

APPLICATION OF THE SUPRAVITAL STAINING METHOD TO THE STUDY
OF THE SORPTIVE PROPERTIES OF CERTAIN ORGANS IN
EXPERIMENTAL TUBERCULOSIS

PART IV. SORPTIVE PROPERTIES OF KIDNEY AND SUPRARENAL GLAND TISSUES

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The objective and comparative evaluation, both quantitative and qualitative, of the degree of injury suffered by the cells of various tissues of animals during the course of tuberculosis is of great importance for the study of the pathogenesis of this disease. We have shown that such an evaluation may be achieved by means of the supravital staining method of D. N. Nasonov and V. Ia. Aleksandrov, in conjunction with the histological examination of the tissues. Our investigations of the sorptive properties of the cells of the cerebral cortex, skeletal muscles, spleen, and lungs of guinea pigs have revealed changes in the functional state of tissue elements, which are evident within a few hours of inoculation of the animals with tuberculosis, and have also shown how these changes develop as the disease progresses.

Our results showed the possibility of investigating the changes in sorptive properties of other tissues of experimental animals infected with tuberculosis.

The present paper describes the results of a study of the sorptive properties of the kidneys and adrenals of animals suffering from experimental tuberculosis, of different degrees of severity.

We have been unable to trace any literature references to any similar researches. The published material deals with the evaluation of specific and unspecific changes in these organs in various forms of tuberculosis (these papers are both of the clinical and of the experimental type [5, 6, 7, 10, 12, 19, 20, 21, 23, 25]). They give only a qualitative evaluation of the changes observed, and apply mostly to advanced stages of the disease [1, 8, 9, 11, 13, 15, 16, 22]. Physiological investigations were restricted to overall renal function, and more recently to adrenal function [2, 3, 4, 14, 17, 18, 24].

EXPERIMENTAL METHODS

We used 410 guinea pigs, and carried out 800 examinations (400 of the kidneys, and 400 of the suprarenal glands). The animals were divided into 3 groups, of which the first was inoculated subcutaneously (in the region of the right axilla) with a suspension of *M. tuberculosis* (bovine type, Strain 109), at a dosage level of 0.01 mg in 1 ml of physiological saline (the most severe degree of infection); the animals of the 2nd group were given subcutaneous injections of BCG culture, at a dosage level of 1 mg in 1 ml of physiological saline (the mildest degree of infection), while the animals of the 3rd group were inoculated with the virulent culture after having

*Deceased.

previously been immunized with BCG culture (two vaccinations; the disease was of a medium grade of severity in these animals).

The kidneys and adrenals were removed together with the capsules, and were placed on muslin moistened with Ringer's solution. The capsule was carefully stripped from the kidneys. The organs were then immersed for 10-15 minutes in Ringer's solution, in order to 'refresh' them. They were then immersed for 30 minutes in 0.01% neutral red solution at 20-22°; the kidneys were suspended in the dye solution. After staining, the organs were rinsed in Ringer's solution, stumps of blood vessels were removed, and the organs were suspended in a fixed volume of acidified alcohol (70% alcohol, acidified with 2% sulfuric acid; the volume was the same for each organ). The organs were removed after 24 hours, dried to constant weight, and weighed on a torsion balance. Optical absorption of the extracts was measured in a Pulfferich step-photometer, and was recorded per gram dry weight of the organ. The determinations were made at the same time intervals after inoculation as in our previous researches.

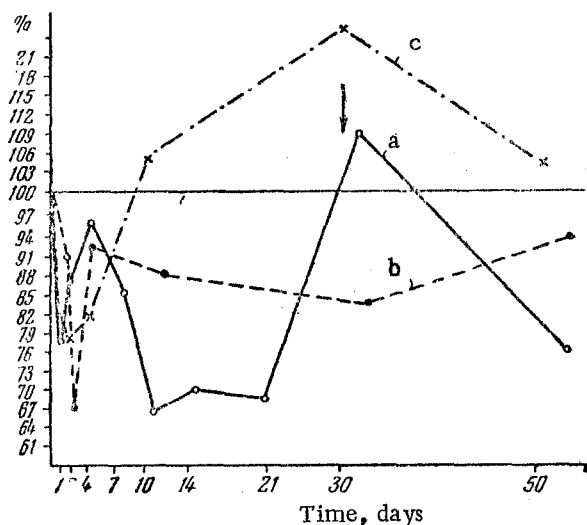


Fig. 1. Changes in sorption of neutral red by the kidneys of guinea pigs suffering from experimental tuberculosis. Abscissae: time in days after inoculation; ordinates: amount of neutral red bound by the organ, expressed as percentages, taking 100% as the amount bound by a kidney of a control animal. The arrow indicates the day on which solitary tubercles were first observed. a) After inoculation with a virulent culture of bovine type tuberculosis germs; b) after inoculation with an attenuated BCG culture; c) after inoculation with virulent culture, following previous immunization (two vaccinations).

in the staining capacity of the brain and muscles, and particularly of the spleen and lungs, we now observed a marked fall in sorptive capacity of the kidneys and adrenals of similarly infected animals.

Sorption of neutral red by kidney tissue proceeded differently in each of our three experimental groups. In the group inoculated with BCG culture the sorption level remained below that of the control group over the whole period of observation. In the group infected with virulent culture the sorptive capacity fell to a very low level until the 21st day after inoculation, when it rose somewhat (to +9%) on the 30th day. It is of interest that the peak value coincided in time with the appearance of tubercles, and with a rise in the dry weight curve. In the third group, in which the guinea pigs were given virulent culture after having previously been immunized by two vaccinations with BCG culture, the sorption level rose above that of the control group on the 10th day

Parallel with determinations of sorptive capacity we made histopathological examinations of the organs taken from guinea pigs which had been set aside for this purpose. We found (although not in every case) that solitary tubercles appeared in sections of the kidneys on the 30th day after inoculation with virulent cultures of *M. tuberculosis*. No pathomorphological changes could be seen in the kidneys before then, or in the adrenals at any time after inoculation.

EXPERIMENTAL RESULTS

It was necessary, in order to evaluate the sorptive properties of kidney and adrenal tissues of animals suffering from tuberculosis, to know how the dry weight of these organs varied during the course of the disease. Our experiments showed that significant variations in the dry weight of these organs were only encountered when the animals were inoculated with virulent culture. Increase in the dry weight of the kidneys was first noted on the 30th day after inoculation, and by the 50th day it was 50% higher than in the control animals.

We found (Figs. 1 and 2) that the supravital staining of the kidneys and adrenals showed considerable fluctuations during the course of the tuberculous process. The sorption curves for the kidneys (Fig. 1) and the adrenals (Fig. 2) of the animals of all 3 groups of experiments merit special attention, since they differ radically from those previously found by us for the brain, muscles, spleen, and lungs (Parts 1, 2, and 3 of this series). Whereas we then found an increase

after inoculation; by the 30th day it exceeded this level by 25%, and was 16% higher than that of the group inoculated with virulent culture alone.

The first phase of lowering the sorptive capacity of the kidneys is identical with that found earlier for brain, muscle, spleen, and lung tissues. Sorptive capacity fell to a minimum of -35% on the 10th day after inoculation, in the first group of experiments. In the second and third groups minimum values were found after 48 hours and 24 hours, respectively. As is evident from Fig. 2, the sorption curves are of an undulating nature.

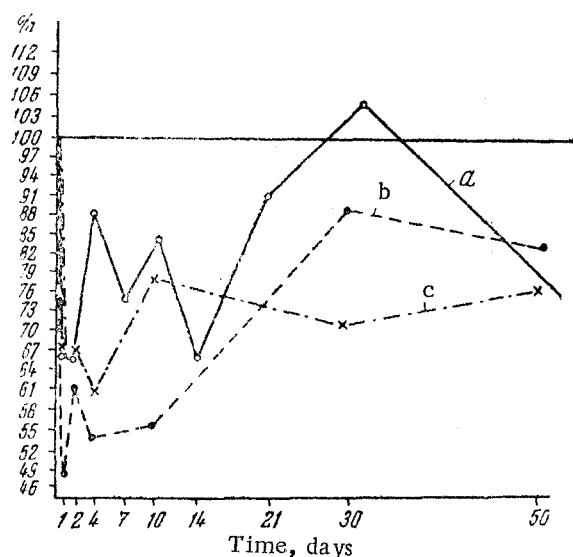


Fig. 2. Changes in sorption of neutral red by the adrenals of guinea pigs suffering from experimental tuberculosis. Abscissae: time in days after inoculation; ordinates: amount of neutral red bound by the gland, expressed as percentages, taking 100% as the amount bound by the glands of control animals. Designation of curves as for Fig. 1.

The differences in the nature of the reactions of the kidneys to infection are not restricted only to quantitative differences in the degree of lowering of sorptive capacity in the three groups. At no time after inoculation with BCG culture did we observe a sorptive capacity higher than that of the control group. The sorptive capacity of the kidneys of guinea pigs infected with virulent culture exceeded that of the controls on one occasion only (on the 30th day), by only 8.6%. It was at this time that the appearance of solitary tubercles was noted. The sorptive capacity of the kidneys of guinea pigs inoculated with virulent culture after having been immunized exceeded the control level on the 10th and the 30th days (unfortunately no determinations were made between these days), attaining a fairly high level on the 30th day (+ 25%). Binding of neutral red by adrenal tissues was much less than in the control series for all three groups (see Fig. 2). The lowest values were encountered in the group inoculated with BCG culture, being 52% of the control level 24 hours later. The sorption curves for all three groups resembled those found for the kidneys. Very slight increase in sorptive capacity of the adrenals was found only in the group given virulent culture, reaching +5% on the 30th day, after which it again fell below the control level. In the remaining two groups it was at all times lower than in the control series.

It may be concluded from our results that the adrenals possess extraordinarily high powers of adaptation to the changed conditions prevailing during the course of tuberculous infection; this did not apply to any other organ examined by us.

In concluding this paper, which is the last of the series of communications, we think it desirable to summarize our findings, the more so as they apply to a number of different organs of animals of the same species.

As is known, it has been shown by D. N. Nasonov and his co-workers that injuries inflicted on the living cells of a number of isolated organs by a wide variety of physical and chemical agents lead to enhancement of the affinity of protoplasm for basic and acidic stains. It has been shown that this unspecific reaction varies in intensity parallel with the gravity of the injury inflicted on the cell.

As far as we are aware, our researches are the first in which it has been attempted to investigate the reactivity of the tissues of whole organisms during the course of a pathological process; our results show that the supravital staining method can be applied to the quantitative evaluation of the damage suffered by tissue elements of different organs at different stages of development of the tuberculous process in experimental animals. Marked changes in the sorptive properties of the tissues were found to occur within the first few days after inoculation, before there were any anatomical signs of the disease, both in organs which were subsequently affected by the specific inflammatory process (lungs, spleen, kidney), and in those not so affected (brain, muscle,

adrenal glands). As observed in isolated organs, these changes vary in intensity, according to the gravity of the disease, which differs from group to group of animals, and exhibit fluctuations as the disease progresses. These fluctuations have the common feature of being of a phasic nature; the changes in reactivity seen in the first phase of lowered affinity for the dye reflect the process of adaptation of tissue elements to the complex of noxious factors (direct and reflex) which come into play during the progress of the morbid process. Paraneurotic changes then supervene, associated with rise in sorptive capacity, and, judging from our results, these changes may be reversible. Only in the advanced stages of the grave form of the disease, and in those organs which are the most susceptible to injury by the given pathogen, do substantial irreversible changes appear in the protoplasm.

We would emphasize that the supravital staining procedure of D. N. Nasonov and V. Ia. Aleksandrov has permitted of the establishment of the following: 1) the existence of changes in the functional state of the tissue elements of organs in which such changes cannot be perceived by any other procedure, or in which they only appear at an advanced stage of the disease (brain, muscles, adrenal glands); 2) the existence of changes in the functional state of all the organs examined following vaccination with BCG culture, although at no time subsequent to vaccination could any specific morphological changes be detected; 3) the existence of qualitatively and quantitatively dissimilar changes in the sorptive properties of different organs, side by side with features common to all of them; this permits of the drawing of conclusions regarding the dynamics of the morbid process, and of the order in which these organs are involved in the tuberculous process.

We conclude from our observations that at the stage at which extensive anatomical changes make their appearance in a given organ (changes in its weight and in the qualitative composition of its tissue elements), the study of sorptive properties becomes pointless, as the data obtained are not comparable with those of the controls or with the preceding state of the organ.

Although we are not inclined to consider that the results of our study of the physiological state of cells of animals suffering from experimentally induced tuberculosis are necessarily specific for this disease, the possibility cannot be excluded that they may have some pathognomonic significance for tuberculosis.

SUMMARY

Sorption of neutral red by the isolated kidneys and adrenals of guinea pigs has been determined, following inoculation of the animals with virulent or BCG cultures of bovine type M. tuberculosis, as well as after inoculation of immunized animals with virulent culture. In contrast to other organs, the sorptive capacity of these organs falls considerably after inoculation, although similar fluctuations are seen subsequently in the sorption — time curves as with other organs (brain, muscle, spleen, lungs) during their phase of heightened affinity for the stain. Maximum sorption of neutral red coincides in time with appearance of tubercles in the kidneys, spleen, and lungs. In the kidneys, inoculation of immunized guinea pigs with virulent culture is followed, after an immediate fall in sorptive capacity, by a sustained rise to levels higher than those found in the control series or in nonimmunized animals infected with virulent culture.

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