logical destruction of the placental tissues, leading to the onset of fetal pathology or death.

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HISTOCHEMICAL CHARACTERISTICS OF EXPERIMENTAL MONILIASIS UNDER SPECIFIC SENSITIZATION CONDITIONS

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The role of specific sensitization in the pathogenesis of experimental moniliasis was studied histochemically. Activity of several enzymes of the fungus and of infiltrating inflammatory cells was determined and assessed quantitatively. Tissue changes in visceral moniliasis were found to correspond to changes in the active substances in cells of the fungus and of the focus of inflammatory infiltration. In the early stages of parasitism increased activity of fungal enzymes was observed, followed by a decrease, which coincided with a decrease in the number of vegetative forms. Preliminary administration of monilial allergen induced increased sensitivity to subsequent infection. Changes in specifically sensitized rats developed against the background of marked vascular disorders and followed a hyperergic type of course.

KEY WORDS: *Moniliasis; sensitization; enzyme activity.*

To establish a basis for effective specific therapy and laboratory control over immunotherapy of the mycoses elucidation of the role of specific sensitization in the pathogenesis of monilial infection is of great importance [3-5, 7]. A special role belongs to histo-enzymological investigations of *Candida albicans* under tissue-parasitism conditions. However, few such investigations have been described [1, 8].

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The object of this investigation was to assess preliminary specific sensitization as a pathogenetic factor in experimental visceral moniliasis.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250-300 g. The experimental model of visceral moniliasis was obtained by intravenous infection of the animals with a suspension of a virulent strain of *Candida albicans* (1 million cells in 0.5 ml physiological saline). Some animals were first sensitized by two subcutaneous injections of debris of killed cells of the same strain mixed with Freund's incomplete adjuvant. The effectiveness of sensitization was assessed from the increase in antibody titer in the agglutination test with blood serum of sensitized rats and also in allergic skin tests with passive transfer of allergy to guinea pigs.

The kidneys, lungs, and spleen of animals killed after 1, 3, 7, 14, 21, and 23 days and also of animals which died were investigated. The same organs of intact rats, and also of rats which were sensitized but not infected with a virulent strain of the fungus, acted as the control.

Repeated seedings were made from tissue homogenates of the test organs in order to obtain retrocultures of the agent. Tissue sections were stained with hematoxylin-eosin, Sudan Black, and methyl green—pyronine. The fungus was detected by the PAS reaction and by the Gomori—Grocot and Gram—Weigert methods. Activity of succinate (SD) and lactate dehydrogenases (LD) and NAD- and NADP-diaphorases of the fungus and of the cells of the inflammatory focus of infiltration was determined histochemically during development of the moniliasis. The enzyme activity was assessed quantitatively by a cytophotometric method using the MUF-6 and MF-4 instruments. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The tissue reaction to the agent took the form of the development of suppurative granulomatous inflammation located predominantly in the kidneys. Vegetative forms of the fungus caused intensive emigration of leukocytes. The exudative-necrotic reaction reached its maximal intensity on the 3rd-7th day. In the rats which died the kidney lesions were more widespread in character. By the 14th day intensive fibrosis of the granulomas was observed. Fungi were found in the efferent system of the nephron. By the 21st-23rd day the fungi could be found only in the renal pelves as clusters of yeast-like and filamentous forms.

Besides changes connected with the direct action of the fungus, activation of elements of the reticuloendothelial system and foci of lymphohistiocytic infiltration also were found. In the glomerular apparatus of the kidneys the basement membranes of the glomeruli were thickened, loose in texture, and homogeneous in appearance and PAS-positive masses were deposited between the capillary loops, possibly as a result of the localization of the antigen in the tissues [2, 6].

In sensitized rats the monilial infection followed a more severe course than in the intact rats. Vegetative forms of the agent invaded the inflammatory cell barrier. The kidney lesions bore the character of multiple abscesses. Signs of membranous glomerulonephritis were more clearly defined. The newly formed granulomas consisted of large number of epithelioid, plasma, giant multinuclear, and lymphohistiocytic cells. Plasma cells appeared in the pathological foci sooner than in rats with unaltered immunoreactivity. Phagocytosis of the fungus by giant multinuclear cells was often incomplete in character. Organization of the granulomas was noticably later than in the unsensitized animals.

Morphological manifestations of increased sensitivity of the sensitized tissues consisted of rapidly developing and spreading tissue necrosis, profound necrobiotic changes in the parenchyma of the organs, the formation of large foci of lymphoid-macrophagal infiltration in the stroma of the organs and in the perivascular tissues, and vaso-necrotic reactions with signs of hyperemia and exudation.

Preliminary sensitization of the experimental animals thus led to an immune response, as shown by the intensive cell reaction detected histologically and by the formation of antibodies revealed by the agglutination test. The blood serum agglutinin titer of the sensitized rats reached 1:60. However, the antibodies not only did not protect the animals against subsequent infection, but they aggravated the course of the moniliasis.

Investigation of the enzyme activity of tissue forms of the fungus showed that activity of LD, SD, and NAD- and NADP-diaphorases varied depending on transformation of the parasitic agent associated with its viability in the different stages of the moniliasis. Growth and multiplication of the agent in the early stages of parasitism were accompanied by a sharp increase in the activity of the enzymes studied. During interaction with the tissues of the host the fungi underwent considerable morphological changes and these were reflected in the intensity of their metabolism.

The steady decrease in enzyme activity in the cells of the fungus could be connected with a decrease in the number of vegetative and viable forms of the agent on the 7th day in the unsensitized rats and considerably later in the sensitized animals. Activity of the enzymes was low in destructive and phagocytosed fungal cells. Fungi found in isolated cavities in the renal pelves (21st-23rd days) multiplied vigorously. This process was accompanied by increased activity of all enzymes of the agent tested. The higher LD activity of the fungus at all times of observation indicated intensive processes of glycolysis in the cells of the agent, maintaining its viability under anaerobic conditions both in the pathologically changed tissue and during intracellular parasitization.

During the development of experimental moniliasis close correspondence was observed between the degree of the morphological changes in the tissues and the activity of oxidoreductases in the cells of the focus of inflammatory infiltration. As the necrobiotic changes progressed in the tissues the activity of the enzymes fell sharply, and none whatsoever could be found in the foci of necrosis. After the end of the acute period of the disease, when the exudative-necrotic phase was followed by the productive phase, parallel with the increase in the number of cells participating in granuloma formation, increased activity of all enzymes was observed, probably in connection with active synthesis in the cells. The lowering of enzyme activity during the period of marked fibroblastic changes evidently reflected some degree of return to normal metabolism in the tissues.

It follows from this description that preliminary injection of monilial allergen into experimental animals induces a state of increased sensitivity to subsequent infection in them. The changes arising as a result of infection in specifically sensitized rats follow a hyperergic type of course. Phagocytosis of the fungus by giant multinuclear cells is often incomplete in such sensitized animals. As a result, on destruction of the phagocytes, the fungus could again behave as a pathogenic agent. High activity of oxidoreductases in the cells of the inflammatory focus of infiltration is evidence of the intensity of metabolism in the tissues in response to penetration of the agent and is evidently a unique protective mechanism with a compensatory role. The marked morphological changes in the tissues were accompanied by a sharp decrease in enzyme activity, reflecting the low level of energy metabolism in the affected tissues.

The results of this investigation show that during immunotherapy of the moniliases it is undesirable to use complete allergens capable of increasing the sensitivity of the host to subsequent infection and of aggravating the course of the moniliasis.

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