STUDY OF THE SORPTIVE PROPERTIES OF SOME ORGANS IN EXPERIMENTAL TUBERCULOSIS, USING THE SUPRAVITAL STAINING METHOD

COMMUNICATION III. SORPTIVE PROPERTIES OF THE SPLEEN AND LUNGS

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We have applied the supravital staining method to the evaluation of the degree of injury suffered by tissue elements at different stages of development of experimental tuberculosis. Parts I and II of this series dealt with changes in the sorptive properties of the cerebral cortex and skeletal muscles of guinea pigs. Our observations confirmed the possibility of applying this method to the detection of early, premorphological changes taking place in the tissue elements of the brain and the muscles of infected animals, as well as to the quantitative comparative assessment of the degree of injury suffered by these organs at different stages of the tuberculous process, and to the study of the specific features of the development of the lesions of the tissue elements in time. As is known, the organs investigated by us are not greatly affected by the specific tuberculous process. It was therefore necessary to extend our investigations to such tissues as the spleen and the lungs, which suffer severe injury as a result of the specific inflammatory lesions of tuberculosis.

EXPERIMENTAL METHODS

We investigated the sorptive properties of the lungs and spleen of guinea pigs, the brain and muscles of which had been studied in our earlier researches.

For determination of the sorptive properties of the lungs, we opened up the thoracic cavity, removed the contents, and placed them on a muslin napkin moistened with Ringer solution. We removed the heart, the pleura, and the esophageal stump. We ligated the trachea, and at the same time attached a weight (a glass sphere) to the preparation, which was then washed free of blood in a stream of Ringer solution. The spleen was similarly freed of surrounding tissues, and washed in Ringer solution. The organs were then immersed for 30 minutes in 0.01% neutral red solution at 20-22°. The stained organs were then rinsed in Ringer solution, any remaining pleural tissue was removed, as well as the lymph glands of the root of the lungs, and the spleen was suspended in a test tube, and the lungs in a large cuvette, containing a fixed volume of acidified 70% alcohol (2% solution of sulfuric acid), for extraction of the dye. Extraction of the sorbed dye was complete within 24 hours.

The determination of the amount of sorbed dye, the weighing of the dry organs, and the statistical treatment of the results of each experiment and of the control series, were done according to the same general procedures for all the experiments (Part I).

We performed 800 determinations in 3 series of experiments (400 for each organ). In the first series the animals were inoculated with a virulent culture of M. tuberculosis, bovine type, in the second series with an attenuated BCG culture, and in the third series with the virulent culture, following previous immunization with BCG culture (two vaccinations).

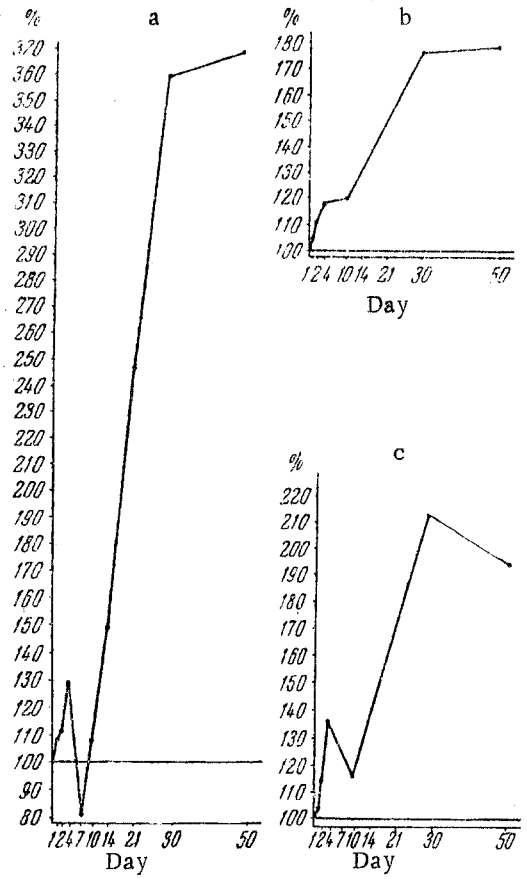

Fig. 1. Curves expressing change in dry weight of guinea pig spleens during the course of experimental tuberculosis.
Ordinates: dry weight of the spleen, taking 100% as the weight found in healthy control animals; a) inoculation of the animals with a virulent bovine type culture of M. tuberculosis, b) inoculation with an attenuated bovine type culture, c) inoculation of previously immunized animals (two vaccinations) with virulent bovine type culture.

The lungs and spleen were examined 1, 2, 4, 7, 10, 14, 21, 30 and 50 days after infection with virulent culture, and 1, 2, 4, 10, 30 and 50 days after infection with attenuated BCG culture, and after infection with virulent culture following BCG vaccination.

Pathological examinations of the viscera of a group of animals, specially set aside for this purpose, were made at the same time intervals, and parallel with the above experiments.

EXPERIMENTAL RESULTS

Solitary tubercles appeared in the lungs 10 days after inoculating guinea pigs with virulent mycobacteria; they consisted mostly of epithelial cells. On the 50th day of infection the lungs of all the animals contained multiple, confluent tubercles, made up of epithelioid cells. Necrosis was evident at the center of many of the tubercles. Solitary epithelioid tubercles made their appearance 30 days after inoculation of virulent culture into previously immunized animals. The number of tubercles was somewhat greater towards the 50th day of infection. At no time were any necrotic changes found in the tubercles of the animals of this series.

Solitary epithelioid tubercles were found in the spleen of guinea pigs inoculated with virulent culture, from the 7th day of infection. By the 30th day the tubercles were multiple and confluent. Solitary epithelioid cell tubercles made their appearance between the 10th and the 30th day of inoculation of previously immunized guinea pigs with virulent culture. Multiple, confluent tubercles were present on the 50th day.

In order to evaluate the results of our study of sorptive properties of the lungs and spleen of the experimental animals we had first to ascertain how the dry weight of these organs varied, bearing in mind the considerable intensification of inflammatory processes with them, as the disease progressed.

Figure 1 shows the changes in dry weight of the spleen at various times (abscissae) after inoculation of the various groups of animals. A steep rise is found in the dry weight of the lungs, and particularly of the spleen, of animals inoculated with virulent cultures, and this rise is most pronounced in the advanced stages of the disease. A considerable rise in the dry weight of the spleen is also evident in the two other series of experiments, being more pronounced when the previously immunized animals were inoculated with virulent culture; the differences in the weight of the lungs are much smaller in these series.

Our results show that changes in dry weight of the organs must be taken into account in evaluating the changes in sorptive properties at certain definite stages of development of the morbid process. For infection with virulent cultures of M. tuberculosis this stage is reached on the 14th day of infection, in the spleen, and on the 21st day for the lungs. Inoculation of nonimmunized animals with BCG culture, and of immunized animals with virulent culture, is followed by change in the dry weight of the spleen from the 30th day on, and by change in the dry weight of the lungs at the same time, but only in animals infected with virulent culture.

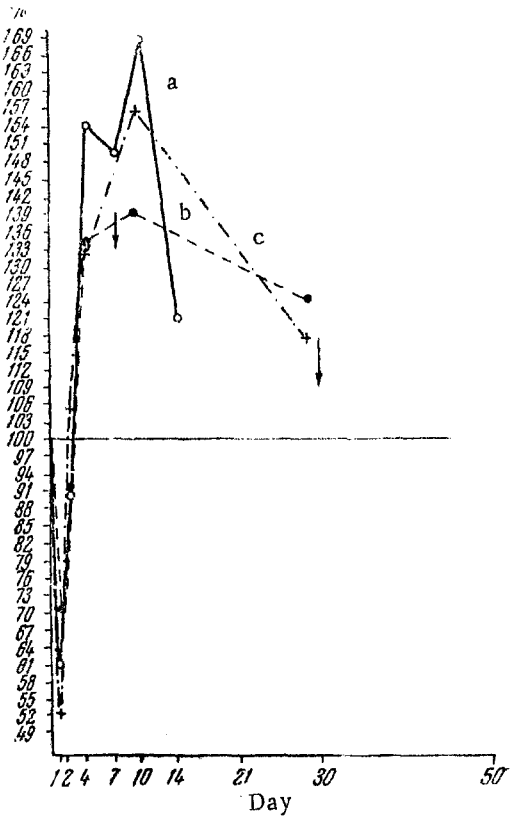

Fig. 2. Changes in sorption of neutral red by the spleen of guinea pigs suffering from experimental tuberculosis.
Ordinates: amount of sorbed neutral red, taking as 100% the amount taken up by the spleen of control (healthy) animals; a) after inoculation with virulent bovine type M. tuberculosis culture, b) after inoculation with attenuated BCG culture, c) after inoculation with virulent culture of previously twice immunized animals. The arrows indicate day of appearance of tubercles.

Results of Supravital Staining Experiments

The results of the experiments on sorption of neutral red by the spleen and lungs of the three groups of animals at different times after infection (abscissae axis) are illustrated in Figs. 2 and 3.

Tables 1 and 2 show the differences between the amounts of dye bound by the spleen and lungs of the experimental and the control animals, at different times after infection, and also the mean square error of the numerical results.

Comparison of the sorptive properties of the lungs and spleen shows that in all three series of experiments the sorptive capacity of these tissues undergoes a series of fluctuations during the course of the infection, the amplitude of the fluctuations being greater with virulent than with attenuated cultures.

The sorptive capacity of the lungs and spleen of the experimental animals varies biphasically. The first phase is distinguished by a fall in sorptive capacity, taking place within a few hours of infection. The duration of this phase is the same in all three series of experiments, but its magnitude varies according to the experimental conditions.

The second phase is characterized by a rise in sorptive capacity, to a different extent for each series of experiments. Transition to the second phase takes place with an abrupt rise in the curves (Figs. 2 and 3), beginning with the 2nd day after inoculation. The curves reach a first peak on the 4th day after inoculation. A second peak in the curves for the spleen and lungs is seen on the 10th day after inoculation with virulent mycobacteria; when the animals are inoculated with BCG culture the curve falls for the lungs as from the 4th day, but rises for the spleen.

The curves continue to rise, for both lungs and spleen, after the 4th day following inoculation of previously immunized animals with virulent culture, after which they both fall smoothly.

The sorption curves for the spleen and lungs are of the same shape as those found previously for other organs (brain, muscles), with the same biphasic course of the process, characteristic of all organs during the progress of the tuberculous infection; quantitative differences in the reaction of each organ are, however, present.

Comparison with the results of our earlier work shows that the sorptive capacity of the lungs and spleen is not only higher than that of the animals of the control series, but that it also exceeds that of the brain and muscles, under comparable experimental conditions. The greatest differences between the sorption curves are found in the organs of animals inoculated with virulent culture.

The results of histopathological examination of the lungs and spleen, considered in conjunction with their sorptive properties, showed that whereas microscopic evidence of specific inflammatory processes could be perceived in the spleen only from the 7th day, and in the lungs from the 10th day, after inoculation, the supravital staining method revealed profound changes in the protoplasmic substance of the cells of these organs from the first day of the infection. The supravital staining method thus permits of the detection of changes in the cell

TABLE 1

Binding of Neutral Red by the Spleen of Healthy Guinea Pigs and of Guinea Pigs Infected with Tuberculosis Germs, Expressed in Terms of Percentages, and the Mean Square Error.

Infected with	No. of expts.	Number of days after inoculation								
		1st day	2nd day	4th day	7th day	10th day	14th day	21st day	30th day	50th day
Virulent M. tuberculosis	10—30	—40±8.32	—9.8±2.26	+54.0±6.8	+49.2±11.1	+68.8±11.5	+25.8±4.55	+63.6±10	+44.2±11.7	+19.0±4.16
BCG	3	—29.3±1.85	—8.7±2.35	+34.7±4.1	—	+39.0±6.9	—	—	+24.7±3.7	+3.3±1.2
BCG + virulent M. tuberculosis	3	—48.3±2.01	+5.3±13.56	+32.0±8.06	—	+56.7±6.88	—	—	+18.3±2.9	+36.7±11.0

Note: the figures preceded by the signs + or - signify increase or decrease in the amount of dye bound by the spleen of the inoculated animals, as compared with the amount bound by the spleen of healthy controls, taken as 100%. Figures preceded by the sign ± are the mean square errors. The values given in the columns represent the arithmetic means of the total number of determinations for the given day.

TABLE 2

Binding of Neutral Red by the Lungs of Healthy Guinea Pigs and of Guinea Pigs Infected with Tuberculosis Germs, Expressed in Terms of Percentages, and the Mean Square Error.

Infected with	No. of expts.	Number of days after inoculation								
		1st day	2nd day	4th day	7th day	10th day	14th day	21st day	30th day	50th day
Virulent M. tuberculosis	10—30	—24.3±1.45	—15.0±2.09	+67.2±19.2	+49.0±28.6	+72.8±10.4	+15.3±3.2	+15.6±2.6	+19.6±7.8	+14.2±4.5
BCG	3	—21.3±6.9	—24.7±6.38	+33.3±5.1	—	+18.0±3.0	—	—	+8.0±5.67	+10.3±3.17
BCG + virulent M. tuberculosis	3	—36.3±3.84	—30.3±4.66	+49.3±11.05	—	+56.7±16.0	—	—	+26.7±12.7	+10.0±5.0

Note: the figures preceded by the signs + or - signify increase or decrease in the amount of dye bound by the lungs of the inoculated animals, as compared with the amount bound by the lungs of healthy animals, taken as 100%. Figures preceded by the sign ± are the mean square errors. The values given in the columns represent the arithmetic means of the total number of determinations for the given day.

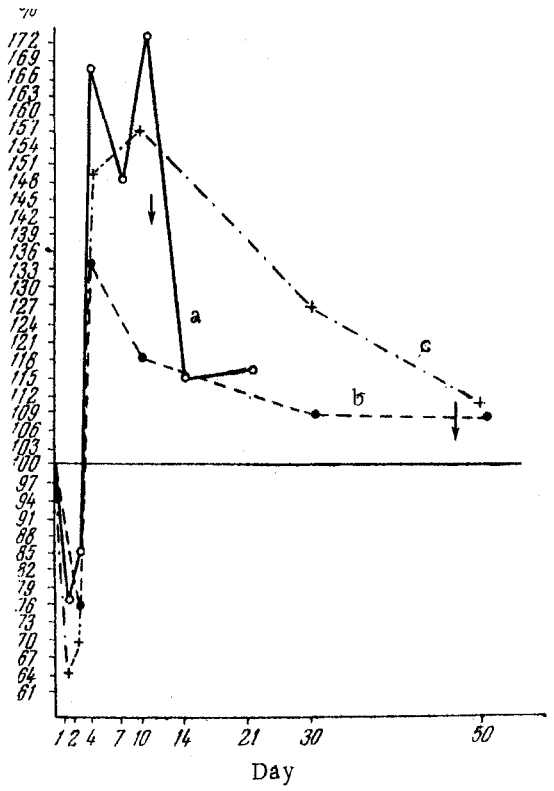

Fig. 3. Changes in sorption of neutral red by the lungs of guinea pigs suffering from experimental tuberculosis.
Ordinates: amount of sorbed neutral red, taking as 100% the amount taken up by the lungs of control (healthy) animals; a) after inoculation with virulent bovine type M. tuberculosis culture, b) after inoculation with attenuated BCG culture, c) after inoculation with virulent culture of previously twice immunized animals. The arrows indicate day of appearance of tubercles.

protoplasm of the lungs and spleen much earlier than the appearance of histopathological changes. It hence follows that the changes in the sorptive properties of the protoplasm, which reflect the physiological state of the cells are among the very earliest and most delicate indications of the reaction of the protoplasm to the very minute changes in the environmental conditions of the cells, due to the tuberculous infection. These changes in sorptive capacity also enable one to make a comparative quantitative evaluation of the changes occurring in the functional state of tissue elements. The appearance of tubercles coincidently with the peak of the sorption curves of both the organs examined points to the interdependence of these phenomena.

SUMMARY

Inoculation of guinea pigs with virulent or attenuated cultures of M. tuberculosis is followed by an immediate fall in the sorptive capacity for neutral red of the spleen and the lungs, to a minimum at the end of the first day, followed by a steep rise to a peak value, which coincides in time with the appearance of tubercles in histopathological preparations. The sorptive capacity fluctuations vary in amplitude parallel with the severity of the tuberculous process, in the ascending order: BCG inoculation, inoculation of immunized animals with virulent culture, inoculation of nonimmunized animals with virulent culture. The changes in sorptive capacity are a far more delicate indication of the very earliest stages of tuberculous infection than are histopathological methods.