THE ISOLATION OF AN O SPECIFIC SUBSTANCE FROM GASTRIC JUICE OF SECRETORS AND CARBOHYDRATE-LIKE SUBSTANCES FROM GASTRIC JUICE OF NON-SECRETORS

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The nature of the blood group specific substances has been studied by many investigators, among them Brahn and Schiff (urine and saliva) (1, 2), Brahn, Schiff, and Weinmann (pepsin) (3), Freudenberg, Eichel, and Dirscherl (urine) (4), Freudenberg and Westphal (peptone) (5), Jorpes and Norlin (urine) (6), Hallauer (blood) (7), and Meyer, Smyth, and Palmer (pig gastric mucosa) (8). Some of these authors have stressed the carbohydrate nature of the substances with which they were working. Landsteiner (9) isolated from horse saliva a purified carbohydrate-like substance exhibiting strong A potency. The original material used for the isolation of the group specific substances seems to play an important rôle in these investigations. Their concentration in the tissue cells and body fluids varies considerably. Putkonen (10) gives the following relative figures in regard to the concentration of group specific substances in various materials of human origin: Saliva, 108 to 1024 units; blood, 8 to 32 units; urine, 2 to 4 units. In a thorough study of the quantitative occurrence of water-soluble group specific substances in tissues and secretions, Friedenreich and Hartman (11) agree principally with Putkonen's findings.

In a previous communication (12), the isolation of a carbohydrate-like substance from human gastric juice of patients belonging to group B has been reported. This substance exhibits strong B potency and is as active as the A preparations obtained according to methods recently employed (*cf.* Landsteiner and Chase (13), Landsteiner and Harte (14), Goebel (15)). The investigations to be reported here deal with the question as to whether it is possible to isolate an O substance from gastric juice of human beings of group O. According to the original belief human cells belonging to group O are characterized by the absence of the A and B substances rather than by the presence of a specific O factor. Some normal animal sera, particularly beef sera, as well as certain immune sera, agglutinate human red blood cells of group O in somewhat higher dilutions than cells of other groups (*cf.* Schiff (16)). The treatment of such normal beef sera with A_1B cells removes agglutinins acting on the blood cells of groups other than

group O and thus renders the sera more specific for the agglutination of O cells. Anti-O serum of this type agglutinates human red blood cells belonging to group O and, in addition, cells belonging to group A_2 but not to A_1 (*cf.* Landsteiner and Levine (17), Friedenreich and Zacho (18)). The problem arises whether the agglutination of O cells by a suitable normal beef serum is based upon the presence of a specific O factor comparable to the A and the B factors, or whether this agglutination can be explained on an entirely different basis. The demonstration of a specific O substance would be of obvious importance for the theory of the blood groups and their inheritance.

Methods and Materials

Preparation of Carbohydrate-Like Substances from Gastric Juices of Human Beings Belonging to Different Groups.—The method used for the isolation of the carbohydrate-like substances from gastric juice is practically the same as described in the previous paper (12). This method was employed by Goebel (15) in his isolation of the A substance from commercial peptone. Essentially, the procedure consists in a series of precipitations of a crude polysaccharide fraction with 2.5 volumes of alcohol in the presence of sodium acetate. The partially pure carbohydrate fraction is deproteinized following Sevag's (19) procedure. After dialysis a protein-free polysaccharide fraction is recovered by precipitation in 10 volumes of acetone.

The isolation of a polysaccharide fraction from gastric juice of persons of group O was accomplished as follows:—

1000 cc. of pooled gastric juice from secretors belonging to group O were obtained. Solid particles were removed and the supernatant heated in a boiling water bath for 5 minutes. The material was then cooled and clarified in a centrifuge. 135 gm. of crystalline sodium acetate were dissolved in the clear fluid and a crude polysaccharide fraction precipitated with 2.5 volumes of alcohol. The precipitate was allowed to settle for 24 hours and then separated by decantation and centrifugation. This precipitate was redissolved in water and insoluble material discarded after centrifugation. 75 cc. of clear supernatant were recovered. The carbohydrate fraction was reprecipitated after the addition of 12 gm. of crystalline sodium acetate and 2.5 volumes of alcohol. This precipitate was redissolved and subsequently subjected to two more precipitations, using sodium acetate and alcohol in the same proportions. The resulting precipitate was then dissolved in 25 cc. of water containing 5 gm. of sodium acetate. The solution was centrifuged at high speed and the perfectly clear supernatant adjusted to a pH equal to 4.8 by the addition of 30 per cent acetic acid. This solution was deproteinized by shaking with chloroform and butyl alcohol according to Sevag's procedure. 19 cc. of clear solution were obtained after centrifugation and separation. Addition of 2.5 volumes of alcohol to this solution precipitated the active fraction which was redissolved in 20 cc. of water and dialyzed through cellophane against successive changes of distilled water. The solution was finally added to 10 volumes of acetone and the carbohydrate fraction precipitated from the colloidal suspension after the addition of a few crystals of sodium chloride. The precipitate was washed repeatedly with acetone and finally dried over sulfuric acid *in vacuo*. The total yield was 185 mg.

The preparation consists of a white hygroscopic substance. Reactions for protein in a 1 per cent solution could not be obtained with such reagents as trichloracetic acid and sulfosalicylic acid. The Molisch reaction was strongly positive. A micro Kjeldahl determination gave a total nitrogen value of 2.8 per cent corrected for ash content which was found to be 7.5 per cent. Following acid hydrolysis, the copper reduction value corresponded to 39.8 per cent glucose calculated on the ash-free basis. Phosphorus in inorganic or organic form was not present.

Thirty-eight specimens of gastric juice have been subjected to fractionation essentially following the above procedure. (There were 18 B specimens, 4 of them non-secretors; 10 O specimens, 2 of them non-secretors; 8 A specimens, and 2 AB specimens.) In many cases the yield was only a few milligrams which did not permit chemical studies beyond the protein tests and the Molisch reaction.

Of particular interest were carbohydrate fractions obtained from four persons belonging to group B but of the non-secretor type. Of these four, two were obtained in sufficient amounts to be subjected to preliminary chemical analysis. The first one of these specimens contained no demonstrable nitrogen by the micro Kjeldahl method and its copper reduction after hydrolysis was equal to 76.2 per cent glucose calculated on an ash-free basis. The second preparation gave 2.73 per cent nitrogen and a glucose value of 30.3 per cent.

So far our experience indicates large variations in total nitrogen and copper reduction values not correlated to the biological activity of the isolated fractions. In our previous report on the isolation of the blood group specific B substance, we stated that the total nitrogen content of the isolated fraction on an ash-free basis was 1.5 to 1.6 per cent. Recent preparations of the B substance have yielded nitrogen values in the same range but also values between 3 and 4 per cent. Our original B preparation exhibited copper-reducing activity corresponding to 75 per cent glucose after acid hydrolysis. The corresponding figures in subsequent preparations have ranged from 40 to 70 per cent. Phosphorus has not been demonstrated in any of these substances.

It should be pointed out that the above nitrogen figures are all based on micro Kjeldahl determinations. The true values are probably somewhat higher corresponding to the usual difference in the values obtained by the micro Kjeldahl and the micro Dumas procedures.

Preparation of the Anti-O Beef Serum.—Various samples of beef blood of 100 to 200 cc. were collected from the slaughter house. The blood was kept overnight in the ice box. A small amount of serum was drawn off and examined for its capacity to agglutinate human red blood cells. Occasionally a serum was found that agglutinated human red blood cells of group O somewhat stronger than those belonging to other groups. Such serum seemed to be most suitable for the preparation of an anti-O reagent. However, for the purpose of obtaining an anti-O reagent it is also possible to use beef serum that agglutinates human blood cells of various groups at about the same degree, although it usually does not give a very strong anti-O reagent (*cf.* Sasaki (20)).

After the serum to be treated had been selected, the entire amount of serum available was separated from the clot and subjected to treatment with A_1B cells in the following way: To 50 cc. of a mixture consisting of equal amounts of normal active beef serum and physiological saline solution was added 1 cc. of a 50 per cent suspension of human blood cells. The mixture was kept in the ice box for a half hour and then centrifuged. The supernatant fluid was tested against human red blood cells belonging to various blood

groups. The absorption was repeated if red blood cells other than O still were agglutinated too strongly. A potent anti-O reagent usually agglutinates, at least slightly, human blood cells belonging to the various groups. If the absorbed serum was found to be satisfactory, it was dried in amounts of 1 to 10 cc. per ampoule or kept in the refrigerator in the presence of 0.01 per cent merthiolate.

TABLE I

Agglutination of Human Red Blood Cells of Various Groups by Normal Beef Serum

Dilutions of beef sera	Beef sera				
	2	3	7	11	12
			Group O Cell Suspension		
(1) 1:10	++	++	+++	+++	+++
(2) 1:20	+	+	+++	+++	+++
(3) 1:40	−	±	++	+++	+
(4) 1:80	−	−	+	++	+
(5) Saline	−	−	−	−	−
			Group A Cell Suspension		
(1) 1:10	++	+++	+++	+++	++
(2) 1:20	+	++	+++	+++	++
(3) 1:40	±	++	++	++	+
(4) 1:80	−	+	+	++	±
(5) Saline	−	−	−	−	−
			Group B Cell Suspension		
(1) 1:10	+	++	+++	++	++
(2) 1:20	±	++	+++	+	+
(3) 1:40	−	+	+	±	+
(4) 1:80	−	±	+	±	±
(5) Saline	−	−	−	−	−

− = no agglutination.
± = faint agglutination.
+ = slight agglutination.
++ = marked agglutination.
+++ = strong agglutination.
++++ = very strong agglutination.

Tests of Beef Sera.—The agglutination of human red blood cells belonging to different blood groups by various normal beef sera is shown in Table I. The experiment was carried out in the following way:—

Decreasing amounts of beef serum, volume 0.2 cc., were mixed with 0.2 cc. of a 1 per cent suspension of human red blood cells belonging to the groups O, A, and B respectively. These mixtures were kept at room temperature for 10 minutes and then centrifuged for 1 minute at medium speed. The tubes were then shaken up slightly and the degree of agglutination determined.

Table I shows that different types of normal beef sera occur although the differences may not be too marked (*cf.* Witebsky and Okabe (21), Schiff and Sasaki (22), Sasaki (20),

Friedenreich and Zacho (18), Dahr (23)). Some beef sera contain only weak agglutinins for all types of human cells while the titers of others are definitely higher. Some show a preference for red blood cells of group A. An additional difficulty consists in the fact that different blood specimens, even of the same group, show considerable variation in the degree of agglutinability. Among the beef sera given in Table I, serum 11 was selected and absorbed with cells belonging to the group A_1B. It should be stated in this connection that not all cells of group A_1B are equally suitable for absorption for reasons that need further clarification.

Table II shows an experiment demonstrating the anti-O agglutination by beef serum 11 after its treatment with A_1B cells. The experiment itself was carried out in the following way:—

Decreasing amounts of absorbed beef serum 11, volume 0.2 cc., were mixed with 0.2 cc. of: (*a*) 1 per cent suspension of human A_1B cells, (*b*) 1 per cent suspension of human O cells. The first tube contained a 1:2 dilution of the absorbed beef serum corresponding

TABLE II

Agglutination of Human Red Blood Cells by Means of Absorbed Normal Beef Serum

Absorbed beef serum 11	Group AB cell suspension	Group O cell suspension
(1) 1:2	+	+++
(2) 1:4	±	+++
(3) 1:8	−	+++
(4) 1:16	−	++
(5) 1:32	−	++
(6) 1:64	−	+
(7) 0	−	−

to a 1:4 dilution of the original native serum. The test tubes were kept at room temperature for 15 minutes and then centrifuged at a medium speed for 1 minute. The resulting agglutination was observed after the tubes had been shaken up. In the case of O agglutination, the tubes should be shaken only slightly. Too violent shaking might break up the agglutinated cells completely.

The agglutination of human red blood cells of group O by means of the absorbed beef serum 11 is quite definite when compared with the agglutination of A_1B cells. Beef serum 11 was the strongest anti-O serum found in over one hundred blood specimens examined so far for this purpose. However, it is possible to use beef serum that does not contain such a strong anti-O agglutinin as beef serum 11. Experiments to be referred to in this paper have been carried out not only with beef serum 11 but also with other anti-O beef sera.

EXPERIMENTAL

Large amounts of group specific substances are present in the saliva and gastric juice of human beings. As far as we know, these secretions contain the highest concentration of group specific substances occurring within the human body. There are, however, certain persons whose saliva is more or

less free of group specific substances. Roughly 20 to 30 per cent of all people belong to the group of "non-secretors." The others constitute the large group of "secretors" (Putkonen (10), Lehrs (24), Schiff and Sasaki (22)). The demonstration of the group specific substances in saliva and gastric juice is accomplished by means of the "inhibition of agglutination" test. This inhibition is perfectly group specific, inasmuch as secretions containing the A substance inhibit the potency of the isoagglutinin anti-A while B-containing specimens counteract the isoagglutinin anti-B. Among the individuals belonging to group O, secretors as well as non-secretors are

TABLE III

Agglutination of Human Red Blood Cells Belonging to Group O by Means of Absorbed Beef Serum Anti-O after Treatment of the Latter with Specimens of Gastric Juice and Saliva Respectively

Dilutions of gastric juice and saliva	I Gastric juice specimens from			II Saliva specimens from		
	Patient 1 Group O	Patient 2 Group O	Patient 3 Group A	Patient 1 Group O	Patient 2 Group O	Patient 3 Group A
(1) Undiluted	+	−	−	+++	X	X
(2) 1:3	+	−	−	+++	−	−
(3) 1:9	++	−	−	+++	−	−
(4) 1:27	++	−	+	+++	+	+
(5) 1:81	+++	+	+	+++	+	+
(6) 1:240	+++	+	+	+++	+	+
(7) 1:720	+++	++	++	+++	+	+
(8) 1:2200	+++	+++	++	+++	+	+
(9) 0	+++	+++	+++	+++	+++	+++

X = missing.

found. However, inhibitory potency toward the O agglutination is not only exhibited by specimens from O individuals but is present more or less in those of secretors belonging to other blood groups (*cf.* Sasaki (20)). Table III shows an experiment in which the inhibitory potency of gastric juice specimens of three different individuals toward the O agglutination is compared with that of saliva. The experiment itself was carried out in the following way:—

Specimens of gastric juice and saliva were heated in a boiling water bath for 10 minutes immediately after being collected and then centrifuged. The clear supernatant fluid was used in the following experiment.

Decreasing amounts of gastric juice (I) and saliva (II) respectively, volume 0.2 cc., were mixed with 0.1 cc. of an absorbed beef serum anti-O and kept at room temperature for 15 minutes. Then 0.1 cc. of a 1 per cent suspension of human red blood cells of group O was added. The mixtures were again kept at room temperature for about 15 minutes and then centrifuged. The resulting agglutination can be seen from Table III.

Table III shows that the saliva and gastric juice specimens obtained from patient 1 do not, or only slightly, inhibit the agglutination of O cells by anti-O beef serum. In contradistinction, the saliva and gastric juice specimens of patients 2 and 3 exhibit definite inhibitory activity. Attention is drawn to the fact that the first two patients belong to group O and the third patient to group A. The experiment clearly indicates the existing difference between secretors and non-secretors recognizable even as far as group O is concerned. However, the inhibition of O agglutination by secretions containing the O factor is much weaker than the inhibition of agglutination of A and B cells by means of secretions containing the A and B factors respectively. The difference in degree of inhibition is considerable. It must be kept in mind, of course, that the inhibitory power of specimens from different individuals varies a great deal. This variation rarely creates any diagnostic difficulties as far as the specimens from A or B patients are concerned because the inhibitory potency in these instances is usually marked. But in the case of the O agglutination it is our experience that difficulties may be encountered in determining whether or not one is dealing with a secretor or a non-secretor because of the relatively high concentrations of saliva and gastric juice necessary to demonstrate the inhibition. Consequently, the question as to the specificity of the inhibitory potency of O-containing specimens deserves special attention.

The best approach to the problem of the existence of an independent substance exhibiting the O property would be through the isolation of such a substance in a chemically pure state. In order to achieve this purpose, the isolation of the O substance from gastric juice of human beings belonging to group O was attempted, using the technique described above. In this manner the carbohydrate fractions from the gastric juices of several people of group O were isolated and examined for their O potency. In regard to the question of specificity, it was important to obtain a satisfactory control preparation. The corresponding carbohydrate fractions obtained from gastric juices of people of the non-secretor type constitute, without doubt, the most suitable controls. Several gastric juices from people of this type were analyzed and their carbohydrate fractions isolated. Because of our interest in the isolation of the B substance from gastric juices of human beings belonging to group B as reported in a previous paper, mainly non-secretors belonging to group B were used in our investigations so far.

The inhibitory activity of these carbohydrate-like substances isolated from the gastric juices of human beings of various groups on the isoagglutination of O, A, and B cells was examined in the following way:—

Four different carbohydrate-like substances, namely, (1) A specific substance (No. 74), (2) B specific substance (Nos. 23/24 pooled), (3) O specific substance (No. 34), and (4) the carbohydrate fraction isolated from the gastric juice of a non-secretor belonging to group B (No. 36) were used in the experiment. Decreasing amounts of these substances, volume 0.2 cc., were mixed in part I, with 0.2 cc. of undiluted serum of group B; in part II, with 0.2 cc. of 1:2 diluted serum of group A; in part III, with 0.2 cc. of anti-O beef serum 11 diluted 1:4. Relatively strong serum dilutions were used in this experiment in order to obtain clear-cut results rather than to determine the end point of inhibitory power of the group specific substances under investigation. The mixtures were allowed to stand for 15 minutes at room temperature. Then 0.2 cc. of a 1 per cent suspension of human red blood cells was added as follows: to part I, cells belonging to group A; to part II, cells belonging to group B; to part III, cells belonging to group O. After standing at room temperature for another 15 minutes, the tubes were centrifuged. The readings obtained are given in Table IV.

The following conclusions can be drawn from the experiment:

1. Only the B substance inhibits agglutination of B cells by serum of group A.

2. Only the A substance inhibits agglutination of A cells by serum of group B.[1]

3. All three substances, A, B, and O, on the other hand, inhibit agglutination of O cells by the anti-O beef serum.

4. The carbohydrate fraction isolated from the gastric juice of a non-secretor belonging to group B lacks any inhibitory potency toward any of the three group specific sera.

The results of these experiments indicate that it is possible to isolate a carbohydrate-like substance exhibiting O potency from the gastric juice of human beings belonging to group O. Because the results of the O agglutination are usually not as clear-cut as the results of the agglutination of A and B cells by their respective isoagglutinins, the specificity of the inhibition of O agglutination might be questioned. However, the failure of the carbohydrate fraction isolated from the gastric juice of a non-secretor to show O potency serves as a negative control and points to the fact that the O potency as exhibited by the respective isolated carbohydrate-like fractions is based upon the presence of an actual substance. The gastric juice of the non-secretor apparently contains a carbohydrate-like substance quantitatively and chemically similar to that isolated from the gastric juice of a secretor. However, the carbohydrate-like substance isolated from the gastric juice of the non-secretor differs from that of a secretor in regard to its serologic activity. That is the reason why it constitutes a most suitable and nec-

[1] Some B preparations give a slight cross reaction with the A preparations and *vice versa*. This cross reaction needs further investigation.

TABLE IV

Agglutination of Human Red Blood Cells Belonging to Blood Groups O, A, and B Respectively by Means of Group Specific Serum Treated with Carbohydrate-Like Substances Isolated from Human Gastric Juice

Dilutions of substances 1:1000	A substance	B substance	O substance	Substance from non-secretor Group B
		Part I		
		Agglutination of Human Cells Belonging to Group A by Serum of Group B Treated with the Group Specific Substances		
(1) Undiluted	−	++	++++	++++
(2) 1:3	−	++++	++++	++++
(3) 1:9	−	++++	++++	++++
(4) 1:27	−	++++	++++	++++
(5) 1:81	−	++++	++++	++++
(6) 1:240	±	++++	++++	++++
(7) 1:720	++	++++	++++	++++
(8) 1:2200	+++	++++	++++	++++
(9) 1:6600	++++	++++	++++	++++
(10) 0	++++	++++	++++	++++
		Part II		
		Agglutination of Human Cells Belonging to Group B by Serum of Group A Treated with the Group Specific Substances		
(1) Undiluted	++++	−	+++	+++
(2) 1:3	++++	−	+++	+++
(3) 1:9	++++	−	++++	+++
(4) 1:27	++++	−	++++	+++
(5) 1:81	++++	−	++++	+++
(6) 1:240	++++	±	++++	++++
(7) 1:720	++++	++	++++	++++
(8) 1:2200	++++	+++	++++	++++
(9) 1:6600	++++	+++	++++	++++
(10) 0	++++	++++	++++	++++
		Part III		
		Agglutination of Human Cells Belonging to Group O by Anti-O Beef Serum Treated with the Group Specific Substances		
(1) Undiluted	−	−	−	+++
(2) 1:3	−	−	−	+++
(3) 1:9	−	−	−	+++
(4) 1:27	−	+	−	+++
(5) 1:81	+	+	+	+++
(6) 1:240	+	++	+	+++
(7) 1:720	++	+++	++	+++
(8) 1:2200	++	+++	++	+++
(9) 1:6600	+++	+++	+++	+++
(10) 0	+++	+++	+++	+++

essary negative control in experiments demonstrating the activity of the O substance.

So far about 40 specimens of gastric juice have been examined and their carbohydrate fractions isolated. In some instances only a few milligrams of the isolated substances were obtained, sufficient to carry out a few tests. Table V shows the inhibitory potency toward the O agglutination of six different carbohydrate-like substances isolated from human gastric juices of persons of various blood groups.

TABLE V

Agglutination of Human Red Blood Cells Belonging to Group O by Absorbed Beef Serum after Treatment of the Latter with Various Carbohydrate Fractions Isolated from Human Gastric Juice

Dilutions of carbohydrate fractions 1:1000	Carbohydrate fractions diluted 1:1000 belonging to groups					
	(*a*) O	(*b*) O	(*c*) A	(*d*) AB	(*e*) Inert B	(*f*) Inert B
(1) 1:3	−	−	−	−	++	+
(2) 1:6	−	−	−	−	++	+
(3) 1:12	−	−	−	−	+++	++
(4) 1:24	−	−	−	−	+++	++
(5) 1:48	−	−	−	−	+++	++
(6) 1:96	−	−	−	−	+++	+++
(7) 1:190	±	−	+	±	+++	+++
(8) 1:380	+	±	+	+	+++	+++
(9) 1:760	+	+	+	+	+++	+++
(10) 1:1520	++	+	+	+	+++	+++
(11) 1:3040	++	++	++	++	+++	+++
(12) 0	+++	+++	+++	+++	+++	+++

Decreasing amounts of the carbohydrate-like substances diluted 1:1000, volume 0.2 cc., were mixed with 0.1 cc. of an absorbed normal beef serum 7 diluted 1:4. After being kept for 4 hours at ice box temperature,[2] 0.1 cc. of human red blood cells belonging to group O were added. The tubes were kept for 15 minutes at room temperature and then centrifuged. The resulting agglutination shown in Table V was obtained after the tubes had been shaken up, this time rather thoroughly. The carbohydrate fractions examined in the experiment were as follows: (*a*) O substance (No. 34), (*b*) substance from non-secretor belonging to group B (No. 36), (*c*) O substance (No. 45) (*d*) substance from non-secretor belonging to group B (No. 46), (*e*) AB substance (No. 47), (*f*) A substance (No. 49).

The inhibitory activity of the group specific substances toward the agglutination of human cells belonging to group O by anti-O beef serum is evident. On the other hand, the carbohydrate-like substances isolated

[2] In later experiments it proved to be unnecessary to keep the tubes at ice box temperature for 4 hours. Instead, they were kept for 15 to 30 minutes at room temperature.

from the gastric juice of non-secretors belonging to group B do not, or only slightly, inhibit the agglutination of O cells by anti-O beef serum. It is noteworthy that the carbohydrate-like substance isolated from the gastric juice of a patient of group AB (specimen 47) exhibits definite O potency. When examined for A and B activity, it proved to be of marked and approximately equal potency for both. In a second AB case (specimen 75) the carbohydrate-like substance isolated from the gastric juice behaved similarly to the first one. However, in this case the inhibition of the O agglutination, although definite, appeared to be somewhat weaker.

DISCUSSION

The agglutination of human red blood cells belonging to group O by normal animal sera as well as by certain immune sera has been observed and confirmed by several investigators. The problem arises whether or not there is a group specific substance O chemically and serologically comparable to the group specific substances A and B, or whether the O factor is something different and does not constitute an independent group specific substance as such. Schiff and his coworkers maintained the conception of an O substance analogous to the A and B substances. There are, however, several differences between the A and B agglutination on the one hand and the O agglutination on the other hand. For instance, the isoagglutination of human red blood cells of groups A and B by their respective isoagglutinins, provided potent sera are used, is usually stronger and more specific than the agglutination of O cells by anti-O beef serum. The most striking difference becomes evident when the inhibitory potency of secretions containing the O factor is compared quantitatively with secretions containing the A and the B factors. Saliva and gastric juices of human beings belonging to the groups A and B may inhibit the isoagglutination of the respective blood cells in dilutions up to 1:10,000 or more. On the other hand, the same secretions of human beings belonging to group O inhibit the O agglutination in dilutions up to one in several hundred, or sometimes less. Because of the relatively high concentration of material necessary to demonstrate the O effect in the cases of saliva and gastric juice, which are very viscous, the interference of non-specific factors has to be kept in mind. Schiff (25) in 1934 thought that it was possible to overcome this difficulty by using anti-O agglutinins found in the serum of a goat immunized by Eisler in Vienna with Shiga dysentery bacilli. When this immune serum was used as an anti-O reagent instead of the usual normal beef serum, dilutions up to 1:10,000 and more of saliva and gastric juice belonging to group O proved to be active in the inhibition of the O agglutination. It is obvious,

however, that further light on the problem can be obtained only by studying the isolated O substance.

Freudenberg and Eichel (26) in 1934 isolated carbohydrate-like substances from the urine of human beings belonging to the three different groups O, A, and B. However, only the A activity of the material was examined by means of the hemolysis inhibition test. Hallauer (7) in 1934 prepared extracts from washed blood cells of groups A, B, and O and obtained substances that were insoluble in alcohol and acetone. He pointed to the possible carbohydrate-like nature of all three blood factors. The material obtained in each instance, rich in phosphorus and sulfur, was perfectly specific in its inhibitory power also as far as group O is concerned.

The carbohydrate-like substance isolated in the above described manner from the gastric juice of human beings of group O manifests its potency in dilutions up to one in several hundred thousand and more. The greatest difficulty in accepting the theory of the existence of an independent O substance analogous to the A and B substances arises from the observation that the A and B substances isolated from the gastric juice of human beings as well as from commerical peptone and pepsin exhibit definite O potency. According to Bernstein's conception of the inheritance of blood groups, one might be tempted to explain these findings on the basis that the A and B substances were obtained from heterozygotes AR or BR, the letter R representing the recessive gene for the O substance. This explanation, although most reasonable, is rendered questionable because the carbohydrate-like substances isolated from the gastric juice of two AB persons exhibited not only A and B, but also O potency. One may argue that the similarity in chemical structure might be the reason for the O activity of the isolated A and B substances. On the other hand, the possibility could be entertained that there is a basic substance constituting the bulk of the group specific substances, somewhat incorrectly referred to as O substance, on top of which the A and the B property might be present or absent.

CONCLUSION

1. The isolation of a group specific O substance from the gastric juice of human beings belonging to group O is described. Absorbed normal beef serum constitutes the anti-O reagent.

2. The O specific substance inhibits the agglutination of human red blood cells belonging to group O. The agglutination of A cells by the isoagglutinin anti-A, or B cells by the isoagglutinin anti-B, is not influenced by the O substance.

3. The A and B specific substances isolated from human gastric juices

inhibit the agglutination of O cells by absorbed normal beef serum, frequently to about the same extent as does the O substance itself.

4. The carbohydrate fraction isolated from the gastric juice of nonsecretors belonging to group B does not inhibit the agglutination of human red blood cells of group B by the isoagglutinin anti-B nor the agglutination of O cells by the anti-O reagent. The significance of these findings for the conception of the nature of the blood groups and their inheritance is discussed.

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