneration of normal and depressed isolates have been outlined in Fig. 18. The activity has been expressed in terms of per cent change with respect to the control. There was a very high activity of Na⁺,K⁺-ATPase during regeneration as is evident from Fig. 18. In the regenerating isolates obtained from normal hydras, 3 peaks of activity of Na⁺,K⁺-ATPase were observed. The activity at 4 hr was 240% that of the control and it formed the first peak. The highest activity was observed at 16 hr, which was as high as 2160% that of the control. There was a sharp decline in activity till 20 hr, following which, a rise was seen leading to the third peak at 24 hr. The activity at this point was 1860% that of the control. Thereafter, the activity decreased till 32 hr, increased till 40 hr and remained stationary till 44 hr.
The pattern of activity during regeneration of isolates excised from depressed hydias was the same as that of normal in the second half of regeneration, but differed in the first 28 hr (Fig. 18). There were two peaks of enzyme activity at 8 and 28 hr. The activity started decreasing from 0 hr till it reached a low level at 4 hr. The activity at 8 hr was 1400% that of the control forming the first peak. There was a gradual decrease in activity till 16 hr. The second peak, smaller than that of the first one, was observed at 28 hr, where the activity was 700%. After 28 hr, the pattern was same as that seen in normal isolate regeneration.

6.5. Effect of Enzyme Inhibitors on Normal Isolate Regeneration

The isolates were treated with inhibitors of alkaline phosphatase, acid phosphatase and ATPase to study their nature of regeneration. Observations were made on isolates treated with inhibitors from the beginning of first or second peak till the highest point of activity. Data on continuous treatment of inhibitors were also obtained.

6.5.1. Determination of Optimal Dose of Inhibitors

For the treatment of isolates with the various inhibitors, the optimal concentration was selected on the
basis of dose tolerance test to avoid lethal effects on isolate. L-Homoarginine, an inhibitor of alkaline phosphatase, produced lethal effects at 8 mM. L-Leucine, another inhibitor of alkaline phosphatase was not lethal even at a concentration of 20 mM.

The inhibitors of acid phosphatase were also tested for the maximum nontoxic concentration. Potassium fluoride showed lethal effects at 6 mM and tartaric acid at 0.1 mM.

Ouabain and sodium orthovanadate were used as inhibitors of ATPase. Ouabain was nontoxic even at 6 mM, while sodium orthovanadate was toxic at a concentration of 40 mM.

The concentrations of various chemicals finally used for inhibition studies were the following: L-Homoarginine, 4 mM; L-leucine, 10 mM; Potassium Fluoride, 4 mM; Tartaric acid, 0.06 mM; Ouabain, 1 mM and Sodium Orthovanadate, 30 μM.

6.5.2. Inhibition of Alkaline Phosphatase

Table 9 provides data on the effects of alkaline phosphatase inhibitors on the regeneration of isolated anterior gastric annuli. In normal untreated isolate, regeneration time was 22 and 26 hr for hypostome and basal disc, respectively.
6.5.2.1. **L-Homoarginine**

First peak inhibition: The first peak (1-6 hr) inhibition studies performed with 4 mM L-homoarginine, which brings about an inhibition of 42.38% in specific activity of alkaline phosphatase, showed a delay in hypostome (28 hr) as well as basal disc (35 hr) regeneration (Table 9). The appearance of the first tentacle rudiment was delayed by 5 hr (Fig. 19A). By the end of 32 hr, the hypostome had been reconstituted in 87% of the isolates. But 100% regeneration was achieved only by 47 hr.

The regeneration of the basal disc was inhibited to a greater extent. Though, the initiation of basal disc regeneration took place 1 hr later than control, the successive appearance of the basal discs was slower. Maximum regenerations achieved were 64% by 50.5 hr (Fig. 19B).

Second peak inhibition: Inhibition of the second peak (13-28 hr) of alkaline phosphatase activity by L-homoarginine affected the regeneration of hypostome to a comparatively lesser degree (21 hr; Table 9). Though the restitution of hypostome started 3 hr later, the process was completed 2.5 hr earlier than the control (Fig. 19A).

A greater delay was, however, observed in basal disc regeneration (42 hr; Table 9). Initiation of basal disc regeneration took place 12.5 hr later than the control.
By 49 hr, a maximum of 40% regeneration was attained (Fig. 19B).

Continuous inhibition: Continuous treatment with the inhibitor slowed down the hypostome regeneration time to 27 hr. The emergence of the first pair of tentacle rudiments was delayed by 5 hr. The number of regenerated hypostomes increased to a maximum of 90% gradually by 37 hr (Fig. 19A).

Maximum inhibition of the basal disc regeneration was resultant of the continuous L-homoarginine treatment after the isolation. There were only 10% isolates that formed basal disc successfully after 43 hr (Table 9; Fig. 19B).

6.5.2.2. L-Leucine

Treatment with 10 mM L-leucine brought about a 39.28% fall in the specific activity of alkaline phosphatase.

First peak inhibition: The inhibition of first peak of the enzyme by L-leucine slowed down the appearance of the first tentacular pair by 5.5 hr. But the successive appearance of the hypostomes was faster than that of the control. With the result 100% restitution was obtained 2 hr earlier than the control (Fig. 19C). The approximate time for hypostome regeneration was 25 hr (Table 9).
The basal disc regenerated at 25 hr. Initiation of the regeneration of basal disc took place 1 hr in advance to the control, but the number remained constant after reaching a value of 67% at 32 hr (Fig. 19D).

Second peak inhibition: Inhibition of the second peak of enzyme activity affected the regeneration in the same way as the other inhibitor of alkaline phosphatase. A comparatively negligible delay was observed in hypostome formation (22 hr) and a significant delay in basal disc regeneration (36 hr; Table 9). The delay in the appearance of the first hypostome was 4.5 hr and the 100% regeneration was seen 2.5 hr earlier than that of the control (Fig. 19C).

The basal disc initiation started 1 hr later than the control, and the rate of their reappearance was very slow. No more basal discs were seen after reaching a value of 60% by 49 hr (Fig. 19D).

Continuous inhibition: The delay in regeneration was even more apparent after continuous treatment with L-leucine. The hypostomes were noticed at 29 hr in 100% isolates and basal discs at 47 hr in 78% isolates (Table 9). There was an initial delay of 7 hr in the reconstruction of the first hypostome. The appearance of successive hypostomes was slow and 100% regeneration was perceptible only at 43 hr (Fig. 19C).
The first basal disc appeared after 30 hr and till 45 hr only 20% of the isolates had reconstructed their basal discs. After that, there was a sharp enhancement in basal disc regeneration, which reached a maximum value of 78% at 54 hr (Fig. 19D).

6.5.3. Inhibition of Acid Phosphatase

Potassium fluoride and tartaric acid were used to inhibit acid phosphatase activity. The inhibition studies showed that potassium fluoride at its maximum nontoxic concentration (4.0 mM) brought about an inhibition of 60.9% in the specific activity of acid phosphatase, while tartaric acid showed 29.7% inhibition at its highest nontoxic dose (0.06 mM). As mentioned in the previous section, the regeneration of isolates was monitored after inhibiting acid phosphatase continuously or at the first (1-6 hr) or second (18-28 hr) peak.

Table 10 demonstrates the effect of inhibition of acid phosphatase on hypostome and basal disc regeneration time and the maximum percentage of successful regenerations obtained. In control, all the isolates regenerated successfully and the time for the restitution of hypostome and basal disc was 22 hr and 26 hr respectively.
6.5.3.1. Potassium Fluoride

First peak inhibition: Inhibition of the first peak of acid phosphatase by potassium fluoride resulted in the appearance of tentacle rudiments at 29 hr and the basal disc at 38 hr (Table 10). The first pair of tentacles was observed 3 hr after the control (Fig. 20A). By 34 hr all the isolates had restituted hypostome with a pair of tentacles.

The first basal disc appeared 3 hr later than the control and the basal disc number in treated isolates lagged behind the control ones with time. After 45 hr there was a marked increase in basal disc regeneration till 50 hr, i.e., from 50% to a maximum of 77% (Fig. 20B).

Second peak inhibition: After the inhibition of second peak, the time for the restitution of hypostome decreased (24 hr), whereas that of basal disc remained almost same (38 hr) as that in the first peak inhibited isolates (Table 10). The appearance of first hypostome was delayed by 2 hr. Though the hypostome was formed in all the isolates, a delay of 5 hr was observed for achieving complete regeneration (Fig. 20A).

However, no basal disc was observed till 30 hr, i.e., 9 hr after its appearance in control. A maximum of 40% regeneration took place by 49 hr. No more regeneration was seen thereafter (Fig. 20B).
Continuous inhibition: Continuous treatment with potassium fluoride delayed the appearance of hypostome to 28 hr and basal disc to 53 hr (Table 10). There was an overall delay of 6 hr in the initiation of first pair of tentacle rudiments. Complete regeneration was achieved 5 hr later than control (Fig. 20A).

The basal disc was most severely affected by continuous treatment. The first basal disc was observed after 47 hr. The basal disc was seen till later stages of regeneration only in 10% isolates (Fig. 20B).

6.5.3.2. Tartaric Acid

Tartaric acid treatment yielded results similar to those obtained with potassium fluoride treatment (Table 10).

First peak inhibition: Inhibition of the first peak delayed regeneration of both the extremities. All the hypostome rudiments made their appearance at 31 hr (Table 10). Till 29 hr, the extent of hypostome regeneration by inhibition of first peak of enzyme activity with tartaric acid remained the same as that resulted with potassium fluoride, whence about 50% regenerations had taken place. After that the tentacle rudiments appeared more slowly. All the isolates were beset with tentacle pair and hypostome by 42 hr (Fig. 20C).
The regeneration time of basal disc was affected significantly (39 hr; Table 10). The first basal disc appeared later than that in the control, the number increased gradually and finally a maximum of 93% isolates had developed basal disc by 48 hr (Fig. 20D).

Second peak inhibition: The reconstruction time of the hypostome was unaffected when the isolates were treated with tartaric acid from 18-28 hr (22 hr; Table 10). The hypostome initiation in second peak inhibited isolates was slightly slower than that in the controls, but after 23.5 hr, there was a steep increase in the number of regenerations. With the result, regeneration in all the isolates was achieved 2 hr earlier than the control (Fig. 20C).

The reappearance of basal disc was delayed, but the delay was less than that seen in the first peak inhibited isolates (33 hr; Table 10). However, the percentage of successful attempts to form basal disc was much lower (18.2%) in this treatment (Fig. 20D).

Continuous inhibition: Continuous treatment with the inhibitor further delayed the regeneration process, affecting the basal disc to a greater extent. The regeneration time for hypostome and basal disc was 30 hr and 44 hr respectively (Table 10). There was a 6 hr delay in the appearance of the first pair of tentacle rudiments.
The number of regenerated hypostomes increased rapidly till 25 hr and gradually after that, reaching a value of 100% by 43.5 hr (Fig. 20C).

On being treated with tartaric acid continuously, the first isolate was bestowed with a basal disc 10 hr later than the control. The number of successful regenerates increased gradually and by 49 hr the number reached 100% (Fig. 20D).

6.5.4. Inhibition of ATPase

Two inhibitors were used to study their effect on regeneration by means of their inhibitory role on ATPase activity. Ouabain inhibited the Na\(^+\),K\(^+\)-ATPase, whereas sodium orthovanadate inhibited all the plasmalemma ATPases without any effect on mitochondrial ATPase. As in earlier experiments, the first peak (1-6 hr), second peak (12-17 hr) and continuous inhibition studies were performed for this enzyme too. Table 11 represents the effects of ouabain and sodium orthovanadate on regeneration time of treated and control isolates. The control isolates were beset with tentacles at 22 hr and with basal disc at 26 hr.

6.5.4.1. Ouabain

First peak inhibition: Treatment with 1 mM of ouabain from 1-6 hr resulted in a delay in both hypostome and basal disc regeneration. The hypostome appeared at about 26 hr in all (100%) and basal disc at 32 hr in 75%
isolates (Table 11). The first peak inhibition resulted in a 5 hr delay in the appearance of hypostome. There was a gradual increase in the regeneration of successive tentacles, till 46 hr (Fig. 21A).

The first basal disc was seen 0.5 hr later than the control. The number increased till 42.5 hr and reached a stable value of 75% (Fig. 21B).

Second peak inhibition: The inhibition of second peak of enzyme activity did not show much delay on average time of the restructuration of hypostome (23 hr), but the restitution of basal disc was delayed (34 hr; Table 12). The treated isolates showed a delay of 2.5 hr, as compared to the control, in the appearance of first hypostome. Full regenerations were observed at 34 hr; 18 hr later than the control (Fig. 21A).

The appearance of first basal disc was perceived 1 hr later than the control in isolates treated from 12-17 hr with ouabain. The number of basal discs increased till it reached 27%. No additional basal discs were formed from 26 to 36 hr, after which the percentage was elevated to 54% at 40 hr. This remained the maximum number of basal discs regenerated (Fig. 21B).
Continuous inhibition: On being treated continuously with ouabain, altogether different results were obtained. There was an acceleration in the process of hypostome regeneration (21 hr) though not successful in all the cases (80% successful; Table 11). There was a delay of only 1 hr in the appearance of the first pair of tentacles, but later, the rate of restitution of hypostomes was quite high (Fig. 21A).

The basal disc in treated isolates was reconstituted later than that in the control (47 hr; Table 11). The regeneration of first basal disc started after a delay of 21 hr. There was a gradual increase in the number of regenerations and it reached 50% by 49 hr (Fig. 21B).

6.5.4.2. Sodium Orthovanadate

Sodium orthovanadate did not show any remarkable effect on time of regeneration (Table 11), but the percentage of successful regenerations was affected.

First peak inhibition: After the first peak inhibition, a slight delay in hypostome regeneration time (23 hr) was observed. The regeneration of the first hypostome started 5 hr later than the control. The highest point of regeneration was achieved by 32 hr, i.e., 3 hr later than the control (Fig. 21C).
The basal disc regeneration showed a different trend. The first isolate was bestowed with basal disc 4 hr later than the control; the successive regenerations were gradual and a 100% success in basal disc restitution was observed by 42 hr, thus causing a significant delay in the average time of basal disc regeneration (32 hr; Table 11; Fig. 21D).

Second peak inhibition: After the inhibition of second peak, the regeneration of hypostome was perceived at 23 hr (Table 12). The regeneration of the first hypostome started 4 hr later and were accomplished in all the isolates 5 hr later than that seen in the control (Fig. 21C).

The first basal disc appeared 1.5 hr later than that observed in the control isolates, and the increase in their number was much slower. After reaching a maximum of 60% at 40 hr, the number remained constant (Fig. 21D). The average time of basal disc regeneration was 34 hr (Table 11).

Continuous inhibition: After continuous treatment with the inhibitor, the regeneration was faster than noticed in the regenerates otherwise treated with sodium orthovanadate, but the number of successful attempts was reduced. Figure 21C depicts that in the appearance of first hypostome a delay of 4.5 hr was observed. The hypostomes appeared quite regularly, but stopped doing so after reaching a value of 80% by 26 hr. The average time of hypostome formation was 23 hr (Table 11).
The basal disc formation was inhibited to a greater extent. Though in the first few generates, the time of restitution was just 1 hr more than the control, but no more than 30% isolates could regenerate their basal disc till 50 hr, the average regeneration time being 24 hr (Fig. 21D; Table 11).

6.6. **Effect of Enzyme Stimulators on Depressed Isolate Regeneration**

Bromodeoxyuridine, formic acid and noradrenaline bitartarate show a stimulatory effect on alkaline phosphatase, acid phosphatase and Na⁺,K⁺-ATPase respectively.

6.6.1 **Determination of Optimal Dose**

Bromodeoxyuridine produced toxic effects on hydra at a concentration of 1 mM; so 0.8 mM concentration was used for experimental purposes. Formic acid was toxic at 8 mM, thus 5 mM concentration was used for experiments. Noradrenaline bitartarate was used at a concentration of 0.1 mM, because at concentrations higher than 0.12 mM, it produced lethal effects on hydra.

6.6.2. **Effect on Regeneration**

The activators were tested for their effects on depressed isolates during regeneration. Table 12 represents the time and percentage of successful regenerations in the
depressed isolates after continuous treatment with bromodeoxyuridine (0.8 mM) formic acid (5 mM) and noradrenaline bitartarate (0.1 mM).

In control isolates, the time taken for hypostome and basal disc regeneration was 24 hr and 30 hr respectively. The successful regeneration was 100% for hypostome and 80% for basal disc. Continuous treatment with bromodeoxyuridine decreased the time of regeneration for hypostome as well as the basal disc. In both types of regenerations, 90% success was observed. The time required for appearance of hypostome was 21 hr and for basal disc was 26 hr.

Treatment with formic acid had a delaying effect on hypostome regeneration (25 hr) and a slightly stimulatory effect on basal disc regeneration (27 hr). With this treatment, the hypostome appeared in all, whereas the basal disc was restituted only in 61% of the isolates.

Noradrenaline bitartarate treatment did not affect hypostome regeneration (24 hr; 100% successful) and hastened the basal disc regeneration (26 hr; 90% successful) (Table 12).

The percentage of successful regenerations with respect to time has been plotted in Fig. 22.

Bromodeoxyuridine delayed the appearance of first tentacle rudiment by 1.5 hr. By 20 hr, the number of hypostomes in treated isolates was more than those in control
and it remained so till 24 hr, when 80% of the hypostomes had been formed. The maximum success obtained was 90% (Fig. 22A). On the other hand, the basal disc regeneration was strongly stimulated by this treatment. Its initiation was seen 5 hr in advance to the control. A total of 90% isolates could restitute their basal discs successfully (Fig. 22B).

Formic acid, a stimulator of acid phosphatase did not show a very remarkable effect on regeneration of hypostome in depressed isolates. The tentacle rudiments appeared at the same time as in control. Till 22.5 hr, the number of hypostomes was a little higher than the control, but after that the percentage decreased and 100% regeneration was seen 10 hr later than that in control (Fig. 22A). The basal disc regeneration was also faster than control till 32 hr, but they were never restituted with success in more than 61% isolates (Fig. 22B).

The Na\(^+\),K\(^+\)-ATPase stimulator, noradrenaline bitartrate, delayed the appearance of the first hypostome by 1 hr, but the rate of regenerations was faster than the control, so that by 23 hr, 63% regenerations had occurred. After 23 hr, the rate of appearance of hypostomes decreased again. Complete regeneration was observed 10 hr later than the control (Fig. 22A). The basal disc regeneration was highly stimulated by this chemical. Its initiation was seen 5 hr prior to the control and a maximum of 90% basal discs were seen by 37 hr (Fig. 22B).