Human A- and B-Like Antigens on Red Cells of Marmosets.* (29718)

N. GENGOZIAN†
School of Aerospace Medicine, Aerospace Medical Division (AFSC), Brooks Air Force Base, Texas

Studies are being conducted in this laboratory on the use of the South American marmoset, *Tamarinus nigricollis,* for total-body irradiation effects(1). Since we are interested in the therapeutic value of bone-marrow transfusions in lethally irradiated animals, it is desirable to know whether red cell antigenic differences exist among these primates that would permit identification of any transplanted donor elements in the irradiated host. The tests reported here show the presence of B-like antigens on the marmoset red cells and also suggest that these cells have an A-like antigen specificity. In terms of the human A and B antigens, however, no difference could be detected among the 40 marmosets tested that would distinguish one animal from another by the red blood cells.

Materials and methods. Commercial human anti-A and anti-B antiserum reagents obtained from Knickerbocker Laboratories (New York) were used in all tests. After absorptions of these reagents with either human or marmoset red blood cells (rbc), eluates containing specific agglutinins were prepared from the absorbing cells by the technique of Landsteiner and Miller(2). Agglutination titrations were made by doubling dilutions of antiserum or eluate in physiological saline followed by the addition of an equal volume of 1% red blood cells. After incubation at room temperature for 10 minutes, the tubes were centrifuged at 1000 rpm for 1 minute in a clinical centrifuge. The highest dilution of reagents giving a definite macroscopic agglutination was defined as the titer, which was then converted to $\log_2$ titer. Inhibition tests with marmoset saliva for A, B, and H substance were made by the procedure described by Wiener(3). Titrations of the saliva were done as follows: To the serially diluted saliva was added an equal volume of anti-A, anti-B, or anti-H (*Ulex europaeus* extract) reagents of appropriate titer and the reaction mixture was incubated at room temperature for 10 minutes. The red cells (A, B, or O) were added, and the reaction mixtures were incubated another 10 minutes at room temperature after which they were centrifuged and the agglutinations were read. The inhibition titer was recorded as the first dilution ($\log_2$) giving a definite macroscopic agglutination.

Results. The unabsorbed anti-A serum reacted with the marmoset cells although to a lesser degree than with the human A cells (Table I). Reaction of the unabsorbed anti-B serum with marmoset cells showed the same titer as that obtained with human B cells. After absorption of these reagents with the respective cell types, it was found that the absorbed anti-A showed the same degree of reactivity with the marmoset cells. Absorbed anti-B, however, showed a significant reduction in titer when tested with the marmoset cells.

The previous absorption tests suggested

TABLE I. Reaction of Anti-A and Anti-B Reagents with Marmoset Cells Before and After Specific Absorptions.

Reagent	Agglutinin reaction with: A rbc	B rbc	M* rbc
Antiserum:	Titer ($\log_2$)		
Anti-A			
Unabsorbed	9	—†	4
Absorbed with A rbc	0	—	4
Anti-B			
Unabsorbed	—	9	9
Absorbed with B rbc	—	0	5
Eluate:			
Anti-A			
Unabsorbed	6	—	0
Absorbed with A rbc	0	—	—
Absorbed with M rbc	6	—	—
Anti-B			
Unabsorbed	—	5	4
Absorbed with B rbc	—	0	0
Absorbed with M rbc	—	0	0

* M = marmoset.
† Test not performed.

* Research supported by U. S. Air Force Contract AF 41(609) 2186.

† Medical Division, Oak Ridge Inst. of Nuclear Studies, Oak Ridge, Tenn.

the presence of B-like antigens on the marmoset rbc. However, since the presence of known heteroagglutinins in our two reagents obviated any definitive test, specific eluates of anti-A and anti-B activity were prepared following absorption of the reagents with A and B cells. As also shown in Table I, the anti-A eluate failed to react with the marmoset cells. In contrast, the anti-B eluate reacted with the marmoset cells almost to the same titer as with the B cells. Absorption of this anti-B eluate with either B cells or marmoset rbc removed completely the agglutinin activity for both red cell antigens. Subsequent tests on cells obtained from 40 marmosets showed reactivity only with the anti-B eluate. All were negative with the anti-A eluate.

The human anti-A and anti-B antisera were next absorbed with marmoset rbc. For the absorptions, rbc from 4 tamarins previously tested with the anti-A and anti-B eluates were pooled. One milliliter of the anti-A and anti-B sera were absorbed 4 times with 1 ml packed marmoset cells. After each absorption, the supernates were tested for any remaining activity to human or marmoset cells. As shown in Table II, the 4 consecutive absorptions of anti-A serum reduced its activity for A cells by only one dilution tube. Similar absorptions on the anti-B serum showed a considerably greater reduction in the anti-B titer. The absorptions were discontinued when no further titer reductions were obtained. With each antiserum, the heteroagglutinating activity for the marmoset cells was completely eliminated after the first absorption. After the first absorption the packed cells were washed carefully and eluates were prepared. Testing of the eluates obtained from the marmoset cells used to absorb the anti-A serum (anti-M(A)), showed it to contain strong agglutinating activity to A cells and no reaction to the marmoset cells (Table II). The anti-M(B) eluate reacted with both the B cells and the marmoset rbc, the agglutinating activity being greater for the human B cells. Two similar experiments with cells obtained from an additional 8 animals yielded the same result.

Inhibition tests for A, B, and H substances were made on the saliva obtained from 8 marmosets. As shown in Table III, the saliva of all marmosets inhibited the anti-A activity while failing to affect the anti-B and anti-H reagents.

TABLE II. Reaction of Anti-A and Anti-B Reagents Following Absorption with Marmoset Red Blood Cells.

Reagent	Agglutinin reaction with: A rbc	B rbc	M* rbc
Antiserum:	Titer (log_2)		
Anti-A			
Unabsorbed	9	—†	4
1st absorption	8	—	0
2nd "	8	—	—
3rd "	8	—	—
4th "	8	—	—
Anti-B			
Unabsorbed	—	9	9
1st absorption	—	7	0
2nd "	—	6	—
3rd "	—	5	—
4th "	—	5	—
Eluate:			
Anti-M(A)‡	5	—	0
Anti-M(B)‡	—	5	3

* M = marmoset.
† Test not performed.
‡ Eluates prepared after absorptions of anti-A (A) and anti-B(B) reagents with marmoset rbc.

Discussion. Although the anti-A and anti-B reagents used in this study clearly had heteroagglutinins reactive with the marmoset cells, the absorption data of Table I suggested the presence of a B-like antigen with the absence of any A antigen on the marmoset rbc. This was further confirmed by the failure of these cells to react with the specific anti-A eluate and their strong reaction with the specific anti-B eluate. While the data of

TABLE III. Tests for A, B, and H Substances in Marmoset Saliva.

Animal No.	Saliva inhibition titer (log_2)* A	B	H
1	6	0	0
2	5	0	0
3	7	0	0
4	8	0	0
5	9	0	0
6	8	0	0
7	6	0	0
8	8	0	0

* Saliva titrations begun at a dilution of approximately 1:8 ($log_2 = 3$).

Table I suggested the lack of any A-like substance on marmoset rbc, surprising results were obtained with eluates prepared by absorption of the antiserum reagents with the marmoset cells. Thus, the strong anti-A activity of the eluate anti-M(A) prepared from the absorbing marmoset cells suggests that these cells may also have some A substance (Table II). Why this antigen was not evident when the marmoset cells were allowed to react with the specific anti-A eluate (Table I) is not clear. That the anti-M(A) eluate did not react with the marmoset cells is expected because the heteroagglutinating activity of the anti-A serum from which the eluate was made was weak. Evidence for the presence of a B-like antigen was further indicated by the absorptions with the marmoset cells, which caused a significant reduction in titer of the anti-B reagent. Also, the anti-M(B) eluate had agglutinating activity for both B and marmoset cells.

The results of the saliva inhibition tests in some respects directly contrasted with the results for the red cell antigens. Thus, if an animal is a secretor, one would expect to find the same antigen in its saliva as on the red cells. But the B antigen was not detected in the saliva under the conditions of our tests. The strong inhibitory effect exhibited on the anti-A reagent indicated the presence of an A-like antigen in the saliva, which correlates with the eluate data of Table II suggesting the presence of some A substance on the red cells. Failure to detect any H substance in the saliva was surprising in that among humans all secretors regardless of blood type normally have the H antigen in their saliva (2).

Our results indicating a B-like antigen on the marmoset red cells agrees with the observation of Landsteiner and Miller reported in 1925(4). Wiener *et al*(5) have recently reported the presence of A antigen and the absence of the B and H antigen in marmoset saliva. Our inhibition tests have confirmed his observations. The evidence suggestive of some A-like antigen on the red cells as found in this study has to our knowledge not been reported earlier. Certainly, the genetic control of the human A, B, and H antigens on red cells and in saliva of the marmosets would appear to be quite different from that of the human. Thus far tests in our laboratory have failed to detect naturally occurring agglutinins to human A and B cells in the sera of these animals. Similarly, natural isohemagglutinins among these animals have as yet not been found. The latter observation is not surprising in view of the established hematopoietic chimerism known to exist in these primates, a phenomenon that would lead to suppression of any development of isohemagglutinins through immunologic tolerance(6).

Summary. Absorption studies with human anti-A and anti-B sera suggested the presence of B-like antigen on marmoset red blood cells and the absence of any A-like antigen. Reaction of specific anti-A and anti-B eluates with marmoset cells confirmed the absorption tests. Eluates prepared from the absorption of the anti-A and anti-B reagents with marmoset red cells suggested, however, the presence of an A-like antigen in addition to the B-like antigen on their red cells. Inhibition tests on marmoset saliva suggested the presence of the A antigen and the absence of B and H antigenic substances in their saliva.

ADDENDUM. In current iso- and heteroimmunization studies aimed at obtaining reagents capable of differentiating marmosets by their red cell antigens, the presence of anti-B agglutinins ($\log_2$ titer range 1-5) to human B cells has been detected in the sera of 18 of 30 marmosets tested. Weaker reactions to human A cells have been found in two. The presence of the anti-B agglutinins in their sera correlates with the A-like substance on the marmoset red cells and in the saliva, and suggests further the non-identity of the B-like substance found on their cells to that on human B cells. Wiener *et al*(5) have noted the lack of any anti-A agglutinins in 11 marmoset sera tested and the presence of anti-B agglutinins in four.

1. Gengozian, N., Batson, J. S., Smith, T. A., In *Proc. of International Symposium on Bone Marrow Therapy and Chemical Protection in Irradiated Primates,* Aug. 15-18, 1962. Radiobiol Inst. T.N.D., printed by Krips, Rijwijk (Z.H.) The Netherlands,

p245.
2. Landsteiner, K., Miller, C. P., *J. Exp. Med.*, 1925, v42, 853.
3. Wiener, A. S., *Blood Groups and Transfusion*, 3rd Ed., Charles C Thomas, Springfield, Ill., 1943.
4. Landsteiner, K., Miller, C. P., *J. Exp. Med.*, 1925, v42, 863.
5. Wiener, A. S., Moor-Jankowski, J., Gordon, E. B., *Am. J. Phys. Anthrop.*, in press.
6. Gengozian, N., Batson, J. S., Eide, P., *Cytogenetics*, in press.

Received September 2, 1964. P.S.E.B.M., 1964, v117.

Studies on Glycolytic Enzymes: Glucose-6-Phosphatase, Phosphorylase and Phosphoglucomutase. Effect of Glucose Cycloacetoacetate (Hydrolysed). (29719)

VIDHYA KRISHNAMURTHI, S. G. NAYUDU AND M. C. NATH
Department of Biochemistry, Nagpur University, Nagpur, India.

The activities of the hepatic glycolytic enzymes, glucose-6-phosphatase, phosphorylase and phosphoglucomutase have been found to be altered in disturbed carbohydrate metabolism. An increase in liver glucose-6-phosphatase activity has been reported in alloxan diabetic and fasting animals(1-5). A parallelism between liver glycogen and activity of phosphoglucomutase in physiologic and pathologic conditions was indicated by Weber and Cantero(6). An increase in hepatic phosphorylase in obese hyperglycemic mice(7) and a decrease in fasting rats have been observed (8).

Glucose cycloacetoacetate (GCA) has been found to have an ameliorating effect in alloxan diabetes; it cured alloxan diabetes in rabbits when given with estrogen and reduced blood glucose concentration when given alone (9). As it has also been reported to maintain glycogen synthesis and breakdown in equilibrium in the liver(10), it was considered interesting to study its effect on the activities of these hepatic enzymes in alloxan diabetic and fasting rats.

Materials and methods. Male albino rats weighing 100-150 g were divided into 5 groups as follows:

Group I—Normal fed rats; Group II—24 hr fasted rats; Group III—24 hr fasted rats injected GCA(h); Group IV—Alloxan diabetic rats; and Group V—Alloxan diabetic rats injected GCA(h).

The animals were fed a normal diet of the following composition:—wheat flour 60%, fats 10%, protein 20%, salt mixture 5%, yeast powder 5%, throughout the experimental period. Rats were made diabetic by subcutaneous injection of alloxan monohydrate (dose—130-150 mg/kg). Some rats died in 5 to 7 days. Those surviving and having a blood glucose level above 200 mg/100 ml were used in the experiment. GCA prepared by the method of West(11) as modified by Nath *et al*(12) was hydrolyzed with 2 N HCl for 20 minutes in a boiling water bath and extracted with ether to remove the resinous material formed. The aqueous solution was adjusted to pH 7.2 with 2 N NaOH. This was injected into the rats of Groups III and V intraperitoneally in 3 equal doses of 600 mg/kg at 10 a.m., 5 p.m. and again at 10 a.m. next morning. Two hours after the third injection, the rats were killed and their livers removed for determining the enzyme activities. The animals of Groups II and III were fasted for 24 hours before killing whereas the animals of other groups were fed.

The rats were stunned, decapitated and bled. The livers were quickly excised, pooled in a beaker standing on cracked ice, chilled for 5 minutes, blotted on filter paper and weighed. A 10% homogenate was prepared in ice cold water for glucose-6-phosphatase and phosphoglucomutase activity and in .1 N NaF for phosphorylase activity. Glucose-6-phosphatase activity was assayed according to the method of Swanson(13). Phosphorylase was assayed by measuring the rate of liberation of inorganic phosphate from glu-