

possibly associated with the advanced age of individual animals. The rise in molybdenum may also reflect changes in the enzyme activity of the liver with age.

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¹ Richert, D. A., and Westerfeld, W. W., *J. Biol. Chem.*, **203**, 915 (1953).

² Dick, A. T., and Bingley, J. B., *Aust. J. Exp. Biol. Med. Sci.*, **29**, 461 (1951).

³ Underwood, E. J., "Trace Elements in Human and Animal Nutrition", p. 125 (Academic Press, New York, 1956).

Serological Test for Determination of Parentage in Cattle

THE antigenic individuality of red cells of cattle was noted in 1910¹, and studies conducted since 1940 by many workers have made it possible to identify more than fifty blood factors. The number of blood-group combinations is very large, and in practice it is difficult to find two individuals with the same combination. Identification of red cell antigens in cattle and knowledge of the mode of inheritance of these characters have been found to have a practical application among others in the exclusion of paternity and the control of pedigree. A condition for the efficacy of these tests is the possession by the laboratory of a large number of immune sera (reagents).

In 1950 Löns², basing his argument on antigenic individuality of human red cells, proposed the method of determining paternity by the use of polyvalent immune serum. He assumed that the polyvalent serum absorbed by the red cells of the father and the mother would react with the red cells of all other persons with the exception of the red cells of a child of the couple the red cells of which were used for the absorption. This method did not yield the expected results and control tests proved it to be useless.

Prof. F. Milgrom suggested to us that an analogous method might be successful for the determination of parentage in cattle.

In order to obtain polyvalent sera, rabbits were injected with red cells of 20 different cattle. The resulting sera hæmolyse in high titre the red cells of all cows, and the absorption of the serum with red cells of one animal removed the antibodies for the red cells used for absorption and left antibodies for all other samples of blood. Out of nine immune sera obtained, two (Nos. 919 and 345) could be successfully used in further tests; their absorption was relatively easy. The absorption conditions were specific for each serum. The best absorption formula was determined by a procedure similar to that used in determining the method of absorption of rabbit anti-*M* or anti-*N* antisera (of the *MNSs* system in humans). Sera Nos. 919 and 345 were absorbed in dilutions of 1:25 and 1:45 respectively. The volume of red cells used for absorption ranged from 1/1 to 1.5/1, depending on the antigen power of the red cells. The absorption time for serum 919 was 2.5 hr. and for serum 345, 4 hr. Further details concerning the technique employed will be described elsewhere by one of us (J. R.).

For absorption, a mixture of red cells of the mother and the father was used. After absorption, a hæmo-

Table 1. RESULTS OF TESTS WITH THE USE OF TWO POLYVALENT SERA

Material tested	Results obtained with serum No.					
	919		345		919+345 together	
	Number tested	Parent-age excluded	Number tested	Parent-age excluded	Number tested	Parent-age excluded
Offspring	41 <i>1.000*</i>	0 <i>0.000</i>	41 <i>1.000</i>	0 <i>0.000</i>	41 <i>1.000</i>	0 <i>0.000</i>
Related individuals	142 <i>1.000</i>	80 <i>0.563</i>	146 <i>1.000</i>	81 <i>0.554</i>	140 <i>1.000</i>	86 <i>0.614</i>
Unrelated individuals	663 <i>1.000</i>	528 <i>0.796</i>	662 <i>1.000</i>	527 <i>0.796</i>	650 <i>1.000</i>	579 <i>0.891</i>

* Figures in italics denote the percentage of the number tested.

lysis test was made with the red cells of the offspring. As a control, tests were performed with red cells used for absorption and with red cells of about twenty other cattle. These red cells came from: (1) the offspring of only one of the couple the red cells of which were used for the absorption—these cattle are denoted in Table 1 as 'related'; and (2) cattle regarded as 'unrelated'. These were unrelated or showed a lesser degree of relationship than the cows in group 1. As a complement, guinea pig serum diluted 1:10 was used. This serum was previously controlled to see that it did not contain antibodies for cattle red cells.

After absorption the sera never reacted with the red cells employed for absorption nor did they react with the red cells of cows that were the offspring of the couple the red cells of which were employed for absorption. In contrast to this, these sera reacted with the majority of control red cells both with the samples from the 'related' and 'unrelated' heads of cattle. 35 families with 41 offspring were studied. Control tests were made with 140 'related' and 650 'unrelated' individuals. Table 1 gives the results of our tests.

From these results it appears that the use of two polyvalent sera makes possible the exclusion of about 89 cows out of 100 that were not the offspring of the couple the red cells of which were used for absorption. When the tested individual is an offspring of only one of the heads the red cells of which were used for absorption, exclusion of parentage is still possible in 61 per cent of cases. The first of the figures given, 89 per cent, is almost the same as giving positive indication of the proper pair of parents in the case when it was not possible to exclude the parentage.

It seems that the method described will be of practical use in checking doubtful parentage since the method is incomparably more simple and more within the scope of an ordinary laboratory than the method employed hitherto in which many reagents difficult to obtain are used. The efficiency of the method is quite good; this method could be increased if a third polyvalent serum were used.

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¹ Todd, D., and White, M. B., *J. Hyg.*, **10**, 185 (1910).

² Löns, M., *Z. Hyg.*, **131**, 371 (1950).