CHAPTER 5

HISTOMORPHOLOGICAL CHANGES IN SPLEEN DURING TAIL REGENERATION IN THE GEKKONID LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

Histophysiology of reptilian lymphoid organs has been investigated on a number of fronts. A wide variety of lymphoid organs and aggregates are found to be present in reptiles which include thymus, spleen and lymph nodes (Borysenko and Cooper, 1972). Nevertheless, spleen is the most prominent of peripheral or secondary lymphoid organs in terms of size, morphological complexity and immunological importance (Cohen, 1976). Stimulus leading to repair and regeneration in the lizard, *Hemidactylus flaviviridis* elicits a considerable vascular response (chapter 4) which also can be studied from changes in the spleen profile. Since white pulp of the spleen is concerned with lymphocytopoiesis and involvement of these blood components in the body response to regeneration has been documented (chapter 4), the role of spleen in the body response to tail regeneration is worth considering. With this purpose in mind, present investigation was undertaken which embodies the results of gravimetric and histophysiological changes in spleen along with the localization and pattern of reactivities of certain hydrolytic enzymes, *viz.*, acid and alkaline phosphatases during the

process of tail regeneration in the lizard, H. flaviviridis. These non-specific phosphatases which are implicated in divergent cellular functions like functional differentiation and phagocytosis were studied histochemically in this context. Tail regeneration was also studied in splenectomised lizards to evaluate the extent of involvement of the spleen in general body response to the process.

MATERIALS AND METHODS

Adult healthy lizards weighing 12 to 13 gms, irrespective of sex collected from the University campus were maintained on a diet of insects. Autotomy was induced by pinching off the tail leaving 2 - 3 basal segments intact next to vent. The normal lizards with intact tail as well as at different stages of tail regeneration (Shah and Chakko, 1968a) were sacrificed, and following studies were carried out.

Group I : After sacrificing under hypothermic anaesthesia, spleens from normal lizards and those at different stages of tail regeneration were removed immediately, blotted free of body fluids, weighed and fixed in Bouin's fixative. 5 to 7 μ thick paraffin sections were cut and stained with haematoxylin - eosin and Jenner-Giemsa stains for histological examinations (Gurr, 1956).

For histochemical demonstration of acid and alkaline phosphatases, spleens were removed from normal lizards and those at different stages of tail regeneration, and fixed on cryostat microtome chuck maintained at -20°C. Fresh frozen sections of 12 to 18 μ thickness were cut and fixed in cold acetone. The sections were washed thoroughly in several changes of distilled water. Method of Burstone (1962) was employed.

Suitable controls were run in media devoid of the respective substrates.

Group II : Splenectomy was performed in normal lizards as well as those with different stages of regenerating tail, by making a paramedial incision on the left side of the abdomen. For controls, sham operations were performed on similar sets of lizards. The incisions were sutured with silk thread and necessary post-operative care was taken. Splenectomised lizards with intact normal tail were subjected to tail autotomy after 10 days when the operation wound was found to be completely healed. After splenectomy on normal lizards and those with different stages of regenerating tail, effects of splenectomy on either the wound healing or the rate of growth of the regenerate was observed and recorded.

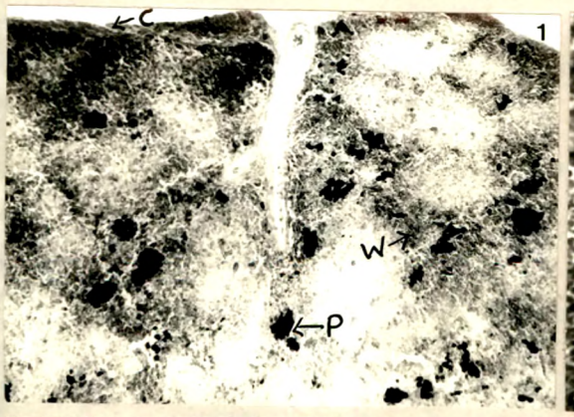
RESULTS AND OBSERVATIONS

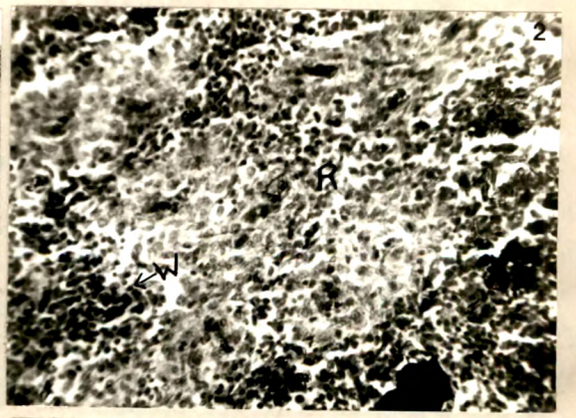
Spleen weight taken during the process of tail regeneration revealed a remarkable increase during wound healing phase which was about 14 fold. By the time when the blastema was formed there was a sudden drop in its weight, nevertheless, it was more than that in the lizards having intact original tail. Its weight dropped further during differentiation phase to the weight recorded for the normal lizards (with original tail) and remained so thereafter (see Table 1).

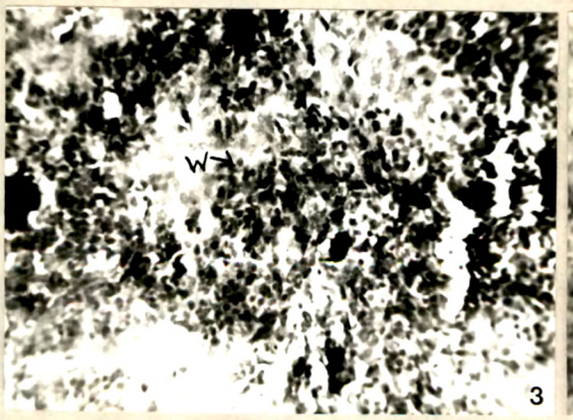
Histological observations on the spleen of the normal lizards (with original tail) revealed that spleen is encapsulated by connective tissue (Fig. 1). There are well differentiated regions of red and white pulps, though the white pulp is more extensive consisting of lymphoid cell aggregates surrounding central arterioles. Typical germinal centers are absent. Nevertheless, numerous large cells with pale nuclei resembling reticular cells are present immediately adjascent to the central arterioles. During wound healing phase, an increase in white pulp was observed (Fig. 3). However, when blastema was formed, there was a significant decrease in the amount of the white pulp (Fig. 4) and as the regenerate progressed towards differentiation, the spleen picture gradually

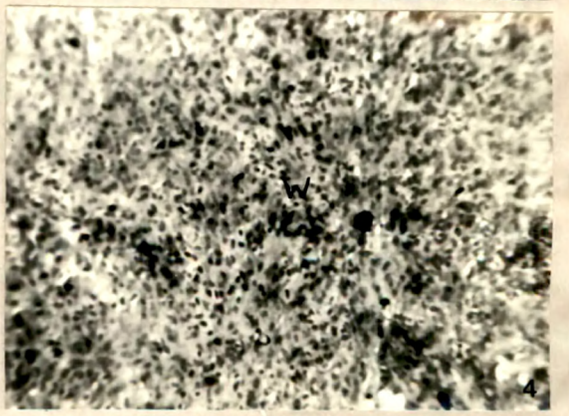
Table 1 : Relative weight of spleen per 100 gms body weight during different phases of tail regeneration in H. flaviviridis

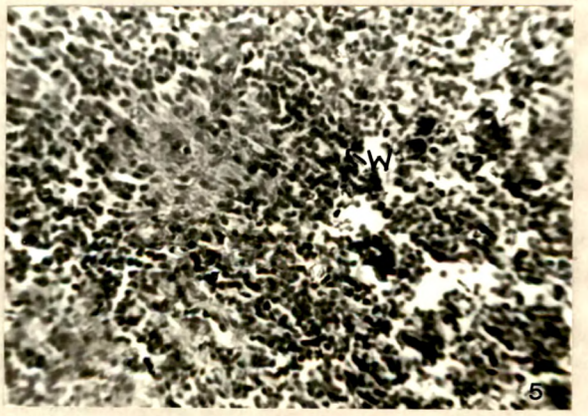
Normal tail and phases of regeneration	Spleen weight X 100 / Body weight
Normal tail	0.004
Wound healing	0.057
Blastema	0.012
Differentiation	0.005
Fully regenerated tail	0.005


C
W
P
1


R
W
2


W
3


W
4


W
5

EXPLANATION FOR FIGURES

Fig. 1 : Photomicrograph showing section of spleen of normal lizard with intact original tail. 63 X

Fig. 2 : Photomicrograph showing section of spleen of the normal lizard with original tail. 250 X

Fig. 3 : Photomicrograph showing section of spleen during wound healing phase. Note an increase in the white pulp. 250 X

Fig. 4 : Photomicrograph showing section of spleen during blastema phase. Note a decrease in the white pulp. 250 X

Fig. 5 : Photomicrograph showing section of spleen during differentiation phase attaining near normal histological features.

ABBREVIATIONS

R - Red pulp

W - White pulp

C - Connective tissue capsule

P - Pigments

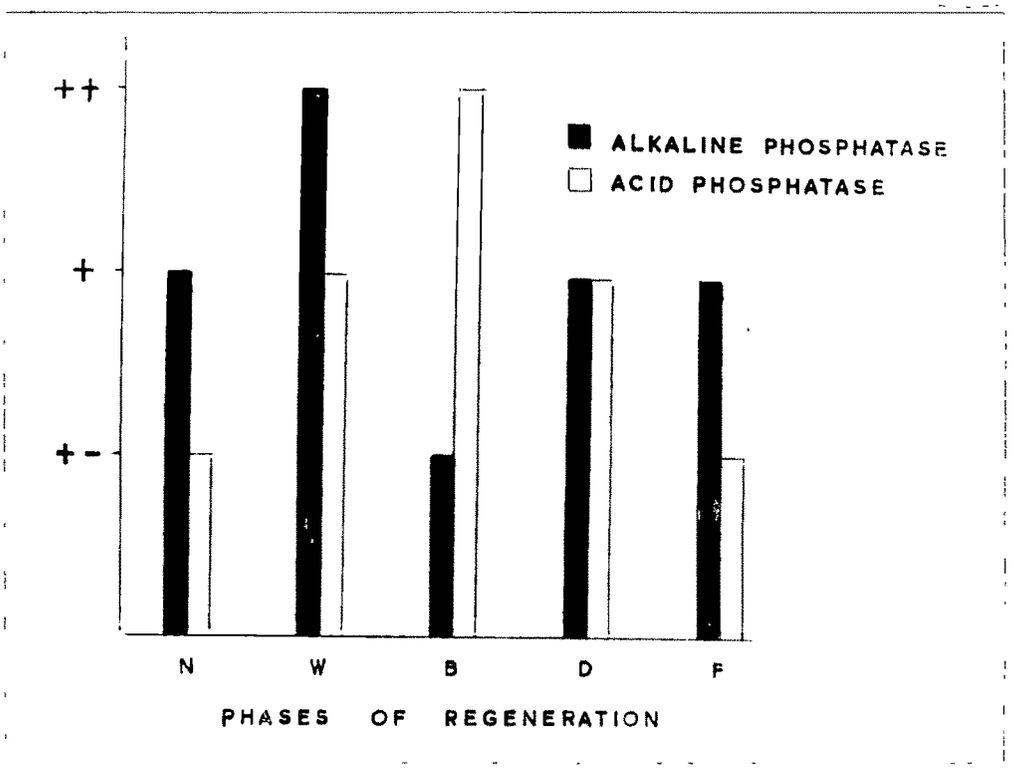


Fig. 6 : Histogram depicting activities of phosphatases in spleen during different phases of tail regeneration in H. flaviviridis.

N - Normal tail
W - Wound healing phase
B - Blastema phase
D - Differentiation phase
F - Fully regenerated tail

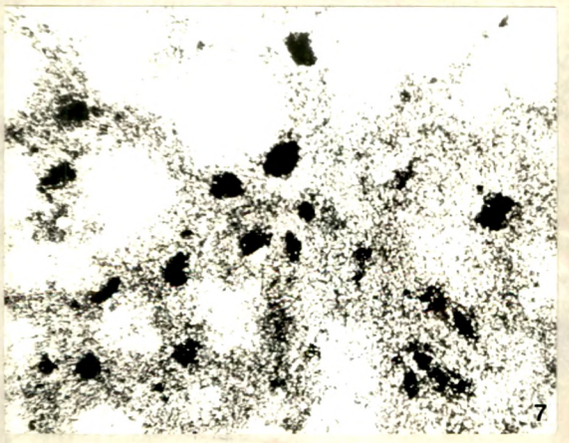
+- Very low, + Low, ++ Moderate

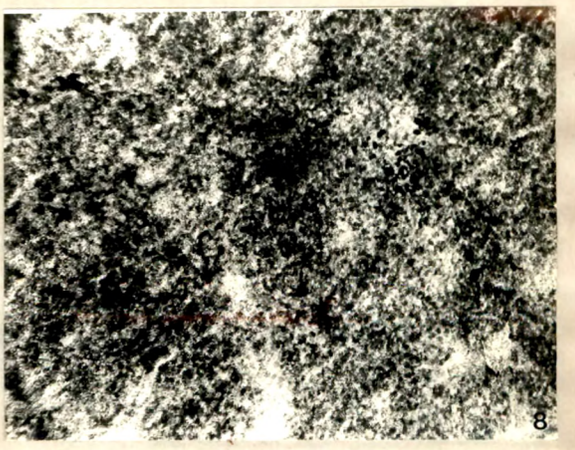
resumed almost normal features (Fig. 5) as were observed in the lizards with intact original tail. With regards to tail regeneration, the red pulp region of the spleen did not show any noticeable change.

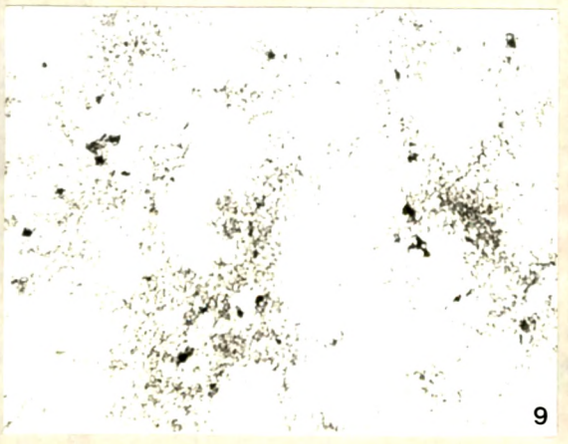
Phosphatases : In the spleen of normal lizards (with intact original tail) alkaline phosphatase activity was more than that of acid phosphatase (Fig. 6). Both these enzymes were present in the white pulp cells only whereas their activity was virtually absent in the red pulp cells (Fig. 7 & 11).

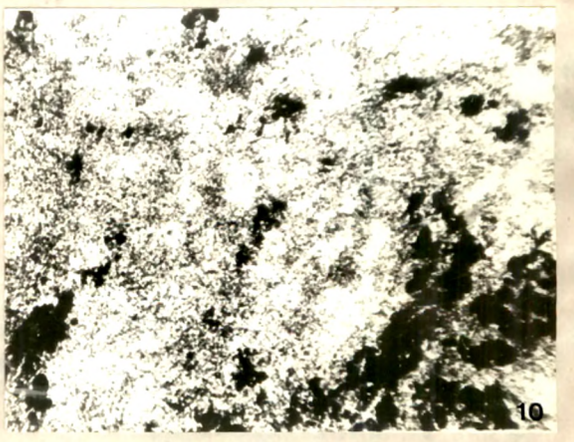
During wound healing phase, there was an increase in the activity of both the phosphatases in the cells of white pulp (Fig. 8 & 12), but alkaline phosphatase reactivity declined below the normal level when the blastema was formed (Fig. 9). However, at this stage the activity of acid phosphatase remained at a higher level (Fig. 13). Nevertheless, during the next phase of tail regeneration, i.e. differentiation, alkaline phosphatase reactivity in spleen reached its preautotomy level (Fig. 10), whereas that of acid posphatase gradually decreased reaching its preautotomy level when the regenerate reached its full grown state (Fig. 15).

Splenectomy: Splenectomy did not prove fatal to the lizards and all the operated animals remained in good health. It


7


8


9


10

EXPLANATION FOR FIGURES

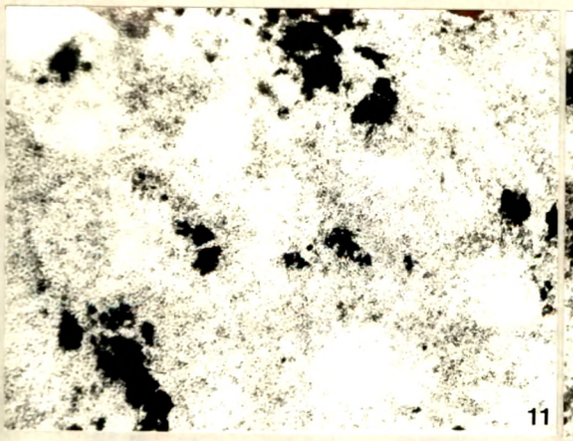
Photomicrographs showing distribution pattern of alkaline phosphatase in the spleen during various phases of tail regeneration. Magnification 63 X.

Fig. 7 : Distribution of the enzyme during preautotomy period. Note activity in the white pulp.

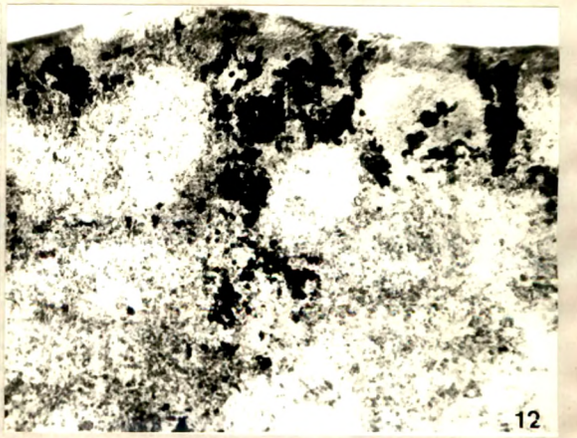
Fig. 8 : Increased activity of the enzyme in white pulp during wound healing phase is discernible.

Fig. 9 : Blastema phase: Note low intensity of reactivity of the enzyme in white pulp.

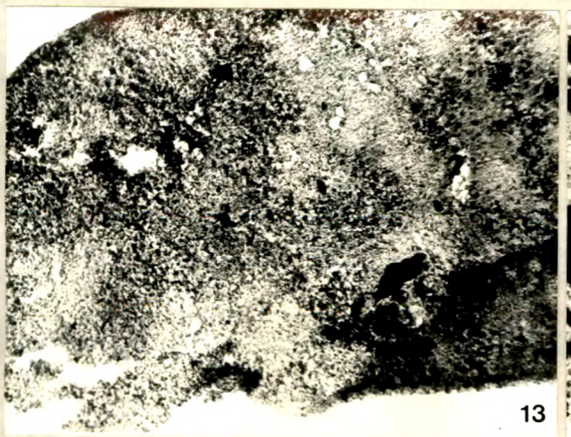
Fig. 10 : Photomicrograph depicting attainment of near normal pattern of distribution of the enzyme during differentiation phase.



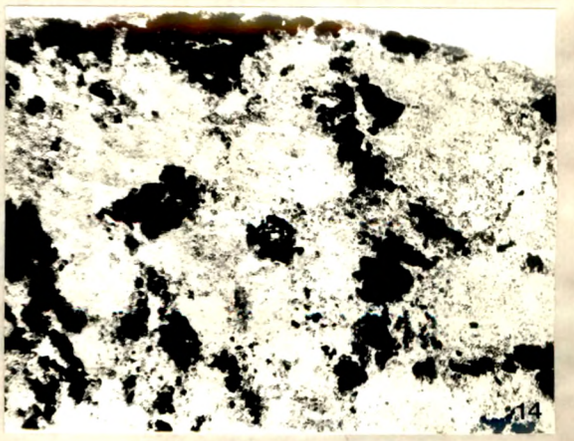
11



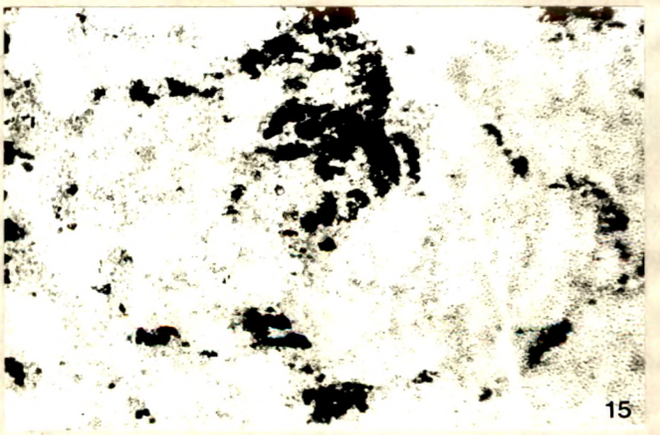
12



13



14



15

EXPLANATION FOR FIGURES

Photomicrographs showing distribution pattern of acid phosphatase in the spleen during various phases of tail regeneration. Magnification 63 X.

Fig. 11 : Distribution of the enzyme during preautotomy period. Note activity in the white pulp.

Fig. 12 : Note increased activity of the enzyme during wound healing phase.

Fig. 13 : Photomicrograph showing attainment of peak enzyme response during blastema phase.

Fig. 14 : Differentiation phase : Note a decline in the enzyme activity from that seen in the previous phase.

Fig. 15 : Distribution of the enzyme in spleen of a fully regenerated animal attaining near normal pattern.

was observed that splenectomy caused a delay of about 1.5 ± 0.331 days in the healing of the autotomised wound of the tail. However, once the wound healed, the rate of growth of the regenerate remained unaltered as compared to controls. Splenectomy preformed at later stages of regeneration i.e. at wound healing, blastema and differentiation did not affect the rate of growth of the regenerate.

DISCUSSION

The spleen of H. flaviviridis shows well differentiated regions of red and white pulps, however, they are not easily recognizable (Kanakambika and Muthukkaruppan, 1973). The bulk of the organ is composed of the white pulp which in turn is composed of lymphocytes aggregated around central arterioles. Each arteriole in white pulp is surrounded by reticular cells; but these reticular cells are not easily identifiable in the histological preparation (Pitchappan and Muthukkaruppan, 1977). There are no germinal centers in the spleen of this lizard. Pollara et al. (1969) have reported lack of germinal centers as the characteristic of the spleen of all ectothermic vertebrates. The absence of germinal centers in the lizards Tiliqua rugosa and Calotes versicolor have been reported by Wetherall and Turner (1972)

and Kanakambika and Muthukkaruppan (1973) respectively; in tuatara by Marchalonis et al. (1969) and in turtle by Borysenko and Cooper (1972).

The spleen is found to be the major site of immune response in the lizard Calotes versicolor (Kanakambika and Muthukkaruppan, 1973) and in tortoise (Ambrosius and Hoheisel, 1973). Involvement of spleen in the general body response during early phase of tail regeneration in the present study is indicated quantitatively by a tremendous increase in its weight. Such an increase noted herein could be suggested to the hyperfunction of this lymphoid organ which is to be expected during the wound healing phase when there is a greater demand of lymphocytes at the wound site. Holzbach et al. (1964) have reported that increased spleen size is an index of its hyper-function. Hence, the presently observed increase in spleen weight could be a response to the increased demand of lymphocytes at the wound site and in general for the immune response of the body. This is further substantiated by an increase in the total leucocyte count and the differential count of lymphocytes during the same phase (chapter 4). Histological observations on the spleen during this phase of tail regeneration revealed an increase in the white pulp, indicative of greater lymphocyte production.

However, when the wound was healed and blastema was formed, there was a drastic reduction in the weight of the spleen, nevertheless, it was more than that in the animals with original intact tail. Histological observations on the spleen during the blastema phase indicated a reduction in the white pulp tissue. These changes in the weight and structure of spleen were partially reflected in the reduced count of lymphocytes in the blood observed during this period (chapter 4). These observations clearly indicate a potential involvement of the spleen in the healing process but not so in the later phases of tail regeneration.

As the regenerate progressed towards differentiation, spleen almost attained its weight as was in the lizards with intact original tail and its histological features were also comparable to those observed in the normal lizards (with intact original tail). By this time lymphocyte count of the blood also had reached its preautotomy value (chapter 4).

Phosphatases: Activities of both the hydrolytic enzymes viz., acid and alkaline phosphatases were discernible in the cells of white pulp only. An increase in the activity of both these phosphatases in the white pulp during wound

healing phase implies an augmentation of lymphocytopoietic function of the spleen. Alkaline phosphatase which is considered as an inducible enzyme is known to accumulate in tissues or organs before the onset of their functions (Moog, 1965). The process of wound healing would undoubtedly demand increased turnover of lymphocytes which would result in enhancement of cell proliferation in the white pulp of spleen that would be reflected in the increased alkaline and acid phosphatase activities. Phosphatases have been implicated in cellular proliferation (Olsen and Nordsquit, 1966; Shah and Menon, 1974) and also phagocytosis (Fry et al., 1973). Hence, an increase in reactivities of these two phosphatases in the spleen in the present context could be considered to be aiding the cell proliferative and lytic activities occurring at the wound site during the early period of regeneration.

The decline in reactivity of alkaline phosphatase to a subnormal level during blastema phase could be construded to be due to the completion of wound healing and less involvement of lymphocytes in the regenerative process. However, an increase in acid phosphatase activity during the same period indicates lytic activity occurring due to cellular debris from the regenerate and tail stump being brought there.

During differentiation period, alkaline phosphatase activity reached its preautotomy level and remained so thereafter, which indicates the normalisation of spleen function. At this stage, histological features of the spleen were comparable to those observed in lizards with intact original tail. Acid phosphatase decreased gradually in reactivity and reached its preautotomy level in animals when the regenerate reached its full grown state.

Splenectomy: It is obvious from the present study that splenectomy caused a delay in the healing of autotomy wound, nevertheless, the time taken for the attainment of subsequent phases of regeneration remained unaltered and unaffected once the wound healing was completed. These observations indicate that the organ plays a significant role during wound healing phase as a general body response to autotomy. Further, splenectomy performed at later stages of regeneration, viz., at wound healing, blastema and differentiation did not cause any deleterious effect on rate of growth of the regenerate. These observations further underscore the fact that splenic involvement is important mostly during wound healing phase and not so in the subsequent phases of tail regeneration. Considering the lymphocytopoietic function of the spleen, it appears that the organ exerts its influence on wound healing through the production of these cell types.