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Effect of the Purified Blood Group A Substance on Permeability of the Capillaries.

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(Introduced by Albert E. Sobel.)
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In a previous communication,[1] it was shown with the aid of a new method that a number of substances increase the permeability of the capillaries to a very high degree. Very active were commercial peptones. Some of the constituents of commercial peptones were examined, therefore, and in the course of these investigations it was found that A and B substances of Witebsky had a strong effect on capillary permeability. It could not be decided, however, whether this effect was due to the blood substances themselves or to impurities. To answer this question we first tried to make use of the observation that enzymes in saliva and feces destroy the blood group substances.[2-5] We, therefore, treated the AB substance of Witebsky with extracts of feces and the saliva of nonsecretors and examined whether the effect on capillary permeability disappeared with the blood group activity. These attempts, however, were frustrated by the fact that the extracts of feces and the saliva of nonsecretors themselves had a very strong effect on capillary permeability. A new avenue of approach was opened by the work of Kabat, Bendich and Bezer[6,7] who succeeded in purifying the blood group A substance. The capillary effect of this substance will be described in the present paper. Included are investigations on an inactive substance, likewise isolated by Kabat, which is chemically and physico-chemically indistinguishable from the A substance but differs from it serologically. The nature of this substance will be discussed below.

Methods and Materials. The method used in this investigation for the demonstration of the capillary effect is the indirect intracutaneous test with diphtheric toxin and the indirect intramuscular test with tetanic toxin. Both procedures have in common that the increased capillary permeability manifests itself in the increased effect of the antitoxin. The details of this method, and the evidence for this interpretation of the experimental results were presented in our previous communication.[1]

The preparation of the purified A substance has been described by Kabat, Bendich, and Bezer.[6,7] Gastric mucin or stomach linings of hogs were digested with pepsin and purified with the aid of the method of Morgan

TABLE I.
Capillary Effect of the Purified Blood Group A Substance as Demonstrated by the Indirect Skin Test with Diphtheric Toxin.

Dilution of toxin	Series A (control)	Series B
1:50	N	—
1:100	N	E++
1:200	E++++	E+
1:400	E+++	E+
1:800	E++	O
1:1,600	E++	O
1:3,200	E+	O
1:6,400	O	—
1:12,800	O	—

N: necrosis.
E: erythema.
Number of + indicates extent of erythema.
O: no reaction.
—: not done
Results read after 72 hr.

1 Friedemann, U., Traub, F. B., and Langstadt, D., *J. Immunol.*, 1946, **54**, 197.

2 Schiff, F., and Akune, M., *Münch Med. Wochensch.*, 1931, 657.

3 Stimpfl, A., *Z. f. Immunitätsf.*, 1932, **76**, 159.

4 Schiff, F., and Weiler, G., *Biochem. z.*, 1931, **235**, 454.

5 Schiff, F., and Weiler, G., *Biochem. z.*, 1931, **239**, 489.

6 Kabat, E. A., Bendich, A., and Bezer, A. E., *J. Exp. Med.*, 1946, **83**, 477.

7 Bendich, A., Kabat, E. A., and Bezer, A. E., *J. Exp. Med.*, 1946, **83**, 485.

TABLE II.
Capillary Effect of the Purified Blood Group A Substance and of a Preparation Containing 60% A Substance and 40% "Inactive Substance" as Demonstrated by the Indirect Test with Tetanic Toxin.

Dilution of antitoxin	Series A (control)	Series B	Series C
1:20	+6, L.T. 1	—	—
1:40	+6, L.T. 1	—	L.T. 2, O
1:80	+3, +3	—	L.T. 2, O
1:160	+2, +6	L.T. 1 L.T. 2	L.T. 2 L.T. 5
1:320	—	L.T. 1 L.T. 4	+5, +8
1:640	—	+2, G.T. 4	+3, +6
1:1,280	—	+5, G.T. 4	+3, +3

—: not done.
O: no symptoms.
L.T.: local tetanus.
G.T.: general tetanus.
+: indicates death
Numerals indicate day of onset of tetanus or of death.

and King.[8] The stomachs of some hogs yielded the pure A substance while from other stomachs a second substance was obtained, chemically identical with the A substance but serologically inactive. Finally, from the pooled hog stomachs was obtained a product composed of 60% of A substance and 40% of inactive substance. These 3 preparations were kindly placed at our disposal by Dr. Kabat. In the following experiments 1% solutions of these substances in saline were used.

Experiment 1. One cc of diphtheric antitoxin 874 (1600 units per mil.) in a dilution 1:50 was injected intravenously into a white rabbit weighing 2500 g. Immediately afterward 0.1 cc of serial dilutions of diphtheric toxin 1116 were injected intracutaneously. The serial dilutions of toxin were injected in duplicates. In Series A the toxin was diluted in saline solution, in Series B it was diluted in a 1% solution of the purified A substance. The results are recorded in Table I.

It will be seen from Table I that at least 8 times more toxin has been neutralized in Series B than in Series A. This result we interpret as indicating that the purified A substance increases the permeability of the capillaries of the skin to antitoxin approximately 8 times.

Experiment 2. 0.5 cc of serial dilutions of tetanic antitoxin 327 (1200 units per mil.) was injected intravenously into guinea pigs weighing 250 g. Twenty lethal doses of tetanic toxin 1556 (0.1 mil. of a dilution 1:25) were injected intramuscularly. The experiments were carried out 3 times. In Series A the toxin was diluted in saline solution, in Series B in a 1% solution of the purified A substance and in series C in a solution of the preparation containing 60% A substance and 40% inactive substance. The results are recorded in Table II.

Again in Series B the antitoxin is approximately 8 times more effective than in Series A. In Series B the minimal concentration which protected both animals against death was 1:320 while in Series A even a concentration 1:40 protected only one animal. In Series C the antitoxin was approximately 4 times more effective than in Series A. These results indicate that a 1% solution of the purified A substance increased the permeability of the capillaries to antitoxin about 8 times while with a 1% solution of the preparation containing 60% A substance and 40% "inactive" substance the increase was about 4 times.

Experiment 3. One cc of diphtheric antitoxin 874 (1600 units per mil.) was injected intravenously into a white rabbit weighing 2500 g. 0.1 cc of serial dilutions of toxin 1116 was injected intracutaneously. In Series A the toxin was diluted in saline, in

[8] Morgan, W. T. J., and King, H. R., *Biochem. J.*, 1943, **37**, 640.

TABLE III.
Capillary Effect of the "Inactive Substance" as Demonstrated by the Indirect Skin Test with Diphtheric Toxin.

Dilutions of toxin	Series A (control)	Series B
1:50	N	—
1:100	N	N
1:200	E++++	E++++
1:400	E+++	E+++
1:800	E++	E+
1:1,600	E+	O
1:3,200	E+	O
1:6,400	O	—
1:12,800	O	—

N: necrosis.
E: erythema.
No. of + indicates extent of erythema.
O: no reaction.
—: not done.
Result read after 72 hr.

Series B in a 1% solution of the "inactive" substance. The results are recorded in Table III.

As may be seen from Table III, the inactive substance increases the permeability of the capillaries 4 times.

Discussion. The scarcity of the material at our disposal made experiments on a larger scale impossible. The quantitative data, therefore, require confirmation when larger quantities of the purified blood group substances will be available. The indirect intracutaneous test with diphtheric toxin as well as the indirect test with tetanic toxin, however, leaves no doubt that the purified A substance increases the rate of neutralization of toxins, presumably resulting from the increase of the permeability of the capillaries. The fact that the "inactive" substance likewise increased the rate of neutralization and thus presumably had affected the capillary permeability, although to a lesser degree, seems at first to detract from the significance of our findings. Dr. Kabat, however, has authorized us to publish the following personal communication:

9 Personal communication.

"Kabat, Bendich and Bezer[9] have found that Type XIV antipneumococcal horse serum, which has been shown to agglutinate cells of groups A, B and O,[10] gives precipitin reactions with both the active and the inactive hog substances, leading them to the inference that the inactive substance may be associated with blood group O activity." It is highly probable, therefore, that the capillary effect of the "inactive" substance is due to its relationship to the blood group O substance. The blood group B substance is not available in a purified form and nothing can be said, thus far, as to its effect.

As shown in our previous communication,[1] the capacity of increasing capillary permeability is common to a wide variety of substances. To these we now have added the purified blood group substances. This finding assumes additional significance in view of the presence of these substances in the tissue fluids of secretors. Schiff,[11] and Friedenreich and Hartman[12] have discussed the question whether these substances may fulfill any physiological function. On the basis of the results reported in the present paper, the possibility must be considered that they may play a role in the regulation of capillary permeability. From this point of view the division of human beings into secretors and nonsecretors gains added interest.

Summary. With the aid of a new method it was found that the purified blood group A substance considerably increases capillary permeability. Similar results were obtained with the "inactive" substance purified by Kabat *et al.* from hog stomach, and probably closely related to the group O substance.

10 Weil, A. J., and Sherman, E., *J. Immunol.*, 1939, **36**, 139.

11 Schiff, F., *Über die blutgruppenspecifischen substancen des menschlichen Koerpers*, Gustav Fischer, Jena, 1931.

12 Friedenreich, V., and Hartmann, G., *Z. f. Immunitatsf.*, 1938, **92**, 141.