

OBSERVATIONS ON THE STABILITY OF THE ERYTHROCYTES OF THE OX, PIG, AND SHEEP

MARCUS W. LYON, JR.

WASHINGTON, D. C.

In doing a parallel series of Wassermann reactions by the Noguchi method with acetone insoluble extract of heart as antigen, and by the drop modification of the original Wassermann with alcoholic extract of heart as antigen, difficulty was experienced in obtaining sheep blood owing to the fact that no sheep were being killed at the local abattoir. Ox blood was readily obtainable and it was decided to make an anti-ox erythrocyte amboceptor. A working amboceptor was made after 5 intravenous injections into a rabbit. Several others have been made since, but never with less than 5 intravenous injections. The ox cell system worked so well and was in such close agreement with the Noguchi method that the latter was gradually abandoned and the ox system used exclusively, employing 2 antigens, alcoholic extract of human heart and cholesterinized extract.

After reading the account by Kolmer and Casselman¹ on natural hemolysins in human serum and Gradwohl's account² of his modification of the Bauer and Hecht-Weinberg method of performing the Wassermann reaction, I became curious to try out a series of human sera on the corpuscles of the 3 animals commonly found in abattoirs.

For 10 weeks the corpuscles of as many different oxen, pigs, and sheep were tested with a varying number of human sera, 123 in all. The various bloods were collected in sterile citrate solution once a week at the abattoir. As all 3 animals were not always killed on the day before doing the Wassermann tests, the bloods were often kept on ice at the abattoir or in the laboratory for 1 to 3 days before they were used. The weakening effect on the corpuscles by keeping them 3 days is shown in Table 2, Animals 4. The effect is insignificant, but is least marked on the ox corpuscles. Following the regular Wassermann tests 3 series of tubes were set up and to each tube was added 0.5 c.c. of salt solution, 1 drop of fresh guinea-pig serum, 1 drop of the various sera to be tested, and 0.5 c.c. of a 3% suspension of the 3 kinds of cells being tested. The drops of sera were delivered with a capillary pipet of 1 mm. outside diameter. The guinea-pig serum was known not to be hemolytic for the various cells being tested and as the human sera had all been previously used in the regular test they were known to be anticomplementary. The 3 series of tubes were done in duplicate, 1 set for the unheated

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¹ Jour. Infect. Dis., 1915, 16, pp. 441-447.

² Jour. Amer. Med. Assn., 1917, 68, p. 514.

active sera and the other set for those portions of the sera which had been inactivated at 56 C. for use in the regular Wassermann tests. The tubes were incubated in a water bath for 1 hour at 37 C., then placed in the ice box for 2 or more hours for the corpuscles to settle out, when the results were read. The degrees of hemolysis were estimated by the eye. Five per cent. is a mere trace and is negligible and was only used to distinguish tubes in which the supernatant fluid was absolutely white. Ten per cent. is a pronounced trace, 25%, 50%, 75%, and 100% represent increasing grades of hemolysis. These degrees of hemolysis correspond with the following Wassermann readings: 0% and 5%, + + + +; 10% and 25%, + + +; 50%, + +; 75%, +; 100%, negative, provided that the readings had been made from the use of only 1 tube and 1 volume of serum. In actually performing the test the degrees of hemolysis are determined from the use of 2 tubes, 1 containing a single and the other a double quantity of the serum to be tested. Table 1 gives a summary of the results.

TABLE 1
PERCENTAGES OF HUMAN SERUM CAUSING INCREASING GRADES OF HEMOLYSIS WHEN ACTING ON THE ERYTHROCYTES OF THE OX, PIG, AND SHEEP IN THE PRESENCE OF FRESH GUINEA-PIG SERUM

Extent of Hemolysis	Active Human Serum			Inactivated Human Serum		
	Ox	Pig	Sheep	Ox	Pig	Sheep
0%	40	47	0	69	78	3
5%	13	15	0	15	6	1.6
0 and 5% together	53	62	0	84	84	4.6
10%	15	16	1.6	11	5.7	3
25%	14	8	5.7	3	5.7	7
50%	8	5	7	2	3	26
75%	7	6	18.7	0	0.8	17
100%	3	3	67	0	0.8	42

This table shows that all the corpuscles are more resistant to the inactivated sera than they are to the active sera, a result naturally to be expected, although in many cases there are no differences or no appreciable differences. The pig corpuscles are relatively more resistant to the active sera than are the ox corpuscles, but not so far as the inactivated sera are concerned. The active sera produced essentially no hemolysis of the ox cells in 53% of the cases; of the pig cells in 62%, while all of the sheep cells showed some grade of hemolysis with active sera. With the sera inactivated the percentages are for the ox cells, essentially no hemolysis, 84%; with the pig cells, 84%; and with the sheep cells, 4.6%. Complete hemolysis by the active sera on the ox cells occurred in 3% of the cases; on the pig cells in 3%; and on the sheep cells, 67%. When the inactivated sera were used the figures are 0% for the ox cells, 0.8% for the pig cells, and 42% for the sheep cells.

In no instance were the sheep cells more resistant than those of the ox or pig. In a very few instances, sera numbers 12 inactivated, 17 inactivated, 20 active, 31 active, 101 active, 104 active, 111 inactivated, 113 inactivated, the cells of all 3 animals showed the same degree of resistance. The pig and ox cells parallel one another very closely. Any difference in resistance between pig and ox is usually in favor of the pig. In a few instances, the ox cells were more resistant than those of the pig, sera numbers 29, 35, 54, 58, 61, 62, 64, 65, 66, 69, 71, 119, active; and 20, 22, 29, 31, 32, 35, 47, 62, 64, 65, 66, 68,

71, 119, 120, 122, inactivated, and in these cases the difference in degree of hemolysis was usually not marked.

No satisfactory explanation of these facts can be offered. To say that human serum contains a natural antisheep erythrocyte amboceptor is simply another way of expressing the observed facts. Zoologically, sheep and oxen are closely allied and one would expect their corpuscles to behave similarly under the influence of a serum obtained from as zoologically remote an animal

TABLE 2

DEGREE OF HEMOLYSIS OF THE ERYTHROCYTES OF THE OX, PIG, AND SHEEP, EXPRESSED IN ESTIMATED PERCENTAGES, WHEN PLACED IN HYPOTONIC SALT SOLUTIONS. NO. 4A
REPRESENTS THE SAME CORPUSCLES AS THOSE FROM ANIMAL 4, BUT THEY WERE TESTED THREE DAYS LATER AFTER HAVING BEEN KEPT ON ICE

Hypotonic Salt Solutions	Ox Number						Pig Number						Sheep Number					
	1	2	3	4	4a	5	1	2	3	4	4a	5	1	2	3	4	4a	5
0.75%	0	0	0	0	0	0	0	5	5	0	0	0	5	0	0	0	0	0
0.70%	0	0	0	0	0	0	5	5	10	0	5	0	10	0	5	0	0	0
0.65%	0	0	0	0	0	0	10	10	25	5	10	0	50	5	10	0	5	0
0.60%	5	0	0	5	5	0	25	25	25	5	25	10	75	10	25	5	10	0
0.55%	10	5	0	10	10	0	50	50	50	25	75	50	100	25	50	25	75	0
0.50%	75	25	5	10	25	0	75	75	75	50	100	50	100	75	75	100	100	5
0.45%	100	75	10	75	75	5	100	100	100	100	100	100	100	100	100	100	100	50
0.40%	100	100	50	100	100	10	100	100	100	100	100	100	100	100	100	100	100	75
0.35%	100	100	100	100	100	50	100	100	100	100	100	100	100	100	100	100	100	100
0.30%	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

TABLE 3

DEGREE OF HEMOLYSIS OF THE ERYTHROCYTES OF THE OX, PIG, AND SHEEP, EXPRESSED IN ESTIMATED PERCENTAGES WHEN TREATED WITH ONE DROP OF GUINEA-PIG SERUM AND VARYING QUANTITIES OF INACTIVATED SERA OF EEL, SHAD AND ROCK

Quantities of Fish Serum	Ox Number			Pig Number			Sheep Number		
	1	2	3	1	2	3	1	2	3
Eel, <i>Anguilla chrysypa</i>									
1 drop	100	100	100	100	100	100	50	75	75
$\frac{1}{2}$ drop	100	100	100	100	100	100	5	50	75
$\frac{1}{4}$ drop	100	100	100	100	100	100	0	25	75
$\frac{1}{8}$ drop	75	75	100	100	100	100	0	25	10
Rock, <i>Roccus lineatus</i>									
1 drop	5	0	0	50	25	0	25	25	5
$\frac{1}{2}$ drop	5	0	0	25	10	0	5	25	5
$\frac{1}{4}$ drop	5	0	0	5	0	0	5	25	5
$\frac{1}{8}$ drop	5	0	0	0	0	0	0	25	5
Shad, <i>Alosa sapidissima</i>									
1 drop	0	5	50	0	25	50	0	25	50
$\frac{1}{2}$ drop	0	5	25	0	5	50	0	25	50
$\frac{1}{4}$ drop	0	5	5	0	0	50	0	25	25
$\frac{1}{8}$ drop	0	0	5	0	0	25	0	25	5

as man. The zoologically unrelated ox and pig behave similarly so far as their erythrocytes are concerned under the influence of human serum. Kolmer and Casselman's series of 10 different animals showed an entire lack of zoological association. The well-known ease with which an antisheep cell amboceptor can be prepared appears in part at least to depend on some inherent weakness of the sheep erythrocyte.

In Table 2 are shown the results of subjecting the erythrocytes of 5 different oxen, pigs and sheep to the decreasing strengths of salt solution. The different animals showed considerable individual variation as to the behavior of their erythrocytes in these solutions. Thus Ox 5 and Sheep 5 had resistant corpuscles, while Pigs 2 and 3, and Sheep 1 had weak corpuscles. The average strength of solution where no hemolysis occurred with the ox corpuscles was 0.59%, with the pig corpuscles, 0.74%, and with the sheep corpuscles, 0.69%. Thus the ox cells were decidedly the most resistant, and the pig cells the least. One would expect that it might be an easy matter to prepare an anti-pig cell amboceptor. Such was not the case, though perhaps the rabbits tried were not adapted to making good amboceptor. The average point where the corpuscles of the 3 animals showed complete hemolysis in the hypotonic salt solutions are for the ox 0.39%, for the sheep 0.46%, and for the pig 0.45%, showing the ox cells to be most resistant and the sheep and pig cells to be essentially alike. Hypotonic salt solution does not have the same effect on zoologically related animals, but the effect is not in the same direction as in the case of the action of human serum.

Table 3 shows the behavior of the erythrocytes of 3 different oxen, pigs and sheep when treated with 1 drop of guinea-pig serum and an equal and less amounts of 3 different fish sera, those of the eel, rock, and shad. The results here are quite astonishing and not at all in accordance with what one would expect from the zoological relations or from the results seen in Tables 2 and 3. The pig cells so resistant to human sera are very unstable in the presence of eel serum and the ox cells almost equally so, while sheep cells have a comparatively high degree of resistance. Rock and shad sera seem to have about the same effect on the 3 kinds of corpuscles, but rather less on the ox cells, and most on the sheep cells.

CONCLUSIONS

The ease with which the erythrocytes of the ox, pig, and sheep are broken up by the same agent bears no relation to the zoological position of the 3 animals. The erythrocytes of the ox on the whole are comparatively stable in most circumstances and appear to be well adapted as an indicator in complement fixation tests with human serum, much better than sheep corpuscles are. Sheep erythrocytes are comparatively unstable, tho relatively resistant to eel serum. Their use in complement fixation tests with human serum introduces a variable factor which is undesirable, altho it has probably little effect on the final value of the tests. Erythrocytes obtained at random from abattoir animals on the whole behave similarly from week to week, and appear to be quite suited for hemolysis experiments, and indicators in complement fixation tests, as are the corpuscles from a single animal. Ox erythrocytes seem to have better keeping qualities than the erythrocytes of the pig or sheep.