

## THE HISTOCHEMICAL STUDY OF THE PHOSPHATASES DURING IMMUNOGENESIS

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The phosphatases, the acid and alkaline phosphomonoesterases, are enzymes capable of catalyzing hydrolysis of phosphoric acid esters. The inorganic phosphorus formed as a result of the action of these enzymes is essential for various processes associated with metabolism of carbohydrates, proteins, nucleotides, and so on [1,2,4,6,7]. It is especially interesting to discover the role of the phosphatases in immunogenesis, when several cell systems become activated and the synthesis of specific proteins (antibodies) is intensively stimulated.

The object of the present investigation was to make a histochemical study of the enzyme activity of acid and alkaline phosphatases during immunogenesis produced by a living brucellar vaccine, and also when this process was influenced by the action of hormones.

## EXPERIMENTAL METHOD

Four series of experiments were carried out on male guinea pigs weighing 300-400 g, immunized with a living vaccine culture of *Brucella* 19BA in a dose of 250 million bacterial cells subcutaneously in the region of the left thigh. The animals of series I (27 guinea pigs) received a single injection of the brucellar culture, the animals of series II (18 guinea pigs) received two such injections at intervals of three months, while the animals of series III (18 guinea pigs) received cortisone daily during the three days before immunization and the 7 days thereafter, and the animals of series IV (18 guinea pigs) received desoxycorticosterone (DOCS) three times before and three times after immunization at intervals of two days. Cortisone acetate was injected in all the experiments intramuscularly in a dose of 12.5 mg and DOCS in a dose of 10 mg per guinea pig. The animals of series I were investigated periodically at intervals of 2, 6, 24, 48, and 96 h and 10, 20, 30, and 90 days, and the guinea pigs of the remaining series until the 10th day after immunization (in series II, after revaccination). The controls were healthy (4) animals, and animals (16) receiving only the hormones. At each of the periods of the investigation, 2-4 guinea pigs were examined. Material for the investigation was taken from the regional (left inguinal and para-aortic) and distant lymph glands, the spleen, the liver, and the adrenals. Gomori's method was used for detecting acid and alkaline phosphatase. In parallel tests, pieces of the same organs were stained to obtain survey preparations and to detect RNP, glycogen, amino acids, protein SS- and SH-groups, and so on. The antibodies in the extracts of the lymph glands and spleen and in the blood serum were detected by a method described previously [3].

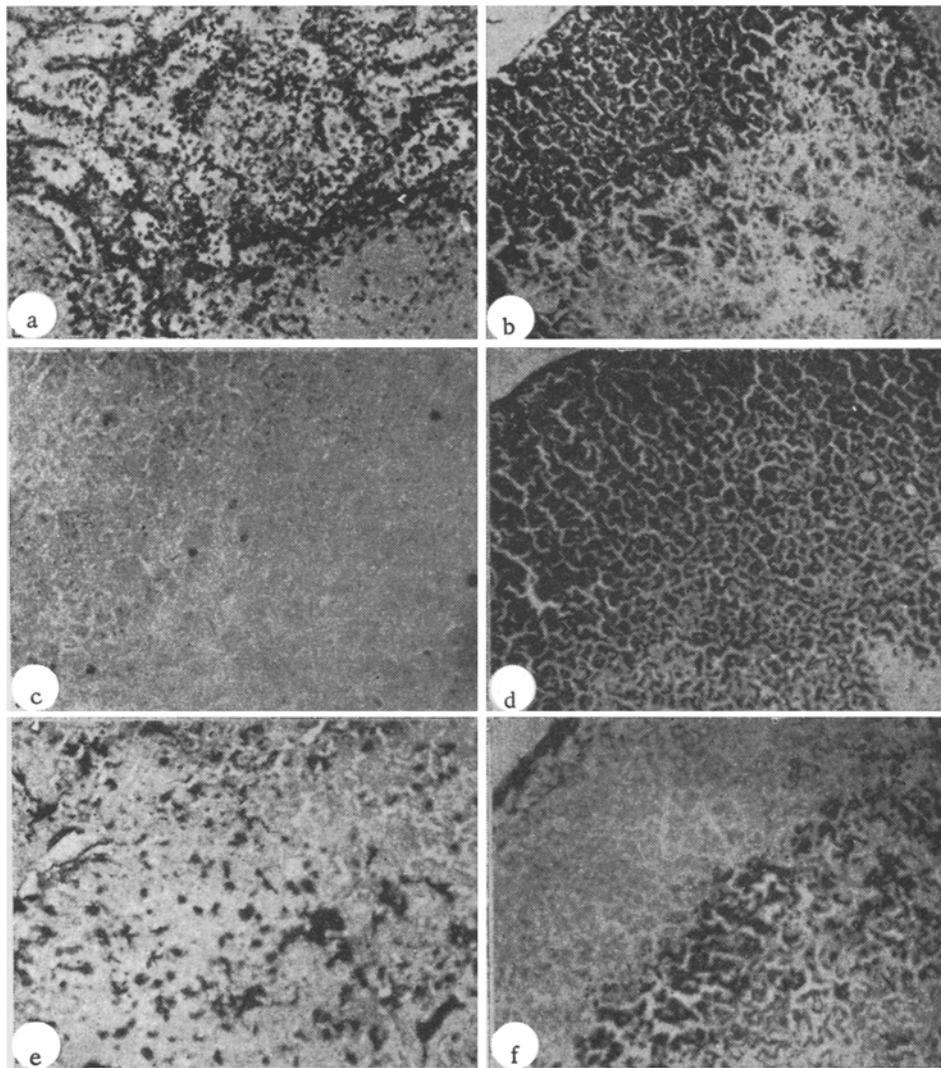
## EXPERIMENTAL RESULTS

The acid phosphatase activity in the follicles of the lymph glands and spleen of the healthy guinea pigs was very low. Comparatively higher activity of the enzyme was observed in the red pulp of the spleen, the medullary cords, and the sinuses of the lymph glands. The distribution of alkaline phosphatase activity in these organs was almost identical to that of acid phosphatase, but it was found mainly in the cytoplasm of the cells. In the liver acid phosphatase activity was found in the parenchymatous cells and the Kupffer cells, being particularly high in the latter. Alkaline phosphatase was found in individual Kupffer cells, its activity varying from high to very low (see figure, b). In the adrenal cortex, the highest acid phosphatase activity was found in the zona fasciculata, followed by the zona reticularis (the widest in guinea pigs) and the zona glomerulosa. The alkaline phosphatase activity was highest in the cells of the zona glomerulosa and zona fasciculata, while in the zona reticularis the distribution of the enzyme was irregular (see figure, d). The adrenal medulla was characterized by high activity of the phosphatases.

In the lymph glands, spleen, and liver of the healthy guinea pigs, the activity of the phosphatases was depressed by cortisone but stimulated by the action of DOCS.

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Activity of phosphatases in organs of guinea pigs in normal conditions, during immunization, and under the influence of hormones: a) high acid phosphatase activity in sinususes of a lymph gland on the 10th day of immunization; b) alkaline phosphatase activity in the liver in normal conditions is seen in individual Kupffer cells; c) high alkaline phosphatase activity in hyperplastic Kupffer cells in the liver on the 20th day of immunization; d) high alkaline phosphatase activity in the zona glomerulosa and zona fasciculata of the adrenal cortex in normal conditions; e) high alkaline phosphatase activity in the widened zona reticularis of the adrenal cortex on the 20th day of immunization; f) decrease in alkaline phosphatase activity in the zona fasciculata and zona glomerulosa and increase in its activity in the zona reticularis of the adrenal cortex on the 5th day during a combination of immunization with injection of DOCS. Gomori's stain. Magnification: a) objective 8X, ocular 10X; b and c) objective 10X, ocular 10X; d-f) objective 10X, ocular 7X.

In the lymph glands, spleen, and liver of the healthy guinea pigs, the activity of the phosphatases was depressed by cortisone but stimulated by the action of DOCS.

Between 2 and 6 h after immunization, the activity of both acid and alkaline phosphatases in the lymph glands and spleen fell appreciably. The decrease in enzyme activity was marked in the follicles, although it was also clearly defined in the elements of the pulp. In the liver at these times the acid phosphatase activity also fell slightly, but the alkaline phosphatase activity was not appreciably affected. From the second day after immunization, the activity of the phosphatases rose gradually in the red pulp of the spleen, the medullary cords, and the sinususes of the

lymph gland — in the macrophages and reticulum cells. Very high enzyme activity was found from the 5th day after immunization. At these times the sinuses were grossly distended and congested with macrophages and plasma cells, the nuclei and cytoplasm of which showed very high activity of the enzymes. In the process of immunogenesis, the activity of the phosphatases in the follicles was not appreciably increased, although in some cases an increase in the activity of the enzymes was observed in some cells, and sometimes also in a group of cells in the center of the follicle, around the blood vessels (see figure, a). The histochemical changes in the activity of the phosphatases were clearer in the regional lymph glands than in the distant glands and spleen. At the height of immunogenesis, the acid phosphatase activity in the liver was sharply increased in the nuclei, and the alkaline phosphatase activity in the cytoplasm of the hyperplastic Kupffer cells (see figure, c). In the process of immunogenesis, the medullary layer gradually diminished in size in the adrenals on account of widening of the cortical layer, mainly of the zona fasciculata, characterized by high activity of the phosphatases (see figure, e). On the 90th day after immunization, the structure of the organs investigated, and also the histochemical findings relating to the content, distribution, and activity of the acid and alkaline phosphatases showed no appreciable abnormality.

Following revaccination of the guinea pigs, the dynamics of the histochemical picture of the phosphatases was the same as after primary vaccination, although the changes in activity of the enzymes were more marked and they reached their maximum earlier than after primary immunization. This demonstrated the higher intensity of the process.

When immunization was combined with administration of cortisone, the activity of the phosphatases in the lymph glands was significantly lowered, although the activity in the elements of the pulp showed a tendency to increase, and on the 10th day the activity of the enzymes was almost the same as after immunization. In the spleen the acid phosphatase activity showed similar changes to those in the lymph glands, but the alkaline phosphatase activity fell only very slightly. In the liver there was no change in the activity of the phosphatases under the influence of cortisone. Administration of DOCS in conjunction with immunization caused an increase in the activity of the phosphatases in the lymph glands, spleen, and liver. In the adrenals, under the influence of cortisone and DOCS, the cortical layer was narrowed, mainly because of the increase in width of the zona fasciculata, and the activity of the phosphatases fell here and in the zona glomerulosa. The alkaline phosphatase activity rose in the zona reticularis (see figure, f). These facts are of definite interest because they indirectly demonstrate the synthesis of DOCS in the zona fasciculata and zona glomerularis of the adrenal cortex.

Hence, after both primary immunization and revaccination, the activity of the phosphatases in the lymphoid organs was on the whole stimulated in the elements of the pulp, the reticulum cells, the cells lining the sinuses, the macrophages, the cells of the plasma series, and the Kupffer cells, i.e., in the cells responsible for the seizure of foreign antigenic material and for antibody production. Against the background of the changes noted above, the phosphatase activity of the follicles was very slight, emphasizing their subsidiary role in the process of immunogenesis. As the authors showed earlier [9], the changes in the activity of the phosphatases in the organs studied preceded changes in the metabolism of nucleoprotein, proteins, and polysaccharides, and these in turn preceded the appearance of antibodies in the extracts of the organs and blood serum. The high enzyme activity of the lymphoid organs during brucellar immunization indicates the important role of phosphatases in the processes of immunogenesis and, in particular, in the mechanism of antibody formation.

The important role of the phosphatases in immunogenesis was emphasized also by the results of the experiment in which administration of hormones was combined with immunization: as a rule, the depression of antibody formation by cortisone and its stimulation by DOCS were preceded by a decrease in activity or by activation of the enzymes in the lymphoid organ.

A feature of particular interest is the temporary depression of phosphatase activity in the lymph glands and spleen after primary immunization and also after revaccination.

In response to various external environmental stimuli disturbing the homeostasis of the organism, a state of stress may arise, the initial phase of which is characterized by an increase in the secretion of glucocorticoids, and also by involution of the lymphoid apparatus [5,8,10,11,13]. This involution of the lymphoid tissue also arises after administration of exogenous cortisone [12,14]. In the present experiment in which cortisone was given to guinea pigs, a marked decrease in the enzyme activity was observed in the lymphoid organ. A similar fall in activity of the phosphatases was also observed in the initial stages of immunization and revaccination. On the ground mentioned above, it may be postulated that the fall in activity of the enzymes in the early periods after immunization was caused by endogenous activation of the secretion of glucocorticoids. Hence, in the earliest periods of its existence in the organism (whether by primary immunization or revaccination), besides causing changes in the specific

immunological reactivity, the antigen must also give rise to phenomena of stress in the system of the pituitary and adrenal cortex, and these characteristic signs of a state of stress are important for the rapid mobilization of the protective forces of the organism.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.

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