ON THE DISTRIBUTION AND INHERITANCE OF A NATURAL ANTIBODY IN CATTLE¹

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THE origin of so-called normal or naturally occurring antibodies has provided a fascinating material for discussion almost since the beginning of immunology. There is ample evidence to indicate that many naturally occurring antibodies have arisen as a consequence of inapparent immunization, (BAILEY 1928), and certain investigators contend that all antibodies result only as a consequence of immunization (DUPONT 1934; WEINER 1951). Others propose that certain regularly occurring natural antibodies, as, for example, the isoagglutinins of man, are genetically conditioned (LANDSTEINER 1945; FURUHATA 1927). There have, until recently, been few if any situations involving natural isoantibodies which lend themselves to genetic analysis.

In 1951, STORMONT discovered that certain cattle possess natural hemolysins which act selectively on the red cells of group O sheep. He designated these hemolysins as natural bovine antisheep O. Serum samples from hundreds of cattle were screened for additional examples of antisheep O but none were found outside of the original family—a Hereford bull and three of his seven daughters. This suggested that natural, bovine antisheep O is conditioned by the action of a single dominant gene (STORMONT 1953). In the foregoing report it was shown that antisheep O also reacts with a soluble, specific blood group substance (s), called Oc, in the blood of certain cattle, thereby permitting in conjunction with the use of anti-J, the recognition of four phenotypes **J**, **JOc**, **Oc** and “—” in the J or J-Oc system of cattle (SPRAGUE 1958; SPRAGUE and STORMONT 1957). The present report is concerned with the influence of the phenotypes **J**, **JOc** and **Oc** of the J-Oc blood group system on the appearance of anti-O and on additional data bearing on the inheritance of natural, bovine antisheep O.

MATERIALS AND METHODS

Each sample of normal bovine serum was tested for the presence of both anti-J and antisheep O. The samples were diluted 1:4 and 1:16, based on a previously established optimum of 1:4 to 1:16, heated for 30 minutes at 56° C and were then tested with the red cells of four group R sheep, four group O sheep and one group i (i.e., non-R, non-O) sheep. Usually, but not invariably, J-positive and J-negative

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cattle red cells were included in the tests to help serve as an indicator of the presence of anti-J but this was not a necessary procedure in view of the fact that anti-J has considerably greater avidity for the red cells of group R sheep than it has for those of J-positive cattle.

The tests were performed as follows: to 0.10 ml (two drops) of the diluted serum in a 10×75 mm test tube was added one drop of a 2.5 percent suspension of washed red cells of a given individual. This mixture was shaken and allowed to stand at room temperature (22 to 27° C) for 10 minutes at which time one drop of guinea pig complement (diluted 1:10 to 1:20) was added. The mixture of serum, red cells and complement was shaken and the first reading of the test was made approximately 30 minutes later. The tests were again shaken and a second reading was made after an interval of two to three hours.

If the red cells of the four group R sheep were lysed to the exclusion of those of the group O and i sheep, the serum was classified as anti-J. Conversely, if the red cells of the four group O sheep were lysed to the exclusion of those of the group R and i sheep, the serum was classified as anti-O. If the serum lysed the red cells of all group R and all group O sheep, but not those of group i, this was indicative of the presence of both anti-R and anti-O but, nevertheless, absorptions were performed in order to demonstrate the presence of each of two populations of antibody. A number of the cattle sera contained heterohemolysins for the red cells of all sheep and these heterohemolysins often obscured or masked hemolysis due to either or both anti-J and anti-O. Consequently, it was necessary to perform absorptions on sera of that order.

Where absorptions were necessary, each serum was diluted 1:4 and divided into three aliquots. One of the aliquots was absorbed twice with group R red cells using a ratio of red cells to serum of 1:2 for the first absorption and 1:1 for the second. The first absorption was run for 15 minutes at room temperature and the second for 30 minutes. It was then chilled at 4° C for 30 minutes just prior to centrifugation. The second aliquot was treated with group O red cells and the third with group i using the same procedure as that given for the aliquot treated with group R red cells. The three absorbed samples were heated for 30 minutes at 56° C and tested with the same panel of nine sheep bloods. The sera so treated were then classified on the basis of the antibodies remaining following the absorptions.

0.91 NaCl solution was used throughout these procedures.

RESULTS

As already mentioned, no further examples of natural, bovine antisheep O were encountered outside the immediate family of the Hereford bull whose serum served as the original source of anti-O used in the foregoing study (SPRAGUE 1958) as well as in the studies by RENDEL *et al.* (1954), SORESENSEN *et al.* (1954) and RENDEL (1957). This particular bull, California Stanway 3rd registration number 4965229, was obtained from the University of California (U.C.) beef herd and was used in one of the experimental herds of this school.

Over the course of this study, 453 blood samples were collected from animals in the U.C. beef herds. Table 1 summarizes the results of the classification of these animals with respect to the occurrence of anti-J and anti-O, and also with respect to their **J-Oc** phenotypes, according to procedures given in the foregoing report (SPRAGUE 1958). Of the 453 samples, a total of 128 proved to have demonstrable anti-O and 17 of these 128 animals also possessed anti-J.

Table 2 is a summary of the inheritance data on anti-O. Because of the inhibitory effect of the phenotypes **J**, **JOc** and **Oc** on the appearance of anti-O, the critical matings (lines 4, 5 and 6 of Table 2) are restricted to parents of phenotype "—" in the J-Oc system. In these critical matings, it is seen that the inheritance of anti-O is consistent with the postulate of a single dominant gene. It remains to be seen whether more extensive data will bear out these observations.

The pattern of parentage information available for the entire herd (which included some offspring, one or both of whose parents was not tested) demonstrated that 100 animals with anti-O were related directly as descendants of either Dandy Domino the 46th 1573523 or California Stanway 2471801. The

TABLE 1

Classification of 453 Herefords (U.C. Beef) with respect to anti-J and anti-O antibodies and J and Oc soluble substances found in their sera

Phenotypic class	Number of animals	Number with anti-J and anti-O in serum	Number with anti-J in serum	Number with anti-O in serum	Number lacking both antibodies
JOc	29	0	0	0	29
J	93	0	0	0	93
Oc	58	0	33	0	25
—	273	17	60	111	85

TABLE 2

*Summary of 277 matings with respect to the phenotype anti-O and the combined phenotypes **JOc**, **J**, **Oc** here denoted as SSS (soluble specific substance) and the phenotype "—" (absence of SSS)*

Parental phenotypes		Offspring phenotypes		
SSS	SSS	Anti-O	Non-anti-O	SSS
1. non-anti-O × non-anti-O	SSS —	7	3	42
2. non-anti-O × non-anti-O	SSS —	6	18	27
3. non-anti-O × anti-O	— —	26	46	65
4. anti-O × anti-O	— —	12	6	0
5. anti-O × non-anti-O	— —	9	17	0
6. non-anti-O × non-anti-O	— —	0	11	2

— represents phenotype non-sss.

remaining 28 animals with anti-O were related to these same two lines of descent but were not directly related to the two sires.

Some anti-O animals have Oc-positive offspring as well as anti-O offspring. Assuming that the allele J^{Oc} (see preceding report) is a recessive, the segregation of Oc-positive animals from matings involving an anti-O parent indicates that the anti-O parent is heterozygous for the allele J^{Oc} . The question arises, is there a regular or necessary relationship between $J^{Oc} j$ heterozygotes and the phenotype anti-O? Since neither J-positive nor Oc-positive animals exhibit the phenotype anti-O, and there is no phenotypic test for $J^{Oc} j$ heterozygotes, an indirect test to determine a relationship between Oc and anti-O must be employed.

Let us call the class of J-negative-Oc-negative (i.e., $J^{Oc} j$) dams of Oc offspring X. Let us call the class of all J-negative-Oc-negative animals which are not X, Y (i.e., $J^{Oc} j$ and $j j$).

The proportion of X with anti-O and without anti-O may be compared with the proportion of Y with anti-O and without anti-O. If there is a necessary relationship between $J^{Oc} j$ heterozygotes and anti-O, the proportion of anti-O's among X should be different from the proportion of anti-O's among Y.

The data are X, anti-O 12; Y, anti-O 112; X, non-anti-O 9; Y, non-anti-O 148. The chi-square value for the significance of the difference between proportions is not regarded as significantly different and there is, as a consequence, no compelling evidence in favor of a regular relationship between the genotype $J^{Oc} j$ and the phenotype anti-O.

DISCUSSION

In the present study anti-J occurred in the serum of some J-negative cattle (Oc and "—" of Table 1) but not, of course, in the serum of J-positive cattle (JOc and J). On the other hand anti-O was only observed to occur within the phenotypic class "—". The reason for the "exclusion" of anti-O from J-positive phenotypes lies in the fact that all J-SSS sera tested to date exhibit weak cross reactions with anti-O. Erythrocytes from animals of these J-positive Oc-negative phenotypes will act in absorptions to remove anti-O antibodies. The converse situation, that is, cross reaction between Oc-SSS and anti-J, does not pertain since anti-J and Oc-SSS are frequently found to co-exist in the same serum. The cross reactions between J-SSS and anti-O are of such a degree that they rarely present a problem of classification in the routine inhibition tests.

The isoagglutinins of man are the most extensively studied natural antibodies because of their importance in human transfusions. The question as to whether or not these antibodies are inherited cannot be resolved as long as the very regular relationship between the agglutinins and the agglutinogens pertain. However, the cattle JOc system provides ideal conditions for the study of the genetic origin of isoantibodies because additional "degrees of freedom," as it were, are available. The critical classes of non-SSS with antibody and without antibody are commonly found as shown in Table 1. This critical observation permitted a study of the antibodies of segregants from matings of animals without detectable SSS with the results for anti-O shown in Table 2. The data avail-

able have also been examined with regard to anti-J and it has not been possible to formulate any generalization which might specifically account for the occurrence of this antibody fraction.

Two previous papers dealing directly with the genetic basis of normal antibody fractions are of particular interest.

The first, that of STUART *et al.* (1936), reported the investigation of normal rabbit sera containing agglutinins reactive with "group-specific A" substance of man. These workers investigated nine inbred strains of rabbits and concluded that "group-specific" agglutinins were inherited as the products of a mendelian recessive.

Further work by WHEELER *et al.* (1939), reveals that the presence of the *alpha* agglutinin may best be understood in terms of a human A (like) substance regularly found in *alpha* negative animals. Thus, this situation appears to be similar in form to that of the ABO system of man.

The second, that of STERN *et al.* (1956), presents statistical evidence for genetic control in mice of natural agglutinins for sheep erythrocytes.

At present it seems probable that although uncontrolled immunization will account for many examples of natural antibodies as reviewed by WEINER (1951) there may exist in addition cases of *bona fide* natural antibodies under direct genetic control.

SUMMARY

The origin of a natural antibody of cattle designated anti-O is ascribed to the action of a dominant gene.

The relationships between the phenotypes in the J-Oc system of cattle blood groups and anti-O are discussed.

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