

EXPERIMENTAL REPRODUCTION OF HEMOLYTIC DISEASE OF THE NEWBORN IN LOWER MONKEYS

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L. S. Volkova, M. Sh. Verbitskii, and A. V. Andreeva

Laboratory of Pathological Embryology (Head, Docent L. S. Volkova),
Institute of Experimental Biology (Director, Professor I. N. Maiskii)
of the Academy of Medical Sciences of the USSR, Moscow, and Institute
of Experimental Pathology and Therapy (Director, Professor B. A. Lapin)
of the Academy of Medical Sciences of the USSR, Sukhumi
(Presented by Active Member of the Academy of Medical Sciences of the USSR
N. N. Zhukov-Verezhnikov)

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There are no reports in the literature of hemolytic disease of newborn monkeys in natural or experimental conditions.

The experimental reproduction of hemolytic disease of the newborn in lower monkeys is difficult because of the scarcity and the contradictory nature of the information concerning differentiation of the blood groups in animals of this species.

The object of the present investigation was to attempt to reproduce hemolytic disease in newborn monkeys.

EXPERIMENTAL METHOD AND RESULTS

In recent years the authors have made a serological investigation of different species of monkeys (baboons, macaques, mandrills, green guenons, mangobeys, geladas, and hybrids: Papio hamadryas and gelada, P. hamadryas and P. anubis), kept in the Sukhumi nursery, and have established the presence of intra- and interspecies blood differences [1, 2]. On the basis of these results pairs of parent monkeys were chosen which were antigenically incompatible (the male's erythrocytes contained an antigen not present in the female; the female's blood serum agglutinated the male's erythrocytes) in order to obtain offspring with hemolytic disease.

Experiments were conducted on baboons (P. hamadryas). The degree of immunological incompatibility between the embryo and mother was increased by immunizing the female with the erythrocytes of the male (before mating and during pregnancy).

The outcome of pregnancy, and also the results of serological, hematological, and pathological investigations which were made, confirmed the development of hemolytic disease of the newborn in the young of these parents.

The results of these observations are described below (taken from actual case notes).

1. The female Taksa was immunized with the erythrocytes of the male Koldun, with whom she was subsequently mated.

In the first cycle three intramuscular injections were given, each of 2.5 ml of a 50% suspension of triple-washed (in physiological saline) erythrocytes at intervals of 2-3 days. On the 59th day of pregnancy (the mean length of pregnancy in P. hamadryas is 184 days) a further two injections of erythrocytes of the same male were given in the same dose, at an interval of 2 days.

Labor occurred at term, but the mother gave birth to a stillborn fetus with icterus of the skin and the visible mucous membranes. Hematological investigations revealed an extremely high bilirubinemia — 409.6 mg % (nor-

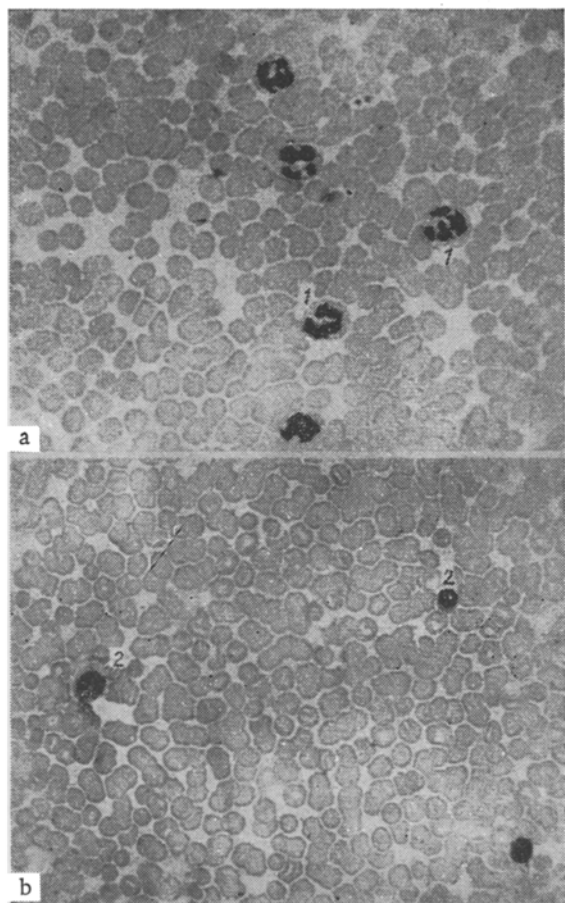


Fig. 1. Film of peripheral blood of a normal newborn *Papio hamadryas* (a) and of a stillborn animal dying from hemolytic disease of the newborn (b). 1) Leukocytes; 2) erythroblasts. Stained by May-Gruenwald method. Objective 90 \times , ocular 10 \times .

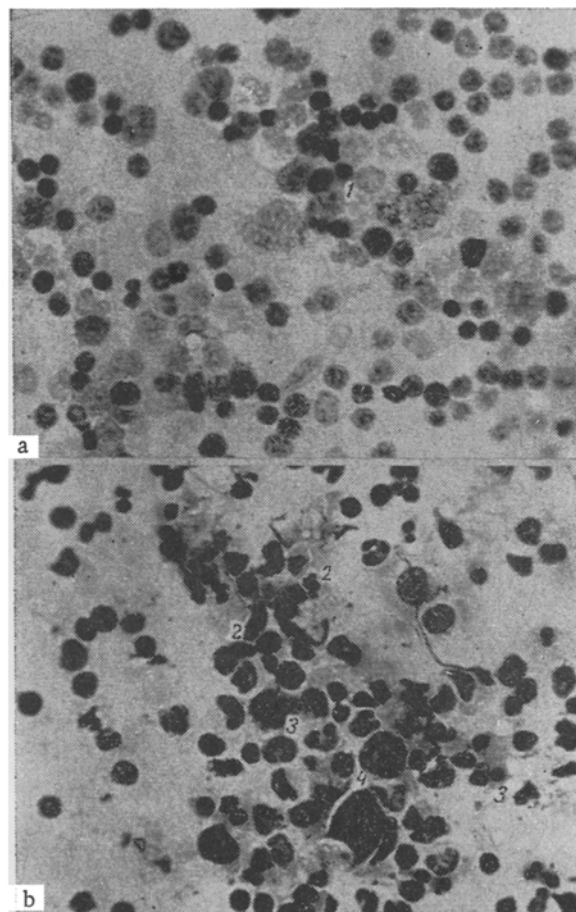


Fig. 2. Film of bone marrow of a stillborn *P. hamadryas* dying from birth trauma (a) and from hemolytic disease of the newborn (b). 1) Erythroblast (normoblast); 2) erythroblasts in stage of karyorrhexis; 3) proerythroblasts; 4) normoblasts. Stained by May-Gruenwald method. Objective 90 \times , ocular 10 \times .

mal 12.8 mg%). The hemoglobin concentration (120%) and the erythrocyte count in the blood of the newborn fetus (5,920,000 per mm^3) were normal. Anisocytosis and poikilocytosis with polychromatophilia and marked erythroblastosis were observed in the peripheral blood; many erythroblasts with karyorrhexis were found (Fig. 1a, b). In the bone marrow erythroid hemopoiesis predominated over myeloid (Fig. 2a, b). In impressions of the tissues of the liver, spleen, and lymph glands foci of extramedullary hemopoiesis were found. Crystals of brown pigment were seen in the liver cells (Fig. 3a, b). The serological findings were as follows: in the ABO system the mother's blood was $O\alpha\beta$ and the father's AO. Before immunization of the female with the male's erythrocytes the agglutination reaction between her serum and these erythrocytes was negative.

One week before labor the agglutination reaction in a saline medium likewise was negative, while in a colloidal medium (gelatine) the titer of anti-erythrocyte (against the male's erythrocytes) antibodies was 1:4, and in the reaction with trypsinized erythrocytes it was 1:16. On the day of labor, and 1 week later, antibodies were detected in the mother's serum in the saline medium (in a titer of 1:8), while in the gelatine medium they were found on the day of labor in the following titers: with the father's erythrocytes 1:32, and with the erythrocytes of the fetus 1:8.

Complete antibodies to the father's erythrocytes were found in the colostrum. The fetal erythrocytes gave a positive direct Coombs' test. In addition, A antigen, present in the father's erythrocytes but absent from the mother's serum, was detected in the blood of the newborn fetus. It is interesting that a week after birth, incomplete antibodies were found in the mother's blood in a titer of 1:64, indicating additional sensitizing of the mother taking place at the moment of labor. Similar results were obtained in other investigations.

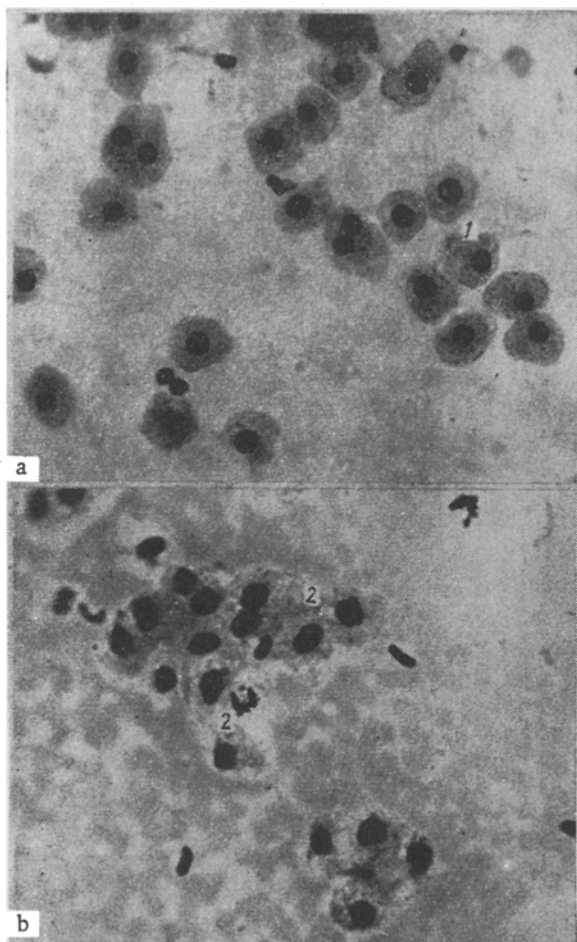


Fig. 3. Impression from the liver of a stillborn P. hamadryas fetus dying (a) from birth trauma, and (b) from hemolytic disease of the newborn: 1) liver cell; 2) crystals of pigment in cytoplasm of liver cells. Stained by May-Gruenwald method. Objective 90 x, ocular 10 x.

At the end of the first half of pregnancy the female had a blood-stained discharge. On the 89th day of pregnancy she aborted. The skin and visible mucous membranes of the fetus was icteric. Serological and hematological analysis of the fetus could not be carried out. However, the changes in the titer of antibodies in the mother's blood serum also confirmed beyond doubt that death of the fetus was due to its immunological incompatibility with the mother. For instance, the titer of antibodies in the mother to the erythrocytes of this male 2 days after abortion was 1:2, 1.5 months after — 1:32, and 2 months after — 1:8.

Hence, for the first time, hemolytic disease of the newborn has been produced experimentally in Papio hamadryas (and in monkeys in general).

SUMMARY

The article describes the author's experiments with artificial induction in lower monkeys (Papio hamadryas) of a hemolytic disease of newborn. The experiments were made on monkey couples — sires and dams — incompatible in the isoantigenic respect. An increase in the immunologic incompatibility between the fetus and the maternal body was achieved by immunization of females with blood erythrocytes from the males with which they had been mated. The possibility of induction of hemolytic disease of newborn in lower monkeys has been proved for the first time.

Analysis of the results described shows that a diagnosis of hemolytic disease of the newborn could be made in the stillborn fetus. It is important to note that all five previous pregnancies of this female with other males had terminated in the birth of a healthy offspring.

2. The female baboon Andaluziya was immunized with the erythrocytes of the male Karabas, with whom she was subsequently mated. The first pregnancy of this female had taken place in 1961 (with another male) and had ended in the birth of a normal offspring at term. The present pregnancy was the second. In the ABO system the mother's blood was Oo and the father's also Oo, but the mother's serum agglutinated the father's erythrocytes even before immunization. A living fetus was born at term, but died 24 h later. Its skin and visible mucous membranes were icteric. In the pathologist's opinion the fetus died as a result of a cerebral hemorrhage.

Antibodies against the father's erythrocytes were found in the mother's blood serum in a titer of 1:8, and antibodies against the fetal erythrocytes in a titer of 1:16, whereas in a control test with the erythrocytes of other monkeys no agglutination took place.

Hence, in this case also, the clinical picture and serological analysis gave grounds for the diagnosis of hemolytic disease of the newborn, from which the fetus died.

3. The female Pura was immunized with erythrocytes of the male Koldun with whom she was subsequently mated.

In the ABO system the mother's blood was O α and the father's Ao. Before immunization the titer of antibodies in the mother's serum to antigens of the father's erythrocytes was 1:2. The history showed that this female had two previous pregnancies with this male, the first of which (in 1960) had ended in still birth on the 147th day of pregnancy, and the second (also in 1960) in birth of a healthy offspring at term. The present pregnancy was her third.

LITERATURE CITED

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