MORPHOHISTOCHEMICAL STUDY OF THE RAT PLACENTA AFTER EXPOSURE TO CARBON TETRACHLORIDE AT DIFFERENT STAGES OF PREGNANCY

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Intraventricular injection of CCl_4 (0.3 ml/100 g body weight) into female rats causes marked injury to the tissues of the placenta and, in particular, to the chorionic epithelium of the labyrinthine portion, with maximal development of degenerative and necrobiotic changes on the 14th-16th day of pregnancy (if the poison is injected 48 h before sacrifice). An increase in the number of mitotically dividing epithelial cells is observed at all times and the dimensions of the nuclei and nucleoli of the cytotrophoblastic cells are increased on the 17th-18th day of intrauterine development of the fetus. Injection of CCl_4 leads to a decrease in the content of histochemically detectable glycogen in the cytoplasm of the chorionic epithelium and a decrease in the content of amylase-resistant glycoproteins, protein, and RNA in the degenerating cells.

KEY WORDS: *Placenta; CCl_4; necrobiotic changes.*

There is no dispute now about the protective function of the placenta, which is not restricted to its property of selective permeability [1, 6, 7, 9]. In some cases the chorionic epithelium metabolizes certain hormones and converts them into less active compounds [12], and so maintains the homeostasis of the feto-placental system. As regards the influence of hepatotropic substances on the function and structure of the placenta, the data are contradictory [8, 10, 15].

In connection with the role of the placenta in maintaining a high degree of exchange between mother and fetus, it is interesting to study the histochemical characteristics of the placenta in conjunction with the administration of CCl_4 at different stages of pregnancy.

EXPERIMENTAL METHOD

Experiments were carried out on 80 noninbred albino rats weighing initially 230-260 g. The phases of the estrous cycle and the date of the beginning of pregnancy were determined by examination of vaginal smears and the discovery of spermatozoa in the genital passages [5]. The experimental animals (48) received CCl_4 as a 50% oily solution, in a dose of 0.3 ml of the pure substance per 100 g body weight, by intragastric injection through a polyethylene tube at different stages of pregnancy (12th, 14th, 16th, and 18th days). The rats were decapitated 48 h later and the placentas fixed in 10% formalin, Carnoy's fluid, and alcohol-formalin. Dewaxed sections were stained with hematoxylin-eosin, with Schiff's reagent by McManus's method, with bromphenol blue, with Schiff's ninhydrin reagent, gallocyanin, and fast green at pH 2.0-2.2 and 8.2-8.5. Other sections were impregnated with silver by Foot's method or stained with picrofuchsin and colloidal iron by Hale's method. The staining methods and the corresponding chemical and enzymic control methods are described in Pearse's (1962) textbook. The diameter of the nuclei and nucleoli was measured with an ocular micrometer (objective 90, ocular 15) and the number of mitoses was counted in 6000 cells and expressed in promille.

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Fig. 1. Destruction and elimination of chorionic epithelium in labyrinthine portion of placenta of an albino rat 48 h after intragastric injection of CCl_4 into the mother. Hematoxylin–eosin, 600×.

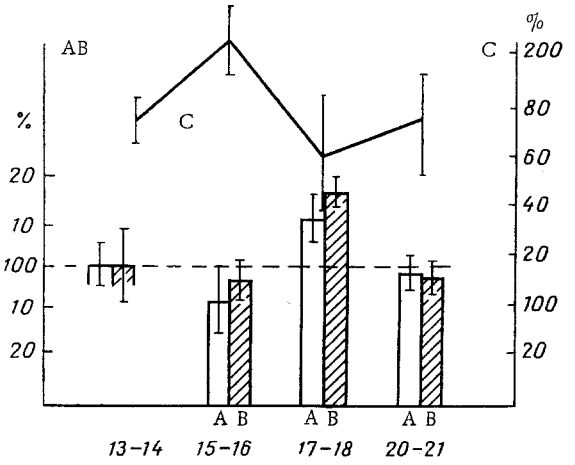

Fig. 2. Changes in dimensions of nuclei (A) and nucleoli (B) and in the number of mitotically dividing cells (C) of cytotrophoblast of labyrinthine portion of placenta of albino rat 48 h after administration of CCl_4. Ordinate, dynamics of various indices (in %); initial value in control series taken as 100%; abscissa, time of pregnancy (in days). Confidence limits calculated for $P=0.05$.

EXPERIMENTAL RESULTS

Administration of CCl_4 to the pregnant animals caused injury to the cells of the placenta: Destructive and necrobiotic changes appeared in the chorionic epithelium and, in particular, in its cytotrophoblastic component. Development of the destructuve processes reached a maximum in the labyrinthine portion of the placenta on the 14th-16th day of pregnancy, i.e., when the poison was given on the 12th-14th day of fetal development. In some places in the labyrinthine portion of the placenta maternal leukocytes accumulated in contact with destroyed placental structures. The process of destruction at that time could be so intensive that, as a result of destruction and elimination of the chorionic epithelium,

areas appeared in the spongioblast of the placenta where fetal blood cells were separated from the maternal blood stream by a single layer of endothelial cells (Fig. 1).

Injection of CCl_4 into the pregnant animals at all the above times caused a marked increase in the number of mitotically dividing cells of the chorionic epithelium, which reached a maximum in the placenta on the 14th-16th day (Fig. 2). In the later stages of development, compared with the control animals, the number of cells with large nuclei and of binuclear cells similar to diplokaryocytes of the desmochorial placenta is greater in the labyrinthine part of the placenta. A characteristic feature was the more severe damage of the labyrinthine portion of the placenta, in contact with cells of the intermediate zone.

Whereas at the beginning of the third week of pregnancy administration of CCl_4 caused destructive and necrobiotic changes to appear, after the 17th-18th day the response to injection of CCl_4 was an increase in the quantity of connective tissue in the labyrinthine portion of the placenta, where it was localized in the region of blood vessels, filling the space between the chorionic epithelium and the endothelium of the fetal capillaries and distinguished by histochemically detectable acid mucopolysaccharides and by the presence of modified reticulin fibers. In addition, cytotrophoblastic islands (18th-19th day) and nucleated fetal erythrocytes were found in the spongioblast. The size of the labyrinthine portion of the placenta was smaller at all stages of development than in the control and the zone of intermediate cells was relatively enlarged.

Histochemical study of the glycogen distribution in the placental tissue showed a marked reduction in its content 48 h after administration of CCl_4 at all times of pregnancy studied and, in particular, on the 15th-16th day. A decrease in the content of neutral glycoproteins and RNA was found in the cytoplasm of the chorionic epithelium in foci of degenerating cells. At the same time the intensity of the reaction for acid proteins increased in the structures of the labyrinth.

The dimensions of the nuclei and nucleoli of the cytotrophoblastic cells of labyrinthine portion of the placenta increased significantly 48 h after administration of CCl_4 to the mother only on the 17th-18th day of pregnancy (Fig. 2).

A characteristic feature of the lesion in the rat placenta 48 h after administration of CCl_4 to the mother was "acellular" destruction of the placental tissues, revealed by the histochemical reactions for neutral glycoproteins and acid proteins. A special feature of this destruction was the presence of positive granules of various sizes, the number and size of which grew as the degree of destruction intensified, on the surface of the placental tissue in contact with maternal blood. Considering that CCl_4 is completely eliminated from the body within 24 h after a single dose, and also the absence of cellular infiltration in the region of destruction, the destructive process described above can be interpreted as a manifestation of humoral immunological conflict, induced by a primary disturbance of the function and structure of the chorionic epithelium by CCl_4. A short latent period and the clear manifestation of the response are evidence, first, of the presence of antiplacental antibodies in rats under normal conditions in the second half of pregnancy, as other investigations have confirmed [2, 3, 11, 13] and, second, that the antibodies cannot exert their action if the structure of the chorionic epithelium of the placenta, the normal function of which is also to ensure the antigenic neutrality of the chorion in relation to the mother [2], is undamaged.

Intragastric injection of the heterotropic poison into the mother thus causes considerable injury to the placental tissues and, in particular, to the chorionic epithelium of the labyrinthine portion; this is indirect evidence of the detoxicating ability of the placenta, which reaches the maximum of its activity on the 14th-16th day of pregnancy. Incidentally, CCl_4 causes changes not only in the microsomes of liver and kidney cells, but also of placental cells [10]. The existence of a metabolic system responsible for inactivating toxic substances in the placenta is of great biological significance, for in this way the fetus is protected from toxic agents penetrating from outside into the mother or formed in her body. This is particularly important in the early stages of pregnancy, before the fetal liver has acquired any detoxicating powers.

The biological peculiarity of the histophysiology of hemochorial placentas (the group to which the human placenta belongs) also plays an important role in the development of antenatal pathology; brief exposure to a toxic substance which causes even a trivial disturbance of function of the chorionic epithelium can give rise to a "chain" reaction of immuno-

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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