CHAPTER - 2

SECTION: I HAEMATOPOIESIS AND REGENERATION: HAEMOPOIETIC CHANGES IN LIVER, SPLEEN AND BONE MARROW DURING TAIL REGENERATION IN THE SCINCID LIZARD, MABUYA CARINATA

Though the process of regeneration has been under intensive investigation from different angles by many workers, curiously enough, haematopoiesis in relation to regeneration has been totally overlooked. In this context, the previous investigation on blood during tail regeneration in *Mabuya carinata* (Chapter - 1, Section - 1) revealed significant changes with regard to the total number of circulating blood cells (both R.B.C and W.B.C) as well as the haemoglobin content. The most noteworthy feature that emerged from the study was a tremendous increase (more than double the normal) in the total number of erythrocytes, and, the appearance of large number of lymphocytes and monocytes during the first week of tail regeneration. This sudden and high haemopoietic insurgency observed in the blood, necessitates a detailed study of the haemopoietic centres such as liver, spleen and bone marrow, as these three organs represent the most important postnatal haemopoietic organs of a vertebrate body. Such an investigation taken as a follow up of the previous observations on blood, would not only validate the earlier observations but also enable to gain an insight into the mechanics of haematopoiesis in relation to the process of regeneration. Apart from this, the study could also provide useful information about haematopoietic aspects in reptiles in general, as very little is known about it at present.

MATERIAL AND METHODS

Adults Mabuyas obtained from Karnataka, India, and maintained in the laboratory on a diet of insects were used as the experimental animals. The animals were first allowed to acclimatize to the laboratory conditions by keeping them for 2 to 3 weeks before the commencement of the work. The tails were autotomised as mentioned in the previous chapter and samples of liver, spleen and bone marrow (from femur) were collected by sacrificing the animals at fixed intervals of 3, 5, 7, 12, 25 and 60 days in correspondence with the various phases of tail regeneration. Bouin's fixed liver and spleen samples of both normal as well as regenerating animals embedded in paraffin wax, and sectioned at 5μ thickness were stained with haematoxylin - eosin and Jenner - Giemsa stains respectively. Smears of bone marrow were also stained with these two stains. Quantitative estimation of the iron content of the liver samples was carried out by the method of Elvehjem (1930) and Kennedy (1927) as described by Hawk et al., (1957).

RESULTS

Development of many haematopoietic nodules in the liver during regeneration was the most significant observation. The nodules varying in shape and size were seen scattered hapazardly all over the liver mass, though, quite a few could be found developing in close proximity to the blood vessels. Samples of 3rd day showed slight individual variations as far as the pace of development of the nodules were concerned. The earliest visible change appeared to be an influx of erythrocytes into the liver, as could be made out by the presence of large number of these cells within the sinusoids (Figures- 2, 4). The next stage, marked by

FIG. 1; TABLE. I. CHANGES IN THE HISTOSOMATIC INDEX (HSI) DURING TAIL REGENERATION IN MABUYA CARINATA

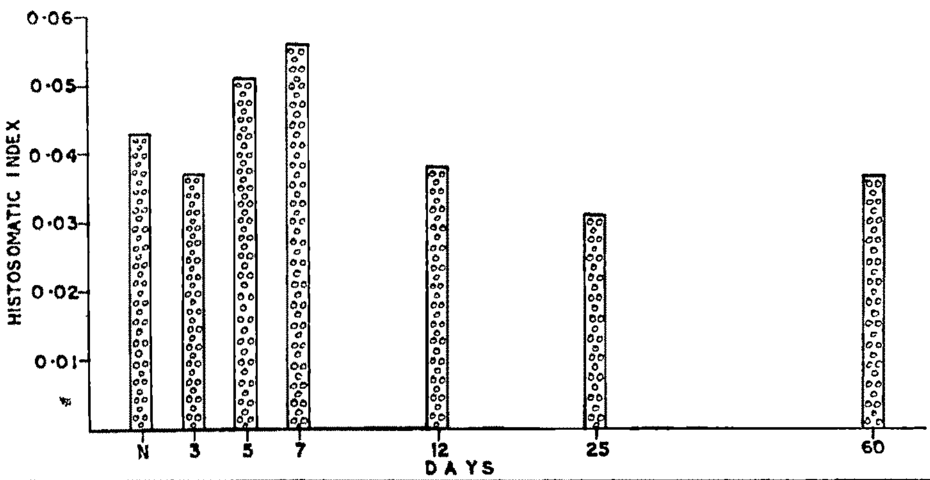
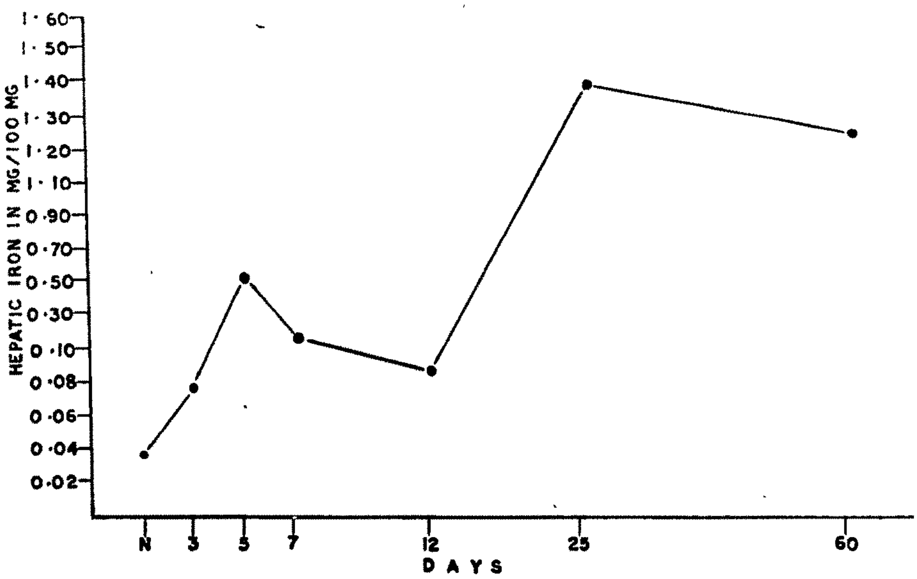


TABLE. I	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	25TH DAY	60TH DAY
H S I SPLEEN	0·043± 0·012	0·037± 0·017	0·051± 0·013	0·056± 0·016	0·038± 0·012	0·031± 0·011	0·037± 0·010

FIG·2· TABLE·2· CHANGES IN THE HEPATIC IRON CONTENT IN MABUYA CARINATA



TABLE·2	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	25TH DAY	60TH DAY
HEPATIC IRON	0·036± 0·007	0·076± 0·005	0·530± 0·054	0·170± 0·026	0·087± 0·044	1·580± 0·194	1·250± 0·232

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EXPLANATION TO FIGURES

Fig. 3. Histological picture of the liver of normal Mabuya (with intact original tail). 125 X.

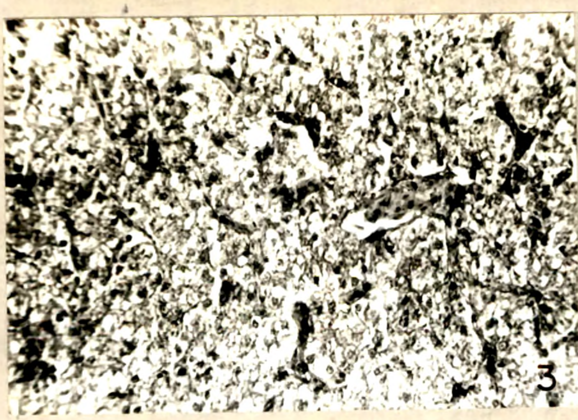
Fig. 4. Photomicrograph of the 3rd day (after tail amputation) liver of Mabuya showing influx of R.B.Cs within the sinusoids. 125 X

Fig. 5. Another 3rd day liver sample showing R.B.Cs in the vascular channel and adjacent sinusoids (arrows). 125 X.

Fig. 6. Enlarged version of Fig. 5. Arrows indicate R.B.Cs. 250 X

Fig. 7. A slightly later stage of 3rd day showing the formation of presumptive nodules marked by R.B.C destruction and pigment deposition. 125 X.

Fig. 8. Many presumptive nodules of 3rd day showing better demarcation. 125 X.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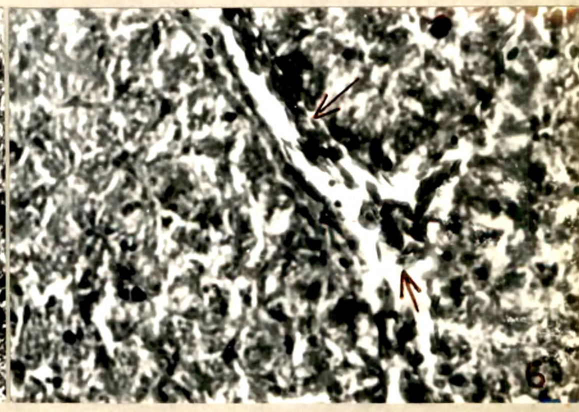
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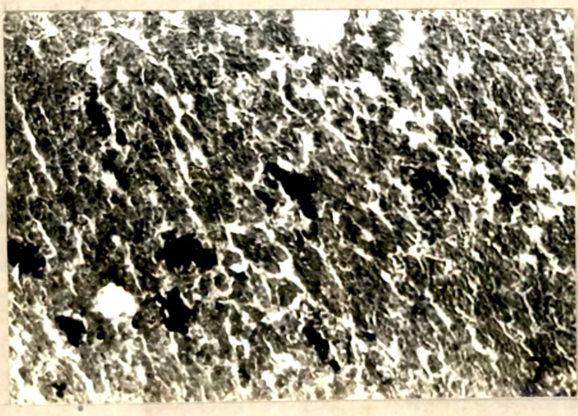

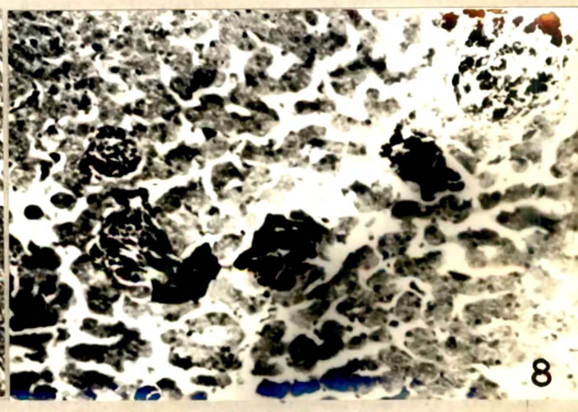
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EXPLANATION TO FIGURES

Fig. 9. Another 3rd day sample showing one clear cut and a few ill defined presumptive nodules. 125 X.

Fig. 10. Enlarged version of the presumptive nodules. Note the R.B.Cs in the sinusoids. 200 X.

Fig. 11. One single early nodular area enlarged to show the advanced stage of R.B.C destruction and blotches of pigment accumulation. 500 X.

Fig. 12. Later stage of nodule development showing the appearance of clear cut cellular assemblage within the nodules as the pigment material gets cleared. 125 X.

Fig. 13. Incfeasing basophilia of the cells within the nodules. 125 X.

Fig. 14. One single nodule which is well advanced in development and lined by R.B.Cs. 125 X.


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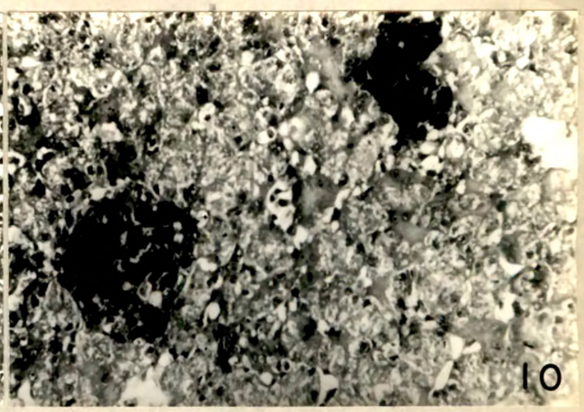
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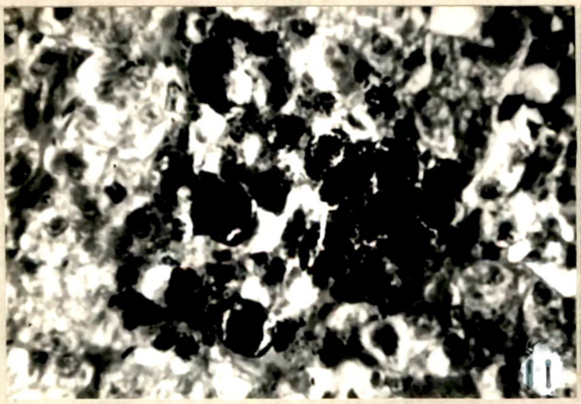
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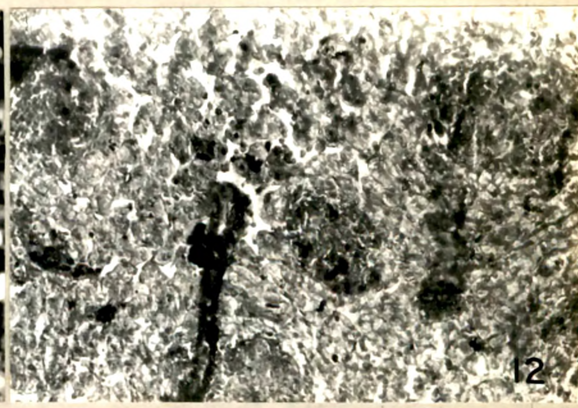
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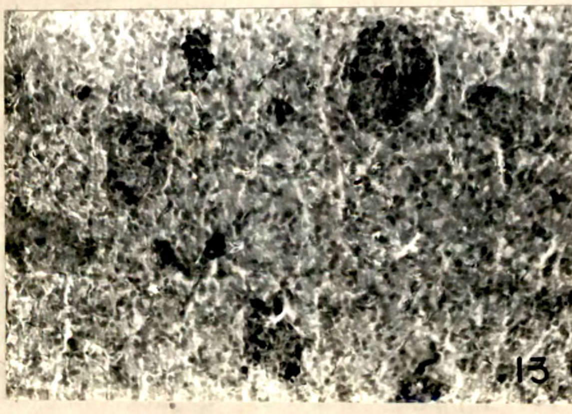
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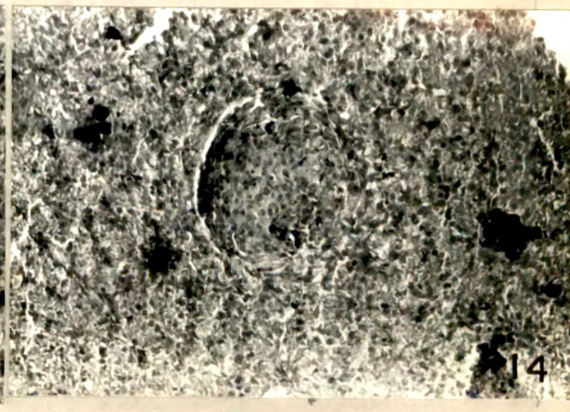
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EXPLANATION TO FIGURES

Fig. 15. Enlargement of the nodule in Fig. 14. Note the boundary being formed by the linear alienation of R.B.Cs. 250 X.

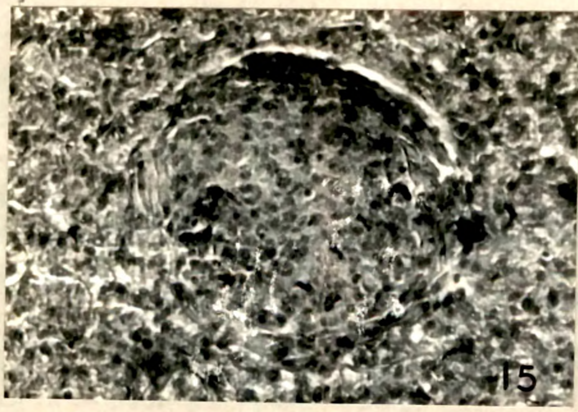
Fig. 16. A single nodule in the midstage of development enlarged to show the diffusion of pigment material, nuclear pycnosis of R.B.Cs and the appearance of cells of the lymphocyte series. 500 X.

Fig. 17. A magnified view of a nodule about to release the lymphocytes. 200 X.

Fig. 18. A 7th day liver sample showing a developmentally advanced nodule showing the cells (lymphocytes) ready to be released. 125 X.

Fig. 19. Same nodule in Fig. 18. enlarged. 250 X.

Fig. 20. One mature nodule in close proximity to a blood vessel ready to release the cells. 200 X.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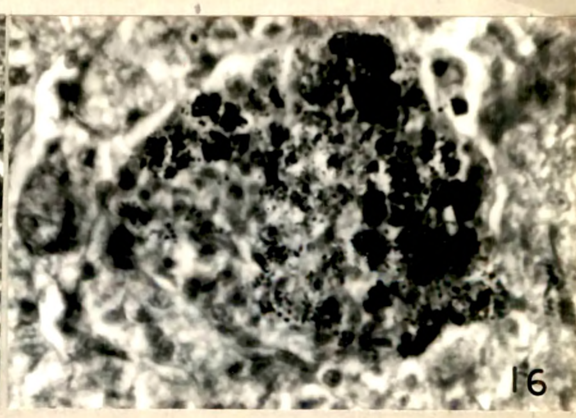
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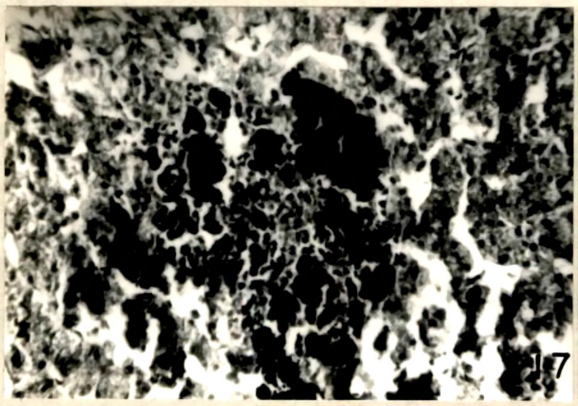
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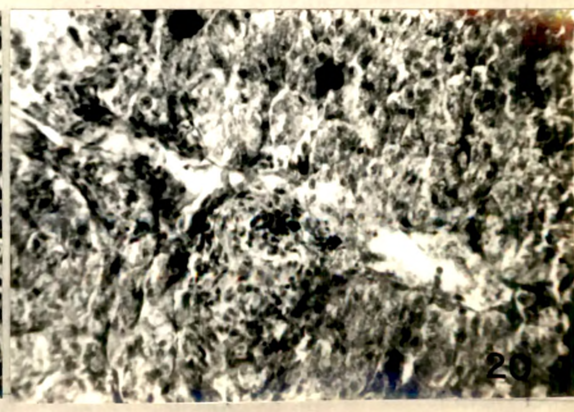
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EXPLANATION TO FIGURES

Fig. 21. A 12th day liver sample of Mabuya showing a mature nodule boardering a blood vessel. Also note another nodule which is already disrupted with the released contents lining the periphery of the vascular channel. 125 X.

Fig. 22. The nodule in Fig. 21. magnified. 200 X.

Fig. 23. Nodules and lymphocytes in relation to a large blood vessel. 125 X.

Fig. 24. Another field depicting the same. 125 X.

Fig. 25. Photograph depicting the clear cut migration towards blood vessel of many basophilic cells (lymphocytes) from all over. Note a nodule which has already opened out and has blotches of pigment material within it. 125 X.

Fig. 26. 12th day liver showing lymphocytes in the process of migration towards blood vessels. 125 X.


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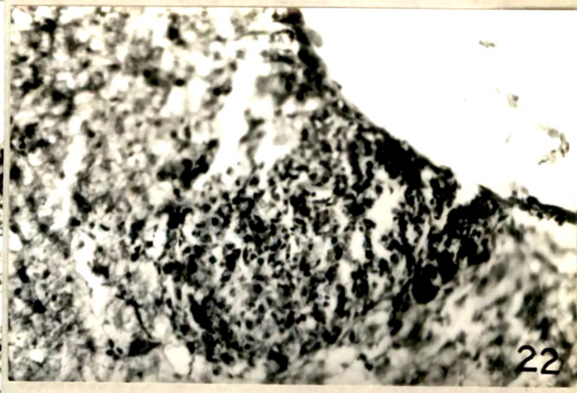
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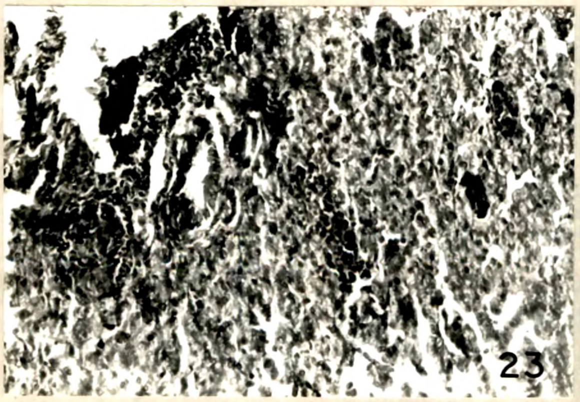
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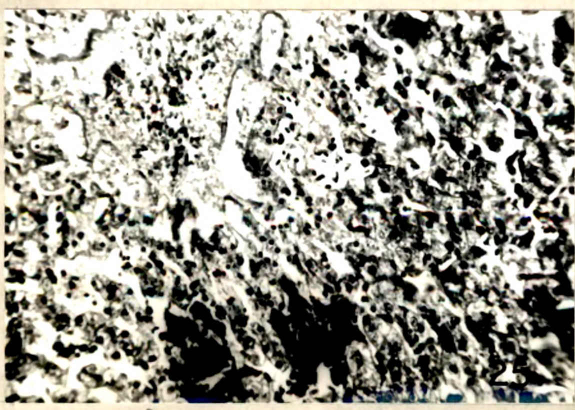


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EXPLANATION TO FIGURES

Fig. 27 and 28. Enlargements of the two blood vessels seen in Fig. 26. depicting the linear stream of lymphocytes approaching the blood vessels. 250 X.

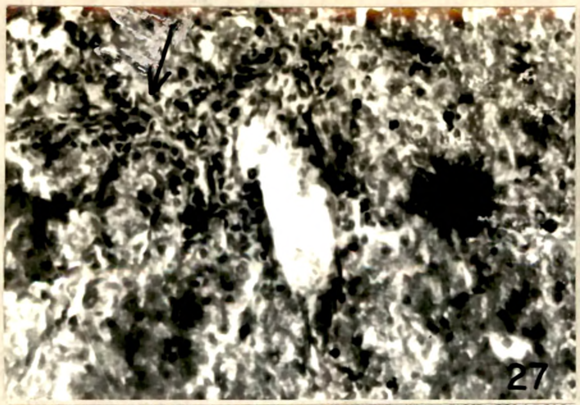
Fig. 29. Increased R.B.C destruction within the blood vessels as well as in the sinusoids as seen on the 25th day. Also note the many presumptive nodules with arrested development. 125 X.

Fig. 30. A higher magnification of Fig. 29. Note the enlarged sinusoid spaces closer to the blood vessel showing R.B.C. destruction. 250 X.

Fig. 31. Photograph depicting R.B.C influx on the 25th day. Also note one mature nodule (top) and one which has already liberated its contents and is now filled with left over cellular debris (bottom). 125 X.

Fig. 32. Two nodules demarcated from the adjoining liver mass by connective tissue encapsulation (see text). 125 X.

Fig. 33. A high magnification of an enlarged sinusoidal space of a 25th day liver sample showing R.B.C destruction. 500 X.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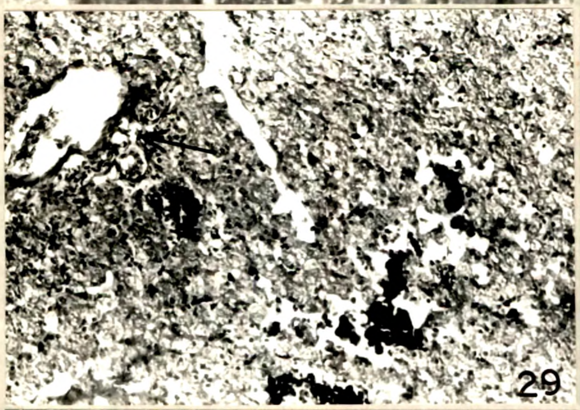
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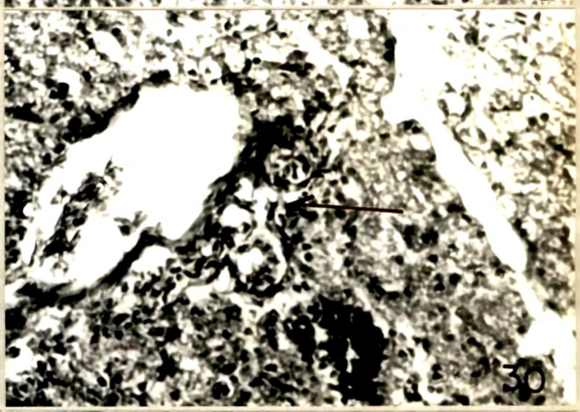
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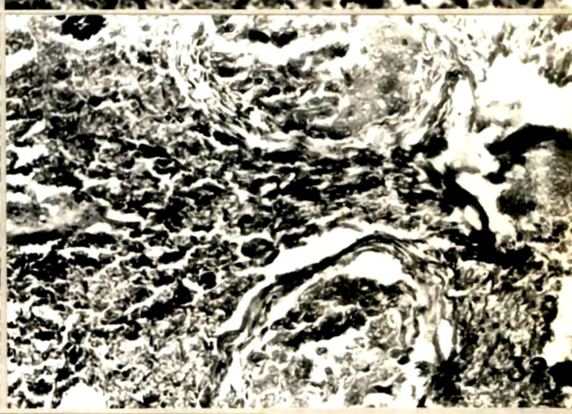

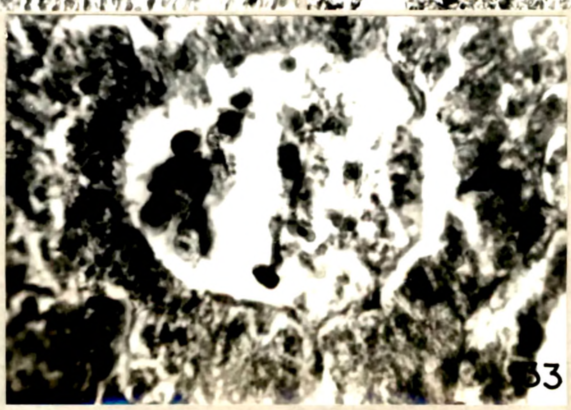
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EXPLANATION TO FIGURES

Fig. 34. Photomicrograph of spleen of normal Mabuya (with intact original tail). 125 X.

Fig. 35. Splenic structure on the 3rd day after tail autotomy showing hyperplasia of the white pulp. 125 X

Fig. 36. An enlarged version of the same. 200 X.

Fig. 37. R.B.C influx and their destruction as seen on the 5th day postautotomy. 200 X.


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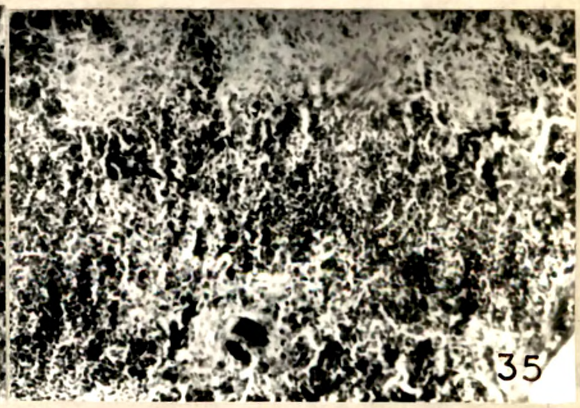
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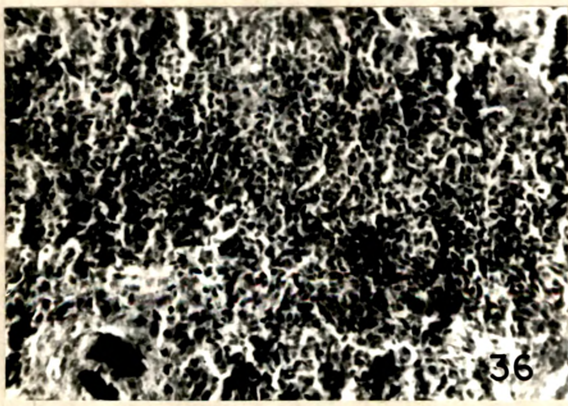
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EXPLANATION TO FIGURES

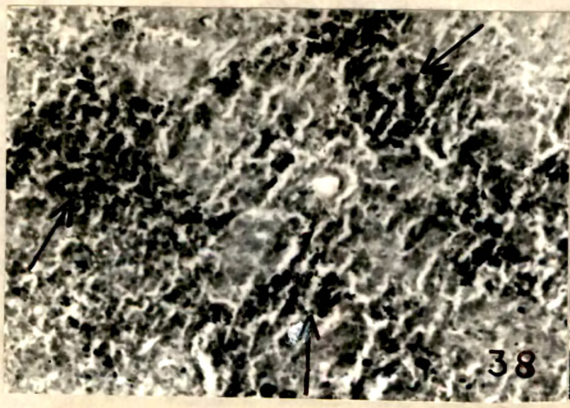
Fig. 38. 7th day spleen sample showing increased R.B.C destruction and pigment accumulation. 125 X.

Fig. 39. Another 7th day sample depicting the same (dark areas). Note also the increasing spaces within the tissue denoting possibly the release of lymphocytes. 125 X.

Fig. 40. Loose, space filled structure of spleen as observed on the 12th day indicating the release of lmyphocytes. 200 X.

Fig. 41. Partial recovery of the spleenic structure visible on the 25th day postautotomy. The dark patches represent the second phase of R.B.C destruction. 200 X.

Fig. 42. A 60th day, spleen sample showing a near normal histological appearance. 125 X.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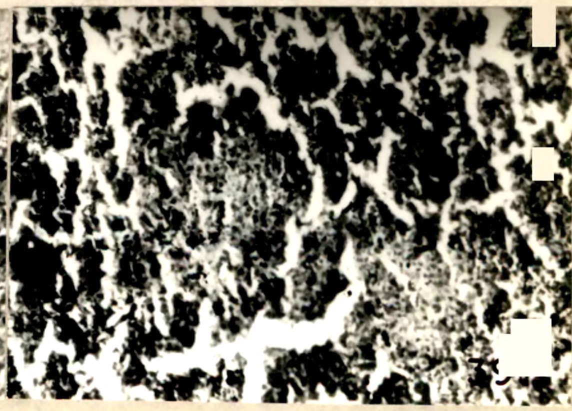
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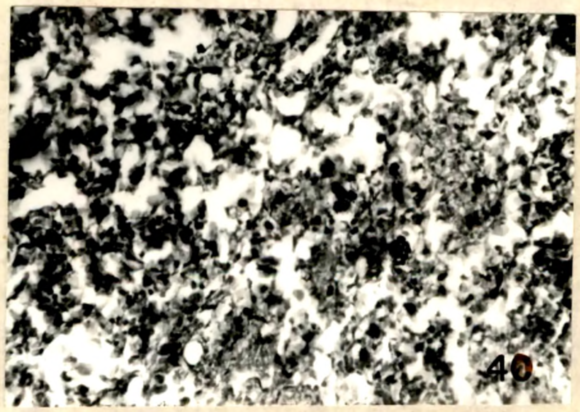
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EXPLANATION TO FIGURES

Fig. 43. Picture of bone marrow of a normal Mabuya, (with intact original tail). 200 X.

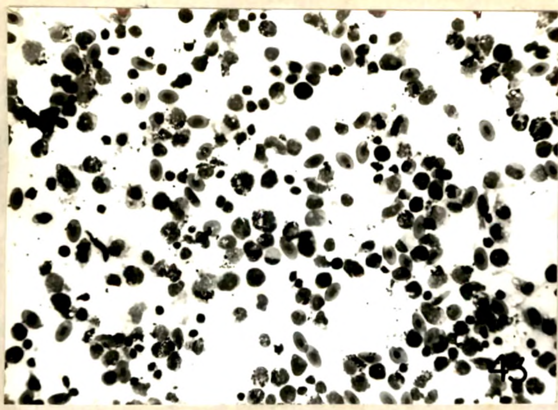
Fig. 44. A part of Fig. 43 enlarged to show the various cell types. E - Erythrocytes; G - Granulocytes; H - Haemocytoblasts; L - Lymphoblasts; M - Monoblasts; PE - Preerythrocytes. 300 X.

Fig. 45. Bone marrow picture on the 5th day postautotomy showing the presence of innumerable immature erythrocytes. 200 X.

Fig. 46. An enlargement of a part of Fig. 45. 300 X.

Fig. 47. Increased haemopoietic activity as noted on the 7th day postautotomy. 200 X.

Fig. 48. A higher magnification of Fig. 47. denoting the increase in the number of haemocytoblasts (H) and erythroblasts (E). 300 X.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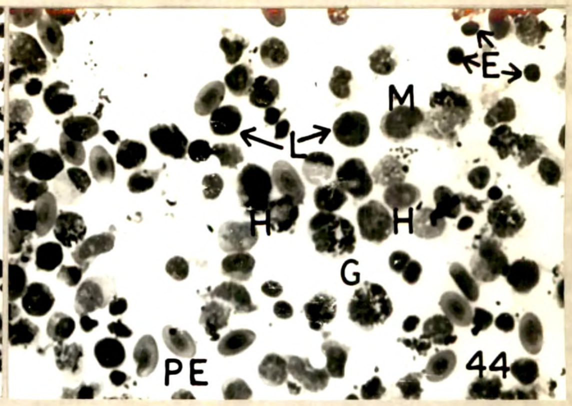
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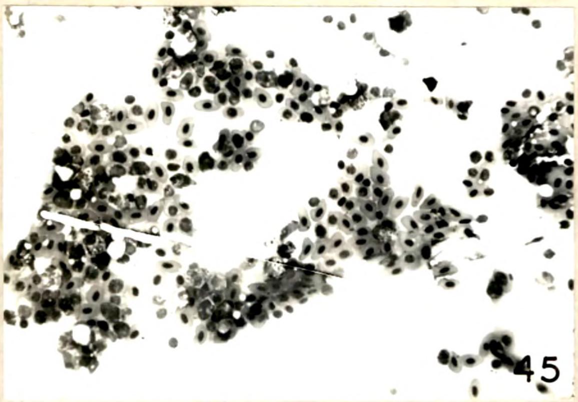
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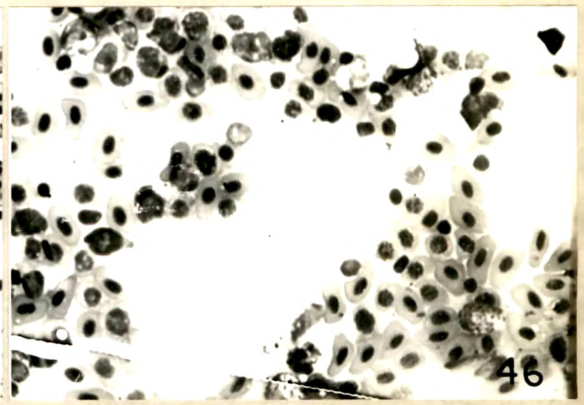
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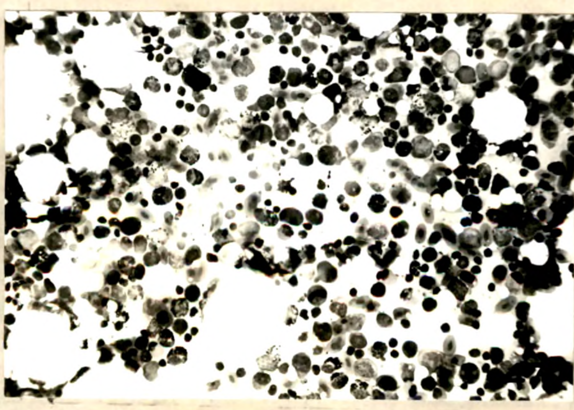

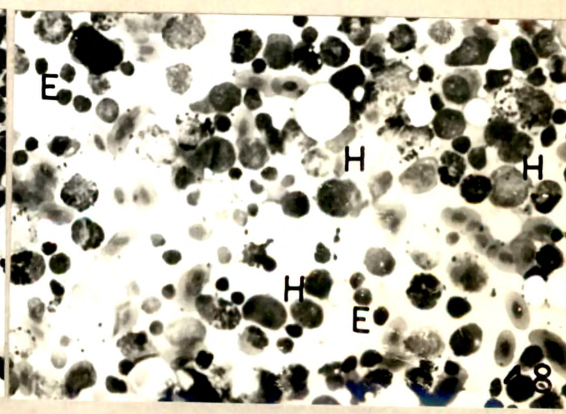
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EXPLANATION TO FIGURES

Fig. 49. Another field of the 7th day sample showing the depleted outlook of the marrow due to increased release. Note the presence of a few immature erythrocytes (bottom). 200 X.

Fig. 50. Fig. 49. at a slightly higher magnification. 300 X.

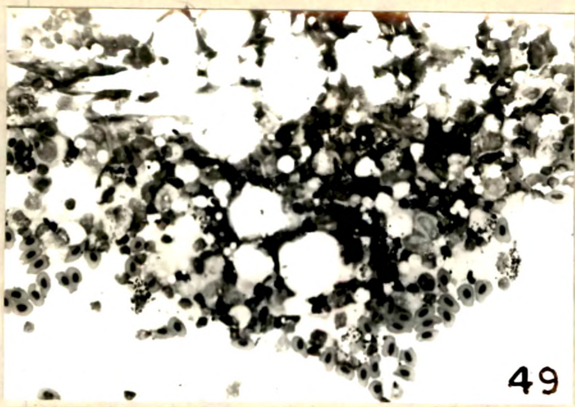
Fig. 51. Bone marrow of Mabuya as seen on the 12th day of tail regeneration showing the persisting stepped up haemopoietic activity. 200 X.

Fig. 52. A higher magnification of a part of Fig. 51. Note the presence of many monoblasts (M) as well as immature erythrocytes (IE) and granulocytes (G). 300 X.

Fig. 53. Another field of the 12th day sample showing an accumulation of innumerable immature erythrocytes due to the suspended release. 200 X.

Fig. 54. A part of Fig. 53 magnified. 300 X.

Fig. 55. A 25th day bone marrow sample showing progressive maturation of the immature blast cells accumulated due to the suspended release. 200 X.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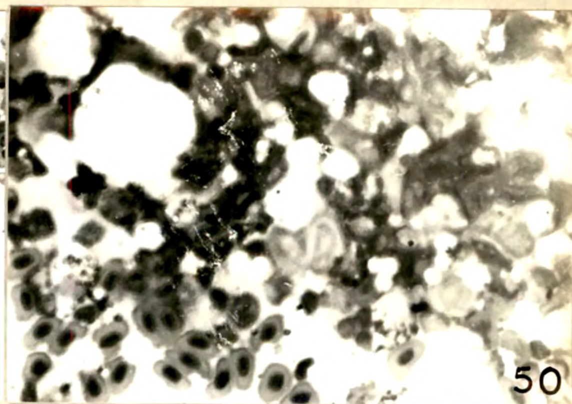
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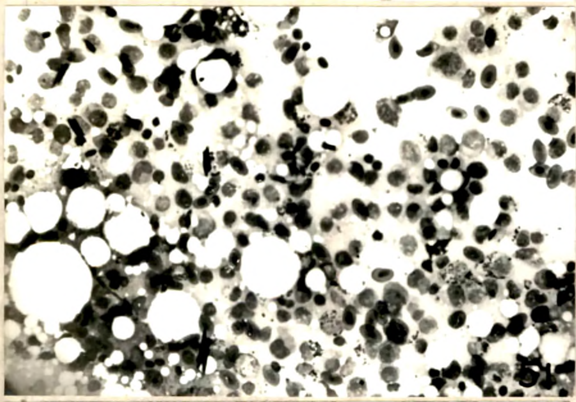

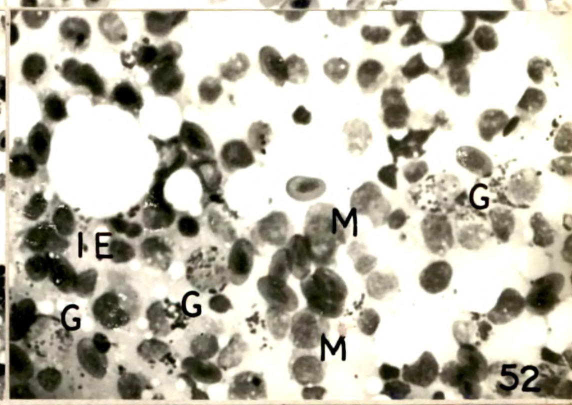
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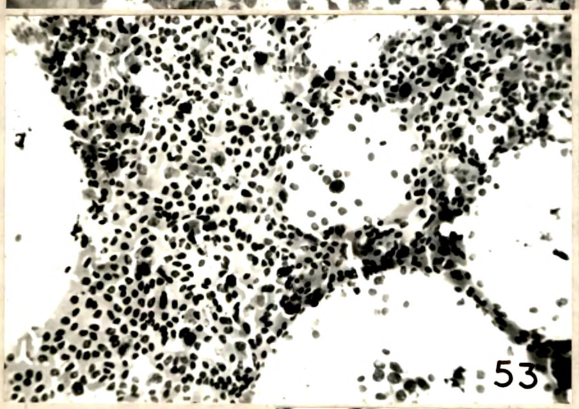
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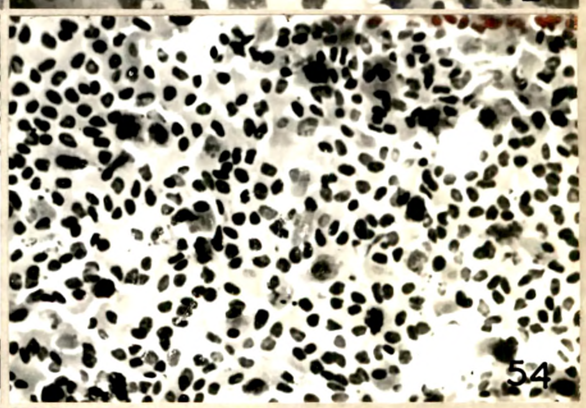
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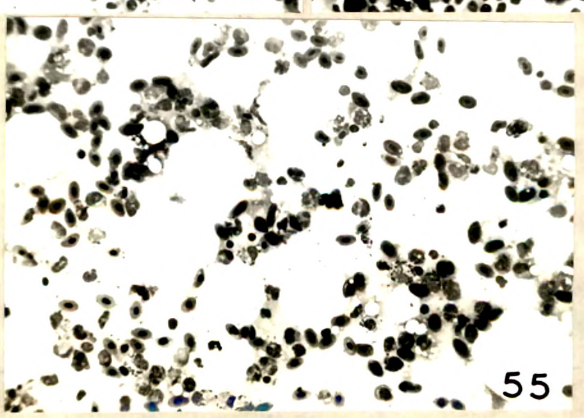
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EXPLANATION TO FIGURES

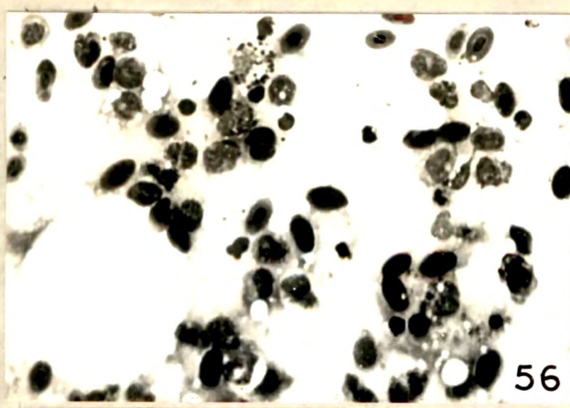
Fig. 56. Enlarged version of Fig. 55. 300 X.

Fig. 57. Another 25th day bone marrow picture showing the accumulated erythroblasts having differentiated upto the polychromatic stage. 200 X.

Fig. 58. Enlargement of Fig. 57. 300 X.

Fig. 59. and 61. Bone marrow samples of Mabuya taken on the 60th day of tail regeneration denoting the presence of many immature orthochromatic erythrocytes and preerythrocytes. 200 X.

Fig. 60 and 62. Higher magnifications of figures 59 and 61 respectively. 300 X.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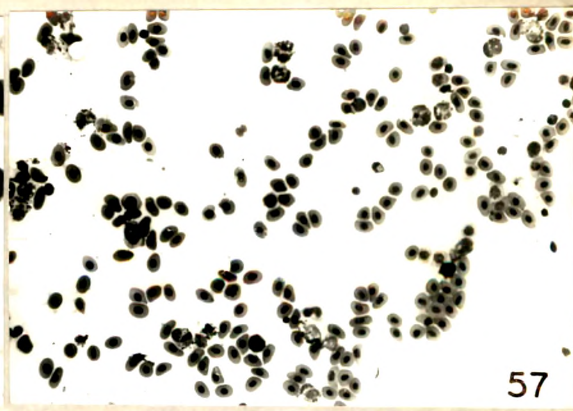
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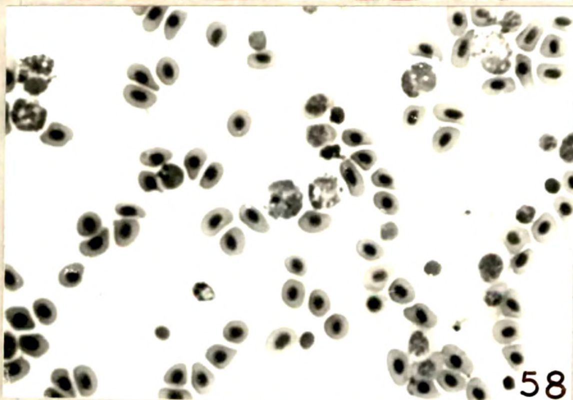
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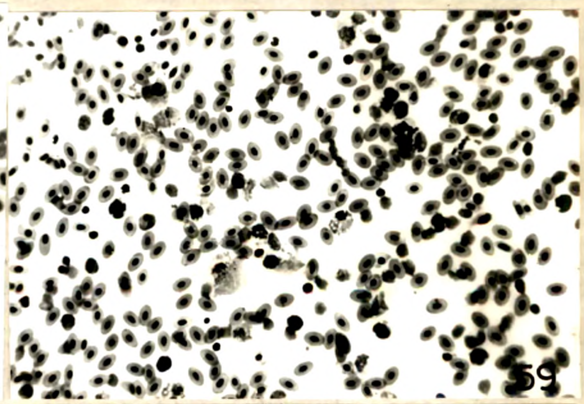
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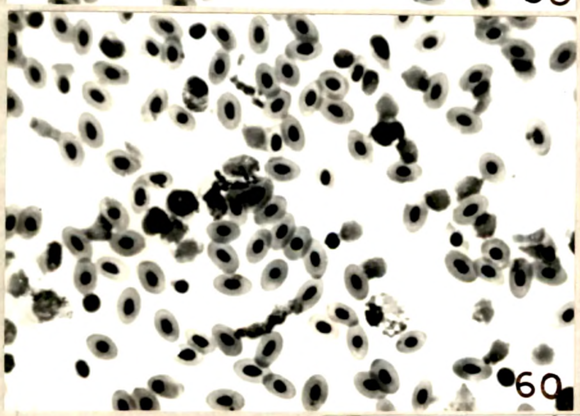
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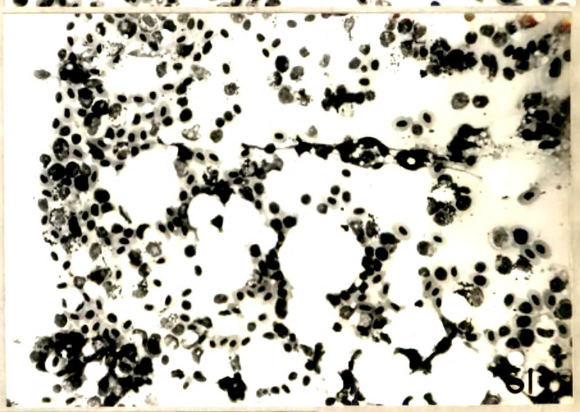
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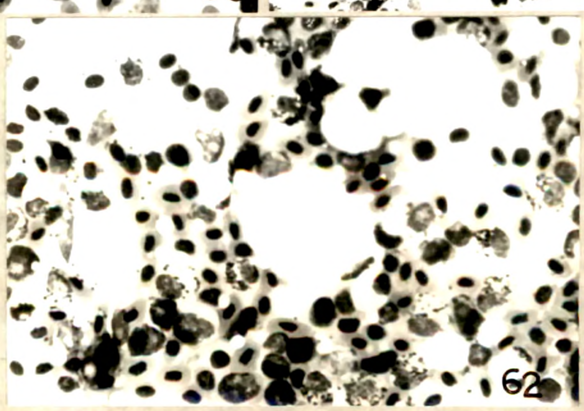
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by the destruction of erythrocytes and depostition of pigment material (probably derived from the disintegrating erythrocytes) could be observed in many of the samples which were the presumptive areas of development of the nodules. In some of the 3rd day samples, these areas even assumed the full fledged nodular appearance though such areas still remained obscured by the blotches of pigment material that were present. Most of the samples of the 5th day (did not present varied picture as was the case earlier) had such fully developed nodules, and, in many of the nodules, appearance of lymphoblasts could be visualised. In a few individuals, by this stage (.i.e., day 5) a number of fully grown and mature nodules filled with both lymphoblasts and lymphocytes, and about to release the contents could also be seen. All the liver samples of the 7th day stage were seen to contain many mature nodules, of which a sizable number of them were either about to release their contents or had already done so. Those nodules which were in the close vicinity of blood vessels opened into them, whereas the others which were in the parenchymatous mass of the liver just liberated their contents, and the liberated cells then traversed through the sinusoids so as to reach the vascualr channels. However, many presumptive areas as well as early developing nodules could also be seen at this stage. A similar appearance was exihibited by the liver samples of all 12 days old tail regenerating individuals. All the 25th day samples showed a total lack of mature nodules though a few developing nodules could still be discerned. A notable feature at this stage, however, was the renewed infiltration of erythrocytes and their destruction in marked areas of varying sizes, both closer, to, as well as away from the blood vessels. In the lizards bearing 60 days old regenerates, the liver assumed a more or less normal appearance excepting for the presence of a few nodules of early developing type as in the case of the 25th day samples. Presence of such nodules in both the cases could be presumed to be the remnants whose developmental progression had been suspended. Since only lymphoblasts and lymphocytes and no developing erythrocytes could be detected within the developing and developed nodules, these hepatic nodules in Mabuya carinata could be best designated as of the lymphocytopoietic type. The nodules in the present case, however, differed, from those in the case of birds (Shah et al., 1969) in the lack of, both, a connective tissue capsule on the outside, as well as the distinct zonation within. The nodules in Mabuya appeared to be demarcated from the neighbouring parenchymatous tissue by the erythrocytes and possibly the reticuloendothelial cells of liver itself by forming a covering layer on the outside. However, some of the 7th and 12th day samples showed a few encapsulated nodules with a few cells within, entangled in a connective tissue meshwork. Such nodules by their very rare occurence, their closeness (as they are found in a cluster in a limited area of the liver) and the appearance, could be considered as nodules which probably had failed to undergo normal development and hence were encapsulated to cordon them off from the surrounding areas of liver. If viewed in this light, it is quite possible, that these nodules are of the atropic type which are being marked off for gradual demolition and disposal. In correspondence with the influx of erythrocytes and their destruction noted above during the early and late periods of regeneration, the hepatic iron content was also found to show an increase during these periods. In between these two periods the hepatic iron content recorded a decrease. The hepatic iron content during various periods of regeneration is given in Table - 2, Figure - 2.

The observations in the case of spleen showed an increased content of erythrocytes in the 3rd and 5th day samples as compared to the normal ones. On the 7th and 12th days, the erythrocyte population, though higher than normal, was, however, reduced in comparison to the 3rd and 5th days. Once again there was a further increase on 25th day and a more or less normal appearance was regained by the 60th day. The only other interesting change as far as spleen was concerned, was the hypertrophy of white pulp areas as could be made out by the increased number of lymphoblasts having greater basophilic character. Unlike the established pattern of increased number of nodules with germinal centres in the spleen of mammals and birds during enhanced lymphocytopoiesis, in the present case, the enhancement appeared to be registered as a marked hypertrophy of white pulp area as a whole and was more evident during the 5th and 7th days of regeneration. These histological changes in the spleen appeared to be well corroborated by the changes in spleen weight observed during these periods (Table - 1.) The 12th day spleen showed numerous spaces and reduced amount of white pulp, by which it could be surmised that the release of lymphocytes must have occured by now. By day 25 and thereafter the spleen assumed a more or less normal appearance. The assumption of a major haemopoietic function by the bone marrow could well be envisaged by the present observation in *Mabuya carinata*. The increased number of both the circulating erythrocytes as well as monocytes observed between the 3rd and 12th days of regeneration (Chapter - 1, Section - 1) were well supported by the presently noted increased haemopoietic activity in the bone marrow druing the same periods. Increased number of haemocytoblasts, erythroblasts as well as monoblasts appeared to corroborate the fact (Figures- 5, 12).

GENERAL OBSERVATIONS ON BONE MARROW

The most significant aspect was the low level of lymphocytes in the marrow of the normal lizards, and still very much reduced level of these in the marrow of regenerating animals. Increased haematopoietic changes in bone marrow were denoted by the overall incfease in the number of haemocytoblasts and the appearance of immature erythrocytes of the blast series. These changes were well evident between 3rd and 7th days of regeneration. However, on day 12, the marrow was found to contain large number of immature erythrocytes of the early type. Similarly, the marrow on the 25th and 60th days too were found to have large aggregations of erythrocytes of the late type, (polychromatic cells), and, the mature ones respectively. (Figure -).

DISCUSSION

Currently observed changes in bone marrow, liver and spleen are indicative of the apparent involvement of the haemopoietic machinery in the regenerative phenomenon. The marrow picture clearly demonstrates an increase in haemocytoblasts, erthroblasts and monoblasts through 3rd, 5th and 7th days of tail regeneration. Haemocytoblasts which are the multipotent stem cells appear to be triggered into action by the stimulus of regeneration and appearance of large numbers of even smaller haemocytoblasts are indicative of this. Such smaller cells are known to appear during

Increased or quick mitotic activity (Harris, 1974). The corresponding increase in the number of differentiating erythroblasts of various stages as well as monoblasts are also suggestive of the fact, that, concurrent to the increased mitotic activity of the stem cells, they are also stimulated at an equally faster rate to differentiate into these two cell types. The previous observations of a highly elevated level of erythrocyte count and the concomitant presence of large number of immature erythrocytes of the blast series in the blood during the 5th and 7th days of regeneration (Section-1, Chapter-1) together with the presently observed changes in the marrow are conclusive evidences in favour of an increased necessity of erythrocytes during the early periods of regeneration. Obviously, the critical need of such large number of cells within a short period of 5 days after autotomy being more acute, the marrow in its endeavour to cope up with the demand is apparently forced to release cells at a comparatively immature stage. This becomes all the more pertinent, when the fact that it takes about 5 to 7 days for normal maturation of marrow erythrocytes at the steady state level of erythropoiesis, is taken into consideration.

However, with a reduced necessity of red cells during the later phases of regeneration as noted by the fall in cell count through 12th and 25th days of regeneration (Chapter - 1, Section - 1), the marrow also appears to show corresponding changes as noted by the presence of large number of erythrocytes in ascending series of development and differentiation during the 12th, 25th and 60th days of regeneration. This is evidently indicative of the subtle operation of a physiological regulating mechanism whereby with a reduced necessity, the marrow ceases to release the erythrocytes and in this condition of suspended release, the large number of blast cells already formed and committed under the initial stimulus of regeneration undergoes progressive differentiation and remain stored within the marrow. The role of marrow in producing the monocytes is also well exemplified by the observed increase of the monoblasts during early period of regeneration. The functional significance of monocytes during regeneration has been discussed in the previous chapter (Section - 1). Presence of an almost equal number of both granulocytes and monocytes in the bone marrow could be explained by a common stimulating factor and common ancestral cell for these two cell types (Harris, 1974). Development of lymphocytopoietic nodules in the liver is the most striking revelation of the present investigation. Presence of such nodules in the liver of normal birds and their increase during abnormal and/or pathological conditions have been well documented (George and Naik, 1963; Pilo, 1967; 1970; Shah et al., 1969a). Unlike in birds, in the case of Mabuya, such nodules seem to appear only under the stress of regeneration and that too during the early regressive phase of regeneration (i.e. till about 12 days after autotomy). Another interesting observation that is to be taken into consideration is the almost negligible level of lymphocytopoiesis in the bone marrow during this period. Concomitant to the lymphocytopoietic nodule development in liver, the spleenic white pulp also undergoes hyperplasia, These two factors are significant and highlight the high demand of lymphocytes imposed by the regenerative process. Another organ associated with lymphocyte production is thymus, and in the present context, the possible involvement of this organ too needs to be ascertained and as such is under experimental scrutiny. Possible involvement of lymphocytes in populating the regeneration blastema was hinted at in the earlier chapter. This suggestion finds ample support not only by the noticeable lymphocytopoietic activity in both liver and spleen but also from the time factor of release of the lymphocytes from these two organs. The appearance of empty nodules as well as release of lymphocytes into the liver sinusoids on the 7th and 12th day samples and the loose space filled structure of spleen on the 12th day of all the experimental lizards examined, are further evidences in favour, as this most critical period between the 5th and 12th days after autotomy is marked by the formation of a regeneration blastema filled up with a mass of multipotential mesenchymal cells. In this context, the known multipotentiality of lymphocytes is an added asset that qualify them as the most potent contributing source of cells for the regeneration blastema. This assumed fact, however, needs further experimentation to establish its validity.

The influx of erythrocytes into both liver and spleen and their destruction immediately after autotomy (3rd to 5th) and once again during the later stage (between days 12 and 25) is another significant observation. The increased iron content noted during these two periods (Table - 2) is in good correspondence with the above, and is in itself explanatory. The very high content of iron noted in the 25th and 60th day samples is correlatory with the corresponding observations on liver. Whereas the increased erythrocyte destruction during the later period of regeneration could be well accounted, as an attempt at normalisation of the number of circulating erythrocytes from the high level that was necessary during the early regressive phase of regeneration, the influx and destruction noticeable immediately subsequent to autotomy is totally unexplainable and remains enigmatic in nature. It is interesting to keep in mind at this juncture, that the haemoglobin type is known to change from the embryonic or fetal type to the adult type by a change from the prenatal to postnatal life and also by a change in location of erythropoiesis (Harris, 1974). Recently, similar change over of haemoglobin type has also been reported with reference to metamorphosis in amphibia (Just and Attivan, 1972; Osake et al., 1974; Benbassa, 1974). It is also known that the embryonic or fetal haemoglobin has a higher oxygen affinity and that the same erythroblast can synthesize both the types of haemoglobin simultaneously. In this background, it would be rather interesting to know whether any such smooth transition from adult to embrynnic and back to adult type of Hb takes place in Mabuya during regeneration, in which case the initial influx and destruction of erythrocytes noticed in liver and spleen could well be for the suppression or reduction of cells with adult type Hb. In conclusion, it could be safely surmised that the process of regeneration places a heavy demand on the haemopoietic system, and in the endeavour to meet the requirements under the impetus of regeneration, the haemopoietic centers respond in the most dramatic manner. In the normal animals, the circulating lymphocyte population appears to be maintained by a combined activity of low tone functioning of bone marrow and spleen, whereas under the exigency of an increased demand during regeneration (with the marrow having engaged itself to the task of supplying the required number of erythroblasts/cytes and monoblast/cytes) the burden appears to be shared by an overactive spleen and the newly commissioned liver. It is rather evident that the enhanced haemopoietic demands evoke both, cellular response as marked by the increased cell production and accelarated release, as well as locational response as exemplified by the reappearance of haemopoietic activity in previously active centres. From these studies, certain pertinent queries that crop up, and which need due attention at the present juncture are, (1) What is the nature of the regeneratory stimulus that triggers off haemopoietic mechanisms? Could it be erythropoetin and colony stimulating factor or other endocrinological factors such as thyroxine, testosterone and prolactin (Rall et al., 1964; Pilo and George, 1970)? (2) Waht is the source of cells for lymphocytopoiesis in liver? Are they the circulating stem cells which get seeded under the favourably renewed microenvironment or are they the hepatic reticuloendothelial cells themselves? (3) Whatever be the cellular source, what is the factor that stimulates liver to take up haemopoiesis that too lymphocytopoiesis? (4) What would be the relation between erythrocyte destruction and nodule development in liver? It is known that the presence of some cellular debris could act as a triggering factor for nodular development (Hill and Popisil, 1960; Shah et al., 1969). In this light, could it be possible that the influx of erythrocytes might be for a dual purpose, that of Hb transition as well as providing necessary stimulus for nodule development in liver, or, is it a mere matter of chance incidence? And lastly (5) If it be accepted that lymphocytes play a major role in the formation of a regeneration blastema what could be the significance of dedifferentiation of stump tissues? Could it be possible (though in a hypothetical thinking) that under the neurohumoral stimulus of the nerve cord, some of the stump tissues dedifferentiate after autotomy, and these cells then direct the formation of a typical wound epithelium of the regenerating type and also help in the orientation of newly differentiating tissues by information transfer or induction. These are some of the interesting points which need attention and are being looked into, and the information so gained might help in unravelling some of the still latent mysteries of the phenomenon of regeneration.

SECTION: II HAEMATOPOIESIS AND WOUND HEALING: HAEMATOPOIETIC CHANGES IN LIVER, SPLEEN AND BONE MARROW DURING TAIL WOUND HEALING IN THE AGAMID LIZARD, CALOTES VERSICOLOR

The earlier investigations (Chapter - 1) conducted on the cellular composition and haemoglobin content of blood in the two lizards, Mabuya and Calotes during tail regeneration as well as tail wound healing respectively, have given a divergent pattern of changes. This differential response highlights the significant involvement of haematopoietic system in the regenerative mechanics as compared to the mere wound healing process. Moreover, in the previous section, the haemopoietic organs such as liver, spleen and bone marrow were also seen to undergo specific changes in response to the process of regeneration. In this wake, the possible changes undergone by these organs during normal wound healing in the lizard, Calotes versicolor, too, was deemed fit to investigate not only to serve as a suitable control but also to obtain some information regarding the involvement of systemic factors in mechanics of wound healing in a lizard as nothing practically is known about these aspects.

MATERIAL AND METHODS

Adult Calotes obtained from the local animal dealer and maintained in the laboratory on a diet of insects were used as the experimental animals. The animals were first allowed to get acclimatized to the laboratory conditions by keeping them for 2 to 3 weeks prior to the commencement of work. The tails were amputated at a fixed distance from the vent, and samples of liver, spleen and bone marrow (from femur), were collected by sacrificing the animals at fixed intervals of 3, 5, 7, 12, 15 and 25 days. Bouin's fixed liver and spleen samples of both normal as well as tail wound healing lizards embedded in paraffin wax and sectioned at 5μ thickness were stained with Haematoxylin - Eosin and Jenner - Giemsa stains. Smears of bone marrow were also stained with these two stains.

RESULTS

LIVER

The histological appearance of liver of normal animals with intact tails appeared to be very much similar to that of Mabuya as noted in the previous section, excepting for the cell size which tended to be slightly bigger in the case of Calotes. Subsequent to tail amputation as observed on the 3rd day, the liver appeared to show no histological change in particular excepting for the turgid nature of hepatocytes observable in certain regions. The most significant change that could be noted on the 5th day was the influx of R.B.Cs into the sinusoids and the early stages of their destruction. Concurrently, the hepatic tissue at this stage was marked by the appearance of fat droplets, with the extent and degree of appearance varying from lizard to lizard. Subsequently, on the 7th day, the influx, destruction, the resultant accumulation of pigment material as well as the fat deposition became very much intensified and most of the individuals at this stage appeared to have entered into a pronounced fatty liver condition. The microscopical examination of the liver sections at this stage also revealed in many cases the presence of small groups or clusters of lymphocytes scattered at random and without exhibiting the regular nodular appearance characteristic of Mabuya during its tail regeneration. As late as the 12th day there was still

FIG·1: TABLE·1· CHANGES IN THE HISTOSOMATIC INDEX (HSI) OF SPLEEN DURING TAIL WOUND HEALING IN CALOTES VERSICOLOR

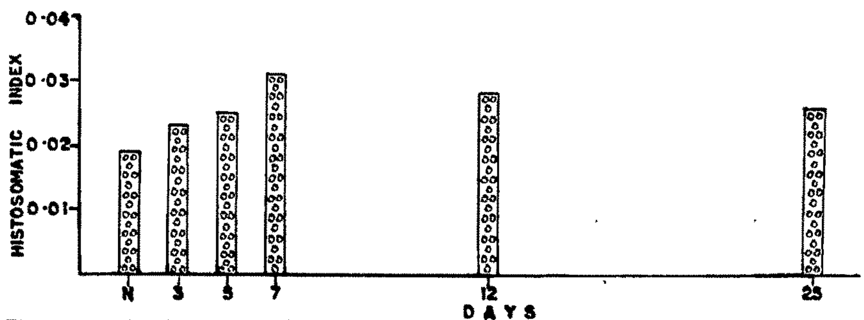


TABLE · 1	NORMAL	3RD DAY	5TH DAY	7TH DAY	12TH DAY	25TH DAY
H S I SPLEEN	0·019± 0·012	0·023± 0·008	0·025± 0·026	0·031± 0·0003	0·028± 0·008	0·026± 0·025

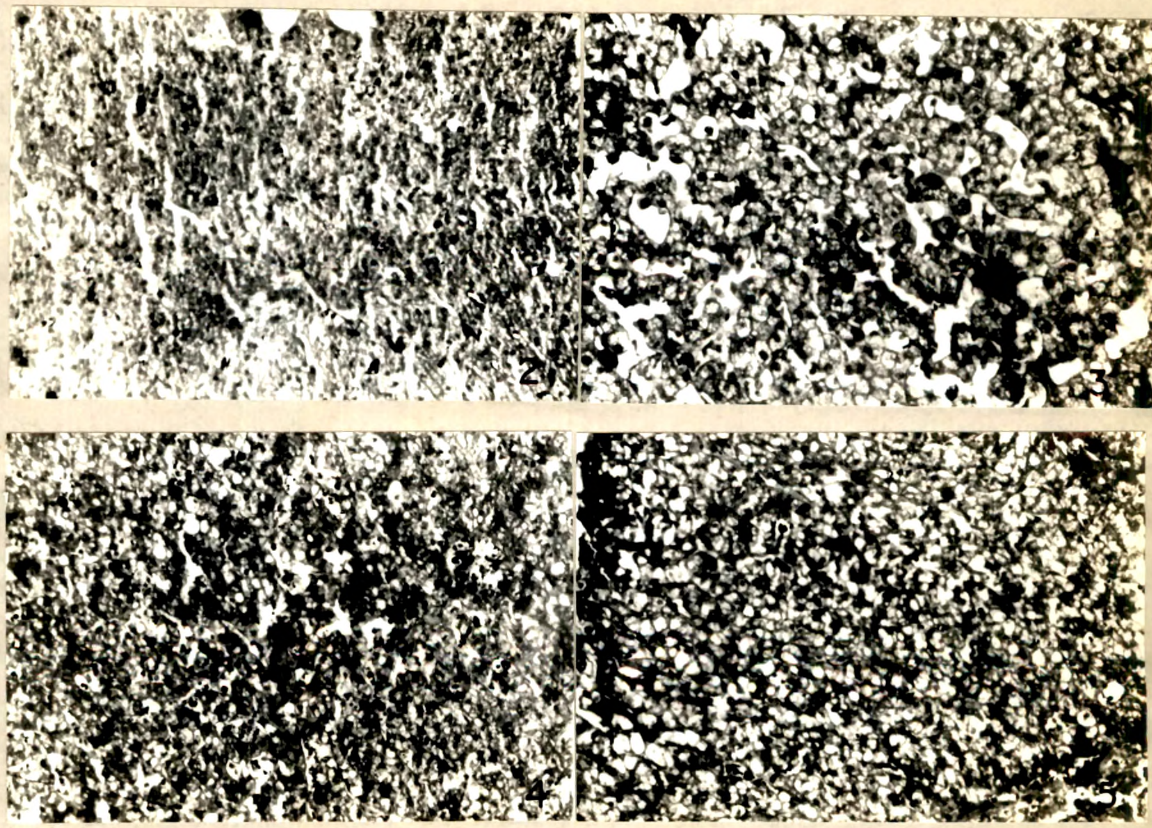
EXPLANATION TO FIGURES

Fig. 2. Photomicrograph of the liver of normal (preamputation) Calotes. 125 X.

Fig. 3. Liver of Calotes as seen on the 3rd day postamputation of its tail. Note the turgid nature of the hepatocytes probably due to steatosis. 125 X.

Fig. 4. Early stages of fat infiltration as seen on the 5th day postamputation. 125 X.

Fig. 5. Another 5th day liver sample of Calotes showing an advanced stage of fat infiltration. 125 X.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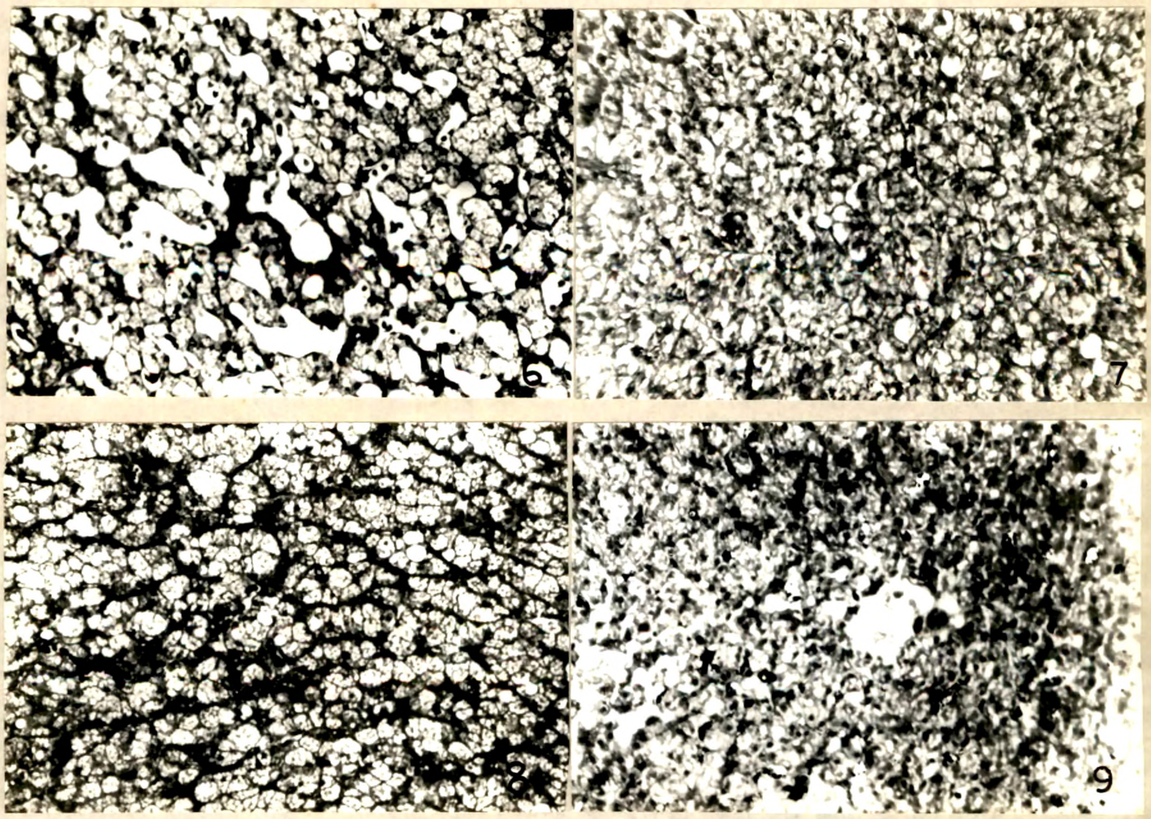
EXPLANATION TO FIGURES

Fig. 6 and 7. Two grades of fatty liver development as seen on the 7th day postamputation of tail in Calotes. 125 X.

Fig. 8. Another 7th day liver sample depicting an advanced fatty liver condition. Note the connective tissue meshwork that is formed. 125 X.

Fig. 9. Yet another 7th day sample denoting the influx and destruction of R.B.Cs (Black patches). 125 X.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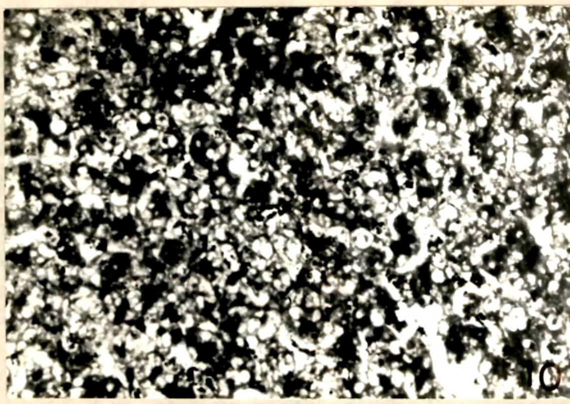
EXPLANATION TO FIRGURES

Figs. 10 and 11. Differing grades of recovery and recoupment manifested during the 12th day of postamputation tail wound healing in Calotes. Also note the persisting destruction of R.B.Cs (dark areas). 125 X.

Fig. 12. A near normal appearance of the liver seen on the 25th day after tail amputation. 125 X.

Fig. 13. Another 25th day liver sample depicting a normalised histological appearance. 125 X.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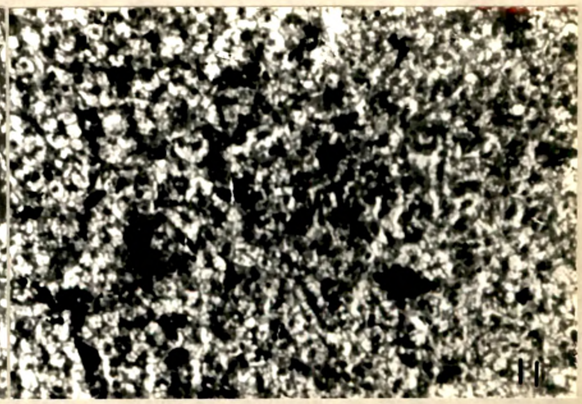
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EXPLANATION TO FIGURES

Fig. 14. Histological picture of spleen of normal (preamputation) Calotes. 125 X.

Fig. 15. Spleen structure as seen on the 3rd day after tail amputation. Note the hyperplasia of the white pulp. 125 X.

Fig. 16. 5th day (postamputation) spleen of Calotes. Note the hyperplasia of white pulp. 125 X.

Fig. 17. A slightly magnified view of Fig. 16. 200 X.


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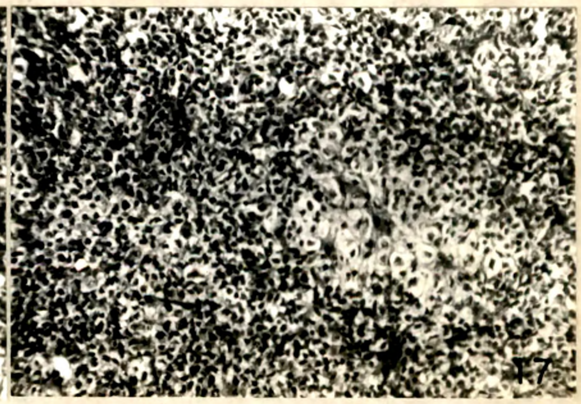
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EXPLANATION TO FIGURES

Fig. 18. Photomicrograph of 7th day (postamputation) spleen of Calotes. Note the reduced intensity of lymphocytes/blasts and the noticeable R.B.C destruction (dark patches). 200 X.

Fig. 19. 12th day (postamputation) spleen sample of Calotes. Persisting R.B.C destruction could still be discerned. 125 X.

Fig. 20. Spleen structure as seen on the 25th day (after tail amputation) in Calotes. Note the more or less normal appearance. 125 X.

Fig. 21. Higher magnification of Fig. 20.


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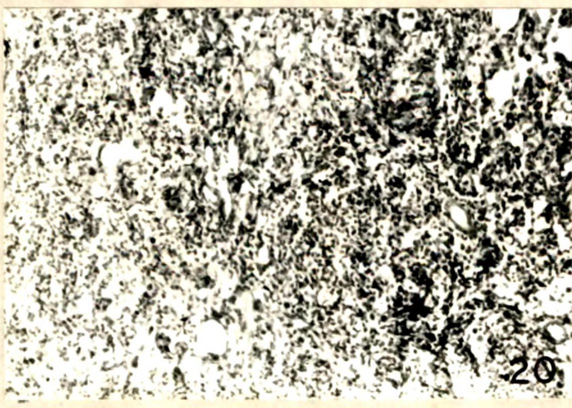
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EXPLANATION TO FIGURES

Fig. 22. Bone marrow picture of normal (with intact original tail) Calotes. 200 X.

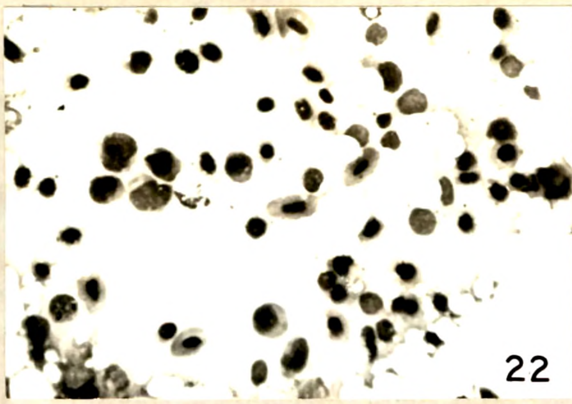
Fig. 23. An area of the 3rd day (postamputation) sample of Calotes showing differentiating R.B.Cs. 200 X.

Fig. 24. Higher magnification of Fig. 23. 400 X.

Fig. 25. Bone marrow of Calotes as seen on the 5th day after tail amputation. Note the increased number of erythroblasts (arrows). 200 X.

Fig. 26. Enlarged version of the same. 400 X.

Fig. 27. Photomicrograph of the bone marrow smear of Calotes taken on the 12th day postamputation tail wound healing. Note the depleted look of the marrow. 200 X.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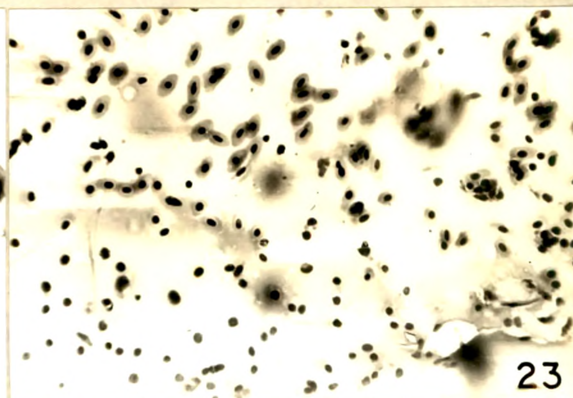
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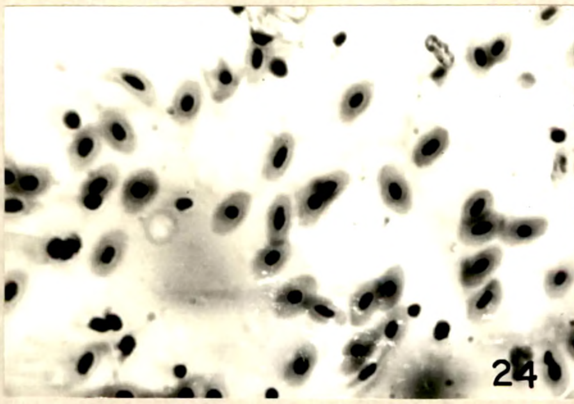
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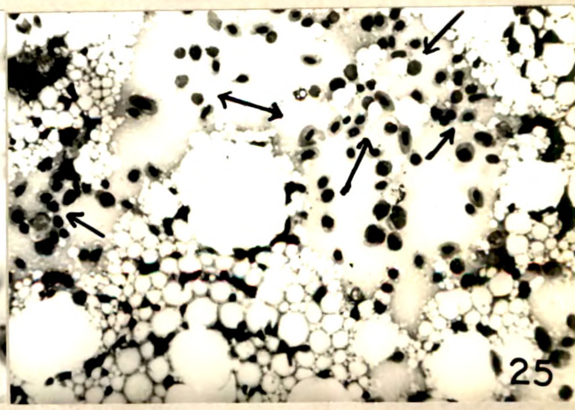
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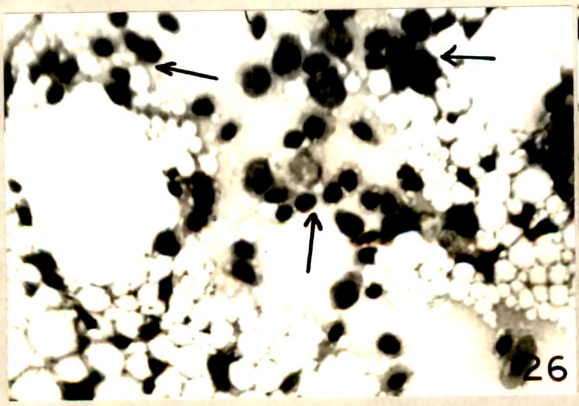
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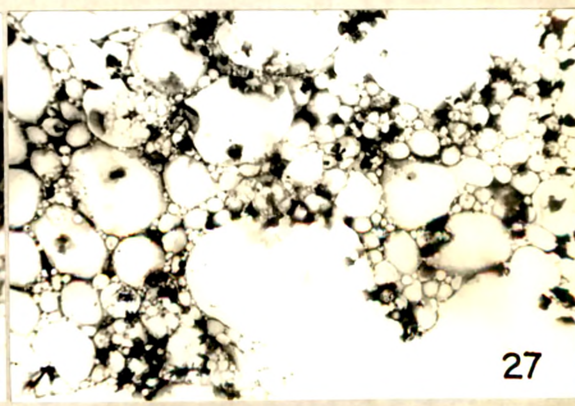
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a persistence of R.B.C destruction and pigment accumulation with a certain degree of recoupment and recovery from the fatty liver condition in a few of the lizards. By about the 25th day, however, the process of recovery appeared more pronounced with a degree varying from a more or less completely recouped condition in some, to a few which was in the early to midstage of recovery.

SPLEEN

Subsequent to tail amputation on the 3rd day, the splenic tissue in Calotes depicted mild symptomatic changes indicative of an activation process whereby certain areas of activity within the white pulp could be visualised. By about the 5th day however, these changes appeared to be more marked and an increased population of lymphocytes within the white pulp became more obvious. Following this, on day 7 almost all the spleen samples showed presence of R.B.Cs in large numbers in both the vascular channels as well as within the splenic tissue itself. A curious observation was the presence of a few fat droplets within the spleen of a few lizards, on the 5th and 7th days postamputation. By about the 12th day, the tissue sections of spleen appeared to show a loose texture with a reduced population of lymphocytes. This was also marked by an advanced stage of R.B.C destruction. By about the 25th day postamputation, the histological appearance of the spleen had more or less reverted to the normal condition.

BONE MARROW

The bone marrow appeared to show little or no change on the 3rd day postamputation as compared to the normal. However, on days 5 and 7 postamputation, there appeared to be a complete depletion of haemopoietic cells from the marrow and the marrow smears showed only reticular fibres and adipocytes with a few blast cells scattered here and there. By about days 12, 15 and 25 postamputation, haemopoietic activity appeared gradually in the marrow and by day 25, more or less a normal pattern and picture was established.

DISCUSSION

At the very outset it could be surmised that the haemopoietic organs such as liver, spleen and bone marrow of Calotes do not respond as dramatically during its postamputational wound healing, as in the case of

Mabuya during its tail regeneratinn (previous section). Unlike in Mabuya, in the case of Calotes the bone marrow depicted no change whatsoever on the 3rd day postamputation thus denoting the relatively insignificant involvement of marrow haemopoiesis in the normal healing mechanics of the lizard. The most significant difference that stands out during the 5th and 7th days postamputation/autotomy respectively in the two cases, is the diametrically opposite pattern of response of the marrow. In the case of Mabuya during these periods, the marrow responded by an actively stepped up rate of haemopoiesis as marked by the division and differentiation of various types of haemopoietic cells and their release, especially of the erythrocyte series, leading to a densely populated appearance of the marrow. On the other hand in the case of Calotes, the marrow responded by a release of the marrow reserves already existent under the normal level functioning, thus depicting a completely depleted outlook. It becomes apparent from these observations that in the case of Mabuya, the triggering of the haemopoietic activity in the bone marrow and the resultant changes in the circulating blood cells (Section - 1 of Chapter - 1) are specifically programmed to meet the functional needs of the regenerating process. However, in the case of Calotes during its tail wound healing, with no such functional needs, the marrow haemopoiesis remains unstimulated, and the depletion in the marrow reserves noticed on the 5th and 7th days, appears to be merely due to an enhanced release necessitated by a fall in the circulating red blood cell count to half (Chapter - 1, Section - 2). Though the reduction in the cell count is adequately compensated by an effective depletion of the marrow reserves, the causative factor and/or the adaptational significance of the fall in the blood cell count and its immediate compensation as well as the underlying mechanisms controlling the release of marrow reserves all remain enigmatic, and, eludes any precise explanation. With the known and purported functions of lymphocytes hinted at in the previous chapter (Elves, 1966; Wasserman, 1965; Specter, 1966; Pilo, 1970; Shah et al., 1970; Harris, 1974; Burwell, 1963) and the increase in the population of circulating lymphocytes observed therein, the lymphocytes were considered to play some functional role in the healing mechanics of Calotes versicolor. In the case of Mabuya, wherein the contributing source (of tremendous numerically increased number of lymphocytes required to meet the exigency of regeneration) ultimately turned out to be the spleen as well as the hepatic tissue, however in the case of Calotes, the sole source of increased lymphocytes appearing in the peripheral circulation seems to be the spleen, as the hepatic tissue in this lizard tended to remain haemopoietically insensitive. In the light of the noted haemopoietic insensitivity of the hepatic tissue in Calotes, the hepatic lymphocytopoietic activity which was secondarily triggered off in the case of Mabuya assumes definite significance and tends to highlight the greater degree of quantitative as well as qualitative functional involvement of lymphocytes in the regenerative process as opposed to mere wound healing. Viewed in this perspective a mere wound healing process in a lizard appears to entail only a low tone requirement of lymphocytes which could be easily and adequately met with by the spleen. Such an involvement of spleen during the postamputational caudal wound healing in Calotes is denoted by both the histological appearance as well as the increase in the histosomatic index of the organ (HSI i.e. Percentage weight) observed in the present study. The appearance of few lymphocytes in the hepatic tissue of Calotes during the 5th and 7th days could be easily attributed to their infiltration from the vascular channels.

However, the 7th and 12th day liver and spleen samples are marked by the influx of R.B.Cs and their destruction as observed in case of Mabuya. Though in the case of Mabuya certain tentative explanations could be drawn in this connection, similar events noted in Calotes apart from emphasizing the obvious relation between the fall in the circulating R.B.C count and their appearance in liver and spleen, does not at this juncture offer any clue and remains more or less enigmatic. The most significant aspect of the changes in the histological feature of the hepatic tissue noted during tail wound healing, is the development of a fatty liver condition marked by the appearance of fat droplets and the gradual but complete transformation of the liver to such an extent so as to give it a pseudoappearance of an adipose tissue by about the 7th day itself. Though the etiology or even the significance involved in such a transformation remain once again a mystery, the response of the fat droplets towards neutral lipid stains (Unpublished observations) as well as the tremendous increase in the total lipid and glyceride content of the liver noted during such a transformation (Section - 2, Chapter - 4) are all the compelling evidences in favour. Though a number of specific and nonspecific factors are known to contribute towards the etiology of fatty liver condition in mammals (Popper and Schaffner, 1957) it is rather impossible to even guess of any possibility in the present case at the present juncture. A cursory review of this aspect, however, is presented in later chapters (4, 5, 6, and 8) based on the investigations on the changes of other biochemical and metabolic parameters.