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N. R. MOUDGAL  
CHOH HAO LI

Hormone Research Laboratory,  
University of California,  
Berkeley.

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### Occurrence of an Rh-like Antigen in Red Cells of the Sheep

Landsteiner and Wiener<sup>1</sup> discovered that the sera of rabbits immunized by the erythrocytes of the rhesus (macacus) monkey would agglutinate those of certain human subjects irrespective of their ABO groups. They added, therefore, the Rh-antigen to the list of antigens characterizing the human red cells. Some human red cells, therefore, must be assumed to possess a receptor-substance for antibodies which rabbits or still better guinea pigs can form when immunized with rhesus erythrocytes. Later, Wiener and Peters<sup>2</sup> demonstrated the antigenicity of the Rh-receptor of human erythrocytes in man, thus establishing the principal cause for erythroblastosis in the new-born.

Apart from the Rh-antibody obtained by immunization of guinea pigs with rhesus erythrocytes, there are antibodies formed in women eventually immunized when bearing a fetus possessing Rh-erythrocytes or possibly formed in human males and females following transfusion of blood incompatible with Rh.

As is known, Rh denotes a group of antigens distinguishable in man and the Rh-antigen marked D according to Fisher<sup>3</sup> is the most common in man, and human D-erythrocytes are also agglutinated by an anti-Rh guinea pig immune serum.

Therefore, two anti-D sera occur, one the human anti-D and the other an animal immune anti-Rh serum which can be used as anti-D. These sera, however, are different: the human anti-D serum contains only human Rh-antibodies, for example, anti-D, besides other human isohæmagglutinins of the ABO and other systems, while the guinea pig immunized with rhesus erythrocytes produces, besides antibodies against the rhesus D, those which are able to react with some receptor-substance on human erythrocytes very similar to the rhesus D, and when immunized with human D-erythrocytes the guinea pig (or rabbit) may produce besides anti-D also human species-specific antibodies. The latter may explain the observation<sup>4</sup> that animal anti-Rh immune

serum can react with human foetal (Rh-) erythrocytes. Thus, there are many differences between the animal and the human anti-D sera. But the anti-D itself appears to be different in that the rhesus D cannot be identical to the human D, since rhesus erythrocytes, while able to absorb the anti-D antibodies from a guinea pig anti-rhesus erythrocyte immune-serum, cannot absorb the anti-D from human anti-D serum, which itself can be absorbed easily by human D-erythrocytes. Even by washing three times the rhesus erythrocytes sediment which is in 1:1 ratio to human anti-D serum, we did not succeed in nine cases in decreasing the anti-D titre, but to one dilution step only. This failure confirms earlier observations<sup>5</sup> where there was no success in absorbing a guinea pig anti-rhesus erythrocytes immune serum by human D red blood cells.

On account of the immunizing effect, however, D-antigen must be present in both human and rhesus erythrocytes, but apparently in such a way masked or concealed in the stroma that its presence cannot be proved serologically, but by immunization only. Murray and Clark<sup>6</sup>, by immunizing guinea pigs with human dd-erythrocytes, are believed to have succeeded in stimulating the formation of anti-D antibodies.

In our experiments, boiling in distilled water did not free enough D from rhesus erythrocytes to enable the boiled cells completely to absorb human anti-D antibody, but, however, to decrease its titre to one or, in some experiments, to two more dilution steps as had been possible in the experiments mentioned by using fresh rhesus red cells. Boiling red cells, however, had proved effectual in demonstrating the presence of D antigen in experiments with sheep erythrocytes.

When sheep red cells, suspended in distilled water (not in saline solution), are boiled, they can absorb completely anti-D antibodies from both anti-D sera from patients as well as from commercial anti-D test sera, while human erythrocytes, treated in the same way by boiling, were not able to absorb anti-D to a similar extent. A more detailed account of these experiments will be published elsewhere<sup>7</sup>. Here only some details may be mentioned which require further experimental research: (1) Whether the presence of the D antigen in the red cells of sheep bears any relation with other blood grouping characteristics in sheep is not ascertained as yet. (2) Why boiling must be done in distilled water instead of saline remains to be shown. (3) The Rh-antigen in sheep red cells appears to represent only that portion which is common to both C and D antigens, since the absorbing power of boiled ovine red cells, investigated so far, does not distinguish between C and D antibodies. (4) Experiments are in progress to show whether immunization of guinea pigs or rabbits with boiled red cells of the sheep will produce, besides Forssman antibodies, Rh-(D?)-antibodies.

B. URBASCHEK  
K. TRAUTWEIN

Tierhygiene Institut der Universität,  
Freiburg i. Br.

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