CHAPTER - 6

SECTION: I CHANGES IN THE VISCERAL FAT BODIES ASSOCIATED WITH HAEMATOPOIESIS AND LIPID METABOLISM, IN RELATION TO TAIL REGENERATION IN THE SCINCID LIZARD, MABUYA CARINATA: A HISTOMORPHOLOGICAL ANALYSIS

Visceral fat bodies in poikilotherms are known to function as efficient storage organs of depot fat made use of under different physiological necessities such as starvation, breeding, aestivation etc. Regeneration with its tremendous metabolic turnover and metabolic intricacies may be presumed to have an influence on the adipose tissue. Previous studies (Shah and Ramachandran, 1972, 1973, 1975, 1976; Radhakrishnan, 1972; Chakko, 1968; Magon, 1970; Hiradhar, 1972) have reported a significant local involvement of lipids in the molecular ecology of the regenerating lizard tails. The observations made during the present course of studies have also indicated an intrinsic involvement of various visceral organs especially the hepatic tissue in response to the stimulus of regeneration (Section - I of Chapters 3, 4, 5, 6, 7, 8,); and thud lend further motivation to understand the possible involvement of visceral fat bodies in the regenerative mechanics. Moreover, the adipocytes are also known to be suspected of undergoing changes and get transformed into blood cells (Wassermann, 1965; Pilo, 1970). In this light, as well as the increased haemopoietic activity associated with tail regeneration in Mabuya carinata, (Section-I of chapters 1 & 2), the visceral fat bodies with their abundant store of fat, attract attention, and might also be construed to play some role, thus necessitating atleast a preliminary investigation. It is with these considerations as well as the observation of a shrinkage of fat bodies, subsequent to tail autotomy and the progression of regeneration, that, a histomorphological analysis of the visceral fat bodies in Mabuya carinata were deemed fit to be undertaken.

MATERIAL AND METHODS

Adult Mabuyas obtained from Mysore, Karnataka State, India, and maintained on a diet of insects were used as the experimental animals. The selected animals were allowed a period of acclimatization to the laboratory cinditions and the tails autotomised at the end of the period. The autotomised animals with tails of varying stages of regeneration as fixed for the present course of study were then sacrifised under mild anaesthesia and the fat bodies removed, blotted and weighed on a Mettler balance and were then fixed in Bouins fluid for histological preparation. The weights of the entire fat bodies obtained were calculated in terms of percentage weight in relation to the body weight (Histosomatic index; HSI).

RESULTS

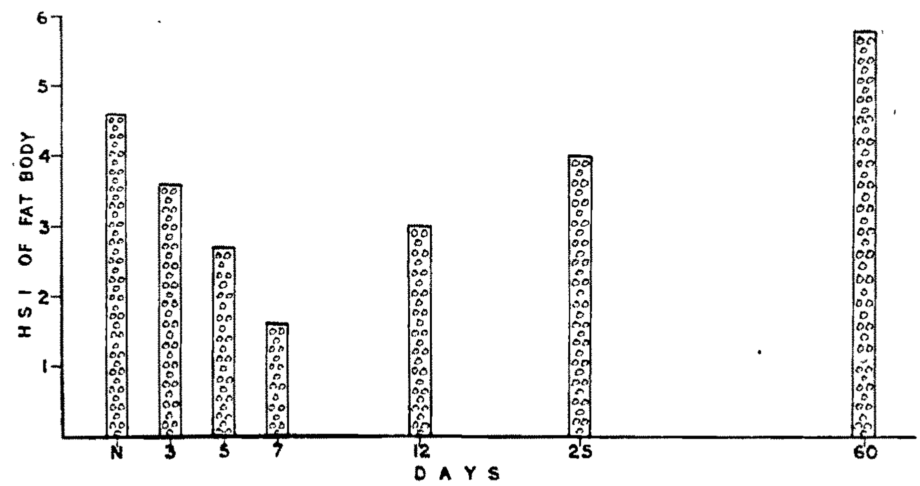
The HSI of the fat bodies was seen to show consistent fall from 4.65 in the normal animals with intact tails to a low value of 1.66 on the 7th day after tail autotomy, through the 3rd and 5th days. However, subsequent to this, the HSI was noted to show a reversed continuous upward trend through the 12th and 25th days till it attained a slightly above normal value on the 60th day (5.84). These changes in the HSI of the fat bodies are depicted in Figure - 1, Table - 1.

HISTOLOGICAL OBSERVATIONS

NORMAL

The histological feature of the fat bodies

FIG·1: TABLE·1· CHANGES IN THE HISTOSOMATIC INDEX OF FAT BODY IN MABUYA CARINATA



TABLE·1	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	25TH DAY	60TH DAY
HSI FAT BODY	4·65± 2·28	3·66± 1·74	2·76± 0·68	1·66± 0·88	3·04± 1·22	4·05± 1·82	5 84± 1·93

EXPLANATION TO FIGURES

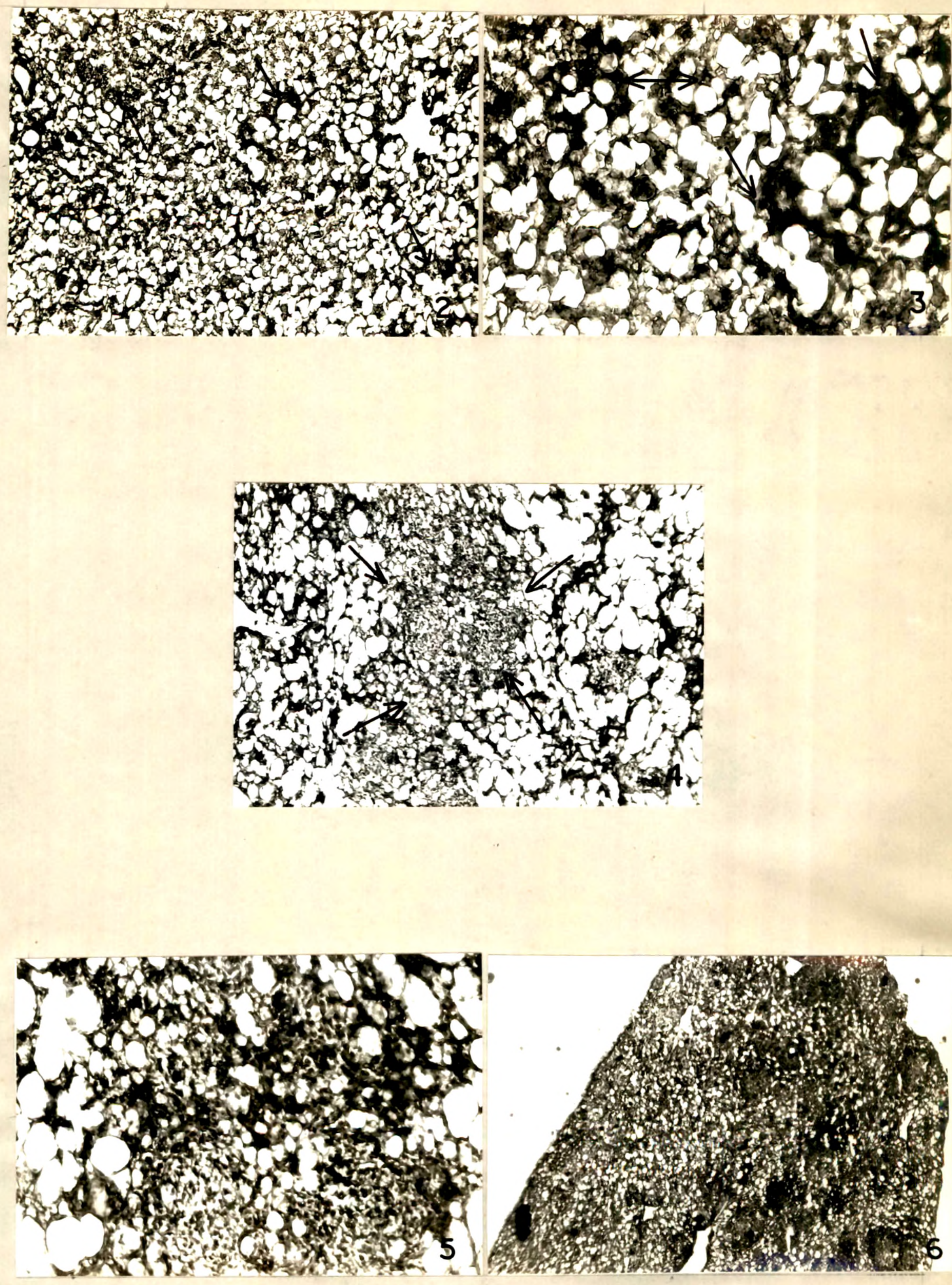
Fig. 2. Histological feature of the fat body of Mabuya prior to its tail autotomy. Note the presence of haemopoietic cells. (arrows). 50 X.

Fig. 3. Higher magnification of Fig. 2. 125 X.

Fig. 4. Note the increased haemopoietic activity as seen on the 3rd day postautotomy of the tail. 50 X.

Fig. 5. Higher magnification of Fig. 4. 125 X.

Fig. 6. Fat body of Mabuya as seen on the 5th day after autotomy. Note the masking of the fat body by haemopoietic cells. 50 X.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EXPLANATION TO FIGURES

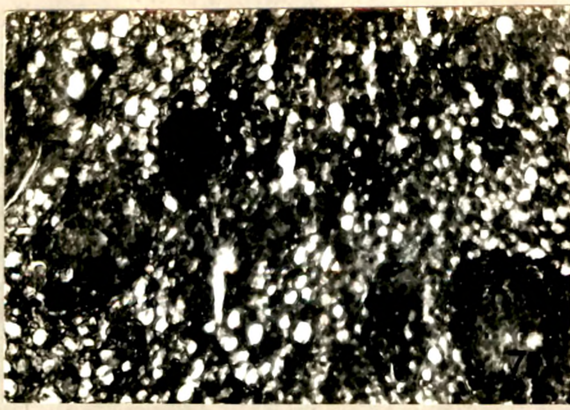
Fig. 7. Higher magnification of Fig. 6. Also note the presence of a few nodule like structures. 125 X.

Fig. 8. Histological appearance of the fat body of Mabuya on the 7th day of tail regeneration. Note the complete masking of the adipocytes and the pseudo appearance of a lymphoid organ. 50 X.

Fig. 9. Higher magnification of Fig. 8. Presence of both lympocytes as well as R.B.Cs could be discerned. 125 X.

Fig. 10. 12th day (postautotomy) fat body which is totally cleared of all haemopoietic cells. Note the presence of haemopoietic cells towards the periphery and the mutilated structure of the adipocytes. 50 X.

Fig. 11. An enlarged version of the same. 125 X.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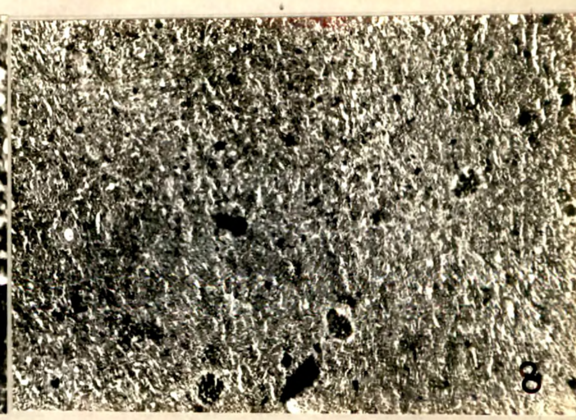
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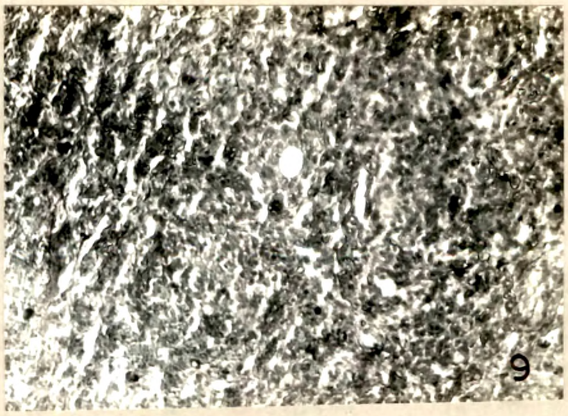
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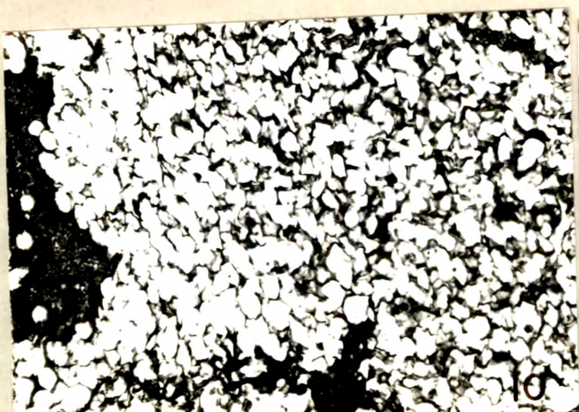
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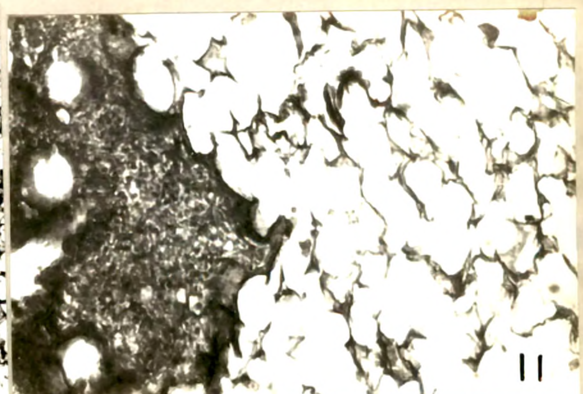
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EXPLANATION TO FIGURES

Fig. 12. Another 12th day fat body sample depicting a partial recovery. 50 X.

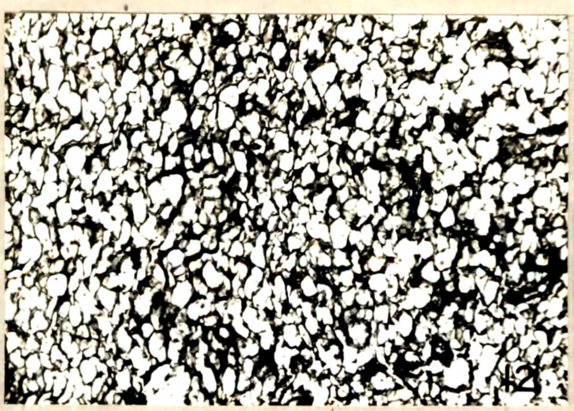
Fig. 13. Higher magnification of same. 125 X.

Fig. 14. Histological picture of fat body as seen on the 25th day of tail regeneration. Note the marked recovery of the adipocytes and the appearance of haemopoietic activity. 50 X.

Fig. 15. Higher magnification of same. 125 X.

Fig. 16. Fat body of Mabuya as seen on the 60th day of tail regeneration. Note the clear cut normal appearance of the adipocytes and the normal grade of haemopoietic activity. 50 X.

Fig. 17. Higher magnification of same. 125 X.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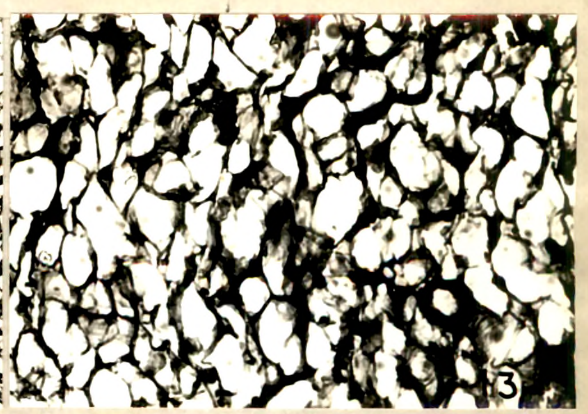
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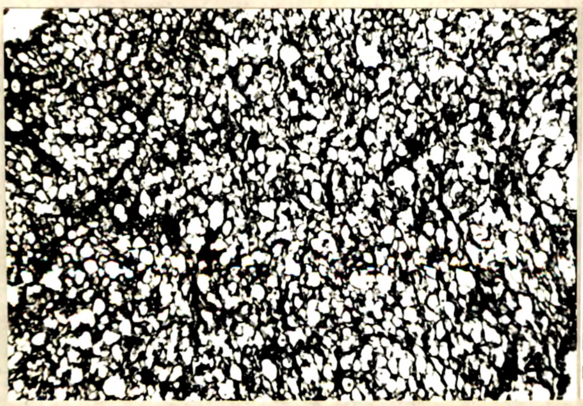

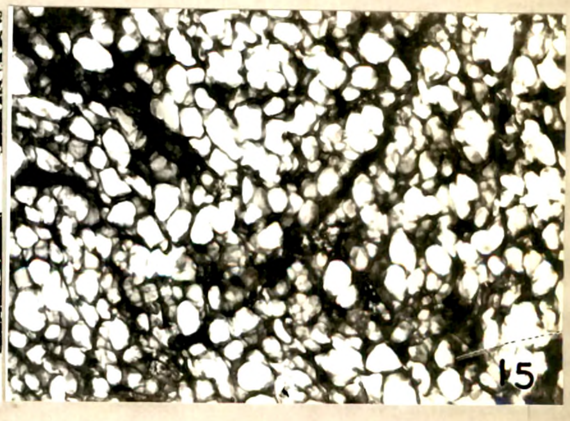
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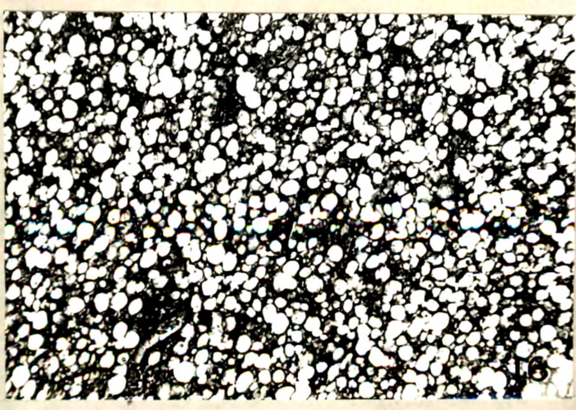
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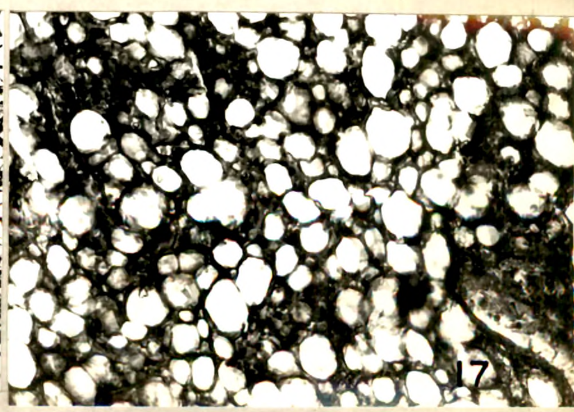
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appeared to give an impression that the fat bodies in Mabuya carinata might well be satelite haemopoietic organs. The sections showed localised areas, where there were concentration of immature haemopoietic cells. These areas appeared to be more of focalised centres containing cells of haemopoietic series with many R.B.Cs in the late differentiation stage.

LIZARDS WITH REGENERATING TAILS

The 3rd day post autotomy samples of the fat bodies tended to show a slightly different histological picture as compared with those of the normal. The localised concentration of the haemopoietic cells seen in the normal cases appeared to disappear at this stage, and the cells were more or less spread all over the adipose tissue.

By about the 5th and 7th days of tail regeneration, the adipose tissue appeared to get completely transformed giving an appearence more or less of a lymphoid organ. The adipose tissue seemed to be swarmed over by haemopoietic cells chiefly lymphocytes. Also seen were relatively a few R.B.Cs at various stages of differentiation. Concentration of lymphocytes resembling something like lymphocytopoietic nodules were also apparently observable in the sections of the adipose tissue on the 5th day. Though all the 5th day samples showed an intense concentration of haemopoietic cells more or less masking the adipocytes completely, some of the 7th day samples showed a reduced intensity of such cells. Such samples also revealed the reduced size of the adipocytes, as well as an obliteration of their structural integrity. However, by about 12th day, the fat bodies appeared to get more or less cleared off the blood cells. Sections of the 12th day adipose tissue samples showed haemopoietic cells only scattered, here and there. As far as the adipocytes were concerned, though certain regions did show signs of recovery marked by the increased size and turgidity of the adipocytes, other areas still depicted the shrunken appearance of the adipocytes.

Onset of recovery evidenced on the 12th day seemed to get further strengthened and, the adipose tissue assumed a semblance of normality by about the 25th day with a few haemopoietic cells remaining scattered especially at the periphery. The 60th day samples showed completely recovered appearance with scattered localised haemopoietic areas and large adipocytes, thus resembling more or less the normal adipose tissue picture excepting for a slightly lesser concentration of haemopoietic cells.

DISCUSSION

The changes observed in the visceral fat bodies in the present study appear to project a very crucial and significant involvement of the adipose tissue in Mabuya carinata as one of the systemic responses during its tail regeneration, especially during the early phases. The functional role of the fat bodies in the lizard can be considered to be of a dual one; as a subsidiary haemopoietic organ as well as a ready source of high metabolic energy. From the observation of only a few patches of haemopietic cells, chiefly made up of blast cells and a few differentiating R.B.Cs, the visceral fat bodies in the case of adult Mabuya carinata may be construed to serve as only secondary or stand by haemopoietic organs in the normal animals, with a low tone activity. However, immediately subsequent to tail autotomy and the initiation of the regenerating process, the haemopoietic activity of the fat bodies appears to get a sudden spurt as observed by the changes on the 3rd, 5th and 7th days of tail regeneration (Figures - 4 - 9), thus emphasizing or strenghthening the contention that they are emergency haemopoietic organs put into use under acute physiological need. Though there is no relevent information regarding the involvement of fat bodies in haemopoiesis amongst the poikilotherms, there are reports regarding the observation of blood cells and their formation in the primitive organs (embryonic structures destined to develop into adipose tissue) in human embryos (Wasserman, 1926; Gruber, 1921; Alfejew, 1924; Godina, 1939; Pretl, 1947; Hoffman, 1950).

Concurrent to the haemopoietic changes, the visceral fat bodies were also seen to undergo a reduction in size marked by not only a visible shrinkage, but also substantiated by a fall in HSI (Table - 1, Figure - 1). The fall in HSI appears to be swift and continuous right from the time of autotomy, and is maintained so through 3rd and 5th days, till the lowest HSI is recorded on the 7th day postautotomy (Table - 1, Figure - 1). However, thereafter the HSI starts rising (as seen on the 12th day), till a near normal level is attained on the 25th day and an above normal value on day 60 after autotomy. Such a set of reverse changes clearly give an indication, that, during the early regressive periods of regeneration, lasting upto the preblastemic phase, there is an acute need for energy rich fuels and in this period of necessity the energy vaults (fat bodies) pay out their stores of long term bonds; and later during the progressive phases of regeneration, the reservoirs of energy replenish their stores. The tremendous depletion of lipids from the fat bodies during the early periods of regeneration may be due to the operation of a strange but significant admixture of hormonal as well as intrinsic factors, striking an effective balance, and appears to emphasise the fact, that in the wake of the initiation of the regenerative process, a highly stepped up rate of oxidative metabolism is the feature. Some significant evidences in favour of this contention can be drawn from the observations of geared up activities of various visceral organs such as liver, spleen, bone marrow and kidney (Section - I, of chapters - 2, 3, 4, 5, 7, 8, 9), as well as the elevated haemopoietic activity (Section - I, Chapters - 1 & 2). Further, the two fold increase in the number of circulating red blood cells as well as haemoglobin content during this early period of regeneration (Section - 1, chapter - 1), as well as the unpublished observations on the thyroid activity, lend added credence to the concept. Moreover, previous studies on tail regeneration in *Mabuya carinata* had indicated the dependence of the regenerating system on oxidative metabolism from the wound healing phase onwards (Shah and Ramachandran, 1970, 1973, 1974, 1975, 1976; Shah *et al*., 1976; Radhakrishnan, 1972). When viewed in the perspective of these compelling evidences, it is tempting to assume that the regenerative process on its initial wake, triggers off a series of systemic activities involving a high incidence of oxidative metabolism, requiring the participation of energy rich lipid moieties, and that, in this phase of physiological need, the fat bodies serve as the easily accessible storehouse from which withdrawal of funds in substantial quantities can be availed of. Though a major share of the lipids so mobilised can be considered to be channelised towards meeting the energy requirmments of the stepped up activities of the body as a whole, a part of it could also be considered to be transported to the site of regeneration itself, thus contributing in part at least to the building up of the blastemic store of lipids. A very significant observation in this context, is the transformation of fat bodies seen on the 5th and 7th days of regeneration, whence they appear more like lymphoid organs, packed with haemopoietic cells especially lymphocytes. Though a reasonable increase in the number of haemopoietic cells may be explained on the basis of a stimulated rate of haematopoiesis, the currently recorded increase, is however, intriguing, and in this light, the possible infiltration of blood cells especially lymphocytes from the vascular channels into the fat bodies appears rather tenable. If it be assumed so, these lymphocytes may be ascribed the role of fat transport from the fat bodies to the blastema, as the lymphocytes have been implicated in the formation of regeneration blastema (chapter - 2). It may thus be presumed that the lipids which were known to play an important role in the molecualY ecology of regeneration blastema are in fact compiled by both an actual transport from fat bodies through lymphocytes during their transit to the site of regeneration to participate in the formation of blastema as well as by a local biosynthesis as brought out by the various investigations (Shah and Ramachandran, 1973, 1974, 1975, 1976; Radhakrishnan, 1972). Such an assumption is supported by the facts, that, the lowest percentage weight of the fat bodies is recorded on the 7th day, that the fat bodies get totally cleared of the blood cells immediately after the 7th day and, that blastema takes shape during this period. Moreover, the possible functions of lymphocytes in lipid transport has been already hinted at by some workers (George and Naik, 1963; Pilo, 1970).

From the data obtained on the changes in the HSI of the fat bodies (Table - 1, Figure - 1), as well as from the morphological and histological appearance (Figures - 10 - 16) it becomes evident that, with the commencement of histodifferentiation in the regenerate by about the 12th day and the progression of the processes of cell division, differentiation and growth in the regenerate through the 25th to the 60th day <u>i.e.</u>, during the progressive phases of regeneration, the requirement by the various tissues in the body having come down, the fat bodies enter into a phase of recoupment and replenishment, marked by the rapid deposition of lipids. Though there might still be a persistent requirement for lipids by the regenerating tail tissues, it would be rather meagre (as compared to the overall requirement of the animal in the earlier period) which could be easily availed of from the circulating pool of the lipid fractions; and as such the earlier enzymological and metabolic studies on tail regeneration (Shah and Ramachandran, 1970, 1972, 1973, 1974, 1975, 1976; Radhakrishnan, 1972) have indicated such a condition (metabolic changes over, from a lipid oriented one to a carbohydrate oriented one by the tail tissues of the regenerate from the late differentiation phase onwards). The observations of a fall in red blood cell count and haemoglobin content of the blood after the 7th day (Section - 1, chapter - 1), and reduced haemopoietic activity (Section - 1, chapter -2) as well as the changes in the thyroid histology (Unpublished), are all further convincing evidences which lend credence to the above conclusion when reflected on to the present observations on the visceral fat bodies. However, it is rather important to note at this juncture that alongwith the deposition of lipids in the fat bodies, the content of this metabolite in the liver also shows an increase above normal value on the 25th day, and by day 60, the level attains its maximum, whence the process of regeneration comes towards its close (Section-1, chapter-4). When viewed in this background, the prevalence of an anabolic influence during the later half of regeneration, possibly mediated by endocrine factors (such as insulin, prolactin and androgens) and/or intrinsic factors could well be purported to be in operation. Incidentally, this period (later half of regeneration) is also marked by the laying down of the submuscular and subcutaneous adipose tissues in the regenerating tail. The observations of very low levels of blood lipid during the 12th, 25th and 60th days of regeneration (Section - 1, Chapter - 4), too, are pertinent in this connection and help in substantiating the contention that there is an all pervading lipogenic force in operation in the lizard body. Possibility of an increased dietary intake and its contribution to this process of lipogenesis can well be within the realm of possibilities and cannot be overlooked.

On the whole, the present study has clearly brought out that there is a period of lipid catabolism during the early phases followed by a period of lipid anabolism during the later phases of tail regeneration in the lizard Mabuya carinata. In this pattern of lipid metabolism, the visceral fat bodies serve as the efficient store houses from which withdrawals as well as remittance of the energy rich bonds could be easily made.

SECTION: II A HISTOMORPHOLOGICAL ANALYSIS OF THE VISCERAL FAT BODIES IN RELATION TO TAIL WOUND HEALING IN THE AGAMID LIZARD, CALOTES VERSICOLOR

The primary role of fat bodies is the storage of triglycerides as a potent source of energy. In general, adipose tissue may also serve other functions, such as insulating against cold, or as a mechanical buffer or lubricating system, as in joints and in the socket of eye. It may also be a site of heat production during exposure to cold and especially during awakening from hibernation. These functions, however, although important in special circumstances are nevertheless secondary to the primary role of this tissue in energy storage. To be of use, an energy store must be easily accessible for withdrawals or deposits of energy. Though two types of adipose tissue are recognisable in the case of mammals, the white one and the brown one - no such distinction is applicable to poikilotherms. The brown type of adipose tissue is thought to be of common occurence in embryos as well as hibernating animals and is thought to be metabolically more active than the white (C.F. Joel, 1965; Wassermann, 1965; Napolitano, 1965). Adipose tissue as reserve storage for lipids appeared amongst vertebrates in amphibians. The fishes have no specialised fat bodies and most of the lipids are stored in the liver and also muscle. The fat bodies which appeared in amphibia are also present in a well developed form, amongst the reptiles too. The abdominal fay bodies of amphibia as well as reptiles are documented to supply nutrition during adverse circumstances and breeding (Dessauer, 1955a, b; Fox and Dessauer, 1957; Wicht and Jones, 1967; Zain and Zain, 1967; Smith, 1968, Licht and Gerbman, 1970, Walker and Withoff, 1970; Minnich, 1971). Moreover, in the previous section of this chapter, the fat bodies were also shown to play an important role during tail regeneration in the lizard, Mabuya carinata. In this light, the possible effect of tail wound healing on fat bodies in the lizard Calotes versicolor was deemed worthwhile to investigate not only to get an idea of the involvement of fat bodies in the normal process of injury and healing but also to help serve as a control to the study on regeneration, as Calotes does not regenerate its 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. After allowing a fortnight of acclimatization to the laboratory conditions, the tails of the animals were amputated. Since then, animals both normal with intact tails, as well as the amputated ones during various periods such as 3, 5, 7, 12, and 25 days post amputation were sacrificed under mild anaesthesia and their fat bodies taken out. The weights of the fat bodies were recorded and then fixed in Bouin's fluid for histological preparation. The Bouins fixed tissues processed and sectioned in the routine fashion were stained with Haematoxylene-Eosin. From the weight of the fat bodies, and, the total body weight, the percentage weight (Histosomatic index; HSI) was calculated.

RESULTS

In contrast to the changes observed in the case

FIG. 1: TABLE 1 CHANGES IN THE HISTOSOMATIC INDEX OF FAT BODY IN CALOTES VERSICOLOR

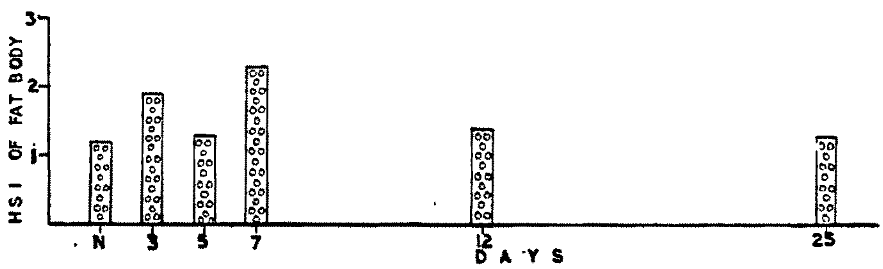


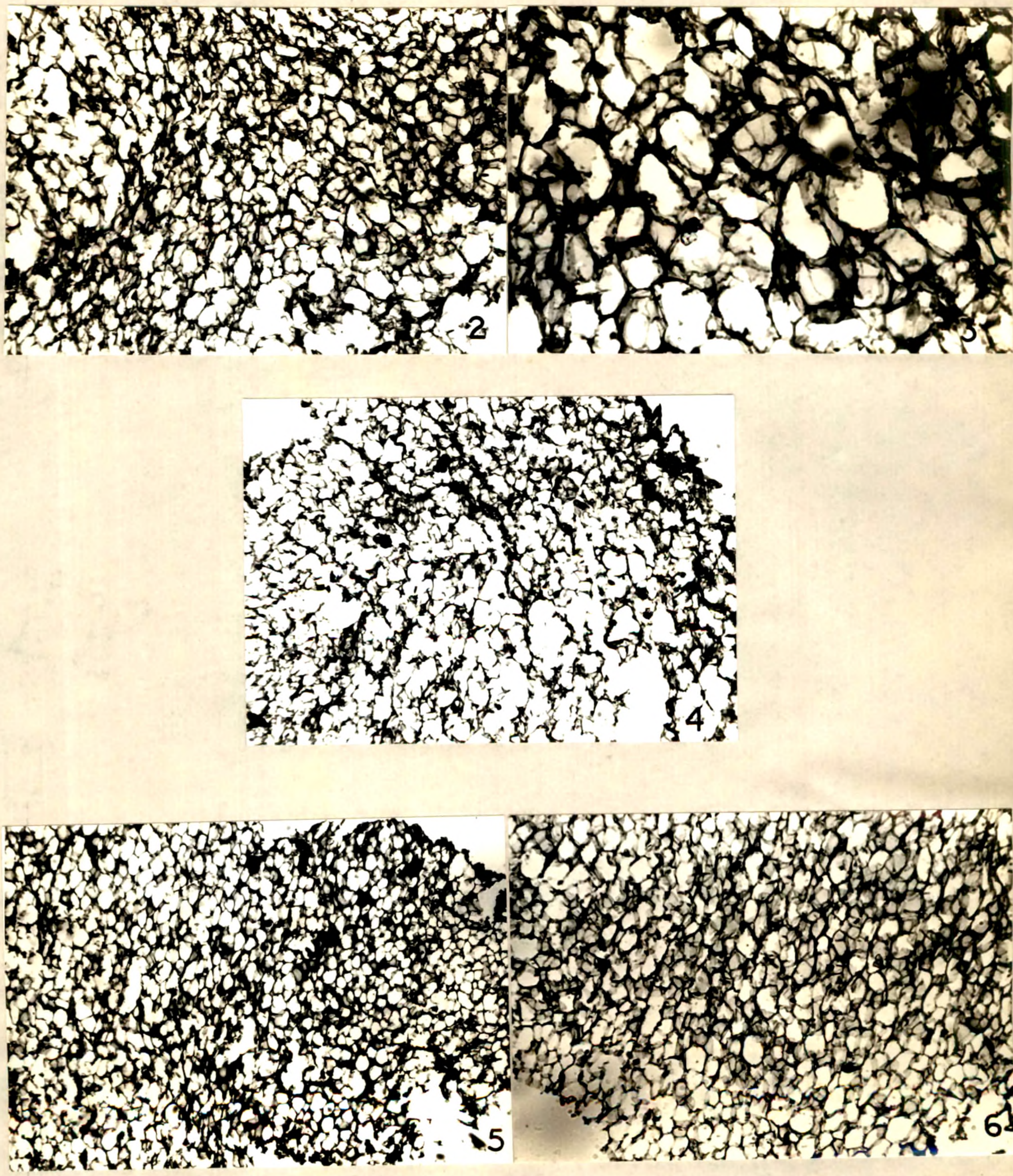
TABLE 1	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	25TH DAY
HSI FAT BODY	1·29± 1·18	1·99± 0·57	1·30± 0·93	2·32± 1·10	1·40± 0·83	1·32± 0·18

EXPLANATION TO FIGURES

Fig. 2. Photomicrograph of fat body of Calotes prior to its tail amputation. Note the absence of haemopoietic activity. 50 X.

Fig. 3. Higher magnification of Fig. 2. 125 X.

Fig. 4 - 6 Fat body of Calotes as seen during the 3rd, 5th and 7th days of wound healing after its tail amputation denoting no change at all. 50 X.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of Mabuya, in Calotes, the percentage weight (HSI) of the fat bodies did not show any appreciable change during the various periods of healing after tail amputation. However, a perceptible change was observed on the 7th day whence the HSI showed an increase from the normal. Since then, the HSI showed a declining trend and reached the normal level by about the 25th day, with a slightly above normal level on the 12th day. The changes in the HSI of the fat bodies in Calotes are depicted in Table - 1, Figure - 1.

Examination of the histologival sectiond of adipose tissue also revealed no change at any time during wound healing or even during post healing, excepting for an increased size of the adipocytes on the 7th day. The picture of the fat bodies during tail wound healing in Calotes versicolor is depicted in the Figures- 2. Unlike in Mabuya, the fat bodies in Calotes do not possess any haemopoietic characteristics.

DISCUSSION

Apparently there appears to be no change whatsoever either in the morphological or histological appearance nor in the HSI of the fat bodies in Calotes during wound healing after amputation of its tail. Since there is no trace of haemopoietic cells at any stage of tail wound healing or in the normal ones, it may be easily construed that unlike in Mabuya, in Calotes, the fat bodies have no heamopoietic function at all. Moreover, mobilization of fat from the fat bodies too can be overruled during the healing process in Calotes owing to the more or less unchanged percentage weight of the fat bodies. However, the minor changes noticeable in the HSI of the fat bodies (Table - 1, Figure - 1) appear to parallel the more significant changes in the total lipid content of the liver observed during the various periods of tail wound healing in Calotes (chapter - 4). On the 3rd day after tail amputation, the hepatic lipid content as well as the HSI of the fat bodies showed an increase. It appears that the factors in operation at this stage of wound healing in Calotes, is such that, they tend to favour lipid deposition in both liver as well as fat bodies, either, by synthesis within, or else, by emphasize the operation of a strong force of lipogenesis and/or lipid mobilization and deposition on about the 7th day, whose influence leads to a depostion of lipids in the liver and the fat bodies. The changes observable in both these organs during the later periods are indicative of cessation of operation of such a force or influence, and in this wake, the lipid content of both the liver as well as the fat bodies tend to attain the normal levels gradually. The adaptive significance or even the pathological significance of such series of changes remain enigmatic. And further, whether these changes are of a specific nature elicited by only lizards in response to a loss of a part of their body, or, whether they are characteristic of even other groups of vertebrates, and also the underlying controlling mechanisma are all difficult to fathom at this juncture and would warrant further explorations to unshroud the mystery.

On a comparative basis, the present observations on Calotes versicolor during its postamputational tail wound healing, when equated with those on Mabuya carinata during its postautotomic tail regeneration, emphasizes the fact that the systemic factors elicited in these two cases especially with regard to lipid metabolism are quite divergent and totally unrelated. This, thus leads to the conclusion that the animals with a potential capacity to regenerate their lost parts are endowed with certain inherent intrinsic visceral responses as well as adaptive (physiological and metabolic) changes; and the capacity to elicit or express these potential responses might be, one of the controlling mechanisms underlying the phenomenon of regeneration.