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# Blood Group Antigens in Butanol Extracts of Animal Tissues[1] (40175)

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It has been shown by Wiener *et al.* (1) that a wide variety of animal species have the I and i blood group antigens on their erythrocytes. These workers reported that rabbits were I-positive and i-negative and that Rhesus monkeys were I-negative and i-positive. Although Marty *et al.* (2) had failed to demonstrate the Ii antigens on the red cells of one guinea pig. Zelenski and Lambert (3) showed by absorption and agglutination experiments that rabbit erythrocytes have I, H and HI but no i blood group substances, that all four specificities were present on the red cells of guinea pigs, and that monkeys have only the i specificity on their red cells. This report presents the results of attempts to recover H, HI, I and i substances from the erythrocytes of rabbits, guinea pigs and monkeys to demonstrate these subtances in extracts of organ tissues of the same species of animals.

*Materials and methods. Antisera.* The anti-I (Hal) and anti-i (Obr) sera were obtained from human group A donors, the anti-HI (Hil) serum from a group B donor, and the anti-H (Pec) and anti-H (Pol) sera were from group $O_h$ (Bombay) donors.

*Test cells.* The human group OI (adult) and Oi (umbilical cord) red cells used as test cells in the inhibition of agglutination experiments had been collected as whole blood into ACD, PHS formula B, anticoagulant solution. The red cells were prepared for freezing, frozen in the liquid phase and stored in the vapor phase of liquid nitrogen, thawed and maintained at 4° by the method of Mohn, Cunningham and Bowman (4).

*Animal red cells and tissues.* Animal blood was collected from the heart as whole blood and placed into ACD, PHS formula B, anticoagulant solution. Red cells were promptly washed free of plasma at 4° with PBS (phosphate-buffered saline solution, pH 7.35). Twenty vols of 10% suspensions of red cells in PBS were lysed with one vol of 1:200 digitonin in PBS. Stromata were washed free of hemoglobin at 4° and resuspended in 2 vol of distilled water for extraction.

Small pieces (10 $mm^3$) of organ tissues were carefully washed in cold distilled water, weighed and minced. Ten percent suspensions (w/v) used for extraction were prepared by homogenizing the minced tissues in predetermined vols of cold distilled water.

*Extraction.* Extraction of stromata and tissue suspensions were made by the method of Rosse and Lauf (5). Four vols of the suspensions of stromata and tissues were extracted for 30 min at 4° with *n*-butanol. After centrifugation at 4° for 15 min at 2790*g* the lower aqueous phase was removed and lyophilized. The dry extracts were dissolved in PBS in concentrations of 5 mg/ml and used for serologic analysis.

*Inhibition of agglutination. Preliminary titrations of antisera.* Preliminary titrations of the human antisera by direct agglutination were performed each day that inhibition experiments were carried out to determine the dilution that would provide slightly less than maximum agglutination (++++) for the inhibition test systems.

*Inhibition of agglutination.* Serial twofold dilutions in PBS of the extracts (5 mg/ml) were made in 0.1 ml vol in 10 × 75 mm tubes. An equal vol of the appropriate dilution of antiserum was then added to each tube of the row and the mixtures incubated 30 min at 4°. A third vol of 3% test cell suspension was added to each tube of the row, the tubes shaken and incubated 30 min at 4°. The tubes were centrifuged 3 min at 600*g* in the cold and then returned to the water bath at 4°. Tubes were examined for agglutination directly from the water bath and the results graded and recorded. Scoring of reactions was by the method of Mohn *et al.* (6). Maxi-

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mum agglutination of ++++ was scored as 8, +++(+) as 7 and so on to weak agglutination (+) scored as 1 and no agglutination scored as 0 (6). Inhibition scores represented the amount of agglutination inhibited.

*Results.* Stromata of erythrocytes and selected organ tissues of rabbits, guinea pigs and monkeys were studied for the presence of H, HI, I and i blood group substances. Aqueous phase fractions of single butanol extractions (5) were examined in inhibition of agglutination experiments at 4° using human test systems composed of group OI (adult) and group Oi (cord) red cells and anti-H, anti-HI, anti-I and anti-i sera.

*Rabbits.* The inhibition scores obtained with the extracts of tissues and red cell stromata are presented in Table I. Human anti-A and anti-B sera with human group A and group B test cells were included in these experiments as control test systems since it is well known that A and B blood group antigens are present in rabbits (7). None of the three extracts of red cell stromata of rabbits yielded any group A inhibitory activity, and the tissue extracts of only one (#1) of the three rabbits revealed the presence of group A substance. This is consistent with previous observations that "humanlike" A substance is not present on rabbit red cells but is present in the tissues of some but not all rabbits (8–10). In contrast, group B substance was recovered in the extracts of red cell stromata as expected (7) and in those of gastric mucosa, lung and submandibular gland of all three rabbits but in none of the extracts of parotid gland, pancreas and liver.

Blood group H substance was not demonstrated in any of the extracts with the possible exception of trace amounts in the extracts of red cells and gastric mucosa. Unequivocal recovery of HI activity was obtained only in the extracts of gastric mucosa, although its presence in stromal extracts was suggested. Blood group I substance was recovered from the red cells, gastric mucosa and lung of all three rabbits but not from submandibular and parotid glands, pancreas and liver. No i activity was found in any of the extracts of rabbit red cells or tissues.

*Guinea pigs.* Group H, HI and I substances were recovered unequivocally in the extracts of red cell stromata, gastric mucosa, lung, pancreas and kidney of all guinea pigs studied (Table I). Low levels of I and marginal levels of H substances were observed with the extracts of submandibular gland. No H, HI or I inhibitory activity was seen in any of the extracts of liver or brain. Evidence of i substance was not seen in any of the guinea pig extracts.

*Monkeys.* The i blood group substance was readily demonstrated in all of the extracts of erythrocytes of monkeys but not in those of gastric mucosa, lung, pancreas, liver and brain (Table I). All extracts of the four monkeys studied were devoid of any H, HI and I activity with the exception that H substance was recovered from the gastric mucosa of all four monkeys.

*Discussion.* Unlike the crossreacting anti-A, B (anti-C) sera of group O individuals, which react with group A erythrocytes in the absence of B substance and with group B erythrocytes in the absence of A substance, anti-HI sera react only with the red cells having both H and I substances and not with cells with either the H (adult group Oi) or I (group $O_hI$) antigens alone.

It was expected that the inhibition of the anti-HI serum in this study would be dependent on adequate amounts of both H and I substances in the extracts. The absence of either one or both specificities would preclude the presence of HI activity. The results were consistent with expectations but with two exceptions: (1) the extracts of gastric mucosa of rabbits had quite good levels of I and HI activity but only low or trace levels of H activity at the concentration (5 mg/ml) of extracts examined; (2) the extracts of submandibular gland of guinea pigs had low levels of both H and I substances but no demonstrable HI activity.

It appears that in humans the I specificity is in the interior of A, B, H, $Le^a$ or $Le^b$ carbohydrate chains associated with the precursor type of molecule and is masked by the sugars of the other specificities (11). The anti-HI sera could be reacting broadly with the combined HI region of the complete chain or with the complete H chain (with hidden I determinants) and the incomplete chains having only I specificity. The increased I activity of group $O_hI$ (Bombay) red cells over that of normal adult group OI cells (12) could be explained by the greater amount of I determinants detectable when H determinants are

TABLE I. INHIBITION OF AGGLUTINATION SCORES BY H, HI, I, i BLOOD GROUP SUBSTANCES IN AQUEOUS PHASE FRACTIONS OF BUTANOL EXTRACTS OF ERYTHROCYTES AND ORGAN TISSUES OF RABBITS, GUINEA PIGS AND MONKEYS.

| Human Antisera | Human control extracts: Red cell stromata: Adult O | A | B | AB | Cord O | B | AB | Animal tissue extracts[a]: Red cell stromata 1 | 2 | 3 | 4 | Gastric mucosa 1 | 2 | 3 | 4 | Lung 1 | 2 | 3 | 4 | Submand gland 1 | 2 | 3 | 4 | Parotid gland 1 | 2 | 3 | 4 | Pancreas 1 | 2 | 3 | 4 | Kidney 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Rabbits* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Anti-A | | 17 | | | | 0 | | 1 | 0 | 0 | | 12 | 1 | 0 | | 15 | 0 | | | 9 | 0 | 0 | | 9 | 0 | 0 | | 11 | 9 | | | 0 | 0 | | |
| Anti-B | | 0 | | | | 32 | | 38 | 41 | 41 | | 28 | 29 | 29 | | 31 | 34 | | | 26 | 18 | 32 | | 0 | 0 | 0 | | 0 | 0 | | | 3 | 2 | | |
| Anti-H | | | 17 | | | | 0 | 6 | 2 | 1 | | 3 | 5 | 6 | | 0 | 1 | | | 1 | 1 | 0 | | 0 | 0 | 0 | | 0 | 1 | | | 0 | 0 | | |
| Anti-HI | | | 26 | | | | 0 | 5 | 7 | 9 | | 15 | 18 | 17 | | 0 | 0 | | | 0 | 1 | 0 | | 1 | 0 | 0 | | 0 | 0 | | | 0 | 0 | | |
| Anti-I | | | | 24 | | | 1 | 22 | 25 | 25 | | 18 | 25 | 18 | | 21 | 17 | | | 0 | 0 | 0 | | 1 | 0 | 3 | | 3 | 6 | | | 1 | 0 | | |
| Anti-i | | | 0 | | | | 18 | 0 | 0 | 0 | | 0 | 1 | 0 | | 0 | 0 | | | 0 | 0 | 0 | | 0 | 0 | 0 | | 0 | 0 | | | 0 | 0 | | |
| *Guinea pigs* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Anti-H | 42 | | | | | | 5 | 18 | 21 | 27 | 35 | 52 | 50 | 51 | 46 | 24 | 28 | 29 | 25 | | 8 | 5 | 4 | | | | | | 30 | 29 | 30 | | 24 | 23 | 21 |
| Anti-HI | 46 | | | | | | 6 | 28 | 29 | 28 | 28 | 35 | 29 | 37 | 20 | 23 | 22 | 19 | 20 | | 2 | 2 | 1 | | | | | | 26 | 28 | 33 | | 24 | 25 | 23 |
| Anti-I | 33 | | | | 20 | | | 30 | 30 | 29 | 31 | 29 | 31 | 28 | 31 | 22 | 27 | 29 | 29 | | 10 | 9 | 9 | | | | | | 11 | 23 | 21 | | 24 | 23 | 20 |
| Anti-i | 1 | | | | 24 | | | 2 | 4 | 2 | 4 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | | 0 | 0 | 1 | | | | | | 0 | 0 | 0 | | 0 | 0 | 0 |
| *Monkeys* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Anti-H | 38 | | | | | | 3 | 0 | 1 | 1 | 1 | 10 | 15 | 15 | 11 | 0 | 0 | 0 | | | | | | | | | | 1 | 1 | 0 | 0 | | | | |
| Anti-HI | 31 | | | | | | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | | | | | | | | | | 0 | 0 | 0 | 3 | | | | |
| Anti-I | 28 | | | | 3 | | | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | 0 | 0 | 0 | 0 | | | | |
| Anti-i | 3 | | | | 21 | | | 22 | 27 | 31 | 28 | 3 | 2 | 2 | 0 | 2 | 1 | 1 | | | | | | | | | | 1 | 1 | 0 | 0 | | | | |

[a] Blood group activity was not recovered in any of the extracts of liver (rabbit, guinea pig, monkey) and brain (monkey).

not added to the chains of group $O_hI$ cells.

The results of this study of rabbits are consistent with the foregoing immunochemical studies. The low levels of H activity observed with the extracts of red cell stromata and gastric mucosa of rabbits may reflect (1) the low concentrations of extracts used in this study or (2) some difficulty of the human anti-H serum of group $O_h$ individuals in recognizing H substance of rabbits.

*Summary.* Aqueous phase fractions obtained after extraction with *n*-butanol of erythrocytes and organ tissues of rabbits, guinea pigs and monkeys were examined for H, HI, I and i blood group activity in inhibition of agglutination experiments using red cells and anti-H, anti-HI, anti-I and anti-i sera of human origin.

Rabbit and guinea pig extracts of erythrocytes and some but not all organ tissues studied were shown to have H, HI and I but no i blood group activity. Monkeys had i substance only in the extracts of red cells and H substance only in the extracts of gastric mucosa. The I and HI substances were not demonstrated in any of the extracts of monkey tissues or red cells.

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