

# INFLUENCE OF TUBERCULOUS ANTIGEN ON BLOOD SYSTEMS OF ANIMALS

## COMMUNICATION IV. REFLEX NATURE OF LEUCOCYTOSIS AND REACTION OF BONE MARROW TO INTRODUCTION OF BCG CULTURES IN THE ORGANISM

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In previous communications [3, 4, 5] we showed that the tubercle bacillus and the products of its vital activity, introduced parenterally by exciting the peripheral sensory tips of the nervous system, produce reflex changes in the composition of the peripheral blood.

The experiments showed that, as a rule, after intramuscular administration of the attenuated tubercle bacillus - BCG culture emulsion - in the peripheral blood of a cat, a growing increase in the number of leucocytes, occurring after a temporary leucopenic phase, is observed. In connection with this there arose the necessity of clarifying whether the leucocytosis induced by introduction of the BCG culture was a result of redistribution or a genuine increase. It was also necessary to establish whether the bone marrow takes part in this reaction or whether all changes in the number of formal elements of the peripheral blood depend only on the redistribution of the leucocytes from some organs to others within the confines of the vascular system.

The increase in the number of young forms of neutrophils prompted the hypothesis that the leucocytic reaction of the cat to parental introduction of BCG culture is, to a considerable degree, genuine leucocytosis. In order to confirm this hypothesis observations were made on the changes in the number of leucocytes in different regions of the vascular system arising immediately after injection of BCG culture emulsion in the muscles of the femur of the hind limb of the cat.

### EXPERIMENTAL METHODS

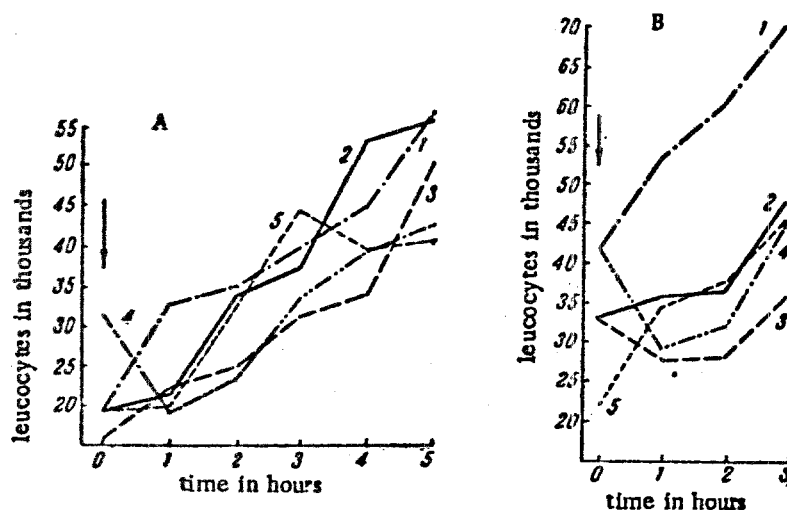
The experiments were performed on 38 cats. Altogether 238 investigations of the number of leucocytes and 16 investigations of the bone marrow were carried out. Blood specimens were taken from the vessels of the ear, left and right paws, spleen and small intestine (through incision). Several series of experiments were conducted.

### EXPERIMENTAL RESULTS

It was possible to take blood specimens from the vessels of the internal organs of the narcotized animal. Consequently, at first we had to establish how narcosis (Nembutal) influences the blood system. It was established in three animals that under the influence of nembutal narcosis no significant changes take place in fluctuations in leucocyt content in the peripheral blood.\*

\*A similar fact is noted in a paper by K. G. Malysheva [2].

In the following series of experiments we studied the character of the leucocytic reaction to injection of BCG culture (15 mg in 3 ml physiological saline) in the muscles of the femur in conditions of narcosis. For this purpose observations were conducted on 11 cats in a state of narcotic sleep (solution of Nembutal was introduced intramuscularly on the basis of 0.22 g nembutal per cat of 3 kg weight). In 9 out of 11 anesthetized animals a leucocytic reaction to injection of the BCG emulsion was absent. Only in two cats, 3 and 4 hours after injection of the BCG culture, was an increase in the number of leucocytes noticed. In these experiments narcosis was less deep than in the former ones. Convinced that narcosis was unsuitable for our investigations since it contributes to suppression of the leucocytic reaction to BCG, we used the method of decerebration for immobilizing the animal. In three experiments we made an observation on the change in the number of leucocytes of the decerebrated cat for 5 hours after the operation with an interval of 1 hour without any supplementary influences. The fluctuations in the number of leucocytes in this case were small – altogether up to 2-3 thousand in one cubic millimeter of blood (i. e. up to 11% in relation to the original number of leucocytes). After establishing this fact we injected the following 13 animals with the BCG culture in the muscles of the femur of the hind limb one hour after the decerebration operation.



Fluctuation in number of leucocytes in blood of cat in various vascular regions after injection of BCG emulsion into left hind limb. Significance: 1) left paw; 2) ear; 3) small intestine; 4) spleen; 5) right paw – ↓ moment of injection. A and B – different experiments.

The blood, taken from the vein of the ear, the right and left paws, spleen and small intestine of the experimental animal, was investigated before injection and then 1, 2, 3, 4, and 5 hours after injection of the BCG culture. In these experiments we noted that the original content of the leucocytes in the investigated regions of the vascular channel differed. It was found that in the vessels of the peripheral regions (ear, right and left hind limbs) the leucocyte changes showed parallel development. A gradual increase in the number of leucocytes starting one hour after injection and growing for 4-5 hours was noted. The number of leucocytes reached a considerable figure. Thus, in Experiment No. 8 (Fig. A), in the vessels of the ear the number of leucocytes rose from 18,950 to 55,300 per  $\text{mm}^3$ , in the vessels of the right paw the number increased from 18,950 to 41,000 and in the vessels of the left paw from 19,050 to 56,000.

Analogous findings were obtained in other experiments, in particular, in Experiment No. 12 (Fig. B).

As regards the changes in the number of leucocytes in the vessels of the organs of the abdominal cavity, at first we noted in a series of experiments (7 out of 13) a phase of reduction of leucocytes, continuing for two hours, giving way to growing leucocytosis. Thus, in Experiment No. 8 the number of leucocytes in the spleen one hour after injection dropped from 31,400 to 17,900 per  $\text{mm}^3$  of blood, then in 2 hours rose to 23,000, in 3 hours to 33,300, in 4 hours to 38,700 and in 5 hours reached 42,700. In Experiment No. 12 the number of

leucocytes in the vessels of the spleen before injection was 42,000, one hour later 28,600, in 2 hours 32,400, in 3 hours 45,200. In the vessels of the small intestine before injection there were 32,350 leucocytes, in one hour there were 27,450, in 2 hours 27,900 and in 3 hours 35,200. In the remaining experiments developing leucocytosis in the vessels of the abdominal cavity was noted only one hour after introduction of the culture. These findings suggest that the first phase of the reaction of the blood system to introduction of BCG culture — an increase in the number of leucocytes in the peripheral vessels — takes place through redistribution mechanisms, i. e. decrease in the content of the leucocytes in the vessels of the organs of the abdominal cavity. In those cases where no leucopenic phase was observed in the vessels of the organs of the abdominal cavity, redistribution, obviously, took place through other vascular regions — liver, lungs.

TABLE 1

Changes in Myelogram in Response to Intramuscular Introduction of BCG Culture in

Blood cells	June 4, 1954		June 25, 1954		July 2, 1954		July 7, 1954		July 9, 1954	
	Before injection	5 hours after injection	Before injection	4 hours after injection	Before injection	4 hours after injection	Before injection	4 hours after injection	Before injection	4 hours after injection
Myeloblasts	7.4	10	1.2	2.6	4.2	8.6	8.2	11.6	7	12.2
Promyelocytes	5.6	7.6	2.4	5.6	2.4	4.2	5.4	5.6	5.8	5
Myelocytic neutrophils	3	5	1	2	1.8	4.8	1.4	2	2.8	4
Myelocytic eosinophils	3.8	3	0.2	1.4	0.4	2.8	2.2	1.8	4.2	2
Myelocytic basophils	1.4	1	0.2	0.6	0.6	1.6	1.2	0.2	0.6	1
Metamyelocytic neutrophils	16.6	22.2	8.4	11.6	9.2	21.4	21.8	26	36.4	32
Metamyelocytic eosinophils	2.4	2.6	0.4	1.6	2	1.8	1.8	2.2	2.6	1.6
Neutrophils — rod nuclear	12.6	8.8	13.6	20.4	12.8	14.8	25.8	20.2	16.2	12.6
Neutrophils — segment nuclear	7.2	2.2	22.4	16.2	21.4	13	1.2	0.6	1.6	1.8
Lymphocytes	0.4	0.4	13	3	4	0.4	0.2	0.2	0	0.2
Monocytes	0.4	0.2	3	1	3	0.6	0.4	0.2	0.2	0.2
Basophils	0.2	0.2	0	0	0.4	0	0	0.2	0.6	0.6
Eosinophils	8.6	5	3.6	4.2	9	7.6	2.4	6.2	5.2	5.6
Proerythroblasts	2.4	3.2	2.4	2.6	4.4	4.8	4.2	4.4	4.4	3.8
Erythroblasts I	5.2	5	4	3.6	4.4	8	2.4	3.8	2	2.2
Erythroblasts II	6.2	6	6.8	5.6	6.2	9	6.2	3.6	3.8	3.8
Erythroblasts III	12.8	13	14.6	16.6	12.2	3	13.6	9.2	5.2	7.2
Plasmatic cells	1.4	1.4	1.6	1	1	0.8	1	0.8	0.6	3
Megacaryocytes	1	0.6	0.6	0.2	0.2	0	0	0.2	0.2	0.2
Hematohistioblasts	0.8	1.2	0.2	0.2	0	0.8	0.4	0.4	0.2	0.2
Reticulo-endothelium cells	0.6	1.4	0.4	0	0.4	1	0.2	0.6	0.4	0.8
Total number of nucleated elements	89 500	143 500	69 500	116 500	45 000	127 000	221 000	199 000	185 000	165 000
Leucocytosis of peripheral blood	10 350	15 150	17 500	27 950	10 100	16 150	20 800	25 400	20 900	24 800

In the second phase of the reaction, after only a relatively short time interval (2 hours) leucocytosis assumed genuine characteristics, i. e. the leucopoietic activity of the bone marrow was intensified. This is born out by a parallel increase in the number of leucocytes in all the investigated vascular regions.

Similar results were obtained by E. L. Kan [1] in study of the mechanisms of the leucocytic reaction in response to stimulation of the mechanoreceptors of the stomach.

Our findings may serve only as indirect proof of the participation of the bone marrow, of its leucopoietic system, in the reactions of the blood system to parenteral introduction of emulsion of BCG culture.

The results of study of the bone marrow blood formation after introduction of BCG culture gave final and direct evidence of this fact.

In the last series of experiments, concluding this section, we investigated the bone marrow in 8 unanesthetized cats before injection, and 4-5 hours after injection, of emulsion of BCG culture into muscles of the femur of the hind limb. The BCG culture was injected in the deafferentated limb in two of the animals. In the experimental animals we investigated fluctuations in the number of leucocytes and changes in the leucocyte formula in the peripheral blood, taken from the ear. At the start and at the end of the experiment (i. e. in 4-5 hours) the bone marrow was extracted with a thick needle from the tibia, alternately from various limbs. The number of nucleated cells in one mm<sup>3</sup> was calculated and the myelogram of the bone marrow determined.

As is shown by Table 1, 4-5 hours after injection of the emulsion of the BCG culture in the muscles of the femur of the hind limb of the cat, in the peripheral blood, taken from the ear, an increased number of leucocytes was noted. As was shown earlier and also in our experiments this increase in the number of leucocytes takes place chiefly on account of the neutrophils.

In the bone marrow of the experimental animals (see Table 1) we observed an increase in the number of myeloblasts and in the majority of experiments, of promyelocytes. In the greater part of the investigations the number of myelocytes rose. The content of the rod-nuclear and segment-nuclear neutrophils remained unchanged or even relatively declined in relation to the original figure. A similar picture of changes in the myelogram may be described as a "shift to the left", i. e. rejuvenation of the bone marrow. The changes established testify to stimulation of the myeloid series of the bone marrow. The changes on the part of the erythrocytic series in the majority of experiments were insignificant.

TABLE 2

Changes in Myelogram After Introduction of BCG Culture in Deafferentated Limb of Cat  
(July 13, 1954)

Blood cells	Animal				Blood cells	Animal			
	№ 1		№ 2			№ 1		№ 2	
	before injection	4 hours after in- jection	before injection	4 hours after in- jection		before injection	4 hours after in- jection	before injection	4 hours after in- jection
Myeloblasts	5.2	5.6	5.6	5	Eosinophils	4	6.8	2.8	4.2
Promyelocytes	8.2	7.2	10.4	7.2	Proerythro- blasts	3.4	3.4	2.8	2
Myelocytic neutrophils	4	3.8	2.6	2.8	Erythroblasts I	1.6	4.6	2.4	1.8
Myelocytic eosinophils	3.2	2.2	2.8	2	Erythroblasts II	4.4	4.4	2.2	4.6
Myelocytic basophils	1.4	2.2	0.6	0.4	Erythroblasts III	7.2	9	7.8	9.6
Metamyeloe- cytic neutro- phils	24.4	19	17.8	15.2	Plasmatic cells	2.4	1.6	3.2	3
Metamyelo- cytic eosino- phils	1.4	3	2.2	2.8	Megacaryocytes	0.4	0.2	0	0.2
Neutrophils - rod nuclear	24.8	21.6	34.6	35.2	Hematohistiob- lasts	0.6	1.8	0.4	1.2
Neutrophils - segment nuclear	1.2	1.6	0.8	1.4	Reticulo-endo- thelium cells	1	1	0.2	0.8
Lymphocytes	0.2	0.4	0.2	0.2	Total number of nucleated elements	180 000	186 000	168 500	177 500
Monocytes	0.2	0.6	0.2	0.2	Content of leu- cocytes in vessels of ear	17 250	15 750	23 600	20 600
Basophils	0.8	0	0.4	0.2					

Thus, injection of BCG culture leads to intensified removal of mature neutrophils from the bone marrow to the peripheral blood and also to stimulation of its myeloid component (which was expressed in our experiments in intensified myelopoiesis).

Our findings (Table 2) were obtained in investigation of the bone marrow in the cats after introduction of emulsion of BCG culture in the deafferentated limb (in these experiments the bone marrow was taken from the limbs with maintained innervation).

As is clear from Table 2 the introduction of the BCG culture in the deafferentated limb of the cat does not produce a reaction on the part of the bone marrow, as was observed after BCG injection in a healthy limb. As has already been pointed out, in this event in the peripheral blood no leucocytosis, characteristic of the reaction to BCG was observed.

The results of the investigations suggest the conclusion that intramuscular injection of a BCG culture leads to a reflex increase in the number of leucocytes in the peripheral blood. It was established that the observed first phase of the leucocytic reaction depends on redistribution of the formal elements — transfer of them from the vascular regions of the internal organs to the peripheral blood stream. In the second phase of the reaction there occurs a genuine increase in the number of leucocytes in all vascular regions of the body as a result of activation of blood formation in the bone marrow.

The reflex nature of the reaction of the bonemarrow and leucocytes of the peripheral blood is borne out by experiments undertaken in conditions of Nembutal narcosis, and also by observations on the animals subjected to the operation to remove the afferent nerves of the limb.

#### LITERATURE CITED

- [1] E. L. Kan, Material for Study of the Influence of the Nervous System on Blood Composition, author's abstract dissertation\* (Leningrad, 1953).
- [2] K. G. Malysheva, Mechanisms of Pathological Reactions,\* (Leningrad, 1950), Nos. 16-20, pp. 128-137.
- [3] M. D. Shkolnikova, Byull. Eksptl. Biol. i Med. 1952, No. 12, pp. 24-26.
- [4] M. D. Shkolnikova, Byull. Eksptl. Biol. i Med. 1953, No. 6, pp. 22-24.
- [5] M. D. Shkolnikova, Byull. Eksptl. Biol. i Med. 1956, No. 2, pp. 33-36.

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\*In Russian.