

# EFFECT OF SMALL DOSES OF CAFFEINE ON THE DEVELOPMENT OF EXPERIMENTAL TUBERCULOSIS IN WHITE MICE

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As shown in our previous investigation [1], excessive excitation of the central nervous system unfavorably affects the development of experimental tuberculosis. In the present work we studied the effect of moderate excitation of the central nervous system on the development of the indicated pathological process.

Such a condition can be produced with the help of caffeine injections. Prolonged administration of small doses of caffeine, V. K. Fedorov points out, leads to increased excitability of the cerebral cortex and, through induction, to an increase in internal inhibition and an increase in the mobility of the nerve processes [V. K. Fedorov, 2].

We studied the effect of small doses of caffeine on the course of experimental tuberculosis in 207 white mice weighing from 17 to 20 g. Caffeine (0.05 mg for each mouse) was administered to part of them (89); subcutaneously daily for 7 days prior to infection; caffeine was not administered to 86 mice, which composed the control group. After 7 days, the experimental and control mice were infected intravenously with a culture of tuberculosis bacteria (strain 109 of the bovine type) in a dose of 0.015 mg in 0.25 ml of physiological solution. Daily caffeine injections of the experimental mice were continued until the 41st day from the time of infection. Then caffeine administration was discontinued for 33 mice of the experimental group, since in the above-mentioned work of V. K. Fedorov it was established that the effect of small doses of caffeine, administered for 40-45 days, remains during the subsequent 20 days.

Out of 34 which went into the second control group, 4 were killed before the beginning of the experiment as a control over the original state of the internal organs. The effect of prolonged use of caffeine on the internal organs was studied on the remaining 28 uninfected mice. Experimental and control mice were killed during the course of the experiment, on the 6th, 18th, 24th, 30th, 41st, 50th and 60th day after infection, with 12-15 animals in each group. The mice were weighed before autopsy. The lungs (together with the heart) and the spleen were weighed and the coefficient of the weight of the organs was determined. At autopsy, the pathological anatomical changes were found, evident as the appearance of greyish-yellow nodes in the lungs and as an increase in the size of the spleen. Visible changes were not observed in the other internal organs.

Microscopic appraisal of the tuberculous changes in the lungs was made on the basis of a count of the number and of a determination of the size of the tubercles.

# EXPERIMENTAL RESULTS

Beginning on the 18th day after infection (macroscopically determinable changes were not evident at earlier periods) a difference was found in the number of tubercles formed in the lungs of animals in the control group. (Table 1). Thus, on the 24th day after infection, a considerable number of tubercles was found in 10 out of 13 control animals. At the same time, only in two cases out of 13 was a great number of tubercles found among the experimental animals. On the 24th, 41st and 60th day after infection, tubercles in greater numbers and of a greater size were found in the lungs of the majority of mice of the control group, while at the same times after infection, tuberculous changes in the lungs of the experimental mice were found in the form of single tubercles, and only in a few mice was a large number of tubercles found ("Table 1").

During the period from the 41st to the 60th day of illness, when the caffeine infections were stopped, a large number of tubercles was discovered in the lungs of part of the experimental mice: in 5 case out of 15 by the 50th day, in 4 animals out of 18 on the 60th day after infection. At those same times, pathological anatomical changes in the lungs of the control animals were incomparably more evident.

TABLE 1

Number of Tubercles in the Lungs of White Mice  
(according to autopsy data)

Time of observation (in days)	Control					Experiment				
	number of mice	-	+	++	+++	number of mice	-	+	++	+++
6	1	1	—	—	—	11	11	—	—	—
18	10	5	3	2	—	12	10	2	—	—
24	13	1	2	4	6	13	3	8	2	—
30	10	1	5	3	1	10	2	1	4	3
41	11	—	1	1	9	10	—	6	4	—
50	14	6	5	1	2	15	3	6	1	5
60	17	3	2	3	9	18	1	13	—	4

Symbols: — absence of tubercles, + single tubercles, ++ small number of tubercles, +++ large number of tubercles.

The results of the experiment on all autopsy days were as follows: a large number of tubercles were found in the lungs of 27 out of the control group of 86 mice, single tubercles were found in 18 cases. Single tubercles were observed in the lungs of 36 mice out of 89 experimental animals. A large number of tubercles was observed in only 12 observations (Fig. 1).

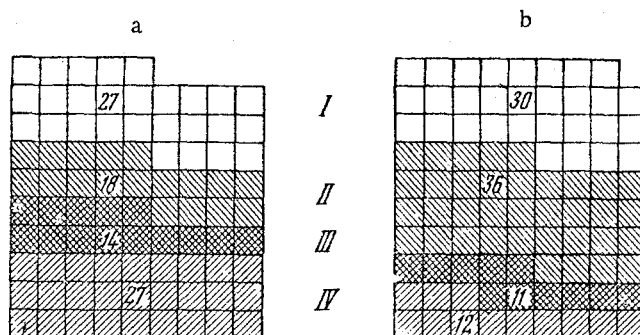


Fig. 1. Number of tubercles in the lungs of mice of the control (a) and experimental (b) groups.

I) absence of tubercles, II) single tubercles, III) average number and IV) very many tubercles.

A difference in the weight increase of the lungs and spleen was also observed in mice of both groups. Thus, the average weight of the lungs of the control mice by the 60th day of illness was 833.2 mg, of the spleen - 713 mg; by the same time the average weight of the lungs of the experimental mice was 622.5 mg, of the spleen - 322.5 mg.

The weight of the lungs of healthy mice which received caffeine for a long time did not change substantially, varying between 530 and 584.5 mg on the average, the weight of the spleen was not more than 240 mg. The coefficient of the weight of the organ was taken as the indicator of the weight of the organs of animals in the control and experimental groups. It was established that the weight coefficients of the lungs and spleen of mice in the experimental and control groups changed in the same way as the absolute weight of these organs (Table 2).

TABLE 2

Combined Weight Data in Experiments Studying the Dynamics of the Tuberculosis Process in Mice

Experimental Group	Average Weight			Average Coefficient	
	of the body ( in g)	of the lungs (in mg)	of the spleen (in mg)	of the weight of the lungs	of the weight of the spleen
Healthy mice:					
before the experiment	23.2	635	350	24.8	15.3
receiving caffeine 6 times	22.4	530	240	23.8	8.8
After infection:					
6 days after					
control .....	23.8	461.6	238	20.1	9.6
experiment .....	23	525	211	22.2	8.9
18 days after					
control .....	22.3	620.9	467.5	27.9	20.9
experiment (caffeine 21 times)	23	551.2	355	23.7	15.4
healthy mice at this time	27	616.5	370	22.6	13.6
24 days after					
control .....	20.9	603.7	509.3	28.8	24.3
experiment (caffeine 24 times)	22.8	576.8	394.3	25.2	17.7
healthy mice at this time	23.8	570	200	23.9	8.3
30 days after					
control .....	21.5	608	472	28.2	21.9
experiment (caffeine 32 times)	23.6	584	322	24.7	13.2
healthy mice at this time	-	-	-	-	-
41 days after					
control .....	21.6	705	511	31.2	22.6
experiment (caffeine 40 times)	25.5	601	244	23.5	9.5
healthy mice at this time	29	566	209	19.5	7.2
50 days after					
control .....	27.2	542.6	324.6	19.9	11.9
experiment (caffeine not administered)	25.5	611.1	454.7	23.9	17.8
60 days after					
control .....	25.1	833.2	713.3	33.1	28.4
experiment (caffeine not administered)	25.1	622.5	322.5	24.7	12.8

From the data of Table 2 it is evident that the weight of the lungs, spleen, and also the weight coefficients were higher in animals of the control group.

Thus, a difference in the degree of affection with tuberculosis of mice of the control and experimental groups was established. The number and size of the tubercles in the lungs, the absolute weight of the lungs and spleen, the coefficients of the weights of these organs were considerably larger in the control animals which did not receive caffeine than in the experimental ones.

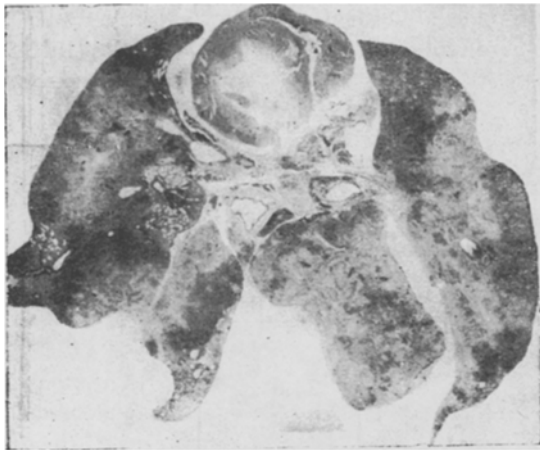


Fig. 2. Histotopogram. Total affection of the lungs.  
Mouse No. 611-control. Magnification 5x.



Fig. 3. Histotopogram. Merged tubercles, surrounded by airy lung tissue.  
Mouse No. 589 - experiment. Magnification 5x.

The lungs of animals of all three groups were taken for histological examination (including the lungs of healthy mice, receiving caffeine); sections were stained with hematoxylin-eosin, Sudan III (for fat) and were stained for tubercle bacilli. Part of the sections were made with the freezing microtome; some organs were first imbedded in paraffin.

From the 6th day of illness until the 60th day, morphological nonspecific changes in the form of lymphoid infiltrates, surrounding the bronchi and blood vessels with a broad band were observed in the lungs of all the mice. The tubercles visible at autopsy in the lungs of the control and experimental mice represented areas of catarrhal-desquamating pneumonia histologically. The alveoli in the affected part of the lung contained exudate, consisting primarily of alveolar macrophages - large cells with foamy protoplasm, an eccentrically located nucleus and fatty inclusions in the protoplasm. In addition to the alveolar macrophages, lymphoid cells and polymorphonuclear leucocytes were found in the exudate. Such tubercles were enclosed at the beginning of the illness, but as the tuberculous process developed, they merged, forming massive diseased areas which contained tubercle bacilli, the latter were either located inside the macrophages or lay free. The sizes of the tubercles in the control mice, according to microscopic data, were considerably larger by comparison with the same measurements in the lungs of the experimental animals. Merged tubercles were most often seen in mice which had not had caffeine administered. They appeared earlier than in the experimental animals. On the 60th day after infection the lung parenchyma of 10 animals out of 17 in the control group was affected throughout, with the exception of a few alveoli free from exudate (Fig. 2). In 4 cases, wide areas of necrosis were observed, the affected parenchyma had the appearance of a homogeneous structureless pink mass with an accumulation of nuclear powder. Merged tubercles were observed by the 60th day of illness in only 4 cases out of 18 experimental mice. However, in distinction to what was observed in the control animals, in these cases considerable areas of airy pulmonary tissue occurred around the merged tubercles. Evident necrosis

was not observed in the affected part of the lungs of the experimental mice (Fig. 3).

Microscopic examination of the lungs of healthy mice which received caffeine showed the presence of pulmonary tissue with unchanged structure in the majority of cases. The alveoli were free, the alveolar walls thin. In part of the cases peribronchial and perivascular lymphoid infiltrations, thickening of the alveolar walls (due to proliferation of the histiocytic cells) were observed. Also observed were areas of emphysema; the bronchi were distended, the bronchial mucosa was smoothed, the alveolar walls thinned and torn.

Evaluating the changes observed quantitatively, on the basis of macro- and microscopy, it can be noted that the number and sizes of the tubercles, the weight of the lungs, spleen and coefficients, of weight and of these organs were greater in mice of the control group. Tubercles of average sizes and of a coalescent nature were found more frequently in this same group of animals. Coalesced tubercles appeared considerably later in mice which has been administered caffeine a long time; necrotic affection of the lungs of the experimental mice was absent. Summarizing the above results of observations, we can note that the prolonged administration of small doses of caffeine to white mice infected with tuberculosis leads to a distinct mitigation of the development of the specific process. Consequently, moderate reinforcement of the processes of excitation and internal inhibition, as well as an increase in the mobility of the nerve processes, has a positive effect on the state of natural immunity to tuberculous infection. It is also possible to reach the conclusion from these data that changes in the functional state of the central nervous system have a considerable effect on the development of experimental tuberculosis.

### SUMMARY

The action of moderate stimulation of excitatory process in the central nervous system upon development of experimental tuberculosis was studied.

Mice infected with tuberculosis received caffeine during 7 days before and 41 days after infection (0.05 mg subcutaneously).

Macro- and microscopic research has shown that the number and size of tubercles, weight of lungs and spleen and weight coefficients of these organs are larger in mice not injected with caffeine. These mice also revealed some necrotic lesions in lungs which were not observed in mice injected.

Thus repeated injections of caffeine increases resistance of animals against experimental tuberculosis and leads to attenuation of the specific process.

### LITERATURE CITED

[1] K. A. Poletaeva, in the book: Study of the Role of the Nervous System in the Pathogenesis and Therapy of Tuberculosis, \*Medgiz, 1954, pp. 270-280.

[2] V. K. Fedorov, Farmakologiya, 1952, No. 1, pp. 7-13.

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\* In Russian.