On the "J" Classification of Rabbits and Production of Anti-J in "J-Negative" Rabbits. (26031)

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One of the chief problems in blood typing the J system of cattle is that of obtaining J antibodies which will lyse the red cells of all J-positive cattle to the exclusion of those bloods that are truly J-negative. The reason for the difficulty is that there are many genetic intergrades of J-positive bloods ranging from those in which the "J-coated" red cells have relatively little affinity for J antibody, and may not be lysed even by the best J reagents available, to those which have marked affinity for and are readily lysed by anti-J. Consequently, it has often been necessary to resort to inhibition tests for soluble J substance in blood plasma to distinguish the "lowest" intergrades of J-positive bloods from those that are truly J-negative. Until recently, the only source of anti-J has been the normal serum of J-negative cattle, but it is an unusual normal serum, if one exists, which will promote lysis of all J-positive cattle bloods. Various investigators(1,2,3,4,5) have attempted to enhance the titers and broaden the specificity of J isolysins by isoimmunization and some of these investigators have also attempted to produce anti-J by heteroimmunization. Although an immune response to cattle J substance has been claimed(2), most workers are convinced that there is little or no effect(1,3,4,5). It is now found that "J-negative" rabbits immunized with human red cells of type A regularly produce antibodies which cross react rather strongly with J-positive cattle bloods, and some of the J reagents obtained in this manner are, in the experience of the authors, equal to or superior to the best J reagents obtained from J-negative cattle. This report is concerned with production of such antibodies and with the "J" classification of rabbits.

Materials and methods. Mature, commercial rabbits of both sexes and of different breeds, primarily New Zealand Whites and Californians, were used. Prior to the immunizations, a sample of normal serum was collected from each rabbit and these samples were tested for their ability to inhibit lysis of J-positive cattle blood by anti-J, according to the following procedure. Samples of rabbit normal serum (inhibitant) were heat-inactivated for 1 hr at 56°C. To each of a series of 5 tubes (10 x 75 mm) was added 1 drop (app. 0.05 ml) of a rabbit's serum, diluted 1:1 in the first tube, 1:2 in the second, 1:4 in the third, 1:8 in the fourth and 1:16 in the fifth. To the one drop of diluted inhibitant in each of the 5 tubes was then added 2 drops of anti-J in a dilution sufficient to promote nearly complete hemolysis of J-positive red cells within 1½ hr, as judged by the positive control. (In the present study, we used our C86 normal serum diluted 1:50.) The tubes were then shaken and, after an interval of 10 minutes, 1 drop of a washed, 2.5% suspension of red cells from a J-positive donor was added to each tube. The tubes were again shaken and, after another interval of 10 min., 1 drop of freshly thawed, undiluted rabbit complement (a pool of fresh-frozen serum from 40 to 60 selected rabbits) was added to each tube. The tubes were again shaken and readings of hemolysis were recorded at 1½ hr and again after another 1½ hr. Controls consisted of a positive control (J antibody + red cells + 1 drop of saline in place of inhibitant), a complement control (saline in place of antibody and inhibitant) and a saline control. 0.91% NaCl solution was used as saline. All tests were performed at room temperature ranging from 23.5°C to 28.5°C.

The direct blood-typing tests were performed in much the same manner except that no inhibitant was used and that guinea pig complement diluted 1:15 was substituted for rabbit complement in the tests employing rabbit antisera or J reagents derived from rabbit antisera. In fact, the substitution of guinea pig complement for rabbit complement, which has heretofore been considered the comple-

ment of choice with all cattle blood-typing reagents, was the most crucial step in demonstrating the cross reactions of rabbit anti-human A with cattle J. The particular cross reactive lysins in such antisera are poorly activated with rabbit complement in contrast with guinea pig complement.

For the immunizations, each rabbit received a series of 6 intravenous injections, 2 per week, of 0.5 ml of a 50% suspension of red cells per injection. Antisera were collected and frozen 6 days after the last injection.

Six of 21 rabbits which were immunized with human red cells of type A received a series of 3 booster injections on alternate days beginning 54 days following collection of the antisera of the primary series, and these antisera of the secondary series (coded #2 in Table I) were collected 5 days after the third booster injection.

Results. Sera of 110 rabbits were examined for their ability to inhibit the lytic reactions of J antibody with J-positive cattle red cells. Serum of 54 of the rabbits showed no inhibition and were accordingly classified as "J-negative." Serum of each of the remaining 56 rabbits, classified as "J-positive," showed complete inhibition in the 1:1, 1:2 and 1:4 dilutions and most showed partial to complete inhibition in the 1:8 dilution but none completely inhibited lysis in the 1:16 dilution. The results were remarkably uniform in contrast with comparable inhibition tests using cattle serum as inhibitant. (Some J-positive cattle serums show only partial inhibition in the 1:1 and 1:2 dilutions in contrast with some which show complete inhibition through dilutions as high as 1:256.) Serums of 29 J-positive and 29 J-negative rabbits were examined for presence of natural agglutinins for human red cells. Fifteen of the J-positive rabbits possessed no agglutinins for human red cells whereas the remaining 14 possessed certain weakly expressed agglutinins which reacted approximately equally with all 4 groups. Only 3 of the J-negative serums failed to agglutinate human red cells. Seven had rather weakly expressed agglutinins reactive only with group A and AB red cells; 11 had weak agglutinins reactive with group A, AB and B red cells, whereas 8 had weak agglutinins which reacted with the red cells of all 4 groups. In the latter 2 classes, however, reactions with A and AB red cells were usually more intense than were those with cells of group B or group O.

In view of the indication of natural anti-human A agglutinins in the majority of the J-negative rabbits but in none of the J-positive rabbits, we are of the opinion the J classification described here parallels the classification(6,7) of "A-positive" and "A-negative" rabbits by more difficult technics involving both complement fixation and inhibition. We did not attempt to confirm this opinion by use of those technics(6,7). Nevertheless, we note at this point a further parallel, *i.e.,* that it was only the J-negative rabbits of the present study, like the A-negative rabbits of previous studies (see 7), which produced strongly reactive agglutinins specific for A of man on immunization with group A red cells.

In Table I are summarized the results of titration of 27 antisera, engendered by immunization with human group A red cells, in tests with J-positive and J-negative cattle bloods. Six of 12 antisera (including 2 obtained following booster injections) produced in J-positive rabbits contained no hemolysins for cattle red cells, whereas the remaining 6 contained some hemolysins (titers of 1:4 to 1:256) which failed to distinguish between J-positive and J-negative cattle as confirmed by absorptions. On the other hand, 14 of 15 antisera (including 4 obtained following booster injections) produced in J-negative rabbits contained hemolysins which cross reacted specifically with the red cells of J-positive cattle as indicated by the marked differences in titer shown in Table I. In fact, 6 of the antisera had titers ranging from 1:64 to 1:2048 with J-positive red cells but did not cross react at all with J-negative red cells. In any event, the lysins which did cross react with J-negative bloods (antiserums numbered RH28, 31, 33, 35 and 37) could be readily removed by absorption with J-negative bloods without appreciably affecting either degree of reactivity or titer of the antisera in tests with J-positive bloods. Conversely, however, J-positive bloods absorbed all the hemolysins

TABLE I. Cross Reactions of Rabbit Antisera against Human Red Cells of Group A in Lytic Tests with J-Positive and J-Negative Cattle Red Cells.

Antiserum No.*	"J" classification of rabbit	Lysins for cattle in normal serum and their titer	Antiserum titer in tests with cattle red cells: J-pos	J-neg
RH 9	J-neg	Yes,† 4	512	0
RH10	"	None	64	0
RH11	"	"	0	0
RH12	J-pos	"	32	32
RH13	"	"	0	0
RH14	"	"	0	0
RH15	"	"	0	0
RH16	"	"	0	0
RH17	J-neg	"	64	0
RH18	J-pos	"	4	4
RH19	"	"	0	0
RH28	J-neg	"	256	16
RH29	J-pos	"	0	0
RH30	"	"	32	32
RH30 #2			128	128
RH31	J-neg	Yes,† 16	256	16
RH32	J-pos	None	16	16
RH32 #2			256	256
RH33	J-neg	Yes,‡ 4	256	16
RH33 #2			2048	0
RH34	J-neg	Yes,† 16	256	32
RH35	"	" , 4	64	8
RH35 #2			256	16
RH36	J-neg	None	1024	0
RH36 #2			1024	0
RH37	J-neg	"	512	64
RH37 #2			2048	32

* #2 indicates antiserum of secondary series.
† Species heterolysins.
‡ Lysins specific for the A factor of cattle.

for J-negative bloods, thereby indicating that all cattle have at least one common blood factor which, like blood factor J (see 1), is serologically related to the A substance of man.

Tests with several of the antisera (RH9, RH36 and RH37 #2 of Table I) have now been performed in parallel with our best J reagents (C86 and C100) obtained from normal cattle serum and also our best J antiserum obtained by immunizing J-negative cattle with human red cells of group A. In general, RH9 and RH36 antisera proved to be equivalent or nearly so to the C100 J reagent and to the J antiserum produced by immunizing a J-negative heifer with group A red cells. They closely approached but did not quite measure up to the C86 reagent in ability to lyse weakly reactive J-positive bloods. On the other hand, the RH37 #2 antiserum has so far proved to be superior to all of our other J reagents in its ability to lyse the red cells of weakly reactive J-positive bloods. Whether this reagent will lyse the red cells of all truly J-positive bloods cannot, of course, be proved. Nevertheless, we have yet to find J-positive cattle blood, as measured by inhibition tests, in which the red cells cannot be lysed by the combined action of RH37 #2 reagent and guinea pig complement. This is the more remarkable considering that the RH37 #2 reagent was used in dilutions of 1:50 and 1:100.

J-positive and J-negative rabbits were also immunized with a variety of other bloods including human red cells of groups B and O, sheep red cells of groups R and O, and both J-positive and J-negative cattle and goat blood. With the exception of 2 out of 4 antisera produced in J-negative rabbits by immunizing with red cells of group R sheep, there was no indication of antibodies which would differentiate the red cells of J-positive from J-negative cattle. The J hemolysins in the 2 rabbit anti-sheep R serums were considerably weaker than those engendered by human red cells of group A, but like the latter were most effective with guinea pig complement.

Summary. It is shown that certain antibodies engendered in "J-negative" (or A-negative) rabbits by immunizing with human red cells of type A will selectively lyse red cells of J-positive cattle. Some of the "J" reagents so produced have proved to be either equal to or superior to the better J reagents obtained from J-negative cattle in their ability to promote lysis of the more weakly reactive intergrades of J-positive blood. The key to recognition of these specific cross reactions was substitution of guinea pig complement for rabbit complement. The method of classifying J-positive and J-negative rabbits is described and data are presented on distribution of the J types in 110 rabbits.

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Effects of Antiserum in Bioassay of Thyrotropin and Thyroid Activator of Hyperthyroidism. (26032)

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Recently evidence has accrued that there is in the blood of patients with Graves' disease a substance which can stimulate the thyroid gland of guinea pig(1,2), mouse(3), and presumably the patient's own gland. It has been suggested that this substance may not be thyrotropin of pituitary origin(4); this was based on several qualitative differences shown to exist between the activity found in the blood in hyperthyroidism and that found either in pituitary extracts or in the blood of patients with myxedema. A further distinguishing feature is furnished in the present study, wherein antisera to bovine (pituitary) thyrotropin, produced in rabbits, were found to inhibit thyrotropic activity of bovine pituitary extracts and that in the blood of patients with myxedema, but not the thyroid activator in serum of patients with hyperthyroidism.

Methods. Antiserum (A) to commercial bovine thyrotropin (thyrotron, Nordic Biochemicals) was prepared by administering intramuscularly, 3 weeks apart, 2 injections of 2.5 mg of this preparation emulsified in Freund's adjuvant (complete), to totally thyroidectomized rabbits. Antiserum (B) to U.S.P. standard (bovine) thyrotropin was prepared by administering intradermally 5 weekly injections of 1 mg of this preparation emulsified in Freund's adjuvant (complete), in the interdigital webs of intact rabbits. These antisera were tested for titer and specificity using the bis-diazotized benzidine hemagglutination test, as previously described (5,6,7).

Thyrotropic effect was assayed by a method previously described(8); mice with thyroidal iodine labelled with I^{131} were injected intravenously with serum or other test solution and response measured as an increase in circulating radio-activity. This was expressed as a percentage of pre-injection concentration of I^{131} in the blood and a positive response was thus greater than 100%. Four to 6 mice were injected with each test solution. Thyrotropin of pituitary extracts or that found in the blood in myxedema produced maximal effect within 2 hours, but the thyroid activator in the blood in hyperthyroidism had maximal effect only after about 9 hours(3).

Human serum for assay was obtained from 3 patients with spontaneous myxedema and 3 with hyperthyroidism (Graves' disease). Diagnosis had been made previously by conventional criteria of physical examination and measurement of protein bound iodine in serum and thyroid uptake of I^{131}. Serum was separated from the blood within an hour of its being obtained, and stored at 4°C until used for assay, within one week in all instances.

Mixtures of serum or thyrotropin standard (U.S.P.) with antiserum or control serum were kept at room temperature for 30 minutes then centrifuged. Although no gross precipitate was seen the upper portion of fluid in the centrifuge tube was always used for assay.

Results. The hemagglutinating antibody titer of these thyrotropin antisera ranged

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