IMMUNOCHEMICAL STUDIES ON BLOOD GROUPS

XI. Species Differences among Blood Group A Substances*

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The presence of blood group substances in any secretion or tissue is generally established by the capacity of the secretion or of extracts of the tissue to inhibit hemagglutination of erythrocytes of a given blood group by its corresponding isoantibody (*cf.* references 1, 2). By this technic human secretions and tissues of persons of blood group A (1, 2), the stomach linings of some individuals of various species notably hog (3–5), horse (6–8), and cow (9–11) have been shown to contain blood group A substances; similarly the presence of blood group B substance in stomach linings of some horses (7, 8) and cows (11, 12) and of blood group O substances in the stomach linings of certain hogs (4, 5) and cows (11) has been established. As these various blood group substances were purified and their chemical and immunological properties studied, it became more and more apparent that substances of comparable purity from different species showing a given blood group activity were not identical. For instance, A substance from hog stomach was found to be a better antigen in human beings of blood groups O and B, than was A substance prepared in a similar manner from human saliva, and indeed a good antibody response was obtained in two individuals who received hog A substance after they had failed to respond to the same quantities of human A substance and to crude A saliva (13). Purified blood group substances from individual hog stomach linings (A and O) varied in methylpentose content from about 6 to 13 per cent with an average of 9 per cent (14) while substances of similar purity from human saliva (A, B, and O substances) and cyst fluid (A substance) varied from 9 to 18 per cent with an average of about 13 per cent (14, 15); blood group substances from large pools of horse stomach linings averaged about 7 per cent (8) while those from cow stomachs ranged from 1.5 to 5.2 per cent (11). The horse blood group substances contained somewhat less glucosamine and reducing sugar and more

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non-glucosamine nitrogen than did the substances from the other species (8). Morgan and Watkins (16) have found differences in the reactivity of various samples of human O substances with cattle, rabbit, and human anti-O; some human O substances behave similarly to hog O substances and have been termed H substances by Morgan (16), while others react only with a particular type of naturally occurring human anti-O and Morgan designated these as "true" O substances. It has also recently been found that certain cow stomachs yield a material which shows very little capacity to inhibit hemagglutination of B cells by anti-B but is relatively effective in precipitating the anti-B from an antiserum prepared by immunization of individuals of blood group A with horse B substance (11). The present study of the reactions of hog, human, and horse blood group A substances with homologous and heterologous antisera formed in man as measured by the hemagglutination inhibition and quantitative precipitin methods provides additional evidence of species differences in these materials. By the use of antibody to human A substance, formed by isoimmunization during pregnancy, to precipitate hog and human blood group A substances, and analysis of the washed specific precipitates for hexosamine (*cf.* reference 4), further evidence for the purity of these substances was obtained.

EXPERIMENTAL

The hog and horse blood group substances from individual stomachs and the human saliva substances were those prepared and described previously (4, 8, 13). In addition a sample of human A substance from ovarian cyst fluid (17) was supplied by Dr. W. T. J. Morgan; analytical properties of this preparation determined in this laboratory have been reported (15). Saliva sample A.B.$_5$ 10 per cent was prepared as previously described (13) except that after peptic digestion and alcohol precipitation, the material was redigested with pepsin prior to purification with phenol. The properties of the commercial samples from pools of horse stomachs designated Lederle 35P1A, prepared from a pool of horse stomachs all of which showed some B activity, but some of which also possessed A activity, and Lilly prepared from a random pool of horse stomachs, have also been described (8). Material (CL phenol-insol.) was also obtained from the saliva of a non-secretor of blood group A (*cf.* reference 18) by the methods used for obtaining blood group substances from saliva (13).

Antisera to hog A substance were produced in two individuals of blood group B, Lach and T.R., by injection of A substance from hog mucin sample 2A and from hog 10 respectively (*cf.* reference 4). Subscripts represent the number of the blood sample obtained at varying intervals following immunization. Samples Lach$_5$ and Lach$_6$ and T.R.$_5$ were used. Antiserum to human A substance was obtained from two individuals. One of these, Ru, was a woman of blood group O who had become isoimmunized to A substance during pregnancy and had delivered a baby of blood group A with erythroblastosis fetalis. The erythroblastosis was very probably due to the anti-A since after absorption of the mother's serum with A_1-cdecde erythrocytes, the serum failed to agglutinate the father's red blood cells and the Coombs test on the baby's cells was negative. The second sample, P.M., was produced in an individual of blood group B by immunization with blood group A substance from human saliva; data on the antibody response are given in a previous paper (13). Antibody to horse A substance was obtained by injection of an individual of blood group B, Ra, with the Lederle sample, 35P1A of horse blood group substance.

Hemagglutination inhibition tests were carried out and the various samples were compared by determining the minimum quantity of substance capable of inhibiting completely in a volume of 1 ml., the hemagglutination of 0.1 ml. of a 4 per cent suspension of washed human A cells by 10 to 20 hemagglutinating units of antiserum. All samples listed in Table I were assayed simultaneously with a given antiserum.

Quantitative precipitin curves were obtained by adding increasing quantities of the various blood group substances to a measured volume of antiserum (usually 0.5 to 2.0 ml.); the total volume was kept constant by the addition of saline when necessary. The contents of the tubes were mixed, incubated for 1 hour at 37°C., and the tubes placed in the refrigerator for 6 to

TABLE I

Hemagglutination Inhibition Tests on Hog, Horse, and Human Blood Group Substances with Homologous and Heterologous Anti-A Formed in Human Beings of Blood Groups B and O

Blood group substance used	Minimum quantity of substance inhibiting hemagglutination				
	Antiserum used				
	Anti-hog A		Anti-human A		Anti-horse A
	$Lach_6$ 1:8	T.R.$_5$ 1:5	Ru 1:25	P.M.$_2$ 1:10	Ra_2 1:15
	μg.	μg.	μg.	μg.	μg.
Hog 3 (A)	0.2	0.5	2	2	0.5
" 4 (A)	0.2	0.2	1	0.5	0.5
" 5 (A)	0.2	0.2	2	1	0.5
" 10 (A)	0.2	0.5	2	2	1
" 6 (O)	50	50	>50	50	>50
" 25 (O)	25	50	>50	50	>50
Human cyst A	0.5	0.5	0.5	1	2
G.C. 10% (saliva A)	1	0.5	1	2	5
A.B.$_5$ 10% (saliva A)	1	1	1	1	5
Lederle 35P1A (horse)	25	25	25	25	2
Lilly (horse)	5	10	25	5	1
Be 10% (saliva O)	>50	>50	>50	>50	>50
F.P. 10% (saliva O)	>50	>50	>10	>50	>50
C.L. phenol-insol. (saliva A non-secretor)	>50	>50	>25	>50	>50

8 days. The precipitates were centrifuged off in a refrigerated centrifuge, washed twice in the cold with saline, and analyzed for nitrogen by the Folin-Ciocalteu tyrosine method as described for small amounts of antibody by Heidelberger and MacPherson (19, *cf.* reference 2). Supernatants were tested for anti-A by their capacity to hemagglutinate A erythrocytes and for the presence of A substance by the ability of the supernatant to inhibit hemagglutination of A cells by anti-A (20).

RESULTS

Table I summarizes the data obtained in hemagglutination inhibition tests. With hog, human, and horse anti-A, hog A substances (hogs 3, 4, 5, and 10), the human cyst and saliva (G.C. 10 per cent and A.B.$_5$ 10 per cent) A sub-

stances, and the Lilly horse blood group substance showed high A activity, while the two hog O substances (hogs 6 and 25), two samples of substances from O saliva (Be 10 per cent and F.P. 10 per cent) as well as a sample of blood group substance prepared from the saliva of a non-secretor of blood group A (C.L. phenol-insol.) showed no activity. The other sample of horse substance (Lederle 35P1A) prepared from stomachs selected for B activity but containing A and presumably also the inactive substance (8) showed little A activity except with its homologous anti-horse A.

With the two samples of anti-hog A, the human A substances were slightly but consistently less effective than the homologous hog A substances in hemagglutination inhibition, amounts of from 0.5 to 1.0 μg. of the former being required as compared with 0.2 to 0.5 μg. of the latter. Much larger amounts, 5 to 10 and 25 μg of the horse blood group substances, Lilly and Lederle 35P1A, were required to inhibit hemagglutination.

With the two samples of anti-human A, the hog and human A substances were approximately of equal potency; as with anti-hog A much larger quantities of the horse substances were required.

Hemagglutination inhibition tests with the antiserum to horse A substance showed the homologous horse substances to be much more potent. Although the activity of the four hog A substances appears slightly higher (Table I), it must be remembered that both the Lilly and Lederle samples contain considerable quantities of B and probably inactive substances and hence on the basis of their content of A substance are probably as reactive or even somewhat more reactive with anti-horse A than is the hog A substance. The human A substances appear to be the least effective of the three species of A substances in the hemagglutination inhibition tests with anti-horse A.

Table II and Figs. 1 and 2 show the quantitative precipitin curves obtained with two samples of anti-hog A, $T.R._5$ and $Lach_5$, respectively. The specificity of the sera for anti-A is evidenced by the failure of hog O substance to precipitate with either antiserum; in addition $T.R._5$ gave no precipitate with either horse B substance or a horse substance showing neither A, B, nor O activity (*cf.* reference 8); serum $Lach_5$ was exhausted before these last two products were available.

With serum $T.R._5$ (Fig. 1), hog A (curve 1) and human cyst A (curve 2) precipitated the same quantity of antibody N at the point of maximum precipitation. The cyst A substance, however, appears to have a slightly but probably not significantly greater capacity to precipitate throughout the antibody excess region than the sample of hog A substance (hog 5) used. The horse A substances, however, were much less effective in precipitating anti-hog A from $T.R._5$, the best sample, Lilly, precipitating only about one-half of the antibody (curve 3) and horse 1, 15 per cent and Lederle precipitating but one-sixth of the antibody (curve 4).

TABLE II

Precipitation of Antibody to Hog A Substance by Hog, Human, and Horse Blood Group Substances

Antigen added	Antibody N precipitated									
	Hog A	Human cyst A	Human saliva A ($A.B._5$ 10%)	Human saliva A (G.C. 10%)	Horse Lederle 35P1A	Horse Lilly M336	Hog O (hog 6)	Horse A (horse 1, 15%)	Horse B (horse 4, 15%)	Horse inactive (horse 6, 15%)
μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.
2.0 ml. serum $T.R._5$; total volume 3.0 ml.										
4.3	9.4*									
5		11.4					0.0			
8.6	14.1									
10		17.7			3.7	6.5				
12.8	19.4									
15		23.4‡						4.7		
20					5.0	8.2				
21.3	24.2									
25		28.6						4.7	0.3	0.0
30					5.0	9.2				
40					4.4	10.6	0.0			
42	28.5									
50		27.8						4.7		
60					6.1	12.2				
75								4.7		
86	27.8									
100		25.5			5.2	13.8		5.7		
150									0.3	0.2
1.0 ml. serum $Lach_5$; total volume 3.0 ml.										
10	16.6§	16.1	14.4	11.6	1.6	3.2				
20	19.1	17.4	15.5	13.6	1.8	5.8	0.2			
30	20.5	17.5	13.9	14.5	1.9	7.7				
40	20.5	17.7	13.6	15.2	1.2	8.4				
60	18.9	15.5	12.1	14.6	2.2	11.2				
100	16.4	11.6			2.5	10.8	0.1			

* Hog 5.

‡ One determination discarded.

§ Hog 4.

With the $Lach_5$ antiserum, however, (fig. 2), the hog A substance (curve 1) precipitated about 3 μg. more antibody N than did the human cyst A substance (curve 2). The latter in turn precipitated about 2 μg. more of antibody N per 2 ml. serum than did two samples of human saliva A substance, $A.B._5$ 10 per cent(curve 4) or G.C. 10 per cent (curve 3). $A.B._5$ 10 per cent was considerably

more effective than G.C. 10 per cent in precipitating anti-A in the antibody excess region; this is probably attributable to the additional peptic digestion which it received since a previous saliva sample A.B.$_4$ 10 per cent from the same individual prepared as was G.C. 10 per cent yielded a product giving a quantitative precipitin curve identical with that of G.C. 10 per cent (13). As with T.R.$_5$, horse A substances precipitated much less antibody, the Lilly (curve 5)

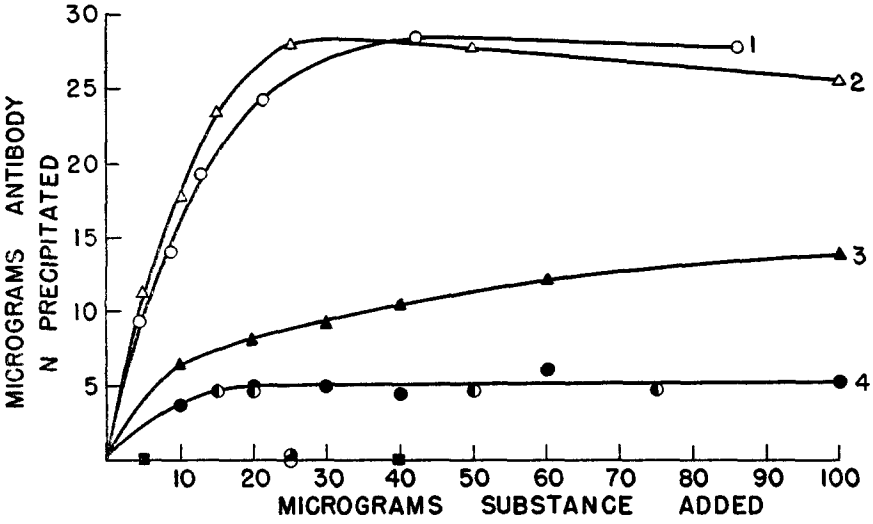

FIG. 1. Antibody N precipitated from 2.0 ml. antiserum T.R.$_5$ by various blood group substances. Antiserum T.R.$_5$ prepared by injection of individual of blood group B with hog A substance (4).

Curve 1 ○ = hog A substance.
Curve 2 △ = human cyst A substance.
Curve 3 ▲ = horse substance (Lilly).
Curve 4 ● = horse substance (Lederle 35P1A).
◐ = horse A (horse 1, 15 per cent).
■ = hog O.
◑ = horse B (horse 4, 15 per cent).
◓ = horse inactive (horse 6, 15 per cent).

and Lederle (curve 6) samples precipitating about one-half and one-tenth of the total antibody N respectively.

The findings with the two samples of antibody to human A substance, Ru and P.M.$_2$, are shown in Table III and Figs. 3 and 4. With serum Ru, in which the anti-human A was formed as a consequence of heterospecific pregnancy, essentially the same maximum amount of antibody (Fig. 3, Table III) was precipitated by hog A (curve 1) and by human cyst (curve 2) and saliva A substances. In the region of antibody excess the human A substance was slightly more effective in precipitating anti-A than was the hog substance. The point

of maximum precipitation was reached with somewhat less human cyst or saliva A substance than with hog A substance. The significance of these relatively small differences is difficult to assess. A small amount of antibody was precipitated by horse A substance and only negligible quantities by hog or human saliva O substances, and horse B and inactive substances.

With antiserum P.M.$_2$ (Table III, Fig. 4), hog O and human saliva O substances failed to precipitate, again indicating the A specificity of the antiserum.

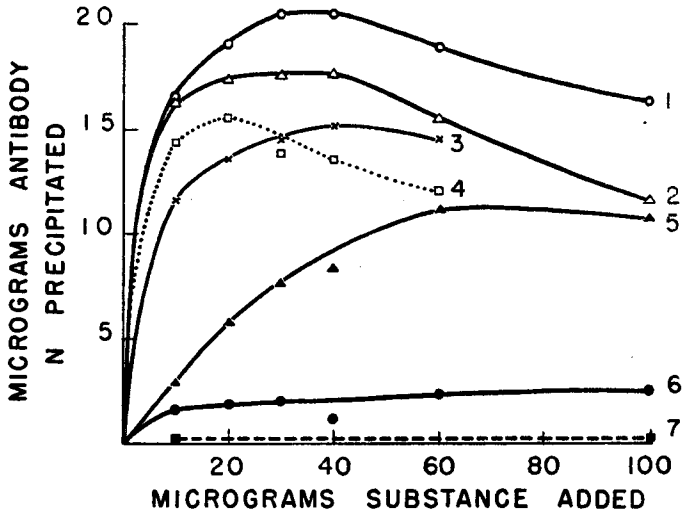

FIG. 2. Antibody N precipitated from 1.0 ml. antiserum Lach$_5$ by various blood group substances. Antiserum Lach$_5$ prepared by immunization of individual of blood group B with hog A substance.

Curve 1 ○ = hog A substance.
Curve 2 △ = human cyst A substance.
Curve 3 × = human saliva A substance (G.C. 10 per cent precipitate).
Curve 4 □ = human saliva A substance (A.B.$_5$ 10 per cent precipitate).
Curve 5 ▲ = horse substance (Lilly).
Curve 6 ● = horse substance (Lederle 35P1A).
Curve 7 ■ = hog O substance.

Identical quantitative precipitin curves were obtained with two different samples of hog A substance. At the point of maximum precipitation, the hog A substance precipitated about 1.2 μg. N more than the human cyst A substance and 1.7 μg. N more than the saliva A substance; it is doubtful whether these differences are of significance. The cyst substance (curve 2) appears to be somewhat more effective per unit weight throughout the antibody excess region than the hog A substance (curve 1) or the saliva substance (curve 3). Only very small quantities of antibody N were precipitated by horse blood group substances (curves 4 and 5).

The results with the antiserum to horse A substance, Ra, are given in Table IV and in Fig. 5. Of the 50 μg. of antibody N precipitable by homologous horse A substance, only very small quantities (0 to 2.8 μg. N) could be obtained with

TABLE III

Precipitation of Antibody to Human A Substance by Hog, Human, and Horse Blood Group Substances

Antigen added	Antibody N precipitated								
	Hog A (hog 8)	Human cyst A	Human saliva A ($A.B._5$ 10%)	Horse A (horse 1, 15%)	Hog O (hog 6)	Human saliva O (Be phenol-insol.)	Horse B (horse 4, 15%)	Horse Lederle 35P1A	Horse inactive (horse 6, 15%)
μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.
0.5 ml. serum Ru; total volume 1.0 ml.									
2	4.1	6.7							
4	8.9	12.0							
5	11.9	13.1	13.2		0.7				
6.3	12.1	13.2							
10	13.0	13.4	14.3			0.8			
15	13.4; 14.0*	12.7	13.2						
20	13.5	11.5; 11.6	12.0						
25				3.2			1.3		0.8
30	12.9	10.7	12.8						
50	11.2		11.1		0.5				
0.5 ml. serum P.M.$_2$; total volume 1.0 ml.									
5	7.4; 6.6*	10.0	7.4						
10	12.2; 12.1*	12.7	11.3						
15	13.7; 14.6*	13.5	12.9						
20	14.7; 14.6*	13.1	13.0	0.6	0.0	0.0			
25								1.8	
30	14.7	12.7							
40	14.6*		10.8	1.0					
50	13.1	11.2						2.8	
60				1.1					
80				2.1					
100					0.5	0.5		3.1	

* Hog 4.

hog and human O substances, with horse B substance, or with the inactive horse substance, indicating that at least 95 per cent of the antibody was anti-A-specific.

Horse A substances were the most effective per unit weight in precipitating the anti-A, and precipitated the largest quantities of antibody. The sample of

horse A substance (horse 1, 15 per cent) from an individual horse stomach was most effective (curve 3) and the Lilly sample from the random pool of horse stomachs was the next most effective precipitant (curve 5); both of these substances precipitated the same maximum quantity of antibody. Comparison of the relative quantities of the horse 1 and Lilly samples required to precip-

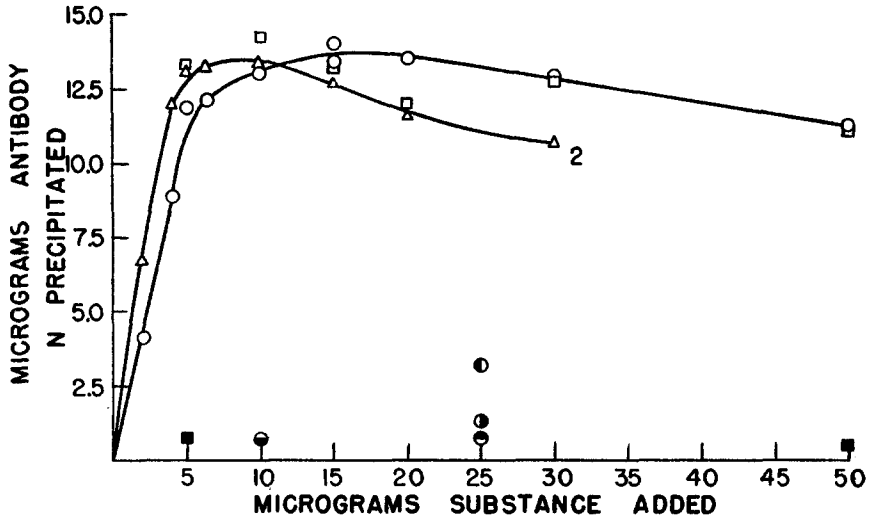

FIG. 3. Antibody N precipitated from 0.5 ml. antiserum Ru by various blood group substances. Antiserum Ru contains antibody to human blood group A substance induced in a woman of blood group O during pregnancy with a fetus of blood group A.

Curve 1 ○ = hog A substance
Curve 2 △ = human cyst A substance.
□ = human saliva A substance (A.B.$_5$ 10 per cent).
◐ = horse A substance (horse 1, 15 per cent).
■ = hog O substance.
◑ = horse B substance (horse 4, 15 per cent).
◓ = horse inactive substance (horse 6, 15 per cent).
◒ = human saliva O substance.

Points for curve 2 drawn for cyst A substance; no curve drawn for A substance from saliva.

itate a given quantity of antibody N (21, *cf.* reference 2) at various levels in the antibody excess region from 15 to 45 μg. of antibody N precipitated, indicated that the Lilly sample had about 40 per cent of the capacity of the horse 1 sample to precipitate anti-horse A. It is of interest, however, that the Lilly sample was found to have somewhat greater activity in inhibiting hemagglutination than did the horse 1 sample (8). The Lederle sample of horse substance was less potent in precipitating anti-horse A than the horse 1 or Lilly sample. This would be expected since the material was prepared from horse stomachs

selected for the presence of B substance and hence homozygous A horses as well as completely inactive horses (8) would have been eliminated and the only A substance introduced would have come from heterozygous AB horses. A peculiar finding is the failure of the Lederle sample to precipitate all of the anti-horse A. From curve 4, Fig. 5 and Table IV, it is evident that the Lederle 35P1A sample precipitated only 40 of the 50 μg. of antibody N in the serum.

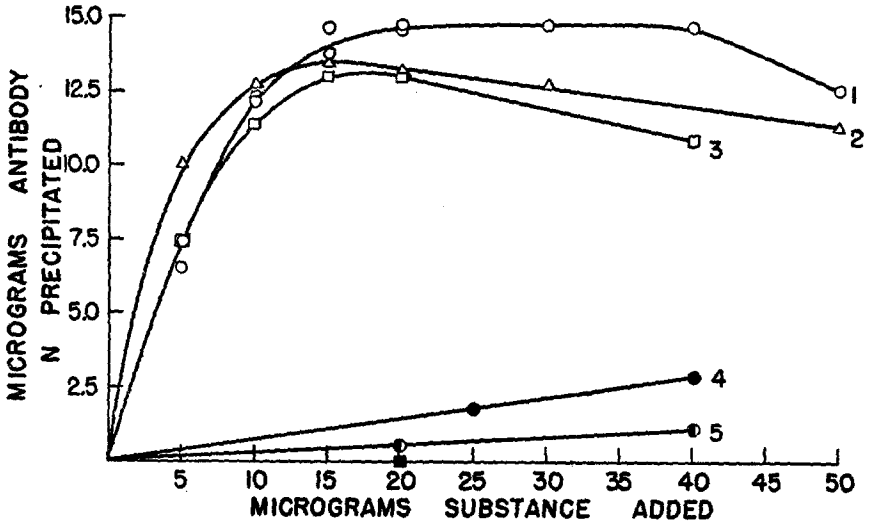

FIG. 4. Antibody N precipitated from 0.5 ml. antiserum P.M.$_2$ by various blood group substances. P.M.$_2$ prepared by immunization of individual of blood group B with human saliva A substance (13).

Curve 1 ○ = hog A substance.
Curve 2 △ = human cyst A substance.
Curve 3 □ = human saliva A substance (A.B.$_5$ 10 per cent precipitate).
Curve 4 ● = horse substance (Lederle 35P1A).
Curve 5 ◐ = horse A substance (horse 1, 15 per cent precipitate).
■ = hog O substance.

Since the Lederle sample does not precipitate all of the antibody, a precise comparison of its precipitating power in relation to the horse 1 or Lilly sample is not possible. However a very rough estimate obtained in the region of relatively large antibody excess (15 to 30 μg. N) in which the effect of differences in the total antibody precipitated by the various samples would be minimal, indicates that the Lederle sample shows an order of A activity of about 25 per cent of that of the horse 1 and 70 per cent of that of the Lilly sample.

The heterologous blood group substances, hog A (curve 1) and human cyst A (curve 2), precipitated only a fraction of the total antibody; the former precipitating a maximum of about 30 μg. N and the latter precipitating only about 20 μg. N of the 50 μg. present (Table IV).

TABLE IV

Precipitation of Antibody to Horse A Substance by Hog, Horse, and Human Blood Group Substances

Antigen added	Antibody N precipitated								
	Hog A (hog 3)	Human cyst A	Horse A (horse 1, 15%)	Horse B (horse 4, 15%)	Horse Lederle 35P1A	Horse Lilly M336	Horse inactive (horse 6, 15%)	Hog O (hog 25)	Human O (F.P. phenol-insol.)
μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.	μg.
1.5 ml. serum Ra_2; total volume 3.0 ml.									
5								0.2	
10	8.6	8.5							
15			26.3			11.1			
20	12.6	11.2						1.5	
25				1.8	13.9	19.8			
30	15.4	12.0	44.6						
40	19.3	13.1							
45			49.2						
50					24.2	32.8			0.7
60		19.0	50.0						
75			50.0		31.9	42.8			
100	26.7*; 27.0	19.0	47.4		40.5	48.5	2.8	2.1	
150				1.7	38.5	44.3		2.8	
200	30.3	19.6			30.3				

* Hog 4.

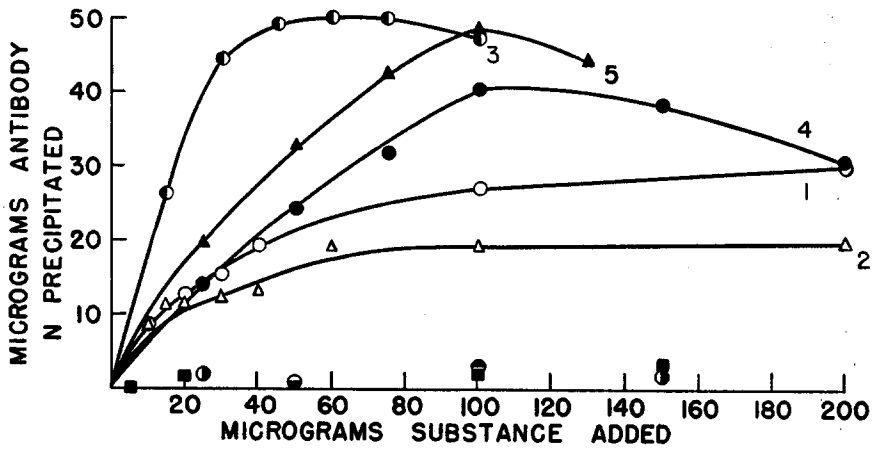

FIG. 5. Antibody N precipitated from 1.5 ml. antiserum Ra_2 by various blood group substances. Antiserum Ra_2 prepared by injection of individual of blood group B with horse substance (Lederle 35P1A).

Curve 1 ○ = hog A substance.
Curve 2 △ = human cyst A substance.
Curve 3 ◐ = horse A substance (horse 1, 15 per cent).
Curve 4 ● = horse substance (Lederle 35P1A).
Curve 5 ▲ = horse substance (Lilly).
◑ = horse B substance (horse 4, 15 per cent).
⊖ = horse inactive substance (horse 6, 15 per cent).
■ = hog O substance.
⊖ = human saliva O substance.

With all antisera, antibody not precipitated by heterologous A substances could be recovered from the supernatant by precipitation with homologous A substances.

The availability of serum Ru, containing antibody to human A substance induced as a result of heterospecific pregnancy, made possible a further test of the purity of the hog and human A substances. Specific precipitates formed in the region of excess antibody were washed and analyzed for hexosamine as previously described (4); the proportion of the hexosamine of the added A

TABLE V

Hexosamine Analyses on Specific Precipitates of Purified Hog and Human Blood Group A Substances and Anti-Human A Formed during Heterospecific Pregnancy

1	2	3	4	5	6	7	8	9	10	11	12	13	14
A substance	Amount used	Glucosamine in added A substance	Volume serum Ru used	Total volume	Glucosamine found in specific precipitate	Found / Added Glucosamine	Antibody N precipitated	Antibody N precipitated corrected for solubility	Solubility correction factor*	Glucosamine found in specific precipitate corrected for solubility‡	Glucosamine due to antibody§	Glucosamine in precipitate due to antigen‖	Proportion of glucosamine of added A substance precipitated¶
	μg.	μg.	ml.	ml.	μg.	%	μg.	μg.		μg.	μg.	μg.	%
Hog 8	60	20	2.0	4.0	22	110	54	60	1.11	24	4	20	100
Human cyst A	60	20	2.0	4.0	22	110	51	56	1.10	24	4	20	100
A.B.$_6$ 10%	60	20	2.0	4.0	19	95	53	58	1.10	21	4	17	85

* Column 9 divided by column 8.

‡ Column 6 multiplied by column 10.

§ Antibody N precipitated (corrected for solubility) multiplied by 0.07 (ratio of hexosamine to N in human gamma globulin).

‖ Column 11 minus column 12.

¶ Column 13 divided by column 3.

substance in the specific precipitate was calculated by applying correction factors for the solubility of the specific precipitates (21, 13) and for the hexosamine in the specific precipitate due to antibody. The data in Table V, arranged as in previous papers, show that essentially all of the hexosamine of the three samples was specifically precipitated by the anti-human A.

DISCUSSION

The data presented (Tables I to IV) clearly establish that purified hog, human, and horse A substances are not identical in their blood group A specificities but show definite species differences detectable by hemagglutination inhibition and by quantitative precipitin determinations. These species differ-

ences are generally similar to species differences among other serum, tissue, or bacterial antigens (*cf.* reference 2) in that the blood group substances react best with their homologous antibodies. Among the three species of blood group A substances the hog and human substances appear to be very similar and indeed many of the differences are very close to or just beyond the limits of error of the methods. However, with one anti-hog A serum, $Lach_5$, the human substances precipitated a significantly lower amount of antibody than did the homologous hog substances. Taken together with the previous finding (13) that individuals would produce antibody to hog A after having failed to respond to injections of human saliva A substance, this indicates that the two substances despite a very close similarity are not identical.

By both technics the horse A substance appears to be distinctly different from those of the other two species and appears to be more closely related to the hog than to the human substance. This is evident from Fig. 5 and Table IV in which the hog A substance precipitates considerably more anti-horse A from serum Ra_2 than does the human cyst A substance as well as by the hemagglutination inhibition data with serum Ra_2 in Table I.

These species differences are undoubtedly a manifestation of differences in chemical constitution of the blood group A substances of different species. A similar situation has also been shown to exist between horse and cow blood group B substances (11) in which the cow substance had relatively little inhibiting effect on the hemagglutination of B cells by anti-horse B but was relatively highly effective in precipitating most of the anti-horse B. In view of these findings it would appear that the distinction by Morgan and Watkins (16) between the H and O substances may be another manifestation of differences among these substances.

These species differences introduce further complexities into a group of substances which from the analytical and immunochemical data previously accumulated appear to be an extraordinarily heterogeneous group of substances centering about a fundamental pattern of chemical composition. Blood group A, B, and O substances, within a given species, constitute a family of substances varying in their methylpentose (fucose) content but otherwise not differing significantly in other analytical or physicochemical properties thus far determined. Despite their sharply specific blood group activities, they show a close similarity of structure as evidenced by their cross-reactivity with Type XIV antipneumococcus serum; in all species studied the fucose end groups reduce the reactivity with Type XIV antiserum as indicated by the increased amount of Type XIV antibody precipitated after mild acid hydrolysis. In at least one species, the hog, there is an inverse correlation between the cross-reactivity with Type XIV antiserum and fucose content. The heterophile or Forssman activity associated only with the blood group A substance has also been shown to be increased by similar mild hydrolysis (22).

The implications of some of the data presented are not yet clear. For instance, the failure of the Lederle substance to precipitate the maximum quantity of antibody from the antiserum to horse A, and the superiority in precipitating power of horse 1, 15 per cent to both Lilly and Lederle samples with anti-horse A (Fig. 5) in contrast to its inferior precipitating power to the Lilly and similarity to the Lederle material with anti-hog A serum T.R.$_5$ (Fig. 1), are obscure. It is possible that they may be related to the genetic factors determining the inheritance of the A, B, and inactive horse substances and perhaps they will be explained by a detailed quantitative immunochemical study of the substances from individual horse stomachs and their antisera which is now in progress.

The data in Table V provide additional corroborative evidence, on a weight basis, for the high degree of purity of the blood group substances. While previous data using antisera prepared by immunization with hog A substance had established that essentially all of the glucosamine and fucose of certain hog and human blood group substances was specifically precipitable by excess of anti-hog A (15), the data now presented show exactly the same result with an antiserum to human A substance arising as a consequence of heterospecific pregnancy. This finding effectively disposes of the theoretically possible objection that the precipitin formed on injection of hog A substance was antibody to some impurity and not anti-A; this objection was not too cogent in view of the evidence previously accumulated (4, 13, 15) as well as that presented in Tables I to IV indicating that the antibody produced was specifically anti-A. Analyses of specific precipitates for their glucosamine content are not affected by Aminoff and Morgan's (23) finding that blood group A substance contains both chondrosamine and glucosamine since both substances give equal color intensity by the analytical method used. If chondrosamine is actually present the data would merely represent total hexosamine and not glucosamine.

SUMMARY

The reactions of hog, human, and horse blood group A substances with antibodies produced on injection of these substances into individuals of blood groups B and O have been studied by hemagglutination inhibition and by quantitative precipitin technics.

Species differences in reactivity with antisera exist among these blood group A substances. Hog and human A substances are very closely related chemically as evidenced by their extensive cross-reaction while horse A substance cross-reacts much less with antisera to both hog and human A substances.

Analysis for hexosamine of specific precipitates of hog and human A substances and antibody to human A substance formed as a consequence of heterospecific pregnancy has shown that essentially all of the glucosamine of the blood group substance is precipitated.

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