ANALYSIS OF THE CONDITIONS DETERMINING PENETRATION OF PROTEINS THROUGH THE PLACENTAL BARRIER

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Sensitization of pregnant and nonpregnant females with horse serum and injection of pyrogenal into these animals were accompanied by the appearance of Cx-reactive protein in their blood. After normal pregnancy, the blood of physiologically mature young rabbits born from females sensitized with horse serum contained no Cx-reactive protein, while that of physiologically immature rabbits did. Injection of pyrogenal only into pregnant females likewise led to the appearance of Cx-reactive protein in the blood of the pregnant females and of physiologically immature young rabbits born from them.

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The question of the passage of proteins through the placental barrier still presents unsolved problems. Special investigations in our laboratory have shown that bacteria, toxoplasmas, hormones (insulin, thyroxin, adrenalin, etc.), and the low-molecular weight substance trypan blue do not pass from the maternal to the fetal blood during normal pregnancy [1-5]. However, these same investigations showed that the substances studied may pass through the placenta during pathological pregnancy when the "gestation dominant" is inhibited. Inhibition of the gestation dominant may be produced by various stress factors acting on pregnant animals, as a result of which the intrauterine development of the fetus takes place abnormally and terminates with the birth of dead, or of living yet physiologically immature, young animals [1, 4, 7]. A factor of definite interest in the analysis of placental permeability to proteins is the Cx-reactive protein formed endogenously in the body. Special investigations have shown that the appearance of Cx-reactive protein in the blood accompanies the formation of antibodies against various antigens [6, 10-13], including horse serum [6]. Injection of pyrogenal can also give rise to the formation of Cx-reactive protein [6, 11].

The object of the present investigation was to study the conditions determining the appearance of Cx-reactive protein in the blood of pregnant rabbits and of their young after injection of horse serum and pyrogenal into the mothers during normal pregnancy and when the gestation dominant is inhibited.

EXPERIMENTAL METHOD

The following series of observations were made and experiments carried out to determine Cx-reactive protein in the blood of pregnant females and newborn rabbits: 1) control group: normal pregnancy and physiologically mature newborn rabbits; 2) after sensitization of females with horse serum; 3) after sensitization of females with horse serum and creation of experimental neurosis in the animals; 4) after injection of pyrogenal into females; 5) after sensitization of females with horse serum and injection of pyrogenal into them. Two series of observations on pregnant rabbits were also made to analyze the effect of horse serum and pyrogenal on the formation of Cx-reactive protein. Cx-reactive protein was estimated by precipitation in capillary tubes [8]. The test was carried out with antiserum against Cx-reactive protein prepared by Pashinin's method [9]. Relative quantitative differences were assessed from the size of the column of precipitate in the capillary tubes in millimeters. Horse serum was injected in some experiments subcutaneously, in others intravenously in doses of 0.1-0.2 ml/kg body weight. In the experiments on nonpregnant and pregnant females, horse serum was injected 3, or occasionally 4 times at intervals of 2-3 days. The pregnant animals were sensitized during the last ten days in such a way that the last sensitizing dose was given 1-3 days before parturition. Cx-reactive protein was determined daily after each

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TABLE 1. Results of Determination of Cx-Reactive Protein in the Blood of Pregnant Rabbits and Their Young

Test object			Number	Presence (+) or absence (–) of Cx-reactive protein
Female rabbits	Nonpregnant	Sensitized with horse serum	17	+
		After injection of pyrogenal	5	+
	Pregnant	Normal pregnancy	8	–
		Sensitized with horse serum (including 10 females in which experimental neurosis was produced)	21	+
		After injection of pyrogenal	8	+
		After sensitization with horse serum and injection of pyrogenal	8	+
Newborn rabbits	Physiologically mature	From normal pregnancy	45	–
		From 7 pregnant animals sensitized with horse serum	32	–
	Physiologically immature	From 4 pregnant animals sensitized with horse serum	20	+
		From 6 pregnant animals sensitized with horse serum and in which experimental neurosis was produced*	36	+
		From 6 pregnant animals receiving pyrogenal	32	+
		From 16 pregnant animals sensitized with horse serum and receiving injection of pyrogenal	85	+

* The young rabbits from the remaining pregnant animals of this group were stillborn.

injection, the first determination being made after 24 h. Pyrogenal was injected intramuscularly in doses of 0.1, 0.2, and 0.5 mg/kg body weight once or twice. Experimental neurosis was produced by the method adopted in the Laboratory of Age Physiology and Pathology [2, 3, 5, 7]. Newborn rabbits were classified into physiologically mature and physiologically immature by the diagnostic methods adopted in the laboratory, and the animals were investigated on the 1st day after birth and also later during ontogenesis [7]. To analyze the subsequent ontogenesis, animals were left from each litter. The experiments were carried out on 22 nonpregnant and 59 pregnant rabbits, from which 250 newborn rabbits were obtained and studied.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1. Cx-reactive protein was not present in the blood of pregnant females during normal pregnancy or in the blood of physiologically mature newborn rabbits. After injection of horse serum into both nonpregnant and pregnant females, Cx-reactive protein with precipitate measuring 1-2 mm appeared in the blood next day. Intravenous injection of horse serum gave a long precipitate after 24 h, and subcutaneous injection did so more frequently on the 2nd day. On subsequent days, after reaching a maximum, the concentration of Cx-reactive protein fell and on the 3rd-4th day it could no longer be detected by the method used. After the 2nd injection the same pattern was repeated as after the 1st, although in some experiments the length of precipitate at the maximum showed a slight increase. After the 3rd and 4th injections the quantity of Cx-reactive protein increased to 3-4 mm, and although its concentration fell on subsequent days, it could still be determined in the blood for a comparatively long time, even for 10-15 days in some special cases.

After injection of pyrogenal into both nonpregnant and pregnant females, Cx-reactive protein also appeared, the length of the precipitate being 1-2 mm; the dynamics of its concentration was the same as after a single injection of horse serum. Injection of pyrogenal into a rabbit already sensitized with horse serum led to an increase in the concentration of Cx-reactive protein by 0.5-1.5 mm in the length of precipitate.

In the experiments on pregnant rabbits, 43 females were sensitized with horse serum (Table 1). In every case of normal pregnancy, no Cx-reactive protein was present in the blood of the physiologically mature newborn rabbits from sensitized females, but it was present in the blood of physiologically immature young rabbits. This was found in experiments in which an experimental neurosis was produced in sensitized pregnant females, after which the concentration of Cx-reactive protein in their blood as a rule was doubled. In four experiments sensitized females gave birth to physiologically immature young without any special additional procedures. Evidently the experimental conditions themselves had caused inhibition of the gestation dominant in these females. Experiments in which one or two injections of pyrogenal were given to the pregnant rabbits unsensitized with horse serum showed that in every case they gave birth either to dead or to physiologically immature young, with Cx-reactive protein present in their blood to give a precipitate 0.5-2 mm in length. Usually Cx-reactive protein with a precipitate of 1-3 mm was found in the blood of newborn rabbits born from pregnant females sensitized with horse serum and also receiving an injection of pyrogenal or in which an experimental neurosis was produced. A special analysis showed that the Cx-reactive protein formed in the newborn rabbits on the first day after birth continued to appear in them in some cases on the 3rd, 5th, and even the 12th day. Often the concentration of Cx-reactive protein was higher on subsequent days after birth than on the first day. These facts suggest that the Cx-reactive protein in the blood of young rabbits born in these cases may be of two types: one obtained from the mother antenatally, and one endogenous. The endogenous is formed as a result of penetration of protein antigen (horse serum) into the fetal blood. Special investigations using injection of turpentine into newborn rabbits have shown that no endogenous Cx-reactive protein is formed in them. However, as development of the young rabbits takes place, Cx-reactive protein is formed by them in larger quantities than on the first day, as other authors have also reported [10].

The results thus demonstrate that in normal pregnancy proteins present in the maternal blood do not pass through the placenta into the fetal blood. If the normal course of pregnancy is disturbed, however, the placenta becomes permeable to proteins contained in the maternal blood, and the physiologically immature young animals born under these conditions have this protein in their blood.

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