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STUDIES ON *GECKO GECKO* (L.) (LACERTILIA: GEKKONIDAE)

I. Blood Cells

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Miss G. S. Gazdar and S. P. Karmarkar (1982). Studies on *Gecko gecko* (L.) (Lacertilia: Gekkonidae) I. Blood Cells. *Bull. Inst. Zool., Academia Sinica* **21**(1): 51-60. Blood cells of *Gecko gecko* have been studied. The erythrocytes show a tendency towards anisocytosis and a trend towards enucleation. Meager erythropoiesis is present in peripheral blood. The heterophilic cell is similar to that of bird and its granules are stable at pH 6.8. The other cell types are typical except that the basophiles are abundant. The presence of peculiar giant cells in the buffy coat preparations is reported. Their similiarity to megakaryoblasts is speculated and their functional significance is discussed.

The important position reptiles occupy in evolution is well known. Since they are the first egg laying land dwelling animals, their various adaptive changes in body structure has been a subject of intensive study. Associative changes in the internal mileu are likewise necessary to keep pace with the demands of the changing physiological requirements of the body. This is evident from studies which reveal that it is in reptiles that for the first time the marrow found an ideal location for the blood cell production in the epiphysis of the long tubular bones and the spleen became an organ of lesser importance as regards its blood cell forming capacity which is taken over by the marrow tissue. Another significant evolutionary advancement regarding blood and its formation is that most of the lymphatic organs have first made their appearance in the reptilian group. The lymph nodes first appeared in the mesenteries of the crocodiles and so is also the case with the definite tonsilar organs, the pharyngeal tonsils which lie in the median dorsal wall of the naso-pharynx, which again are present only in some groups of reptiles like the alligators (Crispens[1]).

Though haematological studies in reptiles began early in the 19th century, contributions of some value began in 1909 by Eberhardt[2] who was the first to work out the cell types in blood and connective tissue of the European tortoise *Emys*. Significant contributions followed, and among these worth mentioning are Jordan and Flippen[5], Ryerson[9], Graziadei[4] and of recent, Pienaar[8], Taylor and Kaplan[11] and Efratti *et al.*[3].

These attempts mainly reveal work done on European species where interpretation of cell types are based chiefly on fixed cell studies. Also few workers have attempted to co-relate the differences in blood picture of the different vertebrate species, with their phylogeny and evolution.

The present work is an initial step towards the understanding of the cellular elements found in the peripheral blood of one species of Indian lizard *Gecko gecko*. Keeping in mind that just morphological identification and classification of cells is insufficient to prove developmental

relationships and that too much reliance on morphological differences only can lead to erroneous interpretations, the present research also involves the study of the behaviour of live cells. Phase contrast microscopy gives definite advantages in showing existing cell structures without the disadvantage of using toxic dyes which very often bring about artifacts introduced during killing and staining the cells which are both confusing in interpretation and morphology. Live cell studies are verified with the conventional smear preparations which reveal tinctorial reaction of various cell inclusions especially those of granulocytes. Furthermore, the chance discovery of the presence of 'giant cells' in live preparation strengthens the phylogenetic link between the reptilian and higher vertebrate groups. An attempt is also made here to clarify and iron out the discrepencies in nomenclature associated chiefly with cells of the granulocytic series.

MATERIAL AND METHODS

Material

The animal chosen for the present investigation was a group of lizard from the subclass Lepidosouria:—

Gecko gecko ..... Linne. 1758.

The lizards were brought in bulk from Calcutta suburbs in West Bengal. They were confined in a special cage in the laboratory, and the experimental animals were isolated out from time to time as and when required for study. A bi-weekly feeding schedule was maintained and the individuals were fed on insects and mealworms.

Methods

Blood was obtained by cardiac puncture from a point mid-ventral and anterior to the pectoral girdle from where a long needle was inserted from the anterior aspect of the heart. The animals were lightly anesthetized with ether prior to blood aspiration and strict aseptic conditions were maintained throughout the procedure to avoid risk of contamination. Sodium citrate was the anticoagulant of choice.

A drop of aspirated blood, compressed just sufficiently between the slide and coverslip revealed details of thrombocytes and erythrocytes. The former cell type being fragile and prone to distortions; necessitated immediate observation prior to subjecting the blood to centrifugation. In order to obtain a sufficient quantity of leucocytes at a time, a modified technique of Shelly[10] was followed to procure buffy coat preparations. Buffy coat yielded a host of live and undistorted leucocytes other than thrombocytes. The cells were then compressed inbetween the slide and coverslip by even and controlled pressure just sufficient enough to reveal the necessary details within. The coverslip was sealed off with petroleum jelly. Conventional blood smears were simultaneously prepared and stained with the normal Romanowsky blood stains in combination with Giemsa. Standardization of pH of the staining media was found to be a crucial factor necessary for good staininge specially of the heterophiles.

RESULTS

Erythrocytes

Typically elliptical and nucleate, the cell measures 18.34 $\mu \times 11.29\ \mu$ (Fig. 1). It is biconvex with blunt and rounded ends. The nucleus appears phase dense due to the presence of dense compact chromatin block, which are more evident on the inner side of the nuclear envelope. The nuclear outline is clear but irregular due to protuberances arising from the nuclear membrane. The thick cytoplasmic layer shows up phase dense and phase luscent areas within an otherwise dull gray cytoplasm. No cytoplasmic inclusions are present in normal cells.

It is not uncommon to encounter anisocytes and poikilocytes, the nuclei of which conform to the cell shape. A large number of immature stages are also present among which are the proerythroblasts (Fig. 2), basophilic (Fig. 3) and polychromatophilic (Fig. 4) erythroblasts, and juvenile (Fig. 5) erythrocytes. A dividing erythroblast in prophase (Fig. 6) is also seen.

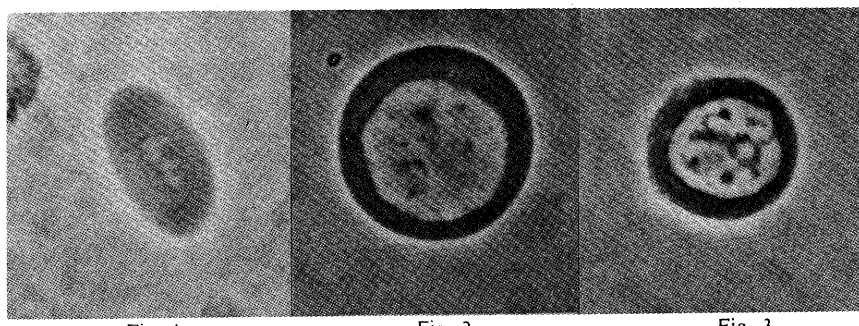

Fig. 1 Fig. 2 Fig. 3

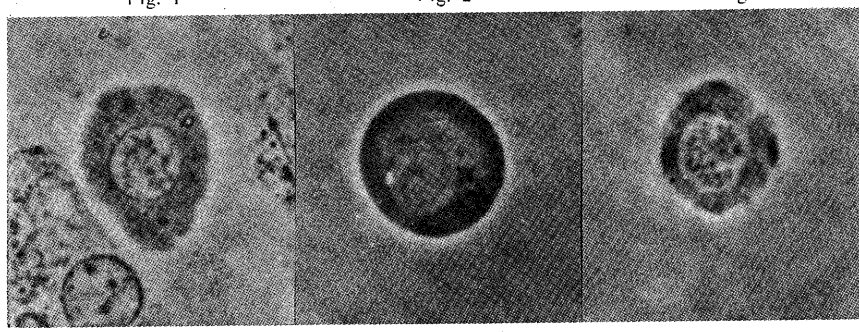

Fig. 4 Fig. 5 Fig. 6

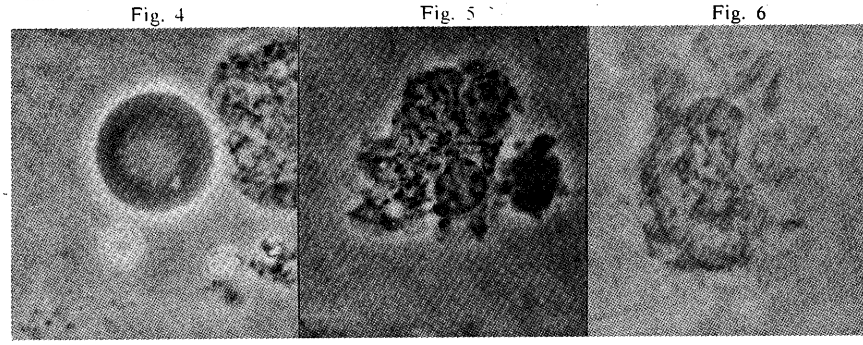

Fig. 7 Fig. 8 Fig. 9

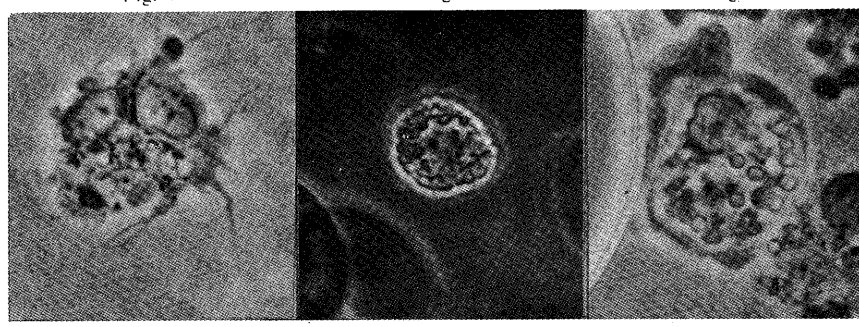

Fig. 10 Fig. 11 Fig. 12

10 μ

Erythroplastids (Fig. 7) though comparatively few are encountered both in live and smear preparations.

Fixed smears exhibit the normal cell picture.

Heterophiles

These polymorphonuclear cells are fairly abundant and measure approximately 14.5 $\mu \times$ 14.81 μ. Almost round in contour the cell has two to five nuclear lobes, but cells with four nuclear lobes are more common (Fig. 8). Each lobe has a distinct nuclear membrane with the contained chromatin in the form of clumps. The nuclear lobes are interconnected with thin chromatin strands. The cytoplasm is abundant with rod shaped granules mostly arranged in an asteroid configuration around a cytocentrum (Fig. 9). The cell is highly motile and exhibits great plasticity, moving in a peculiar flowing movement with a simultaneous change in shape of the cell as well as the granules which assume the form of fat spindles or spheres, eventually changing back to their original form. The proneness of such granular distortions seem to be due to their fluid nature. The movement of the cell is accompanied by cytoplasmic emissions in the form of flat continuous veillike pseudopodia extending over several microns. In day old live preparations the granules seem to coalesce and round up and the hyaloplasm gets drawn out into long thin dendritic projections (Fig. 10) extending over several microns.

In conventional smear preparations the use of an acidic buffer solution for staining is a requisite to retain the original identity of the acidophilic granules, which otherwise break down giving a blurred appearance to the cytoplasm. The granules stain a deep pink colour.

Eosinophiles

They are small round to oval cells measuring 10.58 $\mu \times$ 10.34 μ (Fig. 11). The cell is mononuclear with a central nucleus almost always masked by the highly refractile cytoplasmic granules. The nucleus in flattened preparations exhibit a nuclear envelope and dense chromatin material. The cytoplasmic granules are round and highly refractile under phase contrast. They show a variation in size from tiny round to large ones (Fig. 12). The cell on the whole exhibits slow sluggish movement and moves by emitting tiny round globular pseudopodia. In degenerate and dying cells the granules fuse together presenting a clumpy appearance.

In fixed preparations the granules take up a dull brick red colour. Many cells exhibit a ruptured cell wall with the granules scattered. This is perhaps due to the over-crowding of the granules, which, due to stress applied in smear preparations, forces open the cell wall scattering the granules.

Basophiles

An abundant cell type, it is slightly larger than the intact eosinophile. Basophiles are round, mononulear (Fig. 13) and measure approximately 13.23 μ in diameter. The nucleus is round, slightly indented and centrally located in the cell. The chromatin is a loose fine meshwork on the interstices of which are tiny chromatin granules. The cytoplasm has rounded granules which are slightly smaller in size as compared with the eosinophilic granules. A few normally overlie the nucleus (Fig. 14). If the nucleus shows an identation, no granules are present in its 'hof' or 'bay'. The granules under phase contrast appear dull dark and opaque. In flattened cells vacuolations are common which increase in numbers with a lapse of time. Cell movement is of a slow gliding type by a gradual speading of the cytoplasm in wave-like motions (Fig. 15). During movement the granules remain more or less concentrated around the nucleus and move along with it. In day old preparations the cell disintegrates rapidly and the granules exhibit Brownian movement.

Permanent smears show the basophile as a highly chromophilic cell with dark blue to purple staining granules.

Figures 16 to 20 depict a sequence of movement of an eosinophile and a basophile.

Thrombocytes

Typical cells are seen only in fresh live

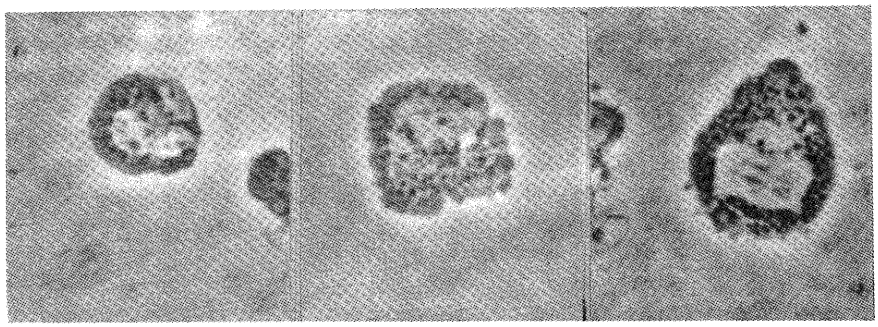

Fig. 13 Fig. 14 Fig. 15

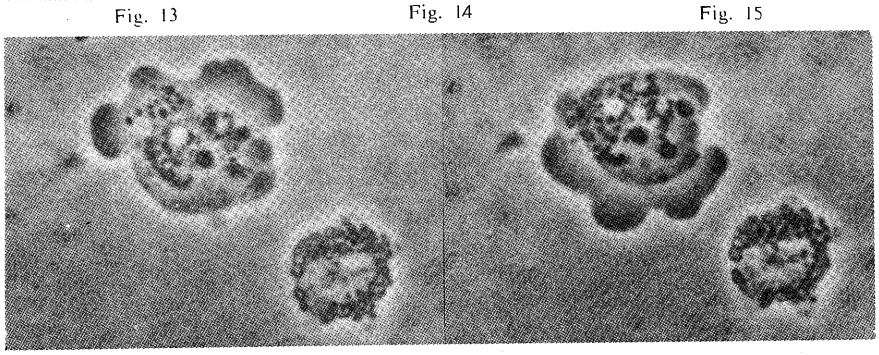

Fig. 16 Fig. 17

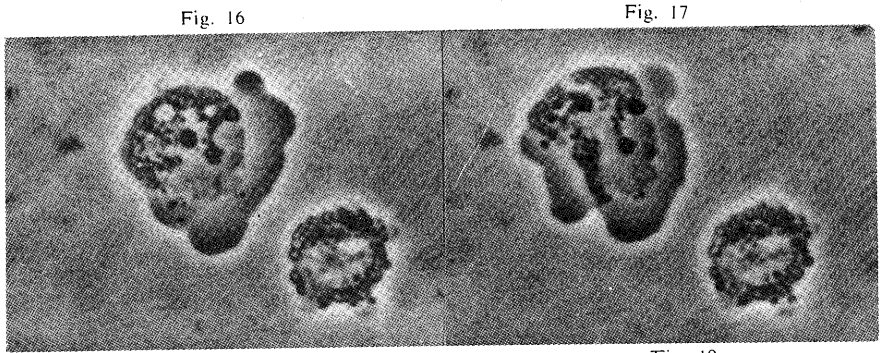

Fig. 18 Fig. 19

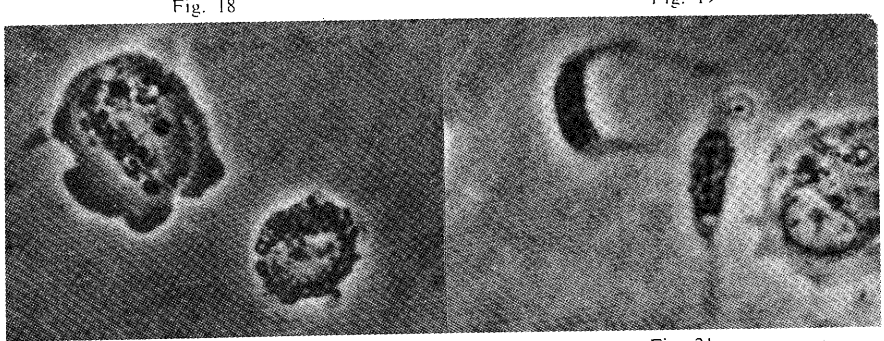

Fig. 20 Fig. 21

10 μ

blood preparations in which they are very common. It is a spindle shaped cell with its two ends tapering to fine points (Fig. 21) and measuring 24.14 $\mu \times 4.85\ \mu$ approximately. The nucleus is central, compact with distinct blocks of phase dense chromatin and a distinct thick nuclear membrane. Flattened cells exhibit a large central karyosome. The cytoplasm is a thin even layer around the sides of the nucleus and is prolonged and tapers to fine points at the two poles in cells which are elongate. These cells when live assume different forms (Fig. 22) due to the spontaneous spreading out tendency of their cytoplasm on a foreign surfac. Thus some cells appear spread out and flattened as their cytoplasm gives out veil-like projections in all directions. The fixed smear shows these cells in a retracted condition in clusters.

Immature thrombocytes in the form of prothrombocytes (Fig. 22) frequently make their appearance in peripheral blood which demonstrates that the cells have a differentiating capacity in the circulating blood.

Lymphocytes

An abundance of small lymphocytes are present in the circulating blood. It is a small cell with a large nucleo-cytoplasmic ratio and measures 5.7 μ in diameter (Fig. 23) approximately. It is round to very slighly oval with a central nucleus. The nucleus conforms to the cell shape and occasionally has a small indentation on one side. The chromatin is clumpy and the nuclear membrane distinct. The cytoplasm is scanty in the form of a thin rim around the nucleus and appears gray under phase contrast. Some cells show vacuolation and a few dull granules randomly strewn. When stationary, the cell has a round contour. They move actively by means of rounded pseudopodia (Fig. 24) and very often assume the typical hand-mirror shape.

The medium sized lymphocytes (Fig. 25) show similar structural details as mentioned for small lymphocytes except that they possess a nucleolus and are slightly larger in diameter.

In preparations made after repeated bleedings, large lymphocytes also make their appearance (Fig. 26). That these cells are the earlier precursors can be evidenced from their angular nucleoli. They show a striking resemblance to the haemocytoblasts and are throught to be so by many authors. In both, the medium and the so called large lymphocytes, the cytoplasm surrounding the nucleus is a thick band and in the stained slide exhibits a sky blue colour.

Monocytes

Monocytes are not an abundant cell type seen in peripheral blood and are found scattered singly and far between. However, when encountered they are easily recognizable from their characteristic nucleus. The cell is large round to oval and measures 13.93 $\mu \times 13.75\ \mu$. The nucleus is large, eccentric and reniform (Fig. 27) often extending from one edge of the cell to the other. The nuclear envelope is distinct and folded with the chromatin arranged in a more or less linear fashion, or, as in the case of slightly younger cells, in the form of large clumps. The nucleus appears transparent and hazy in live cells viewed under phase contrast. The cytoplasm is abundant and appears a transparent gray. A fine deposit of granules corresponding to the azurophilic granules can be seen throughout most of the cytoplasm (Fig. 28). The presence of a vacuole is not an uncommon occurence. Movement is effected by means of small veil-like extensions given off from the cytoplasm in the direction of movement. Monocytes exhibit sluggish and spontaneous movement.

"Megakarvoblast like" cells

These cells are seen in live buffy coat preparations. The cell is voluminous with a usually bilobed appearance (Fig. 29). It measures approximately 47.05 $\mu \times 42.32\ \mu$ (and the younger stage measures 23.5 $\mu \times 25.55\ \mu$ approximately.) The nuclear lobes are placed at the cell edges and exhibit indentation. The nuclear envelope is distinct and the chromatin is arranged in the form of a thick mesh work on which are seen chromatin blocks. The nucleoli which number 1-2 are very prominent, large, angular and of an irregular shape. In younger

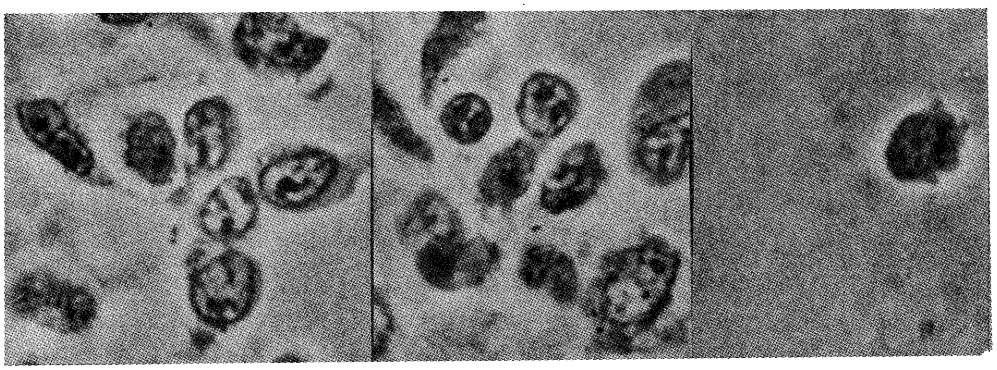

Fig. 22 Fig. 23 Fig. 24

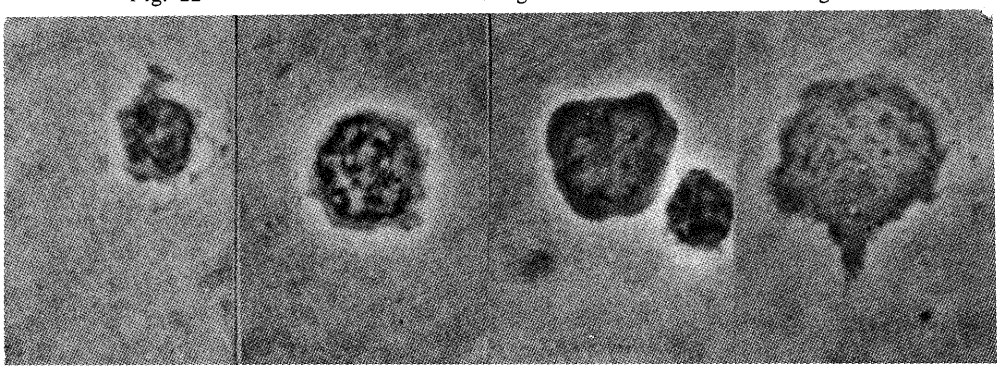

Fig. 25 Fig. 26 Fig. 27 Fig. 28

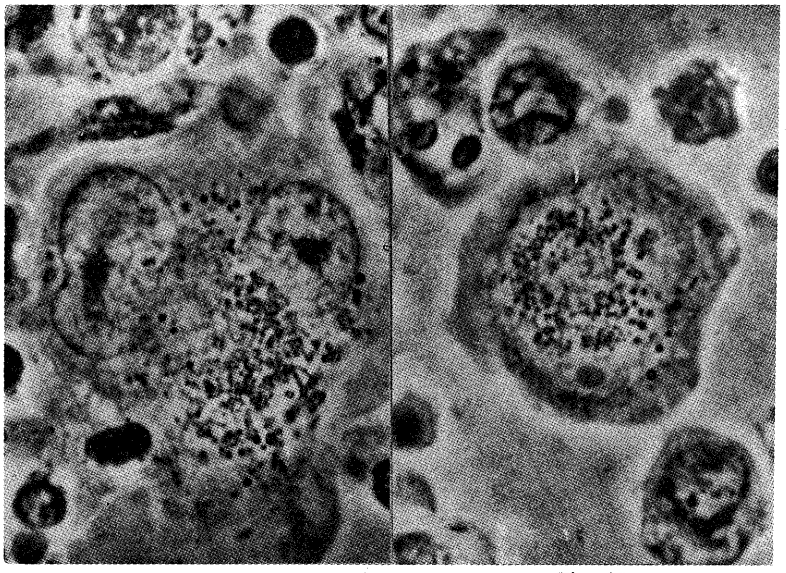

Fig. 29 Fig. 30

10 μ

cells (Fig. 30) the cytoplasmic volume is less which increases with the age of the cell. The cytoplasm appears dull gray under phase contrast and exhibits undulatory margins. The peripheral cytoplasmic zone is without any inclusions and appears more or less clear. The cytoplasm also shows an achroplasmic clear zone situated somewhat in the center of the cell inbetween the nuclear lobes. Around this clear 'functional area' are situated the cytoplasmic granules which appear phase dense and dull. The granules are of varied shapes and sizes.

Since these cells were not observed in fixed smears their staining properties cannot be listed here.

DISCUSSION AND CONCLUSION

The study reveals the presence of all the cell types expected to be present in the blood of higher vertebrates, besides the presence of a peculiar type of giant cell in the peripheral blood of *Gecko*. There is a noticeable tendency of the erythrocytes towards anisocytosis with size variation between the largest and smallest, varying between $18.8\,\mu \times 12.3\,\mu$ to $16.47\,\mu \times 10.00\,\mu$. Besides, a sizable number of erythroplastids are found with a variation in size and shape, which indicates a trend towards enucleation. Blood of normal individuals reveal the presence of juvenile erythrocytes which is a common occurence in the blood of all lower vertebrates. However, their numbers are seen to increase after haemopoietic stimulus, by way of bleeding, was applied. The presence of an occasional erythroblast in mitosis further indicates the process of meagre erythropoiesis in peripheral blood.

Among the leucocytes the morphology of eosinophiles and basophiles seems fairly constant in almost all vertebrate class. *Gecko* eosinophiles and basophiles also conform to the cell picture presented by mammalian homologues. The normal picture of a neutrophile being a polymorphonuclear cell with dust-like granules as seen in human blood changes considerably as one studies its cell homologue in vertebrates lower than mammals. Kyes[6] suggested that the term 'heterophile' be used for a group of neutrophiles other than those found in man, in which specific inclusions show great diversity in form and staining reaction. Heterophiles have been equated with mammalian neutrophiles by many workers like Ryerson[9]. The active motility of the heterophile which is comparable with the mammalian neutrophile further strengthens the close similarity between the two cell homologues. The heterophile cell type of *Gecko* is similar to that described for bird (Lucas and Jamroz[7]). The present cell study reveals the heterophile as a fragile cell prone to distortions. With the normal staining techniques the granules are seen to break down and dissolve imparting an orange-red colour to the cytoplasm. The pH of the staining media seems to be a crucial factor and the granules are seen to be stable at pH 6.8. Another point of similarity with that in birds is the polymorphonuclear nature of the *Gecko* heterophile. Besides, the particular way in which the cell moves is seen to be common with those in birds and mammals.

The other two granulocytes are typical and therefore warrant no special mention except that, unlike the rarity of the mammalian basophiles the basophiles of the reptile under consideration are abundant. Of the agranulocytic series, the lymphocytes are typical and most abundant. The monocytes are, however, few as compared with the other cell types.

Buffy coat preparations of *Gecko* blood reveal the presence of peculiar giant cells unusually large. That these cells are young and the precursors of some cell series is obvious from their large angular nucleoli present within their bilobed large nucleus. Due to the presence of cytoplasmic granules they bear superficial resemblance to granulocytes. A follow up study on the bone marrow of these animals revealed giant cells befitting the description of promegakaryocytes. It is presumed that these giant cells circulating in the blood could be the precursor forms of megakaryocytes. It is probable that these forms are accidentally released

from their site of formation in the bone marrow and swept in the general circulation. This assumption is based on the fact that 20–50% megakaryocytes in human blood are known to enter general circulation (Wintrobe[12]).

The presence of megakaryoblast like cells can only be speculated here. If their identity is established for certain, the presence of these cells would prove another major link in evolution and show the reptiles as a definite step further towards vertebrate evolution. Since platelet like formations are not observed in live cell study, the function of these cells remains shrouded in mystery. The occurence of thrombocytes side by side along with the promegakaryocytes further places their functional aspect in jeopardy since thrombocytes are considered as homologous to megakaryocytes. As these cells fit more or less the description of basophilic megakaryocytes and since they are quiescent, not known to bud off platelets until they reach their full maturity, their counterpart seen in reptilian bloon are naturally unproductive. It could, however, be possible that platelet forming cells have made their first appearance in some of the reptilian groups and have remained dormant within these animals. Further work in this field is bound to reveal interesting findings which might unravel their functional aspect and thereby establish their identity.

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蛤　蚧　之　研　究

I. 血　球

G. S. GAZDAR and S. P. KARMARKAR

本文記述蛤蚧之紅血球研究結果，紅血球大小不均勻且有逐漸去核之趨勢，瘦弱紅血球之生成多發生於外圍之血球。此種異型之細胞與發現於鳥類者相似，同時其所含之顆粒大致維持在 pH 6.8。除了嗜鹼性細胞數量較多外，其他各類型之細胞概屬典型。本篇同時報導，在經緩衝液處理過之蛤蚧血液，可發現異常之巨型細胞，經推測測其與成巨核細胞 (Megakaryoblasts) 有多少相似性，並同時在本篇之結尾討論其機能。