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Differences in Sera of Human Subjects with Respect to Heteroagglutinins for Mouse Erythrocytes.*

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In attempting to demonstrate and induce agglutinins in the blood of 9 types of hybrid mice and in mice of 5 strains inbred for many generations, suspensions of mouse erythrocytes in human sera were routinely used as control material.[1] It was thus noticed that mouse erythrocytes were agglutinated by the sera obtained from human subjects of all 4 blood groups. There seemed to be no special affinity of the erythrocytes of mice for sera of any one type as such. It was apparent, however, that not all the samples of sera from human subjects possessed the property of agglutinating mouse erythrocytes. The negative reactions were heterospecific with respect to the blood groups in human subjects. The most obvious conclusion, therefore, was that there existed an antigen in mouse erythrocytes, the serological analog of which was present in most, but not all, human sera. Subsequent experiments showed that this serological component was specifically adsorbed by homologous antigen. For the sake of convenience, "Mo agglutinin" will henceforth be used to designate this component of human serum.

Gorer[2-5] demonstrated that isoagglutinins were present in the sera of mice in which tumors had recently regressed. This work was independently duplicated by Lumsden[6] who induced isoagglutinins in rats by means of normal or malignant nucleated cells. Both Gorer[3] and Lumsden[6] concluded that the natural resistance of a rat or a mouse to a tumor transplantation is directly proportional to the capacity of the animal to produce agglutinins.

In this paper we have been concerned with an agglutinin for mouse erythrocytes which occurs in most human sera and which is similar to or identical with that developing in mouse sera when transplanted tumors regress. While this is probably not related to the factors that govern susceptibility to cancer in the human subject, it is of interest that it was not present in approximately 14% of human subjects. It is the purpose of the present paper to present the work on the attempts to determine the approximate incidence and data on the adsorption and specificity of the Mo agglutinin in human sera.

Method and Materials. In these experiments, mice† of the following inbred strains were employed: C_3H, C_{57} black, I, A, and JK. The following types of hybrid mice of F_1 and F_2 generations were also used: $C_{57}xC_3H$, C_3HxC_{57}, C_3HxA, $C_{57}xCBAN$, $C_{57}CBANxA$, $C_{57}xA$, AxCBAN, $AxC_{57}CBAN$. In general, the technique used was similar to those described by Gorer.[2] The samples of human sera were obtained commercially, or from ourselves, our colleagues, and the Baltimore Rh Typing Laboratory. All sera were inactivated by heating in a water bath at 56°C for 30 minutes. 1:5000 merthiolate solution was used as a preservative.

The agglutination tests were carried out as follows: One drop of blood was taken from the mouse tail and suspended in 2 cc saline-citrate solution. The sera were diluted 1:2. Two drops of diluted serum and 4 drops of cell suspension were placed in a small test tube and mixed. The tubes were then centrifuged slowly for 30 seconds and then

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1 Figge, F. H. J., Cohen, L., and Winokur, G., submitted for publication.

2 Gorer, P. A., *J. Gen.*, 1936, **32**, 17.

3 Gorer, P. A., *Brit. J. Exp. Path.*, 1936, **17**, 42.

4 Gorer, P. A., *Brit. J. Exp. Path.*, 1937, **18**, 31.

5 Gorer, P. A., *J. Path. and Bact.*, 1937, **44**, 691.

6 Lumsden, T., *Am. J. Cancer*, 1938, **32**, 395.

† All the mice used in these experiments were the progeny of mice obtained from Dr. L. C. Strong in 1941.

TABLE I.
Agglutination of Mouse AxCBAN (7) Erythrocytes with Various Human Sera.
(Numbers in parentheses are for identification of sera.)

A (1)	3+	A (57)	3+	B (21)	2+	AB (20)	3+	O (65)	2+
A (46)	2-3+	A (58)	2+	B (22)	—	AB (277)	3+		
A (50)	2+	A (59)	2+	B (23)	3+	AB (141)	3+		
A (51)	2-3+	A (60)	1+	B (24)	3+	AB (142)	—		
A (52)	3+	A (61)	2+	B (25)	2+	O (60)	2+		
A (53)	2+	A (62)	2+	B (26)	3+	O (61)	3+		
A (54)	—	A (63)	3+	B (27)	3+	O (62)	3+		
A (55)	2+	A (64)	3+	AB (1)	3+	O (63)	—		
A (56)	3+	B (1)	2+	AB (10)	3+	O (64)	—		

allowed to stand for 30 minutes after which they were read with the naked eye and checked microscopically. The readings were recorded as –, 1+, 2+, and 3+, depending on the degree of reaction.

The following adsorption technique was employed: 5 cc of oxalated blood of the desired type was centrifuged and the cells were washed 3 times with physiological saline solution. After the final washing, the supernatant saline was drawn off with a pipette and 10-12 drops of the corresponding serum to be used was added to the cells. The cells and serum were then mixed, centrifuged slowly for 30 seconds, and then allowed to stand 3 hours. The tube was then centrifuged at high speed for 5 minutes, after which the serum was removed and tested against a suspension of the blood cells used in the adsorption. If there was no agglutination of these cells, it was assumed that all the agglutinins had been removed from the serum.

Results. Human type A, B, AB, and O sera were found to agglutinate mouse erythrocytes. The agglutinogen was invariably present in the erythrocytes of mice of all of the 5 strains inbred and the 8 types of hybrid mice mentioned previously.

The data in Table I show that the Mo agglutinin was not present in 5 of 37 samples (approximately 14%) of human sera. It may also be observed that the negative reactions occurred in each of the four major blood groups so that the absence as well as the presence of the Mo agglutinin is not specifically associated with any of the standard human blood groups.

In the adsorption studies (Table II), the Mo agglutinin was shown to be a specific agglutinin which could be adsorbed from human serum by mouse erythrocytes, but not by human erythrocytes of any blood group (Table II). It was found to be entirely independent of α and β agglutinins as indicated by the fact that it was present in 4, but absent in 2, sera of the O group (Table I). Moreover, the α and β agglutinins could be adsorbed on the appropriate human erythrocytes without removing the Mo agglutinin. Conversely, the adsorption of the Mo agglutinin on mouse erythrocytes did not remove the α or β agglutinins or a heterospecific agglutinin for rabbit erythrocytes.[7,8] (Table II.)

The Mo agglutinin was also found to be present in both Rh+ and Rh– blood, and group A Rh+ cells failed to adsorb the Mo agglutinin. This data indicated that there was no apparent relationship between Mo agglutinin and Rh agglutinin. It is, perhaps, needless to point out that Rh sensitization is an induced phenomenon in Rh-persons who have had previous transfusions with Rh+ blood; or in some Rh– mothers who have been delivered of Rh+ children, the total incidence being comparatively low, at least in the latter group; whereas the Mo agglutinin is concerned with a hitherto unnamed naturally occurring agglutinin present in most, but not all, human sera examined thus far.

Discussion. It is necessary to clarify the contribution made by this work in relation to the advances made by others. Sievers[7] has presented a case of heteroagglutination with human serum and has shown that the agglutinins vary in different species of animals.

[7] Sievers, O., *Acta Path. et Microbiol. Scand.*, 1937, **14**, 553.

[8] Wiener, A. S., *Blood Groups and Transfusion*, 3rd ed., C. C. Thomas, Springfield, 1943.

TABLE II.
Cross Adsorption Experiments.
Specific Adsorption of Mo Agglutinin and Failure of Mouse Erythrocytes to Adsorb Rabbit Erythrocyte Agglutinins from Human Sera.

Human serum type	Erythrocytes					
	Human				Mouse	
	A-Rh+	A-Rh—	B	Rabbit	AxCBAN (7)	A (813)
A (50)	—	—	3+	2+	2+	
A (46) before adsorption	—	—	3+	3+	2-3+	
A (46) after adsorption with B cells	—	—	—	3+	2+	
B (22) before adsorption	3+		—	3+	—	
B (23) before adsorption	3+	3+		3+	3+	3+
B (23) after adsorption with A-Rh+ cells	—			3+	3+	3+
B (23) after adsorption with A-Rh— cells		—		3+	3+	3+
A (46) adsorbed with mouse (AxCBAN 7) cells	—	—	3+	3+	—	
B (23) adsorbed with mouse (C_3H 1534) vells	3+	3+	—	3+	—	

The original discovery of the Rh factor[8] was made by testing human beings with antiserum from rabbits which had been injected with Rhesus blood; in this fashion 85% of the individuals tested were positive. Gorer's[2] differentiation of the blood types in mice by reactions with human A serum was based on the quantitative or qualitative differences in the agglutinogens present in mouse erythrocytes. Since Gorer used himself as a source of type A serum, it is evident from his results that his blood contained Mo agglutinin. He apparently did not use a sufficiently large number of other individuals as sources of A serum to encounter one which did not contain Mo agglutinin. It is almost certain however, that the antibody in human A serum that he used, to study the differences in the agglutinogen content of mouse erythrocytes, was Mo agglutinin. At least, the agglutinin in human sera that he used was absorbed by mouse erythrocytes and it was shown in this work that mouse red cells absorb only Mo agglutinin and not α or β agglutinins or the rabbit cell agglutinin.

Summary and Conclusion. The performance of agglutination tests, using the blood cells of mice of 5 inbred strains and 8 types of F_1 and F_2 hybrid mice, have shown that human sera can be differentiated, apart from the usual intra-group reactions, by their ability to agglutinate mouse erythrocytes. The differentiation of human sera on this basis depended on the presence or absence of a specific agglutinin (Mo agglutinin). It was found to bear no specific relationship to either the A-B-O blood groups or the Rh factor. Five of the 37 human sera tested (approximately 14%) did not contain the Mo agglutinin. Mouse erythrocytes adsorbed the Mo agglutinin but not α and β agglutinins or the rabbit cell agglutinin. Human erythrocytes of the α and β group and rabbit red cells adsorbed their specific agglutinins, but not the Mo agglutinin.