

AN EXPERIMENTAL STUDY OF HAEMOLYTIC DISEASE
OF THE NEWBORN DUE TO ISOIMMUNIZATION
OF PREGNANCY

II. REPORT OF AN ATTEMPT TO PRODUCE ANTI-RED CELL
ISOANTIBODIES IN GUINEA-PIGS, TOGETHER WITH A NOTE
ON THE BLOOD PICTURE OF ONE-DAY-OLD GUINEA-PIGS

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INTRODUCTION

In a previous paper (Heard, Hinde & Mynors, 1949) an attempt was made to produce the syndrome of haemolytic disease of the newborn in rabbits, with the object of studying experimentally some of the features manifest in the naturally occurring human disease. Although *in vivo* sensitization of the red cells of newborn rabbits was produced, the young rabbits appeared clinically unaffected and developed normally.

Before undertaking further experiments with the rabbit, it was thought expedient to repeat the investigation in another animal species. The longer gestation period of the guinea-pig and the greater maturity of its haemopoietic system at birth, together with its general convenience as a laboratory animal, commended it for the purpose.

The experimental procedures adopted with the guinea-pig were modelled closely on those used with the rabbit. Guinea-pig does were mated to bucks with whose blood they had previously been inoculated. After each course of inoculations, and each pregnancy, the sera of the does were examined for antibodies against the red cells of the corresponding buck. The red cells of their offspring were also tested for evidence of *in vivo* sensitization by maternal antibody. A full blood count was carried out on all newborn guinea-pigs between 12 and 24 hr. after birth, and they were kept under observation for any clinical manifestations of disease.

In no case could isoantibodies be detected in the post inoculation or post partum sera from these guinea-pig does, nor, save in one litter, was there any evidence of

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in vivo sensitization of the red cells of their offspring at birth. Even in the single exception in which the direct anti-globulin test on the red cells of a litter of guinea-pigs was positive at birth, no isoantibodies could be detected in the maternal serum and the doe died soon after from advanced pasteurellosis.

There was no sign of clinical illness or anaemia in any of the newborn guinea-pigs.

EXPERIMENTAL STUDY

(a) *Outline of experiments*

The guinea-pigs used were apparently unrelated animals selected from the department stock. Their ages ranged from 6 to 18 months. For the first experiment twelve does were divided into three groups of four and each group received a course of intraperitoneal injections of citrated whole blood from the particular buck with which that group was subsequently mated. The experiment was carried through three pregnancies, a fresh course of inoculations being given during each period of gestation.

In the second experiment a group of twenty-five does was used. Each doe received two courses of intraperitoneal injections of citrated whole blood from at least ten bucks, the second course commencing 10 weeks after the last injection of the first course. Each doe was then mated with the buck from which she had received most blood. A further course of inoculation of the same buck's blood was given during the period of gestation.

In both experiments a course of inoculations consisted, with minor differences, of eight intraperitoneal injections at 2-day intervals of citrated whole blood in amounts increasing from 0.25 to 2.0 ml.

Samples of blood were obtained from each doe by cardiac puncture on the tenth day after the completion of each course of inoculation and on the fourth day of each puerperium. Serum from each of these samples of blood was examined for antibodies against the red cells of the corresponding bucks.

Samples of blood were also obtained, by cardiac puncture, from all the newborn guinea-pigs when they were between 12 and 24 hr. old, and the red cells examined for evidence of *in vivo* sensitization by maternal antibodies. A complete blood count was also carried out on each of these samples of blood.

(b) *Methods*

(i) *Serological methods.*

The sera of the does were examined for red cell immune isoantibodies by the following methods:

(a) Direct agglutination.

(b) Indirect antiglobulin sensitization test, using a rabbit anti-guinea-pig globulin serum.

(c) The trypsin method for detecting antibody sensitization.

(d) Immune haemolysis, using both guinea-pig and rabbit complement.

The red cells of the newborn guinea-pigs were examined for *in vivo* isosensitization by:

(e) The direct antiglobulin sensitization test.

(f) The trypsin method. (This test was used on the blood of the offspring of the second experiment only.)

The techniques of the direct agglutination, indirect and direct anti-globulin sensitization tests were those described in the previous paper (Heard *et al.* 1949), except that, in the present experiments, a solution of 0.9% sodium chloride was used for suspending the red cells and diluting the sera. In the previous experiments with rabbits, 3.33% solution of magnesium sulphate had been used as the diluent. All tests were performed at 37° C., and many were also repeated both at room temperature and at 4° C.

The rabbit anti-guinea-pig globulin serum was prepared by inoculating rabbits with guinea-pig serum and absorbing the resulting antiserum with well-washed guinea-pig red cells, until free of agglutinins for these cells. The ability of this serum to detect guinea-pig antibodies adsorbed on bovine red cells is described elsewhere (Gleeson-White, Heard, Mynors & Coombs, 1950).

The technique used for the trypsin test (Morton & Pickles, 1947) is also described in the above-mentioned paper (Gleeson-White *et al.* 1950). The trypsin reagent itself was controlled with tests using human red cells sensitized with 'incomplete' Rh antibody.

The tests for immune haemolysins were performed with 4–8 M.C.D. of the complements as standardized with a system of sheep red cells and three minimal haemolytic doses of a horse anti-sheep red cell haemolysin.

(ii) *Haematological methods.*

Blood was obtained by cardiac puncture and added to a bottle containing a suitable amount of Wintrobe's oxalate mixture. Total red and white cell counts were performed in the usual manner. For the haemoglobin estimations 0.1 ml. of the oxalated blood was diluted with 20 ml. 0.4% ammonia solution and the haemoglobin content determined with a photoelectric colorimeter. Blood smears were stained with Leishman's solution and reticulocyte smears with brilliant cresyl blue.

RESULTS

(a) *General observations*

In the first experiment, all twelve does were apparently in good condition at the end of their second pregnancy. One guinea-pig (no. 1856), however, died 3 months later without becoming pregnant again, and three other does died during or after their third pregnancy, from causes unconnected with this experiment.

The second experiment was complicated by an outbreak of infection affecting nearly half of the experimental does and a high proportion of stock does housed in the same room. The infected animals either aborted shortly before term or gave birth to full-term still-born offspring. Retention of placentae and products of conception was common and many animals showed delayed involution of the uterus. Of the twenty-five experimental does, eleven aborted and seven died. *Salmonella typhimurium* was isolated from the heart blood of one of these and from two of the stock does.

Post-partum samples of serum from the twelve does in the first experiment and twenty-two of the twenty-five does in the second experiment were examined for immune isoantibodies.

There were eighty-seven live and two still-births in the first experiment, and twenty-four live and twenty-eight still-births in the second. Direct antiglobulin sensitization tests were carried out on samples of blood from each of the one hundred and eleven live births and on twenty-two of the thirty still-births.

(b) Serological results

In no case could an immune anti-red cell isoantibody be demonstrated in a doe's serum by any of the methods used.

The direct sensitization test performed on the red cells of their offspring was always negative except in the case of one family of three, all of which gave a positive test which remained positive for over 3 weeks. The affected litter was one in the second series of pregnancies of the first experiment. The doe (no. 1856) never produced any demonstrable antibodies against the buck's cells. She had no young after the first mating and she died from advanced pasteurellosis 3 months later without becoming pregnant again.

The interpretation of the positive direct sensitization test in the three offspring of this doe is not clear. It may well be related to the maternal infection. It would not appear to be due to sensitization by maternal anti-red cell antibodies as no such antibodies could be detected in the serum of the doe.

(c) Haematological findings

The offspring from the three series of litters of the first experiment may be considered together with the offspring born during the second experiment as the same examinations were made.

Blood smears and reticulocyte counts were made from all the live offspring, and blood smears from ten of the thirty stillborn young were stained and examined. Table 1 records the results of the full blood counts made on ninety-eight of the live offspring. The results are compared in the same table with figures obtained from the examination of fifteen newborn guinea-pigs aged between 12 and 24 hr., and born of untreated mothers. These fifteen control animals were killed and differential counts were made on smears of their bone marrow.

The figures for the haemoglobin and red blood cell counts are significantly lower in the experimental than in the control animals, but there is no rise in the reticulocyte count and no appearance of nucleated red blood corpuscles in the peripheral blood to indicate that this is due to rapid blood destruction or replacement. The apparent increase in the figures for the white blood corpuscles of the experimental group is not significant. The three young guinea-pigs of doe no. 1856 giving positive serological results had further haematological examinations at 3 and 10 days, but nothing abnormal was found.

Klieneberger (1927) gives a full account of the haematological findings in the adult guinea-pig, but does not discuss the picture in the newborn animal. It is

partly for this reason that we have included our findings in this small series of newborn guinea-pigs.

Table 1. *Blood levels in guinea-pigs aged between 12 and 24 hr.*

Blood	Normal guinea-pigs			Experimental guinea-pigs		
	No. of observations	Average	Standard error	No. of observations	Average	Standard error
Haemoglobin (g./100 ml.)	13	15.48	0.35	95	14.33	0.13
Red blood cells (millions/c.mm.)	16	5.92	0.18	96	5.34	0.06
White blood cells (c.mm.)	16	1575 Polymorphs, 54 % Lymphocytes, 45 % Monocytes, 1 %	207	91	1865	89
Reticulocytes (% of red blood cells)	—	0.8	—	—	0.8	—
Nucleated red blood cells in blood film	—	None	—	—	None	—
Marrow smears						
Erythroid cells (%)	15	70 %	—	—	—	—
Myeloid cells (%)	15	30 %	—	—	—	—

DISCUSSION

The interesting observation which has resulted from this study has been the failure to produce demonstrable anti-red cell isoantibodies in thirty-six guinea-pigs by repeated inoculations of random bloods.

When no antibodies were produced in the first series of animals the second experiment was planned in an endeavour to exclude the possibility that only compatible blood had been given in Exp. 1. It was thought that if each doe received injections of blood from at least ten donors the chances that all the injections would be compatible would be considerably lessened.

The guinea-pigs used were unlikely to have originated from a small nucleus of inbred animals as the department stock from which they were taken had been collected over a period of years from many different sources, many of these not being in the immediate neighbourhood of Cambridge.

There do not seem to be many published references to the study of guinea-pig blood groups. However, Hektoen (1907), Weszeczky (1920) and Lawson & Redfield (1930) all failed to demonstrate naturally occurring red cell isoagglutinins in guinea-pig sera. Again Boyd & Walker (1934) failed to show blood-group differences in guinea-pigs with the use of 'immune' rabbit anti-guinea-pig red cell sera, but only very few animals were used.

If further work confirms this apparent lack of intra-species blood groups in the guinea-pig, a detailed study of the antigenic constituents of their red cells will become a matter of considerable theoretical and practical importance.

SUMMARY

1. Repeated inoculation of thirty-six guinea-pig does with random blood of guinea-pig bucks failed to produce any demonstrable anti-red cell isoantibodies.
2. A note is included on the blood picture of a small series of newborn guinea-pigs aged between 12 and 24 hr.

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