DNA SYNTHESIS IN HYDRA

DISSERTATION SUBMITTED TO THE JAWAHARLAL NEHRU UNIVERSITY
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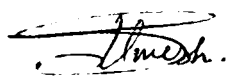
UMESH KUMAR

SCHOOL OF LIFE SCIENCES
JAWAHARLAL NEHRU UNIVERSITY
NEW DELHI-110067
INDIA

CERTIFICATE

The research work embodied in this dissertation entitled, "DNA Synthesis in Hydra" has been carried out in the School of Life Sciences, Jawaharlal Nehru University, New Delhi. This work is original and has not been submitted in part or full for any other degree or diploma at this or any other university.



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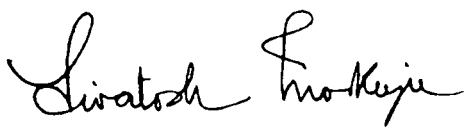

(SIVATOSH MOOKERJEE)
Supervisor

(P.C. KESAVAN)
Dean

School of Life Sciences,
Jawaharlal Nehru University,
New Delhi - 110067, India.

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(UMESH KUMAR)

School of Life Sciences,
Jawaharlal Nehru University,
New Delhi - 110067, India.

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INTRODUCTION

Regeneration is a term capitalized long back, denoting an outstanding area of intense biological interest. Regeneration in many ways is a simulation of a normal development. Like normal development, specific instructions from the genome are given in signalling and guiding the morphogenetic processes to rebuild a lost structure. It has been shown that prior to regeneration, cells undergo dedifferentiation which later redifferentiate to all other types of cells. Differentiated cell is that state of an embryonic cell in which its genome is selectively activated to synthesize structural and enzymatic cell specific proteins. In other words, differentiation is an accumulative progressive loss of potentials of innumerable probabilities inscribed within the genomic household of a mature cell. The macromolecules like DNA, RNA and proteins are not only supposed to modulate the metabolism of cells but also to control their morphogenetic destiny. The encoded information in DNA is expressed through the process of transcription into mRNA (Monod and Jacob, 1961), which are transported to polyribosomal sites (Goodman and Rich, 1963) for final translation. Thus the cellular differentiation can be controlled both at transcriptional and post-transcriptional level (MacLean and Hilder, 1977).

The present upsurge of interest has been stimulated mainly by recent remarkable advances in the cellular and molecular aspects of regeneration and at present time, regeneration has acquired a new look holding a new promise. In this connection intensive studies have been made on the simpler of the more readily available and maintainable animals, namely, lower organisms which are endowed with unique property of regeneration. The tremendous capacity of regeneration in hydra has attracted the attention of many scientists who evidently selected it as a test system and formulated some interesting models of differentiation (Child, 1941; Turning, 1952; Burnett, 1966; Mookerjee and Sinha, 1967; Wolpert, 1969; Goodwin and Cohen, 1969; MacWilliams et al., 1970; Webster, 1971).

Hydra is a small (5-10 mm) fresh water coelenterate containing about 100,000 cells distributed among fifteen cell types (Bode and David, 1978). Its body resembles a tube, consisting of two epithelial cell layers, ectoderm and endoderm, surrounding a gastric cavity. In the ectoderm, the epithelio-muscular cells make up the tissue layer. Lodged among these are the interstitial cells, their differentiated derivatives, nerves and nematocytes, and differentiation intermediates. The endoderm of body column is made up primarily of gastrodermal digestive cells with a small number of gland and mucous cells interspersed among

them. In between the ectoderm and the endoderm a non-cellular mesoglea is present.

Perhaps the most important area of interest in hydra has concerned the experimental analysis of growth and regeneration. Much of what is known today about organization,induction, polarity, gradients and other developmental phenomena has arisen from work on hydra (Lentz, 1966). All these aspects have been studied mostly at morphological and cellular and some at molecular levels. One of the most important theories of bipotentiality concerning the growth patterns of hydra has been proposed by Tripp (1928). The concept of bipotentiality has been further developed by Mookerjee and Sinha (1967) on the dual expression of the middle piece as explant. Issajew (1926) has noted that occasionally a hydra has a Y shaped fork in one of its tentacles and fork gradually moved out to the end of its tentacle and finally disappeared. Brien and Reniers-Decoen (1949) have grafted a vitally stained distal portion of a hydra to an unstained proximal portion. Gradually, the stained cells of distal portion moved proximally reaching the base. These observations led all these workers to same conclusion that the hydra possesses a subhypostomal growth region and that its an immortal animal due to constant renewal of cells

from growth region replacing those cells at the extremities that are lost because of aging and death. The cells of hydra are renewed approximately every 45 days. Under an impaired condition of hypostome, the subhypostomal growth zone in hydra cannot function normally to generate downward cell flow. Ingeniously Mookerjee and Roy (1971) have developed an experimental protocol where immobilization of implant has been explained on the basis of non functional state of subhypostomal growth zone. Autoradiographic studies of digestive cavity after injecting tritiated thymidine into it revealed that labelled cells and mitotic figures were distributed equally along the entire body column (hypostome, stomach and budding region) of hydra (Campbell, 1965). These observations appear contradictory to the concept of a localised growth zone. However, Shostak et al. (1965) reported that mitotic figures are more abundant in the growth region. Evidently it appears that the concept of growth zone seems not invalid although its without a sharp lower boundary.

Transformation of a portion of the adult organism into full components of another individual must necessarily involves such phenomena like determination, differentiation and individuation, the well organized instances of embryogenesis. Budding in hydra has excited biologists since it

was first discovered. Among the earliest workers Lang (1892), Tannreuther (1909), Hadzi (1910), Gelei (1924) and Kanajew (1930) have reported accumulation of interstitial cells initiating bud development at the presumptive budding site and after dividing, caused outpushing of body column which ultimately produced the bud. The differentiated cells of developing buds have been thought to be derived primarily from the interstitial cells. Brien and Reniers-Decoen (1955) have employed X-irradiation and Diehl and Burnett (1965) have employed nitrogen mustard to remove the interstitial cells and observed that the hydra continues to bud for a short period thereby rejecting the primary role of interstitial cells in bud formation. Braem (1910) and Gelei (1924) have stated that materials of bud are derived predominantly from epitheliomuscular cells of epidermis and digestive cells of gastrodermis. Unfortunately, there is a no work correlating their presence and mitotic activity within the event of budding. An isolate from the stem about 1 mm in length transforms directly and completely into hydranth structures thus showing the involvement of all types of cells of regenerating region (Steinberg, 1955).

Quantitative analysis of population of epitheliomuscular, interstitial and cnidoblasts during bud development have revealed that frequency of these cells changes

from a basic homogeneous pattern in early stage to an elaborate heterogeneous one characteristic of different regions of hydra (Sanyal, 1967). The fate map of developing bud of hydra shows no major difference between recruitment patterns of cells from above, below and lateral to the bud tip. The tissue is recruited in concentric rings around the young bud tip and is distored directly outward into the bud column. The recruitment ends at the time that tentacle rudiments appear on the bud (Sanyal, 1966; Otto and Campbell, 1977). Li and Lenhoff (1960) have observed that protein content of bud is 1/3 that of parent but the DNA/protein synthesis ratio of bud being 3 times that of parent hydra. When a bud is excised early in its development, the remaining part still attached to parent can regenerate suggesting the occurrence of cell divisions within bud tissue. The bud bears tentacles only after its cells start undergoing cell division (Burnett, 1961). Recently, Venugopal and Mookerjee (1976) have shown that the inhibition of RNA synthesis in hydra resulted in complete suppression of bud morphogenesis. A partial inhibition allowed the bud formation, but affected all other cells. These results indicate that bud morphogenesis is associated with DNA dependent RNA synthesis.

The ability of hydra to reconstitute itself from tissue pieces and even single cells (Gierer et al., 1972) has stimulated a large number of workers to develop and test theoretical models to explain the pattern formation in hydra (Child, 1941; Turning, 1952; Burnett, 1966; Mookerjee and Sinha, 1967; Wolpert, 1969; Goodwin and Cohen, 1969; MacWilliams et al., 1970; Webster, 1971). In most models diffusible substances are proposed which are unequally distributed along the body axis and which are presumed to control growth and morphogenesis. Views extended in the process of regeneration by different workers are not always in agreement and sometimes definitely confusing. Lang (1892), Hadzi (1910) and Zawarzin (1929) were of the opinion that the presence of active totipotent interstitial cells being the only and primary factor for regenerating cut edge. Kanajew (1926) remarked that interstitial cells are responsible for head regeneration only and not for foot regeneration. He showed that stalk of Pelmatohydra being poor in interstitial cells could regenerate foot but failed to regenerate tentacles. He also reported the occurrence of mitosis in ectoderm cells throughout the body of regenerating hydra instead of local proliferation of interstitial cells. Brien and Reniers-Decoen (1955) have shown that the irradiated hydras deprived of intestitial

cells can regenerate for sometimes before death. Brien (1953) and Moore (1952) showed extension and migration of ectodermal layer over the cut surface to form a thin sheet of flattened cells with dense cytoplasm and subsequent migration of interstitial cells into endodermal layer to give rise to other cell types. Mookerjee and Bhattacharjee (1966) have shown that rebuilding of lost pattern concerns with activity and participation of existing cells in endoderm at cut surface which first heal the wound as layer. Same layer subsequently gives rise to a second layer, namely, the ectoderm. Interstitial cells play their role at later stage during the tentacle formation.

During head and foot regeneration the first detectable change in cell distribution is an increase in number of nerve cells at the site of morphogenesis (Bode et al., 1973). Biochemical studies have shown that interstitial cells are the chief source site of RNA synthesis (Tardent, 1954; Mookerjee and Sanyal, 1962). Shostak (1975) has reported that antimetabolites like colcemid seemed to have effect on head formation. Clarkson and Wolpert (1967) have given quantitative measurements of DNA synthesis along the body column of intact and regenerating hydras suggesting that there

exists essentially a uniform pattern of DNA synthesis. DNA, RNA and protein synthesis were studied during first hour of regeneration of decapitated hydra (Clarkson, 1969a,b; Datta, 1968; Datta and Chakraborty, 1970) in which no appreciable change in DNA synthesis, a large increase in RNA synthesis and a slight increase in protein synthesis was marked, Nucleic acid inhibitors like hydroxyurea is found to delay the hypostome regeneration which is small compared to inhibition of ^{3}H-thymidine incorporation so that DNA synthesis did not appear to play a major role in hypostome regeneration (Clarkson, 1969a,b). There is 3 fold delay in hypostome regeneration by 5-fluorouracil which is also accompanied by inhibition of label into DNA and RNA. He also remarked that initial hypostome regeneration is due to total turnover of stable mRNA. Incorporation of ^{3}H-uridine and ^{3}H-isoleucine into RNA and protein respectively has shown that during the act of regeneration an enhanced rate of synthesis is noted and both RNA and protein follow essentially a similar pattern. These observations have been correlated with variations in cellular activity during regeneration (Venugopal and Mookerjee, 1980).

From the foregoing review of literature on various aspects of the morphogenetic phenomena in hydra it is quite clear that so far the major emphasis has been placed

largely at cellular level of understanding the phenomenon of regeneration. A couple of years back, for the better understanding of the morphogenetic phenomena in hydra a serious probe at the molecular level has been undertaken. A thorough study of RNA metabolism and protein synthesis in normal and regenerating hydra was undertaken by Venugopal and Mookerjee,(1977, 1980a,b,c). Two phases of RNA metabolism have been shown after feeding i.e. synthetic and degradative phase. During regeneration immediately after amputation there is a bursting of RNA synthesis and within 36 hrs of regeneration the capacity of hydra in synthesizing RNA varies from region to region along with varying half life of RNA. The half life of mRNA in hypostome differentiation is about 8 hrs whereas in basal disc differentiation it is about 18 hours.

In the present work, it is intended to study the pattern of DNA synthesis during various morphogenetic phenomena of hydra. The different facets of DNA synthesis during its life's economy will cover the following aspects :

1. DNA synthesis in normal, non-regenerating hydra following the feeding
2. DNA synthesis in different segments of same hydra at specific points following feeding
3. DNA synthesis during bud morphogenesis in hydra

4. DNA synthesis in hydra during hypostome and basal disc regeneration
5. The capacity of regenerating hydra to synthesize the macromolecules during specific hours of regeneration

Since it is well known that the growth and regeneration are greatly influenced by the feeding habits of hydra, so before going into molecular details of DNA synthesis, mere curiosity stretched the problem to study some aspects of the feeding behaviour. Some data have also been presented on the behaviour of gastrodermal cells during the process of digestion. These data would be of much interest as feeding invariably leads to DNA synthesis.

MATERIAL AND METHODS

Hydras, initially procured from a local pond in Santiniketan, West Bengal, and cultured in the laboratory for past couple of years, constituted the experimental material in the present study.

Culture technique

A steady state culture of hydra was maintained in the laboratory in a chemically defined BVT medium as proposed by Loomis and Lenhoff (1956). The culture medium was prepared as follows :

Stock solution A : 7.5 g of $CaCl_2$ in 500 ml of distilled water.

Stock solution B : 10.0 g of $NaHCO_3$, 5.0 g of EDTA } in 500 ml of distilled water

10 ml each of stock A and B were mixed and the volume was made upto 2000 ml using boiled and filtered water. EDTA (Disodium ethylene diamine tetra acetate) was added which selectively chelates the toxic copper ions. The bicarbonate was added to buffer the solution in the region of pH 7.5-8.0. Calcium inns added were specifically required by hydra.

Live nauplii of Artemia salina (Brine shrimp) were used to feed hydras. To bring about the hatching of larvae, each dish was seeded with a pinch of artemia eggs which hatched after 1-2 days of incubation at room temperature ($21^{o}C$). Living nauplii were separated from floating eggs by their settling down at the bottom and phototropic migration. The larvae were pipetted out and washed thoroughly in running tap water. Each hydra was given 8-10 larvae. The culture was cleared twice a day with BVT solution to remove traces of the uningested remnant of brine shrimps.

Selection of Hydras

In all the experiments, 24 hours starved hydras of equal size and health were selected from culture stock. Selected hydras were without gonads and buds. Any one showing any sign of bud or gonad development were discarded from the experimental series.

Feeding behaviour

To study the feeding behaviour, 24 hours starved hydras were given 8-10 artemia larvae each. Their immediate reaction to food was observed under stereobinocular. Different stages of hydra during its feeding and digestion were also fixed in hot bouin's fixative for whole mounts

and histological preparations. The hydras were fixed at a regular interval of 1 hr, 2 hr, 3 hr, 4 hr, 5 hr and 7 hr of the ingestion of the artemia till they excreted out the material from their enteron after completing the digestion.

Tracer incorporation and counting methods

To study the DNA synthesis, ^{3}H-thymidine (activity 1 mCi and specific activity 15,200 mCi/m mole) was used as a radioactive precursor. DNA having incorporated the labelled thymidine was precipitated with trichloroacetic acid at $0^{o}C$ and collected on maxflow membrane filter paper. The extracted material was counted by liquid scintillation counter (Packard Tricarb Liquid Scintillation Spectrometer, Model, 3380) using a toluene based liquid scintillation fluid (8 ml per vial) containing 4 g of PPO and 50 mg of POPOP per liter of toluene. The quenching correction was done for all the samples according to sample channel ratio (SCR) method (Packard Instruction Manual 2136) and counts per minute given by the counter were expressed in terms of disintegrations per minute with the help of standard efficiency-correlation curve. All the measurements were expressed in dpm per 3 hydras in all the samples (whole hydra or regenerate depending upon the type of experiment). Each vial was counted for two minutes and counting was done twice. Average cpm was taken for the calculation. All the

experiments were repeated three times and their average value was taken into account. In regeneration experiments both continuous and pulse labelling experiments were done.

DNA synthesis

DNA synthesis in normal, budding and regenerating hydras was studied by using radioactive precursor (^{3}H-thymidine). In all the experiments, the same concentration of ^{3}H-thymidine - 10 µCi/ml (^{3}H-thymidine, Activity 1 mCi and specific activity 15,200 mCi/m mole) was used.

The chase medium was prepared with unlabelled thymidine (5 mM) in fresh hydra medium and was kept at 5°C.

(i) DNA synthesis following feeding

Hydras without gonads and buds were selected and fed on freshly emerged artemia larvae (8-10 each). After five hours of ingestion when hydras had thrown out their remnants of undigested material they were thoroughly cleaned and transferred to big glass cavity blocks having hydra medium containing ^{3}H-thymidine (10 µCi/ml) in it. 30-40 hydras were put in each ml of radioactive medium. Hydras were squeezed and made to open their mouth with the help of a needle so that their enteron was flooded

with the radioactive medium. At regular intervals of 3 hr, 3 hydras were pipetted out with the help of a pasteur pipette and washed thoroughly with sufficient hydra medium in petridishes. The 3 hydras were transferred to the glass cavity blocks containing 5 ml of chase medium (medium with unlabelled thymidine with concentration 5 mM), kept for 10 minutes and again transferred to another cavity block with chase medium and kept for another 10 minutes. After chasing for 20 minutes hydras were washed and put in Elvehjem glass homogenizer. Hydra medium was completely taken out and the hydras were homogenized in one ml of 2% sodium lauryl sulfate. During homogenization approximately 50 µg of DNA was added as a carrier for DNA precipitation and then equal volume of 20% TCA was added to the homogenate and the whole homogenate was shaken well. The precipitation was done by keeping the homogenate having TCA and carrier at 0^{o}C for 10-12 hours. The precipitates were separated with the help of maxflow membrane filter and washed several times with chilled 5% TCA under oil suction pump. The filters were dried at 60^{o}C in an oven for 1 hour and kept in glass scintillation vials (Packard). The radioactivity in terms of counts per minute was measured by a Packard

Tricarb Liquid Scintillation Spectrometer using toluene based liquid scintillation fluid as mentioned earlier. The cpm was expressed in terms of dpm. The entire experiment was repeated three times from 6 to 45 hr of food ingestion.

(ii) DNA synthesis in different regions of the same hydra

DNA synthesis in different regions of hydra was studied at 18 and 27 hr of feeding(being the peak points in DNA synthesis profile of a normal hydra after feeding) by continuous labelling method. At the above mentioned peak hours, hydras were taken out of label, washed with sufficient hydra medium and transferred to chase medium for 20 minutes. Once again the hydras were washed with medium and cut into four fragments (Hypostome along with subhypostomal region, gastric region, budding region and peduncle along with the basal disc). 10 similar fragments of each region were taken together for homogenization. DNA precipitation and counting was done as detailed earlier for half of the homogenate and remaining half was used for protein estimation.

The protein estimation was done by the Lowry's method (1951). 5 ml of protein reagent (mixed 0.5 ml of

1% $CuSO_4 5H_2O$ and 0.5 ml of 2% COOK.CHOH.CHOH.COONa.$4H_2O$ in 50 ml of 2% Na_2CO_3 in 0.1N NaOH), was added to 0.1 ml of homogenate. After 10 minutes, 0.5 ml of F.C. reagent (Folin-Ciocalteu) was added. Colour intensity was read after 30 minutes at 660 nm. Bovine serum albumin was used as a standard protein. The DNA synthesis was expressed in terms of dpm/mg protein.

(iii) DNA synthesis during bud development

Continuous feeding resulted in bud formation. Hydras having buds at different stages as designated by (Sanyal, 1966) were selected from culture stock and were fixed in hot bouin's fixative for whole mount preparations. The stages fixed were stage I (fully grown hydra without any bud), stage II (hydra with small protrubrance), stage III (Hydra with well developed secondary axis) and stage IV (hydra with hydranth elements).

In order to study DNA synthesis during bud morphogenesis hydras having buds at stage II, when they had just made their appearance like a small conical protrubrance, were selected from the culture. Hydras were properly washed with medium and transferred to the big glass cavity blocks containing the radioactive medium. Hydras were sequeezed to ensure the filling of their

enteron with radioactive medium. At regular interval of 5 hr, 3 hydras were pipetted out and their DNA precipitation and counting was done as detailed earlier. The experiment was carried out for a period of 50 hr during which all the buds got detached. The DNA synthesis was expressed in terms of dpm/3 hydras.

(iv) DNA synthesis during regeneration

Hypostome regeneration : In order to study the hypostome regeneration, the hydras were amputated at subhypostomal level. All the amputations were done very carefully by taking hydras on the cavity slides so that the transverse cuts were made in extended form of hydra. Sharp and pointed sterlized needles were used to cut the hydras at a specific region with a single stroke. For morphological studies the amputated hydras were kept in small petridishes having fresh hydra medium and during its regeneration they were fixed at different stages of regeneration.

DNA synthesis during hypostome regeneration was studied both by the continuous and pulse labelling methods. In continuous labelling experiments the amputated hydras were put into radioactive medium immediately after cutting and at a regular interval of 3 hrs they were pipetted out and DNA precipitation and counting was done by method

mentioned earlier. In pulse labelling experiments, amputated hydras were kept in fresh hydra medium to let them regenerate in that only. Hydras were treated with labelled precursor for 1 hr at different specific times (e.g. 2nd-3rd, 5th-6th hour etc. upto 27th hour). DNA precipitation and counting was done by same method.

Basal disc regeneration : Hydras were amputated at basal disc level and during its regeneration different stages were fixed in hot Bouin's fixative for whole mount preparations.

DNA synthesis during basal disc regeneration was studied by continuous and pulse labelling methods. In the former, hydras were kept in radioactive medium immediately after cutting and its DNA precipitation was done after every 3 hrs from the moment of amputation upto 21 hours. In the later, hydras were treated with radioactive medium at specific times for one hour and DNA precipitation and counting was done by the above mentioned method.

Whole mounts and histological preparations

The hydras were fixed in hot bouin's fixative for 3-4 hr, washed with 50% and 70% ethanol successively for 5 minutes each and then put in saturated solution of

lithium carbonate in 70% ethanol to remove excess of fixative. The hydras were stained with borax carmine, dehydrated with ethanol, cleared in xylene and mounted in DPX.

For histological studies, the hydras were fixed in hot Bouin's fixative and impregnated with paraffin wax (58-60°C) after complete dehydration. The sections were cut with rotary microtome. The sections were deparaffinized with xylene, stained with Ehrlich's haematoxylin and mounted in DPX. Photomicrographs were taken under high power to elucidate the details.

Isotopes and Chemicals

The radioactive precursor thymidine (specific activity 15200 mCi/m mole) was procured from Bhabha Atomic Research Centre, Bombay. PPO, POPOP, thymidine and DNA were obtained from Sigma Chemical Company, MO., U.S.A. The maxflow membrane filters were obtained from Maxflow, Bombay, India.

EXPERIMENTAL RESULTS

Feeding behaviour

Before the study of the profile of DNA synthesis during the different aspects of the life's economy of hydra, some additional data have been provided first about the mechanism of food capturing, manner by which the food is engulfed and about the active participation of cells of the body wall in materialising the digestion of the food substances within its body system.

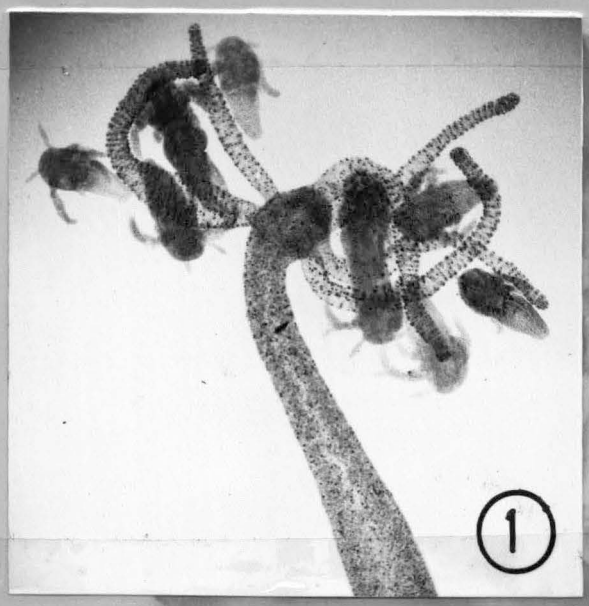
Hydras showed striking feeding behaviour when studied under a stereobinocular. The process of feeding reaction seemed to be dependent upon physiological state of the organism which was conditioned by the time lapse of the last feeding. The highly starved hydras for two days showed immediate reaction to their prey by ushering their tentacles; moderately hungry specimens for one day showed comparatively less tentacular manoeuvres and the well fed one did not at all respond to the food with the tentacles.

The feeding activities in hydra were composed of a series of complex behavioural sequences. When the artemia larvae were brought to the hydras, they struck to them, usually on the outstretched tentacles and became

there hooked by nematocyst discharge (Fig. 1). The portion of tentacle proximal to the prey then contracted, often spirally inward giving a curvature to hold the prey well as seen in Fig. 2. Further retraction of the curved tentacle mechanically brought the prey closer to the hypostome. The time of tentacle movement was very short which started within seconds of attachment of the prey. As the prey came near the mouth, all the tentacles bent towards the oral direction concertedly. This resulted in adjacent tantacles to come in closer contact with prey. The final process of engulfing the artemia larvae was mechanised by literally pushing them into the enteron through the hypostome by using the tips of the tentacles. This tucking in of the food by the tentacle tips was seen to be repeated several times even after all the prey were totally engulfed. The later stage of feeding behaviour in hydra showed mouth creeping around the prey and closing about it. It slowly stretched itself over the food. During this process of encroachment often parts of tentacles were also seen to be swallowed. Once the food was swallowed neither the tentacles showed any new response to fresh artemia nor the mouth showed any creeping movement. Throughout this later stage, the contraction of tentacles and body column were inhibited.

Fig. 1 : Number of artemia larvae being hooked by tentacles of 24 hrs starved hydra by nematocyst discharge X 110.

Fig. 2 : Retraction of tentacles to bring the hooked larvae closer to mouth. Note the tentacular curvatures to hold firmly the prey X 355.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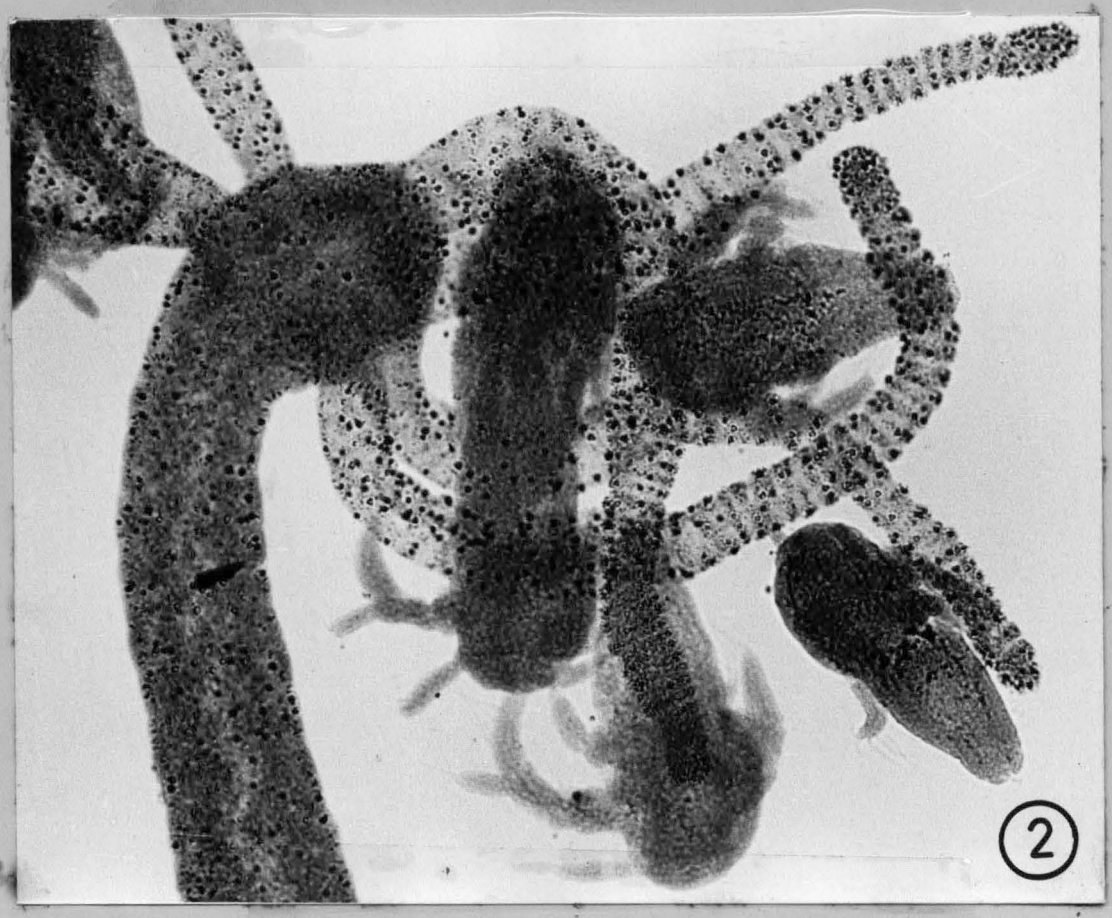
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After the ingestion, the food was accumulated in the enteron. Its accumulation made the gastric region swell out like a balloon (Fig. 4) which could easily be compared with that of starved hydra (Fig. 3) where the entire body presented a cylindrical appearance. Shortly after this accumulation, the food was dissolved and distributed evenly throughout the enteron of hydra (Fig. 5). Food underwent liquefaction within next couple of minutes and after around five hours of ingestion the hydra started throwing out a viscous liquid material at regular intervals by repeated contractions of the body. Undigested food in the form of a small ball was made to roll up towards anterior end of the enteron again by repeated contractions of the body column till that was also ejected out of the body. The contraction exerted pressure on the excreta which had to make its way out through mouth. The mouth remained open for couple of seconds after the undigested food was excreted. The hydra assumed its normal shape within next few minutes (Fig. 6).

Cellular behaviour during digestion

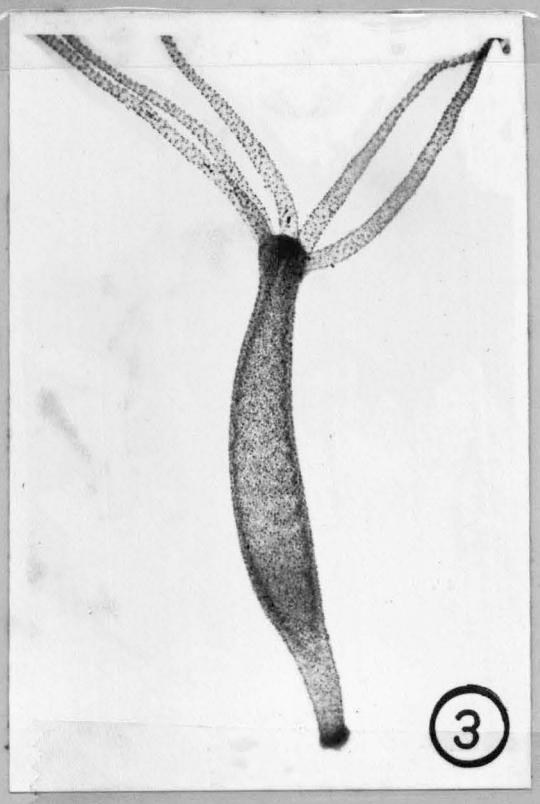
Besides the gross morphological responses of a hydra towards capturing of the prey, the internal cell

Fig. 3 : A normal 24 hrs starved hydra. Note the typical cylindrical gastric region X 110.

Fig. 4 : The same hydra after ingestion of food seen after 20 minutes. Note the inflated gastric region X 110.

Fig. 5 : The same hydra after 40 minutes of ingestion of food showing even distribution of food material after its final liquefaction X 110.

Fig. 6 : The same hydra after 5 hrs of ingestion of food (after excretion). Note the assumption of its normal cylindrical appearance X 110.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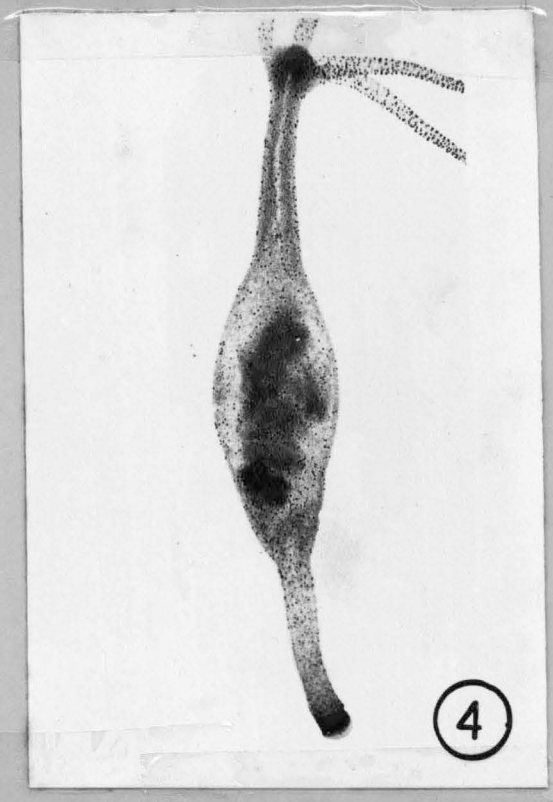
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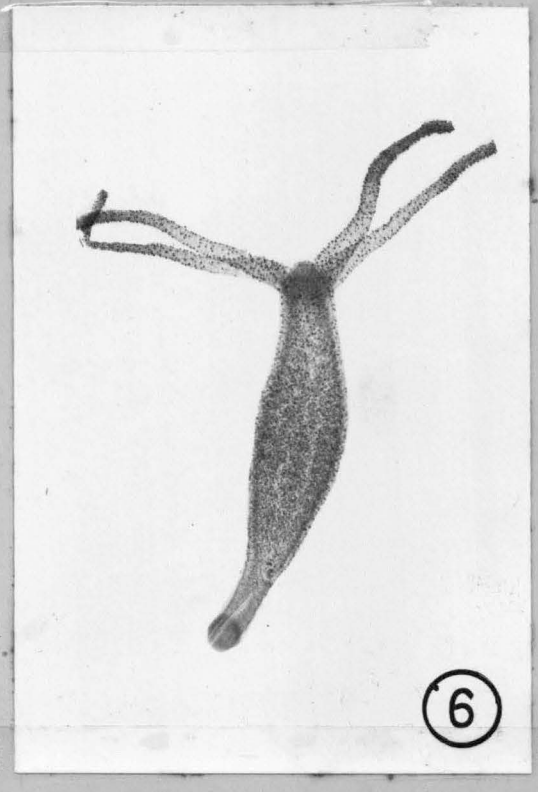
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layers during the process of digestion also presented remarkable behaviour pattern. Histological sections revealed a good of hitherto unknown properties of cells during the different phases of digestion. The entire process of digestion took 4-5 hours after which the remnants of artemia were regurgitated.

After 1 hr of ingestion

Fig. 7 depicts in L.S. the entire hydra after it had engulfed artemia. One could easily identify about 8 broken body pieces of artemia inside the enteron. The most interesting feature was the presence of a number of pieces of tenacles flanked by the artemia tissues, which were undergoing a rapid disintegration. Closely knitted cellular matrix of the engulfed pieces of tentacles also presented evidence of undergoing a process of dissolution; thereby letting loose the nematoblasts as loose single cells. Close inspection of the section revealed that some discrete nematoblasts were invading the artemia body and actively participating in extra-cellular digestion by adopting the process of phagocytosis. Still more interesting was the fact that both ectoderm and endoderm of hydra were contributing to the process of digestion. The endoderm throughout its circumference of the enteron showed that the regular normal layering

of the tissue of the hydra had been disrupted. Many endodermal cells had left the body wall by freeing themselves from cell contact and they were entering as free cells into enteron (Fig. 11). Hundreds of cells, throughout the space of the enteron, showed an active participation in digestion; these endodermal body wall cells entered the enteron enmasse and actively involved in phagocytosis by harbouring on the disrupted tissues of artemia. It was difficult to identify the normal endodermal characteristics of the body wall of the hydra at this stage and the relative position of different kinds of cells there in. Another striking phenomenon was both nematoblasts and interstitial cells, belonging to ectoderm were not perceptible. Mesoglea in between the ectoderm and endoderm which imposed a barrier between them had become imperceptible; thereby rendering a high degree of cell to cell migration.

After 2 hr of ingestion

After 2 hr of intake of artemia, the further changes observed in cell layers of hydra had been depicted in a transverse section (Figs. 8 and 12). One could note easily that both from the ectoderm and endoderm large number of cells had left their original positions in body

wall and had migfated in the enteron to participate in the process of digestion (Fig. 8). Infact, due to the disappearance of cells from the body wall, the transverse section showed many spaces in both layers particularly in ectoderm (Fig. 12). The cells which left endoderm showed a tendency of dedifferentiation. It was difficult to identify the typical cell type during this phase in the enteron particularly. The ingested artemia had been pulverised and were undergoing liquefaction. The net result of these catalytic processes was the conversion of artemia body into semifluid state. It was very impressive to see that large number of endodermal cells as single entities were actively participating in the process of digestion.

After 3 hr of ingestion

The two body layers of hydra which were protecting the digestive process continued to present a kind of disorganised texture, without presenting the typical arrangement of cells (Fig. 9). The mesoglea in between them was imperfect and at places completely disappeared. Both the layers appeared rather atypical without showing any characteristic normal histological pattern (Figs. 9 and 13). The absence of nematoblasts and interstitial cells was remarkable feature. After 3 hr of ingestion of artemia, the enteron was characterized by presence of hundreds of

loose endodermal cells, as shown in Fig. 13, actively feeding on the artemia material which had by now lost its cellular structure and had become solute. It gave the impression that the discrete endodermal cells while actively feeding by phagocytosis were undergoing divisions and increasing their numbers.

After 4 hr of ingestion

By 4 hr the digestion seemed to be complete, the artemia material after undergoing a process of hydrolysis made it difficult to observe inside the enteron. However, the behaviour of participating cells was found to be remarkable. A definite tendency of discrete loose hydra cells so far participating as free cells in the enteron now showed a tendency of rejoining the endodermal layer of the body wall. During the process of rejoining of the free cells with the remaining cells of the endoderm, sometimes they assumed crescent shape at the point of joining with endodermal cells. There were several stages of rejoining the cells from enteron back to endodermal layer. This in its turn had an effect of giving impression of restructuration of loose endoderm into a distinct characteristic matrix. At this stage redifferentiation could be observed for the first time. Repacking of cells was not only involving the endoderm but also eventually

the ectoderm which was also returning to its characteristic pattern. The mesoglea became quite delineated.

After 5 hr of ingestion

Rejoining of the endodermal cells into the endodermal layer could be well shown in T.S. (Figs. 10 and 14). Section showed a number of interesting points. A process of redifferentiation had set in. Differentiation of interstitial cells and ultimately their culmination to nematoblast could be seen. However, the layers had yet not fully reorganised. There were many empty spaces which were to be repacked by reentry of cells into body wall within couple of hours.

After 7 hr of ingestion

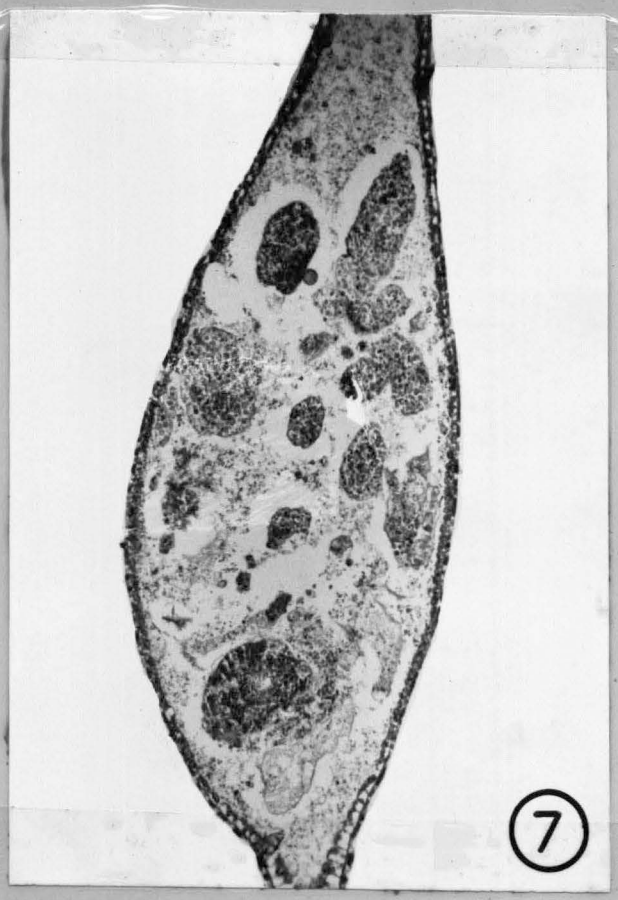
Fig. 15 depicted in L.S., the restructuration of body layers of hydra which had just completed the process of digestion. The ectoderm now consisted of nematoblasts, interstitial and other types of cells. The endodermal cells had regrouped into its own characteristic pattern. Reappearance of discrete cell types including gland cells could be seen. Still there were a few empty spaces left and the rejoining of cells continued. The mesoglea had been well characterized. The most important point was that during the initial phase of digestion, the body wall not

Fig. 7 : Photomicrograph of feeding hydra in L. S. after 1 hr. Note the broken pieces of artemia and portions of tentacles inside the enteron X 355.

Fig. 8 : Photomicrograph of hydra in T.S. after 2 hrs of feeding. Note the presence of number of free cells inside the enteron together with the artemia pieces X 900.

Fig. 9 : Photomicrograph of hydra in T.S. after 3 hrs of feeding. Note the disorganised texture of both ectodermal and endodermal layers of body wall, advanced stage X 900.

Fig.10 : Photomicrograph of hydra after 5 hrs in T.S. Note the absence of food material indicating completion of digestion and rejoining of free cells with endodermal layer X 900.



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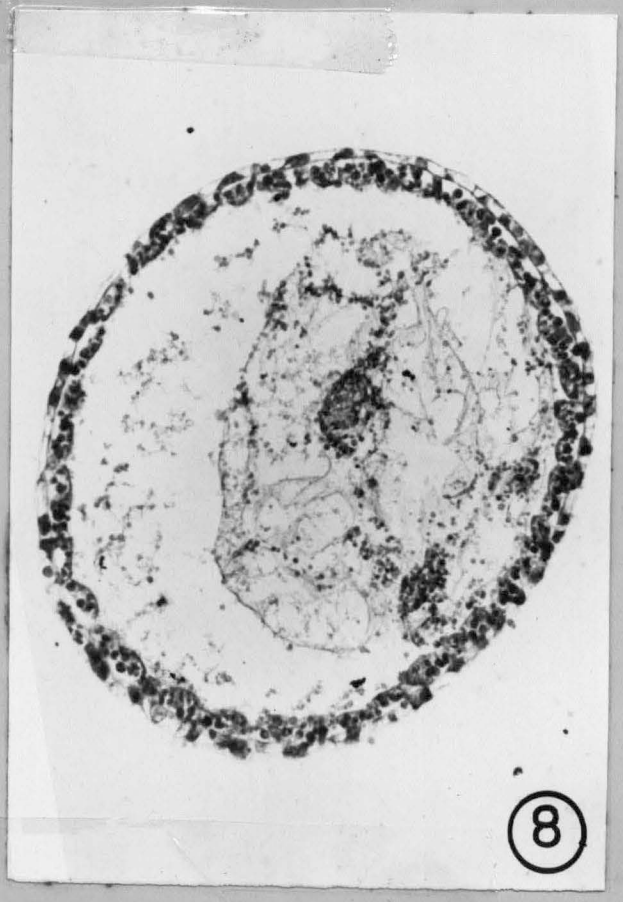

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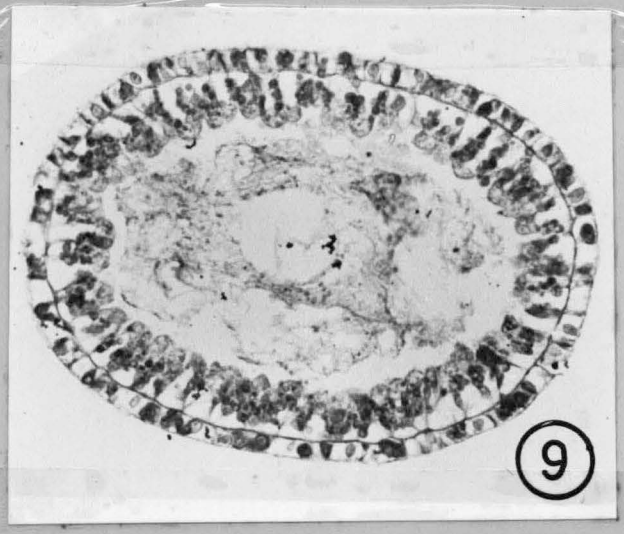

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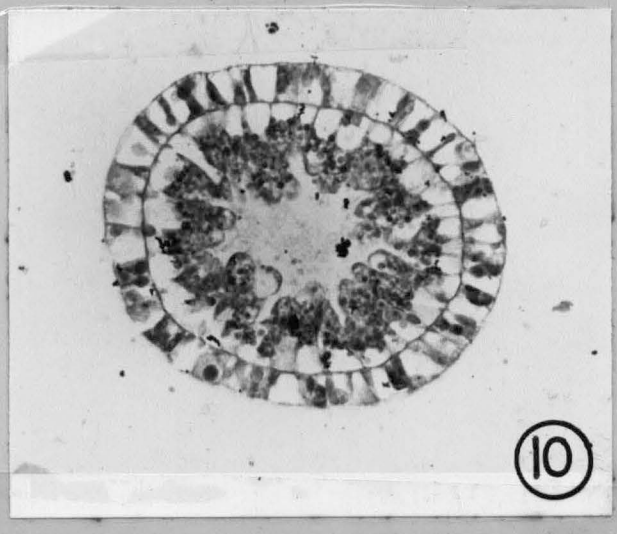

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High powered view of cellular activity during digestion:

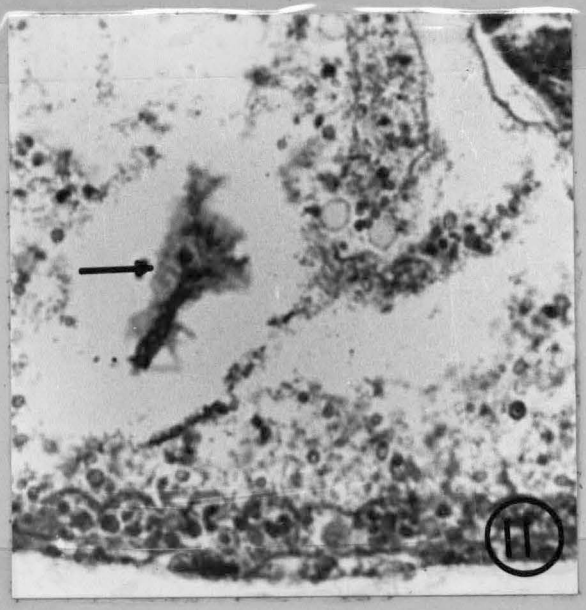
Fig.11 : Photomicrograph of hydra in L.S. after 1 hr of feeding. Arrow indicates the tissue free cells in the enteron X 2250.

Fig.12 : Photomicrograph of hydra in T.S. after 2 hrs of feeding. Note the presence of spaces both in ectoderm and endoderm and also the wondering phagocytic cells in the enteron X 2250.

Fig.13 : Photomicrograph of hydra in T.S. after 3 hrs of feeding. Arrows indicate the presence of large number of tissue free cells in the enteron and loosely arranged layers of body wall X 2250.

Fig.14 : Photomicrograph of hydra in T.S. after 5 hrs of feeding. Arrow indicates the rejoining of free cells with the endodermal layer X 2250.

Fig.15 : Photomicrograph of hydra in L.S. after 7 hrs of feeding. Note the reorganisation of both the body layers. Arrows indicate free cells rejoining the endodermal layer X 900.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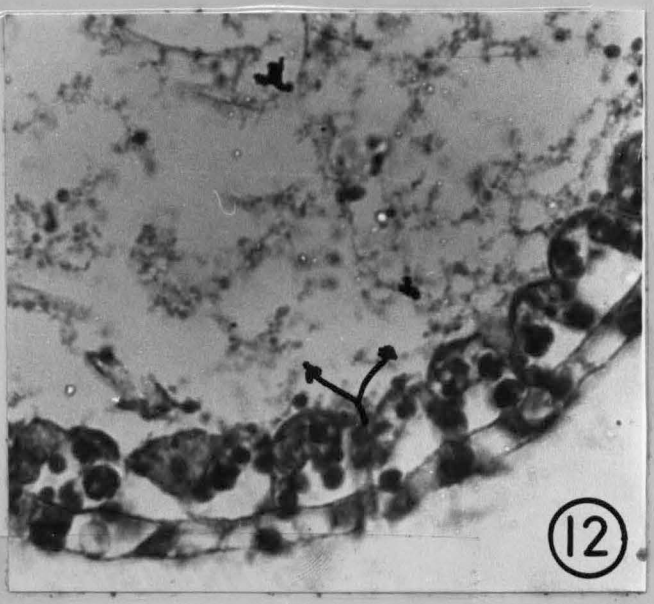
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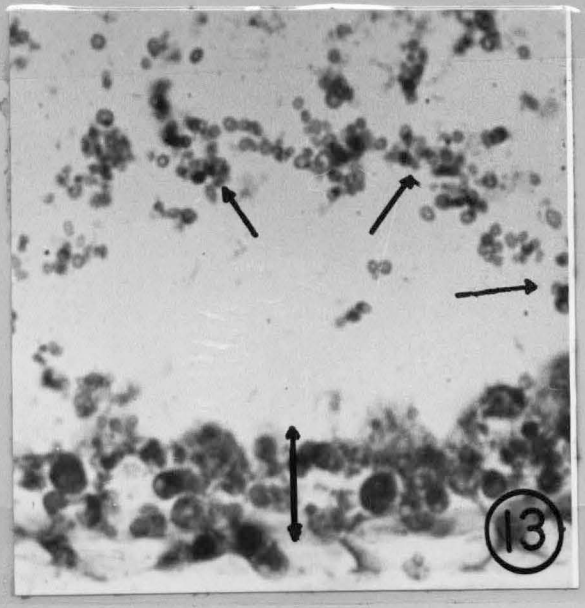
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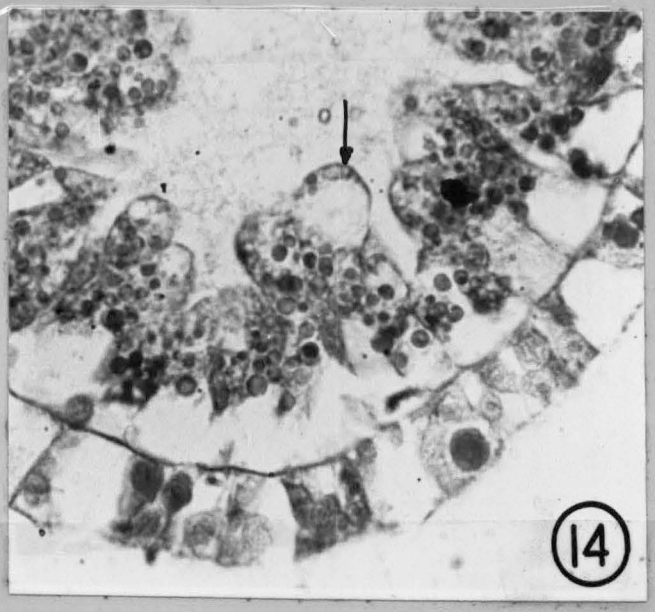
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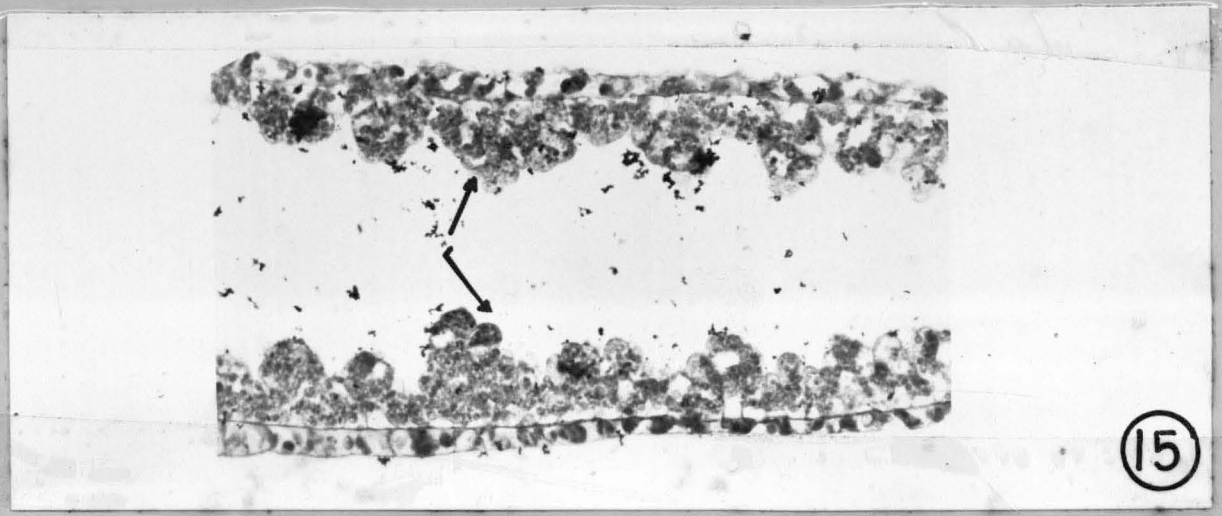
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only lost their cells but also underwent somekind of dedifferentiation which after completion of the digestion ones again became differentiated and rejoined with large number of wandering endodermal cells.

DNA synthesis following feeding

Having described the food capturing mechanism, the consequential DNA synthesis was studied by radioactive precursor (^{3}H-thymidine) incorporation. A graphical representation of ^{3}H-thymidine incorporation into newly synthesized DNA after feeding in normal hydra clearly revealed the positive response for synthesis. The synthetic pattern of DNA was followed upto 45 hr of ingestion (Fig. 16). The overall DNA profile revealed three synthetic peaks at 12, 18 and 27 hr respectively. DNA synthesis started right in the beginning with the result that even at 6 hr appreciable synthesis was found, while there was a significant increase in DNA synthesis from 6 to 12 hrs, a sudden decrease in its synthesis was recorded from 12 to 15 hrs. Similarly an increase in incorporation of radioactive precursor was found between 15 to 18 hours and 24 to 27 hours. However, the former burst was followed by an immediate fall in incorporation while the latter was followed by a plateau showing no change in incorporation. DNA synthesis decreased till

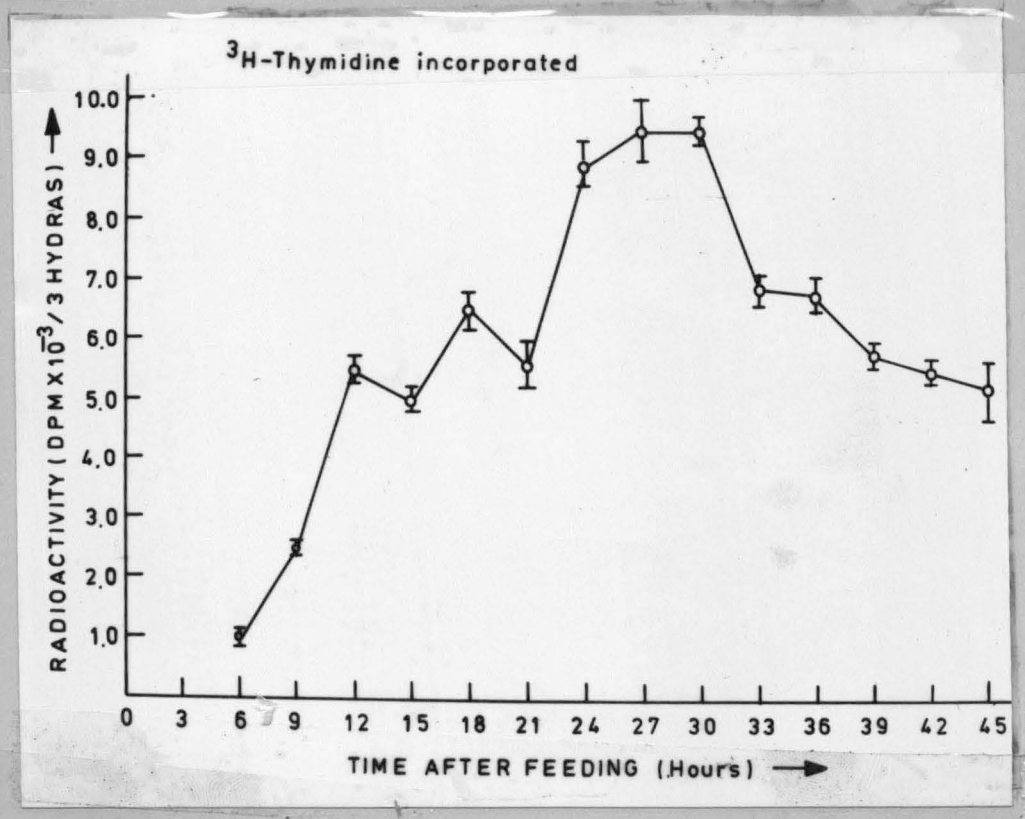


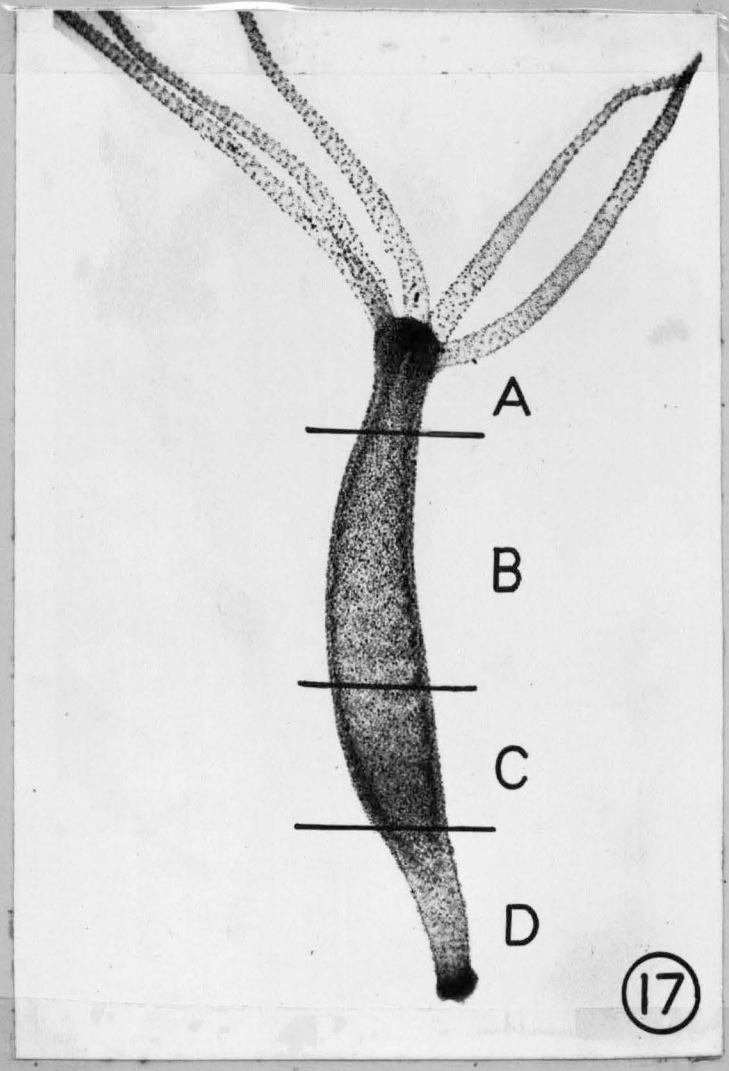
Fig.16 : Incorporation of ^{3}H-thymidine (10 µCi/ml, sp.act. 15,200 mCi/mM) into TCA precipitable DNA in normal non-regenerating hydras following feeding (continuous labelling). Note the three peaks of DNA synthesis, 1st at 12 hr, 2nd at 18 hr and 3rd, the major one, at 27 hr.

45 hrs following this plateau, but the decrease was not gradual. Between 30 to 33 hrs the decrease is quite fast but between 33 to 36 hrs there was no significant change and this was followed by a gradual decrease till 45 hrs. The peaks at 12 to 18 hrs were followed by similar decreasing pattern of incorporation whereas the third peak at 27 hr showed a totally different decreasing pattern. The incorporation at 27 and 30 hr was maximum and was more than 9 times the value at the initial phase.

DNA synthesis in different regions of same hydra

In the last chapter, the total profile of DNA in situ hydra has been shown. In this section an analysis of DNA synthesis at different levels of the body of the same hydra had been taken up as per in four segments as described in experimental procedure. DNA synthesis in different regions of same hydra was studied at 18 and 27 hr of feeding (the two peaks in DNA profile). Four segments (A, B, C and D) were analysed as shown in Fig. 17. The DNA synthesis at both peaks has been depicted by histograms (Fig. 18). It was clearly shown by histograms that DNA synthesis in all four segments was of decreasing order (A > B > C > D) with the anterior most segment (A) having the maximum DNA synthesis and in turn the posterior most had the minimum as compared to one another. Exactly the same pattern was followed at both

Fig.17 : A normal hydra depicting the sites at which the cuts were made to make four fragments of the same hydra. A, B, C and D fragments stand for hypostome along with subhypostomal region, gastric region, budding region and peduncle along with the basal disc respectively. These fragments were used for making ^{3}H-thymidine incorporation studies along the body axis.


A
B
C
D
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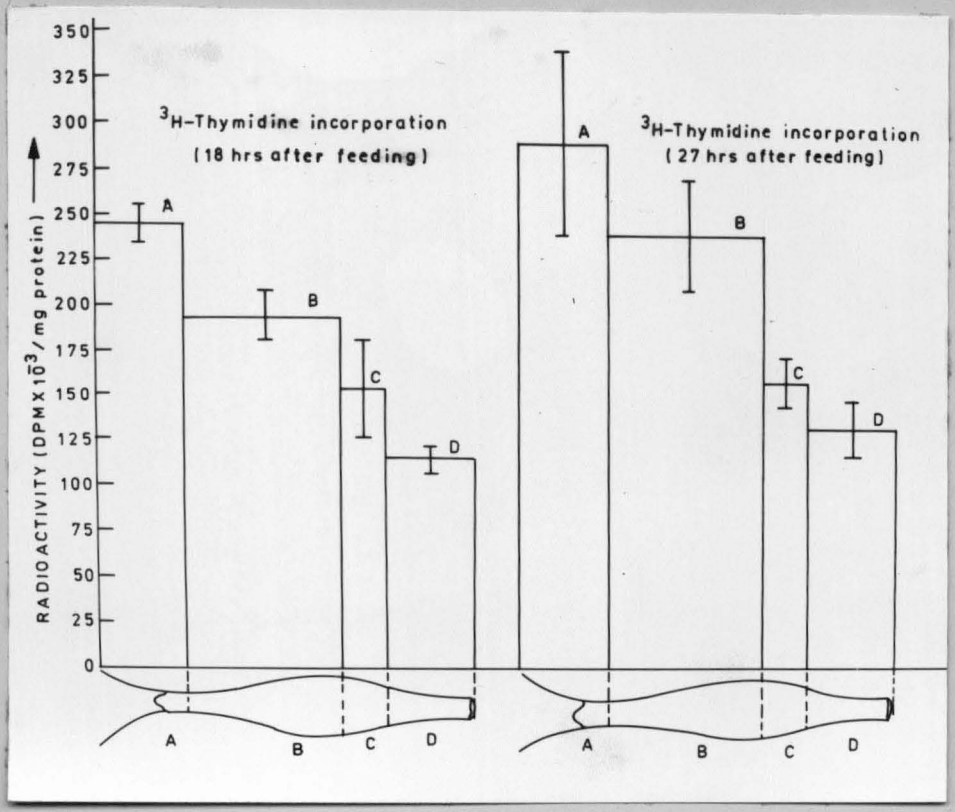


Fig.18 : Histograms showing incorporation of ^{3}H-thymidine (10 µCi/ml, sp.act. 15,200 mCi/mM) into TCA precipitable DNA in different regions of the same hydra at 18 hr and 27 hr of feeding (continuous labelling). Letters A, B, C and D represent hypostome along with subhypostomal area, gastric region, budding region and peduncle along with the basal disc respectively. Note the four fragements of same hydra both at 18 hr and 27 hr of feeding show incorporation of radioactive precursor in decreasing order (A > B > C > D) representing a kind of gradient.

peak hours. It was also revealed that the DNA synthesis in any of the segments at 27 hr was much higher than that of corresponding segment at 18 hr.

DNA synthesis during bud development

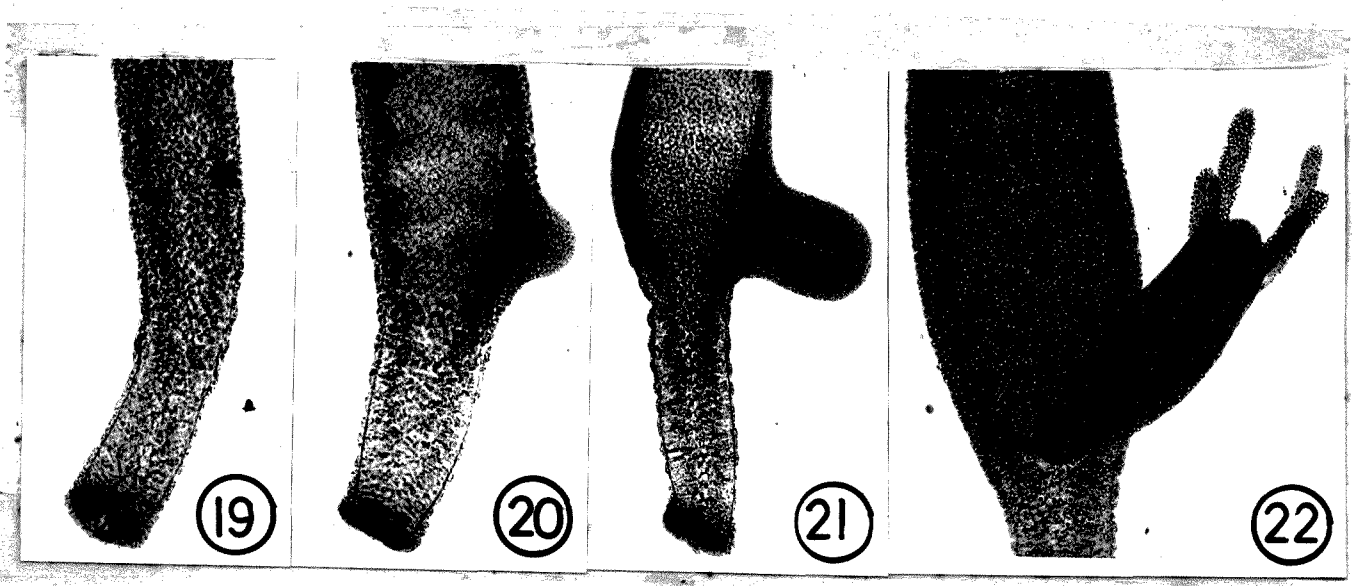
Feeding leads to bud production and in this section, the DNA synthesis at different stages of bud development had been studied. When the fully grown hydras were fed on artemia they started showing buds within 24 hr of feeding. The bud development was studied right from its emergence as a small conical protrubrance till it got detached from the mother. Since there was no obvious demarcation to be identified as the origin of bud and rate of development happened to be affected by individual variation, the different stages with morphological differences as designated by Sanyal (1966) were studied for entire bud development. Fig. 19 depicted the first stage having fully grown individual without any sign of bud formation. The bud appeared as a minute conical projection from the budding region of mother hydra which slowly started growing to become conspicuous as stage II (Fig. 20). The hydras in stage II were selected to study the entire development of the bud at morphological and molecular levels. Within next 5-10 hrs bud started growing as a

secondary axis perpendicular to mother axis (Fig.21). After around 20 hrs the secondary axis started showing signs of hypostome and tentacles development which in turn continued to grow to form fully developed bud (Fig. 22). The buds got detached after around 50 hrs.

The continuous incorporation studies with ^{3}H-thymidine in budding hydra revealed clearly that the process of bud development involved DNA synthesis (Fig. 23). The synthetic pattern was followed upto 50 hrs, the period during which the buds got detached. The spectrum analysis revealed that there was only one peak at 15 hr. DNA synthesis started right in the beginning and increased very fast during the first 15 hrs. The incorporation at 15 hr was maximum and about three times the amount incorporated at 1 hr. It was followed by a very gradual decrease till 30 hr. While there was no appreciable change in synthetic pattern from 30 to 35 hrs a sudden fall in DNA synthesis was recorded from 35 to 50 hrs.

DNA synthesis during regeneration

Hypostome regeneration : The successive changes in a hypostome regenerate from its initial amputation to its ultimate transformation into a normal hydra were followed



Different stages of Bud Development:

Fig.19 : Note the posterior region of a fully grown hydra showing the budding region X 275.

Fig.20 : Note the appearance of bud as a small conical protrubrance X 275.

Fig.21 : Note the elongation of bud as a secondary axis almost perpandicular to mother axis X 275.

Fig.22 : Note the fully grown bud still attached to mother hydra X 275.

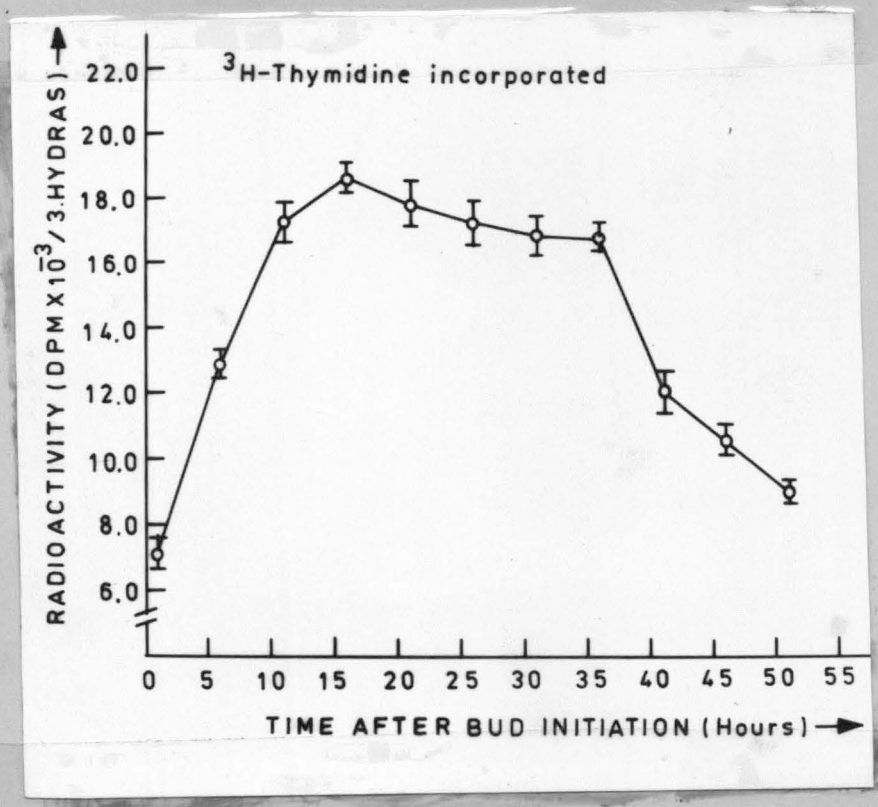


Fig.23 : Incorporation of ^{3}H-thymidine (10 µCi/ml, sp.act. 15,200 mCi/mM) into TCA precipitable DNA in a budding hydra (continuous labelling). Note the sharp increase in incorporation of radioactive precursor during first 15 hrs of bud development followed by a gradual decrease in its incorporation.

closely (Figs. 24-29). Fig. 24 depicted the site at which the cut was made. The cut end became swollen immediately after amputation (Fig. 25). The swelling reduced within first few hours and the anterior end started showing slight elongation till at 15 hr it transformed into hypostome (Fig. 26). Within next 3-4 hrs the rudiments of tentacles made their appearance (Fig. 27) which with the passage of time started showing their elongation (Fig. 28). The elongation continued for next couple of hours till it acquired the typical shape of fully developed hypostome (Fig. 29).

The continuous labelling studies with ^{3}H-thymidine in hypostome regenerating hydras showed that the incorporation of radioactive precursor into DNA began immediately after the amputations were made (Fig. 30). The incorporation increased quite fast during 3 to 9 hrs of regeneration where it gave a peak. The incorporation at this stage was found to be double the incorporation at 3 hr. It was followed by a decrease in synthesis for next 6 hrs. This decrease was very gradual as compared to increase before the peak which was quite fast. This slow down reached a minimum at 15 hr of regeneration, after which the synthesis once again increased rapidly giving another peak at 18 hr. The magnitude of this peak was same as that of the peak at

Fig.24 : A normal 24 hrs starved hydra depicting the site of amputation to study hypostome regeneration X 100.

Different stages of Hypostome Regeneration:

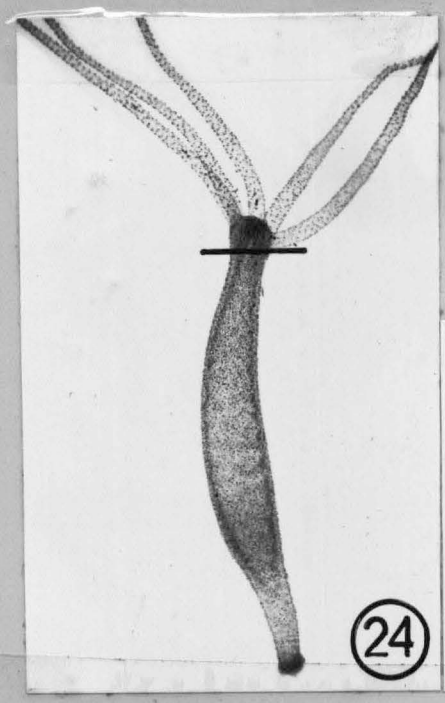
Fig.25 : Anterior region of hydra immediately after amputation. Note the swollen anterior end after wound healing X 275.

Fig.26 : Early differentiation of hypostome formation X 275.

Fig.27 : The appearance of tentacle rudiments X 275.

Fig.28 : The elongation of tentacles X 275.

Fig.29 : Hypostome of the control hydra X 315.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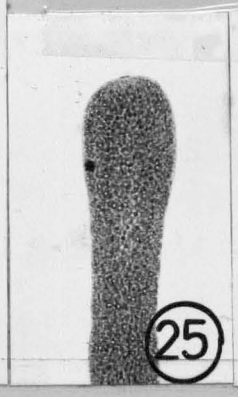
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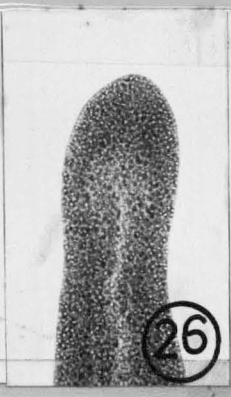
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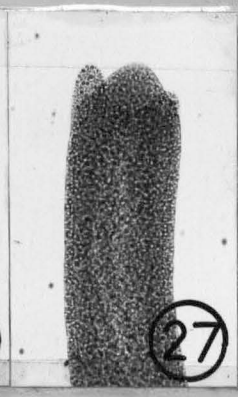
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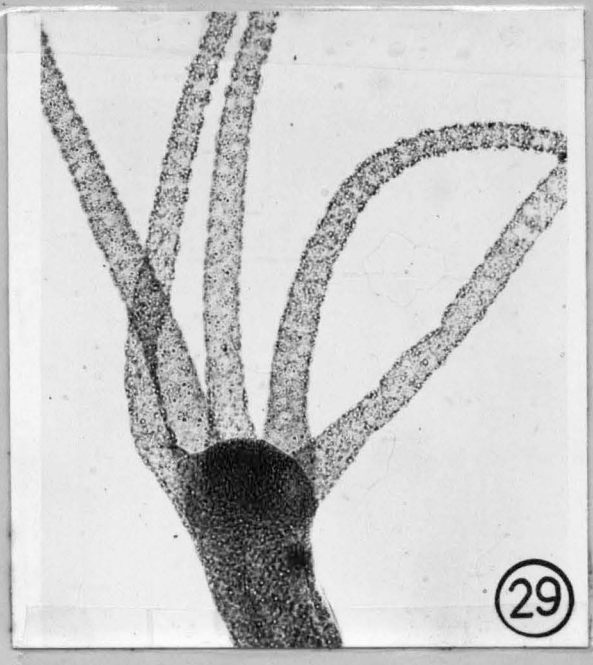
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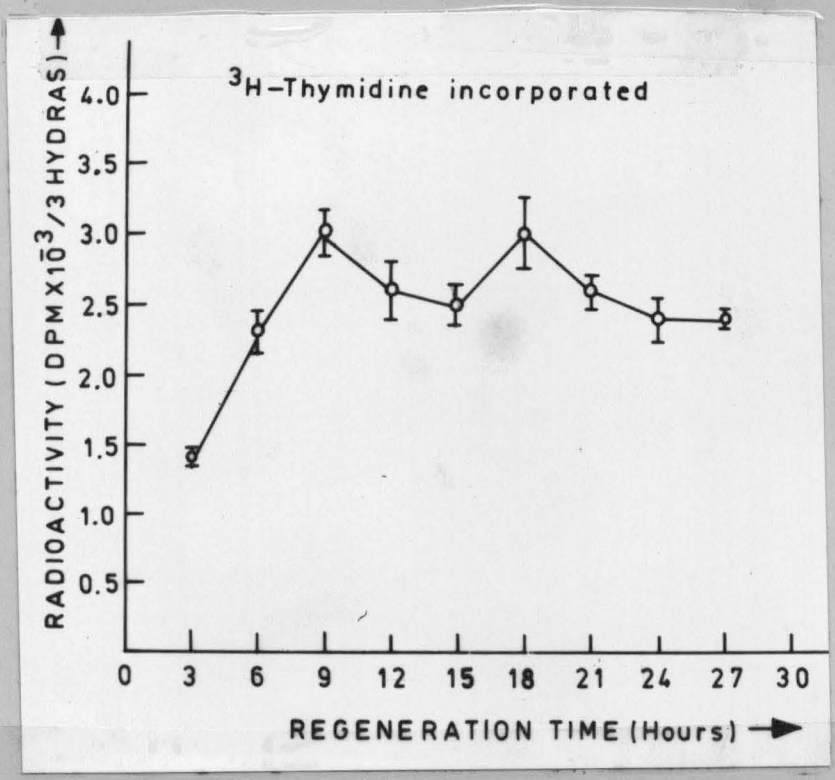

Fig.30 : Time dependent changes in the extent of continuous incorporation of ^{3}H-thymidine (10 µCi/ml, sp.act. 15,200 mCi/mM) into TCA precipitable DNA of hypostome regenerating hydras. Note the appearance of 2 peaks of DNA synthesis at 9 hr and 18 hr of regeneration.

9 hr. The second peak was also followed by a gradual drop in synthesis for next six hrs. After 24 hr a plateau followed showing no change in DNA synthesis. Both the peaks at 9 and 18 hr of regeneration were followed by similar type of decrease in incorporation.

The pulse labelling studies with one hour of labelling done during specific hour of regeneration mainly reflected the capacity of system to synthesize DNA during corresponding periods. Pulse labelling studies during hypostome regeneration gave a similar DNA profile to that given by continuous labelling method (Fig. 31). The profile showed that DNA synthesis began after the amputation was done. Increasing rates of DNA synthesis were found at 3, 6 and 9 hr of regeneration. At 9 hr the incorporation was maximum as given by the radioactivity obtained during this hour. Rate of incorporation at 12 and 15 hr was less as compared to that at 9 hr but it was not very different from each other. Another peak of high synthesis observed at 18 hr having rate of synthesis comparable to that at 9 hr. Following this peak the incorporation went down to the level comparable to the one at 3 hr.

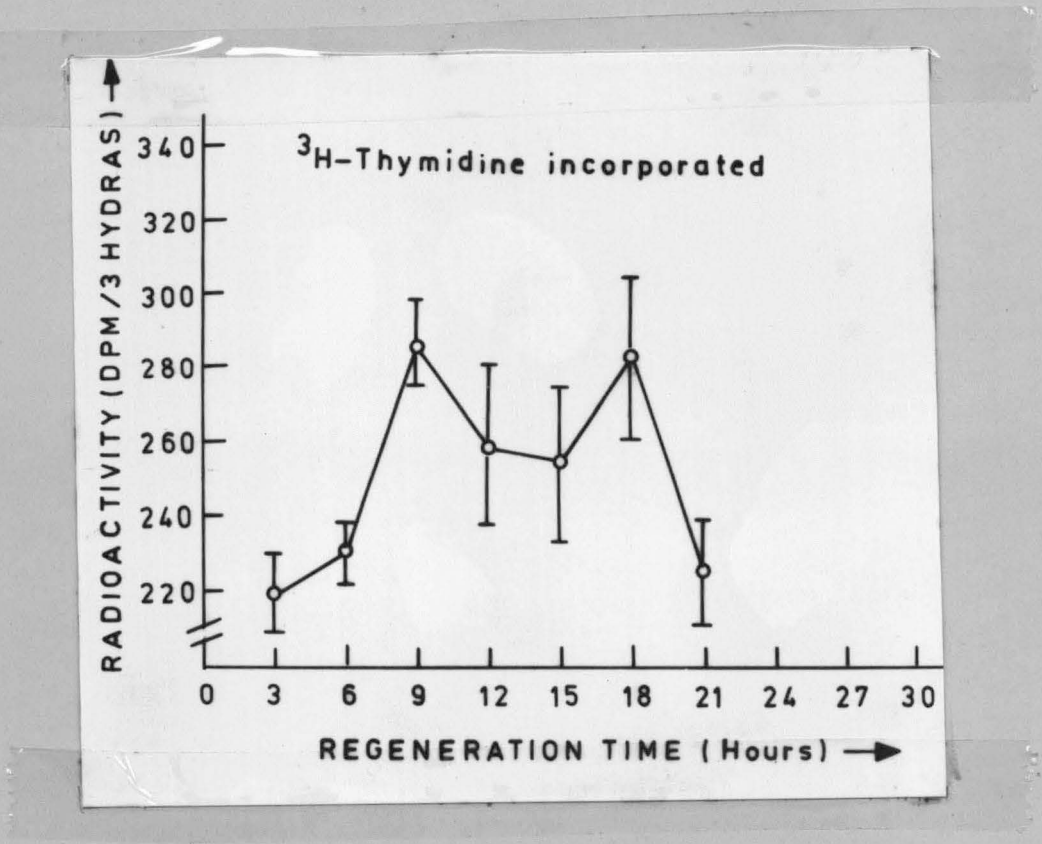

Fig.31 : Time dependent changes in the extent of hourly pulse incorporation of ^{3}H-thymidine (10 µCi/ml, sp.act. 15,200 mCi/mM) into TCA precipitable DNA of hypostome regenerating hydra. Note the appearance of two peaks of incorporation of radioactive precursor at 9 hr and 18 hr of regeneration.

Basal disc regeneration : Hydras amputated at basal disc region took around 16-17 hrs to regenerate. The different stages of regeneration were fixed to study its morphological changes (Figs. 32-36). Fig. 32 depicted the site at which the cut was made. The cut end of hydra became rounded immediately after amputation (Fig. 33) and it was not showing any sticking property. After 15 hrs of amputation the regenerating basal disc started showing basic characteristics of basal disc, like it was having dense population of cell at basal disc area (Fig. 34), and at the same time started showing sticking properties. The basal disc at this state was not developed enough to hold the hydra firmly. Between 16-17 hrs of regeneration the basal disc acquired all the characteristics (Fig. 35) that a normal basal disc shows (Fig. 36). It could easily hold the body firmly.

Incorporation of radioactive precursor in continuous labelling experiments for basal disc regeneration showed that the synthesis started right from the beginning. The synthesis was followed upto 27 hr of regeneration. The overall profile showed two peaks one at 9 and other at 15 hr of regeneration (Fig. 37). The incorporation of radioactive precursor increased quite fast during first 9 hrs of regeneration following which it started decreasing for the next 3 hrs. Another increase in DNA synthesis was observed during 12-15 hrs of regeneration to give a second peak at 15 hr

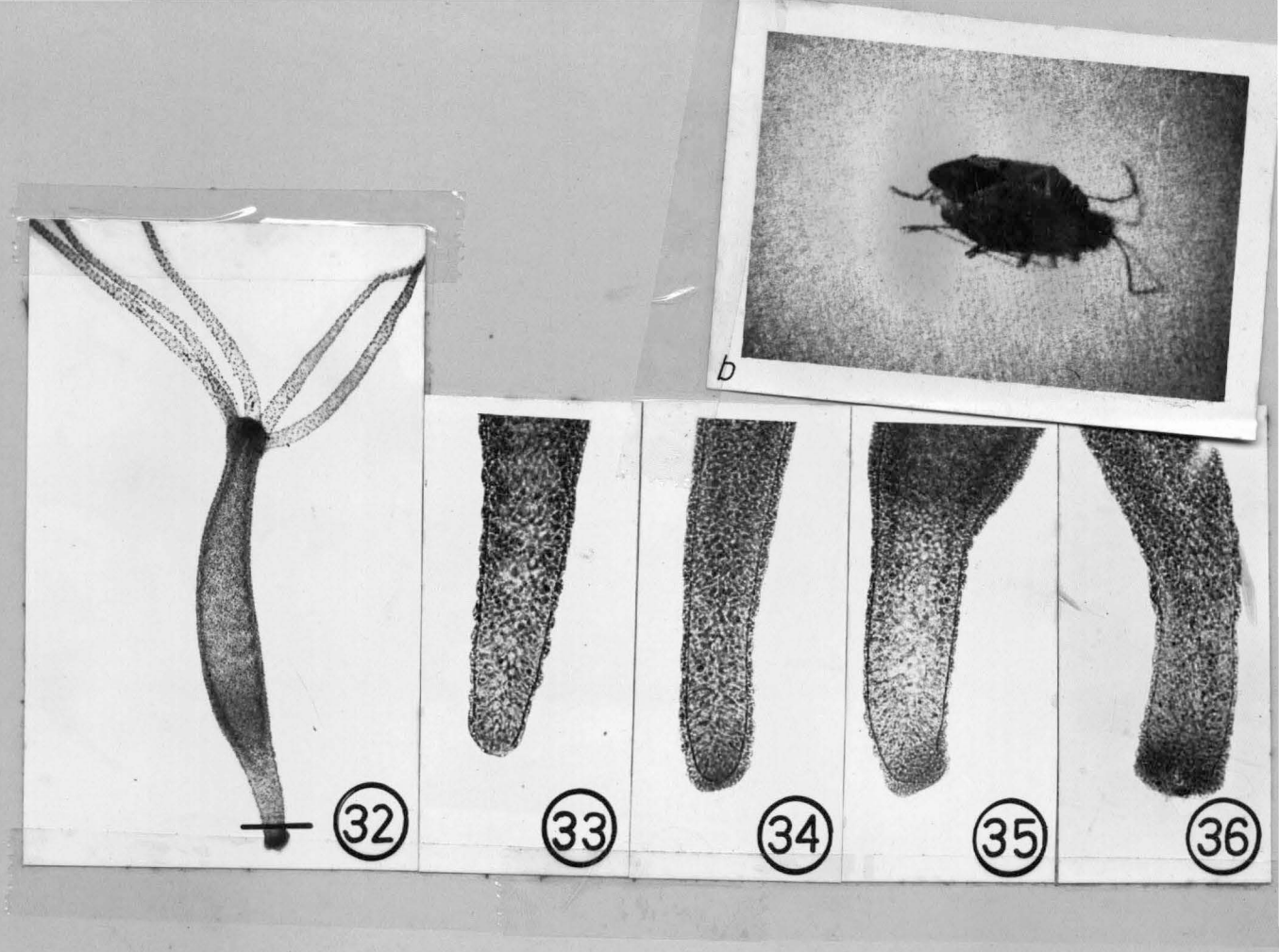

Fig.32 : A normal 24 hrs starved hydra depicting the site of amputation for the basal disc regeneration X 100.

Different stages of the Basal Disc Regeneration:

Fig.33 : The healing of cut end immediately after the amputation X 275.

Fig.34 : The accumulation of large number of cells at the amputated end X 275.

Fig.35 : Fully restituted basal disc X 275.

Fig.36 : Basal disc of the control hydra X 275.

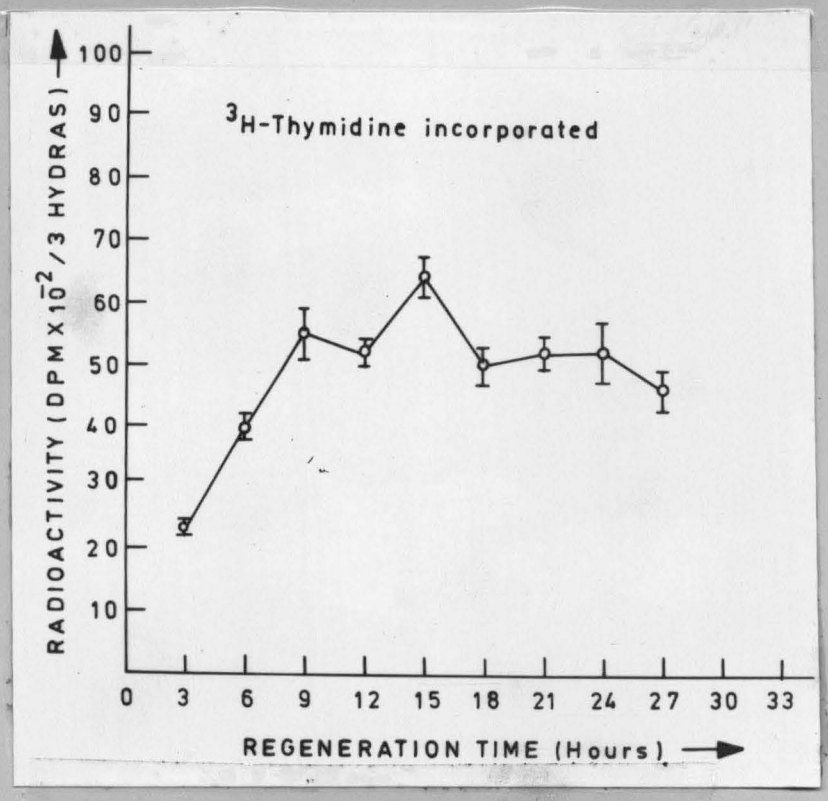

Fig.37 : Time dependent changes in the extent of continuous incorporation of ^{3}H-thymidine (10 µCi/ml, sp.act. 15,200 mCi/mM) into TCA precipitable DNA of basal disc regenerating hydras. Note the appearance of 2 peaks of DNA synthesis at 9 hr and 15 hr of regeneration

which was followed by a decrease for the next 3 hrs. The DNA synthesis did not change appreciably during 18 to 27 hrs of regeneration. The peak at 15 hr showed the synthesis 3 times the synthesis at 3 hr of regeneration.

The DNA synthesis during basal disc regeneration in pulse labelled hydras showed a profile having peak points corresponding to that obtained by continuous labelling experiments i.e. at 9 and 15 hr of regeneration (Fig. 38). The DNA synthesis increased progressively till 9 hr after which it showed declination. Another peak was obtained at 15 hr which was quite low compared to the first peak at 9 hr. The rate of incorporation remained almost same at 18, 21 and 24 hr of regeneration following which it decreased. It was revealed that synthesis at 9 hr was maximum and was crucial for basal disc differentiation.

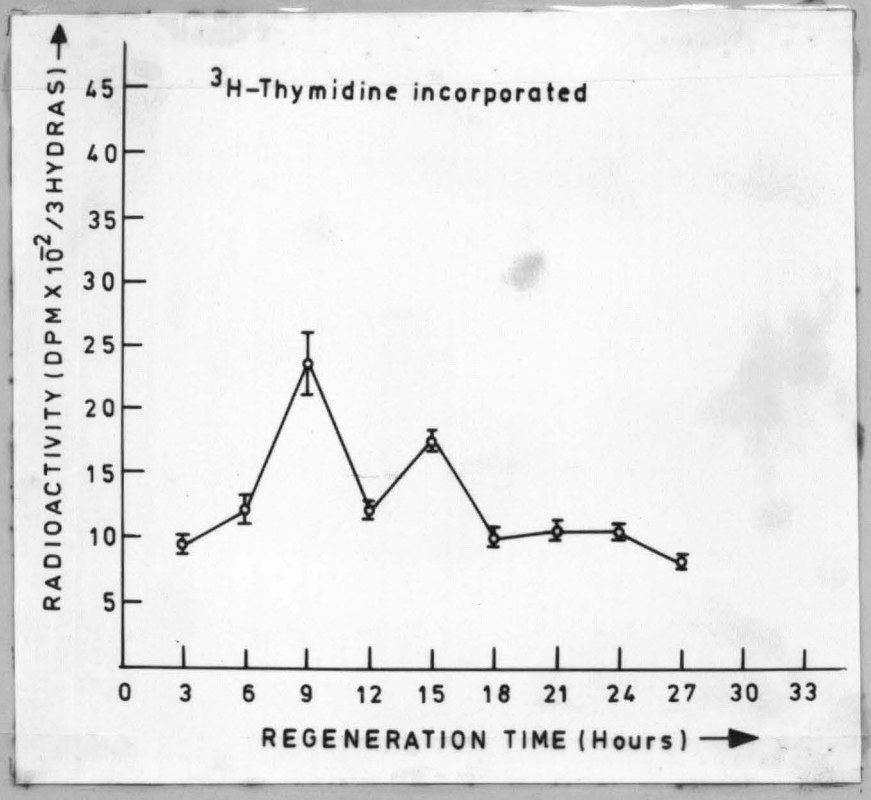

Fig.38 : Time dependent changes in the extent of hourly pulse incorporation of ^{3}H-thymidine (10 µCi/ml, sp.act. 15,200 mCi/mM) into TCA precipitable DNA of basal disc regenerating hydras. Note the appearance of 2 peaks of incorporation at 9 hr and 15 hr of regeneration.

DISCUSSION

Hydra is an ideal system for the study of growth and morphogenetic differentiation because of simplicity of its structure. Different morphogenetic processes in hydra have already been elucidated at the cellular level but not much attention as yet has been paid as far as their molecular mechanisms are concerned. The present study aims at detailing some new information of DNA synthesis during the different aspects of the life of hydra.

Feeding habits of the organism strongly influence both its growth and maintenance. In well fed hydras, the tissue mass doubles in 3-4 days. The organism increases in size upto a point and then the excess tissue is remodelled by budding off young animals and by replacing the exhausted cells. Many specialized cells by their continuous function are exhausted. The digestive cells, gland cells and stinging (cnidoblasts) cells must be replaced periodically if the organism is to retain its regular size and functional integrity. About 15% of the tissue is lost by sloughing at the extremities i.e., through the tentacle tips and basal disc (Bode and David, 1978). The constant proliferation of cells causes a continual displacement of two tissue layers along the body column into buds and towards two extremities where

they form into head and foot structures. The foregoing discussion clarifies the role of feeding in hydra and that is why before going into the molecular events of DNA synthesis during the morphogenetic processes, the present work has been directed to pin point the feeding reactions of hydras to begin with at morphological and cellular level.

Feeding behaviour

The behaviour of hydra shows some striking examples of environmental factors interacting with endogeneously active system within the animal. Feeding response consists of several reflexly linked behavioural sequences (Burnett, 1973). The first element of feeding behaviour is the attachment of prey to an outstretched tentacle by means of nematocyst discharge. Due to mechanical stimulus, caused by contact of tantacle with the prey, two types of nemotocysts, stenoteles and desmonemes, are discharged. The threshold for activation of nematocysts depends upon time lapse of last feeding which has been shown for hydras by Jones (1941) and Ewer (1947). The desmonemes coil around the prey thereby enmeshing it, while the stenoteles pierce its exoskeleton in harpoon like fashion. The discharged nematocysts are released from the tentacles as the prey is enclosed by mouth (Ewer, 1947). After the

attachment of the prey to the tentacle with nematocyst discharge there is a latent period before first observable movement of the tentacle takes place. The tentacle reaction time is usually between 1-2 seconds in duration, but sometimes latency lasts for a minute or more (Burnett, 1973). The coordinated reaction of tentacles mechanically brings the prey to hypostome and this collective movement of tentacles is called "concert." The concerts occur repeatedly till the mouth opens and engulfs the prey. At this stage, the concerts frequently give rise to uncoordinated writhing activities (Ewer, 1947; Lenhoff, 1961). It is believed that the coordinated movements of the tentacles are initiated not only by mechanical stimulus but also by chemical one. These movements occur spontaneously and their frequency is markedly enhanced by dilute extracts of artemia or extremely small concentration of reduced glutathione or its analogues (i.e. below 10^{-8}M). The coordination is accomplished by conduction of impulse or diffusion of chemical factors from point of prey attachment to the hypostome, where presumably coordination takes place.

After the repeated concerted flexions of tentacles, the mouth opens, creeps around and closes about the prey.

The mouth opening response is one in which chemical stimulation seems to play a key role (Burnett, 1973). The starved hydras open their mouths when exposed to GSH. It is a specific chemical stimulator evoking feeding response in hydra. Feeding activities are initiated by GSH released when the prey is pierced by discharged nematocysts. Little is known concerning the mechanisms involved in mouth closing and creeping about the prey. Creeping of mouth may result from stimuli due to contact of the prey on the inside of the mouth. The mechanisms responsible for the mouth closure and termination of the various behavioural sequences are also not clearly known. Once the gastrovascular cavity is filled, it is difficult for hydra to open its mouth because of the internal pressure exerted on the walls of gastrovascular cavity as a result of a large intake of water during early phase of digestion. The feeding responses in hydra are series of linked local responses, each component being initiated by the results of preceding one. These responses suppress the column and tentacle contraction. This is a general phenomenon seen in the coelenterates i.e. inhibition of spontaneously recurring behavioural events by feeding stimuli.

Cellular behaviour during digestion

Initial histological studies made in the present work during the process of digestion have revealed a remarkable cellular behaviour during the time of digestion. The entire cellular set up of the body wall especially that of the endodermal cells seems to be reshuffled. The cells undergo a series of morphological and positional changes during the period of digestion. In order to participate in digestion most of the endodermal cells undergo dedifferentiation and become phagocytic. The digestion in hydra as it is known, is both extracellular and intracellular. While the major portion of digestion is carried on within the cells of gastrodermis, the initial phase occurs within the gastrovascular cavity. As soon as the prey is ingested, its body structure is broken into several pieces both mechanically and enzymatically. Portions of tentacles, engulfed along with the prey also undergo dissolution and their nematoblasts are set free, and thus attain a tissue free state. The nematoblasts change their function to become phagocytic and actively take part in the digestive process. At the same time, the cells from both the body layers of the body wall also start dislocating themselves from the tissue matrix and number

of them enter into the enteron as free cells to participate in the process of digestion. Due to migration of cells from the body wall, it assumes an atypical appearance. The nematoblasts and interstitial cells from their normal location in the ectoderm altogether disappear. The presence of food stimulates the release of contents of zymogenic gland cells into enteron (Semel-van-Gansen, 1954). The enzymes released by these cells initiate the digestive processes and produce small particles of food that are actively phagocytized by the different phagocytic cells actively engaged in digestion. According to Semal-van-Gansen (1954) the release of digestive enzymes is primarily during the first 30 minutes after the entrance of food into gut. Some functions of these digestive enzymes have been studied by Lenhoff (1961). He has found that during the 6-7 hr period between the ingestion of food and egestion of undigested material 90% ^{35}S label (previously incorporated in mouse liver, used as food) is transferred from the gut cavity into gastrodermal cells of hydra. By 4 hrs the digestion seems to be completed. The artemia is no more seen in the solid state but undergoes a complete liquefaction. The wandering cells were released from the body wall of

hydra for accelerating the digestion now are involved in performing the digestive obligation and by 4 hrs they start reentering into the body wall to rejoin with other loosely connected endodermal cells. This in turn gives the impression of restructuration of loose endoderm layer once again into its distinct characteristic matrix. The dedifferentiated cells start redifferentiating into typical cell types. The interstitial cells make their reappearance and undergo differentiation to form nematoblasts. Once the undigested food is thrown out, the body layers start acquiring their typical structure with all types of cells repacked within it as before. The analysis of behaviour of hydra during digestion have clearly revealed that a bulk of hydra tissue undergoes distinct processes of dedifferentiation, migration, subsequent redifferentiation after their reentry into endodermal layer which certainly affects rather reshuffles genetic make up of each of the participating cells. The time lapse between feeding and different morphogenetic processes in hydra seems to be strongly linked. Future work alone will make it known more about the morphogenetic processes that are influenced by feeding habits of this organism.

DNA synthesis following feeding

After restructuration of both the body layers, the

subsequent effects of feeding have been studied at molecular level with the incorporation of ^{3}H-thymidine into DNA by continuous labelling method. It has revealed that DNA synthesis following the feeding shoots up right after restructuration of body layers till 27 hrs of feeding following which it declines (Fig. 16). Rising phase of DNA synthesis shows two small peaks of nearly equal magnitude following which there is a major peak. The phases represent an active synthesis of DNA and it may be considered as the growth phase. It is now believed that the hydra is akin to maintain a definite ratio of different types of cells. When fed, it grows and achieves the optimal ratio of different types of cells. This increase in number of cells is greatly influenced by feeding regime i.e. animals maintained at different feeding regime 1-20 shrimp larvae per day reached different steady states so the growth is directly linked with feeding habits (Otto and Campbell, 1977b). Feeding triggers the cell division and their ultimate differentiation. The newly differentiated cells not only replace the worn out cells but also add to the total number of cells of body resulting in growth.

The DNA profile after feeding shows 2 peaks at 12 to 18 hr and a third one at 27 hr of feeding which

is the most conspicuous. Three peaks of DNA synthesis may represent replication of either specific cell types at specific hours of growth or perhaps it is not so specific as far as the cell types are concerned. The peaks represent non specific replication of all cell types. Only future work can clarify the point. Between the sharp points of peaks - between 12 hr and 18 hr and between 18 hr and 27 hr, the profile shows non replicating phases. Particularly after 27 hrs, no fresh DNA synthesis seems to take place which coincides with termination of the growth phase of the organism. Feeding not only triggers DNA synthesis as evidenced by above discussion but also the RNA synthesis. Venugopal and Mookerjee (1980a) have reported by continuous incorporation studies in normal non-regenerating hydra that there exist two phases with respect to RNA metabolism over a period of 96 hr after feeding. The first phase corresponds to synthetic phase in which a considerable amount of RNA is produced and during the second phase the breakdown of RNA molecules taken place.

DNA synthesis in different levels of same hydra

After the completion of the growth phase, in a normal functional hydra which is given regular feeding, a continuous incorporation studies with ^{3}H-thymidine has

been carried out to pin point the DNA synthesis in the different regions of a situ hydra. All the four regions namely hypostome, gastric region, budding region and peduncle (Fig. 17) show the active DNA synthesis, although the rate at which they synthesize DNA is different when compared to each other. The anterior most region i.e. hypostome along with subhypostomal region shows the maximum synthesis while other regions show synthesis of decreasing order as one proceeds from anterior to posterior region. The basal disc shows the minimum synthesis compared to rest of the fragments. These observations clearly drive one to think that the bulk of the body of hydra is a seat of DNA synthesis. The variation in capability of different regions to synthesize DNA reveals a kind of polarity in hydra as far as the DNA synthesis is concerned i.e. distal part showing maximum and the proximal showing the minimum. These observations allow us to understand the nature of growth zone in a better way. There are two views advanced regarding the working of the growth zone. The first point of view regards that there is a subhypostomal area which has got high mitotic activity and the cells migrate from this region in both anterior and posterior directions (Issajew, 1926; Tripp, 1928; Brien and Reniers-Decoen, 1949; Mookerjee, 1966; Mookerjee and Roy, 1971).

Whereas the other view delineates that the mitotic activity is not restricted to subhypostomal area only but it is extended over to the entire body with subhypostomal area showing greater mitotic activity compared to other areas of the body (Campbell, 1965; Shostak, 1965). The present incorporation studies made on the different levels of the same hydra substantiate the concept that the growth zone is not as localized as it has been thought to be in particular region but it is rather extensive showing a gradient profile high at the anterior region sliding down at posterior, quite consonant with the general gradient concept as such.

DNA synthesis during budding

Often it is believed that budding is a morphogenetic phenomenon, accomplished by morphollaxis. The accumulation of interstitial cells initiates bud development (Lang, 1892; Tannreuther, 1909; Hadzi, 1910; Gelei, 1924). But this is not in concordance with what has been later reported by Brien and Renier-Decoen (1955) and Diehl and Burnett (1965). They have shown that the bud development is possible even after inactivation of interstitial cells with X-irradiation or treatment with nitrogen mustard indicating that interstitial cells are not the only cells responsible for the bud development. The present profile

studies of bud development have shown that it is certainly not by mere morphollaxis alone but an active DNA synthesis is also involved. The DNA synthesis increases rapidly for first 15 hrs of bud initiation. At this stage the bud starts showing signs of tentacle rudiments which means the initial development of bud involves the active DNA synthesis. Following this stage, the DNA synthesis decreases very gradually till the bud is fully developed at about 35 hrs of bud initiation. Venugopal and Mookerjee (1976) have observed that inhibition of RNA synthesis in hydra results in complete suppression of bud morphogenesis. A partial inhibition allows bud formation but affects development of nematocyte, gland cells and interstitial cells which indicate that bud development in hydra is associated with DNA dependent RNA synthesis. Li and Lenhoff (1960) have reported that the part attached to mother hydra after bud excision develops into a full bud which suggests the cell division in bud tissue. The bud bears tentacles only after its cells start undergoing cell division. They have also reported that DNA/protein synthesis ratio of bud is 3 times that of parent. Based on these observations, it is clear that bud morphogenesis involves not only cells of mother hydra but also of developing bud, both dividing actively to form differentiated bud. The last part of our profile study shows a gradual declination

of the synthetic pattern of DNA which corresponds to period during which the fully differentiated bud undergoes detachment. It stays along with mother even after being cut off from the mother enteron and when it can even feed independently. However, the last stages of bud development do not involve any replication of DNA as reflected by profile studies.

DNA synthesis during regeneration

Because of its high power of regeneration, hydra reconstitutes its lost parts within few hours of amputation. Regeneration not only involves cellular rearrangements but also cell proliferation to maintain the relative ratio of different types of cells constant (Bode et al., 1973). The present incorporation studies have revealed that there is a burst of DNA synthesis immediately after amputations. This is true for both hypostome and basal disc regeneration and this burst continues throughout the initial phase of differentiation. Clarkson and Wolpert (1967) and Clarkson (1969a,b) have opined that there is no significant change in DNA synthesis during first hour of regeneration. But our present study, throughout the process of differentiation shows that DNA replication is a very important factor during regeneration. The initial increase in DNA synthesis might be due to excision of hypostome or basal disc of the hydra which acts as a stimulus for induction

of DNA synthesis during initial stages of regeneration. The amputation possibly enhances activation of genes resulting in the DNA replication which is possibly associated with the initial developmental activities of regeneration. The molecular changes during growth and development may coincide with cellular activities. From the profile studies of both hypostome and basal disc regeneration, it is clear that DNA synthesis during both the types of regeneration assumes slightly flexible nature. The hypostome regeneration studies show two peaks at 9 and 18 hr of regeneration and basal disc regeneration shows at 9 and 15 hr of regeneration which suggests that capacity of system varies from time to time in synthesizing DNA molecules along with variations in cellular activities. Number of workers have given possible roles of different types of cells in regeneration of hydra (Long, 1892; Hadzi, 1910; Zawarzin, 1929; Kanajew, 1930; Moore, 1952; Mookerjee and Bhattacherjee, 1966). It has been established that process of hypostome and basal disc regeneration involves activation, migration, multiplication and differentiation of cells (Mookerjee and Bhattacherjee, 1966). They have reported that rebuilding of lost parts requires participation of the existing cells in the endoderm at the cut surface which first heals the would as a layer. As the differentiation proceeds,

the activity of endoderm gland cells also becomes pronounced which can be clearly envisaged during further stages of differentiation, namely the delamination of ectoderm and process of dedifferentiation and redifferentiation. After the wound healing is over the gland cells undergo dedifferentiation and give rise to cells reminiscent of embryonic cells. These dedifferentiated cells, capable of rapid multiplication form nests and subsequently redifferentiate into several types of specialized somatic cells other than the ones they were derived from. Thus, it appears that cellular dedifferentiation followed by redifferentiation is one of the main developmental attributes in rebuilding the lost configuration of hydra. These variations in cellular activities can be correlated with the replication of DNA synthesis during regeneration. The initial DNA synthesis upon amputation and its further increase can be due to active multiplication of the interstitial cells. The initial activity of DNA synthesis continues for first 9 hrs in both hypostome and basal disc regeneration. The declination following initial spurt in DNA synthesis for 9 hrs could be due to fact that the cells might be in the process of division at that time. The second peak of DNA synthesis during hypostome and basal disc regeneration at 18 and 15 hr

of regeneration respectively may be associated with the morphogenetic process of delamination of ectoderm from endoderm, proliferation of interstitial cells and that of ectodermal cells. The declination following this peak might be due to the differentiation of all newly added cells to the regenerating sites. At 18 hr of hypostome regeneration, the hypostome normally acquires a normal form which reveals that DNA synthesis does not take place after the rudiment of the lost parts appear in organism. It could be seen that both cellular and molecular phenomena during regeneration go hand in hand.

The pulse labelling studies made during specific hours of regeneration in hydra have shown the amount of DNA laid down during these periods and also the capacity of the system to synthesize the macromolecules correspondingly. Overall profile with pulse labelling method is almost similar to that given by continuous labelling method in both hypostome and basal disc regeneration. In basal disc regeneration the relative magnitude of two peaks by pulse labelling is just opposite to what is seen by continuous labelling i.e. second peak is of higher magnitude in continuous labelling and lower in pulse labelling. The higher magnitude of second peak in

continuous labelling might be due to the cumulative effect of time preceding that hr. Venugopal and Mookerjee (1980) have shown almost similar role of RNA during hypostome and basal disc regeneration, their results add support to our idea that regeneration is not merely a result of morphollaxis but it also involves new macromolecular synthesis.

One can conveniently sum up the major high lights of the present study. There is ample evidence at hand to have a glimpse of the totipotent nature of hydra cells which are performing different function at different time of exigencies of its life. To illustrate this, one can mention about the role of nematoblast cells, which otherwise are involved for stinging functions, can also be geared for digestive action at the time of feeding. Even some of body wall cells of the trunk region display unique nature of dedifferentiation and phagocytic activity with an enormous power of redifferentiation after they reset into the body wall to perform other physiological functions. In order to appreciate this concept of different strategies evolved by hydra cells at different time of its life, one must seek a flexible genomic control mechanism which at the moment

we can only foresee but do not know much about its mechanism.

The important lesson which one can gain from foregoing study is that invariably DNA synthesis is a major event in the different morphogenetic functions of hydra namely :

a. Feeding results in fresh DNA synthesis
b. Bud morphogenesis involves fresh DNA synthesis
c. Even regeneration necessitates fresh DNA synthesis.

After the completion of the growth phase in a normal functional hydra which is given regular feeding, a continuous incorporation studies with ^{3}H-thymidine has been carried out to pin point the DNA synthesis in different regions of same hydra which indicates a kind of a gradient, quite consonant with the general gradient of hydroid organisation.

SUMMARY

1. The present study has been concerned with DNA synthesis during the different facets of hydroid life by using ^{3}H-thymidine labelling techniques.

2. As feeding initiates DNA synthesis, the mechanisms of ingestion and the cellular involvement in digestion of food has also been given some consideration.

3. The capture of artemia by tentacles is affected by "concerts" of tentacles. During the digestive process some cells like nematoblasts, interstitial cells and gland cells perform phagocytic role and actively participate in intracellular digestion, in addition to their normal functions. Other body wall cells, however, constitute the main elements for carrying on intercellular digestion.

4. The consequence of feeding results in fresh DNA synthesis as shown by incorporation studies with ^{3}H-thymidine in normal non-regenerating hydra. Three peaks of DNA synthesis coinciding at 12 hr, 18 hr and 27 hr respectively have been noticed following a feeding before the emergence of a bud.

5. When DNA synthesis is considered *in situ* dynamic hydra across the anteroposterior axis, the profile

remarkably simulates a gradient, progressively sliding from hypostome to basal disc region. This suggests that the growth zone is not restricted to subhypostomal region alone but it is more extensive along its axis.

6. Bud morphogenesis also involves new DNA synthesis. It is not merely accomplished by morphollaxis but fresh DNA synthesis is involved during first 15 hrs of bud development. The DNA synthesis during bud formation decreases gradually till the tentacle rudiments appear following which there is a steep declination.

7. Both continuous and pulse labelling studies with labelled thymidine during regeneration of hypostome and basal disc have revealed the occurrence of DNA synthesis. In case of hypostome regeneration two peaks of DNA synthesis are observed at 9 hr and 18 hr whereas in basal disc regeneration, it is seen at 9 hr and 15 hr.

8. The study has furnished ample evidence of the totipotent nature of hydroid cells performing more than one functions. Views have been advanced to explain that probably a regulative genomic control is at work.

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