PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

SORBENT PROPERTIES OF CERTAIN ORGANS, STUDIED IN EXPERIMENTAL TUBERCULOSIS BY THE SUPRAVITAL STAINING METHOD

COMMUNICATION I. SORBENT PROPERTIES OF THE CEREBRAL CORTEX

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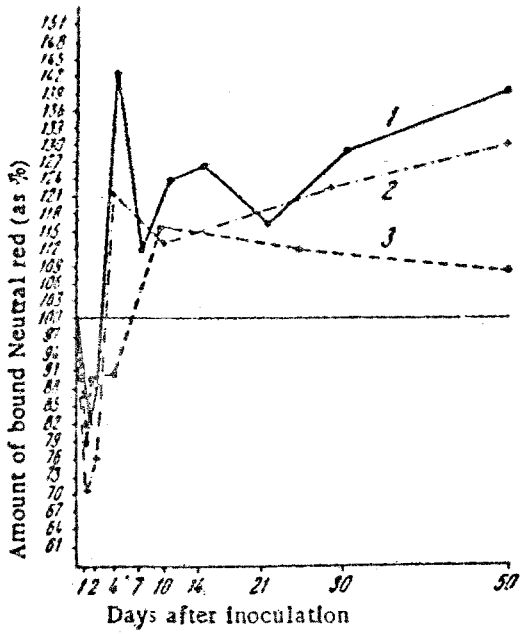
In the study of certain mechanisms of the pathogenesis of tuberculosis, considerable importance is attached to comparative data on the relative resistance of the tissues of different organisms to the pathogen. Although it allows an appraisal of the dynamics of structural lesions of a given organ, the pathomorphological method does not permit the evaluation of early tissue changes, and is still less able to assist in the determination of the precise quantitative extent of injury to the organs at the inception of the morbid process. The necessity arose, in this connection, of finding other methods for the investigation of fine morphofunctional changes in the tissues, arising in the process of development of experimental turberculosis.

It has been shown by D.N. Nasonov and his associates that a variety of injurious factors, differing in their physicochemical nature, give rise in a wide variety of different kinds of cells, to a complex of similar, unspecific changes, to which the term paranecrosis has been applied [7]. One of the most characteristic features of this complex of changes is the more intense staining of the protoplasm of the cells by supravital stains. This unspecific reaction exhibits gradations. The increase in staining capacity of the cells varies directly and quantitatively with the extent of the injury suffered by the cell [1, 2, 4, 5, 6, 8, 9, 10, 11]. It follows that the method of supravital staining of tissues should be applicable to the solution of the problem under investigation. We therefore examined the possibility of applying the method of supravital staining to the evaluation of the extent of injury suffered by the tissue elements of various organs at different stages in the development of experimental tuberculosis. If our results confirmed this possibility, we intended to apply the method to the comparative study of the reactions of the cells of certain organs to tuberculosis infection.

The present paper deals with a study of changes taking place in the sorbent properties of cells of the cerebral cortex of guinea pigs suffering from experimental tuberculosis.

EXPERIMENTAL METHOD

We studied three model forms of experimental tuberculosis, differing in the increasing severity of the pathologic process: disease due to infection with an attenuated BCG culture of M. tuberculosis, disease caused by inoculation of virulent bovine type germs into immunized guinea pigs, and disease due to virulent bovine type germs inoculated into nonimmunized animals.



Curves expressing sorption of dye by tissues of the cerebral cortex of guinea pigs during development of experimental tuberculosis, after inoculation with virulent bovine type bacteria (1), BCG culture (3), and virulent bovine type bacteria after previous immunization (2). The abscissae represent days after inoculation, and the ordinates are amount of Neutral red bound, as percentages. Sorption by brain tissues of control (healthy) animals is taken as 100%.

In the first series of experiments the guinea pigs were inoculated with a suspension of bovine type M. tuberculosis (Strain No. 109) containing 0.01 mg of organisms (in 1 ml of physiological saline), subcutaneously in the region of the right axilla. The animals of the second series were inoculated subcutaneously with 1 mg of BCG organisms (in ml of saline). The guinea pigs of the third series were given two injections of BCG organisms, at an interval of 30 days. Two months after the second injection the animals were given an inoculation of virulent bovine type bacteria (Strain No. 109), at the same dosage level as in the first series.

The animals of the first series were killed 1, 2, 4, 7, 10, 14, 21, 30,and 50 days after inoculation, and in the second and third series 1, 2, 4, 10, 30, and 50 days after inoculation. Healthy guinea pigs, killed at the same times, served as controls. From 5 to 30 animals were taken for examination from the experimental groups, and the same number of controls was taken. We performed 380 examinations in the first series, and 40 each in the other two.

The supravital staining method consisted of the following: the animals were decapitated under ether anesthesia, the cranium was lifted, and the brain was carefully removed and placed first in Ringer's solution for 10-15 minutes, and then into the dye solution (0.01% Neutral red in Ringer solution prepared without sodium carbonate), at 18-20°. The brain was removed after 30 minutes, washed in Ringer's solution, the olfactory lobes and the stump of the spinal cord were removed, and the preparation was immersed for 24 hours in 70° alcohol which had been acidified with 2% sulfuric acid, for extraction of sorbed dye. The brain was then dried to constant weight. The amount of extracted dye was determined by means of a Pulfrich step-photometer, and calculated per mg of dry weight of the brain. The results were subjected to statistical treatment, involving calculation of the mean quadratic error of the deviations.

This procedure permitted the evaluation of the sorbent properties only of the tissue elements of the cerebral cortex, since the dye does not, under the given conditions, penetrate deeper than a few tens of microns below the surface [3]. Microscopic examination of the cortex showed that not only the nerve cells and their processes, but also all the fibers present in this region were stained.

It was necessary, for the evaluation of the sorbent properties of the cerebral cortex of the experimental animals, to know what changes took place in the dry content of this organ during the development of the tuberculous infection. We found, in parallel experiments, that the dry weight of the brain fluctuated only very slightly during the course of the disease. Our findings relating to sorption may therefore be regarded as being trustworthy.

We performed histopathological examinations of internal organs of other guinea pigs parallel with the determinations of sorbent properties of the brain, and at the same times.

EXPERIMENTAL RESULTS

As is evident from our findings (see table and figure), binding of Neutral red by the brains of the experimental animals of all three series very greatly exceeded (by up to 43%) the values found for the control (healthy) animals. This is evidence of a reaction of the tissues of the cerebral cortex,due to the action of a complex of injurious factors (direct toxic and reflex factors), and increasing in intensity as the disease progresses. The greatest intensity of paranecrotic changes in brain cells was found in guinea pigs infected with virulent bovine type

Binding of Dye by Tissues of the Cerebral Cortex of Guinea Pigs Inoculated With Tuberculosis Germs (as percentages of the value found for healthy animals)

Culture taken for inoculation	Time after inoculation (in days)							
	1	2	4	7	10	14	21	30	50
M. tuberculosis	−19.2±4.5	−12.4±3.9	+42.5±6.2	+11.8±3.9	+23.7±5	+26.3±4.6	+16.4±2	+30.2±4.8	+40.6±4.6
BCG	−22±2.8	−11±4.1	−10.3±1.34	—	+15±5.5	—	—	+12±2.3	+8.7±0.66
BCG + M. tuberculosis	−30.3±2.96	−23.7±2.73	+21.3±2.33	—	+14±5.85	—	—	+22.3±3.66	+32±4.61

Note: The symbols + and − represent, respectively, increase or decrease in the amount of dye bound by the cerebral cortex of the experimental animals, as compared with the amount found for healthy control animals. The mean quadratic error is designated by ±. The figures given in each column of the table represent arithmetic means of the results of all the experiments applying to the given time.

bacteria, and the least in those inoculated with BCG culture. Similarly, the most pronounced anatomopathological changes were found in the viscera of animals infected with virulent bacteria; these changes were less pronounced in animals which had been infected with virulent bacteria after immunization with BCG organisms, and were absent from the organs of guinea pigs given BCG vaccinations alone. There was thus a complete parallelism between the severity of the disease and the degree of change in the sorbent properties of the brain.

The change in the sorbent properties of the tissues of the cerebral cortex of the experimental animals was of a biphasic nature. The first phase, seen at the inception of the disease, was characterized by a fall in the capacity of the tissues to bind the dye, while in the second phase there was an increased capacity, shown by the animals of all three experimental series.

The phase in which sorption by the cortical tissues falls below that of the controls, observed within 24-48 hours of inoculation, may, in accordance with the findings of D.N. Nasonov and his associates, be related to adaptation of the brain cells to the action of the complex of factors accompanying the development of tuberculosis. As is evident from the figure, the sorptive capacity of the cortical tissues of animals inoculated with virulent bacteria rose to a value twice as great as in the controls. The first rise in sorptive capacity probably reflects paranecrotic changes which are still partly reversible. In contrast, the second rise in sorptive capacity corresponds with a phase of development of irreversible paranecrotic changes in the brain tissues.

In the experiments involving inoculation with BCG culture, or with virulent bacteria after previous immunization, only one rise in sorptive capacity is seen. This is evidence that the paranecrotic changes occurring in the brain under such conditions differ from those following inoculation with virulent bacteria.

Our findings show that changes in the sorbent properties of the brain originate during the early stages of development of experimental tuberculosis, before any changes in the morphology of the viscera are perceptible (such changes could be perceived seven days after inoculation with virulent cultures, and were not found after BCG inoculation). Our findings show that the supravital staining procedure can serve for the detection of an early reaction of the brain to the development of infection.

We do not consider the changes in supravital staining found by us in guinea pigs inoculated with virulent and BCG cultures necessarily to be specific for tuberculous infection, although the possibility of a certain pathognomicity of these changes for the given pathological process cannot be excluded.

SUMMARY

The method of vital staining was tested in three cases of experimentally induced tuberculosis of varying severity. It was demonstrated that this method was useful in evaluation of the

degree of injury of the tissue elements of the cerebral cortex during the development of this disease. There are no morphological changes which can be detected in the internal organs of animals during the first hours after infection by tuberculosis. However, at that time, substantial changes have already taken place in the cerebral cortex, which are manifested in the change of the sorptive properties of the cells. The degree of change of the sorptive properties corresponds to the severity of the pathological process. The wave-like biphasic character of the changes in the sorptive properties of the cerebral cortical tissue was noted during development of experimentally induced tuberculosis.

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