EXPERIMENTS ON THE RÔLE OF LYMPHOID TISSUE IN THE RESISTANCE TO EXPERIMENTAL TUBERCULOSIS IN MICE.

III. Effect of Heat on Resistance to Tuberculosis.

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In a previous report from this laboratory a series of experiments was recorded which seemed to explain the results of Lewis and Margot,[1] who noted that splenectomized mice had a greater resistance to tuberculosis than normal mice. Murphy and Ellis[2] demonstrated the fact that splenectomized mice, if exposed to suitable doses of x-rays no longer had an increased resistance, but were hypersusceptible to the infection. The interpretation of these results suggested, was that the increased resistance in splenectomized mice was due to the increase in the circulating lymphocytes, which was demonstrated to reach its height about 21 days after removal of the spleen. When the increase in the lymphocytes was prevented by x-rays, the increased resistance to tuberculosis was nullified. It was likewise shown that intact animals could be rendered less resistant to tuberculosis than controls by the use of broken doses of x-rays. The latter observation was confirmed by Morton[3] for the human strain of the organism with guinea pigs. Later Taylor and Murphy[4] showed that mice with a marked increase in the lymphocytes resulting from cancer inoculation in cancer-immune animals had also a marked increase in their resistance to tuberculosis. In this instance also if the increase in lymphocytes was de-

[1] Lewis, P. A., and Margot, A. G., *J. Exp. Med.*, 1914, xix, 187.

[2] Murphy, Jas. B., and Ellis, A. W. M., *J. Exp. Med.*, 1914, xx, 397.

[3] Morton, J. J., *J. Exp. Med.*, 1916, xxiv, 419.

[4] Taylor, H. D., and Murphy, Jas. B., *J. Exp. Med.*, 1917, xxv, 609.

stroyed by x-rays, the animals were rendered highly susceptible to infection.

We regarded these results, coupled with the deductions from observations of the blood count in men with this disease, and the histology of the lesion, as strong direct evidence that the lymphocytes play an important part in the resistance of the animal to tuberculosis. With the development of a new method of stimulating the lymphocytes,[5] namely that of intense dry heat, we have another opportunity to test this conception.

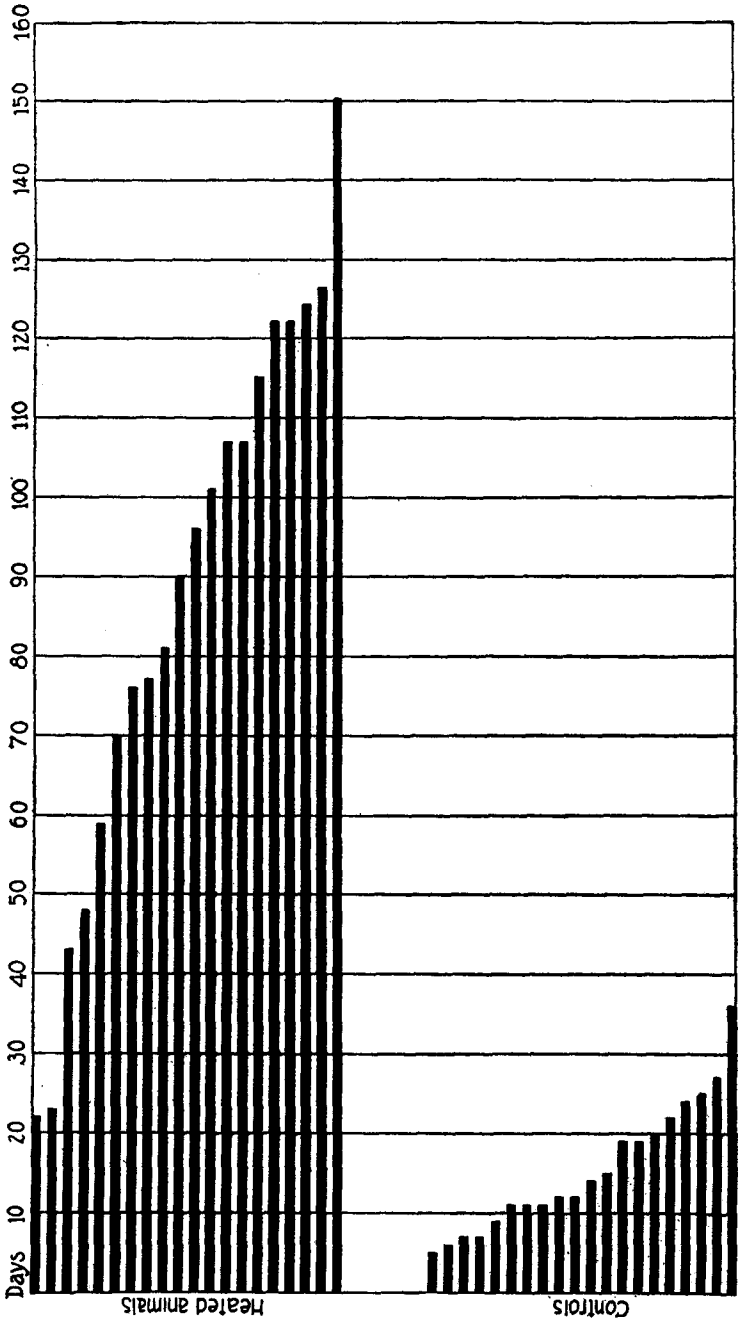
Method.

Mice subjected to dry heat ranging from 55–65°C. over a period of 5 minutes first show a fall in the total white blood count, both the polymorphonuclear cells and, to a lesser extent, the lymphocytes participating in the fall. The lymphocytes, however, show an immediate rebound, followed by a rapid increase which carries the count several hundred per cent above the normal. This rise continues for 14 to 21 days, after which there is a gradual return to the normal level. The polymorphonuclear leucocytes recover slowly and show no stimulation phase. This method gives us an excellent opportunity to test the effect of a marked increase in the lymphocytes on the course of tuberculous infections.

Experiment 1.—Forty mice of the same stock and of about the same age and size were selected for this experiment. Twenty were heated over an electric heat lamp for 5 minutes at a temperature starting at 55°C. and allowed to increase to 65°C. 1 week later these animals, together with the twenty controls, were each inoculated intraperitoneally with 2 mg. of a bovine strain of tubercle bacilli (4 week culture) suspended in 0.5 cc. of normal salt solution. The mice were then placed in individual jars in order to prevent the occurrence or spread of an epidemic. All the mice as they died were carefully autopsied, and films were taken from the peritoneal fluid, and from liver, kidney, lungs, and heart's blood. It was found that all the animals had widely disseminated tuberculosis, which could definitely be accepted as the cause of death.

The control animals died rapidly, the first one on the 6th day, and the last on the 36th day after inoculation. The average number of days of life for this group was approximately 16 days. None of the heated animals died until the

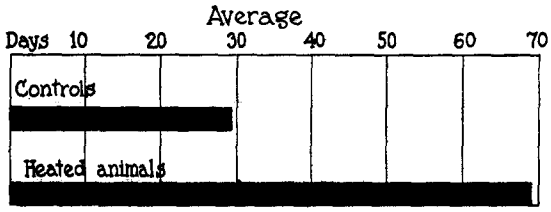
[5] Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, xxix, 1.



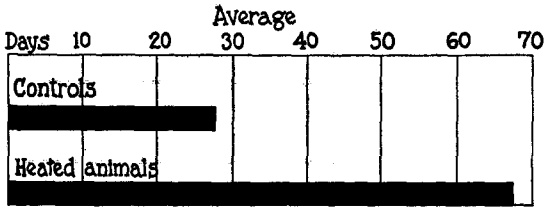
TEXT-FIG. 1. Each horizontal line represents the time of survival of a mouse after inoculation with bovine tubercle bacilli. The first group was subjected to an exposure of dry heat 1 week before inoculation. The second group was untreated.

22nd day after inoculation, and the longest period of life in this group was 150 days, the average length of life for the twenty mice being approximately 88 days. The rate at which the animals died is shown in Text-fig. 1.

Experiment 2.—Twelve healthy mice of about the same age and weight were heated in the same manner as in the previous experiment. A week later these, with twelve control mice from the same stock, were inoculated intraperitoneally with 2 mg. of a bovine strain of the tubercle bacillus suspended in 0.5 cc. of normal salt solution. The animals were segregated in individual jars as in the previous experiment. Autopsies were performed promptly after death and films taken from the principal organs, the peritoneal fluid, and the heart's blood.



TEXT-FIG. 2. The average duration of life of heated mice after inoculation with bovine tubercle bacilli compared with the duration of life of untreated animals. There were twelve mice in each group.



TEXT-FIG. 3. The average duration of life of heated mice after inoculation with bovine tubercle bacilli compared with the duration of life of untreated animals. There were twelve heated mice and fourteen controls.

All these mice showed extensive tuberculosis except one control which lived 89 days, and which was either a highly resistant animal or was not infected because of some accident in the inoculation. In this series the heated animals averaged 69 days of life after inoculation, while the controls averaged only 29 days. The details of this experiment are shown in Text-fig. 2.

Experiment 3.—Twenty-six mice of the same strain and about the same age were selected for the experiment. Twelve were subjected to direct heat ranging from 55–65°C. for 5 minutes. A week later each of the twenty-six animals was inoculated intraperitoneally with 2 mg. of a culture of the bovine tubercle bacillus suspended in 0.5 cc. of normal salt solution. They were segregated in separate

jars as in preceding experiments. Autopsies were performed as the animals died to verify the cause of death. In all, except one of the control animals, there was widely disseminated tuberculosis. In this animal, which lived 117 days, there was no evidence of the disease.

In this experiment the heated animals averaged 67.6 days of life after inoculation, while the controls lived only 27.8 days. The rate at which the mice died is shown in Text-fig. 3. In the last two experiments the same culture of the tubercle bacillus was used for inoculation.

The three experiments described show that animals subjected to one exposure of dry heat and inoculated a week later with a bovine strain of the tubercle bacillus virulent for mice had a greatly enhanced resistance to the organism as compared with that of the untreated animals. The difference in resistance here is much more striking than that seen in splenectomized animals or in the cancer-immune animals.

DISCUSSION.

The impression is gathered from the literature that individuals recovering from tuberculosis develop an increase in the number of circulating lymphocytes, although the point is rarely emphasized. In an analysis of a number of blood counts on rapidly advanced cases of tuberculosis in man a marked decrease in this type of cell is observed. The fact that between these two characteristic findings there is every variation in the type and degree of reaction, with often practically normal blood counts, has led to skepticism in regard to the significance of the blood changes in tuberculosis. In dealing with such a slow, chronic infection, the changes would not be expected in a marked degree except in the two extremes mentioned above. The blood changes, together with the fact that the tuberculous lesion is characterized by an accumulation of large numbers of lymphocytes, are suggestive of the part played by this cell in resistance to tuberculosis. To this evidence we add the results of our experiments; namely, the lowering of resistance to tuberculosis in animals depleted of their lymphocytes by means of x-rays, and the increased resistance in animals with the lymphocytes increased by three widely different methods, splenectomy, cancer immunity, and dry heat.

The chief points which may be brought against this conception is that tuberculosis frequently involves the lymphoid structures, and

they seem, if anything, more susceptible to the infection than many other tissues. The explanation of this fact is not clear, but we do not consider it an overwhelming argument in the face of the mass of evidence which indicates that the lymphocyte is an important factor in the resistance to the tubercle bacillus.

SUMMARY.

Mice with high lymphocyte counts and increased activity of the lymphoid tissue induced by one exposure to intense dry heat exhibit a marked increase in the resistance to large doses of bovine tubercle bacilli as compared with that shown by control animals given a similar inoculation. This resistance, judged by the time of survival after inoculation, is increased from two- to threefold. The average length of life after inoculation for three groups of heated mice was 88, 69, and 67 days respectively, while the control groups averaged 16, 29, and 28 days respectively.