

# INVESTIGATION OF THE CELLULAR COMPOSITION OF THE BONE MARROW IN MONKEYS AFTER REPEATED PUNCTURE

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The study of the dynamics of the morphological composition of the peripheral blood in monkeys [1] has shown changes in the number of leukocytes per  $\text{mm}^3$  after the repeated taking of blood samples, all the experimental conditions being the same. The high leukocyte count figures observed during the first days of the observations were appreciably diminished during the systematic repetition of the procedure of taking blood from the same animal under the same conditions. The fact that a depression of the leukocyte count takes place in monkeys under the influence of repeated blood investigations must be taken into consideration, not only when the physiological normal values for these animals are being established, but also when systematic investigations are being made for the purpose of studying the action of various agents on the blood.

We considered that it would be of interest to examine, in the same way, the dynamics of the cellular composition of the bone marrow in monkeys. In the conduct of our investigation we discovered facts which, although not connected with this depression phenomenon, were nevertheless of essential importance from the point of view of technique.

## EXPERIMENTAL METHOD

The work was carried out on five male monkeys of the species *Papio hamadryas*, aged from one to two years. The animals were clinically healthy and were kept in isolation from the herd.

The site chosen for puncture of the bone marrow was the tibial tuberosity in both lower limbs.

During puncture the monkey sat in the attendant's arms. The leg was fixed vertically, supported on the foot; for convenience in finding the point of puncture the mobile skin surface of the animal's limb was drawn in the direction of the thigh. The skin at the site of puncture was not anesthetized, but after removal of the hair it was painted with alcohol.

Puncture in monkeys presents no special difficulty, for the site of puncture is readily determined. This point

is situated at a distance of approximately 5-10 mm from the articular surface of the tibia.

The site of puncture was palpated with the thumb of the left hand, and with a rotary movement of the right hand a Kassirskii's needle was inserted. The moment of entry of the needle into the medullary cavity was determined by means of the stilet; if, on extraction of the stilet, traces of marrow contents could be seen upon it, it could be considered that the needle was situated within the medullary cavity. After extraction of the stilet, a syringe was attached to the end of the needle for aspiration of the marrow; the contents of the syringe were expelled onto a glass slide, whereupon five films were prepared.

In order to determine the absolute number of leukocytes in the bone marrow, the puncture specimen was collected in a red cell mixing chamber with acetic acid. In the investigation of the myelogram, 500 cells were counted in three films. Furthermore, samples of blood for analysis were taken at the same time from the right ear, and the total and differential leukocyte counts were determined. The experiments were carried out once a week, always during the morning hours before the animals had been fed.

The observations were continued for 6-9 months by the same person, and the experimental conditions remained constant throughout this time.

## EXPERIMENTAL RESULTS

Repeated puncture of the same area of the marrow tissue leads to definite disturbances of the processes of hemopoiesis at that point. These disturbances were shown, in the first place, by a decrease in the number of leukocytes in this particular area of the marrow and by changes in the relative proportions of the cells obtained by puncture.

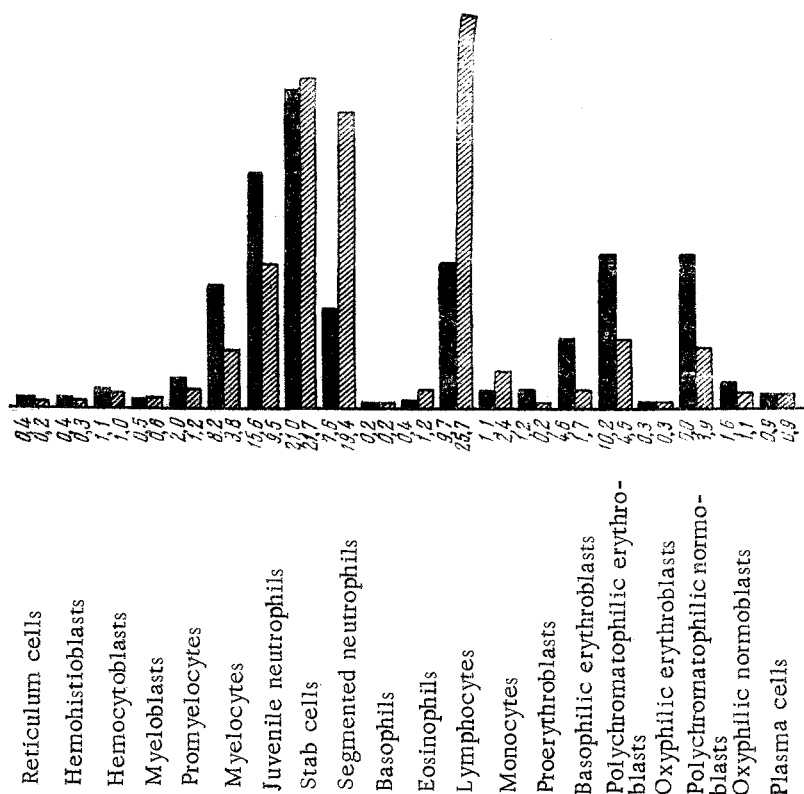
In the table are given the results (obtained in three monkeys: Koster, Vik, and Anyb), showing the absolute number of leukocytes in the marrow specimen as a result of the first and last experiments, and also the

# Dynamics of the Absolute Number of Leukocytes in the Bone Marrow after Repeated Puncture

Name of monkey	Total number of punctures	Duration of experiments (in months)	Absolute number of leukocytes in the bone marrow				P (probability of random variation between mean indices obtained at beginning and end of experiment)
			first day of investigation	mean value in first four investigations	last day of investigation	mean value in last four investigations	
Koster . . . .	31	9.0	353 000	229 000 ±41 000	22 000	27 000 ± 2 700	0.006
Vik. . . . .	24	5.5	137 000	229 000 ±28 000	21 500	35 000 ± 4 000	0.001
Anyb. . . . .	24	5.5	324 000	238 000 ±44 200	23 500	41 000 ±9 700	0.011

number and duration of the investigations carried out. From the figures given, it follows that the absolute leukocyte count in the marrow specimen from the monkeys on the first day of aspiration of the marrow was very high, varying between 353,000 and 137,000. The mean number of leukocytes in the specimens obtained in the first four investigations was in no case less than 229,000. Repeated puncture of the same area of hemopoietic tissue led to an obvious decrease in the number of leukocytes in this area of the marrow, which

varied between limits of 23,500 and 21,500. Statistical treatment of the results showed that the difference between the mean indices obtained at the beginning and end of the investigation was significant. The fall in the number of cells in this area of the bone marrow persisted for 3-4 months, and it was not restored in spite of an interruption of the work for 1½ months. In two monkeys (Antil and Isuden) a fall in the absolute number of leukocytes in the bone marrow appeared in the second or third week of puncture; in the first week of



Myelograms (in per cent) when the absolute number of leukocytes in the marrow was maximum and minimum. Black columns – proportion of cells of marrow specimen with maximum values; shaded columns – the same, with minimum values.

puncture its value was 329,500-157,000, and in subsequent weeks it fell to 24,000-16,000. In this period normal marrow activity was preserved in other areas of hemopoiesis (humerus, ilium) in all the monkeys. Whereas an average of 21,000 leukocytes (3 monkeys) was found in the tibia which was repeatedly punctured, the first puncture of the humerus, carried out at the same time, revealed 194,500 leukocytes, and puncture of the ilium - 105,000 leukocytes. It must be pointed out that normally the absolute number of leukocytes and the quantitative proportions of the cells in the bone marrow in the paired limbs are identical.

Analysis of the changes in the cellular composition of the bone marrow in the experiments described above revealed that this was directly connected with the changes in the leukocyte count. The figure illustrates the mean data of the myelograms (calculated in %) obtained when the absolute number of leukocytes in the bone marrow was maximal (from 350,000 to 100,000) and minimal (from 85,000 to 30,000).\*

It follows from the data shown that in the first weeks of puncture, when the bone marrow is active and rich in cells, the relative proportions of the morphological indices are within the normal limits characteristic of this lower species of monkey. The percentage content of stab cells (21%) in the myelogram is higher than that of segmented neutrophils (7.6%), which is evidence of the rapid expulsion of mature cells into the blood stream.

The normal activity of the cells of the leukoblastic and erythroblastic series in this period is also shown by the following indices: the leuko-erythroblastic ratio was 2.4; the maturation index of the neutrophils was 0.9; the maturation index of the protoplasm of the erythro- and normoblasts, 0.8. The mitotic activity of the cells of the erythroblastic series was 5 : 500, that of the leukoblastic series, 2 : 500.

With the decrease in the number of cells in the marrow obtained by puncture, observed during the days following puncture, the relative cell proportions shown in the myelogram were considerably disturbed. In the first place there was an appreciable fall in the percentage of immature cells of the neutrophil series (promyelocytes, myelocytes, and juvenile neutrophils) and an appreciable rise in the percentage of mature neutrophils (from 28.6% to 41.1%), lymphocytes (from 9.7% to 25.7%) and also of monocytes (from 1.1% to 2.4%). The proportion of cells of the red series was reduced, and the mitotic activity fell - the number of mitoses in cells of the red series was 2.1 : 500, and of the white series, 1.5 : 500.

The functional impairment of hemopoiesis is made apparent by determination of the leuko-erythroblastic

ratio (7.3) and the maturation index of the neutrophils (0.3). Characteristic cells, often in groups of 3-6 at a time, are seen in large numbers in the films at this period, and by their morphology they may be classed as osteoblasts. The appearance of osteoblasts in the marrow specimens is most evident in the 6th-7th week of puncture of the bone marrow, and later their number decreases. Another characteristic feature is that the marrow obtained after frequent aspiration was in no way distinguishable in its external appearance from normal, especially by the presence of a large number of lipid scintillations on its surface.

Changes were also observed in the peripheral blood indices. As the number of punctures increased (to the eight week) the qualitative composition of the blood was modified, as follows: young marrow cells of the type of hemocytoblasts, promyelocytes, myelocytes, reticulum or plasma cells, binuclear lymphocytes, and also erythroblasts, polychromatophilic cells, and erythrocytes with Jolly bodies began to appear in the peripheral blood. The presence of a large number of bare nuclear cells and of cells in a state of leukocytolysis was also noted; all these changes in the blood were clearly shown in the monkeys Koster and Anyb.

It thus follows from the findings described that repeated aspiration from the same area of the bone marrow leads to profound disturbance of the processes of hemopoiesis taking place in that area. This must be taken into consideration when the dynamics of the cellular composition of the bone marrow is being studied.

## SUMMARY

The changes in the cellular composition of the bone marrow were studied in 5 monkeys of the species *Papio hamadryas*. The bone marrow was obtained from the tibial tuberosity of both extremities with the aid of I. A. Kassirskii's needle. Experiments were conducted for a period of 6-9 months in the morning hours, before feeding the animals, once a week. The absolute number of leukocytes in the punctate decreased as a result of frequent punctures; the normal cell ratio in myelograms was disturbed. Qualitative changes also occurred in the peripheral blood. The changes observed were very stable and persisted for a long time.

## LITERATURE CITED

1. I. A. Utkin and M. I. Kuksova, *Doklady Akad. Nauk SSSR* **108**, 981 (1956).†

\* As in original, though these figures seem to be in flat contradiction to those previously cited [Publisher's note].

† See English translation.