CHAPTER - 3

SECTION: I THE EFFECT OF TAIL REGENERATION ON HEPATIC
GLYCOGEN CONTENT AND BLOOD GLUCOSE LEVEL, IN THE
SCINCID LIZARD, MABUYA CARINATA

Previous studies from this laboratory on tail

regeneration (Shah and Chakko, 1967; Magon, 1970;

Radhakrishnan and Shah, 1973; Radhakrishnan, 1972;

Shah and Ramachandran, 1970, 1972, 1973, 1974, 1975)

in both Hemidactylus flaviviridis as well as Mabuya

carinata had established carbohydrate catabolism as

a significant feature during the early regressive

phases of regeneration. Shah and Chakko, (1967), Shah

and Hiradhar,(1974) and Radhakrishnan and Shah, (1973)

based on their studies on glycogen, phosphorylase and

glycogen synthetase had shown that subsequent to

autotomy there is a depletion of glycogen from the

cut end of the tail accompanied by a corresponding

enhanced phosphorylase activity. They further

demonstrated that there is a paucity of glycogen in

the blastema. Shah and Hiradhar, (1974) found a ne

negative glycogen synthetase activity, and,

Radhakrishnan and Shah, (1973) reported histochemical

insensitivity for phosphorylase during this phase of regeneration. Similar reports regarding the paucity of glycogen could be found in the histochemical studies by Schmidt, (1962), Wolf and Cohen, (1963) in newt forelimbs; and in Asellus and planaria (Clement, as reported by Brachet, 1950; Needham, 1952). However, Shah and Ramachandran (1970, 1972, 1973, 1975) based on their studies on enzymes like aldolase, LDH, G.6.PDH and $\propto$-GPDH and the reports of insignificant lipid catabolism had suggested that the blastemal cells are dependant on some alternate source of carbohydrate, possibly blood glucose for their energy necessities as well as for lipid biosynthesis. Moreover, carbohydrate metabolism was again noted by the above authors to play a significant role in the molecular ecology of the regenerating tail during the differentiation phase.

These studies and the assumptions made thereon needed further probings and investigations directed at an understanding of the intrinsic response of the body to meet the differential carbohydrate requirements of the regenerating tail. It is by now well established that glycogen and glucose are the representative examples of polysaccharide and monosaccharide classes of carbohydrate of versatile significance in animal

tissues, not only as potent ready sources of energy but also for the many metabolic interconversions characteristic of innumerable physiological stresses and strains afflicting the animal body. It was in this context that changes in the hepatic glycogen content and blood glucose level, the two kingpins of carbohydrate metabolism in an animal body, were deemed fit to investigate during the various phases of tail regeneration in Mabuya carinata so as to have an understanding of the modulations involved in the central axis of carbohydrate metabolism in relation to regeneration.

MATERIAL AND METHODS

Adult scincid lizards, Mabuya carinata obtained from Mysore, Karnataka State, India, and maintained in the laboratory on a diet of insects were used as the experimental animals. Autotomy of the tails were induced by pinching them off with necessary force. Prior to autotomy, the animals were kept in the laboratory for a fortnight to get them acclimatised to the laboratory conditions. The estimation of liver glycogen and blood glucose was carried out on normal
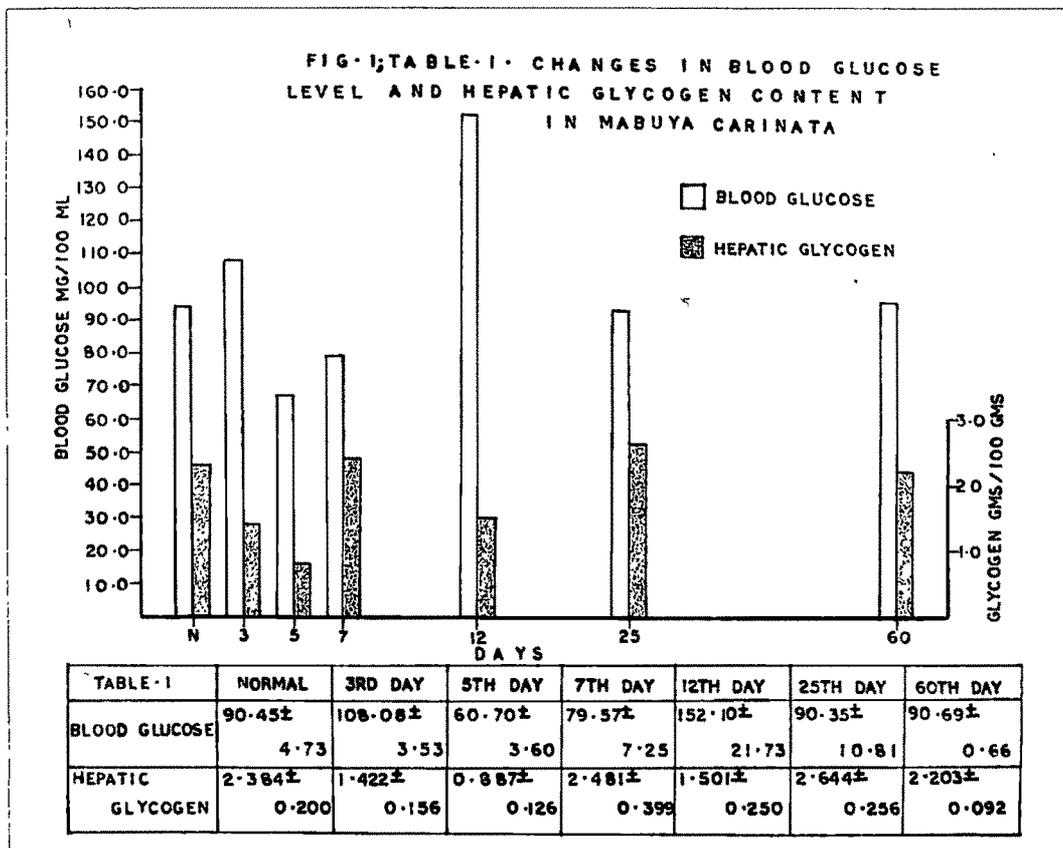
animals as well as on those with the regenerating tails (3rd, 5th, 7th, 12th, 25th and 60th days after tail autotomy respectively).

Estimation of hepatic glycogen was performed by the method of Seifter et al., (1950) and that of blood glucose by the method of Folin and Malmros (1929).

RESULTS

HEPATIC GLYCOGEN

The liver glycogen content of lizards with original tail was found to be 2.384 gms/100 gms. Subsequent to autotomy there was a gradual fall in glycogen content ultimately registering a low value of 0.8872 gms on the 5th day of the tail regeneration. This was soon followed by an increase to the normal level on the 7th day. On the 12th day the glycogen content was again found to be subnormal and stood at 1.501 gms which, however, rose up and registered a slightly above normal level of 2.644 gms on the 25th day. By day 60 of tail regeneration, liver glycogen was once again noted to be in the normal range (2.203 gms). The data on the quantitative estimation of glycogen is presented in Table - 1, Figure - 1.

FIG·1; TABLE·1· CHANGES IN BLOOD GLUCOSE LEVEL AND HEPATIC GLYCOGEN CONTENT IN MABUYA CARINATA

BLOOD GLUCOSE

HEPATIC GLYCOGEN

TABLE·1	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	25TH DAY	60TH DAY
BLOOD GLUCOSE	90·45± 4·73	108·08± 3·53	60·70± 3·60	79·57± 7·25	152·10± 21·73	90·35± 10·81	90·69± 0·66
HEPATIC GLYCOGEN	2·384± 0·200	1·422± 0·156	0·887± 0·126	2·481± 0·399	1·501± 0·250	2·644± 0·256	2·203± 0·092

BLOOD GLUCOSE

The normal glycemic level in Mabuya bearing
original tail during the present course of study
was noted to be 90.45 gms/100 ml of blood.
Immediately after autotomy there was a hyperglycemic
condition with a level of 108.08 mg. Subsequent to
this on the 5th day there was a hypoglycemic
condition with a level of 60.70 mg. Thereafter, the
blood glucose level shot up to a significant
hyperglycemic level of 152.1 mg by 12th day. On both
25th as well as 60th days of regeneration, the
glucose level was found to be exactly identical to
that of the normal animals. Table - 1, Figure - 1
represent the quantitative data on blood glucose
obtained during the various periods of tail
regeneration.

DISCUSSION

Changes in the liver glycogen content and blood
glucose level obtained herein during various periods
of tail regeneration in Mabuya carinata appear to
show a more or less mirror image pattern except for
the period between 3rd and 7th days. From the data it
becomes clear that immediately subsequent to autotomy,

there was glycogen depletion as well as corresponding hyperglycemia as seen on the 3rd day. The glycogen depletion was seen to persist till the 5th day whence the lowest value was registered. However, at this period though the hepatic glycogen content continued to show depletion, the blood glucose level nevertheless was found to undergo a sudden dip registering the lowest hypoglycemic level of the present study. Though the initial glycogen depletion and hyperglycemia observed on the 3rd day could be explained as a sort of stress response subsequent to autotomy, the further decrease in glycogen content in the liver and the parallel fall in blood glucose level are very significant and might indicate the possibility of a high degree of carbohydrate catabolism during the wound healing phase. In this light the previously observed glycogen depletion and increased phosphorylase activity at the cut end of the tail tissues (Radhakrishnan and Shah, 1973; Shah and Chakko, 1967) may be looked upon as an autolytic reaction triggered off by tissue demolition and destruction subsequent to wounding rather than a definite energy yielding mechanism. Such a situation might warrant an extra sourse of ready energy and the

presently observed glycogen depletion in the liver
and hyperglycemia might be viewed in this
perspective. This may also have relevence with the
fact that wound healing is inseperably interlinked
(may be even in a overlapping fashion) with the
continuity of the regeneratory process and hence
there is an exigency of expedited early wound closure
with an associated possible high energy demand. The
control study on Calotes where tail regeneration
does not occur (in the next section) affords some
support to this contention. Another possible reason
for the hypoglycemia at this period might be due to
the channelization of the products of glycogenolysis
towards the energy requirement of the developing
lymphocytopoietic nodules (Chapter - 2, Section - 1)
within the liver itself. Again the role of liver
glycogen and blood glucose in the increased metabolic
needs of various other tissues in the body such as
spleen,marrow, kidney etc., are also factors of
significance and remain at the present juncture as
more of a conjecture. Two other factors of importance
during the regressive phases of regeneration (lasting
up to about the 7th day) are the stimulated synthesis
of ascorbic acid in the renal tissue (Section - 1,

Chapter - 8), and the presence of many immature red blood cells in the blood stream (Section - 1, Chapter - 1). Both these aspects are also worth reflecting in the present context and both might be considered to be dependant on the blood glucose moiety; as it represents the precursor substrate molecules in the former and as instant source of metabolic substrate in the latter case for the maturation. Again the possible diversion of glucose moieties towards lipogenesis also cannot be overlooked as lipid content of liver is known to increase at this period from a reduced content on the 3rd day (Section - 1, Chapter - 4).

The most striking feature of the change in the hepatic glycogen content noted presently is the swift and sudden recovery to the normal level on the 7th day from the lowest level on the 5th day. Of relevence in this context are the reports of a prevalence of anaerobic metabolism and accumulation of lactate during the wound healing and preblastemic phases of regeneration in both reptiles as well as amphibians (Magon, 1970; Shah and Ramachandran, 1970, 1972, 1973, 1974, 1975; Shah and Hiradhar, 1974; Schmidt, 1960, 1962, 1966; Schmidt and Weidman, 1964;

Ryvkina, 1945; Okuneff, 1933). Hence, a coupling of these two observations could lead to the surmise that the lactate produced at the wound surface is being transported to the liver and effectively fed into the glycogenic pathway. This might represent not only an effective and economic mode of replenishing the depleted hapatic glycogen store, but also a conservation of energy as a whole, by the body especially because of the low food intake by the animal during this early period of injury induced stress. The gradual increase in blood glucose from subnormal towards normal level at this period at the same time might be correlated with its reduced demand by the liver (as the lymphocytopoietic activity comes to a close) and that of the circulating blood cells (as indicated by the maximum haemoglobin content of the blood). Another factor of significance in the present context could be the availability of glyceride moieties (Chapter - 6, Section - 1) set free during lypolysis from the fat bodies which could easily contribute to the increase observed at this period in both the parameters.

Further glycogenolysis (as marked by the reduced

hepatic glycogen content) and an intense hyperglycemic

level as noticed on the 12th day are definite

indications of an involvement of carbohydrates of

the systemic origin during this period of

regeneration which roughly corresponds to the blastemic

to early differentiation phases. The previous reports

of a paucity of glycogen and enzymes concerned with

its metabolism (Shah and Chakko, 1967; Shah and

Hiradhar, 1974; Radhakrishnan and Shah, 1973) together

with a lack of lipids and oxidative enzymes (Shah and

Chakko, 1968; Hiradhar, 1972; Radhakrishnan, 1972)

and the intense activity of glycolytic enzymes (Shah

and Ramachandran, 1970, 1972, 1973, 1974, 1975) in

the blastemal cells are all relevent in this connection.

The high hyperglycemic level maintained during this

period thus appears to be of paramouht importance

in facilitating the continuous intake of glucose by

the blastemic as well as the early differentiating

cells as the sole source of metabolite for both energy

purpose as well as for lipid biosynthesis. It is also

quite probable that the maintenance of a high glucose

level in the blood might possibly help in

circumventing partly the absence of capillary

circulation of the regeneration blastema. By seepage

and diffusion, an easy access to a rich source of
glucose in the extracellular environment for the
blastemal cells may thus be continually maintained.
In this phase of high and sole glucose dependency
the maintenance of a three step-system of
concentration gradient involving blood-extracellular
fluid and-intracellular environment of the blastemal
cells could be well visualised; thus ensuring a
continuous effective channelised flow of glucose
along this concentration gradient into the cells of
blastema. The active uptake of glucose by the blastemal
and early differentiating cells is further confirmed
by the high incidence of hexokinase activity noticed
in the regeneration blastema (Chapter - 10).

Subsequent to this by day 25 of regeneration,
whence the process of differentiation is already in
an advanced state, the blood glucose level as well as
the hepatic glycogen content settle down to their
respective normal levels. This could be well correlated
with the metabolic flux reported in the earlier works
from this laboratory (Magon, 1970; Radhakrishnan,
1972; Shah and Ramachandran, 1970, 1972, 1973, 1974,
1975, 1976) wherein a changeover from carbohydrate
to lipid utilization had been suggested during the
progression of differentiation of the tail regenerate

in both *Hemidactylus flaviviridis* as well as *Mabuya carinata*. However, the sharp decline in glucose level of blood from 12th to the 25th day following a sharp rise from 7th to 12th day might also highlight the possible participation of this monosaccharide moiety in many other as yet unknown mechanisms operating within the animal body in conjunction with the varying vagaries and requirements of the regenerating system as well as the coming into operation of the controlling mechanisms. The maintenance of the same normal glycogen and glucose levels in liver and blood respectively on the 60th day and thereafter are definitely indicative of the emancipation of the increased burden placed on the central axis of carbohydrate metabolism from the regenerative mechanics.

It may be appropriate to note that Procassini et al., (1974) based on their quantitative and histochemical studies on newt liver during forelimb regeneration had also concluded that the initial period of regeneration is marked by an enhanced rate of glycogenolysis. Though there is an identity between this and the present work on a general basis, there are, however, certain differences on certain

finer aspects. Whereas in the present work there was
a reversal of glycogen content to the normal level
on the 7th day and its subsequent fall again on the
12th to be followed by a rise and attainment of the
normal level on 25th day, in the case of newt with
regenerating limb, the lowest level of hepatic
glycogen was recorded 24 hours after amputation,
whereafter, there was a gradual increase through first
week to a quick settlement to the normal level by
about the second week itself. This difference between
these two cases may be explained on the basis of
differential metabolic adaptations representative
of the two classes to which the animals belong to.

Finally, if one digresses for a moment and tries
to take a fleeting flash back into the pattern of changes
observed in the present study, the main crux appears
to involve glycogenolysis of hepatic glycogen and
hyperglycemia. It is by now established that lizards
amongst the reptiles have more of alpha cells in
comparison to beta cells in the istlets of their
pancreas (Thomas, 1942; Miller, 1960; Miller and
Wurster, 1956, 1958). Moreover, studies in this aspect
have also established the relative insensitiveness of
blood glucose towards insulin as a result of the
protective action of glucagon-the alpha cell hormone

(Miller and Wurster, 1956, 1958, 1959; Miller, 1961; Dimaggio, 1961, 1961/1962; Vdadesen and Motilica, 1965; Suryavanshi and Rangneker, 1971). Such studies have gone a long way in establishing the effective and adaptive role of glucagon in the metabolic events of lizards. With the known function of glucagon in bringing about hyperglycemia and hepatic glycogenolysis, a possible participation of glucagon during the regenerative process cannot be ruled out. This view point also gains validity from the observation of hypertrophy of alpha cells during regeneration (unpublished) in Mabuya carinata. Moreover, an involvement of thyroxine too in this respect cannot be overlooked as this hormone also is known to bring about similar changes.

SECTION: II THE EFFECT OF TAIL AMPUTATION AND WOUND
HEALING ON HEPATIC GLYCOGEN CONTENT AND BLOOD GLUCOSE
LEVEL, IN THE AGAMID LIZARD, CALOTES VERSICOLOR

The studies on hepatic glycogen content and blood
glucose level during tail regeneration in Mabuya
carinata had indicated a definite systemic response
in the form of glycogenolysis and hyperglycemia. To
have a conclusive evidence that the changes observed
are mainly due to the regeneratory process itself, it
was thought pertinent to explore the possible changes
that might be undergone by hepatic glycogen and blood
glucose during normal wound healing in a lizard with
a nonregenerating type of tail. Moreover, the study
could also establish the role played by these two
carbohydrate molecules in the physiology of the
process of normal wound healing in reptiles.
Information on these aspects in relation to wound
healing in animals in general and particularly in
reptiles is very scanty. Selye, (1950) had concluded
that vertebrates are known to respond to stress by
catabolism of food reserves for energy production.
Studies of some interest in this line are those of
Asnani et al., (1972) on changes in liver glycogen

and LDH during wound healing and repair of pigeon liver, and, Kishnani (1976); Kathuria (1976) on changes of glycogen during normal and diabetic wound healing in liver and skin of rat respectively. Apart from these studies there are no other relevent information available on this topic. It was with this intention the present study on hepatic glycogen and blood glucose was carried out in Calotes versicolor during wound healing of its nonregenerative amputated tail.

MATERIAL AND METHODS

The adult Calotes obtained from the local animal dealer and maintained on a diet of insects were used for the experiment. The animals were allowed a fortnight of acclimatization to the laboratory conditions and the tails of these lizards were then amputated with a sharp scalpel. The cut end of the tails was kept pressed with the fingers for a few minutes to prevemt excessive blood loss.

The estimation of hepatic glycogen content and blood glucose level during the various periods of wound healing as well as in the normal case (nonamputated) were assayed by the methods of Seifter et al., (1950) and Folin and Malmros (1929)

respectively.

RESULTS

HEPATIC GLYCOGEN

The hepatic glycogen content was noted to show a slight decrease on the 3rd day (0.1321) from the normal level (0.1888). This was soon followed by a slightly elevated level of 0.2595 on the 5th day and from 7th day onwards the glycogen content showed the normal level.

BLOOD GLUCOSE

The blood glucose level during the various periods are noted to show a more or less perfect mirror image with that of liver glycogen. The data on glycogen as well as glucose are represented in Table - 1; Figure - 1.

DISCUSSION

In the previous section of the chapter, the depletion of glycogen from the liver and the hyperglycemia noted during tail regeneration in Mabuya carinata on the 3rd day were attributed to a

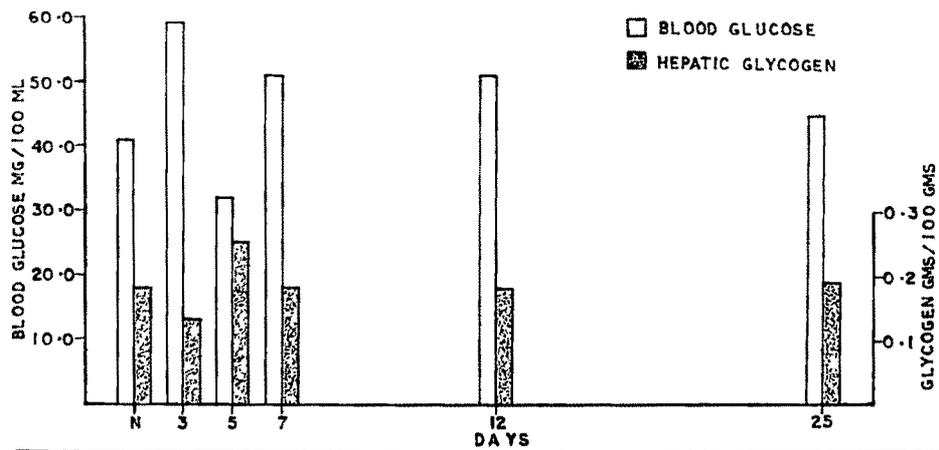FIG·1; TABLE·1· CHANGES IN BLOOD GLUCOSE LEVEL AND HEPATIC GLYCOGEN CONTENT IN CALOTES VERSICOLOR

TABLE·1	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	25TH DAY
BLOOD GLUCOSE	41·09± 4·66	59·28± 4·82	32·29± 2·83	51·75± 1·80	51·77± 1·83	45·26± 2·83
HEPATIC GLYCOGEN	0·18± 0·076	0·13± 0·034	0·25± 0·064	0·18± 0·066	0·18± 0·047	0·19± 0·075

possible shock response. Though a similar drop in
glycogen content is noted in the present case too,
the depletion is however, of a very low magnitude.
It could be easily surmised from this that the shock
induced (due to injury) glycogenolysis in the liver
of Calotes is rather relatively insignificant. In
this light, the substantial glycogen depletion
observed in the case of Mabuya at this period appears
to be of a definite purpose and significance. The
corresponding hyperglycemia observed in both the
cases appears to be of a fundamentally similar type
and could be more of a shock response. However, on a
comparative basis the ratio of hepatic glycogen
depletion to blood glucose elevation appears to be
of a higher order in Mabuya than in Calotes. A
possible explanation in this respect could be that,
whereas in the case of Calotes the glycogenolysis
in liver contributes in toto to the blood glucose
level, in the case of Mabuya on the other hand, the
glycogenolysis contributes on the one hand to blood
glucose and on the other hand to other processes or
mechanisms in operation within the liver itself
(Chapter - 3, Section - 1). In this context, the
need for the products of glycogenolysis in the

hepatic tissue of Mabuya in the light of the previously reported changes within this organ such as increased erythrocyte destruction and associated iron extraction and storage (Chapter - 2; Section - 1) and development of lymphocytopoietic nodules (Chapter - 2; Section - 1) gain further validity and suggests the possible utilization of a part of glycogen for these activities at this period.

These inferences stand verified by the observation of a continued glycogen depletion on the 5th day in Mabuya. However, in the present study in the case of Calotes, the glycogen content was noted to undergo a recovery to a little above normal level with a corresponding fall in the blood glucose level. The two possible explanations in this context are; (1) that the slight hypoglycemic condition developed during this time is due to an effective removal of glucose moieties from blood by the healing tissues of tail as well as by the liver for glycogenesis, and (2) corresponding to the increased glycolytic activity at the wound site during wound healing there is an overproduction of lactate (Okuneff, 1933; Shah and Ramachandran, 1970, 1972) which is being diverted

to the process of glycogenesis in the liver via the vascular channel. Moreover, the higher degree of hyperglycemia observed at this period in the case of Mabuya in comparison to Calotes lends credulity to the fact that there is a hypermetabolic need (during wound healing) in the former due to its regenrative potential as opposed to the latter with its inability for regeneration.

Subsequent to this, on the 7th and 12th days further interesting changes were observed in the case of Mabuya which have been interpreted in terms of the metabolic peculiarities (in the last section) and needs of the animal in the context of the regenerative process in operation. In contradistinction, in the case of Calotes by about 7th day itself the hepatic glycogen content was seen to settle down to the normal level which was found to remain so even on the 12th and 25th days after tail amputation. Correspondingly, the blood glucose level too was observed to settle down to the normal level though with slightly above normal values on the 7th and 12th days. The present observations apart from confirming the active involvement of carbohydrate metabolism

during regeneration in lizards also tend to highlight the comparative insensitivity of this parameter during a normal nonregenerating type of wound healing. This naturally raises the question of the possible energy sources and energetics involved in the wound healing phenomenon in reptiles. The possible alternatives in this connection are that the wound healing process as such (of a nonregenerating type) in a lizard takes place in a low tone metabolic activity involving less metabolic intricacies and adaptations or, that the metabolites of the stump tissues at the cut end might be construed to participate adequately to meet the required energy demands or, probably certain other systemic factors are in operation possibly involving lipids or proteins (Section - II of Chapters - 4 & 7). An investigation into these aspects would be a worthwhile exercise and help in elucidating the metabolic factors in operation during reptilian wound healing.