THE INFLUENCE OF PREGNANCY UPON THE TITRE OF IMMUNE (BLOOD-GROUP) ANTIBODIES IN THE RABBIT

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We have previously described two agglutinogens, $H_1$ and $H_2$, which may be found in normal rabbit red-blood cells. These agglutinogens are determined genetically by allelomorphs or alternative forms of the same gene, and each, when acting alone, behaves in heredity as a simple dominant unit-character. Each of the corresponding agglutinins ($h_1$ and $h_2$) is a specific antibody produced as an immunity response when red-blood cells carrying the agglutinogen are repeatedly injected into a rabbit lacking that agglutinogen in its erythrocytes.

We have also investigated the fate of embryos which had inherited from their fathers red-cell agglutinogens, $H_1$ or $H_2$, antagonistic to the immune agglutinins, $h_1$ or $h_2$, developed in the sera of their mothers before and during pregnancy. During the course of these investigations we discovered in the agglutinin titre of pregnant rabbits certain fluctuations of greater magnitude than those which we had observed in non-pregnant rabbits. For example, rabbit No. 202, which lacked agglutinogens, was mated to an $H_1$ buck and injected bi-weekly with $H_1H_2$ blood. Six days later she possessed both $h_1$ and $h_2$ agglutinins which gave a single + reaction in a 1/1 dilution. In spite of continued injections of $H_1H_2$ blood, neither agglutinin could be detected upon the 20th and 29th days. Injections had been discontinued on the 25th day. Parturition took place upon the 30th day. Upon the 35th day both agglutinins $h_1$ and $h_2$ were again present and again gave a single + reaction in a 1/1 dilution. This doe was then mated to an $H_1H_2$ buck and bi-weekly injections were resumed. Twenty-seven days later she was found to possess neither agglutinin, as was still the case just after parturition upon the 31st day and also 6 days subsequent to parturition.

These results suggested that some factor concerned with pregnancy might be responsible for the loss of titre observed, since agglutinins produced in males were normally retained during long periods of time with little change in titre.

To determine whether the differential factor might be genetic constitution, five test matings were followed, employing three does lacking agglutinogens, mated with bucks possessing a single agglutinogen. The females were injected bi-weekly with the red cells from about 5 cc. of $H_1H_2$ blood, and later their sera were tested for strength of agglutinins, employing a 1/1 dilution. Injection was suspended 5 days prior to parturition. The
results of these experiments are shown in figure 1, in which strength of titre is plotted against duration of pregnancy, or the period of potential pregnancy, 31 days in duration.

In the first experiment (top of Fig. 1), it will be seen that doe A196 developed a 5+ titre of $h_2$ agglutinin and a $3+$ titre of $h_1$ agglutinin, without loss of titre until injections were suspended near the close of the pseudo-pregnant period, the beginning and ending of which are indicated in the figure, each by a vertical line, for it turned out that she was not pregnant. The time at which injections were suspended is indicated by a dot just above the base line in each experiment diagrammed in figure 1.

In the second experiment (Fig. 1, next to top) the doe A5 showed during pregnancy a decrease in $h_2$ titre but with partial recovery of titre without further injection following parturition. In a second mating (diagrammed immediately below the foregoing) she lost $h_2$ agglutinin completely but recovered it to some extent following parturition. Her $h_1$ titre rose during the early part of pregnancy but later dropped, only to rise again following parturition. In the fourth experiment, the doe A89 maintained titre of the two agglutinins rather constantly, with $h_2$ dropping at parturition, and both agglutinins showing an increase following parturition. In a second pregnancy (lowest diagram in Fig. 1) A89 lost much of her $h_1$ titre during pregnancy, but both agglutinins increased following parturition.

If we consider the genetic constitution of the male used in each of these matings, we find that in the first mating of A5, an $H_2$ buck was employed, and the titre of $h_2$ agglutinin was diminished. In her second mating an $H_1$ buck was used, and her high titre of $h_1$ agglutinin was reduced only to in-
crease greatly after parturition. In the first mating of A89 an $H_1$ buck was used, and her $h_1$ agglutinin showed the greatest post-parturitional increase. In her second mating also an $H_1$ buck was employed, and subsequently her $h_1$ agglutinin showed a considerable decrease in titre but recovered after parturition.

These data suggest that possibly the genetic constitution of the male, and hence that of the embryos, may have something to do with the decrease in titre of agglutinins in pregnant rabbits. But how could this be possible? We have shown elsewhere that $H_1$ and $H_2$ bucks such as we have employed in these matings will transmit to their offspring as dominant unit-characters the agglutinogens which they possess, and we have further shown that rabbit embryos develop these agglutinogens very early within their erythroblasts. We have also shown that these maternal agglutinins traverse the placenta and may be found in the circulation of newborn young of the appropriate genetic constitution. If the agglutinin of the mother is neutralized by the antagonistic red cells of the growing embryos in sufficient quantity, we may expect a drop in the mother's titre of that agglutinin only for which her embryos have inherited an antagonistic agglutinogen from their father.

Because the method which we employed of measuring increase in titre will suffice merely for weak titres where no prezone is encountered, and because only one or two points had been determined for each pregnancy, it was decided to use the same three does in further tests of the same nature but employing several refinements of technique. Titres were determined more often. Tests were made in dilution, and the highest dilution at which
a good reaction was observed was taken as a point on the curve of titre strength. Each point so determined was labeled with the strength of reaction at that dilution. This strength was usually single + but might vary up to 3+.

Eleven mating tests were made, employing the refined methods detailed above. Five of these matings proved sterile. The weaker \( (h_1) \) agglutinin was present at the time of mating in two cases only. But in all cases the \( h_1 \) titre rose during the period of potential pregnancy. The stronger \( (h_2) \) serum in these sterile matings rose greatly during the pseudo-

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**TABLE 1**

Strength of reaction for \( h_2 \) in doe A5 when not pregnant.

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**TABLE 2**

Strength of reaction for \( h_1 \) in doe A5 when pregnant.

A good example of this rise is shown in table 1, in which the strength of reaction of the \( h_2 \) agglutinin at different dilutions is given for certain days before, during and after pseudo-pregnancy in doe A5.

For comparison we have shown in table 2 the strength of reaction of the same doe for \( h_1 \) agglutinin before, during and after a real pregnancy. Here it will be seen that the titre steadily decreases during pregnancy, but begins to recover after parturition. In the five sterile matings was found the same tendency to increase in titre to a maximum so long as injections continued, which tendency we had observed previously in the sterile mating of A196, recorded by earlier and less refined methods, and which we have seen also in the production of agglutinins where males and unmated females have been employed as recipients.
During the six fertile pregnancies there was observed a tendency of the titres of both agglutinins, $h_1$ and $h_2$, to decrease, especially during the second and third weeks. This lowering of titre is followed by a rise which may be evident during the last week of pregnancy (injection having been suspended) but is quite marked several days subsequent to parturition. An example of this lowering of titre during pregnancy (already referred to) is given in table 2, in which the strength of reaction of $h_1$ agglutinin at different dilutions is given for certain days before, during and after a true pregnancy in doe A5. This doe was pregnant by an $H_1$ buck, whereas A89 and A196 had been pregnant by an $H_2$ buck. The second pregnancy of each of these three does was by a 0 buck.

The fact that pregnancies produced by matings to 0 bucks show the same lowering of titre as matings to $H_1$ and $H_2$ bucks, proves conclusively that the lowering of titre is a phenomenon of pregnancy per se and is independent of the genetic constitution of the embryos being carried by the mother.

Graphs were prepared for titre strength of agglutinins, $h_1$ and $h_2$, for each of the six pregnancies and also for the five pseudo-pregnancies, plotting titre in highest effective dilution against duration of pregnancy or pseudo-pregnancy in days. From these graphs the average titre was computed for the day of mating, and subsequently for the 5th, 10th, 15th, 20th, 25th and 30th days. This procedure gave us the averages used in figure 2, in which strength of agglutinin is plotted against duration of the pregnant period. The average $h_1$ titre for non-pregnant periods rises from 1.3 to 3.6, and the average $h_2$ titre for non-pregnant periods rises from 6.5 to 27.5. The average $h_1$ titre for pregnant periods drops from 7.7 to 2.0 and then rises, after the suspension of injections, to 13.3. The average $h_2$ titre for pregnant periods decreases from 8.0 to 6.2 and then rises, after the suspension of injections, to 12.4.

Several explanations are possible. One is that the young during their development produce some substance which passes via placenta into the maternal blood stream, capable of preventing the elaboration of agglutinins. Another is that the embryo uses up some substance of the mother’s blood serum which traverses the placenta and is necessary for the production of antibodies. This latter explanation seems more plausible in view of our previous findings that newborn rabbits of the appropriate genetic constitution for producing agglutinins $h_1$ and $h_2$ lack some substance necessary for their production.

In connection with our results, a note of Puccioni is of interest. This author reported a post-parturitional rise in titre of $\alpha$ and $\beta$ normal agglutinins in women regardless of the blood group of the child.

Summary.—Female rabbits decrease markedly in their capacity to produce the immune (blood-group) antibodies, $h_1$ and $h_2$, during the second
and third weeks of pregnancy, but there follows a sharp increase in titre of the antibodies, which is especially evident during the first few days after parturition. That this increase in antibody titre is not directly the result of neutralization by incompatible embryos is shown by the fact that pregnancies in which compatible embryos only are involved, exhibit the same titre decrease.

2 Keeler and Castle, Ibid., 19, 403–411 (1933).
3 Ibid., 20, 273–276 (1934).

RADIOACTIVITY ARTIFICIALLY INDUCED BY NEUTRON BOMBARDMENT

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When certain substances are bombarded with deutons, many and varied nuclear reactions take place. From a target of calcium fluoride, for example,\(^1\),\(^2\),\(^3\),\(^4\) one observes the simultaneous emission of protons, scattered deuterons, alpha-particles, neutrons, particles of electronic mass and gamma rays. In addition it is found that nuclear reactions occur in which radioactive substances are produced.

The very richness of the phenomena renders interpretation difficult and it is therefore desirable to select for separate investigation those nuclear processes that can be made to evidence themselves in most direct fashion. In particular, the recent important discovery of Fermi,\(^5\) that in several substances radioactivity is induced by neutron bombardment, raises an uncertainty in some cases as to the nuclear reactions responsible for the radioactivity induced by deuterons. The question is whether the deuton activity is a primary effect of the type discovered by Curie and Joliot\(^6\) or the result of second order processes wherein neutrons, first liberated by the deuterons, subsequently react with other nuclei.

It is clear that answers to questions of this kind can be obtained from quantitative studies of both the production of neutrons by deuton bombardment and the radioactive effects induced by neutrons. As we\(^3\) had already some roughly quantitative knowledge of the yield of neutrons in several substances, immediately upon receiving Professor Fermi’s first announcement we undertook an investigation of the neutron-induced radioactivity, the first results of which are here reported.