CHAPTER 4

CHANGES IN CELLULAR CONTENTS OF BLOOD DURING TAIL REGENERATION IN THE GEKKONID LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS*

For most of the animals, injury to an organ constitutes a major hazard, and various mechanisms have been evolved by body to combat it as a part of adaptive mechanisms. Any such structural or adaptive changes in animals could reasonably be expected to cause noticeable alterations in the composition of the blood which is endowed to perform a variety of functions. Adaptive changes in the vertebrate reticuloendothelial complex following injury have been evaluated to a great extent in normal healing processes, but there is a surprising lack of information about vascular response during tail regeneration in lizards. Stress is known to cause haematological changes in reptiles (Meints *et al.*, 1975). Hence, it was thought desirable to evaluate any potential involvement of the vascular system as an overall body response to the process of tail regeneration. The present study is an attempt to elucidate the homeostasis of blood cell and bone marrow cell population after tail autotomy and its subsequent regeneration in the lizard, *Hemidactylus flaviviridis*.

MATERIALS AND METHODS

Adult healthy lizards weighing 12 to 14 gms were maintained in the laboratory on a diet of insects. Autotomy was induced by pinching off the tail leaving 2-3 basal segments after vent intact. Following studies on blood and bone marrow were carried out in the lizards with intact original tail and in those with regenerating tail at various phases of regeneration (Shah and Chakko, 1968a). Blood was obtained by cardiac puncture and bone marrow was taken from the femur bone.

Haemoglobin (Hb) content was determined in gm % by Helige Sahli's haemometer.

The number of red blood corpuscles (RBC) and white blood corpuscles (WBC) present per cubic millimeter (mm^3) were determined using a haemocytometer (Neubauer, Germany).

For the differential cell count of blood and bone marrow, smears of these tissues were stained with Leishman's stain (Gurr, 1956).

RESULTS

The data for total count of RBC, WBC and Hb content are given in Table 1 and Fig. 1 and those of differential

counts of WBC and bone marrow cell population are presented in Table 2 and Fig. 2 and Table 3 and Fig. 3 respectively.

The number of RBC in million per cubic millimeter in lizards with intact original tail was 2.130±0.57. During wound healing phase of regenerating tail, its count decreased to half its original value (1.07±0.25) and there was a reduction in Hb content also. However, when the blastema was formed, RBC (including circulating erythroblast) count and Hb content regained the normal (Preautotomy) values, and remained so thereafter (Table 1, Fig. 1).

Number of WBC in thousands per cubic millimeter in lizards with intact original tail was 2.42±0.39. During wound healing phase of the regenerating tail, a three fold increase (7.03±1.07) was observed which gradually declined as the wound healed. Nevertheless, during blastema and differentiation phases of the regenerating tail it was more than that observed in lizards with intact tail. In animals with fully regenerated tail, the leucocyte count reached the value closely comparable to that found in animals with intact tail (Table 1, Fig. 1).

Differential count of WBC in lizards with normal tail revealed that lymphocytes were the most numerous cell type, eosinophils and neutrophils were second and third to

Table 1 : Total RBC, WBC count and haemoglobin content during different phases of tail regeneration in H. flaviviridis.

	Phases of regeneration				
	Normal	Wound healing	Blastema	Differen-tiation	Fully regene-rated
Total RBC $10^5/mm^3$	2.13±0.57	1.07±0.25	2.08±0.29	2.44±0.50	2.11±0.44
Total WBC $10^3/mm^3$	2.42±0.39	7.03±1.07	5.62±0.30	4.20±0.72	2.85±0.72
Hb content in gms	12.2 ±1.39	9.20±0.57	11.5 ±1.91	11.2 ±1.57	13.0 ±1.05

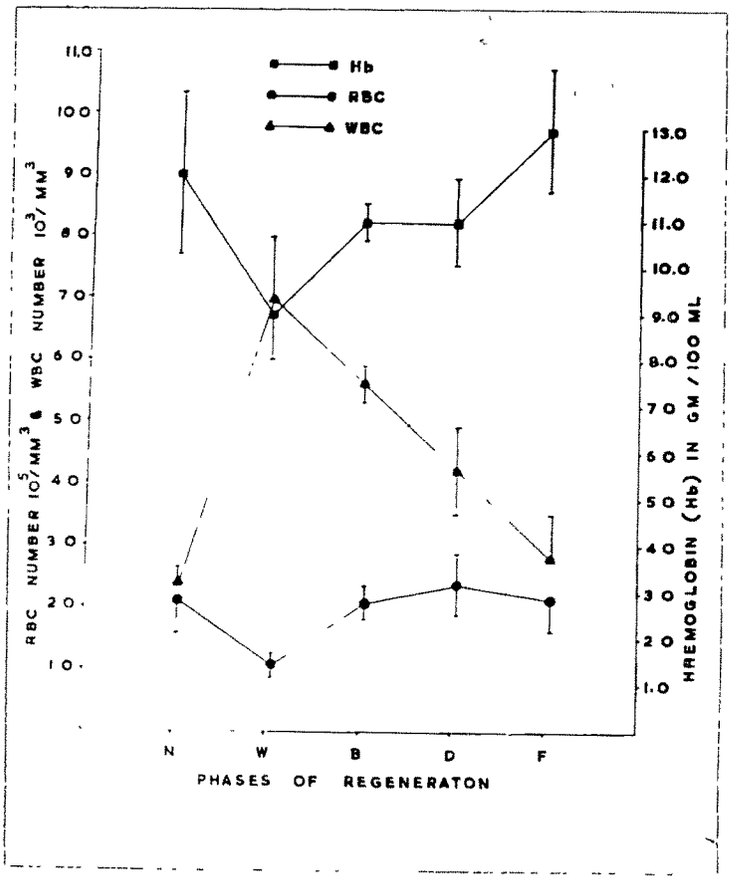

Fig. 1 : Graphic representation of total RBC and WBC count, and haemoglobin content during different phases of tail regeneration in H. flaviviridis.

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

Table 2 : Differential count of white blood cells during different phases of tail regeneration in H. flaviviridis

Phases of regeneration	Differential count of white blood cells				
	Lymphocyte	Eosinophil	Neutrophil	Basophil	Monocyte
Normal	60.3± 9.13	24.12±5.60	10.9 ±3.71	2.10±0.53	4.00±1.91
Wound healing	85.7±15.7	11.00±4.76	2.17±0.97	1.09±0.03	1.13±0.98
Blastema	69.3±12.5	15.5 ±7.31	12.3 ±3.57	2.07±0.56	2.13±0.39
Differentiation	58.3±10.5	18.7 ±7.93	10.0 ±5.76	2.13±0.96	2.00±1.01
Fully regenerated	62.0± 9.76	20.6 ±5.09	10.1 ±6.77	2.00±0.54	3.11±1.91

Table 3 : Differential count of bone marrow cells during different phases of tail regeneration in H. flaviviridis

Phases of regeneration	Differential count of bone marrow cells			
	Hemoblast	Myeloblast	Lymphoblast	Erythroblast
Normal	55.9±13.5	24.2±7.09	5.60±2.34	9.50±4.53
Wound healing	23.8±10.9	23.8±7.97	1.57±0.91	50.6 ±15.5
Blastema	61.9±17.6	30.5±9.65	3.09±1.53	4.48±1.97
Differentiation	51.9±13.6	43.1±10.5	2.70±0.56	1.91±0.37
Fully regenerated	57.3±12.9	25.7±7.44	4.91±1.54	3.50±0.47

Fig. 2

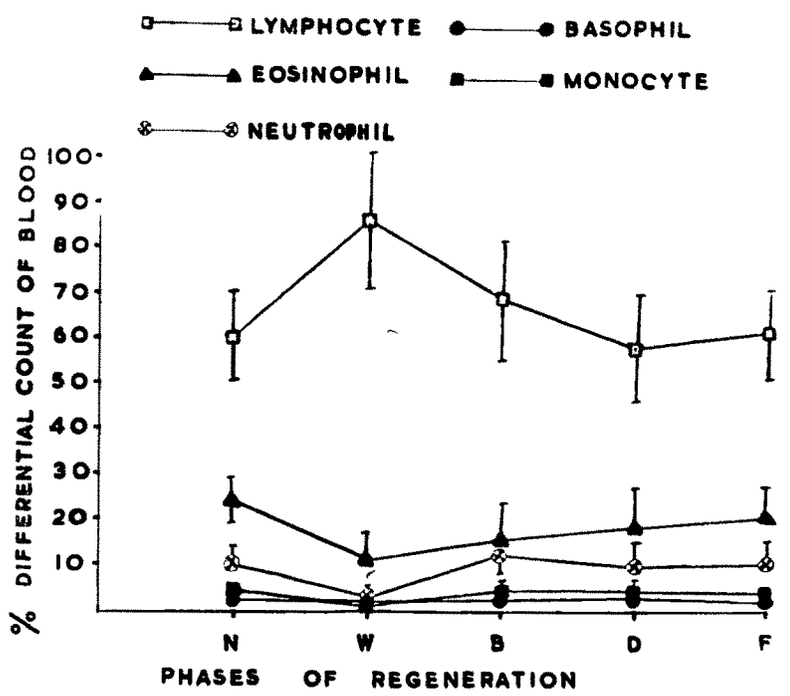
LYMPHOCYTE
BASOPHIL
EOSINOPHIL
MONOCYTE
NEUTROPHIL
% DIFFERENTIAL COUNT OF BLOOD
100
90
80
70
60
50
40
30
20
10
N
W
B
D
F
PHASES OF REGENERATION

Fig. 3

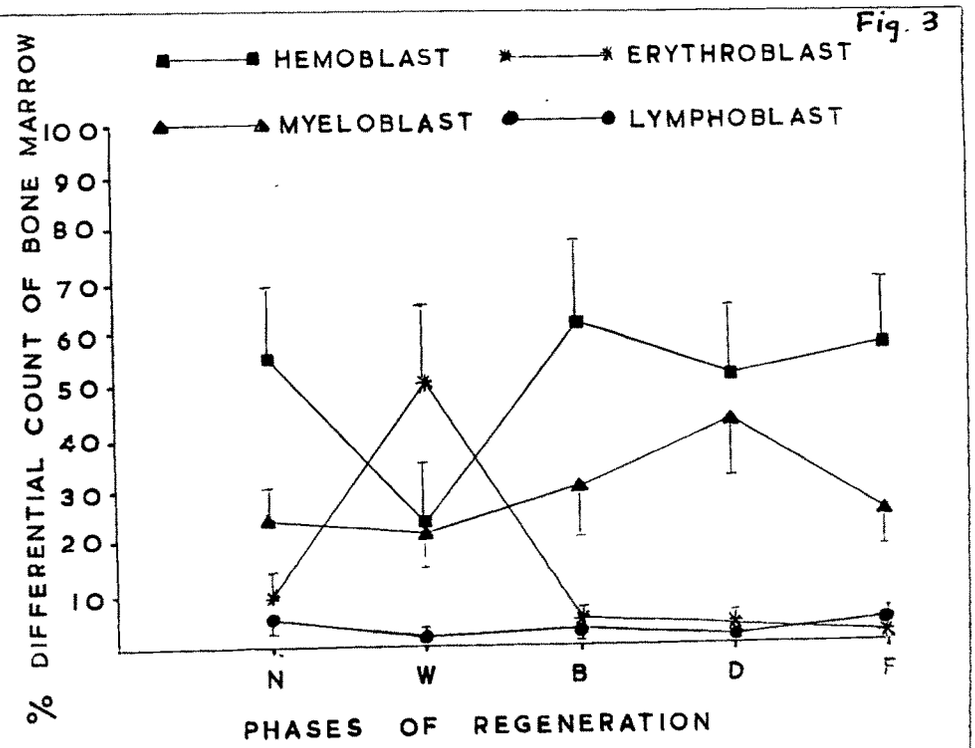
HEMOBLAST
ERYTHROBLAST
MYELOBLAST
LYMPHOBLAST
% DIFFERENTIAL COUNT OF BONE MARROW
100
90
80
70
60
50
40
30
20
10
N
W
B
D
F
PHASES OF REGENERATION

Fig. 2 : Graphic representation of differential count of white blood cells during different phases of tail regeneration in H. flaviviridis.

Fig. 3 : Graphic representation of different count of bone marrow cells 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

lymphocytes; while basophils and monocytes were the least numerous (Table 2). During wound healing period after autotomy, an increase in lymphocytes and a decrease in rest of the types of leucocytes was observed. Once the wound healed, the lymphocyte population gradually decreased, nevertheless during blastema phase, it was more than that observed in animals with original intact tail. During differentiation phase and thereafter, differential count of WBC regained the value observed in lizards with normal intact tail (Table 2, Fig. 2).

In bone marrow of lizards with original tail four types of cells which were identifiable viz., (i) hemoblast (primitive stem cell), (ii) myeloblast, (iii) lymphoblast, (iv) erythroblast (Ham, 1957; Greep, 1966) have been considered for the present study. In bone marrow of lizards with original tail, hemoblasts were the maximum in numbers, while myeloblasts and erythroblasts were second and third to hemoblasts and lymphoblasts were the least in their numbers. During wound healing phase, a decrease in hemoblast population was observed, while erythroblasts had increased in their numbers; no change in myeloblast and lymphoblast number was observed. During blastema and differentiation phases, erythroblasts had decreased whereas myeloblasts had increased in their numbers with almost no change in

haemoblasts and ~~erythr~~lymph oblasts. In full grown regenerate, the bone marrow picture was closely comparable to that in the lizards with intact original tail (Table 3, Fig. 3).

DISCUSSION

Haemodynamic adjustments during tail regeneration in lizard, H. flaviviridis involve certain marked changes in its blood constituents. Reptiles are known to respond hematologically to stress (Meints et al., 1975) and the stress of autotomy in the present study is reflected in the systemic response of vascular system. Andrew (1959) indicated that hemopoietic function of lizards is performed by spleen and bone marrow. All the cells in circulating blood are derived from one multipotent type of cell, hemoblast capable of differentiating in the bone marrow or spleen to produce erythrocyte, granulocyte, monocyte or lymphocyte (Girons, 1970).

In the present study, it is noted that the total RBC count is decreased to half its normal value during wound healing phase of the regenerating tail and a concomitant decrease in Hb content is also observed. This indicated a low oxygen supply in the lizard during this phase. As such oxygen affinity of the blood of lizards is low (Pough, 1969)

and increased energy demands during activity in most reptiles are met primarily by anaerobic metabolism (Bennet and Dawson, 1972; Bennet and Licht, 1972). Hence, it is quite logical to believe that energy needed for the wounded tissue to heal and that of other visceral organs involved directly or indirectly in the process is provided anaerobically, which is reflected in a low RBC count and Hb content. High activity of the enzymes, LDH and OC-GPDH in liver (Chapter 2) during wound healing phase of the regenerate also have revealed an operation of anaerobic glycolysis. Shah and Hiradhar (1974) have also suggested that the healing tail tissues are predominantly showing anaerobic mode of metabolism. In the light of above observations, it is believed that the energy required for regenerative process during the initial phase is generated anaerobically. A remarkable increase in the erythroblast population of the bone marrow but decrease in RBC count during the same phase indicates that the cells are not being released in the blood stream.

Once the blastema was formed and thereafter, RBC count and Hb content showed almost the normal values as seen during preautotomy period. And at this stage, erythroblast population of the bone marrow showed a decrease

in their numbers which is suggestive of the fact that previously depleted RBC count is being made good by the release of erythroblasts into the blood stream.

As for lymphocytopoiesis, poikilotherms are known to lack discrete organs for the purpose and that lymphocytes along with granulocytes and other cells are formed throughout the hemopoietic tissue (Miale, 1972). Healing of the wound is a product of integrated response of several cell types of blood to injury. Schilling (1968) has emphasized the importance of hemostatic mechanism in wound healing. To make good the loss of blood prior to such hemostatic mechanisms become effective, the hemopoietic activity of spleen (Chapter 5) and bone marrow are geared up. Stress is known to cause discharge of lymphocytes from hemopoietic organs (Pickford et al., 1971). A three fold increase in the total leucocyte count is apparently a systemic response for the healing of the exposed surface of the autotomised tail stump. This increase is largely contributed by the lymphocyte population of blood. A concomitant increase in the spleen weight and also in the mass of white pulp (Chapter 5) may be well accounted for, when the increased demand for lymphocytes at the wound site and the multifarious role played by them are considered. Significance of lymphocytes in immune reactions in lizards has been very well understood

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Radhakrishnan, N. 1973. Ph.D. thesis entitled, "Histological and histophysiological studies on the normal and regenerating tail of the scincid lizard, Mabuya carinata", submitted to the M.S. University of Baroda, Baroda-2, India.

Radhakrishnan, N. and Shah, R.V. 1973. Studies on the levels of glycogen and phosphorylase in the normal and regenerating tail of the scincid lizard, Mabuya carinata: A quantitative and histochemical analysis J. Anim. Morphol. Physiol., 20(2): 150-159.

inflammatory response to injury is dominated by accumulation of polymorphonuclear leucocytes at the site of damage (Schilling, 1968). A large number of polymorphonuclear leucocytes in the scab of mouse skin (Tarin and Croft, 1970) and in injured liver of rat (Shah et al., 1974) have been reported. The only well delineated function of neutrophilic leucocyte is that of phagocytosis to combat infection. The eosinophilic leucocyte also can act as phagocytes, although to a lesser extent than neutrophils (Vaughn, 1953). No change in myeloblast population of bone marrow was observed during wound healing phase. The eosinophilic and neutrophilic leucocytes are incapable of division and their constancy in blood stream is maintained by myeloblasts in the bone marrow (Schilling, 1968). The presently observed increase in myeloblast population of bone marrow during the blastema and differentiation phases of regeneration finds support in above contention, when restoration of depleted count of neutrophil and eosinophil in the blood to the preautotomy level is considered.

In animals with fully regenerated tail, all the constituents of blood and bone marrow showed the similar values noted during the preautotomy period. Thus, it could be surmised that blood vascular system plays a significant role during regenerative process.