

STUDIES ON *GECKO GECKO* (LINNAEUS) (LACERTILIA : GEKKONIDAE) II. LEUKEMIA

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Gool Sorabji Gazdar and Sudhakar Premsukh Karmarkar (1983) Studies on *Gecko gecko* (Linnaeus) (Lacertilia : Gekkonidae). II. Leukemia. Bull. Inst. Zool., Academia Sinica 22(2): 193-199. A case of suspected leukemia in *Gecko* is reported. Blood studies are based on the study of peripheral blood smears. An altered blood picture is suggestive of some agranulocytic abnormality. Excessive proliferation of monocytes with altered cytological characters by way of vacuolation and erythrophagocytosis is reported. Presence of excessive numbers of stem cells as compared with the normal blood picture shows another aspect of blood abnormality apart from the abnormal monocytes.

The study of leukemia has received top priority in the field of haematological research. Extensive work in the field is being carried out mostly on mammals chiefly due to its applied importance. With advanced modern techniques in the sphere of staining and cytochemical methods, data is constantly being accumulated shedding new light in the field of identification and cell typing. Erroneous interpretations in the past, chiefly due to the disagreement as regards the nomenclature of cell types—mostly the stem cell types, have led to an increase in controversial literature and to ambiguities in diagnosis. Taking into account the mammalian studies as a basis and a standard work, co-relations are attempted between leukemic conditions found in mammals and those in submammalian species.

In lower cold blooded vertebrates, malignant tumors of genetic and viral origin are reported in a few fishes and amphibians (Heston, 1982). Among the reptiles, so far only one case of leukemia in a lizard, *Agama atricollis* has been reported by Pienaar (1962).

In birds, however, leukemic conditions have been reported in many species and leukemia in fowl have been known and studied in detail, mostly due to the economic importance of these birds.

MATERIALS AND METHODS

The animal under investigation was a full grown adult male which was one of the lizards, *Gecko gecko* received with a consignment of 39 others from West Bengal. It was regularly fed along with the others on roaches and mealworms and showed no outward indication of any diseased condition. The lizard was found free from ectoparasitic infection and was isolated out for experimental purposes. As later blood studies confirmed, it was also free of haemoparasites.

Blood, for initial blood typing studies, 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 animal was lightly anesthetized with ether prior to

blood aspiration. Sodium citrate was the anticoagulant used of choice. Strict aseptic conditions were maintained throughout the procedure to avoid risk of possible infection.

Conventional blood smears were prepared and stained with the normal Romanowsky blood dyes such as Wright's-Giemsa and Leishman's-Giemsa. The staining technique specified by Pienaar (1962) for snake blood was also tried out for these smears.

Blood was aspirated for a total of three times at intervals during the course of its experimental life. The second bleeding was done 18 days after the first and the third bleeding 8 days thereafter.

RESULTS

The initial peculiarity noticed was the dull brown colouration of the aspirated blood, especially at the time of the second "bleeding" which was a clear deviation from the 'blood red' colour. On cursory examination the blood picture revealed massive leucocytosis where the leucocyte types greatly outnumbered the erythrocytes.

Smears prepared after the first bleeding showed a large proportion of haemocytoblast-like cells and in particular monocytes as compared with the normal picture presented by other like animals (Figs. 1 and 2). There were also a large number of lymphocytes when thick portions of the blood smear were observed; a condition reminiscent of mixed leukemias of the "lymphatic monocytic tissue" (Undritz, 1952).

The haemocytoblast-like cells showed a large nucleocytoplasmic ratio (Figs. 3 and 4), with a dark staining large nucleus which usually conformed to the cell shape. The chromatin was in the form of a network with occasional small granules dispersed on its inter-sections. The karyotheca was dark-staining and distinctly visible. Large angular nucleoli were observed in the nuclei of many cells. The cytoplasm stained a clear blue with a thin homogenous perinuclear rim. Some cells were seen with small rounded cytoplasmic blebs on

its periphery.

The monocyte was a large round cell with its typical eccentric nucleus (Fig. 5). The nucleus showed many shapes in these smears, where some were typically reniform, others appeared as stabs and still others were rounded with an indentation or with a constricted centre assuming a figure of 8 (Fig. 6) (Hayhoe and Cawley, 1972). Whatever be the shape of the nucleus the chromatin always seemed to be thready and in a few cells displayed a more or less linear arrangement. The cytoplasm was abundant, and stained a peculiar dull gray or a very dull blue colour. The cells exhibited azurophilic granulation within their cytoplasm.

The lymphocytes were typical and showed no peculiar features (Figs. 4 and 6). So also were the cells of the granulocytic series (Figs. 7 and 8). The thrombocytes were also typical and warrant no special mention (Gazdar and Karmarkar, 1981).

The second bleeding showed an abnormal increase in the two cell types mentioned earlier *i.e.*, the haemocytoblast-like cells and monocytes. The increase had reached such a proportion that these leucocytes greatly outnumbered the erythrocytes (Figs. 4 and 8). The haemocytoblast-like cells showed mitotic figures (Figs. 9 and 10), a majority of them were seen in the prophasic stage. These cells, however, never assumed prolific proportions. A majority of the monocytes exhibited a folded nuclear membrane with engulfed cells within their cytoplasm, especially phagocytosed erythrocytes with their nuclei and haemoglobin content intact (Figs. 11 and 12). The monocytes without engulfed inclusions exhibited a granular cytoplasm, the granules appearing to be mostly azurophilic (Fig. 6). A few of these cells exhibited necrobiotic and fragmented nuclei, due to which they could not be identified with certainty (Figs. 13 and 14). Of the granulocytic series all the cells seemed to have retained their identity except that the heterophiles were seen to be highly polymorphonuclear (Fig. 15).

The third bleeding exhibited a majority of cells in a necrotic and dying state (Fig. 16).

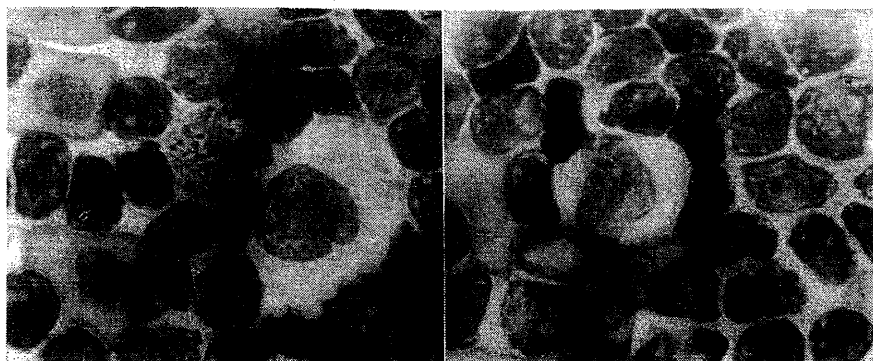


Fig.1.

Fig.2.

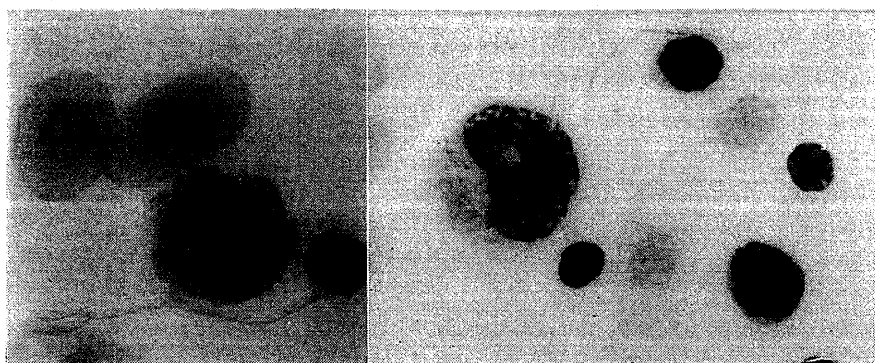


Fig.3.

Fig.4.

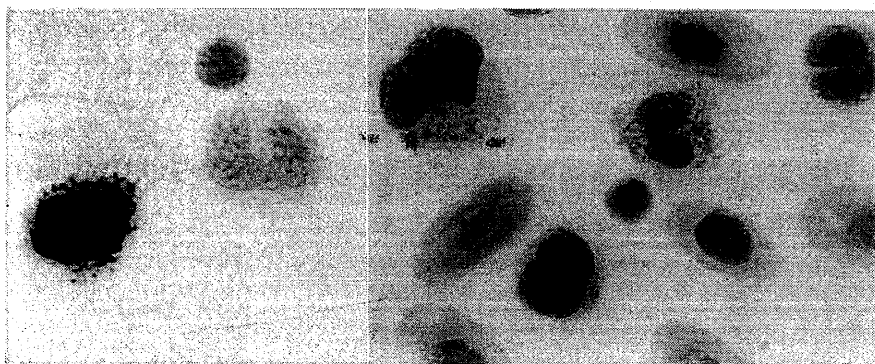


Fig.5.

Fig.6.

10 μ

- Figs. 1 and 2. Smear preparations after first bleeding showing haemocytoblast-like cells with a monocyte in the centre.
- Fig. 3. Haemocytoblast-like cell exhibiting a large nucleocytoplasmic ratio (after first bleeding).
- Fig. 4. Smear after second bleeding showing leucocytosis with prominent haemocytoblast-like cells and a typical lymphocyte.
- Fig. 5. Monocyte with an eccentrically placed nucleus and a typical lymphocyte (after second bleeding).
- Fig. 6. Monocyte exhibiting azurophilic granulation in the cytoplasm and a nucleus with constricted centre. Also seen alongside is a small lymphocyte (after second bleeding).

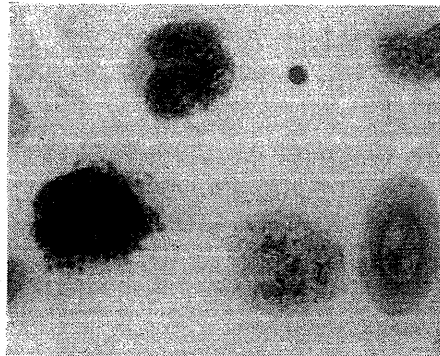


Fig.7.

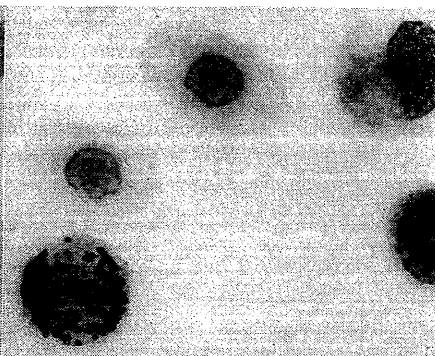


Fig.8.

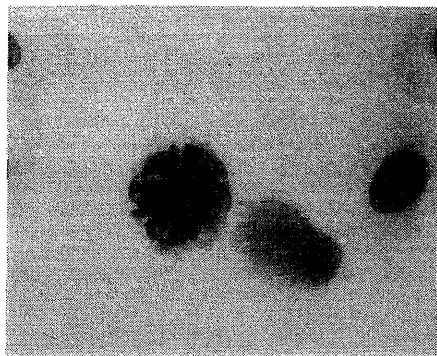


Fig.9.

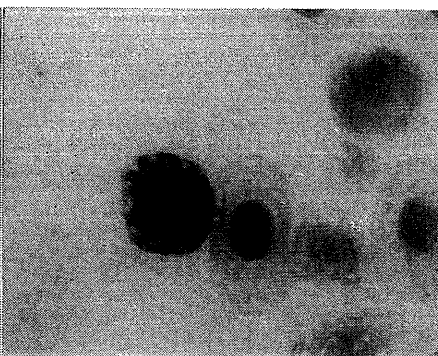


Fig.10

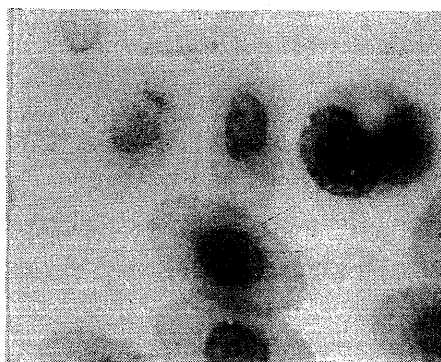


Fig.11.

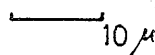


Fig.12.

Fig. 7. An eosinophile of the granulocytic series (after second bleeding).

Fig. 8. A basophile of the granulocytic series (after second bleeding).

Figs. 9 and 10. Haemocytoblast-like cells showing mitotic nuclei in prophase (after second bleeding).

Figs. 11 and 12. Monocytes exhibiting erythrophagocytosis (after second bleeding).

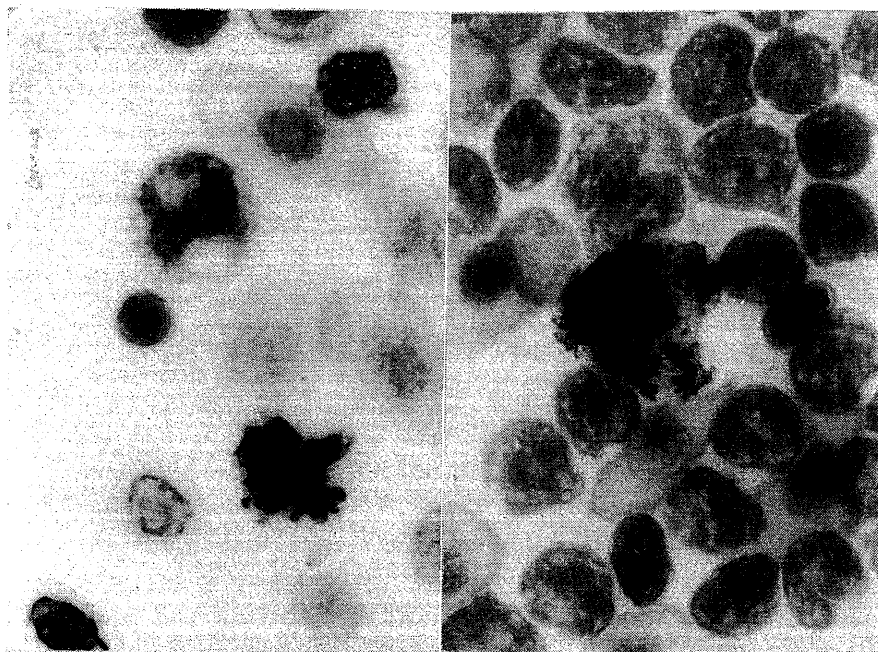


Fig.13.

Fig.14.

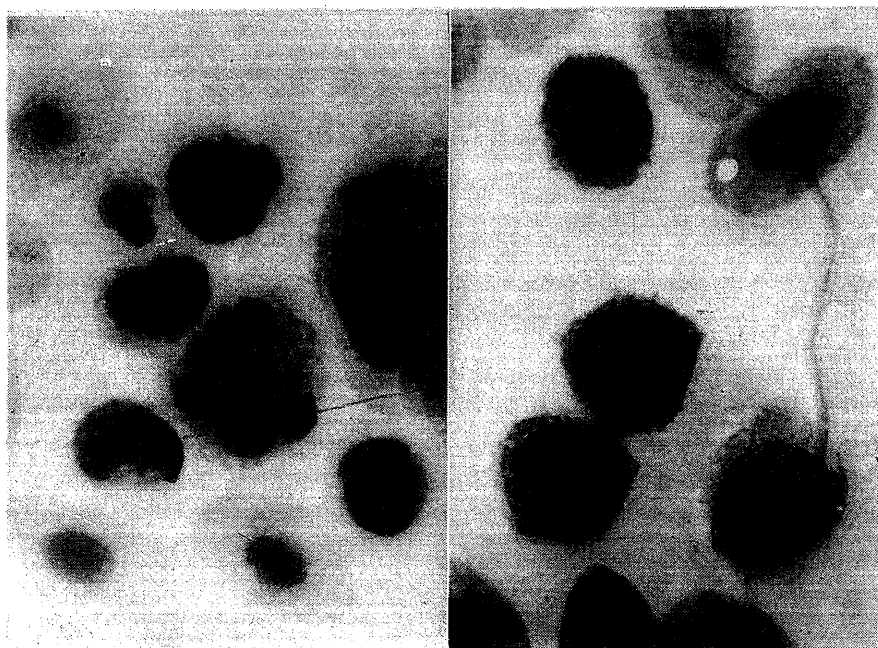


Fig.15.

Fig.16.

— 10 μ

Figs. 13 and 14. Unidentified cells with necrobiotic and fragmented nuclei. (after second bleeding).

Fig. 15. A highly polymorphonuclear heterophile (after second bleeding).

Fig. 16. A cluster of necrotic cells after third bleeding.

Unfortunately, due to the small quantity of blood obtained, only three smears were prepared which may not give concrete evidence to the statement on "dying cells". Barring these cells the blood picture remained unaltered.

No further study could be undertaken on this specimen as the animal died 4 days after the third bleeding from undiagnosed causes.

DISCUSSION AND CONCLUSION

Basing conclusions on the peripheral smear preparations stained with Romanowsky-type dyes, the animal seemed to have agranulocytic leukemia. It may sound vague and non-committal to brand the pathological condition as "Agranulocytic Leukemia", but to name it as either monocytic or lymphocytic would not have been correct as apart from monocytosis a great many stem cells which resembled haemocytoblasts, some with mitotic figures, were present. That the blood was pathologically altered was quite evident, since comparison with normal blood picture revealed a marked deviation towards leucocytosis.

In mammals monocytic proliferation and abnormalities are said to occur in certain infections like tuberculosis, leishmaniasis, endocarditis lenta, typhoid etc. (Bessis, 1956; Goldman, 1972; Undritz, 1952). It has also been found that abnormalities or hyperplasia of the histiocyte a tissue cell, is in a great majority of cases reflected as an abnormality of the monocyte, a cell of the circulating blood (Bessis, 1956).

It was found from the present study that not only was there a great increase in the monocytic cell population, but the cells had deviated from the normal and showed vacuolation and excessive erythrophagocytosis. Moreover, the cells which resembled near normal conditions exhibited excessive granulation in their cytoplasm. This condition of cytoplasmic granulation was suggestive of that of a granular histiocyte as listed under the head "Monocytic Leukemia" by Diggs, *et al.*, 1954. On cursory examination this condition of azurophilic granulation which is often present

in monocytic leukemia tends to make these cells appear similar to myelocytes of the granulocytic series (McDonald, *et al.*, 1965). Statistical evidence gathered from acute leukemic conditions in humans have shown that primitive cells in these types of leukemias have a disordered cell cycle with prolongation of intermitotic phase (Hayhoe, *et al.*, 1964). Thus, mitotic figures have long been noted to be scanty, a condition also noted in the present investigation.

Since the animal was free from ectoparasites and haemoparasites the possibility of any infection from these causes is ruled out; in which case the excessive proliferation of these cells could be attributed to either viral or some other pathological causes, or perhaps even due to some histiocyte malfunctioning in the tissue as suggested by Bessis, 1956. That these cells belong to the monocytic series is further strengthened by the fact that they are concerned principally in the destruction of foreign material or abnormal cells. The high degree of phagocytosis seen after the second bleeding could have been due to this inherent property which was manifested in peripheral blood. Most of the phagocytosed cells were the erythrocytes; erythrophagocytosis being one of the commonest forms of phagocytosis seen in films of haemopoietic tissues, in leukemias and many other diseases (Bessis, 1956).

The presence of excessive numbers of stem cells or haemocytoblast-like cells and a simultaneous release of young erythrocytes could not be attributed to haemopoietic stimuli the animal received due to repeated bleedings, as this technique when tried out with similar animals did not result in any such marked increase in the number of these cells, though of course as expected, a few young and developing erythrocytes did make their appearance.

In order to confirm the leukemic condition, slides were given to three different professional pathologists for identification. Two of these came up with a near similar diagnosis of acute agranulocytic leukemia of whom one said it was a case of acute lymphoblastic leukemia

and the other diagnosed it as a case of some abnormality of the histiomonocytic series. The third pathologist, however, confirmed it as an abnormality associated with the myeloid series. Since these pathologists were not familiar with reptilian blood, slides of blood from normal animals were also provided to them for comparison.

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蛤蜊之研究 II. 白血球過多症

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本文報告一個蛤蜊 (*Gecko*) 的擬似白血病例，由末梢血液抹片的分析發現所謂不正常的血球屬於無粒血細胞異常。文中對於有空泡形成以及紅血球吞噬作用的單核球之過度增殖加以細述。除了不正常的單核球，幹細胞的數目也較正常的多，為另一異常的特徵。

