COURSE OF EXPERIMENTAL TUBERCULOSIS IN ALBINO RATS AFTER PRELIMINARY MUSCULAR TRAINING

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There are many experimental results which show that training may cause profound biochemical and morphological changes in the organism, radically modifying the activity of the central nervous system and perfecting the regulation and coordination of physiological functions in accordance with the work to be performed [3, 7, 8, 12, 14]. It has been found that as a result of training the resistance to a number of unfavorable external environmental factors is increased: hypoxemia [5, 13], toxic substances [2], hyper- and hypothermia [4, 13], ionizing radiation [1, 2, 9, 10], and certain diseases [5, 15, 16].

In the present investigation the study of the physiological mechanism of immunity to tuberculosis was continued. This article describes the findings in respect of the course of experimental tuberculosis in animals subjected to muscular training.

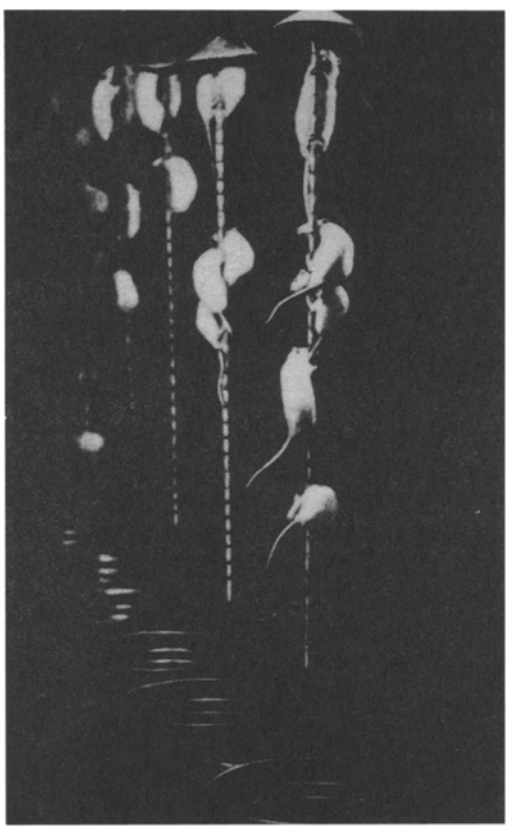

Fig. 1. General view of apparatus used for muscular training.

METHOD

Experiments were carried out on 100 male albino rats, of which 52 received muscular training (Fig. 1) by Shkurdova's method [11]. Immediately after a training period lasting 40 days, the experimental and control rats were tested for their powers of static and dynamic tolerance. This tolerance was defined as the maximal time that the rat could be kept with a load (equal to $\frac{1}{3}$ of its body weight) on a training pole; in the case of the experimental rats it was 14 min 45 sec, and the controls—1 min 10 sec.

At the conclusion of training all the experimental and control rats were inoculated intravenously with a suspension of Mycobacterium tuberculosis, bovine strain No. 109, in a dose of 4.5 mg in 0.5 ml physiological saline. On the 5th, 10th, 20th, 29th, 51st, and 69th days after inoculation the animals were autopsied, 4 from each group. The relative weight of the spleen and lungs was determined. The internal organs were examined with the naked eye and the lungs, liver, and spleen histologically. Pieces of the organs were embedded in paraffin wax and celloidin, and sections were stained with hematoxylin-eosin and for mycobacteria by the Ziehl-Neilson method.

RESULTS

Macroscopic examination of the lungs of the control rats on the 5th day after inoculation showed in every case the presence of numerous submiliary, disseminated, semi-

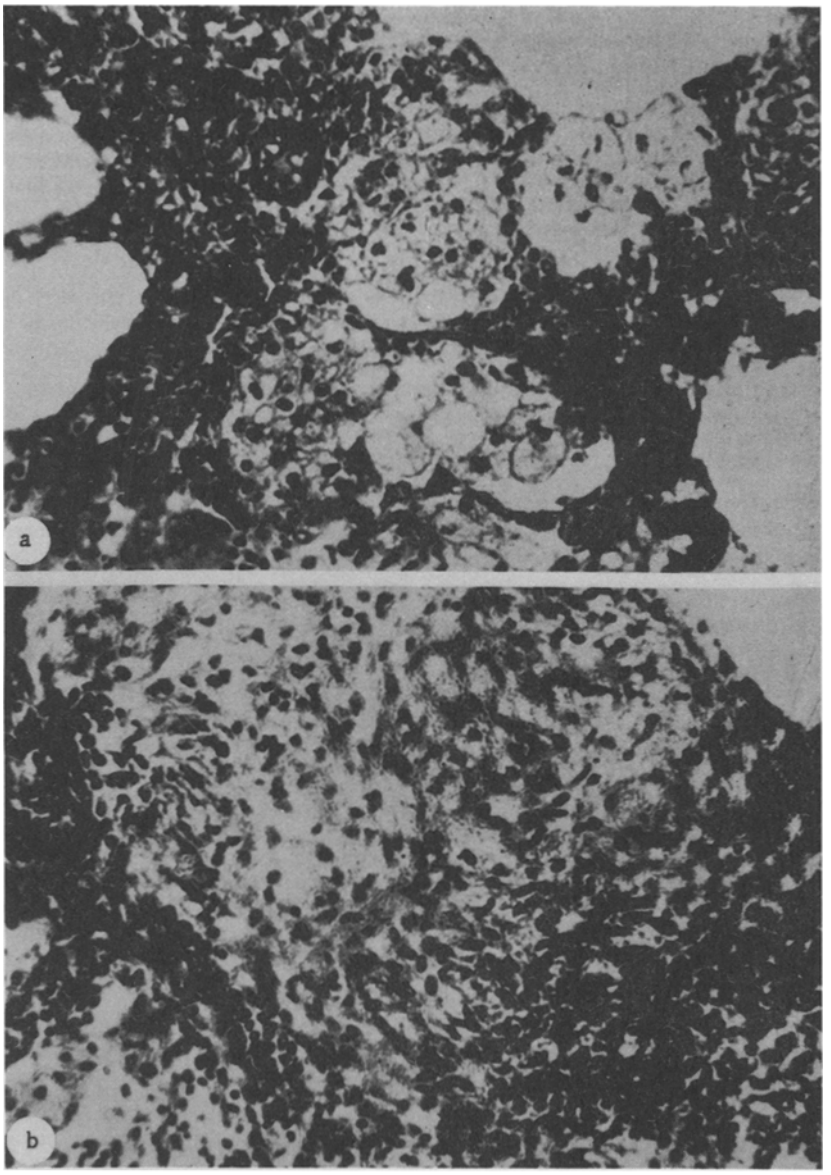

Fig. 2. Lung of a control (a) and experimental (b) rat on the 51st day after inoculation. Control—collection of macrophages in the alveoli; experimental—focus of epithelioid and lymphoid cells. Photomicrograph. Ocular 5, objective 40.

translucent foci, whitish-gray in color. The relative weight of the lungs was 2.03±0.31%. On the 10th, 20th, and 29th days the slightly cyanotic surface of the lungs was almost completely covered by submiliary foci; the relative weight of the lungs was 2.48±0.38, 2.07±0.29, and 1.95±0.08% respectively. On the 51st and 69th days and in the rats which died, confluent foci, gray in color and irregular in shape were found; the mean relative weight of the lungs was 3.34±0.24, 4.1±0.58, and 4.8±0.38% respectively.

Histological examination on the 5th day after inoculation revealed the presence of numerous disseminated perivascular and peribronchial foci of lymphoid cells, macrophages, and epithelioid cells in the lungs of the control rats. Around these focal collections and the interalveolar septa of the lung visible proliferation of histiocytic elements was taking place; in the center of some of the foci were groups of partly disintegrating leukocytes. Small numbers of M. tuberculosis were seen in the foci, both intra- and extracellular in localization, and mainly in the leukocytes. On the 10th, 20th, and 29th days after inoculation a diffuse proliferation of histiocytes was found in the interalveolar septa, accompanied by infiltration of the septa with lymphoid and epithelioid cells, macrophages,

scattered leukocytes, and erythrocytes. In some sections solitary giant, multinucleated cells were found in the peripheral portions of the foci, and edema fluid was present in some areas of the alveoli. On the 29th day of inflammatory foci in the lungs of two rats coalesced to form large foci. M. tuberculosis cells were found in large numbers in the epithelioid cells and histiocytes. On the 51st (Fig. 2) and 69th days after inoculation disseminated foci and large confluent pneumonic foci, bigger than at the earlier periods, were observed; these consisted mainly of macrophages with a large, foamy cytoplasm, sometimes containing inclusions, together with epithelioid cells, lymphoid cells, histiocytes, and occasional giant cells. M. tuberculosis cells were found in large numbers in the foci and the cells of the interalveolar septa. Confluent pneumonic foci were present in the lungs of the rats dying on the 52nd-126th days after inoculation, consisting of epithelioid cells, macrophages, and a few lymphoid cells and erythrocytes. The interalveolar septa were thickened on account of infiltration with macrophages, lymphoid and epithelioid cells, and erythrocytes. Spreading granulation tissue invaded the walls of the bronchi, the submucosa, and the lumen, and in some places granulation tissue invaded the blood vessel walls, and collections of lymphoid cells were seen around the vessels. In the lungs of individual rats collections of leukocytes were seen in the center of the pneumonic foci, some of them disintegrating, together with single giant cells. The number of M. tuberculosis cells was so great that they could be seen under both low and high power of the microscope.

Macroscopic examination of the lungs of the experimental rats on the 5th day after inoculation revealed no changes in 2 cases, and in 1 case single, and in 1 case a few gray submiliary foci were present; the relative weight of the organ was 1.56±0.21%. At later periods a slow progression of the specific changes was observed, and numerous scattered submiliary foci were not found until the 29th day after inoculation; the relative weight of the organ on the 10th, 20th, and 29th days was 1.49±0.05, 1.57±0.17, and 2.12±0.27% respectively. On the 51st and 69th days after inoculation multiple submiliary foci were observed; in a few cases confluent foci were present, and occasionally there were only single submiliary foci. In the rats dying on the 72nd-137th days after inoculation, confluent gray foci were observed on the slightly cyanotic surface of the lungs. The mean relative weight of the lungs at these times was 1.9±0.23, 2.2±0.4, and 6.6±0.51% respectively.

Histological investigation on the 5th day after inoculation revealed no changes in 1 case, single small foci of lymphoid cells and histiocytes, macrophages, and epithelioid cells in 2 cases, and a few scattered foci of the same character in 1 case. M. tuberculosis cells were found in the foci in 2 cases, but were not present in the other 2. On the 10th, 20th, and 29th days very slow progression of the changes was observed, and single or a few scattered foci appeared, consisting of macrophages, lymphoid cells, histiocytes, and epithelioid cells, and not until the 29th day were multiple disseminated foci found in one case. Only a few cells of M. tuberculosis were found, and in some cases there were none. On the 51st and 69th days after inoculation single or a few foci of epithelioid and lymphoid cells were seen (Fig. 2), and in some cases multiple disseminated and confluent foci were present. M. tuberculosis cells were present in small numbers, although in individual cases they were more numerous in the foci and in the cells of the interalveolar septa.

The histological changes in the lungs of the rats dying on the 72nd-137th days after inoculation were of the same character as those in the dying control rats. Large numbers of M. tuberculosis cells were also observed.

No changes could be observed in the liver with the naked eye in the experimental or control groups. Histological examination of the liver of the control rats on the 5th day after inoculation revealed a few scattered small nodules of lymphoid and epithelioid cells, situated perivascularly and in the parenchyma of the organ, accompanied by congestion. Subsequently these changes increased in intensity and they were most marked on the 29th day after inoculation. After the 69th day a decrease in the number of nodules was observed, and in the animals dying on the 52nd-126th days after inoculation only single or a few nodules of lymphoid cells and single epithelioid cells were seen. M. tuberculosis cells were found in much smaller numbers than in the lungs: they were found in the cells of the perivascular collections and nodules, but were completely absent in individual cases.

Histological examination of the liver of the experimental rats on the 5th and 10th days after inoculation revealed numerous disseminated small nodules of lymphoid and epithelioid cells, accompanied by congestion, and in 1 case a few nodules of the same character. The intensity of the changes subsequently diminished appreciably, and after the 51st day single nodules began to be found. The changes in the liver of the rats dying on the 72nd-137th days after inoculation were the same as those in the liver of the dying control rats. M. tuberculosis cells were found in small numbers and not in every case.

Macroscopic examination of the spleen of the control rats revealed no focal changes. From the 5th day after inoculation the mean relative weight of the spleen was increased, and corresponding to the period of investigation its value was 0.72±0.16, 1.66±0.03, and 1.28±0.32% respectively. From the 29th day after inoculation the relative

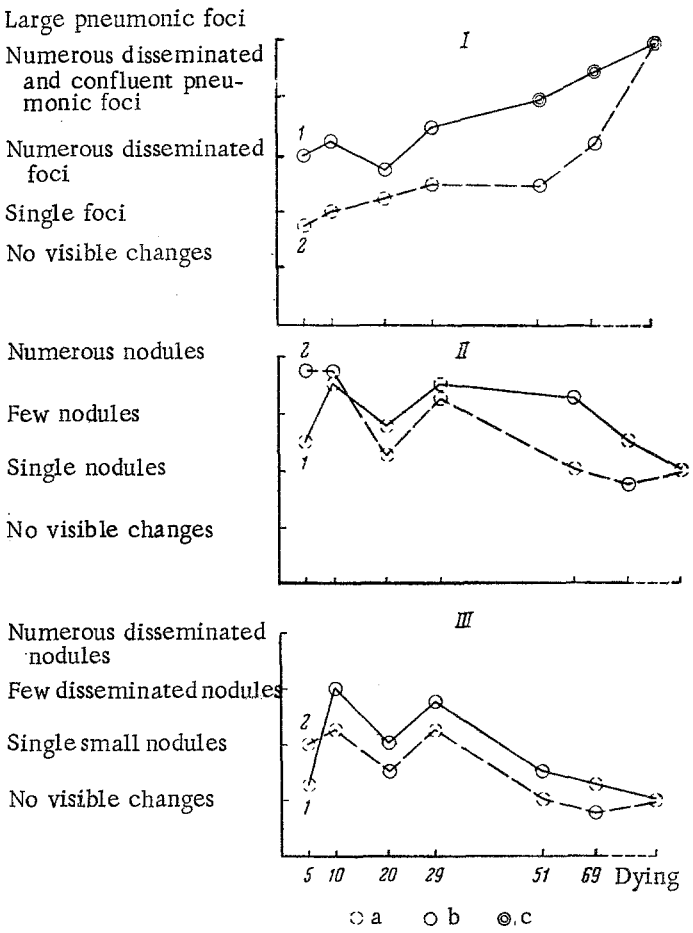

Fig. 3. Dynamics of histological changes and of presence of M. tuberculosis cells in lungs (I), liver (II), and spleen (III) of animals of the control (1) and experimental (2) groups. Along the axis of abscissas—days after inoculation; along the axis of ordinates—severity of histological changes: circles—number of M. tuberculosis cells in sections of organs. a) Single; b) few; c) many.

weight of the organ declined, and its value in the dying rats was 1.03±0.089%. Histological examination on the 5th day after inoculation revealed hyperplasia of the reticulum cells of the pulp and follicles, and the latter contained single, very tiny nodules of epithelioid cells. The intensity of the changes subsequently grew, and they were most marked on the 10th day after inoculation. From the 51st day a reduction in the number of nodules was observed, and on the 69th day only single nodules of epithelioid cells were found in the spleen of the dying rats, together with a sharp decrease in the number of lymphoid cells in the pulp. M. tuberculosis cells were found in small numbers in the epithelioid cells of the nodules at all periods of investigation; on the 69th day and in the sections of the spleens of the dying rats these microorganisms were found only in individual cases.

No macroscopic focal changes likewise could be observed in the spleen of the experimental rats. The mean relative weight was greatest on the 5th day after inoculation (1.11±0.21%) and subsequently its value at different times was 0.88±0.26, 0.74±0.28, 1.08±0.26, 0.58±0.28, and 0.76±0.35% respectively, and in the dying rats 1.06±0.26%. The histological changes in the spleen of the experimental rats were of the same character as those in the spleen of the control animals, but on the 5th day after inoculation they were more marked than in the controls, and subsequently they diminished in intensity. There were no differences in the intensity of the lesions in the dying rats. M. tuberculosis cells were found in small numbers in the nodules at all periods of investigation except the 20th and 69th days, when none were present.

Comparison of the changes in the organs of the experimental and control rats (Fig. 3) shows that by the 5th day after inoculation inflammatory changes in the liver and spleen were more marked in the experimental animals, and in the lungs they were more marked in the controls. Later, however, the disease progressed rapidly in the organs

of the animals of the control group, and from the 52nd day after inoculation the control rats began to die. The course of the tuberculosis in the experimental rats was more sluggish, the extent of spread of the inflammatory changes in the liver and spleen diminished, the volume of the lesion in the lungs increased gradually, and death of the experimental animals began to occur on the 72nd day after inoculation. The survival period of the experimental rats was greater than that of the animals of the control group ($X_c = 74.5 \pm 3.8$, $X_e = 108 \pm 3.9$).

Hence, preliminary muscular training increases the resistance of albino rats to subsequent infection by a virulent strain of M. tuberculosis. The increase in resistance was manifested by a decrease in the severity of the specific changes in animals sacrificed at different periods after inoculation, and also by an increase in the mean survival period of the animals undergoing preliminary muscular training by comparison with those in control animals. The experimental results concur with those of the previous investigation and demonstrate the importance of nonspecific factors in immunity to tuberculosis.

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