DISCUSSION

RNA SYNTHESIS IN NON-REGENERATING HYDRAS

The continuous incorporation studies with $^3$H-uridine in normal non-regenerating hydras showed that in hydras there exist two phases with respect to RNA metabolism over a period of 96 hours after feeding. The first phase corresponds to a synthetic phase in which a considerable amount of RNA is being produced and during the second phase rather the breakdown of RNA molecules takes place. The first phase could be referred to as the phase of growth of the organism as indicated by an acceleration in the RNA synthesis taking place during this period and the second phase can be termed as the phase of starvation. The first period ranges for a duration of approximately 20 hours, from 0 hour after feeding till 20 hours. But the significant feature is that even during the first phase, the synthesis of RNA is not found to be uniformly occurring. There is an initial stationary phase during which practically no synthesis takes place or the rate of synthesis remains more or less constant. Even if any synthesis is accountable, as shown by the nature of incorporation during this period, it is considered to be very meagre as the radioactivity obtained at this stage was practically negligible when compared to the
enormous amount obtained during the later periods. It could be seen that the stationary phase continues soon after feeding till 7 hours and is closely followed by the exponential phase in which the RNA synthesis increases very swiftly. In this period, the maximum incorporation is obtained at about 20 hours and this suggests that a growing hydra synthesizes the maximum amount of RNA near about the 20 hour after feeding, till it is fed next. It could be assumed that after this period, the metabolism is geared towards a pattern of anabolism.

There are several reports suggesting a variable pattern of RNA synthesis during the period of growth and starvation in many organisms. Kinetic studies of incorporation of $^3$H-adenine in yeast S 288C spheroplasts suggest that RNA synthesis continues exponentially for a period of 120 minutes of growth and then decays stochastically. (Hynes and Phillips, 1976). In HeLa cells and also in A9 mouse fibroblasts, it has been shown that an acceleration in ribosomal RNA synthesis takes place as the cells resume growth and mitotic activity (Ringertz, Bolund and Darzynkiewicz, 1970). Alberghina, Sturani and Gohlke (1975) observed that in Neurospora the increase of growth rate requires a proportional increase in the rate of
RNA synthesis and a limit for the maximal growth rate attainable by a cell is given by its inability to increase the rate of RNA synthesis. Hallberg and Smith (1975) suggested that in *Xenopus laevis* progress in oogenesis is coordinated with the progressive synthesis of ribosomal RNA. In the oogenesis of echinoid worms, the growth of the ovum is found to be associated with synthesis of RNA and during the final one third of its growth, there is an increase in the rate of total RNA synthesis (Davis and Wilt, 1972). An enhanced synthesis of ribosomal RNA was observed in growing whole liver (Fujioka, Koga and Lieberman, 1963; Muramatsu and Bus 1965) and also after partial hepatectomy (Stevely and White, 1970). It has been established that liver ribosomal RNA synthesis is enhanced during the growth of whole body after the radiation of the animals (Cammar et al., 1969) and also results in a rise in messenger content (Hidvegi et al., 1970; Fonagy and Hindvegi, 1970). Another example of the definite relationship between synthesis of RNA and growth has been reported by Testa Rudner (1975). They have reported that during sporulation in *Bacillus subtilis*, an increased RNA synthesis occurs as soon as the cells start growing exponentially. The RNA synthesis particularly that of ribosomal RNA increases. An analysis of the nature of RNA synthesized during...
periods of growth in cotyledons revealed that in cells after a period of 24 hours of stationary phase, RNA synthesis rises sharply resulting in a 2 to 3 fold accumulation of ribosomal RNA (Verma and Marcus, 1973). From these studies it becomes clear that the rate of increase in RNA synthesis is undoubtedly related to the rate of growth in an organism in a proportional manner. The fact that among the RNA population majority of the transcriptory product amounts to ribosomal RNA suggests that in hydra also the increase in RNA synthesis observed after feeding might most probably be due to an increase in its ribosomal RNA content. Previously also it has been reported that in hydra histochemically demonstrable RNA increases as the digestion progresses in the organism (Haynes, 1973). Thus, it seems positively suggestive that in hydra, the period in which the RNA synthesis is remarkably noted must correspond to the phase of growth of the organism which ranges approximately over a period of 20 hours i.e. from 0 hour till 20 hours after feeding the organisms. It is also possible that some of the RNA synthesized during this period may remain stable and these templates might get activated at a later stage, perhaps at the onset of biosynthetic activities involved with the stability and maintenance of the organism. However, the lack of an appreciable amount of RNA synthesis till 7 hours
after feeding suggests that during this period practically no growth of cells in the organism takes place. Conversely, the cells might actively be participated in the process of digestion of the engulfed prey, thus resulting in a stationary phase. However, when once this stationary phase is over, the initial RNA pool must be readily available within the growing cells of the organism for the biosynthetic activities to take place as the exponentially growing phase continues.

It is observed that after 19 hours, there occurs a deceleration in the rate of RNA synthesis in the growing hydra. It could be assumed that the gradual declination characteristic of this period may be suggesting the possible breakdown of RNA molecules synthesized previously during the growth phase of the organism. Several lines of evidences focus particular attention on the degradation of RNA molecules during starvation in many organisms, especially in cells starved for various nutrients and amino acids. The rate of accumulation of RNA in bacterial cells is known to be singularly responsive to the nutritional environment; unfavourable growth conditions severely depresses the rate of RNA accumulation (Gallant and Harada, 1969; Gallant et al., 1970; Varney, Thomas and Burton, 1970). Kaplan and Apirion (1975) studied the decay of preexisting ribonucleic acid in Escherichia coli cells subjected to starvation for nitrogen phosphate, amino acids or carbon sources. The
results indicated that degradation of ribosomal RNA by
and large, parallels with that of total RNA and the decay
is dependent on the nutritive content of the cell. Erlich,
Gallant and Lazzarini (1975) showed that in *Escherichia
coli* contraction of the GTP pool results in a 2.5 fold
reduction in the rate of RNA synthesis and in the cessation
of RNA accumulation. Measurements of concentration of
RNA in different strains of *Escherichia coli* shortly
after the imposition of amino acid deprivation indicated
that there is a temporary fall in the amount of all
fractions of RNA, particularly in messenger RNA, relative
to the total cellular content (Midgley and Smith, 1974).
Similarly, effects of starvation of the distribution free
and membrane bound ribosomes in rat liver have been
studied by Ramsey and Steele (1976). The results indicated
that starvation can produce up to a 50% reduction in hepatic
ribosomes and subsequently reducing the protein synthesis.
All these studies suggest that in cells and tissues due to
starvation of nutrients, an appreciable amount of breakdown
of RNA takes place which might have been synthesized and
accumulated in the cells during the period of growth of
the organism. It could be assumed that a similar type of
situation prevails here in hydra also in which most of the
RNA synthesized and accumulated during the phase of growth
of the organism are getting subsequently turned over after
20 hours of feeding. Therefore, the period ranging after 20 hours of feeding could be termed as the phase of starvation of the organism. Histochemical analysis (Mookerjee and Sanyal, 1962) also have shown that during starvation in hydra, a considerable decrease in the total content of RNA may take place. Also, starvation in hydra could be endowed upon by one of its highly morphogenetic phenomenon namely the bud formation. Throughout the development and growth of a bud, a considerable depletion of amino acids and other substances in the mother hydra may take place and this could also enhance the degradation of RNA molecules in addition to normal starvation caused by the lack of feeding.

However, the period of starvation which is found to range between 20 and 72 hours after feeding appears to be a considerably longer period of time. This could be due to the fact that the turnover of RNA molecules synthesized previously during the growth phase of the organism, might be a slower process. A gradual turnover of preformed RNA has been shown to occur during starvation in many kinds of cells (Hackett, Egberts and Traub, 1977). One other possibility for this observed slow decay is that amino acid starvation might have yielded inexact stabilities to the preexisting RNA molecules since it is reported that ribosomal RNA racked up in ribosomes remains more stable during guanine starvation (Erlich, Gallant and Lazzarini, 1975).
It could be seen that in the starved hydra after 72 hours of feeding, a stationary phase is again formed between the 84 and 96 hours, during which practically no synthesis of RNA takes place or probably the degradation of RNA molecule might have completed. A second feeding at this time may result in a new and enhanced synthesis of RNA and subsequently further growth may take place in the organism restoring all the biosynthetic activities. Investigations regarding these lines are in the immediate purview. However, it seems reasonable to argue that it might also be due to the occurrence of the highly morphogenetic phenomenon of bud formation in hydra that a substantially higher amount of RNA is synthesized during the growth phase of the organism which might be utilized later for the normal biosynthetic activities in the mother hydra as well as in the continuously growing bud.

RNA SYNTHESIS IN REGENERATING HYDRAS

One of the remarkable features observed preliminarily during this analysis is the occurrence of a burst in the RNA synthesis taking place immediately after the amputations are done in the organisms. This situation is envisaged in both hypostome as well as in basal disc regeneration and it is further noticed that this abrupt increase in the activity observed after the amputations is continued progressively throughout the initial phase of differentiation. Previous
studies (Clarkson, 1969a, b) have already indicated that there occurs a large increase in the content of RNA during the first hour after decapitation in *Hydra littoralis*. Our results are in conformity with the above report and it further reveals that a similar type of situation prevails during the regeneration of basal disc also. Moreover, the present investigation has been extended to an analysis during further stages of differentiation also. It seems reasonable to assume that the sudden increase in the RNA synthesis during the preliminary hours of regeneration might have taken place as a result of the excision of head or foot of the organism. In other words, it can be said that the mechanical amputation acts as an external stimulus to induce RNA synthesis in hydra to a considerable extent so that a burst of activity is resulted immediately in the postamputational period. Renewed RNA synthesis has been noted during other types of regeneration also such as in lens regeneration of salamander (Reyer, 1962), regeneration of limbs of adult newt (Morzlock and Stocum, 1971) and also in the basophilic neoblasts formed during the regeneration of planaria (Hay, 1968). However, the nature of RNA synthesis during these processes shows certain dissimilarities with what we have witnessed here in hydra in the sense that no sudden and abrupt synthesis is observed after removal of any parts in the former cases. Similarly, if the nature of RNA synthesis is analysed in the case of embryonic development
of many organisms it could be seen that new RNA synthesis
does not begin before the onset of gastrulation (Bell,
1966) and thus fertilization or other type of activation
of the egg does not impose upon a sudden increase in
such an activity. Thus the events occurring even prior
to the actual process of regeneration in hydra gives a
unique status to the organism among large number of systems
undergoing differentiation implicated through molecular
mechanisms. A possible explanation to this novelty is
that a highly enhanced activation of genes must be taking
place soon after the wound is inflicted upon the organism
and as a result of this the level of transcription also
would be stimulated resulting in an elevated plateau of
RNA. It seems reasonable to argue that this increased
gene activity characteristic of the postamputational period
in hydra must essentially be associated with the initial
developmental activities embedded in the restitution
process of lost parts. In this connection it could be
concluded that when hydra is transferred from a metaboli-
cally repressive state to one in which growth and develop-
ment occur, significant biochemical changes can take place
which may coincide with the cellular activities. Hydra
itself is considered to be a system of dynamic instability
(Mookerjee and Sinha, 1967) and therefore, any disturbance
of the normal order in this system may lead to such a
state of dynamic instability which becomes expressive in molecular pathways too.

However, the kinetic studies made during the 36 hours of regeneration in this organisms do not represent a stable pattern of RNA synthesis. Conversely, it is noted that the synthesis during both the types of regeneration assumes a highly flexible and stochastic nature. This discontinuous pattern of synthesis becomes quite apparent when the whole spectrum of RNA synthesis is analysed in the continuously labelled hydra. This pattern is specifically characterised by abrupt elevations in the RNA synthesis occurring during certain hours of regeneration followed by sudden declinations which are subsequent to the elevations. The significant feature is that in any case, whether during the elevation or declination, the synthesis does not remain stationary for a longer period, in the sense that such ups and downs are generally found to be comprised of only short intervals of time. Similarly, if the nature of RNA synthesis during regeneration in the pulse-labelled hydra is analysed it could be seen that the same variable pattern also prevails there. In such situations, the discontinuous pattern suggests that during the period of regeneration the capacity of the system also varies from time to time in synthesizing RNA molecules along with variations in cellular activities.
A large number of studies using a variety of systems have shown that there exists a definite relationship between the cellular activities during development and differentiation in one hand and the synthesis and processing of RNA molecules on the other. Cells growing and dividing rapidly seem to synthesize and accumulate RNA more actively than non-growing cells. It is shown that RNA formation is very much reduced or absent during mitosis in various cell types (Feinendegen and Bond, 1963; Killander, 1965; Shiff, 1965; Mitchison et al., 1969). RNA production is found to increase rapidly during the S phase and there is evidence of a gene dosage effect (Pfeiffer and Tolmatch, 1968; Pfeiffer, 1968). On the other hand, Enger and Tobey (1969) found a steadily increasing RNA synthesis in Chinese hamster cells without a plateau in G1 phase, as did Scharff and Robbins (1965). In Physarum, however, there is a sharp reduction in RNA formation after S phase (Mittermayer, Braun and Rusch, 1964; Mittermayer et al., 1966). Both in yeast (Mitchison et al., 1969) and in ciliates (Rao and Prescott, 1967) the synthesis of RNA continues during the cell cycle particularly during S phase. It has also reported that during the cell cycle of mouse leukaemia cells, the rate of RNA synthesis in S phase is almost double the preceding G1 phase (Meltz and Okada, 1971). In plasmacytoma cells (Eikhom, Abraham and Dowben, 1975), the formation of RNA is
very low during the first three hours of differentiation and a rapid increase takes place during the S phase leading to a maximum. An inactive phase of 24 hours of duration has been observed in plant cell cultures (Verma and Marcus, 1973) before the RNA synthesis rises sharply in the S phase resulting in a 2 to 3 fold increase.

Apart from these demonstrations another line of evidences focuses particular attention on the pattern of RNA synthesis in relationship to the stage of cell differentiation. First of all, it has been shown that stimulation of cells can bring about a considerable change in the pattern of RNA synthesis. In a programmed cell like lymphocyte RNA accumulates always at a very low rate at resting stage and this condition is reversed by stimulation of cell growth (Kay and Handmaker, 1970; Cooper and Gibson, 1971; Wojcierowski, Antosz and Halliop, 1976; Dauphinais and Waithe, 1977). Morphological changes in these cells when stimulated and transformed into blast cells are also accompanied by an increase in RNA synthesis (Monjardino and MacGillivary, 1970). Increase in RNA synthesis subsequent to stimulation and transformation has been reported in other types of cells also (Hirschhorn et al., 1963; Rubin and Cooper, 1965; Schlatterer, 1976; Johnson and Meister, 1977). RNA synthesis has also been found to vary during different stages of differentiation.
Modak and Persons (1971) have reported that during lens cell differentiation, annular pad cells are most active in synthesizing RNA. Later stages of differentiation involve a gradual decrease as cells enter a stationary phase. Synthesis of different RNA species has been found to vary with respect to different stages during the process of biochemical differentiation in erythrocytes (Attardi, Parnas and Attardi, 1970) and also during recovery in ultraviolet irradiated mammalian cells (Nocentiny, 1976). A quantitative change in RNA synthesis is noted in the cultured mammalian myoblasts when they enter into post-mitotic stage prior to fusion (Clissold and Cole, 1973). Similarly, differentiated neuroblastoma cells have been shown to possess a higher content of RNA than undifferentiated cells (Prasad, Bondy and Purdy, 1975). It is also conceivable that cell contact also may bring about a regulation in RNA synthesis as shown in the case of stimulated lymphocytes (Monjardino and MacGillivery, 1970) and also during contact inhibition in human diploid fibroblasts (Levine, Jeng and Chang, 1974). In the latter case, a substantial decrease in the amount of RNA is shown to occur. In developing cells of sea urchin embryo, if cells are prevented from aggregating at early blastula stage RNA synthesis decreases (Giudice, Mutolo and Moscona, 1967; Sconzo et al., 1970). However, an increase in rate is
shown to occur at pregastrula stage (Kobayashi and Kimura, 1976). In sponge cells, RNA synthesis is considerably enhanced when cells are kept in a non-contact condition (Kartha, 1977). However, this trend is reversed along with establishment of contacts being made between cells.

In the light of the above considerations, it seems reasonable to assume that there exists a symbolic relationship between the nature of RNA synthesis and the cellular activities taking place during the process of regeneration. In hydra, it has been established that the process of hypostome and basal disc regeneration involves activation, migration, multiplication and transformation of cells (Mookerjee and Bhattacharjee, 1966). According to these authors, one of the chief highlights in rebuilding the lost pattern concerns with the activity and participation of the existing cells in the endoderm at the cut surface which first heal the wound as a layer. As differentiation proceeds, the activity of the endoderm gland cells also become pronounced which can be clearly envisaged during further stages of differentiation, namely the delamination of ectoderm and the processes of dedifferentiation and redifferentiation. The authors have further shown that the crucial stage of differentiation extends till about 16 hours after the amputation and the transformation...
of gland cells is reversed as the reconstitution of hypostome and basal disc is initiated. After the wound healing is over the gland cells in the regenerating hydra undergo extensive dedifferentiation and give rise to cells reminiscent of embryonic cells. These are the interstitial cells which are indistinguishable from interstitial cells of normal animals and are then capable of rapid division forming nests and subsequently redifferentiate into several types of specialised somatic cells other than from which they are derived of. Thus, it appears that the cellular dedifferentiation followed by redifferentiation is one of the main developmental attributes in rebuilding the lost configuration of hydra.

One would like to advance that the extensive variations in cellular activities occurring during the regeneration in this organism might have been responsible in bringing out the variable pattern of RNA synthesis observed. The spurt in RNA synthesis upon amputation and the subsequent increase during the initial period of differentiation might have occurred as a result of the stimulation and activity of the gland cells. We could see that the initial activity of RNA synthesis lasts for about 6 hours after amputation and this could be well correlated with
the activities of gland cells taking place during the process of wound healing. Wound healing also takes about 5 to 6 hours of duration after the amputations are done. It could be seen that after this initial increase in the RNA synthesis, there occurs a declina-
tion in the rate which attains a minimum at about 8 hours. This could be due to the fact that the gland cells might be in the process of division at that time. The possibi-
li ty that the cells must be in the G1 phase of their cell cycle at about 8 hours of amputation and this phase must necessarily be associated with a reduction of RNA synthesis has been suggested on the light of the evidences cited previously. The second increase in the RNA synthesis, noted as the increase between 8 and 14 hours after amputa-
tion must be associated with the morphogenetic process of delamination of ectoderm from endoderm. As advanced by Mookerjee and Bhattacharjee, it could be noted that during this stage, considerable transformation in the morphology of gland cells take place and also they assume more basophilic in nature. In this regard, one can hypothesize that these factors along with the developmental process of proliferation of a new cell layer from the already existing endoderm might be responsible for bringing up the level of RNA synthesis. It appears that the gland cells at this stage must be in the S phase of their cell
cycle, as noted by the increased rate of RNA synthesis taking place during this time, and also that the delamination process is associated with an elevation in the RNA synthesis. The declination in the synthesis noted hereafter might be due to the dedifferentiation of gland cells and their subsequent proliferation to give rise to the amoeboid interstitial cells. As cited previously, one can notice that during this time, the gland cells in the endoderm tend to revert back to an embryonic state and the new cells in the delaminated ectoderm undergo extensive division also. It could be assumed that the mitosis among the gland cells to produce interstitial cells and the mitosis among interstitial cells themselves might have caused the considerable reduction in RNA synthesis. So also, extensive division among the cells might have resulted in large numbers so that their mutual contact and subsequent contact inhibition also might have reduced the plateau. It could be assumed that once again these cells must be in the G₁ phase of their cell cycle.

In cellular as well as in molecular level, it could be seen that the period ranging between 12 and 20 hours after the amputations represents the period of maximum activity and this must be the crucial stage of differentiation in the organism. Regarding RNA synthesis it could be seen that the maximum synthetic activity during any type
of regeneration in this organism occurs near about the 18th hour after amputation, whether in continuously labelled or in pulse labelled hydra. Similarly, the cellular activities especially those of gland cells and interstitial cells are also found to be mostly enhanced during this period of regeneration (Mookerjee and Bhattacharjee, 1966). The gland cells assume region characteristic cells and the amoeboid interstitial cells undergo transformation and give rise to other types of cells in the system. Thus, it seems reasonable to argue that there exists a definite relationship between the RNA synthesis and the cellular activities taking place during the differentiation and the observed differences in RNA synthesis reflect qualitative differences in the cellular processes. However, during the final stages of differentiation, the RNA synthesis decreases concomitantly and further increases are not shown to take place after 24 hours of regeneration. This must be due to the fact that the process of redifferentiation and the region specific attenuation of cells might have been completed by this time and further progress in differentiation may not be accomplishing an increase in the RNA content. It has been shown that rudiments of tentacles as well as basal disc appear in a regenerating hydra at about 24 hours after amputation.
and they attain a full-fledged stage by about 32 hours (Mookerjee and Bhattacharjee, 1966). In other words, it could be said that profound synthesis of RNA does not take place after the rudiments of the lost structures appear in the organism. However, near about 28 to 30 hours, another small peak of activity is observable and this increase might be attributable to variations in nucleotide precursor pool or due to the fact that some undetected developmental activity is operating at that time. However, if the entire period of regeneration is analysed, it could be seen that the regulation of RNA synthesis, its accumulation and the regulation of cell growth and division are highly integrated processes.

One of the significant aspects of regeneration in hydra is that the processes taking place in the restitution of lost parts resemble embryonic development and differentiation to a great extent. This resemblance is highly reflected when interstitial cells start redifferentiating after having dedifferentiated from an adult cell type, leading to a stage in which totipotency of cells is the main characteristic. If this nature of resemblance is taken into consideration, it could be seen that stage specific transcription and accumulation of RNA is a general phenomenon in many
organisms while accounting for pattern formation. For example, in sea urchin embryos, RNA synthesis is very low during cleavage and the rate is increased proportionally with increase in cell number, cell multiplication and cell activity during development (Eckberg and Ozaki, 1972). During cleavage in Lymnea eggs little or no RNA synthesis is noted prior to the 8-cell stage (Biggerlaur, 1971). Studies on temporal pattern of RNA synthesis in embryos of Xenopus laevis (Lamarca, 1973; Ramage and Barry, 1975) suggest that the rate of RNA synthesis decreases from blastula through gastrula and neurula stages to hatching tadpole. Similarly, in ascidians, detectable RNA synthesis begins only after the larvae start swimming for several hours (Lambert, 1971). Smith and Forrest (1971) have reported that during embryogenesis in the milk weed bug Oncopeltis the synthesis of RNA is discontinuous with a rapid burst at gastrulation and with an almost cessation after 60 hours. Considerable changes in RNA synthesis in the pre-implantation embryo of rabbit also has been shown to occur (Karp, Manes and Hahn, 1973). A shift up transition is noted in the RNA synthesis between preliminary oocyte stage and ovary stage in starfish (Boycan, 1973) and in Drosophila (Mermod, Lorena and Crippa, 1977) suggesting the existence of a lag phase between these two stages. Carp and Whiteley (1973) have reported a distributional difference in the
bulk of RNA content during cleavage in gastropod
*Acmaea scutam* and further stated that there occurs a
difference in the RNA metabolism throughout the progress-
ive developmental stages. Similar reports have been
obtained from studies on development and differentiation
in echiuroid worms (Davis and Wilt, 1972) and in insects
(Shaaya, 1976) also. All these studies emphatically
suggest that stage specific transcription and processing
of RNA is one of the main characteristics of embryonic
development and differentiation and considerable varia-
tions in RNA synthesis may take place with the develop-
mental shifts and transitions. This assumption gains
further support particularly from the studies of
Mookerjee and Bhattacharjee (1966) which suggest that
the process of regeneration in this organism consists of
various definite and interdependent phases. It is also
possible that the increase in RNA content observed during
various hours of regeneration might be due to an elevation
in the rate of synthesis of different classes of RNA also.
In insects it has been shown that an elevation of RNA
synthesis during early period of development is due to an
elevation of ribosomal RNA synthesis while the increase
during transition from larvae to pupa is due to an
elevation of hnRNA (Shaaya, 1976). Similarly, it could
be assumed that the declinations in RNA synthesis occurring
subsequent to the elevations might as well correspond to
the turnover of the accumulated RNA upon attaining confluency, since it has been shown that growing cells do not turnover their RNA and it begins to turnover only when the cells become confluent (Emerson, 1971). Increased template activity (Kostraba and Wang, 1973) and changes of pattern in RNA synthesis (Wallace and Birnstiel, 1966; Church and McCarthy, 1967; Crippa, Davidson and Mirsky, 1967; Abdel-Halim, 1977), during the regeneration of liver have already been suggested. However, a dedifferentiation and subsequent redifferentiation of cells do not take place in such situations.

RNA SYNTHESIS IN REGENERATING HYDRAS: PULSE-LABELLING STUDIES

As it was mentioned previously, short time labelling studies made during specific hours of regeneration in hydra mainly showed the amount of RNA laid down during those periods and also the capacity of the system to synthesize the macromolecules correspondingly. Here also one could note that the stochastic pattern of RNA synthesis prevails, during both the types of regeneration and it could be assumed that the fluctuations in the pattern observed must essentially be corresponding to the processes of wound healing, division of gland cells, delamination of ectoderm from endoderm and especially the dedifferentiation and redifferentiation of cells and
their subsequent proliferation. Thus, a meaningful correlation between the pattern of RNA synthesis and cellular activities could be formulated in this regard.

However, one of the important features observable from pulse-labelling studies is that it suggests mostly the possibility of stability and turnover of RNA molecules synthesized during the period of regeneration, when compared with the continuous labelling studies. It has been reported that short time labelling studies would rather reflect the synthesis of various species of RNA while the longer labelling studies may be more related to the cytoplasmic concentration of RNA classes (Bondy, Prasad and Purdy, 1974; Kern, 1975). By taking these facts into consideration, it does not seem improper to arrive at a conclusion that a marginal difference between the values of RNA in continuously labelled and pulse-labelled hydras during any hour of regeneration might represent the amount of stability of the macromolecule during that particular period and this figure in comparison with that of the preceding hour might as well correspond to the amount of turnover taking place during that period. Figures 22 and 23 represent a comparative assessment made between the two types of synthesis occurring in hypostome and basal disc respectively.
Even though the exact pool size and the rate of turnover of RNA has not been measured so far in regenerating hydras, several possibilities may exist based on the accumulating evidences, so as to what happens to various populations of RNA during differentiation especially with regard to their accumulation and turnover. It has been reported that immediately after transcription among the RNA populations, hnRNA accounts for as such as 75% but has a short half-life and decays rapidly (Darnell, 1968; Rubinstein and Clever, 1972). However, it appears that old RNA is more susceptible to degradation than more recently made RNA (Elicheiri, 1976). Britten and Kohne (1968) have proposed that during the course of differentiation different families of repeated sequences are expressed at different stages thereby causing a change in the pattern of types of RNA synthesis and accumulation. So also, Meier and Brownstain (1976) have shown that some conformational rearrangements of RNA might occur during differentiation and a rapid processing of RNA molecules immediately after the transport have been suggested by Scheer, Trandelenburg and Franke (1973). Papaconstantinou (1967), Papaconstantinou and Julku (1968) have suggested that gradual decrease in RNA synthesis during differentiation is due to defective processing of RNA precursors and an apparent decrease in tRNA occurs when rRNA increases. Similarly, an age-dependent
processing of nuclear and cytoplasmic RNA in eukaryotes has been suggested by Yannarell, Schumm and Webb (1977) and an involvement of timing element in the gene expression and processing of RNA has been proposed by Planta et al. (1975) also. These possibilities cannot be ruled out as suggestive mechanisms operating at molecular level of organization in hydra and analyses in these regards are in immediate purview.

The correlation between RNA degradation and an increased requirement of newly synthesized RNA for protein synthesis would be more easily explained by a precursor-product relationship between hnRNA and mRNA. In this connection, the postranscriptional modifications of messenger RNA during development and differentiation of many tissues and organisms including hydra also must be given significant importance especially with regard to the evolution of poly A containing messenger RNA. It has been shown that the dividing immature cells have high levels of synthesis of poly A containing RNA and the half-life of such RNA increases from 10 hours in the dividing cells to 50 hours in the differentiated muscle cells (Buckingham et al., 1974). Similar type of situation has been noted in differentiating neuroblastoma cells (Prasad, Bondy and Purdy, 1975). A non-coordinate increase in the synthesis rates of informational, polysomal and hnRNAs during
differentiation of Chinese hamster ovary cell has also been shown to occur (Enger and Campbell, 1975). In growing mouse fibroblasts it has been shown that while cells undergo a transition from resting to growing state, the efficiency of poly A transfer is also increased (Johnson et al., 1974; Johnson et al., 1975; Johnson and Meister, 1977). Similarly, in rabbit embryos poly A containing RNAs are synthesized as early as 16-cell stage and it further increases during subsequent stages. During regeneration in liver cells also an apparent alteration in the levels of poly A sequences has been noticed with respect stage of differentiation (Greene and Fausto, 1974). Similarly, during induced differentiation in Euglena gracilis, the kinetics of poly A containing mRNA reaches a plateau within 10 or 20 minutes after differentiation starts (Verdier, 1975). It has already been shown that fertilization of sea urchin ova elicits a 2.5 fold increase in the synthesis of poly A containing RNA it continuous through subsequent development (Slater, Slater and Gillespie, 1972). An increase in the proportion of poly A containing RNA during embryonic development of sea urchin has been reported by Frömson and Duchastel (1975) and Dolecki, Duncan and Humphreys (1977). In this systems a developmental shift in the poly A containing and lacking mRNAs has been shown
by Nemer (1975), Dubroff and Nemer (1976) and Wilt (1977). Involvement of poly A in selective gene expression during differentiation has been suggested by Sarkar, Goldman and Moscona (1973) and poly A size class distribution in mRNAs as a function of time has been shown by Gorsky et al. (1975). All these evidences suggest that significant changes occurring in poly A content in relation to the messenger RNA can bring about a considerable regulation in gene expression during development and differentiation.

The possibility exists here in hydra that since the regeneration process resembles embryonic development and differentiation to a great extent, the changes noted in the pattern of RNA synthesis might have involved considerable variation in poly A containing mRNA. It remains to see so as to how these modifications are attained and regulation occurs at posttranscriptional level during regeneration in this organism.

PROTEIN SYNTHESIS IN REGENERATING HYDRAS

When the nature of protein synthesis was studied in continuously labelled hydra undergoing regeneration, it was observed that like RNA synthesis, proteins synthesis also took place upon the very onset of regeneration and continued progressively during further periods. It has been shown previously that there occurs an increase in protein synthesis during the initial hour of hypostom
regeneration in *Hydra littoralis* (Clarkson, 1969a). The present investigation further reveals that an increase in protein synthesis takes place soon after the amputation of basal disc is made. It is suggested that like the RNA synthesis the removal of the parts by mechanical amputation might be the immediate factor in causing a burst in the protein synthesis. As differentiation proceeds one can see that the rate of protein synthesis is also increased very conspicuously. However, when compared with the RNA synthesis, certain differences could be noted in the general pattern of this synthesis. It is noteworthy that a lesser flexible pattern in observed in the latter case unlike the situation envisaged in the case of RNA synthesis. Similarly, during the basal disc regeneration only two periods of higher synthetic activities are noted, whereas in the case of hypostome regeneration, only one peak is detectable during the entire period of differentiation. It could be assumed that during the process of differentiation degradation of proteins are far less accomplished than degradation of RNA. This could be due to the fact that the molecular processes related to the synthesis and modification of RNA are more pronounced than what occurring in the protein moiety and when once the proteins are synthesized they remain more stable in association with differentiating cells.
and tissues of the organism.

However, a stochastic pattern of synthetic activity becomes more prominent when the nature of protein synthesis is analysed in the pulse-labelled hydra. Like the RNA synthesis, certain elevations and declinations are noted to occur here also. Having the pattern of RNA synthesis in mind, it seems reasonable to argue that the variations in the capacity of the system to synthesize the molecule during different hours of regeneration might positively be associated with the cellular activities taking place during the differentiation processes. In this connection, it has to be remembered that a substantial amount of RNA synthesized during the early periods of differentiation might remain in the cytoplasm as templates which may be activated at a later period of differentiation. Therefore, a homology in the pattern of RNA and protein syntheses especially with regard to a sequence of timing may not necessarily be accomplished at all. This must be one of the reasons by which the process of dedifferentiation and redifferentiation did not result in pronounced variations in the translation process. This assumption holds true more in the case of protein synthesis during hypostome differentiation especially, where one could note the occurrence of only one high peak of synthetic activity. Similarly, in pulse-labelled hydra it could be
noted that the removal of hypostome or basal disc causes a sudden increase in the protein synthetic activity. However, during the basal disc differentiation, a sudden declination is resulted immediately after the tremendous increase which closely follows the amputation. This could be due to the fact that a considerable loss of proteinaceous substances may take place through the open wound at the basal disc region which may result in a considerable reduction in the protein contents also. Such a loss of mucous substances does not take place in the case of hypostome regeneration and therefore a reduction in protein synthesis is not observed in that case. During the later periods of differentiation not much protein synthetic activities are observed and this must be due to the decelerated RNA synthesis taking place during the later hours of regeneration. Similarly, the possibility cannot be ruled out that the variations in protein synthesis might have occurred as a result of variation in the transcription processes. Since the continuous labelling studies show mostly the concentration of the proteins synthesized in the cells, a comparative assessment between the amount synthesized by a continuously labelled and a pulse-labelled hydra might as well reveal the amount of stability of the synthesized proteins.

The transition of cells from resting stage to the growing
state is accompanied by a variety of changes not only in RNA metabolism but also in the amount of protein synthetic machinery (Stanners and Becker, 1971). The rate of protein synthesis closely follows the messenger RNA content rather than the ribosomal RNA content indicating that ribosomes are not rate limiting in cells (Stanners and Becker, 1971) and suggesting that the quantity of messenger RNA determines the rate of protein synthesis immediately (Johnson et al., 1974). Profound variations in protein synthesis soon after growth stimulation of resting 3T6 cells has been shown to take place by Johnson et al. (1974) along with changes in messenger RNA content. This increase appears to result from a more efficient conversion of poly A(+) nuclear RNA into cytoplasmic messenger RNA (Johnson et al., 1975; Johnson et al., 1976a,b) rather than an increase in the rate of transcription (Naukk and Green, 1973) or an increase in the stability of mRNA (Abelson et al., 1974). While taking these possibilities into consideration, it is also noteworthy to mention that the macromolecular metabolism in hydra may be regulated by a control system, which ensures that the components of the protein synthesizing machinery are presented in correct quantities. Existence of such mechanisms operating at the molecular levels of organization has been reported in various other systems also (Mano, Suzuki and Shimatake, 1977; Yamao et al., 1977; Shiokawa et al., 1977; Benz et al., 1977).
STUDIES ON HALF-LIFE OF MESSENGER RNA

The data obtained from studies on protein synthesis in RNA synthesis-inhibited hydras suggests that templates for the first protein synthesis of the onset of hypostome and basal disc differentiation are quite stable in this system. Analysis during subsequent hours reveals that the half-life of messenger RNA in the case of hypostome differentiation is approximately 8 hours, whereas in the case of basal disc it is extended till about 18 hours after the amputation. It could be seen that the protein synthesis in RNA synthesis-inhibited hydra assumes more or less the same patterns as in controls. The effect on RNA synthesis contrasts with the protein synthesis rates, which are approximately equal in both the types of regeneration. The condition persists for many hours and the slow decay of protein synthesis in actinomycin D treated hydras suggests that during this time elaboration of stable templates of messenger RNA occurs. This indicates that although there is a burst of RNA synthesis at the early hours of both hypostome and basal disc determination, it is not really essential for the initial reconstitution processes. The initial differentiation of the proximal and distal ends of hydra cut at hypostomal and basal disc levels is perhaps accomplished by structural proteins, synthesized with help of stable varieties of messenger RNA. It could be assumed that
the release of protein synthesis from the preamputational inhibition is not a result of the synthesis of new classes of messenger RNA. In the case of hypostome differentiation proteins are synthesized till 6 hours of regeneration with the help of already preexisting templates and hereafter, the restitution process would mainly depend upon new DNA-dependent RNA synthesis. This assumption has been supported by data obtained from studies on cellular aspects of regeneration in actinomycin D treated hydrazas in which a complete suppression of hypostome differentiation was observed. (Clarkson, 1969; Datta and Chakrabarty, 1970; Datta and Mitra, 1972). The failure of complete development of hypostome in actinomycin D treated hydrazas suggests that after about 6 hours of differentiation, fresh DNA-dependent RNA synthesis is required for the attenuation of a perfect differentiation. Analysis of cellular nature of basal disc regeneration suggested that on the contrary, the differentiation proceeded to a large extent even though actinomycin D could effectively suppress the RNA synthesis (Discussed elsewhere in this thesis). This suggests that stable template in the basal disc cells could take part in performing active protein synthesis where by the differentiation is accomplished to a much better extent.

The remarkable feature observed during this study is the conspicuous contrasting nature of the two morphogenetic
entres. The potentiality residing in these morphogenetic centres in rebuilding the lost configuration in hydra has been elaborately shown by several authors previously (Discussed vide infra). But the molecular mechanisms by which these differentiations are brought about show essentially a dissimilar pattern with respect to these two different faculties. This has been clearly depicted by the extent of half-lives of messenger RNA associated with the differentiation processes in these two areas and also by their remarkable differences in the decay rates. Studies on cellular aspects of regeneration (Mookerjee and Bhattacharjee, 1966) have previously shown that essentially gland cells and interstitial cells of the same type are taking part in both the types of restitution processes in hydra. In this regard, it is curious to note that the same kind of cells at the vicinity of two different morphogenetic centres contain templates within them with different rates of activation and decay. It could be assumed that the cells descending from the growth zone of the hydra towards the distal end become progressively rich in stable templates presumably during their migration. A differential stability for messenger RNA in developing (Timberlake and Shumard, 1977; Anderson and Smith, 1977), adult (Ramsey and Burton, 1976; Lengyel and Penman, 1977; Gasaryan et al., 1977) and in cell-free translational (Hackett, Egberts and Traub, 1977) systems has also been shown to exist. Moreover,
this discrepancy observed in molecular characteristics with respect to the two opposite morphogenetic centres in hydra suggests that a type of tissue differentiation could be attributed for such a characteristics behaviour. The data tends us to presume that in the evolutionary scale, variations in molecular mechanisms associated with variations in tissue differentiation could be first noted to exist in hydra. This presumption seems tenable when it is conceived that the characteristics of a basal disc is entirely different from the characteristics of a hypostome in the sense that the structural and functional proteins associated with these two centres might basically be different. Basal disc requires more specialized proteins for fibrous and mucous substances which a hypostome does not produce and therefore the protein synthetic mechanism must be more articulate in basal disc region.

Early experiments upon the stability of messenger RNA made use of protocols in which actinomycin D was added to block synthesis of new messengers so that the decay of pre-existing messengers could be followed. The most familiar action of actinomycin D, its inhibition of DNA-dependent RNA synthesis has been mainly considered here in conformity with our results. Actinomycin D inhibits messenger RNA synthesis (Reich, 1963; Reich and Goldberg,
1964) and probably also ribosome RNA synthesis (Reich, 1963; Perry, 1963). It is important to mention that stability of messenger RNA is usually measured indirectly by assuming that stability of the messenger is expressed in dependence of protein synthesis from concomitant RNA synthesis. This is expressed as a decay of protein synthetic capacity upon exposures to inhibitors of RNA synthesis such as actinomycin D. Accumulating evidences suggest that actinomycin D could be used as an effective tool to studies related with the half-lives and stability of messenger RNA. However, in many cases it has been found that actinomycin D would prove to yield inexact estimates of messenger RNA stability (Singer and Penman, 1972; Greenberg, 1972) and that it may itself effect specific messenger RNA half-lives (Tomkins et al. 1969; Stewart, 1975). Similarly, actinomycin resistant RNA synthesis also has been shown to occur in animal cells (Stern and Friedman, 1970; Stern, Amytwanmoh and Cooper, 1973; Sargent and Raff, 1976), which may assert a control role on the transcriptional mechanism. While accounting for the half-lives of messenger RNAs in hypostome and basal disc regenerating hydras, these points have been taken into consideration in expressing the accuracy and therefore the half-lives are denoted only approximately. Singer and Penman (1973) and Sensky, Haines and Rees (1975) suggested alternate methods for measuring accurate half-
lives of messenger RNAs in cell populations and measurement of messenger RNA half-lives in hydra cells based on these methods are in the immediate purview.

In general, it is not known how stability is associated with messenger RNAs and what exactly controls the stability. It has been suggested that poly A itself may be responsible for the high stability of messenger RNA (Greenberg, 1972). This possibility has not been ruled out and it is known that poly A shortens as messenger RNA ages. If the presence of poly A confers stability one could predict that messenger RNA is degraded and lost from the polyribosomes only after the poly A has been reduced to a minimum size as suggested by Sensky, Haines and Rees (1975) and Levy et al. (1975). It is possible that in hydra also messenger RNAs synthesized in the cells could remain itself associated with proteins and remain in the cytoplasm as stable templates without getting degraded quickly. Several reports suggest that RNA and protein complexes may be in fact of considerable importance because they may be involved in determining the metabolic fate of RNA (Auerbach and Pederson, 1975; Komaromy, Molnar and Tigyi, 1975; Irwin, Kumar and Malt, 1975; Molnar and Samarina, 1975; Barrieux et al., 1975; Firtel and Pederson, 1975; Alfageme and Infante, 1975; Fernandez and Singh, 1976). Further investigations regarding messenger RNA stability in hydra cells are being carried out.
INHIBITION STUDIES WITH CORDYCEPIN

Cordycepin when used at a concentration of 100 µg/ml with a pretreatment duration of 12 hours inhibited RNA synthesis in hydra by 90%. This treatment was considered to be the optimum since concentration higher than this proved to be toxic to the system. Concentrations lesser than 100 µg/ml only retarded the incorporation and in such situations a complete suppression of RNA synthesis was not obtained.

Several reports have suggested that cordycepin inhibits RNA synthesis by inhibiting the transport of messenger RNA from nucleus to cytoplasm (Penman, Roshbash and Penman, 1970; Darnell et al., 1971; Slater, Slater and Gillespie, 1972; Adensik et al., 1972; LaTorre and Perry, 1974; Fouquet et al., 1975; Fromson and Duchastel, 1975). These studies suggest that the inhibition resulted probably from the inhibition of posttranscriptional addition of poly A to the growing messenger RNA chains. The hydra also 100 µg/ml cordycepin inhibited RNA synthesis appreciably by affecting this posttranscriptional modification. Studies related with the biosynthesis of poly A messenger RNA suggest that poly A apparently lacks in histone messenger RNA (Adensik and Darnell, 1972; Adensik et al., 1972; Greenberg and Perry, 1972; Sheldon et al., 1972; Sheldon, Jurale and Kates, 1972), and therefore, cordycepin
does not inhibit the synthesis of histone messenger RNA. Nemer (1975) and Dubroff and Nemer (1976) have shown that even among the classes of hnRNA there is a fraction which lacks poly A and there occurs a developmental shift in the synthesis of these class among other classes. Evidently, its synthesis also cannot be blocked by cordycepin, since this RNA does not contain poly A in it. It could be assumed that the fraction of RNA which remained uninhibited by cordycepin in differentiating cells of hydra might contain histone messengers as well as precursor hnRNAs which do not contain poly A in them. A 90% inhibition obtained must be substantiated on the basis that all the messenger RNAs contain poly A must have been inhibited by treatment with cordycepin.

However, when the nature of protein synthesis was studied in cordycepin treated hydras, surprisingly it has been found that the rate of incorporation showed higher yields many times during regeneration than what observed in control. This could be noted to occur especially at least at 4 periods during which the increased activity has resulted in formation of sharp peaks. In normal situation, cordycepin inhibits the messenger RNA synthesis and therefore must bring down the level of protein synthesis also to an appreciable extent. What have been observed here is that cordycepin although inhibited the RNA synthesis by 90%, could not bring down the level of
protein synthesis. On the contrary, the level of synthesis rose to higher degrees after the treatment, very much oppositely to what might occur.

On the basis of prevailing evidences it appears difficult to arrive at a conclusion with regard to this anomaly in the pattern of molecular hierarchy of differentiation in hydra. Moreover, while analysing the cellular aspects of regeneration, certain abnormalities in the pattern formation of basal disc undergoing differentiation after cordycepin treatment, has also been shown to occur simultaneously. This abnormality has taken place in the form of abrupt formation of extra basal discs at the vicinity of the distal end of the regenerating hydra apart from the normally restituted ones after the amputation of basal disc. It could be visualized that the uncontrolled pattern of protein synthesis must necessarily be a simultaneous molecular occurrence concerned with the process of formation of extra basal discs in a regenerating hydra and that these events might have been caused by the cordycepin treatment specifically. It has to be noted that such abnormalities during differentiation were not caused by actinomycin D treatments even though actinomycin D has been shown to create certain side effects on cells and tissues other than its primary function on transcription (Billet, Bowman and Puch, 1971;

One is tempted to advance certain theoretical propositions based on the data obtained and also from the current views of the action of the drug on protein synthesis. It has to be mentioned that 100 μg cordycepin must have inhibited the synthesis of all the poly A(+) messenger RNA when pretreated for a period of 12 hours and this must correspond to the 90% inhibition of the incorporation obtained. The remaining 10% of RNA which were uninhibited by cordycepin must contain histone messenger RNA, poly A(-) hnRNA and other types of RNA, if any, which do not contain poly A in them. Thus, the functions of these poly A(-) RNAs will continue even though the functions of poly A(+) RNAs are inhibited successfully. Now let us assume that during the normal course of gene expression during the process of differentiation in hydra, there exist control mechanisms both at transcriptional as well as at translational level. If it could be conceived that there are one or few translational control RNA (tcRNA) which are lacking poly A in them and which are negatively regulative in function, their functions will continue inspite of the cordycepin treatment since cordycepin inhibits the transport of only poly A(+) messengers. Evidences for the existence of such poly A(-) translational control RNA have been suggested by Bester, Kennedy and
Heywood (1975). They have isolated one of these tcRNAs from initiation factor preparations and has been shown to contain a high percentage of poly U instead of poly A. Further it was shown that this tcRNA, in fact, stimulates the synthesis of proteins coded by the polysomal RNA and the stimulation could result in the synthesis of proteins as much as 3 to 4 times higher than the normal. They have further shown that the utilization of such a negatively functional tcRNA could effectively be blocked by another class of tcRNA which is poly A(+) and the interaction of both these classes of RNA exist in the process of translation. It could be assumed that in normal situation, such a mechanism may be operative in the process of gene expression in hydra in which interaction of two classes of tcRNAs exist in the control of translation process. In such cases cordycepin treatment might have blocked the synthesis of poly A(+) tcRNA which is in nature inhibitory on protein synthesis. However, the synthesis of poly A(-) tcRNA would remain unaffected and as a result this RNA would stimulate protein synthesis especially in the absence of the poly A(+) tcRNA which would act as the counter mechanism in keeping the normal level of translation. Due to such stimulation, the rate of translation would go up and a higher amount of proteins would be yielded. Such negative control on translation
would become expressive only at situations when the synthesis of poly A(+) tcRNA would be inhibited. Such a situation has been caused in the experimental situations only in which cordycepin inhibited all the poly A(+) RNAs, including the poly A(+) tcRNA. Whereas the negatively functional poly A(-) tcRNA remained functional in elevating the rate of protein synthesis to a higher amount that in controls. It could be assumed that the morphological abnormality noted in the form of formation of extra basal disc near the vicinity of the originally regenerating basal disc is resulted due to the uncontrolled protein synthesis. Since the normal functioning of the gene activity underwent an alteration and it paved the way for continuous synthesis of basal disc proteins, nevertheless, the abnormal gene functioning became expressive in the form of production of miniature basal discs in the vicinity of the original basal disc. Existence of low molecular weight RNAs similar to tcRNA has been reported from the embryonic cells of Drosophila (White, 1973) and from the reticulocyte ribosomal wash (Bogdanousky, Hermann and Schapira, 1973); the latter has been found to stimulate α-globin synthesis (Fuhr and Overton, 1975).

The data shows that increased level of protein synthesis are observed at certain hours only and during
the other periods, it remain low and even remarkably lower than the control. This could be explained in the following way. First, the above mentioned mechanism of regulation of protein synthesis might be operating only during particular hours of regeneration such as 4th, 8th, 14th, 22nd, 26th and 30th respectively and these periods represent the crucial stages of differentiation of the basal disc such as the delamination of ectoderm, morphogenetic activities of gland cells, dedifferentiation and redifferentiation of interstitial cells etc. Protein synthesis taking place during these particular acts of differentiation must be given considerable importance when the whole spectrum of regeneration is taken into consideration. During other periods, the synthesis may not be much significantly needed for the restructuration process in the sense that proteins synthesized during periods such as 6th, 10th, 14th, 20th, 24th etc. may not be very essential for the structural accomplishment since these periods do not represent any crucial stages of differentiation. It could be assumed that the protein synthesis during these periods may be functionally less important also that a regulatory mechanism may not be involved with it, resulting ultimately in a low plateau. It is also probable that even if there is any regulatory mechanism operating during these
these periods, the involvement of negatively functioning tRNA is probably absent. Thus, the considerably low amount of protein synthesis during those periods directly reflects upon the inhibitory action of cordycepin on protein synthesis by blocking the messenger RNA transport. However, the period of functionally weaker protein synthesis would ultimately change when a crucial stage of differentiation follows and during that time, since cordycepin does not inhibit the tRNA which is stimulatory, once again the level of protein synthesis would go up. In this way, the concomitant increase and decrease in the protein synthesis would ultimately result in a highly variable pattern as observed. However, it is yet to be analysed whether the stimulation has occurred as a result of alteration in the secondary structure of messenger RNA by the drug as showed by Leinwand and Ruddle (1977), in the wheat germ embryo extract and reticulocyte lysate assays for in vitro protein synthesis. One could also think of a possibility of cordycepin affecting the permeability of cells during certain periods of regeneration thus enhancing the incorporation of the radioactive precursor. But with the prevailing evidences on the mechanism of action of the drug (Rose, Bell, and Jacob, 1977), this possibility could be ruled out as a suggestive mechanism. However, before
accepting this working hypothesis, further analyses have to be carried out to prove that to RNAs exist in differentiating cells of hydra and that they play a regulatory role in the translation process associated with the reconstitution of lost parts.

**INHIBITION STUDIES WITH CYCLOHEXIMIDE**

The present investigation has proved to yield the specific concentration of the drug which would effectively inhibit protein synthesis in differentiating hydas. Numerous reports suggest that cycloheximide is an effective inhibitor of protein synthesis, acting at translational level (Terasima and Yashukawa, 1966; Kim, Gelbard and Perez, 1968; Richards, Ryan and Manning, 1971; Wanka and Schrauwen, 1971; Schneiderman, Dewey and Highfield, 1971; Hyodo, Koyama and Ono, 1971; Drlica and Knight, 1971; Obrig et al., 1971; Rajalakshmi et al., 1971; Kisilevsky, 1972; Farber and Farmer, 1973). The effects of the drug on the cellular process of hypostome and basal disc regenerating hydas have been discussed elsewhere in this thesis.

**POLYACRYLAMIDE GEL ELECTROPHORESIS OF PROTEINS**

A qualitative and quantitative change in the pattern could be inviaged when proteins from normal, regenerating and cordycepin treated hydas were analysed on polyacrylamide gels. It is noteworthy that the accomplishment of
proteins are less achieved in a regenerating hydra than in a normal even though a concomitant increase is noted in the synthesis following amputation of any parts. This has been mainly reflected as considerable reduction in the amount of proteins corresponding to bands 1, 2 and 4 specifically, in a 24 hours hypostome regenerated hydra. In the assortment of proteins in basal disc regenerating hydras, it could be seen that the proteins corresponding to bands 1 and 2 have appeared similarly as in control by about 24 hours of regeneration. This suggests that the accomplishment of proteins, presumably structural, is more achieved in the differentiating basal disc than in the differentiating hypostome. However, here also a significant reduction in the amount could be noted in the fractions corresponding to bands 3 and 4. It appears that an elaborate manifestation of all the kinds of protein are attenuated only after complete differentiation is achieved and the reduction in the amount noted might have been caused by the mechanical removal of the parts. However, it is interesting to note that with the loss of parts, none of the proteins originally present in the normal hydra remain unrepresented. It could be assumed that the restitution process might be involving only an elaboration of proteins present in the regenerating hydra and due to this, a functional difference may not
be attributable between the protein moieties in normal and regenerating hydras.

Most significant was the pattern analysed from regenerating hydras underwent cordycepin treatment. Remarkable differences were obtained in the prominence of the protein fractions suggesting that these involve qualitative and quantitative differences as well. The appearance of these new proteins probably with different molecular weights and their quantitative increase must be resulted due to the uncontrolled protein synthesis after the treatment with cordycepin. Isolation of these new proteins and their characterization are in the immediate purview of study.

There are reports indicating the appearance of new proteins during differentiation. Weeks and Collis (1976) have shown that formation of new tubulin proteins results from deflagellation in *Chlamydomonas reinhardii*. Modifications were observed in the assemblage of proteins during oogenesis (Hallberg and Smith, 1975) and also after partial hepatectomy (Anderson, Grundholm and Sells, 1975). It could be assumed that the wound inflicted mechanically upon the organisms might act as an external stimulus to stimulate the synthesis of hypostome as well as basal disc characteristic proteins. Beekendrof and Kafatos (1976) have shown that in *Drosophila*
the glue proteins are begin to be synthesized at about 106 hours after the egg deposition and synthesis of several new proteins take place at different developmental stages. Similar stage specific protein synthesis and their developmental shifts could be assumed as one of the characteristics of differentiation in hydra.