THE ISOLATION OF THE BLOOD GROUP SPECIFIC B SUBSTANCE

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Thorough studies of the nature of the group specific A substance have been carried out during recent years. Several observations seem to indicate that the A substance occurring in material from human beings may be complex in nature. Following investigations of Brahn and Schiff (1), Freudenberg, Eichel, and Dirscherl (2), Landsteiner (3) first isolated an A specific carbohydrate-like substance from horse saliva in 1932. Since that time, the chemical composition of the A specific substance has been investigated by Landsteiner (4), Landsteiner and Chase (5), Landsteiner and Harte (6), Meyer, Smyth, and Palmer (7), Goebel (8), Freudenberg and Westphal (9), and several others. The study of the A substance is facilitated by its occurrence in relatively large amounts in commercial preparations such as pepsin and peptone.

No data are available as yet as to the nature of the group specific B substance. After several unsuccessful attempts to isolate B substance from blood and various other sources, we finally succeeded in isolating it from stomach juice of subjects belonging to group B, which has been shown to be rich in group specific substances. The isolation of a carbohydrate-like substance possessing B properties was carried out by the following technique which is essentially the same as that used by Goebel (8) for the isolation of the A substance from commercial peptone.

EXPERIMENTAL

110 cc. of gastric juice from two patients belonging to group B were obtained. This was centrifuged and the supernatant fluid was heated for 5 minutes in a boiling water bath. After cooling, the material was again centrifuged and 14 gm. of anhydrous sodium acetate was dissolved in the clear supernatant fluid. 275 cc. of alcohol were added and the resulting precipitate was centrifuged the following day. 10 cc. of normal saline solution were added to this precipitate, and after several hours the mixture was again centrifuged and the undissolved residue discarded. 1.4 gm.

anhydrous sodium acetate followed by 25 cc. of alcohol were added to the supernatant fluid and the mixture was allowed to stand overnight. The precipitate obtained was treated again with normal saline, sodium acetate, and alcohol. The precipitate was taken up in 10 cc. of water and the insoluble material discarded. This solution was adjusted to a pH equal to 4.8 using sodium acetate and acetic acid. The deproteinization was performed with chloroform and butyl alcohol according to Sevag's procedure. After separation the deproteinized solution was dialyzed for 48 hours against successive changes of distilled water. The dialyzate was subsequently evaporated *in vacuo* to a volume of 3.5 cc. The active fraction was obtained as a precipitate after adding this solution to 35 cc. of acetone. The precipitate was collected in a centrifuge tube, washed with acetone, and dried over calcium chloride *in vacuo*. The total yield obtained was 13.5 mg.

The material contained 1.5 to 1.6 per cent nitrogen on an ash-free basis (ash 5.9 per cent) and on hydrolysis gave a reduction corresponding to 75 per cent glucose. A 1 per cent solution gave a strongly positive Molisch test. No precipitation was obtained with trichloracetic acid or sulfosalicylic acid.

Serologic Activity of Material Containing B Substance and of the Carbohydrate-Like B Substance Itself

In order to determine the amount of B substance present in our material, the method of inhibition of isoagglutination was used. The first table shows an experiment in which the amounts of B substance present in saliva and gastric juice were determined.

Decreasing amounts of saliva and gastric juice respectively (total volume 0.2 cc.) were incubated for 15 minutes at room temperature with 0.2 cc. inactivated human serum belonging to group A (dilution 1:3). To this was added 0.2 cc. of a 1 per cent suspension of human blood cells belonging to group B. Agglutination was recorded following centrifugation of the tubes.

Table I shows that the B contents of saliva and gastric juice are practically identical. Dilutions up to 1:2000 completely inhibit agglutination of human cells belonging to group B by A serum because of the preceding combination of the isoagglutinin anti-B with the B substance present in saliva and gastric juice respectively. It should be added that this inhibition is specific in nature inasmuch as specimens of gastric juice belonging to group A and group O are without inhibitory effect in this type of experiment.

The activity of the group specific substance B as isolated from the above described stomach juice specimens is revealed in Experiment II.

Decreasing amounts of a 1:1000 stock solution of B specific substance (total volume 0.2 cc.) were incubated for 15 minutes at room temperature with 0.1 cc. of undiluted inactivated human serum belonging to group A. To this was added 0.2 cc. of a 1 per cent suspension of human blood cells belonging to group B. Agglutination was recorded following centrifugation of the tubes.

As can be seen from Table II, a 1:2000 dilution of the 1:1000 stock solution of the B substance completely inhibits isoagglutination of human cells belonging to group B by serum of group A. The inhibitory power of

TABLE I

Agglutination of Human B Cells by Serum of Group A after Treatment of the Latter with Increasing Dilutions of Saliva and Gastric Juice of Group B Respectively

Dilution of saliva and gastric juice respectively		Saliva	Gastric juice
(1)	1:10	−	−
(2)	1:30	−	−
(3)	1:90	−	−
(4)	1:270	−	−
(5)	1:810	−	−
(6)	1:2,430	−	−
(7)	1:7,290	+	±
(8)	1:21,870	+	+
(9)	1:65,610	+	+
(10)	1:196,830	++	++
(11)	1:590,490	+++	+++
(12)	0	+++	+++

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

the isolated group specific substance B is marked, for even in dilutions of one in several millions a partial inhibition of isoagglutination can be demonstrated. If the potency of the isolated B substance is compared with that of the untreated stomach juice specimens, the concentration of B substance in the original stomach juices examined can be roughly estimated as one in a thousand.

In order to compare approximately the potency of the B substance with that of the A substance,[1] several sera of group A and group B respectively were examined. Two sera of about the same strength were selected for Experiment III.

[1] The A substance was prepared from Difco's neopeptone according to Goebel's technique.

Decreasing amounts of group specific A substance (total volume 0.2 cc.) were pipetted into row 1, while exactly the same dilutions of group specific

TABLE II

Agglutination of Human B Cells by Serum of Group A after Treatment of the Latter with Increasing Dilutions of B Specific Substance

1:1,000 dilution of B specific substance		Degree of agglutination
(1)	1:10	−
(2)	1:30	−
(3)	1:90	−
(4)	1:270	−
(5)	1:810	−
(6)	1:2,430	−
(7)	1:7,290	+
(8)	1:21,870	+
(9)	1:65,610	+
(10)	1:196,830	+
(11)	1:590,490	++
(12)	0	+++

TABLE III

Agglutination of Human A and B Cells by Human Serum of Group A and Group B Respectively after Treatment of the Latter with the Isolated Group Specific Substances

1:1,000 dilution of group specific substances		A substance and serum group B + group A cell suspension	B substance and serum group A + group B cell suspension
(1)	1:10	−	−
(2)	1:30	−	−
(3)	1:90	−	−
(4)	1:270	−	−
(5)	1:810	+	+
(6)	1:2,430	+	+
(7)	1:7,290	++	+
(8)	1:21,870	++	++
(9)	1:65,610	+++	+++
(10)	1:196,830	+++	+++
(11)	1:590,490	+++	+++
(12)	0	++++	++++

B substance were pipetted into row 2. 0.2 cc. of a 1:3 diluted serum of group B was added to row 1, and 0.2 cc. of 1:3 diluted serum of group A to row 2. After standing for 15 minutes at room temperature, 0.2 cc. of a 1 per cent suspension of human blood cells belonging to group A was added to row 1, and 0.2 cc. of a 1 per cent suspension of human blood cells belong-

ing to group B to row 2. After standing for 5 minutes at room temperature, the tubes were centrifuged. Agglutination was recorded following centrifugation of the tubes.

These results indicate that the isolated group specific substances A and B behave almost alike as far as their capacity to inhibit isoagglutination is concerned. They are both very potent. Because of the small amount of B substance obtained so far, definite conclusions as to its chemical composition must be deferred until further material is available.

CONCLUSION

The isolation of group specific B substance from human stomach juice is described. The substance is carbohydrate-like in nature and is as potent as the carbohydrate-like substance group A isolated from commercial pepsin and peptone respectively.

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