CHAPTER-VII

GENERAL CONCLUSION

A final conspectus of the salient features concerning the haematology of Calotes versicolor so far examined together with the various points of difference and conformity with other reptiles may be achieved by the following lines.

The erythrocyte, eosinophil, basophil, lymphocyte, monocyte, thrombocyte and plasma cell are clearly recognized in the peripheral blood of Calotes versicolor. The erythrocyte, eosinophil, basophil, lymphocyte and thrombocyte regularly appear in the circulation. Monocyte frequently becomes absent in the circulation. Plasma cell very rarely appears in the circulation. No pigment cell is found in the blood of Calotes.

Various kinds of immature blood cells also appear in the peripheral circulation of Calotes which put much hardle to the identification of the mature blood cells. Still it is possible with the use of Romanowsky stains like Wright's and May-Grünwald-Geimsa.

The erythrocyte of Calotes versicolor is an elliptical cell with central elliptical nucleus, similar to other lizards. Paranuclear body absent in the erythrocyte. Occasionally, the erythrocyte bears intracytoplasmic dots which may be considered as the consequence of amitotic erythroplastid production. Supravitally stained mature erythrocyte represents no mitochondria or vacuole as it is completely filled up with haemoglobin.

Senile erythrocytes are large, irregular bodies with light staining cytoplasm. They appear in greater number in older Calotes suggesting long life-span of the individual red cell.

Scanty production of erythroplastid in Calotes versicolor where lungs function with their full capacity, favours its relation to the maximum efficiency of the

respiratory system in terrestrial medium.

Anisocytosis is recognized in Calotes versicolor as a normal feature. But there are no specific criteria by which larger erythrocytes may be distinguished as macrocytes and smaller erythrocytes as microcytes.

Morphology of the erythrocyte gradually changes from elliptical to more circular form with the increase of body weight. This morphometric change of erythrocyte in Calotes versicolor may be considered as the consequence of ageing.

Dimensions of erythrocyte and its nucleus are such that they support the inclusion of Calotes versicolor in the family Agamidae and suggest a close relationship of the family Agamidae with the family Iguanidae and Lacertidae.

The average erythrocyte number is 1.11 million per cubic millimetre of blood for Calotes versicolor. The number is almost equal to the erythrocyte number for other lizards of the same group. The principle of "smaller the erythrocyte larger the number" is also applicable here.

Female Calotes represents smaller erythrocyte number, 1.02 million per cubic millimetre of blood. The counts obtained for females never reach the peak figure of counts represented by males. Though the erythrocyte counts in adult males are clearly higher than those of the young males but the count per cubic millimetre of blood falls gradually with the increase of body weight after certain age. Erythrocyte counts made at different months of the year show that it is maximum in the summer months and minimum in the winter months. In the breeding season (summer months) erythrocyte number increases both in male (8.0%) and in female (7.2%). Pregnancy has no special modifying influence on the erythrocyte number. Diurnal variation of erythrocyte count is noted in Calotes versicolor. Erythrocyte number is higher in day time. Count increases not due to increased production of erythrocyte

but due to mobilization of more erythrocytes from the storing organ to meet the demand in increased activity of the animal.

Haemoglobin concentration for the male Calotes versicolor ranges from 6.0-11.0 gm per 100 ml of blood. The average figure is 8.5 gm%. Proportional to the erythrocyte number haemoglobin concentration increases or decreases. Increase of erythrocyte number and haemoglobin concentration in the breeding season is related to the increased metabolic activity of the animal.

The average packed cell volume for the adult male Calotes versicolor is 30%. The value is slightly lower in females, which is 28%. With the increase of body weight packed cell volume does not vary significantly although it is slightly lower in larger animals. During breeding season packed cell volume increases both in male and female. Increase in packed cell volume during the breeding season is definitely related to the increased production of erythrocytes.

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in Calotes versicolor vary round about the average 300/μ^3, 82/$\mu\mu$ gm and 27%, respectively. Mean corpuscular haemoglobin concentration (MCHC) does not vary with the sex and age. Mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) do not vary significantly with the sex but increase with the age of the animal. Mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) increase in old age to compensate the low erythrocyte production.

Average erythrocyte sedimentation rate (ESR) for Calotes versicolor is 4 mm in the first hour. It is well within the reptilian range. In female it is slightly higher. Pregnancy increases the erythrocyte sedimentation rate upto 10 mm in the first hour. Sedimentation rate has no relationship with the size of erythrocyte.

Increase of mean corpuscular volume does not increase the speed of sinking.

The erythrocytes of Calotes are resistant to haemolytic effect of the hypotonic solution similar to other lizards. It appears that there is no relation between the red cell size and haemolytic effect of hypotonic solution. But the medium in which the animal lives has certain relation to the osmotic resistance of the erythrocyte.

Comparatively, the total blood volume of Calotes versicolor is low. During the breeding season blood volume increases (14.8%).

Eosinophils are largest of all the white cells. Two types of eosinophils (a) eosinophil with spindle granules (Espe) and (b) eosinophil with spheroidal granules (Espl) are recognized in Calotes versicolor similar to other reptiles. Eosinophil with spindle granules possesses multilobed nucleus whereas eosinophil with spheroidal granules possesses irregular or spherical or dumble shaped nucleus.

Specific granules for the two types of eosinophils are distinct in shape and size. Eosinophil with spindle granules is actively motile, moves by throwing pseudopodia. Eosinophil with spindle granules represents mitochondria and vacuoles in vital preparation. Eosinophil with spheroidal granules is comparatively less motile, bears few mitochondria and vacuoles. Phagocytic activity for the eosinophil is not detected but the possibility of phagocytic activity resides in eosinophil with spindle granules. Eosinophil with spindle granules is comparatively larger (14.4μ x 12.0μ) than eosinophil with spheroidal granules (12.8μ x 10.4μ).

Basophil is small mulberry in appearance. Average diameter of the cell is 9.6μ. Deeply chromaphilic specific granules densely fill up the whole cytoplasm. So that nucleus remains invisible most of the time. Mitochondria and vacuoles are not visible in vital preparation. Basophil is weakly motile and the movement is occasional.

Lymphocyte is the important class of agranular leucocytes in Calotes and resembles the mammalian counterpart in essential structures. Two types of lymphocytes "small" and "large" are clearly visible in Calotes blood. These two types of lymphocytes can be clearly distinguished by the shape of the nucleus and size of the cell body. Large lymphocyte is larger in size (10.4/u in diameter) than the other lymphocyte (7.2/u in diameter). The nucleus is reniform and ecentrically placed in the large lymphocyte whereas in the small lymphocyte it is spherical and centrally placed. Lymphocytes are motile cell, move slowly giving a "hand mirror" appearance. Large lymphocyte represents mitochondria and vacuoles in vital preparation. Large lymphocyte seems to be an older cell than small lymphocyte.

Monocyte does not appear regularly in the peripheral circulation. It is an irregular cell with irregular nucleus. The cell body measures 11.6/u x 6.8/u in length and breadth generally. In vital preparation mitochondria are visible within the monocyte. It is not found to move with the formation of pseudopodia but the irregular shape of the cell body suggests its motility.

Plasma cell appears in the peripheral circulation very rarely. It is a spherical cell with a central halo. The cell generally measures 11.8/u in diameter. Movement of the cell is not recognized. Plasma cell does not represent mitochondria in vital preparation.

Thrombocyte is a cell with spindle appearance. Greater amount of the cytoplasm occurs at the two ends of the nucleus. The cells are slightly motile. Mitochondria and vacuoles are unrepresented in the cell body. Thrombocyte generally measures 12.8/u x 6.4/u in length and breadth.

Azurophils found in other reptiles by certain investigators seem to be immature cells of the granulocytic series. Neutrophil which appears to be a distinct kind in

other reptiles is recognized as ill preserved, weakly stained eosinophil with spheroidal granules. No pigment cell is found in Calotes versicolor.

The number of leucocytes per cubic millimetre of Calotes blood ranges from 5 to 14.5 thousand. Average number is 9 thousand per cubic millimetre of blood. Total leucocyte is always higher in male. Female has 11% less leucocyte count than male. Young Calotes versicolor represents lower count of leucocyte. But the count gradually increases in adult then declines in old animal. Leucocyte count represents annual variation. Count is highest in winter months and lowest in summer months. Total leucocyte count in Calotes versicolor represents a diurnal variation. From morning till night there is a gradual increase of total leucocyte count, when the minimum count is obtained during early part of the day.

Thrombocyte count for Calotes versicolor ranges from 17.8 to 54.9 thousand per cubic millimetre of blood. Such a wide range of thrombocyte count in Calotes may be indicative of its age variation. Average thrombocyte count is 36.8 thousand per cubic millimetre of blood.

The proportions of the different types of leucocytes in the blood of Calotes versicolor can be clearly observed. Eosinophil (Espe) and lymphocyte are the most numerous kinds in the leucocyte population. Basophil and eosinophil (Espl) are in the second level of abundance. Monocyte and plasma cell are not consistently present in all samples of blood. Sex and age have no modifying influence on the leucocyte formulae. But during the period of activity (summer months) lymphocytes are the most numerous cell type. During winter, although there is no hibernation in Calotes, still eosinophil (Espe) represents highest percentage. Increase in the eosinophil (Espe) percentage is also recognized in pregnant females. No clear cut variation in leucocyte formulae is observed diurnally, although eosinophil (Espe) percentage increase towards evening, probably with the migration of it to the circulation from storing tissue.

Erythrocyte and leucocyte count become lower in Calotes versicolor of larger body weight, suggesting that the animal is growing old and the haematopoietic activity of the haematopoietic loci is gradually regressing. The regularity of the seasonal cycle in total erythrocyte and leucocyte count and also in differential leucocyte count suggests that there are some endogenous factors to control these cyclical changes. Further, the opposite relationship between summer erythrocytosis and summer leucopenia suggests that either there are two distinct groups of stimuli to stimulate erythrocytopoiesis and leucocytopoiesis in different times in different ways or there is a common centre for erythrocyte and leucocyte genesis; when one kind of blood cells is released in greater quantity from the centre of genesis, other kind of blood is released in smaller quantity.

The haematopoietic activity in Calotes versicolor is chiefly confined to the bone marrow and liver. Bone marrow is practically the main centre of haematopoiesis giving rise to erythrocyte, eosinophil granulocytes and large lymphocyte. Erythrocytopoiesis is more prominent than granulocytopoiesis in the bone marrow. Liver regularly gives rise to basophil (mast cell), eosinophil granulocytes but erythrocyte to some extent, occasionally. The change over in myeloid:erythroid ratio from 1.0:1.5 in bone marrow to 1.1-1.7:1 in blood suggests that liver always acts as a subsidiary organ for granulocyte production. No other haematopoietic organ in Calotes is found to produce granulocyte normally. Spleen takes its position next to the bone marrow and liver. Spleen normally gives rise to small lymphocyte only. On some special cases spleen produces erythrocyte.

Intertubular tissue of kidney exhibits only very little haematopoietic activity. Intestine does not represent haematopoietic activity.

Haemohistioblast is identified in the bone marrow of Calotes versicolor with definite haematopoietic activity. Haemocytoblast seems to be derived from this

mobilized "stem cell". Haemocytoblast is structurally similar to the mammalian counterpart. Haemocytoblast gives rise to erythroblast for the erythrocytic series and myeloblast for the eosinophil granulocytic series in different times, in different ways. Erythroblast has deep staining coarsely reticular cytoplasm than myeloblast. Lymphoblast seems to be originated from the haemocytoblast as such there is close structural similarity between the two.

Erythrocytopoiesis continues in the bone marrow and peripheral blood through successive stages like erythroblast, early normoblast, late normoblast, reticulocyte and erythrocyte. Similarly, eosinophil granulocytopoiesis continues in the bone marrow and peripheral blood through the stages like myeloblast, promyelocyte, myelocyte and metamyelocyte. There are no separate kind of promyelocytes and myelocytes for the two types of eosinophil granulocytes. Lymphoblast directly develops into large lymphocyte in the peripheral blood.

Basophil (mast cell) in Calotes versicolor is distinctly a histogenous form. It has different developmental history, neither develops from lymphoid precursor nor from myeloid precursor. The process of maturation chiefly occurs within the liver but may occur in the circulating blood. Basophil (mast cell) are in no way related to young eosinophil and are not degenerated or aborted eosinophil.

Small lymphocyte which takes its origin from the lymphoid cell of the spleen constitute the most important white cell of the circulating blood in Calotes versicolor. The small lymphocyte serves as the direct progenitor of thrombocyte in the peripheral blood. Monocyte and plasma cell are not found in any of the tissues tested for haematopoiesis in Calotes. They are probably originated from lymphoid progenitors (lymphoblast or small lymphocyte) in the circulating blood or haematopoietic tissues.

Multiplication and differentiation of the immature blood cells go on continuously within the peripheral blood of Calotes. Throughout the process of differentiation especially for the erythrocyte and eosinophil granulocytes, increase in the size of the cell body and reduction in the nucleocytoplasmic ratio are recognized.

Heteroplastic origin of lymphocyte is not possible from any other blood cells. On the other hand lymphocyte is the progenitor of so many blood cells in Calotes versicolor. However, monophyletic origin of the different blood cells could not be proved in Calotes, as the origin of basophil (mast cell) at least stands against it.

The haemoparasite that have been found in Calotes versicolor is an intra-corpuscular protozoon supposed to be Gordenella calotesi of family haemogregarinidae. The infection occurs mainly in the summer season and the degree of infestation may be as much as 41% of the collection. Superficially, infection of this parasite can be detected by the presence of mite infestation on the body surface of Calotes. This parasite is not so much harmful to the host. Infestation of the parasite causes minor distortion in red cell structure. Heavy infestation induces anaemia as the red cells are eaten away, when haematopoietic activity of the bone marrow and spleen slightly accelerated to cope with the loss of red cell. Perhaps mild infestation produces a stress response in Calotes versicolor with the production of more eosinophil with spindle granules.

Calotes versicolor can survive long upto 15 days under starvation without mortility, similar to many other reptiles. Mild starvation (5 days) seems to elicit erythrocytopoietic response in this reptile. Reticulocyte percentage does not change under starvation. On the other hand erythroblast number is found to change under starvation. So erythroblast number may be taken as the index of erythrocytopoiesis stimulation. Starvation of longer days (15 days) significantly lowers RBC and Hb count and causes aplasia of the bone marrow. All these suggest that prolonged

starvation acts as a depressant on erythrocytopoiesis. Under starvation when stem cell erythrocytopoiesis from the bone marrow fails, lymphoid erythrocytopoiesis from the spleen starts. Thus lymphoid erythrocytopoiesis from the spleen is a compensatory process.

Single cold exposure at 15°C temperature induces haemodilution in Calotes versicolor. But successive cold exposures at 15°C temperature induces haemoconcentration, when the environmental temperature ranges between 30-35°C. Exposure at more cold temperature (7°C) increases haemoconcentration rather than haemodilution. Cold exposure of 7°C temperature for 4 hours produces shock response in Calotes with the increase of eosinophil (Espe) percentage. Eosinophilia persists in this animal probably due to not or improper functioning of its adrenal gland. Granulocytopoiesis in the summer season due to cold exposure in Calotes indicates that haematopoiesis is not purely under endogenous control, exogenous factors may modify it. Probably there is a good adjustment between the endogenous control and exogenous stimulus in regard to haematopoiesis in Calotes. Finally, Calotes versicolor represents temperature sensitivity and its regulation of nervous nature similar to turtle.

For routine surgical preparation, clove oil has been proved to be an excellent anaesthetic agent having had more advantages over the other usual anaesthetic agents. Clove oil in a very low dose (0.030 ml per 50 gm body weight) produces significant loss of righting reflex and anaesthesia of Calotes versicolor. Clove oil induces deep anaesthesia within three minutes. Deep anaesthesia continues for an hour within which surgical operation can be performed successfully. Recovery from the clove oil anaesthesia is smooth and no gross damage of any system is visible. The therapeutic index ($\frac{LD\ 50}{ED\ 50}$) is 1.8, which represents a good margin of safety in the use of clove oil as anaesthetic in Calotes versicolor. Increase of RBC count during the period of anaesthesia suggests slight but temporary anoxia but haematopoietic loci remain practically undisturbed.