EXPERIMENTAL ANALYSIS OF THE PLACENTAL BARRIER PERMEABILITY IN TOXOPLASMOSIS UNDER CONDITIONS OF NORMAL AND PATHOLOGICAL PREGNANCY

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(Presented by Active Member of the AMN SSSR V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 56, No. 12, pp. 44-48, December, 1963
Original article submitted November 20, 1962

Contradictory opinions are held on the question of toxoplasma transfer across the placental barrier in pregnant women suffering from toxoplasmosis. Some authors maintain that in the latent form of toxoplasmosis, the fetus is infected during the pregnancy [4-8,12-15]. At the same time, in many cases of known latent toxoplasmosis, diagnosed in pregnant women, there has been no infection of the fetus [10,11,16]. The question of the actual state of permeability of the placental barrier continues to remain unresolved, not only in regard to protozoa, but also as pertains to viruses and bacteria. In the investigations of I. A. Arshavskii and coworkers, it was established that in a normal pregnancy the placental barrier is not permeable to staphyllococci or trypan blue (molecular weight 990). In all cases where inhibition of the gestation dominant was caused by the production of experimental neurosis, or by the action of large doses of staphyllococcal culture, leading to illness through sepsis, along with disruption of normal formation of the placenta there occurred disruption of the permeability of the placental barrier [1,2,3,9].

We attempted to approach a resolution of the question of the state of placental barrier permeability in toxoplasmosis, in experiments on pregnant rabbits.

EXPERIMENTAL METHOD

Infection was produced by intravenous or subcutaneous injection of toxoplasma (strain RH). The clinical picture of the illness was appraised from the changes in body temperature, in the leukocyte count, and in the sedimentation rate. In a special series of experiments, non-pregnant rabbits were subjected to a preliminary immunization before being mated. As the vaccine, we used the peritoneal exudate of white mice that had been sacrificed on the 4th day after inoculation with toxoplasma. We used 0.5 ml of the exudate, first treated thermically (at 60° for 10 min); it was injected subcutaneously 6 times, with intervals of 3 days between the injections. Disruption of the pregnancy was produced by two forms of stress actions: 1) the formation of experimental neurosis, caused by the combined (alternated) action of an automobile siren and an electric current, applied to a chamber with a corrugated metal floor; the rabbits were subjected to this action once a day (exposure of 30 min), over a course of 2-3 days; 2) the production of hypoxic hypoxia in a special barochamber, at an altitude of 5000 meters (462 mm Hg); the rabbits were also subjected to this form of action once a day (exposure of 2 h), over a course of 3 days.

Toxoplasma in the infected pregnant rabbits, and in the fetuses (in various organs), was demonstrated both through direct histological investigation, and by the method of biological assays (the test samples were put through serial passages in white mice). Microscopic investigation of smears of the fluids (blood, spinal fluid, peritoneal fluid, ventricular fluid), and also smears and impression-preparations of various organs, were all carried out with the consultation of S. G. Vasina, while the histological investigation was done with the consultation and participation of T. E. Ivanovskaya and T. F. Koga. The placenta of the experimental rabbits was also subjected to histological analysis.

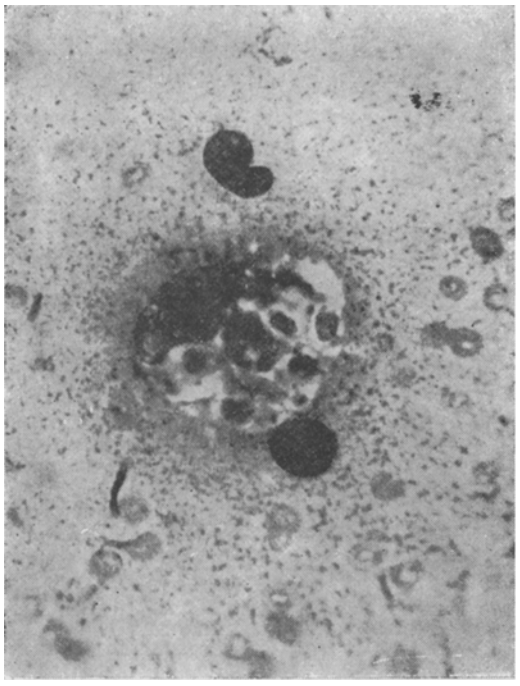

Fig. 1. Pseudocysts containing living parasites in the spleen of mice, after 2 passages from the organs of a fetus (acute toxoplasmosis 72 h after infection). Stained with hematoxylin–eosin. Obj. 90, ocul. 10.

Fig. 2. Uterine wall and maternal portion of the placenta (necrosis) from a rabbit on the 20th day of pregnancy (acute toxoplasmosis 72 h after infection). Stained with hematoxylin–eosin. Obj. 20, ocul. 5.

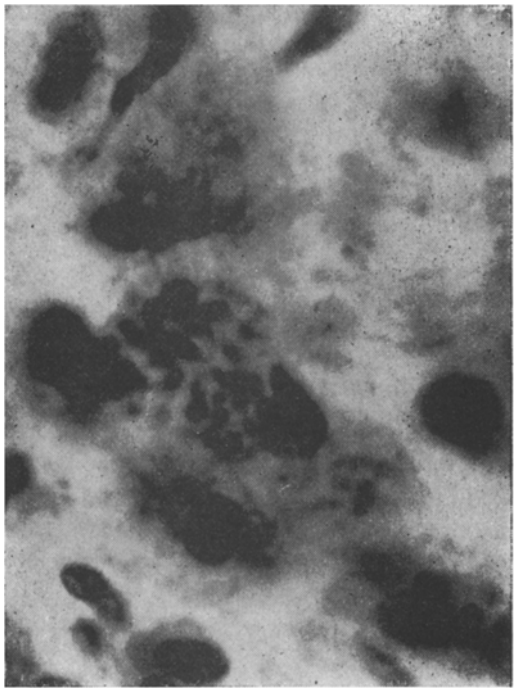

Fig. 3. Pseudocysts containing living parasites in Glisson's Capsule of a mouse liver (passage) from organs of a fetus, associated with experimental neurosis in immunized rabbits. Stained with hematoxylin-eosin. Obj. 90, ocul. 10.

EXPERIMENTAL RESULTS

In the first series of experiments, 10 rabbits with normal pregnancies were infected (acute toxoplasmosis) intravenously, by injection of toxoplasma in a dose of $2 \cdot 10^6$, at different intervals in the pregnancy between the 15th and the 25th days. After 1-2 days, clinical symptoms of the disease arose in the infected rabbits, which progressed with each day (temperature elevation, increase in the sedimentation rate and leukocytosis). Two of the rabbits were opened 24 h after the inoculation. No toxoplasma was observed in the fetuses. In four of the rabbits, 42-70 h after infection, abortions occurred. We did not demonstrate toxoplasmosis in the prematurely born fetuses (after 5 and 7 passages). In 4 rabbits, after 3-4 days, the fetuses were removed by Caeserian section. In all cases, toxoplasma was found in the fetuses only after 72 h had passed since the infection (even after 2 passages) (Fig. 1). These fetuses were characterized, in some cases, by signs of early maceration, and in others, by signs of physiological immaturity.

In the second series of experiments, the pregnant rabbits were subjected to infection (intravenous injection of toxoplasma in a dose of $2 \cdot 10^6$) one day after the preliminary production of experimental neurosis (6 animals) or the preliminary formation of a state of hypoxic hypoxia (7 animals). These forms of action were applied to the

rabbits between the 15th and the 20th days of pregnancy. After infection, the fetuses were removed by Caeserian section. In the fetuses of 12 rabbits, we found toxoplasmosis on autopsy 4 h after inoculation in the 3rd-5th passage, on autopsy 6 h after inoculation in the 3rd-5th passage, on autopsy 6 h after inoculation in the 2nd-4th passage, and on autopsy 12 h after inoculation in the 1st-3rd passage.

In both series of experiments, histological analysis of the placenta in the pregnant rabbits showed that the first signs of inflammatory reaction, in the form of small leukocytic infiltrates in the maternal portion of the placenta, appear only after 24 h following the infection. After 74-80 h, one can already see clearly manifested signs of necrotic damage to the placenta, generally partial but in individual cases, even complete (Fig. 2). From the data of the histological analysis, it follows that the permeability of the placental barrier, in experimental neurosis and in periodically caused states of hypoxic hypoxia, is disrupted long before the arisal in the same placenta of the first signs of inflammatory reaction. Thus, under conditions of normal pregnancy, the placental barrier becomes permeable to toxoplasma 72 h after infection, while by stress forms of action, this occurs only 4 h after the infection.

In the third series of experiments, 9 pregnant rabbits were immunized against the disease of toxoplasmosis prior to their being mated. At various intervals during the pregnancy, they were subcutaneously injected with toxoplasma in a dose of $6 \cdot 10^5$. The symptoms of the clinical illness, toxoplasmosis, did not arise in a single rabbit: temperature, leukocyte count, and sedimentation rate all remained normal. The normal course of the pregnancy was not disturbed. Some of the rabbits were opened at the end of the pregnancy, and the others gave birth at term. The newborn rabbits were normal in weight, and were characterized by the signs of physiological maturity.

After the births, the fetuses and newborns were sacrificed and investigated for the concentration of toxoplasma. Toxoplasma was not observed in the organs of the newborn rabbits and fetuses. Histological analysis of the placenta did not demonstrate any signs of inflammatory reaction at all. Thus, despite latent toxoplasmosis in the investigated rabbits with normal pregnancies, the placental barrier was impermeable to toxoplasma circulating in the blood of the mother.

In the fourth series of experiments, 8 pregnant rabbits, were also immunized against the disease of toxoplasmosis prior to mating, but during the pregnancy we induced experimental neurosis in them. Two immunized rabbits were inoculated subcutaneously with an injection of toxoplasma in a dose of $6 \cdot 10^5$, 30 days prior to pregnancy. On the third day, mildly manifested symptoms of the disease arose in them, which disappeared by the beginning of pregnancy. Experimental neurosis was induced in these rabbits on the 18th and 20th day of pregnancy, and in connection with this we observed the appearance of clinical symptoms of toxoplasmosis. In the remaining 6 immunized pregnant rabbits, experimental neurosis was induced on the 18th-22nd day of pregnancy. One to two days after the formation of the neurosis, they were infected by a subcutaneous injection of toxoplasma in a dose of $6 \cdot 10^5$. The clinical symptoms of toxoplasmosis arose in the rabbits. As in the case of the first 2 rabbits, caeserian section was performed on them at intervals from 1 to 4 days after the inoculation or the formation of experimental neurosis. On autopsy, in a significant portion of the fetuses we found signs of maceration. In the fetuses that were living, we detected toxoplasma (Fig. 3). Signs of inflammatory reaction were demonstrated in the placenta. In the fifth series of experiments, 6 pregnant rabbits were immunized against toxoplasmosis before mating, and on the 15th-18th day of pregnancy they were subjected to the action of hypoxic hypoxia. One day after this, they were inoculated with a subcutaneous injection of toxoplasma in a dose of $6 \cdot 10^5$. Within 1-4 days after the inoculation, we performed a Caeserian section. In the fetuses that were still alive, we discovered toxoplasma. Histological investigation of the placenta demonstrated signs of inflammatory reaction, foci of necrosis, round cell infiltrates, and toxoplasma, distributed in the placenta itself.

Thus, with latent toxoplasmosis in pregnant rabbits, in which the pregnancy was complicated by stress forms of action (experimental neurosis, hypoxic hypoxia), the permeability of the placental barrier to toxoplasma circulating in the maternal blood was disrupted.

In works of our laboratory, it was shown that anemic hypoxia during pregnancy exerts a negative effect on the developing embryo and fetus, not so much by the resultant deficit of oxygen as, above all, by inhibition of the gestation dominant. The latter is caused by the arisal of a new dominant, consisting of adaptive reactions to hypoxia [1-3]. The same thing apparently takes place in the case of hypoxic hypoxia. As shown by the investigations performed in the laboratory, inhibition of the gestation dominant through suppression of the gonadotropic functioning of the hypophysis and the hormonal functioning of the yellow body, disrupts the formation of a physiologically complete placenta. In this case, its permeability is simultaneously disrupted. It is important to note that with latent toxoplasmosis, under conditions of normal pregnancy, the toxoplasma circulating in the blood does not cause an inflammatory

reaction in the placenta. The latter arises only with disruption of the normal formation of the placenta, caused by inhibition of the gestation dominant (acute toxoplasmosis, experimental neurosis, hypoxic hypoxia).

In this report, we have not stopped on a characterization of the changes in development of the embryos and fetuses. With acute toxoplasmosis, and also with a combination of latent toxoplasmosis and experimental neurosis or hypoxic hypoxia, we observed intrauterine death and, in individual cases, deformity and conditions of physiological immaturity. The enumerated three forms of sequelae are also observed with those forms of pregnancy disturbance which are caused by experimental neurosis or hypoxic hypoxia by themselves. As shown by the data of our investigations, these sequelae are intensified in those cases where one of these forms of stress is combined with the toxoplasmosis disease process.

SUMMARY

The work was an attempt to elucidate the problem of placental barrier permeability in toxoplasmosis. Five series of experiments were set up on pregnant rabbits. The chief conclusion drawn was that during latent toxoplasmosis created by infection of pregnant rabbits which have been immunized before being mated, the placental barrier is impermeable to toxoplasma under conditions of normal pregnancy. In those cases with latent toxoplasmosis where the normal course of pregnancy was disturbed by stress (experimental neurosis, hypoxia), the placental barrier became permeable to toxoplasma.

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