CHAPTER - 1

SECTION: I HAEMATOPOIESIS AND REGENERATION: CHANGES IN THE CELLULAR ELEMENTS OF BLOOD AND HAEMOGLOBIN DURING TAIL REGENERATION IN THE SCINCID LIZARD, MABUYA CARINATA

Blood being the most important communicating system in a living body, any normal or abnormal physiological and physical changes in the body, would be reflected on to it and would be manifested in the form of changes in its structure and composition. In fact, the changes undergone by the blood would serve as an index of the disturbance that must be affecting either a part, or the whole organism itself. Further, blood being an important participant in internal homeostasis, any disturbance normal or abnormal in any part of the organism is bound to influence its physiology and show resultant changes either as supporting or aiding measure if it be a normal disturbance, or as a correcting measure if an abnormal one. Varied and extensive changes in the blood of homeothermic vertebrates especially the mammals, in the wake of multifarious physiological and pathological conditions and processes have been well documented.

Though there are a few studies directed mainly to understand the normal blood chemistry and composition in reptiles (Dessauer, 1970; Saint Girons, 1970), investigations comparable to that on homeotherms with respect to physiological changes have received scant attention on poikilotherms in general and reptiles in particular. Previous studies from this laboratory on lizard tail regeneration have reported profound metabolic and biochemical changes in the tail during regeneration (Shah and Chakko, 1967, 1969, 1972; Magon, 1970; Shah et al ., 1971; Shah and Hiradhar, 1974; Hiradhar, 1972; Radhakrishnan and Shah, 1973; Radhakrishnan, 1972; Shah and Ramachandran, 1970, 1972, 1973, 1974; Ramachandran et al., 1975, Ramachandran, 1972). Similar observations have also been reported with regard to amphibian regeneration (Geczik and Wolsky, 1959; Johnson and Singer, 1963, 1964, Niwelinski, 1960; Schmidt, 1966; Wolfe and Cohen, 1963). Moreover, in the present work, it has also been observed that structural and physiological changes occur in other parts of the body as well in relation to regeneration. It is with this background of observable metabolic, physiological, and biochemical changes, both, at the site of regeneration, as well as in other body parts, that the present investigation on the possible changes in the level of haemoglobin as well as the cellular composition of the blood of the Scincid lizard, Mabuya carinata during its tail regeneration has been undertaken. It is deemed that such a study may not only enable us to understand the obligatory changes undergone by blood in response to the regenerative mechanics but could also add to the existing meagre data on the normal blood physiology of reptiles.

MATERIAL AND METHODS

Adult lizards, (Mabuya) obtained from Karnataka, India, and maintained on a diet of insects were used for the experiments. The animals were first allowed to get acclimatized to the laboratory conditions by keeping them for about a fortnight. Autotomy of the tails was performed by pinching off the tails at a fixed distance from the vent. Blood samples were then collected from both normal as well as lizards with regenerating tail by cardiac puncture. The blood samples of the animals with regenerating tail were obtained at fixed intervals of 3, 5, 7, 12, 25 and 60 days respectively after autotomy. These intervals correspond to the various arbitrarily fixed phases of regeneration, such as, wound healing, early and late blastema, differentiation and growth.

For both, the total R.B.C. count as well as W.B.C. count, the standard techniques using haemocytometer were employed. The haemoglobin content was estimated by using Helige Sahli haemometer. For studying the general histological picture of blood as well as for differential count of W.B.Cs, the blood smears were stained by the Jenner-Gimsa technique.

RESULTS

TOTAL RED BLOOD CELL COUNT

The average erythrocyte count in the normal adult Mabuya varied between 5 to 5.5 lakhs (o.5 to o.55 million) per mm^3. From Table - 1; Figure - 1, it is apparent that the early periods of regeneration are marked by an increase in the total R.B.C. count reaching to a more than double the normal level by the end of the first week after autotomy. Thereafter, the count registered a gradual decline and returned to the normal level by the 25th day of regeneration.

MICROSCOPIC PICTURE OF THE BLOOD SMEARS

For the sake of convenience, the immature erythrocytes were arbitrarily divided into two types,

FIG 1. TABLE 1. CHANGES IN THE TOTAL R. B. C. COUNT AND HAEMOGLOBIN CONTENT DURING TAIL REGENERATION IN MABUYA CARINATA.

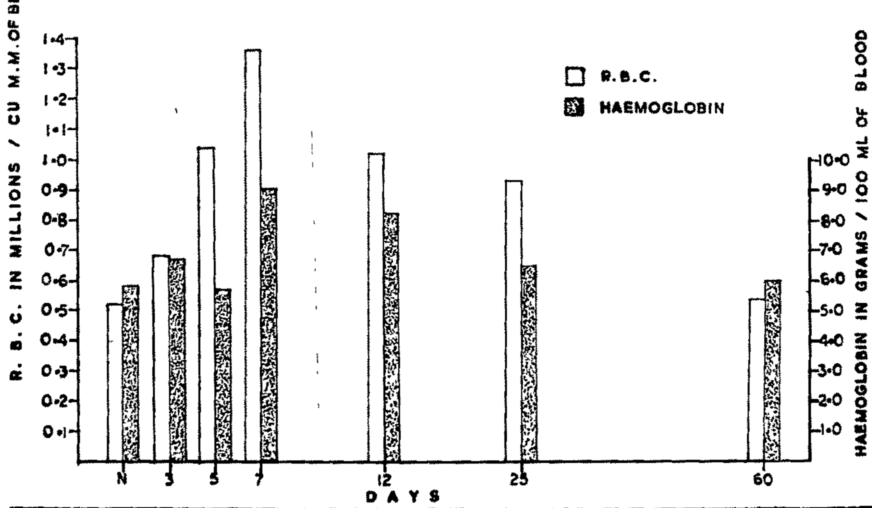


TABLE 1.	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	25TH DAY	60TH DAY
R. B. C.	520000 ± 75277	688000 ± 103053	1040000 ± 50000	1363000 ± 276019	1022000 ± 62230	937500 ± 41932	540000 ± 45460
HAEMOGLOBIN	5.87 ± 0.562	6.74 ± 0.219	5.72 ± 0.914	9.00 ± 1.0	8.20 ± 0.125	6.50 ± 0.5	6.00 ± 0.25

FIG.2. TABEE.2. CHANGES IN THE NUMBER OF IMMATURE ERYTHROCYTES IN MABUYA CARINATA.

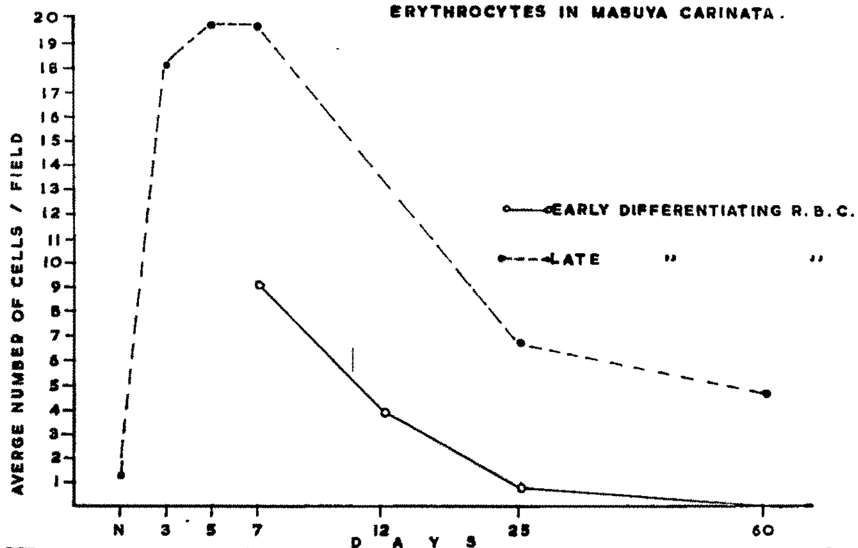


TABLE. 2.	NORMAL	3 RD DAY	5TH DAY	7TH DAY	12TH DAY	25TH DAY	60TH DAY
EARLY DIFF R. B. C.	—	—	—	9.1 ± 5.3	3.96 ± 0.4	0.7 ± 0.122	—
LATB DIFF R B. C	1.38 ± 0.94	18.06 ± 1.5	19.86 ± 2.5	19.88 ± 3.7	13.38 ± 4.1	6.64 ± 1.6	4 7 ± 1 08

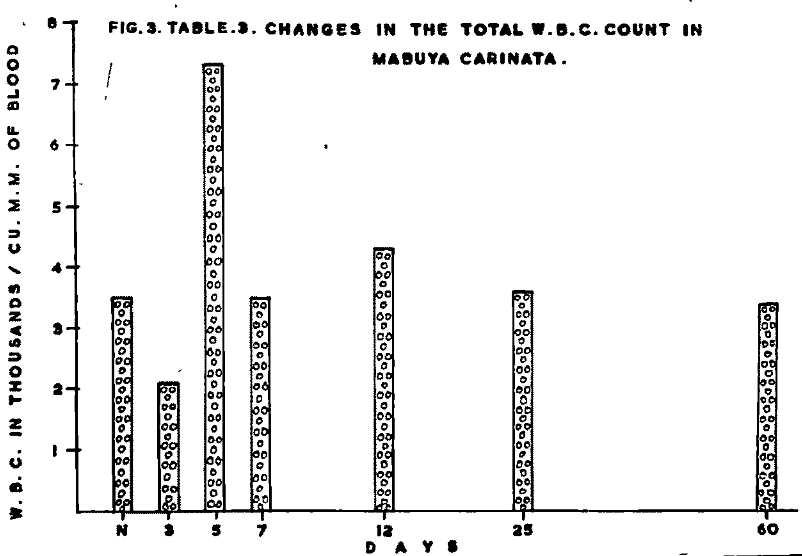


FIG.3. TABLE.3. CHANGES IN THE TOTAL W.B.C. COUNT IN MABUYA CARINATA.

TABLE.3	NORMAL	3RD DAY	5 TH DAY	7 TH DAY	12TH DAY	25TH DAY	60TH DAY
W. B. C.	3500 ± 258	2120 ± 178	7360 ± 517	3550 ± 167	4360 ± 219	3600 ± 365	3400 ± 182

FIG·4: TABLE·4· CHANGES IN THE NUMBER OF GRANULOCYTES IN MABUYA CARINATA

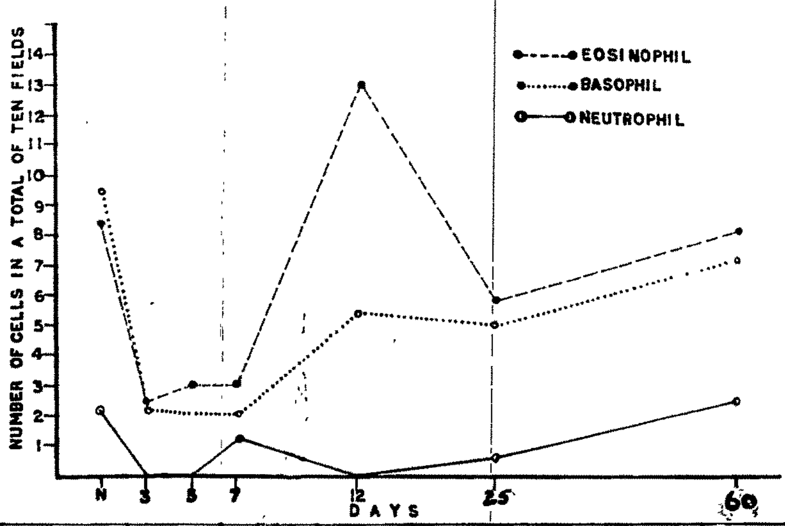
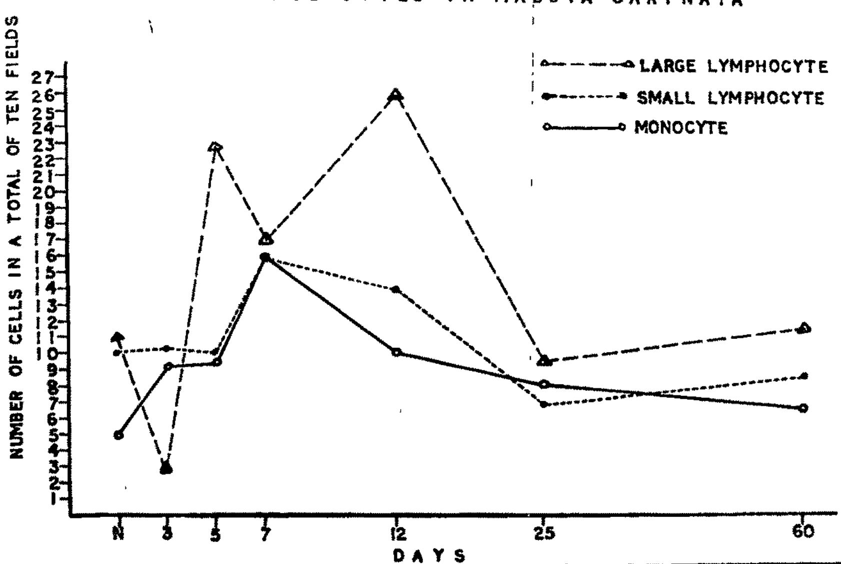


TABLE · 4	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	25TH DAY	60TH DAY
EOSINOPHIL	8·4± 2·5	2·4± 1·1	3·0± 2·1	3·0± 1·7	13·0± 3·3	5·8± 1·3	8·2± 2·3
BASOPHIL	9·5± 2·2	2·2± 0·83	2·0± 2·5	2·0± 1·8	5·4± 5·9	5·0± 4·0	7·2± 1·5
NEUTROPHIL	2·2± 0·97	–	–	1·2± 0·98	–	0·6± 0·80	2·5± 1·20

FIG·5: TABLE·5·CHANGES IN THE NUMBER OF AGRANULOCYTES IN MABUYA CARINATA



TABLE· 5	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	25TH DAY	60TH DAY
LARGE LYMPHOCYTE	11·0± 7·5	2·8± 2·4	22·8± 10·9	17·0± 3·2	26·0± 10·5	9·6± 4·0	11·7± 6·5
SMALL LYMPHOCYTE	10·0± 6·1	10·2± 6·4	10·0± 5·7	16·0± 9·2	14·0± 5·0	6·8± 2·1	8·5± 3·4
MONOCYTE	5·0± 9·8	9·2± 3·0	9·4± 5·1	16·0± 8·7	10·0± 3·2	8·0± 2·8	6·7± 4·1

EXPLANATION TO FIGURES

Fig. 6. Blood smear of normal (with intact original tail), Mabuya. Note the appearance of R.B.Cs. L: Lymphocyte; G; Granulocyte 500 X.

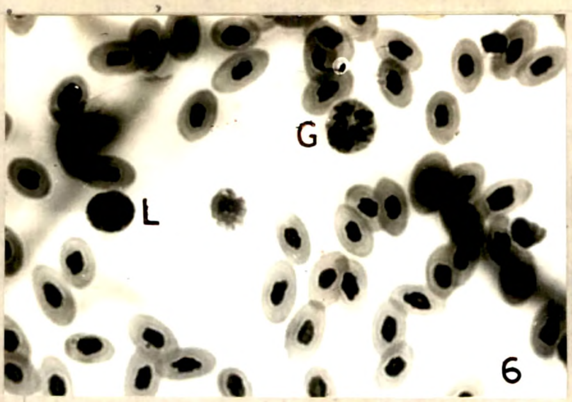
Fig. 7. Blood smear of Mabuya taken on the 3rd day of tail regeneration showing Preerythrocytes (Late R.B.Cs). 500 X.

Fig. 8. Orthochromatic erythrocytes (Early R.B.Cs) seen on the 5th day of tail regeneration. 500 X.

Fig. 9. Blood smear of Mabuya taken on the 7th day of tail regeneration showing early differentiating R.B.Cs (1, 2 and 3). 500 X.

Fig. 10. Polychromatic erythrocytes (Early R.B.Cs) seen on the 7th day of tail regeneration. 500 X.

Fig. 11. Blood smear taken on the 5th day of regeneration showing increased number of large to medium sized lymphocytes (L_1). 500 X.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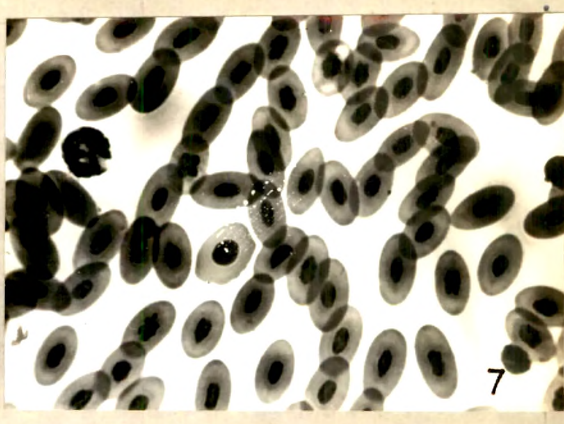
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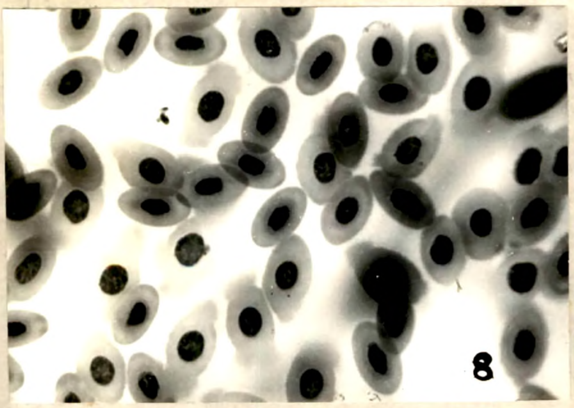
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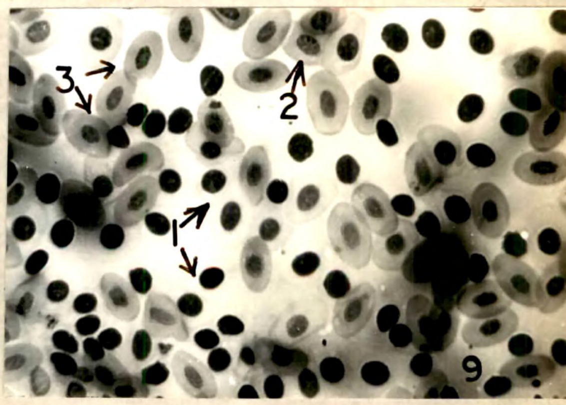
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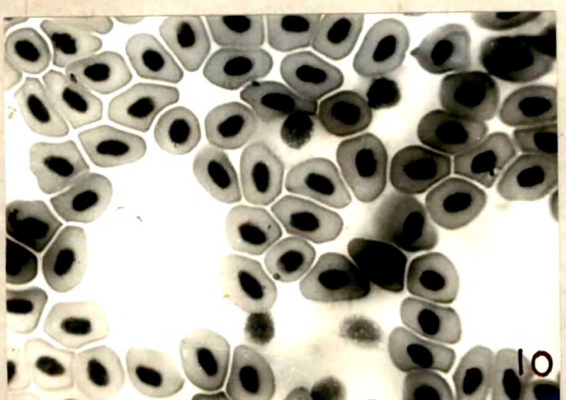
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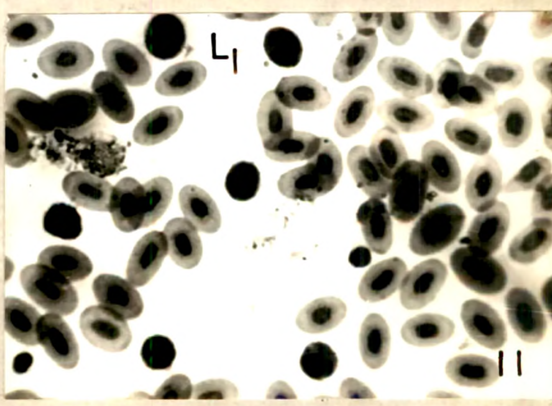
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EXPLANATION TO FIGURES

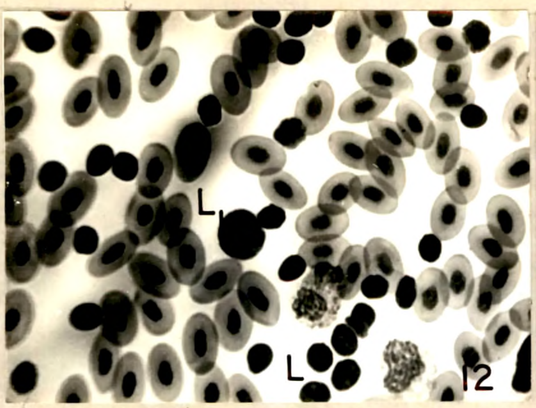
Fig. 12. 7th day blood smear depicting the increased population of small lymphocytes (L). L_1: Large lymphocyte. 500 X

Fig. 13. Near normal R.B.Cs seen on the 25th day of tail regeneration. Note the nuclear condensation. 500 X.

Fig. 14. Photograph depicting the presence of many monocytes (M) with loaded debris material (arrows) and vacuoles in the cytoplasm on the 7th day of regeneration. 1000 X.

Fig. 15. Blood smear of Mabuya taken on the 12th day of tail regeneration showing disrupted monocytes and their liberated contents (arrows). 1000 X.

Fig. 16. One single monocyte with it contents. 1000 X.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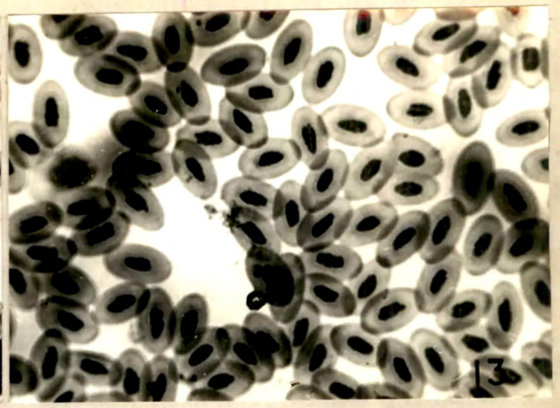
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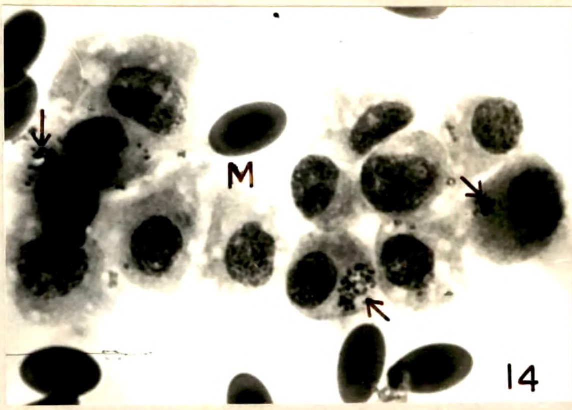
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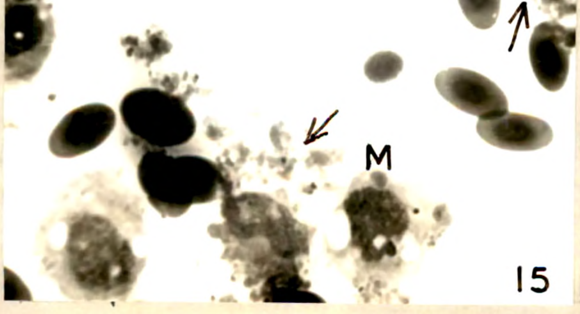
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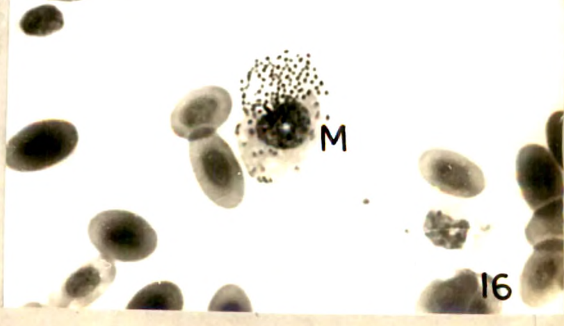
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(I) the early and (2) the late ones, based on their state of development. The normal, fully mature R.B.Cs were oval in shape, with the nuclei which were also oval shaped having their long axis placed parallel to that of the cells. The nuclei were also highly chromophilic (Figure - 6). The early stages of immature erythrocytes on staining were characterised by a rounded form, blue cytoplasm and large nuclei. The prominent nucleus to begin with had a reddish to pinkish tinge which finally acquired its basophilic nature. Similarly the cytoplasm too, which to begin with had a basophilic nature also changed over to the characteristic reddish acidophilic tinge gradually. All these grades of cells, referred to as normoblasts or more specifically as erythroblasts (proerythroblasts, basophilic erythroblasts, polychromatic erythrobalsts and orthochromatic erythroblasts) were grouped under the early types of immature cells in the present study. The late types of immature ones though showing the characteristic mature nuclear and cytoplasmic colorations, were found to be semioval shaped (both cytoplasm as well as nucleus), with masses of chromatin material and less basophilic nuclei (preerythrocytes). The various stages of developing erythrocytes are depicted in Figures - 6 - 11.

In the Table, average number of each type of cells per field observed during normal as well as various stages of regeneration are represented. The maximum number of cells, both late as well as early types of immature cells were seen on the 7th day, being 20 and 9 respectively per field. The early type of cells in fact appeared in significance only by the 7th day (Figures- 9, 10). They were almost absent both in the normal as well as 3 days post-autotomy animals, and on the 5th day, they could be met with occasionally when the smear was viewed as a whole. On days 12 and 25, these cells showed gradual decline and disappeared totally from the smear of the lizards with 60 days old regenerates. The late type of cells starting with an average of 1 per field reached a maximum of 20 per field on the 5th and 7th days post-autotomy. Thereafter, these cells showed a decrease and reached a low level of 7 per field on the 25th day. The same average was more or less maintained in animals even when the regenerate was of 60 days old. This average though low, was nevertheless quite high in comparison to the normal.

HAEMOGLOBIN CONTENT

The levels of haemoglobin in Mabuya during the various periods of study (post-autotomy) are represented in Table - 1, Figure - 1. In accordance with the constant increase in the red cell count immediate to post-autotomy noted above, the haemoglobin content also registered a gradual increase from the normal level of 5.8 gms/100 ml to a maximum level of 9 gms/100 ml on the 7th day. However, there was a slight fall on the 5th day almost to the normal level. After day 7, the haemoglobin content started falling gradually in correspondence to the fall in R.B.C count till it attained a near normal level by day 60 when the regenerative process also was almost completed.

TOTAL W.B.C. COUNT

As could be made out from Figure - 2 the total leucocyte count rose to a maximum on the 5th day (7360) from the normal level (3500). After the 5th day a further increase (4360) was registered on the 12th day after having touched in between the normal level on the 7th day. Thereafter, by the 25th day, the leucocyte count again came down to the normal level and was maintained so even on day 60 when the regenerate was almost fully grown.

DIFFERENTIAL COUNT

It may be mentioned at the very outset that the differential count carried out herein was only with a view to understand the possible changes undergone by the leucocyte population so that these changes may be considered as an index of the demands of the process of regeneration and, correlate these changes in the blood with the regenerative mechanics. In this regard, the present count was only obligatory to the understanding of the phenomenon of regeneration and as such was not an attempt to standardize and establish the percentage of the various types of leucocytes. The various types of leucocytes and their numerical changes noted during regeneration are shown in Tables - 4, 5. The changes being more varied; the general observations (during different phases of regeneration) of the smears are briefly given below.

NORMAL

In the normal (having intact original tail) animals, most of the leucocyte population appeared to be constituted of the granulocytes, chiefly eosinophils and basophils with the neutrophils being almost absent. The lymphocytes and monocytes were very few in mumber.

REGENERATING ANIMALS

3RD DAY AFTER AUTOTOMY

There appeared to be a marked reduction in the granulocyte population at this stage, with only a few lymphocytes and monocytes being the chief representatives of the leucocyte population.

5TH DAY AFTER AUTOTOMY

This stage which corresponds with the completion of wound healing was distinctly marked by a steep increase in the large lymphocyte population. Monocytes were also on the increase though not that well pronounced. However, the granulocytes continued to remain at a low negligible level.

7TH DAY AFTER AUTOTOMY

The granulocyte population remained at the same low negligible level. Whereas the large lymphocytes did not show any further increase, the small lymphocytes appeared to be increased in number. The monocytes appeared to be loaded with debris material and waste products.

12TH DAY AFTER AUTOTOMY

This period was marked by a substantial increase of the granulocytes especially, the eosinophils. The large lymphocytes were at the highest level with the small lymphocytes and monocytes also though at a higher level, were, however, at a slightly lesser number in comparison to that observed on the 7th day. Most of the monocytes were fully loaded with ingested debris material.

25TH AND 60TH DAYS AFTER AUTOTOMY

By day 25, the number of various leucocytes had started declining and in the lizards with 60 day old regenerates all the leucocytes attained more or less their respective normal levels. During the periods 5th, 7th and 12th days, the smear showed large groups of small lymphocytes distributed all over the slide. These areas were not taken for counting. Also observable during the various periods of regeneration were many large undifferentiated leucocytes and other cells which elude precise identification and hence were not taken into account.

DISCUSSION

Present investigations on the cellular composition and haemoglobin content of the blood of the scincid lizard, Mabuya carinata have shown definite changes in response to the process of regeneration. A significant aspect in this connection is the increase in the erythrocyte count, which commences by the 3rd day after autotomy and attains a maximum level on the 7th day. The increase in erythrocyte count is well paralleled by the concomitant increase in the haemoglobin content as well. In the case of Mabuya the problem appears to be somewhat complex and the operation of a dual pattern of change appears to be the feature. In contradiction to Calotes (next section) in this case, the erythrocyte count increased gradually as could be evidenced by the 3rd day itself and reached a double the normal level on the 5th day. In accordance, the haemoglobin content also increased on the 3rd day but showed an immediate abrupt fall on the 5th day, the terminal phase of healing. The fall in haemoglobin content at this period in the face of increase in R.B.C. eount, finds no other explanation excepting that it leads to a reduced O_2 carying capacity of the blood, which might not only enable in supplementing the high anaerobiosis characteristic of wound healing but also help in nullifying the possible setting in of Pasteur effect at the wound site. What precisely controls or regulates this aspect of haemoglobin variation is enigmatic. It is appropriate to note here that Rugh and Somogyi (1968) working on blood cell counts in pre and postnatal mouse have reported a drop in the haemoglobin content from birth through 3 weeks even though the R.B.C. count was registering a progressive increase. The increase in the R.B.C. count observable in the present case appears to be of a totally different pattern as unlike in Calotes, the increase in erythropoiesis is not apparently stimulated by a fall in the number of circulating cells, but presumably by some unknown factor or factors released immediately subsequent to autotomy. When viewed in this perspective, the wound healing of an appendage which ultimately regenerates tends to differ from that of the non-regenerating one. Though the broad mechanics of this process such as tissue demolition, repair and the formation of a wound epithelium subsequent to the loss of tail, remains more or less the same in both the lizards, however, it appears that there are certain specific and intrinsic differences between the two types of wound healing. If it be so recognized that there is a subtle difference between the process of healing in the two cases, it can be assumed that the tail tissues of Mabuya, in awareness of their ordained disposition towards regenerative capacity, release certain signalling factor (s) calling upon the whole organism to attune itself to the major physiological necessities that accrue thereof. Seemingly, one such change appears to be directed towards an increased number of erythrocytes and haemoglobin content and hence an increased oxygen carrying capacity of the blood. Previous studies on tail metabolism during regeneration in Mabuya have indicated a shift in metabolism from anaerobic to aerobic during the early phases (Shah and Ramachandran, 1970, 1973, 1974; Ramachandran, 1972; Shah and Radhakrishanan, 1973; Radhakrishnan, 1972). Interestingly, the present study indicates a peak level of haemoglobin content and R.B.C. count between the 7th and 12th days of regeneration, which corresponds to blastema and early differentiation phases. Thereafter, with the progression of differentiation and a gradual shift back to anaerobic type of metabolism, there is a corresponding gradual decline in both the R.B.C. count as well as haemoglobin content till on day 60 and thereafter (which corresponds with the attainment of a fully almost regenerated condition of the tail), they assume a more or less normal level. The highly augmented level of erythropoiesis can well be gauged by the appearance of large number of immature R.B.Cs in the blood stream during the peak periods, which, in fact tends to be much higher than that observed in the case of Calotes. In fact, the tremendous increase of the early type of immature red blood cells in the blood stream during the 7th and 12th days could in itself be considered as an indication of the fact that the erythropoietic organs somehow fail to keep pace with the sudden and hectic demand made by the regenerating system, and hence are forced to sqeeze out all the available erythrocytes including the immature ones. On a comparative basis, the ratio of haemoglobin to number of red cells appears to be slightly higher in the case of normal Mabuya than in Calotes. This aspect when viewed in the context of the reported observation of the presence of 70% of the total haemoglobin in the form of nonfunctional methemoglobin in certain turtles (Dessauer, 1970) and the presently noted increase in red blood cell count and the resultant haemoglobin content during regeneration, tends to highlight the possible existence of a part of haemoglobin in Mabuya too in the form of nonfunctional methemoglobin and as such could be a factor of significance worthy of consideration. Another significant report in this connection which is worth reflecting at this point, is the one by Pilo and George, (1970) who had shown an elevated level of erythrocyte population and haemoglobin content during the premigratory period in preparation for the increased oxidative metabolism during migration in the migratory starling Sturnus roseus.

The immediate effect of autotomy on the total number of leucocytes appears to be a slight fall on the 3rd day which is not shown by Calotes after amputation of its tail. This discrepency might lie in the fact that the total white cell population in the blood of normal Mabuya is higher than that in Calotes and hence subsequent to autotomy enough cells can be drawn from this common pool to the local site of injury. Also possibly, the exigency of increased erythropoiesis being more acute, the stimulation of leucopoiesis might be taking effect at a slower rate in comparision, and hence, there is a decrease in the leucocyte count on the 3rd day; and by the 5th day (which corresponds to the culminative phase of wound healing) however, the stimulatory effect is fully geared and hence accordingly there is a maximal white cell count at this stage. Subsequently on the 7th day, the count touches down to the normal level only to show a slight increase once again on the 12th day. Thereafter the W.B.C. count touches the normal level with the progression of differentiation as could be seen on the 25th day and is maintained so till the end of the regenerative process. Thus, the significant periods during which W.B.Cs play an important role appears to be during the 5th and 12th days. To ascertain the role(s) played by W.B.C. during regeneration, it becomes pertinent to look into the differential population of the various types of leucocytes (Tables- 4 , 5). From the tables it is evident that the initial drop in cell count observed on the 3rd day is due mainly to a depletion of granulocytes from the total W.B.C population of the blood. This drop in granulocytes is whether due to their migration towards the wound site or due to their withdrawal from the blood is difficult to ascertain. However, the continued low level of these cells during the later phases of regeneration tends to indicate the fact that the granulocytes in general have little functional role if any during lacertilian tail regeneration. In contrast, both the types of agranulocytes show noteworthy changes during regeneration. The monocytes appear to remain at an elevated level all throughout regeneration right from the 3rd day uptil the 25th day. Being phagocytic in nature, their role in removing debris and waste products from the wound site can be well expected. Their peak levels during the 7th and 12th days (corresponding to dedifferentiative and early differentiative phases) and the observation that these cells are loaded with waste materials lead to the surmise that the two processes of regeneration Viz., dedifferentiation and differentiation leave behind substantial amount of cellular debris and perhaps other waste matter which the monocytes remove. Thus they play a pivotal role in the mopping up operation, maintaining a proper environment conducive to the progress of regeneration. The lymphocytes on the other hand could be purported to play a major role between the 5th and 12th days as could be infered from their elevated levels during this period. Lymphocytes are known to have phagocytic properties in many of the vertebrates and in this respect, the increased number of large lymphocytes observable in the blood throughout the period mentioned above is self explanatory. The function of elevated population of small lymphocytes during the same period is, however, debatable. It may be worthwhile to note here that despite the considerable amount of work that has been carried out on amphibian as well as reptilian regeneration by now, there still persists two differing viewpoints regarding the origin of blastemal cells. A long standing and perhaps a major viewpoint holds dedifferentiation of some of the stump tissues as the source of blastemal cells (Towle, 1901; Thronton, 1938; Chalkley, 1954, 1959; Hay, 1959, 1962; Lentz, 1969) while, the opposite viewpoint holds dedifferentiation as the activation of various connective tissue cells or reserve elements (Weiss, 1939; Manner, 1953; Nicholas, 1955; Toto and Annoni, 1965; Schmidt, 1968). This conflicting schools of thought coupled with the light microscopic observation of a nonhomogenous blastemal cell population in Mabuya (unpublished), and the present observation of large groups of small lymphocytes in the blood smears in the period between 5th and 12th days (blastema formation period) are rather interesting and tempt us to suggest the possibility of a probable participation by the small lymphocytes in populating the regeneration blastema, It is quite likely, that the blastemal cell population has a dual origin, contributed in part by the dedifferentiation of stump tissues, and in part by the migrating small lymphocytes and activated connective tissue elements. Moreover, it may also be worthwhile to take congnizance of the reported role of lymphocytes in maintaining the steady state in proliferating tissues (by neutralizing tissue specific factors released from dividing cells; Burwell, 1963), as well as their capacity to act as trephocytes (by donating the DNA which could be reutilized by developing cells; Harris, 1974). In this light, the presently observed increased incidence of lymphocytes as well as the fact that the process of regeneration, essentially a developmental event, involves increased exigency of cell proliferation, are rather significant, and the possible participation of lymphocytes in the above functional garbs too during tail regeneration in *Mabuya carinata* appears rather tenable and as such cannot be overlooked.

SECTION: II HAEMATOPOIESIS AND WOUND HEALING: CHANGES IN THE CELLULAR ELEMENTS OF BLOOD AND HAEMOGLOBIN DURING TAIL WOUND HEALING IN THE AGAMID LIZARD, CALOTES VERSICOLOR

Though the broad mechanics of wound healing and the local metabolic changes during this process have been well established, the possible involvement of the systemic factors in relation to wound healing has received almost no attention at all. The immediate systemic component that might be construed to show any responsive change is the blood vascular system. It was in this context that an investigation of blood in terms of its cellular composition and haemoglobin content has been undertaken in the lizard, Calotes versicolor during its tail wound healing. Moreover, the present study of a comparable nature conducted during tail regeneration in Mabuya carinata has shown certain definite changes. In this light, the current investigation is not only an attempt at understanding the possible changes that might be undergone by blood in response to the wound healing process, but also to serve as a control for the studies on regeneration, as the tail of Calotes unlike Mabuya does not regenerate.

MATERIAL AND METHODS

Adult lizards (*Calotes versicolor*) obtained from the local animal dealer and maintained on a diet of insects was used as the experimental animals. The animals were first allowed to get acclimatized to the laboratory conditions by keeping them for about a fortnight. Amputation of the tail at a fixed distance from the vent was performed with the help of a sharp scalpel. To prevent excessive blood loss, the cut end of the tails was kept pressed for a few minutes. Blood samples were then collected from both normal as well as animals with the healing tails by cardiac puncture. The blood samples of animals with healing tail wound were obtained at fixed intervals of 3, 5, 7, 12, 15 and 25 days respectively after amputation. For both, the total R.B.C. count as well as W.B.C. count, the standard techniques using haemocytometer were employed. The haemoglobin content was estimated by using Hellige Salhi haemometer. For studying the general histological picture of blood as well as for differential count of W.B.C, the blood smears were stained by the Jenner-Giemsa technique.

RESULTS

TOTAL RED BLOOD CELL COUNT

The average erythrocyte count in the normal adult Calotes varied between 8 to 8.5 lakhs (0.8 to 0.85 Million) per mm^3. From Table - 1; Figure - 1, it becomes apparent that the R.B.C. count fell to half the normal level by the 5th day postamputation which was immediately followed by a rapid increase and a settlement to a more or less normal level by the end of the second week of tail amputation.

MICROSCOPIC PICTURE OF THE BLOOD SMEARS

Characterisation of the normal as well as the immature erythrocytes is maintained same as per the descriptions given in the previous section for Mabuya during its tail regeneration. In Table - 3; Figure - 3, average number of each type of cells per field observed in the normal as well as in those at the various periods of tail wound healing are presented. In the present case, contrary to the observations in Mabuya,

FIG I TABLE I. CHANGES IN THE TOTAL R.B.C. COUNT AND HAEMOGLOBIN CONTENT DURING TAIL WOUND HEALING IN CALOTES VERSICOLOR.

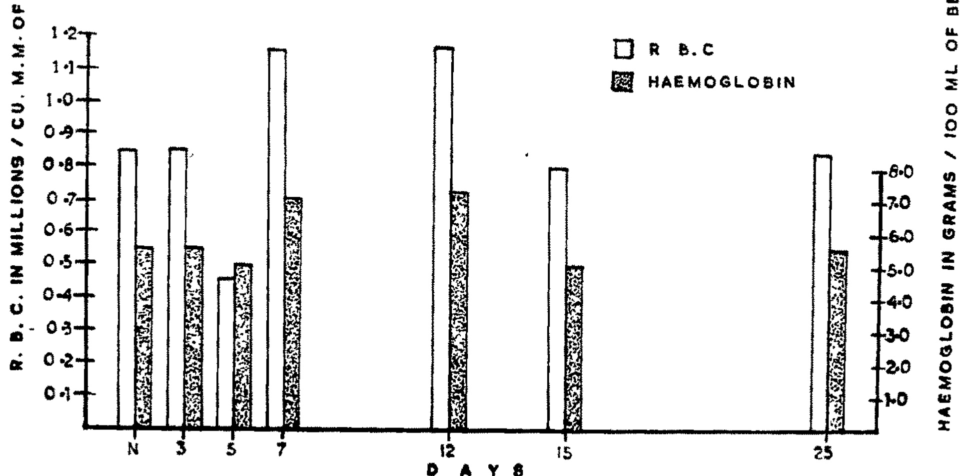


TABLE. I.	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	15TH DAY	25TH DAY
R. B. C.	850000 ± 30550	850705 ± 93966	460205 ± 17078	1160205 ± 73654	1170602 ± 72143	609090 ± 103923	850050 ± 34420
HAEMOGLOBIN	5.50 ± 0.012	5.50 ± 0.012	5.00 ± 0.012	7.00 ± 0.012	7.20 ± 0.28	5.00 ± 0.012	5.50 ± 0.15

FIG.2. TABLE.2. CHANGES IN THE NUMBER OF IMMATURE ERYHROCYTES IN CALOTES VERSICOLOR

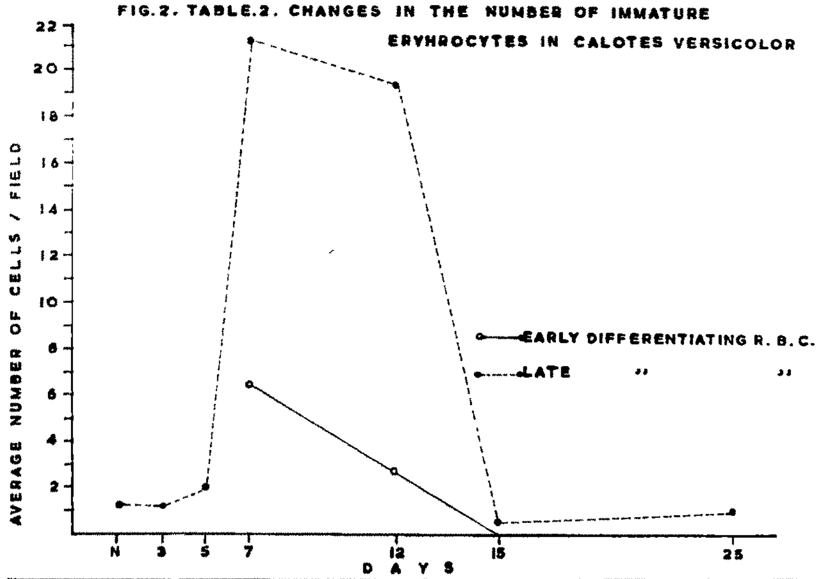


TABLE.2.	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	15TH DAY	25TH DAY
EARLY DIFF R. B. C.	—	—	—	6.47 ± 1·8	2.76 ± 0·6	—	—
LATE DIFF R. B. C.	01.20 ± 0·4	01.18 ± 0·5	02.00 ± 0·7	21.45 ± 5·2	19.63 ± 3·4	00·76 ± 0·14	01.00 ± 0·8

FIG.3. TABLE.3. CHANGES IN THE TOTAL W.B.C. COUNT IN CALOTES VERSICOLOR

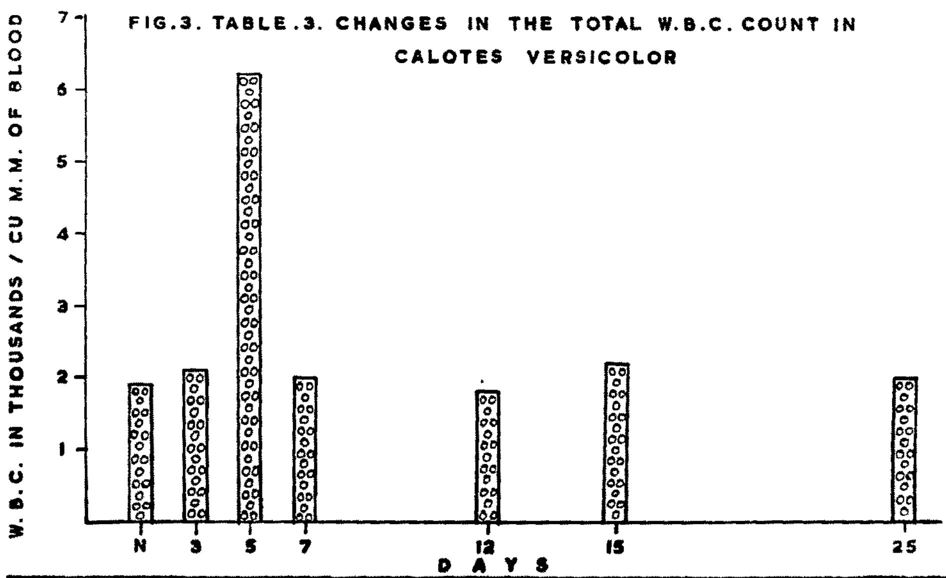


TABLE 3	NORMAL	3 RD DAY	5 TH DAY	7 TH DAY	12 TH DAY	15 TH DAY	25 TH DAY
W B C.	1900 ± 100	2150 ± 191	6205 ± 300	2000 ± 365	1800 ± 200	2200 ± 400	2000 ± 125

FIG·4· TABLE·4· CHANGES IN THE NUMBER OF GRANULOCYTES IN CALOTES VERSICOLOR

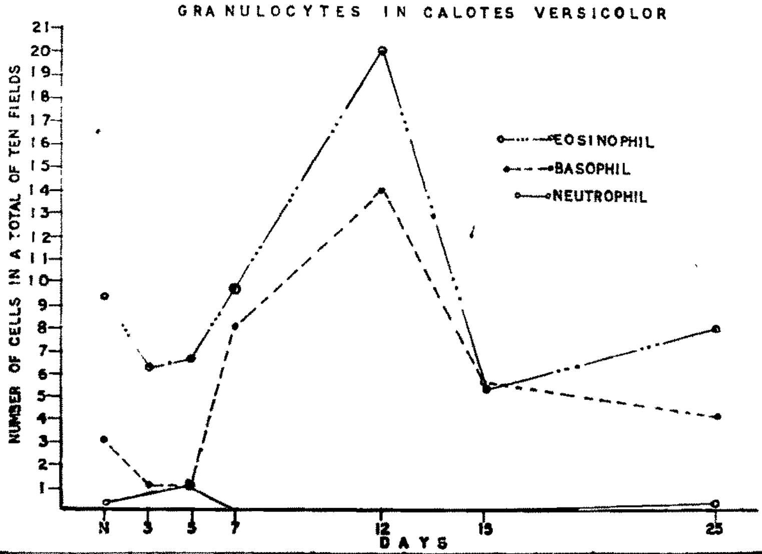


TABLE · 4	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	15TH DAY	25TH DAY
EOSINOPHIL	9·3 ± 3·2	6·3 ± 2·4	6·7 ± 2·2	10·0 ± 3·6	20·0 ± 5·9	5·3 ± 3·3	8·0 ± 3·8
BASOPHIL	3·0 ± 1·4	1·0 ± 0·6	1·20 ± 0·7	8·00 ± 2·9	14·0 ± 4·4	5·6 ± 1·9	4·2 ± 1·1
NEUTROPHIL	0·3 ± 0·1	06 ± 0·3	1·6 ± 0·8	-	-	-	02 ± 0·1

FIG 5: TABLE-5- CHANGES IN THE NUMBER OF AGRANULOCYTES IN CALOTES VERSICOLOR

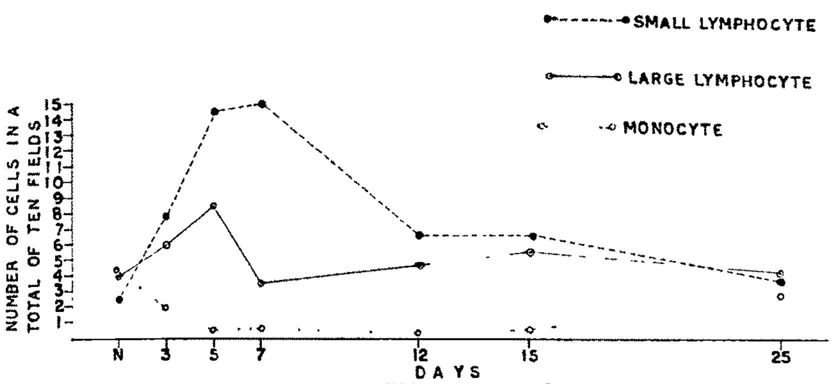
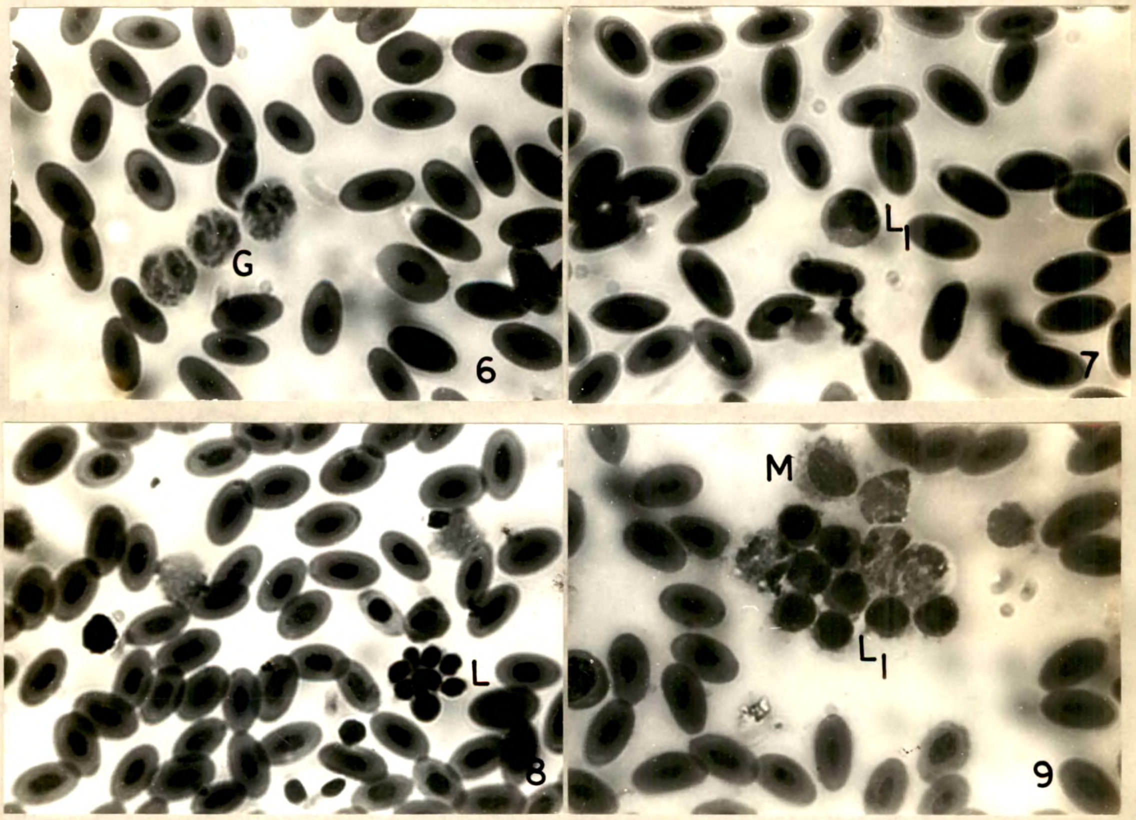


TABLE 5	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	15TH DAY	25TH DAY
LARGE LYMPHOCYTE	4·0± 2·1	6·0± 3·3	8·5± 3·9	3·5± 1·8	4·6± 1·9	5·6± 2·6	4·2± 2·2
SMALL LYMPHOCYTE	2 6± 0·9	7·8± 2·0	14 5± 3·7	15·0± 3·2	6·6± 1·9	6 6± 1·4	3·8± 0·8
MONOCYTE	4·3± 3·1	2 0± 0·4	0·5± 0 1	0·7± 0·1	0 2± 0·1	0·6± 0·2	2·8± 0 7

EXPLANATION TO FIGURES

Figs. 6-9 Blood smears of normal (with intact original tail) Calotes. A : Agranulocytes; L_1 : Large lymphocytes; L : small lymphocytes; M : Monocyte. 500 X.


G
6
L1
7
L
8
M
L1
9

EXPLANATION TO FIGURES

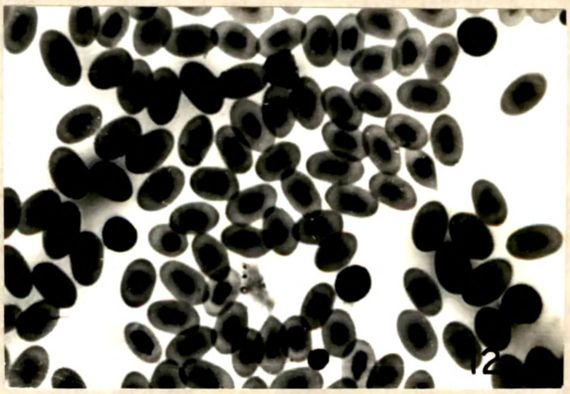
Figs. 10 and 11. Orthochomatic and polychromatic erythrocytes (early R.B.Cs) seen on the 7th day of postamputation tail wound healing in Calotes. 500 X.

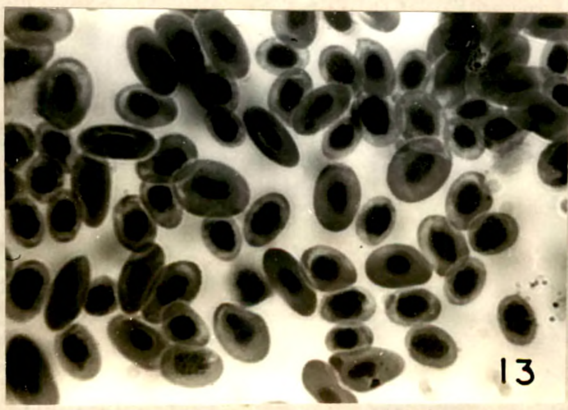
Fig. 12. Late differentiating R.B.Cs seen on the 12th day of tail wound healing in Calotes. Note the nuclear condensation and smaller size of the R.B.Cs. 500 X.

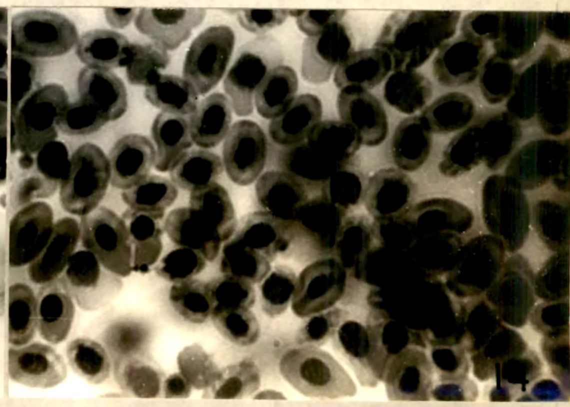
Figs. 13 and 14. Photographs depicting the appearance of many macrocytes (note the larger size of these erythrocytes) on the 5th day of tail wound healing in Calotes. 500 X.


10


11


12


13


14

though there were variations in the number of both the early and late immature type of red cells, the changes were not that well pronounced. The maximum number of these cells per field here were on the 7th and 12th days postamputation in correspondence with the total R.B.C count. However, the period between 5th and 7th days was marked by the presence of many large erythrocytes - the macrocytes.

HAEMOGLOBIN CONTENT

In the case of Calotes the changes in the haemoglobin content was parallel to the changes in the red cell count. The overall change being from a normal level of 5.5 gms/100 ml to 7.0 gms/100 ml and back to the normal level by the 25th day. (see Table - 1, Figure - 1).

TOTAL W.B.C. COUNT

As could be made out from Table - 2, Figure - 2, the leucocyte count in Calotes rose to a maximum on the 5th day (6205/mm^3) from the normal level (1900/mm^3) through the 3rd day (2150/mm^3). After this, the leucocyte count settled down to the normal range by the 7th day itself and this normal range was more or less maintained so thereafter.

DIFFERENTIAL COUNT

It may be mentioned at the very outset that the differential count carried out herein is only with a view to understand the possible changes undergone by the leucocyte population so that these changes may be considered as an index of the demands of the process of wound healing, and correlate these changes in the blood with the healing mechanics. In this regard, the present count is only obligatory to the understanding of the phenomenen of wound healing and as such is not an attempt to standardise and establish the percentage of the various types of leucocytes in the lizard. The various types of leucocytes and their numerical changes during wound healing are shown in Figures- 4, 5; Tables- 4, 5. From the Tables and Figures it becomes evident that the changes chiefly involve the lymphocytes, whose population went up to a maximum level by about the 5th day. The only other observable change in the lizard blood was a slight drop in the number of granulocytes during the 3rd and 5th days of healing. However, the granulocyte population rose up to a peak level (especially of Eosinophils and

Basophils) on the 12th day only to settle down to more or less their corrosponding normal levels on the 25th day.

DISCUSSION

In contrast to Mabuya, in the case of Calotes the erythrocyte count showed a fall to almost half the normal level on the 5th day after tail amputation, which roughly corresponds to the wound healing phase in Mabuya. A drop in R.B.C count in Calotes may be due to a number of factors, of which loss of cells due to accumulation and their destruction at the wound site could be a significant one. Moreover, withdrawal of cells or destruction, or both, in organs such as spleen and liver in response to the local factors from the wounded site released during the healing process also cannot be overruled. Another distinct possibility is the corresponding fall in haemoglobin content leading to a reduced oxygen carrying capacity of the blood, which might not only enable in supplimenting the high anaerobiosis characteristic of wound healing but also help in nullifying the possible setting in of Pasteur effect at the wound site. The increased erythrocyte count noticed on the 7th and 12th days in Calotes appears to be, due to an increased erythropoietic activity triggered by the fall in the cell count on the 5th day acting as a feed back machanism, and in this stimulated condition of erythropoiesis the number of circulating cells tends to overshoot the normal level which however settles down to the normal range by about the 15th day after amputation itself. The increased erythropoietic activity is denoted by the appearance of many immature erythrocytes on these days (7 and 12 ; Figure - 3, Table - 3).

In Calotes, regarding the changes in the total leucocyte count, the only change registered was on the 5th day, whence the total W.B.C count was at its maximum, whereas on all other periods the count remained more or less in the normal range. The increase in the total W.B.C count noticed during the first five days after tail amputation appeared to be mostly contributed by the lymphocytes as could be made out from the differential count (Tables - 4, 5 and Figures - 4, 5). As could be made out from Tables - 4, 5 and Figures - 4, 5 this period of lymphocyte elevation is marked by a corresponding fall in the levels of granulocytes as well as monocytes. Such a fall in the levels of granulocytes as well as monocytes might be considered as due to a migration of these cells to the site of injury for participation in the various autolytic and phagocytic processes associated with the demolition activities characteristic of the healing mechanics in operation at this early period. This, when taken together with the similar response shown by Mabuya with reference to granulocytes during the 3rd and 5th days of tail wound healing (previous section) apparently suggest a possible identity, and might, denote such a change in granulocyte population as a sort of generalised response to wounding or injury amongst lizards. However, in the wake of the known facts that the granulocytes exist under two categories (1) an activity circulating pool (45%) and (2) a marginated pool (55%) in contact with vessel walls or sequestered in sites such as spleen and lungs - and that the distribution between the two pools is determined by physiological and pathological conditions such as exercise and acute inflammation (Harris, 1974), a possible transfer of circulating pool into the marginated pool as part of a response to injury and inflammation in lizards in general also cannot be overlooked.

The increase in the population of circulating lymphocytes noted on the 5th and 7th days postamputation corresponded interestingly enough with the terminal phase of the wound closure in Calotes. It may be appropriate to mention at this juncture, that the nonregenerating type of wound closure wound entail the formation of a heavy scar tissue, or, a copious granulation tissue, which would in turn require the participation of large number of histiocytes, macrophages and fibroblasts. With the purported functional competence and versatality of lymphocytes to get transformed into monocytes, macrophages, histiocytes as well as fibroblasts, both at the site of injury and inflammation as well as elsewhere (Wasserman, 1965; Elves, 1966; Specter, 1966; Pilo, 1970; Shah et al., 1971; Harris, 1974), the possible role of lymphocytes in these transformations at the wound site and their participation secondarily in the healing mechanics of postamputational tail wound healing in Calotes could also be tentatively surmised. The reported requirement of neutrophils in the transformation process of lymphocytes (Harris, 1974) and the currently observed parallel increase of both the circulating lymphocytes as well as neutrophils (see Tables - 4, 5 and Figures - 4, 5) are also rather interesting. Since a mere healing process too entails a certain degree of cell proliferation, a probable functioning of lymphocytes in maintaining a steady state in proliferation (Burwell, 1963) as well as their capacity to act as trephocytes (Harris, 1974), also may be considered as a distinct possibility (though to a meagre extent as compared to Mabuya) in the case of Calotes during its tail wound healing.

The increase in the number of circulating granulocytes observed on the 7th and 12th days interestingly enough correspond to the attainment of peak levels of circulating erythrocytes. In this wake, the reported monophyletic common multipotent stem cells (Harris, 1974), as well as the differential rate of erythropoiesis and granulopoiesis controlled by variation in the marrow vascularity and blood flow, as well as the inhibitory role of one over the other, are worth reflecting here. Such a reflection highlights the fact, that, during the early periods of erythropoiesis (first 5 days postamputation) the granulocytes tend to remain low, whereas with the completion of the process of erythropoiesis and the attainment of a maximal level of circulating erythrocytes (7th day), the granulocytopoiesis comes into operation thus leading to an elevation in the levels of granulocytes during the 7th and 12th days of tail wound healing. Thus the initial suppressed granulocytopoiesis also might be yet another contributing factor in the observed subnormal levels of granulocytes.

Finally, the appearance of macrocytes (on the 5th and 7th days) especially on the 5th day appears to be of an adaptational significance in maintaining an effective (more or less) normal level of haemoglobin content whence the red cell count actually registered a fall to half the normal level. In this context, the suggestions, that in erythroblasts Hb synthesis influences the stage at which mitosis ceases, thus controlling the size of R.B.C, and that, too rapid synthesis causes permature curtailment of mitosis and the resultant production of macrocytes (Harris, 1974), are tenable and worth taking cognizance of. Thus the appearance of many circulating macrocytes on the 5th day postamputation in Calotes, appears to be of a specific purpose in effectively counter checking the drastic fall in the haemoglobin concentration of the blood that could be expected to ensue in the wake of the halving of the R.B.C population observed at this stage.