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## STUDIES WITH ARTIFICIAL FEVER IN EXPERIMENTAL TUBERCULOSIS<sup>1</sup>

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During the past three years studies of the effects of artificial fever on the cellular equilibria in experimental animals have been conducted in the laboratories of the Department of Medical and Surgical Research, Ohio State University, with a high frequency, short wave radio-therm constructed and serviced by Professors Dreese and Byrne of the Department of Electrical Engineering.

That artificially induced fever effects beneficially certain diseased states in man has been rather clearly and definitely demonstrated in recent years. The present task of those interested in this approach toward a better mobilization of the immune forces of the body is to reveal the mechanism or mechanisms by which this desired end is accomplished and to differentiate those conditions in which such therapy may be contraindicated.

There are three avenues by which fever might prove beneficial in combatting infectious disease: (1) by the actual destruction or attenuation of the infecting agent, the thermal death point for the pathogenic bacteria being lower than that for the host cells; (2) by enhancing the development of humoral antibodies; or (3) by stimulating a more effective mobilization of the phagocytic cells of the blood and tissues; or, of course, any combination of these three effects may be hypothesized.

Among those infections mentioned as possibly susceptible to the beneficial results of hyperpyrexia has been tuberculosis. Duncan and Mariette (1) reported the attenuation in vitro of acid fast strains of organisms at human fever temperatures.

<sup>1</sup>Read before the Ohio Academy of Science, April 18, 1935.

Professor W. A. Sterin, of the Department of Bacteriology, however, who has carried on similar studies in co-operation with our group has been unable to demonstrate either lethal or virulence altering effects of fever temperatures upon any of the acid fast group of pathogenic organisms studied under in vitro conditions.

The present report deals with certain observations made in rabbits experimentally infected with virulent tubercle bacilli and subjected to repeated "feverings." Two kilogram rabbits were selected and inoculated with a thirty-day culture of Bovine B-1, undissociated acid fast organisms. Baseline observations for a period of at least two weeks were made on all animals prior to inoculation. Total white cells, supravital differentials, total red cells, and hemoglobin were determined and weights and temperatures were recorded six times during this period. Chest X-rays were made of all animals, and the animals selected for fevering were subjected to a preliminary heating in the radiotherm before inoculation.

A single fevering, in this experiment, consisted of placing one animal at a time, in the radiotherm, and exposing it to a radio frequency of four thermo-ameters, 25 meter wave length, 12,000,000 oscillations per second, for approximately twenty minutes, during which period the rectal temperature rose from 102° to 107°-108° F. This temperature was then maintained by a continuous flow of from 1-2 thermo-ameters, for a period of one hour. At the end of this time the animal was removed from the set and its temperature allowed to return to normal under room conditions. Temperature changes in the animal were followed at intervals of ten minutes while in the radiotherm and every thirty minutes thereafter until the temperature had returned to normal. Blood counts and weights were taken before the rabbit was placed in the set, at the end of one hour of fevering and again when the temperature had returned to normal. The animals selected for fevering were subjected to this elevation of temperature twice a week until death intervened or the observations were terminated. X-rays of the chests of all animals were taken every twenty days. Blood, temperature, and weight changes were followed throughout the experiment, on all animals, at intervals comparable to those established during the base line period.

In the first experiment, fourteen animals were selected for observation. Seven of this group were injected with 0.1 milli-

gram of the Bovine culture, intravenously. Six others received 0.2 milligrams intratracheally. One animal was not inoculated, and served as a normal fever control.

Four animals of the first group and three of the second group were selected for fevering. Fever therapy was begun twenty days after the inoculations. The chest X-rays, taken at this time, showed definite pulmonary lesions. The blood picture also was typical of tuberculous disease (2, 3). The animals injected intravenously survived only one or at most two feverings; the disease process was so acute and the pulmonary congestion so great, that the vital capacities in these rabbits were insufficient to support the added cardiac and respiratory demand during fever treatment. Tuberculous controls inoculated at the same time but not subjected to fevering were killed at the time the others died. At autopsy, all animals showed gross lesions in practically all organs of the body. Gross hemorrhages were found in all of the fevered animals and in but one of the controls. In this group, the amount of the fever therapy was of necessity so limited by the acuteness of the tuberculous process which developed, that it was impossible to draw any conclusions. There is no question but that animals in such a serious and far advanced condition cannot survive an elevation of temperature to the degree mentioned (107–108° F.).

Of the group injected intratracheally, one animal survived three feverings, another eight, and the third nine. The non-fevered controls all outlived the fevered group and were killed by air embolism for comparative autopsy study.

The blood studies showed a definite reversal of the M–L ratio (4, 5) in the fevered animals, in contrast to the non-fevered controls. At postmortem, the dissemination of organisms appeared greater in the fevered group, as indicated by the extent of gross lesions. All fevered animals showed definite gross hemorrhages particularly in the thymus. The normal animal was fevered nine times, and showed no significant cellular changes in the peripheral blood. The weight and temperature of the normal animal remained within normal limits. This animal was not killed at this time but was fevered repeatedly throughout the subsequent experiment as a control.

The above observations indicated the necessity for further study on animals with a less fulminant tuberculosis.

For the second experiment, six animals were selected. Baseline observations were made as indicated above. All of

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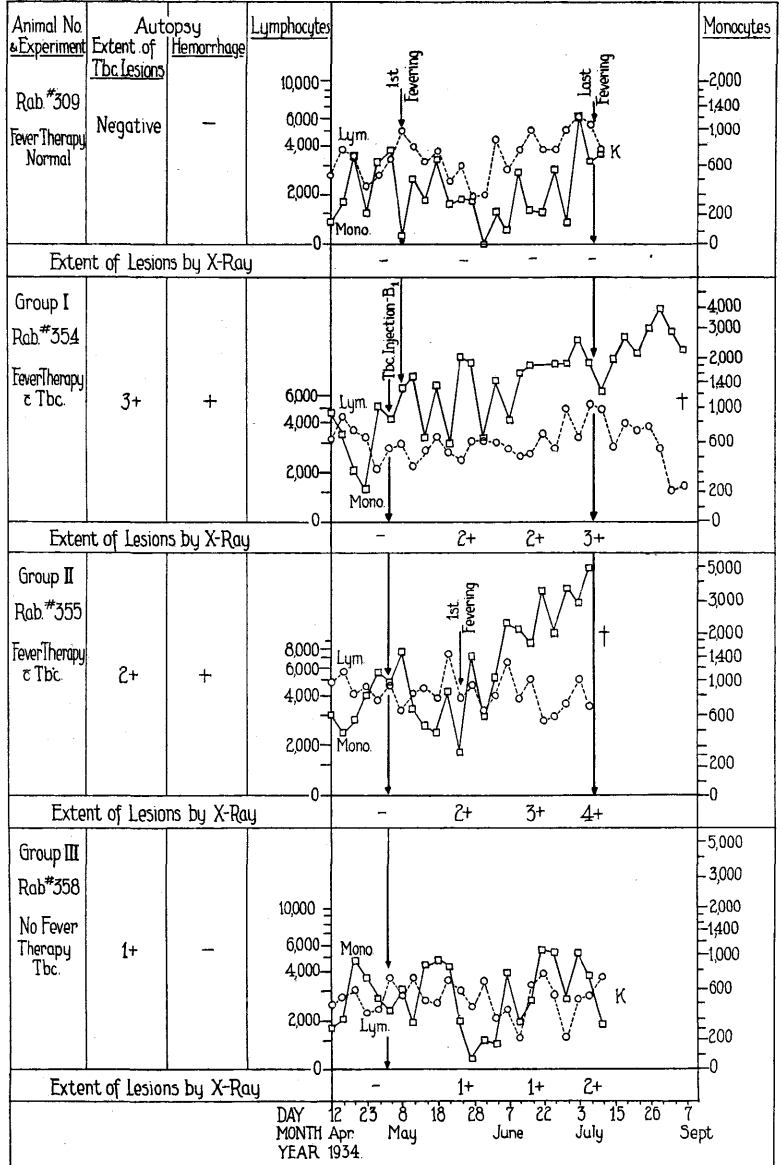


CHART I

these animals were inoculated intratracheally with one-fiftieth milligram of the Bovine B-1 Culture. Two of this group were fevered, beginning three days after inoculation; two others were started twenty days later, and a third group of two was held as tuberculous controls without artificial fever. The normal animal was fevered with this group. Fever was induced two times a week. The animals in Group I were fevered a total of seventeen times. The animals in Group II started twenty days after injection and were fevered twelve times. The animals in Group III received no fever therapy. The normal control was fevered seventeen times in this experiment, a total of twenty-six times during the two experiments.

The chest X-rays were negative on all of the animals before inoculations were made. The extent of the lesions after twenty days was estimated two plus in Groups I and II, and one plus in Group III. Forty days after inoculation, Group I manifested evidence of two plus lesions, Group II three plus lesions, and Group III only one plus. Sixty days after the inoculation, Group I showed three plus lesions, Group II four plus lesions and Group III two plus lesions. X-rays on the normal control were uniformly negative.

One animal from each group, and the normal control were killed at the end of this period of nine weeks. At autopsy, the dissemination of organisms was estimated by the extent and the gross distribution of tuberculous lesions in various organs. The first group showed a three plus dissemination. Group II showed a two plus, and Group III only one plus; that is, tubercles were found only in the lung. Pin point hemorrhages were observed in the thymus glands of the animals in Groups I and II. There were no remarkable temperature or weight changes during this period, in any of the animals. (Slight weight decrease in three out of four fevered animals.) The changes in monocytes and lymphocytes, however, were striking. Chart I shows the relative and absolute changes in the M/L ratio, as found during the course of the experiment in one animal from each of the different groups. With the first induction of artificial fever—regardless of the duration of the disease—a sharp reversal of the M/L ratio in the circulating blood was precipitated. It has been shown (2) and amply confirmed that the M/L ratio in the blood is an accurate index of new tubercle formation in the tissues in tuberculous disease. A persistently rising monocyte curve and a steadily falling

lymphocyte count is of bad prognostic significance. Following the marked vasodilatation and hyperventilation induced by fever therapy an increased dissemination of the disease occurred, reflected most sensitively and accurately by these blood cell changes. The figures from the fevered animals charted on the graphs were taken from the blood counts obtained just before each fevering.

The blood changes occurring in the animals in Groups I and II, kept for longevity studies, continued to reflect an acute tuberculous course, terminating in death, approximately two months after the others were killed. The tuberculous non-fevered control animal survived a longer time with much less change in the blood picture. The degree of dissemination of the disease as indicated by gross lesions, was in the same proportion, in these animals, as in the animals that were killed previously. The microscopic findings were consistent with the gross observations made at the time of autopsy.

The acute blood changes which occurred during the actual fevering were especially interesting from the standpoint of the monocytes and lymphocytes. There was a marked initial depression of circulating cells, both in the tuberculous and in the normal animals during fever. This was followed by a decided monocytic leucocytosis in the tuberculous rabbits only, as the temperature returned to normal. This reaction may be compared to that reported by Geiger (6) upon the injection of colon bacilli into tuberculous as contrasted with normal guinea pigs. The reaction to artificially induced fever, like that to colon bacilli, reveals an excessive tissue storage of monocytes in tuberculous animals, the result of the specific tuberculo-lipoid effect and the tubercle formation (4).

#### CONCLUSIONS

1. Rabbits with primary pulmonary tuberculosis cannot long survive an artificially induced elevation of temperature to 107°-108° F.
2. There is more rapid and extensive lung involvement as shown by X-ray and post-mortem studies in animals that are fevered than in those that are not fevered during the course of the disease.
3. There is a definite increase in dissemination of organisms in fevered rabbits as evidenced by a prompt reversal of the M/L.

index in the blood, by a wider distribution of gross tubercles and by the greater tendency to hemorrhage.

4. Similar elevations of temperatures at repeated intervals in a normal non-tuberculous animal produced no significant alteration in the cellular relationships in the blood or tissues.

5. The blood changes and the physical findings in these experiments suggest a definitely harmful effect of fever therapy in rabbits with primary tuberculosis of bovine type.

6. On the basis of these studies, no human patient with tuberculous disease has knowingly been treated with artificial fever therapy by our group.

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