EXPERIMENTAL REPRODUCTION OF POLYHYDRAMNIOS IN ANIMALS

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Sufficient data have presently been accumulated which indicate that disturbance of the outflow of the amniotic fluid is an important pathogenic factor in the development of polyhydramnios [2, 6, 7].

It was established with the use of radioactive isotopes of deuterium and tritium [8] that about 40% of the amniotic fluid is resorbed through the embryo and its remaining portion enters into the mother directly through the fetal membranes. In an attempt to elicit the role of the fetus in the development of polyhydramnios in experiments with suturing closed the mouth of white rat embryos, it was possible to produce polyhydramnios in ten cases [9]. On the other hand data are available [3, 4] which permit the consideration that in polyhydramnios the quantity and polymeric nature of the acid mucopolysaccharides in the amnion markedly increases, and this leads to disturbance of the system mucinase-mucopolysaccharides and hampers the outflow of the amniotic fluid through the fetal membranes.

In the present work we attempted to reproduce polyhydramnios in white rats by artificially blocking the outflow of the amniotic fluid through the fetal membranes into the uterine wall.

METHOD

Two series of experiments were set up. In the I series, by means of heparin, which, as is known, is an inhibitor of hyaluronidase, we attempted to reduce the activity of the enzyme and thus aggravate the conditions of outflow. In the II series we induced inflammation in the uterine wall of rats, which was accompanied by an accumulation of high-polymeric mucopolysaccharides, which increased the viscosity of the substrate through which resorption was accomplished.

The experiments of the I series were performed on white rats pregnant for 15-16 days. We injected 0.1 ml of heparin (500 IU) into the amniotic cavity by a puncture through the uterine wall and membranes. Simultaneously we injected 0.1 ml of physiological salt solution into the amniotic cavity of a fetus of another cornu for contol. After a day we measured the quantity of amniotic fluid and determined its hyaluronidase activity in conventional units by the viscosimetric method of Sawyer as modified by Natochin [5].

In the II series we induced aseptic inflammation in a small area by inserting a sterile silk thread into the uterine wall of a nongravid animal. After a month we placed the operated animals with males, and then sacrificed them at the stage of the 15-17th day of gestation. We measured the quantity of amniotic fluid and determined its hyaluronidase activity for fetuses which developed in the portion corresponding to the site of insertion of the foreign body and for fetuses from normal portions.

In both series we histologically investigated the uterine wall and membranes (staining with hematoxylin-eosin, iron hematoxylin, etc.) and also used the histochemical methods for eliciting mucopolysaccharides (staining with toluidine blue at different pH values, alcyan blue, by Hale's method, and the PAS-reaction with appropriate controls).

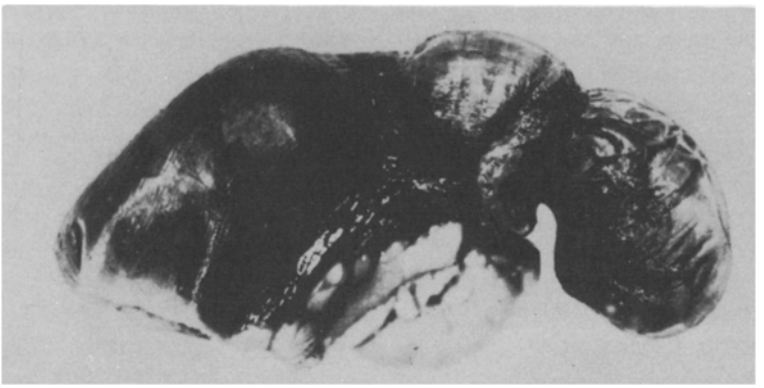

Fig. 1. Uterus of white rat. Pregnancy 15-17 days. Areas of cornu in norm (right) and after injection of heparin (left).

RESULTS

Before carrying out the experiments on reproducing polyhydramnios, in normal animals we determined the quantity and hyaluronidase activity of the amniotic fluid. It turned out that at the 15-17-day stage of pregnancy the quantity of amniotic fluid of one fetus was 0.1-0.3 ml, and its hyaluronidase activity varied within 12-18 units.

In the I series we were able to inject heparin without any side effects in 11 cases out of a large number of experiments (36). But most frequently puncturing of the uterine wall and the injection of the solution led to abortions or (if a certain quantity of heparin entered the tissue of the mother or embryo) to hemorrhage and exsanguination. The results of such experiments were not taken into account.

In 11 cases, which entered into the calculation, a day after the start of the experiment we noted an appreciable increase in the portion of the cornu of the uterus into which we injected heparin (Fig. 1). The neighboring areas, just as the area corresponding to the fetus, into the amniotic cavity of which the physiological salt solution was injected, did not substantially differ from normal.

The measurement data established that for all 11 fetuses which were injected with heparin, the quantity of amniotic fluid increased. In 5 cases the quantity of amniotic fluid was 0.8 ml; in 4, 0.7 ml; in 2, 0.6 ml. The quantity of amniotic fluid of the control fetuses was 0.2-0.3 ml, i.e., it varied within limits established for normal fetuses at the given stage of pregnancy. Thus, in our experiments the injection of heparin caused an increase in the quantity of amniotic fluid by more than two times.

The results of determining the hyaluronidase activity of the amniotic fluid were quite indicative. For fluids into whose amniotic cavity we injected heparin, the activity of the enzyme was zero. For the control fetuses which were injected with physiological salt solution, the hyaluronidase activity in comparison with the norm did not change, 12-18 units. These figures show that under the effect of heparin there is a marked drop of hyaluronidase activity of the amniotic fluid.

In the histochemical investigation of the fetal membranes it was not possible to note any changes in comparison with the norm. As is known, in rodents the extraplacental chorion at early stages is replaced by the wall of the yolk sac which forms the omphaloplacenta. Absorption of the amniotic fluid occurs through the amnion, omphalo-amniotic space (corresponding to the amniochorial space of the human fetus), and wall of the yolk sac. Under normal conditions mucopolysaccharides are elicited in the wall of the amnion which yield a PAS-reaction, and in a smaller quantity are stained by Hale's method, alcyan blue, and toluidine blue. In the wall of the yolk sac are found mucopolysaccharides which react positively when setting up the PAS-reaction. The conducted blocking of the aldehyde and 1,2-glycol groups in reproducing the PAS-reaction gives us grounds to relegate these mucopolysaccharides to the cleavage products or incomplete synthesis of acid mucopolysaccharides [1]. In the omphalo-amniotic space we found small Hale-positive granules which formed as a result of coagulation of protein-carbohydrate complexes of the viscous fluid found here.

This description is also completely valid for fetuses into whose amniotic cavity we injected heparin. We did not note substantial changes in the quantity and distribution of mucopolysaccharides in the membranes through which

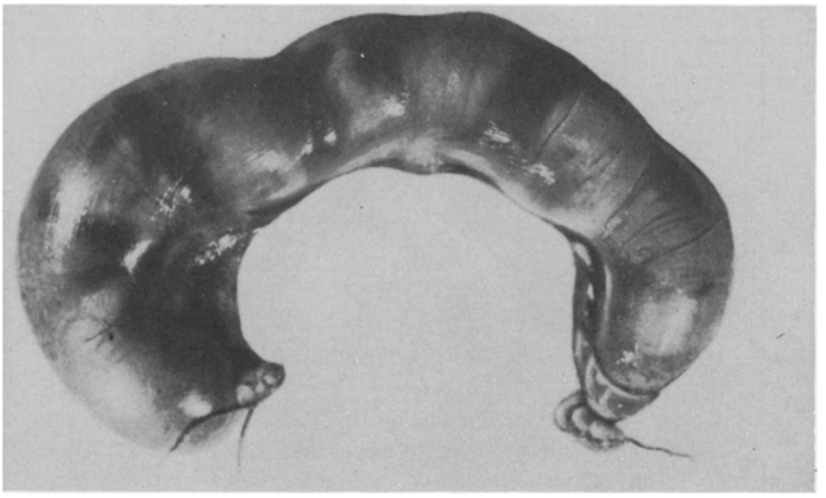

Fig. 2. Uterus of white rat. Pregnancy 15-17 days. Portions of the cornu in the norm (right) and after insertion of foreign body (left).

resorption occurred. Consequently, the delay of resorption of the amniotic fluid is not associated with changes in the membranes or embryo.

In the II series of experiments we ran into a number of difficulties when attempting to induce polyhydramnios. In most cases in the region of the uterine cornu where aseptic inflammation was induced, implantation of the embryo did not occur or the embryos were altered in comparison with the control. In those cases where disturbances in the development of the embryo were observed, the shifts in the resorption of the amniotic fluid could depend not only on changes in the fetal membranes but also on changes of the embryo itself. Convincing data were obtained only in two cases, which are described below.

At autopsy on animals at the 15-17-day stage of pregnancy, when we examined the uterus we noted an increase of that portion of the cornu into which the foreign body was inserted in comparison with neighboring regions (Fig. 2).

A measurement of the quantity of amniotic fluid yielded the following results: the quantity was 0.7 and 0.75 ml for fetuses which developed in the portion where aseptic inflammation was induced; for the remaining fetuses the quantity of amniotic fluid varied from 0.2 to 0.3 ml, i.e., corresponding to the figures characteristic for normally occurring pregnancy.

The hyaluronidase activity of the amniotic fluid for the fetuses under consideration was 16 and 18 units. For the other fetuses the activity of hyaluronidase was 14-15 units. These figures do not greatly differ from the values characteristic for normal pregnancy of white rats. The uterine wall in the region where the thread was inserted was markedly thickened in comparison with the norm, mainly due to development of the connective tissue of the endometrium. An accumulation of young fibroblasts and active macrophages, as well as hematogenic elements of the lymphocyte and special leukocyte type, was noted.

In the histochemical investigation of the fetal membranes and uterine wall significant deviations from the norm were noted in the extraplacental region in both cases. In addition to mucopolysaccharides, stained by means of the PAS-reaction, in the wall of the amnion, yolk sac, and omphalo-amniotic space we elicited, in a considerable quantity, substances which yielded a positive reaction with colloidal iron (Hale method) and β-metachromasia upon staining with toluidine blue. The greatest quantity of these substances was elicited in the omphalo-amniotic space, where under normal conditions only individual Hale-positive granules were demonstrated, whereas in the described cases large accumulations of granules could be noted. We also found a noticeable predominance, in comparison with the norm, of the number of young fibroblasts in the stroma of the membranes and omphalo-amniotic space.

Thus, in the two described methodologically successful experiments, the delay of resorption of the amniotic fluid was not associated with a change of their hyaluronidase activity and is explained, apparently, by the above-described changes in the chemism of the amorphous substance of the fetal membranes.

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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 this issue.**