

S U M M A R Y

CHAPTER - I SECTION : I

In order to evaluate the possible as well as extent of involvement of blood in the process of regeneration, an analysis of blood in terms of its cellular composition and haemoglobin content was undertaken in the Scincid lizard, Mabuva carinata during its tail regeneration. The investigation proved rather interesting and showed definite changes in the form of an increase in red blood cells and haemoglobin, and changes in the white blood cell population, both, total as well as differential. These changes have been construed to indicate the involvement of haemopoietic system in the mechanics of regeneration and the various changes that are observed in the blood are discussed in terms of metabolic intricacies at the site of regeneration, cellular source for the formation of regeneration blastema and the role of monocytes during regeneration.

SECTION : II

In order to evaluate the possible as well as extent of involvement of blood in the process of wound healing, as well as to serve as a control for the corresponding evaluation carried out during regeneration, an analysis of blood in terms of its cellular composition and haemoglobin content has been undertaken in the Agamid lizard, Calotes versicolor during its postamputational tail wound healing. The investigation recorded definite changes in the form of a reduction in the R.B.C. count on the 5th day, and a subsequent increase on the 7th and 12th days, appearance of macrocytes on the 5th and 7th days as well as an increase in the population of lymphocytes and neutrophils and a decrease in the other granulocytes. Though the fall in the red cell count appears enigmatic the appearance of macrocytes has been correlated with the maintenance of a normal level of haemoglobin even in the wake of a fall in the red cell count. The changes in the white blood cell population are discussed and correlated with a number of aspects associated with wound closure.

CHAPTER - II SECTION : I

In response to the observed changes in blood during tail regeneration, a detailed histophysiological study of the haemopoietic organs such as liver, spleen and bone marrow was undertaken. During regeneration, all the three organs were found to undergo histophysiological changes. Increased haemopoietic activity in the marrow and hyperplasia of the white pulp were the important changes shown by the bone marrow and spleen respectively. In the liver, the most marked effect appeared to be the formation of large number of lymphocytopoietic nodules. Another interesting observation was the influx and destruction of red blood cells both in the liver and spleen, once during the early regressive phase and once during the late progressive phase of regeneration. An attempt is made to correlate these changes and observations in the three haemopoietic organs with the possible involvement of lymphocytes in the formation of a regeneration blastema and also a possibility of haemoglobin transition during regeneration. Moreover, the

present observations also correlate well with the previous observations on blood.

SECTION : II

An investigation of the histophysiological changes undergone by the haemopoietic organs (Liver, spleen, bone marrow) during normal wound healing in the Agamid lizard, Calotes versicolor was undertaken not only to serve as a suitable control to the corresponding studies on tail regeneration, but also to ascertain the involvement of systemic factors in the mechanics of wound healing in lizards in general. The liver and the bone marrow were noted to remain more or less insensitive excepting for a depletion of marrow reserves during the early period of wound healing which however is correlated along with the previously observed fall in R.B.C. count as more of a replenishing measure. The response of the spleen is correlated with the increase in the circulating lymphocyte population recorded previously. The most interesting aspect of the present investigation is the development of a

fatty liver condition during the 5th and 7th days postamputation; however, it is rather impossible to offer any explanation in this regard at the present juncture.

CHAPTER - III SECTION : I

Changes in the hepatic glycogen content and blood glucose level were investigated during the various phases of tail regeneration in the Scincid lizard, Mabuva carinata. The investigation revealed an initial glycogen depletion from liver during the wound healing period, a recovery during the preblastemic phase and a further depletion during the early differentiation phase. Thereafter by about the late differentiation phase the hepatic glycogen content attained its normal preautotomy level. The blood glucose level showed an initial hyperglycemia followed by a hypoglycemia and a pronounced hyperglycemic level during the blastemic to early differentiation phases of regeneration. The glyceic condition also touched its normal level by about the late differentiation phase. The results obtained herein definitely indicate an

involvement of hepatic glycogen and blood glucose in the regenerative mechanics and have been discussed in detail in the text.

SECTION : II

Changes in the hepatic glycogen content and blood glucose level were investigated during wound healing after tail amputation in the Agamid lizard, Calotes versicolor. The investigation revealed an initial mild glycogen depletion on the 3rd day and a recovery to the normal level by about the 5th day itself after tail amputation. Correspondingly the blood glucose level showed an initial hyperglycemia on the 3rd day to be followed by a hypoglycemic condition on the 5th day and a recovery to the normal range by about the 7th day itself. These results, taken together with those obtained during tail regeneration in Mabuva carinata have been construed to indicate the relative insignificance of carbohydrate metabolism in Calotes during its tail wound healing and a detailed discussion in this context is undertaken in the text.

CHAPTER - IV SECTION : I

In order to understand the extent of involvement of systemic lipids both direct as well as indirect, a quantitative evaluation of the changes in the hepatic content of total lipids and glycerides as well as the level of blood lipids was undertaken in the Scincid lizard, Mabuva carinata during its tail regeneration. The investigation has shown definite involvement of systemic lipids in the regenerative mechanics as denoted by a fall in the hepatic total lipid and glyceride contents, once during the first three days (early phase of wound healing) and once during the 7th and 12th days (blastemic phase) of regeneration. These falls in the lipid contents are correlated with the appearance and utilization of lipids by the regenerating tail as well as the utilization by the various other body tissues such as liver and blood, in their new functional garbs taken up to support the process of regeneration. The maintenance of a subnormal level of lipids in the blood all throughout regeneration is construed to indicate the fast paced withdrawal and utilization by the

various tissues during the first half of regeneration and the active uptake by organs such as liver, fat bodies and the regenerate in their geared up lipogenesis to attain the normal histophysiological features during the second half of tail regeneration.

SECTION : II

The sparseness of information regarding the metabolic aspects in the repair process in reptiles, as well as the necessity to confirm the changes observed in *Mabuya* as specific responses elicited due to regeneration itself, had stimulated the present investigation on total hepatic lipids, glycerides and blood lipids in the Agamid lizard, *Calotes versicolor* during its postamputational tail wound healing. As compared to *Mabuya*, in the present case, the study tends to indicate an entirely different and diametrically opposite pattern of changes, thus corroborating and strengthening the inherent differences between regeneration and mere wound healing processes. A series of fluctuating changes marked by an initial

steatosis (3rd day) succeeded immediately by a depletion (5th day), again followed by a more pronounced steatosis and development of fatty liver (during the 7th and 12th days), and, followed by a recoument to the normal condition (25th day), is the feature of the present study. The functional significance as well as the associated controlling mechanisms all remain completely enigmatic at the present juncture and more detailed investigations are warranted.

CHAPTER - V SECTION : I

In the light of the previously observed changes on blood lipids, hepatic lipids and glycerides, during tail regeneration in Mabuya carinata, changes in the other lipid fractions too such as phospholipids and cholesterol were assayed so as to have a clear understanding of the involvement of systemic lipids in regeneration. All the three parameters under investigation (hepatic phospholipids, hepatic cholesterol and blood cholesterol) did show certain fluctuations during the course of regeneration. The increase

and decrease in the content of hepatic phospholipids are correlated with lymphocytopoietic activity, influx of R.B.Cs, as well as transport from the wound site, and, release of lymphocytes from the nodules, possible involvement in the formation of serum lipoproteins as well as the formation of bile, respectively. The increased content of cholesterol on the 12th day and its depletion by the 25th day are construed to indicate an active biosynthesis, and its subsequent utilization by the various endocrine organs for the elaboration of various cholesterol based anabolic steroids, as well as by the tail regenerate respectively. The slightly above normal levels of all the three parameters obtained herein are attributed to the operation of a strong anabolic influence during the later half of regeneration.

SECTION : II

A quantitative evaluation of cholesterol in liver and blood, and, phospholipids in liver was undertaken so as to have an idea of the extent of

involvement of systemic factors in mere wound healing and also to serve as a suitable control to the corresponding studies on regeneration. The study revealed the interesting observation of a continuous and precarious fall in the phospholipid content of the liver from the time of tail amputation until the 12th day. Thereafter the phospholipid level registered a gradual increase. On the other hand, the hepatic cholesterol level after an initial slight fluctuation on the 3rd and 5th days postamputation, rose to ^avery much above normal level on the 7th day and, though the above normal level was maintained even thereafter, the level nevertheless recorded slight and gradual fall on the 12th and 25th days. The blood cholesterol level showed a very slight but gradual decline during the first 5 days and thereafter through the 12th and 25th days the blood cholesterol level retraced a gradual return to the normal level. These changes are chiefly correlated with the requirement at the wound site as well as the development of the fatty liver condition and its subsequent recovery.

CHAPTER - VI SECTION : I

In order to understand the role of visceral fat bodies in regeneration, a histomorphological analysis of the fat bodies in Mabuya during its tail regeneration was undertaken. The investigation revealed two interesting aspects; a visible shrinkage and a fall in the histosomatic index, as well as enhancement in haemopoietic activity and infiltration of lymphocytes during the first seven days of tail regeneration. These observations are taken to indicate the involvement of visceral fat bodies in the regenerative mechanics. The appearance of lymphocytes on the 5th and 7th days and their subsequent disappearance together with lipid depletion are construed to indicate the possible functioning of lymphocytes in lipid transport during their passage to the wound site.

SECTION : II

In the light of the observed haemopoietic activity and the involvement of fat bodies in the regenerative mechanics of Mabuya, a histomorphological analysis of the fat bodies in Calotes too was undertaken to

evaluate the possible involvement of fat bodies in the postamputational tail wound healing of lizards. The study, however, yielded no noteworthy changes whatsoever thus leading to the conclusions that (a) the fat bodies of Calotes are not haemopoietic and (b) that the fat bodies do not play any significant role in the normal wound healing mechanics of lizards.

CHAPTER - VII SECTION : I

To assess the extent of involvement of liver in the regenerative mechanics, a quantitative estimation of the hepatic protein, DNA, RNA and water contents was undertaken. The investigations revealed an increase in RNA content, RNA/DNA ratio, as well as protein content during the first twelve days of regeneration. This is believed to indicate the high protein turnover of the hepatic tissue during early period of regenerative process. The possibility of elaboration of regeneration inducer and/or promoter proteins by the hepatic tissue is also envisaged. The high protein content of the hepatic tissue recorded towards the end of

regeneration is attributed to a number of extrahepatic sources and factors.

SECTION : II

A quantitative evaluation of hepatic protein, nucleic acids and water contents was conducted in Calotes during its postamputational tail wound healing to have an idea of the involvement of these factors in the healing mechanics as well as to serve as a suitable control to the parallel studies carried out in Mabuya during its postautotomy tail regeneration. The results accrued herein revealed an insignificant change in the case of nucleic acids, and fall in the protein content, once during the 3rd day and once during the 7th day. These falls in the protein content have been correlated with the possible injury induced catabolic depletion of labile proteins as well as the possible interconversion into lipids (in association with the previously observed increased steatogenesis and fatty liver development).

CHAPTER - VIII SECTION : I

In the wake of the known involvement of ascorbic acid during lacertilian tail regeneration, a quantitative evaluation of hepatic and renal ascorbic acid contents of Mabuya was undertaken during the various periods of its tail regeneration. Excepting for an initial decrease on the 3rd day the hepatic AA content remained more or less in the normal range, thus indicating possible insensitiveness of hepatic AA in the regenerative mechanics. On the other hand, the renal AA content recorded significant changes in the form of an increase on the 3rd day, a fall on the 5th day and an increased above normal level on the 7th through 25th days of regeneration. This is construed to indicate the definite involvement of renal AA content in the regenerative mechanics.

SECTION : II

The changes in the renal, hepatic and caudal AA contents were assayed during the postamputational tail wound healing in Calotes versicolor. Since the

renal AA content never attained an above normal level, it is presumed that the mere wound healing process in a lizard which does not have power of regeneration, does not call for an enhanced AA elaboration. However, the renal AA content recorded a fall twice during the course of wound healing; once during the first five days and once on the 12th day. An attempt is made to correlate these falls with the requirement of the vitamin at the wound site, its utilization by other organs as well as transport to liver. On the other hand, the hepatic AA content recorded a fall on the 3rd day to be immediately followed by an increase to an above normal level on the 5th day. Since then, subsequently on the 7th day, there was a fall to the normal level which was maintained so thereafter. Though the fall observed on the 3rd day is attributed to a generalised stress response, the attainment of an above normal level on the 5th day is correlated with the pathophysiological mechanics associated with the fatty liver development. During wound healing, the caudal tissue was noted

to show an increase in its AA content during the first 5 days and a gradual decrease thereafter, thus denoting a transport of AA to the wound site and its subsequent utilization in the repair of injured tissues and formation of the dermis.

CHAPTER - IX

ATPase was studied histochemically in the normal and regenerating tail of the Scincid lizard, Mabuva carinata. The study revealed a highly elevated level of enzyme activity during the blastemic and differentiation phases of regeneration. Even the wound healing phase was noted to show an above normal level in comparison to the adjoining stump tissues. The high level of enzyme activity during tail regeneration is correlated with processes such as transport, increased energy flux etc. The fully regenerated tail, with the completion of the process of regeneration depicted a level of ATPase activity which was identical in intensity and localization to that observed in the corresponding normal tail.

CHAPTER - X

GDH activity was investigated histochemically in the normal and regenerating tail of the Scincid lizard, Mabuva carinata. The enzyme activity was noted to show an increase subsequent to tail autotomy and a high level of its activity was discernible during the blastemic and differentiation phases of regeneration. Whereas the increased incidence of GDH noted during the wound healing phase at the cut ends of the stump tissues has been implicated in the lytic process leading to the cellular demolition and dedifferentiation, the high enzyme activity registered during the blastemic and differentiation phases of regeneration is correlated with the synthesis of proteins of the glutamate family. With the completion of the process of regeneration, the enzyme activity within regenerate too, settled down to the normal level.

CHAPTER - XI

Histochemical investigations of the hexo and glucokinases was carried out in the normal and

regenerating tail of the Scincid lizard, Mabuza carinata. From the low level of activity characteristic of the normal tail, the tail regenerate during the wound healing, blastemic and differentiation phases depicted a highly elevated level of enzyme activity. The role of hexokinase in glucose uptake and utilization during the blastemic and early differentiation phases have been envisaged. Moreover, the possible functioning of two hexokinases; a nonspecific hexokinase and a specific glucokinase during the blastemic to early differentiation and late differentiation phases respectively has also been discussed. With the completion of the process of the tail regeneration, the enzyme activity in the regenerate attained the characteristic preautotomy pattern of localization and intensity.

GENERAL CONSIDERATIONS WITH A COMPARATIVE
EVALUATION THEREOF-VIS A VIS-THE INVOLVEMENT
OF SYSTEMIC FACTORS, IN REGENERATION AS
OPPOSED TO WOUND HEALING IN LIZARDS.

A preliminary attempt has been made in the present study to evaluate the involvement of systemic responses as mediated by the various visceral factors in the healing mechanics as well as, the repair and regeneration processes of lizards. Such an investigation finds ample justification when viewed in the perspective that the processes of repair and regeneration though essentially constituted of local events cannot, however, be studied in seclusion or in total exclusion of the animal body as the regenerating appendage forms an intergral part of the animal body. An exhaustive analysis of metabolic adaptations and intricacies underlying the process of lizard tail regeneration at the local site has been undertaken previously in this laboratory, (Shah and Chakko, 1967, 1969; Magon, 1970; Shah and Hiradhar, 1974; Hirdhar, 1972; Radhakrishnan, 1972; Radhakrishnan and Shah, 1973; Ramachandran, 1972;

Shah and Ramachandran, 1970, 1972, 1973, 1974, 1975, 1976; Ramachandran et al., 1975). These studies enabled in elucidating the metabolic transformations, characteristic of regeneration, as denoted by a metabolic flux from a carbohydrate oriented one to a lipid oriented one and again back to the carbohydrate one during the progression of the tail regeneration. Moreover, the differential operation of the various metabolic pathways such as the glycolytic pathway, HMP shunt pathway and TCA cycle oxidations as well as the significance and role of sodium and potassium ions and ascorbic acid were also brought out. Such a broad spectrum of local events and responses would necessarily have to be supplemented and supported by the effective participation of many visceral factors of systemic origin. A precisely programmed and specifically synchronised subtle interplay of these factors could be considered to be inseparably interlinked with the various intricate local events and responses associated with the regenerative mechanics. In this perspective a number of parameters such as levels of metabolites

and nucleic acids in the liver, levels of ascorbic acid in the liver and kidney, level of glucose, cholesterol, and total lipids in blood as well as the haematopoietic response have all been analysed and assessed in the present study in terms of the varying vagaries, as well as the stimulus of regeneration in the lizard: Mabuya carinata.

Identical parameters have also been investigated in another lizard, Calotes versicolor, during its tail wound healing under the stress of amputation, so as to bring out the differential responses involved in mere wound healing as opposed to regenerative potential and to establish the controlling mechanisms brought into operation in Mabuya carinata as being specifically due to regeneration.

The data that is accrued from the present investigations, denote the participation of complex but specific set of events (which could be referred to as the systemic responses and as could be made out by the modulation involved in the various parameters studied herein) as accompniment to the regenerative phenomenon. Previous enzymological

studies on tail regeneration in Mabuya carinata (Ramachandran, 1972; Shah and Ramachandran, 1970, 1972, 1974, 1975, 1976; Ramachandran et al., 1975), had indicated, both, an increased exigency of oxidative metabolism as well as the active involvement of lipids in the molecular ecology of blastema and differentiation phases of regeneration. The results of the current investigations too tend to strengthen the above contention and highlight the prevalence of an elevated overall metabolic activity in an induced oxidative environment. The induction and maintenance of a high oxidative environment could be easily visualised from the observations of a tremendous increase in red blood cell count as well as a corresponding parallel increase in the haemoglobin content during the first week of tail regeneration. (Chapter-1). Certain other compelling evidences that could be drawn towards this contention are the changes observed in the colloidal content of the thyroid acini as well as the increase in concentration of certain oxidative enzymes in the liver during the same period. (Unpublished observations). The results obtained

in the case of Calotes wherein neither an increase in R.B.C. count nor the haemoglobin content have been registered postamputation, also lend added support and validity though indirectly, in this connection.

The immediate systemic response to the stimulus of autotomy and the commencement of the regenerative process (wound healing) appears to be the depletion of liver stored metabolites, marked by the decrease in the content of both glycogen as well as lipids (Chapters- 3, 4). Ramachandran (1972) based on the metabolic studies of the regenerating tail of Mabuva carinata had hinted at the possible association of lipids with the regenerative wound healing and queried as to whether lipids could be purported to supply the necessary stimulus or impetus for the process of regeneration? In this wake the currently observed continuous depletion of lipids from both, the liver during the first 3 days of regeneration (Chapter - 4) as well as the fat bodies during first seven days (Chapter-6) are rather noteworthy and signifies the dominant role of lipids in the early phase of the regenerative

mechanics. This becomes all the more clear when viewed in the background of the observations made in Calotes during its post amputational tail healing wherein the lipid content of neither liver nor fat bodies showed any decline whatsoever at any stage during the whole period of the study (Chapter - 4). Such a set of differential responses invoked in the two cases, thus might represent one of the differences involved between the two types of wound healing and emphasizes the fact that lipids are somehow of pivotal importance in giving the necessary impetus to the process of regeneration. This then brings into focus the different set of controlling mechanisms brought into operation and might signify that, in the lizards with regenerative potential either, (a) the storehouses of lipids such as the the liver and fat bodies embody within them some sort of latent inherent sensitivity by which they respond to the stimulus of autotomy and the subsequent repair processes by an immediate and ready release of lipids or, (b) certain specific intrinsic humoral or other controlling factors are elicited which in turn bring about the continuous

systemic release of lipids. Though it would be rather ill founded to conceive the participation in toto of all the lipid molecules in the molecular ecology of the regenerating system (especially in the wake of the depletion of systemic lipids as well as the inherent local synthetic capacity accredited to the regenerating system, Ramachandran, 1972; Radhakrishnan, 1972; Shah and Ramachandran, 1970, 1972, 1973, 1974), it could be easily presumed that quite a bit of the systemic lipids must be getting effectively channelised into the increased energy yielding reactions of the various tissues and organs of the body in the prevailing high aerobic environment. Moreover, the utility value of lipids in many other processes brought into operation specifically to meet the exigency of regeneration such as increased haematopoiesis, hepatic lymphocytopoiesis (Chapters- 1, 2) ascorbic acid synthesis in kidney (Chapter - 8) as well as in many other metabolic transformations involving other metabolites such as proteins, glycogen, glucose etc. (Chapters- 3, 5, 7) also cannot be overlooked.

Though in the case of Calotes there was no comparable systemic lipid depletion, there was, however, an interesting paradox represented by the development of a fatty liver condition. The source of this tremendous hepatic lipid content cannot however, be any extrahepatic organ as even fat bodies are noted to show a slightly increased histosomatic index rather than a decrease (Chapter - 6). However, both the hepatic phospholipids as well as proteins are depicted to show inverse changes with the neutral lipid content during the whole course of fatty liver development in Calotes. Possible involvement of phospholipids and proteins in the process is further strengthened by the observable increase in the value of both these parameters with a concomitant reduction in the hepatic neutral lipid content marking the reversal and recovery from the fatty liver condition during the 12th and 25th days postamputation (Chapters- 4, 5, 7). It could be circumstantially presumed that the hepatic neutral lipids, the phospholipids and proteins are somehow involved in the interconversions leading to both the development as well as recovery of the fatty liver condition that occur during

wound healing subsequent to tail amputation. A more detailed investigation in this direction would be fruitful and is warranted. Such an investigation would not only enable in confirming the validity of the present tentative concept but also would help in understanding the possible involvement of other factors too in the process. It would also enable us in understanding the etiology of fatty liver development, and also underscore the humoral and other controlling mechanisms involved therein in the present case. Moreover, it would also be interesting to know, whether this phenomenon is a species specific individually isolated case, or, whether even other lizards with no regenerative potential would show a similar change in response to the stimulus of amputation and wound healing; in which case, the possible functional significance and/or even the adaptational value of such a biochemical upheaval, if any, would also be of some interest and conjecture.

Glycolysis is known to be the principal feature of the regenerating system from the period immediately subsequent to tail autotomy till the completion of the

wound closure (Radhakrishnan and Shah, 1973; Shah and Ramachandran, 1970, 1972, 1973, 1975, 1976). In this perspective, the continuous depletion of hepatic glycogen noted through the first five days postautotomy, is rather striking, and might indicate the functional significance of carbohydrates of systemic origin in the wound closure associated with regeneration. Furthermore, the hepatic glycogen might also have other additional roles such as immediate energy substrates for the various metabolically activated tissues and organs of the body including liver itself, or even serve as the precursor molecules in the synthesis of ascorbic acid. The observations of a subnormal blood sugar level between the 3rd and 5th days of tail regeneration (Chapter-3), and the increase in the ascorbic acid content of the kidney subsequent to tail autotomy (synthesis^{ing} organ; Chapter-8), are both relevant in this connection. Currently noted depletion of systemic glycogen and lipid contents in the initial periods of regeneration subsequent to tail autotomy, are definitely indicative of the setting up of a complex set of metabolic events

and transformations, as a prelude to meet the increased biochemical requirements of the ensuing regenerative process. Such an exigency of increased involvement of systemic factors in regeneration, is further strengthened^{ne} by the observed relative insensitivity of the hepatic glycogen content in the post tail amputation period in Calotes.

The maintenance of the various systemic responses initiated immediately after tail autotomy, even after the wound healing phase, is well exemplified by the recorded observations of yet another depletion of hepatic glycogen and lipid reserves during the 7th to 12th days postautotomy, which arbitrarily correspond to the blastemic to early differentiation phases of tail regeneration. Both these observations appear relevant, and well in tune, when equated with the earlier studies from this laboratory on tail regeneration; wherein the accumulation of lipids in the blastema and its participation with the commencement of histodifferentiation in the various anabolic as well as energy yielding reactions, as well as the possible involvement of glucose as the sole source

of metabolic substrate for the mesenchymal cells of blastema have been highlighted (Radhakrishnan, 1972; Radhakrishnan and Shah, 1973; Shah and Ramachandran, 1970, 1972, 1973). The loss of glycogen from the liver, and the concomitant, increased blood sugar level during this period of regeneration, are interesting, and the maintenance, of a high level of glucose in the blood might be an effective mode of meeting the requirements of the blastemal cells (as has been outlined in Chapter - 3) especially in the wake of the known poor capillarity of the regeneration blastema. It is quite significant, that in the case of Calotes, no such drastic changes could be visualised at this period. The only feature of any significance at this stage in Calotes, is the observed attainment of a maximal fatty liver condition, with the hepatic glycogen and blood glucose levels remaining more or less at the normal level.

Another aspect of interest and much controversy associated with the process of regeneration, that gains some clue from the present study is, regarding

the source and origin of the blastemal population of the mesenchymal cells. Increased level of lymphocytes in the blood, stimulated activities of haemopoietic organs such as bone marrow and spleen, as well as the most important observation of lymphocytopoietic activity in both liver as well as fat bodies (Chapters- 1, 2, 6) are all tempting and tend to project the lymphocytes of systemic origin, as a major if not the sole source of cells of the regeneration blastema in the lizard, Mabuya carinata. Moreover, the time of release, or disappearance of lymphocytes from the liver, spleen and fat bodies, is also very significant and corresponds well with the formation of the blastema ie between the 7th to 12th days postautotomy. Again, no comparable changes of this nature could however, be recorded in the case of Calotes after its tail amputation, thus emphasizing once again the contrastingly distinct nature of the differential set of systemic responses that are set into motion in lizards on the one hand with regenerative ability as opposed to lizards on the other hand with no regenerative potential.

With the commencement of histodifferentiation and its progression, a gradual process of emancipation from the overtly manifested dependency on the metabolic factors of systemic origin by the tail regenerate, could be easily visualised by the observed attainment of the normal levels of glycogen and lipids in the liver, and glucose in the blood, by about the 25th day of tail regeneration. This when viewed in the light of the reported acquisition by the regenerate, of storing potential (of metabolites) as well as the necessary enzymatic machinery to actively metabolise and bring about various biochemical transformations, during the differentiation phase of tail regeneration (Radhakrishnan, 1972; Radhakrishnan and Shah, 1973, Shah and Ramachandran, 1970, 1972, 1973, 1974, 1975, 1976) tend to indicate the attainment of a greater degree of independence by the regenerate, thus becoming more or less self-sustaining in supporting and maintaining the process of regeneration hereonwards.

Involvement of ascorbic acid too, had been highlighted in the regenerating tail of Mabuaya

carinata (Ramachandran et al., 1975). Quantitative assay^a of ascorbic acid content in kidney and liver (the synthetic and storage organ of ascorbic acid respectively in the lizards) carried out in the present study during the various phases of tail regeneration, to elucidate, possible systemic involvement has given sufficient positive indications to this effect. Comparable evaluations carried out in Calotes during its postamputational tail wound healing have also brought out the divergent responses inherent between regeneration and wound healing. The only similar features identifiable in both the cases appear to be the accumulation of ascorbic acid at the wound site during the wound healing period (Ramachandran et al., 1975; Chapter - 8) and, the role of the hepatic tissue as the immediate source of supply of ascorbic acid, as marked by the depletion of ascorbic acid content noted in both the lizards (Mabuya and Calotes) on the 3rd day postautotomy/ amputation respectively. A relatively higher systemic participation in meeting the increased requirements for ascorbic acid by the regenerate

in Mabuya is denoted by the increased renal ascorbic acid content on the 3rd and 7th days postautotomy. Interestingly enough, in the case of Calotes there was a persistent ascorbic acid depletion from the renal tissue till the 5th day postamputation with no increase to an above normal (preamputation) level at any stage. Though the fall on the 3rd day could be explained in terms of meeting the requirements at the wound site, the further decline in the renal ascorbic acid content noted on the 5th day, which incidentally corresponded with the attainment of an above normal level of ascorbic acid in the hepatic tissue, will have to be viewed in a different context, and might indicate some association of ascorbic acid in the liver with the fatty liver development as discussed in Chapter - 8. However, thereafter the ascorbic acid content in both liver as well as the kidney attained the normal preamputation levels, thus indicating the relatively restricted and nominal participation of the systemic ascorbic acid in the healing mechanics. On the contrary, in the case of Mabuya, the maintenance of above normal levels

(though slight) of renal ascorbic acid content even on the days 12th and 25th postautotomy, is suggestive of an active systemic participation in supplying the necessary ascorbic acid required during the process of tail regeneration. The maintenance of the slightly above normal levels of ascorbic acid in the kidney on the 12th and 25th days of regeneration, more or less in the same level, subsequent to the increased level noted on the 7th day is indicative of an increased participation of the renal tissue, wherein the rate of elaboration and the rate of release keep pace with each other, or perhaps is suggestive of a decreased participation of the renal tissue is debatable. If the latter be the case, then in the context of the five fold increase of the ascorbic acid content reported in the regenerate during this period (i.e. the differentiation phase), the possible acquirement by the regenerating system of the necessary enzymatic machinery concerned with the synthesis of ascorbic acid may have to be conceded. However, the significant increased levels of renal and hepatic ascorbic acid recorded on the

60th day of tail regeneration whence the regenerating process has more or less come towards its termination, rather discounts the above possibility and tends to validate the former contention of an increased renal participation, wherein subsequent to the sudden cessation in the requirement and utilization of ascorbic acid by the regenerate, as well as possibly other visceral organs, there is an immediate piling up of ascorbic acid in both kidney as well as the liver. In any event, more detailed investigations aimed at both, an understand^{ing} of the status of the regenerating system as a possible site of ascorbic acid synthesis, as well as the identification of the control mechanisms involved in the regulation of systemic release of ascorbic acid, would enable in projecting a better picture on this topic. Again, the present study on the ascorbic acid content of the healing amputated tail in Calotes versicolor has revealed an increase to a double the normal level on the 5th day of healing. Similar two fold increase has also been reported in the case of Mabuya (Ramachandran et al., 1975). Eventhough,

subsequently there was a gradual fall in the ascorbic acid content of the healing tail in Calotes, the level even on the 25th day remained above normal. In comparison to this in the case of Mabuya, the ascorbic acid content of the tail fell by half in the blastemic phase, which corresponds to 7th to 12th days of regeneration. This when viewed in the backdrop of the known fact, that ascorbic acid plays an important role in collagen fibrillogenesis, and that, in Mabuya, there is little or no dermal substance formed during wound healing, tends to purport an increased rather than a reduced utilization; thus lending added credence to the earlier suggestion of Ramachandran et al., (1975) that ascorbic acid might be participating in the respiratory mechanics of the regeneration blastema.

Proteins are known to play an integral role in development and differentiation, and in this light, the assay of hepatic protein content carried out during tail regeneration, has also given interesting data. Significantly enough, the hepatic protein content is noted to show a continuous but gradual

increase all throughout tail regeneration. This might appear relevant, when viewed in the light of the known continuous protein turnover, characteristic of regeneration as well as many other events, such as the increased exigency of the formation of blood proteins, carrier proteins for the various hormones etc. The involvement of the hepatic tissue to a greater degree in the elaboration of these various proteins gains added validity from the herein recorded increase in the RNA/DNA ratio from the 3rd to the 12th day of regeneration, which serves as an index of protein synthesis. However, the increasing levels of hepatic proteins observed between the 12th and 60th days of tail regeneration might be attributed to the prevalence of a dominant anabolic influence. The operation of an all pervading anabolic influence during this period of regeneration can be easily envisaged from many other parameters, such as a very much reduced blood lipid level, increased hepatic lipid content, increasing histosomatic index of the fat bodies, as well as differentiation and development of the caudal adipose tissue in the regenerate. The

involvement of certain anabolic steroidal hormones in this context could also be presumed in the wake of the recorded high hepatic and blood cholesterol levels on the 12th day, and the subsequent depleted levels of both the parameters on the 25th day.

More investigations are warranted on these lines, to understand in clear perspectives the role of liver in protein turnover as well as steroidogenesis, and also the possible control mechanisms associated with these aspects. The contrastingly divergent results obtained in the case of Calotes, wherein no increase in protein content of the liver has been noted also, highlight once again the inherent differences involved in the two cases. The changes in the hepatic protein content obtained in the case of Calotes, being, directly related to that of hepatic phospholipids and, inversely related to that of neutral lipids, have been correlated with the mechanisms associated with the fatty liver development (Chapter - 7).

The last part of the thesis involves studies on the histochemical distribution of three enzymes,

ATPase, hexokinase and Glutamate dehydrogenase in the normal and regenerating tail of the scincid lizard, Mabuya carinata. These investigations carried out mainly to complete the earlier enzymological studies of Shah and Ramachandran (1970, 1972, 1973, 1974, 1975, 1976) as well as to validate certain tentative conclusions arrived at thereon, have definitely helped in substantiating such conclusions. The distribution of Mg^{++} dependent ATPase has given a good insight about the increased energy flux in operation during blastemic and differentiation phases of regeneration. However, distribution of other types of ATPase too such as the Na^+ , K^+ and Ca^{++} dependant ones might give a fuller perspective of this aspect. The increased intensity of hexokinase activity observed in the blastemic phase during the same period (Chapter - 3) emphasizes the earlier presumption of a dominant glucose dependant metabolism for the regeneration blastema and signifies the acquisition by the blastemal cells of an effective and competent machinery for the uptake of glucose. Moreover, the possible functioning of two types of hexokinase during regeneration, one

concerned with the catabolism of glucose and the other with the anabolism of glycogen have also been hinted at (Chapter - 10). Shah and Ramachandran (1976) based on their studies on SDH and ICDH in the regenerating tail had suggested an active ICDH centered metabolic activity contributing probably to the elaboration of glutamate. The present study on the distribution of GDH carried out as a sequel to the above line of thinking has also shown an increased activity of the enzymes during the blastemic and differentiation phases of regeneration; phases which are associated with high levels of protein turnover.

Finally it may be concluded that at least a part of systemic responses involved in the process of regeneration have yielded to the pry of the present studies; and have allowed a partial peep into the many veiled and still as yet unknown and well gaurded secrets controlling the most fascinating phenomenon-REGENERATION. It may also be appropriate to state here that during the course of the present discussion a number of bold interpretations and hypotheses have been attempted

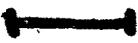
which cut across and venture beyond the safety of established fact with the fond hope of stimulating more detailed investigations on the subject and to attract more challenging critical evaluation on these lines. Inspiration has been drawn in this respect from the introductory remarks of Goss (1972) on " Theories of growth regulation " as quoted below " To propose a hypothesis is to invite the slings and arrows of waiting critics. If it is an honest hypothesis, it is one that can be put to the test, which means that sooner or later someone will probably prove it wrong. Yet in the wake of its demise there often remains a wealth of new information that might not have been sought had there been no hypothesis to challenge in the first place. Such is the debt of gratitude owed to those who have had courage to venture beyond the safety of established fact. Without their inspiration, many a crucial experiment might never have been performed."

EXPLANATION TO CHARTS

Figs. 1-3 Synoptic charts summarising the pattern of changes and involvement of various systemic factors during the wound healing, blastemic and differentiation phases of tail regeneration respectively, in the Scincid lizard, Mabuya carinata.

Figs. 
4-5 Synoptic charts summarising the initiation and involvement of various visceral factors as occurring during postamputation tail wound healing in the Agamid lizard, Calotes versicolor.

PLEASE SEE OVERLEAF FOR THE KEYS TO THE CHARTS.

 NORMAL

 ABOVE NORMAL

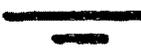
 BELOW NORMAL

 STEADY

 DECREASE

 INCREASE

 RETENTION

 STOP

POSSIBLE EVENTS

STIMULUS OF AUTOTOMY/REGENERATION



PRESENT INDICATION

ENTRY INTO



DURING



RELEASE



* AS PER PREVIOUS STUDIES