BIKEN'S JOURNAL 2, 233–245 (1959)

Studies on Adsorption of Human Serum Components by Bentonite

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(Received for publication, December 14, 1959)

SUMMARY

Lysozyme can be almost completely removed from human serum by adsorption onto bentonite. Furthermore addition of bentonite to the serum causes loss of the properdin activity. This may be due to the fact that properdin in the serum is adsorbed on the bentonite. This was confirmed by the observation that properdin could be separated from the properdin-bentonite complex by the method employed by Pillermer (1956a) to separate properdin from a properdin-zymosan complex.

A serum deficient in properdin (RP) could readily be prepared with bentonite irrespective of the temperature during the adsorption procedures. Since the properdin-bentonite complex however, did not inactivate C'3 even at 37°C, a serum deficient in C'3 (R3) could not prepared by bentonite. The reduction in complement-and C'3 titer in RP (B) (prepared with bentonite) was much less than that in RP (Z) prepared with zymosan).

An amount of bentonite sufficient to adsorb properdin in serum did not adsorb normal antibodies to various types of red cells or to *Escherichia Coli*, nor did it adsorb some of the specific antibodies.

INTRODUCTION

Recently, several components of human serum concerned with natural defense mechanisms have been thoroughly studied and the defense mechanisms and factors in the host have gradually been clarified. Properdin, which is a new antibacterial factor in the serum, has been studied by Pillemer and his co-workers (Pillemer *et al.* 1954, 1956ab, 1957; Wardlaw *et al.*, 1956) it is generally accepted that the third component of complement is inactivated by a properdin-zymosan complex at 37°C, and that properdin, with the aid of complement and Mg, has a bactericidal activity against some gram negative bacilli.

The similarity of properdin to normal antibodies was thoroughly discussed by Skarnes and Watson (1957). Therefore, the differences between the two factors have an interesting problem in immunobacteriology. Inai *et al.* (1958) reported that serum lysozyme was adsorbed by zymosan, and the serum lysozyme which was eluted from the lysozyme-zymosan complex by acidified saline had biological properties similar to egg white lysozyme. In this paper are reported interesting results concerning the adsorption of serum components onto bentonite that had been used as an adsorbent for lysozyme.

MATERIALS AND METHODS

1. *Human serum*

Blood was drawn from healthy human donors and was centrifuged at 3000 rpm for 15 minutes. The separated serum was refrigerated.

2. *Bentonite*

Bentonite (Wako Pure Chemicals Co.) was not treated before use.

Analysis of bentonite (Osaka City Industrial Laboratory)

Loss in quantity by incandescence	17.29 %
SiO_2	57.76 %
Al_2O_3	11.07 %
Fe_2O_3	3.98 %
CaO	4.72 %
MgO	2.63 %

3. *Zymosan*

Zymosan is the insoluble carbohydrate from Fleishman's yeast cells that have been digested with trypsin and extracted with water and alcohol according to the method reported by Pillemer. An appropriate amount of zymosan was boiled in 100 times volumes of physiological saline for 1 hour just before use.

RP and R3 were prepared according to the original method of Pillemer (1956a).

4. *RP*

5 mg of zymosan was added to each 1.0 ml of human serum and after incubation at 17°C for 60 minutes with occasional mixing, it was centrifuged at 3000 rpm for 15 minutes. The supernatant deficient in properdin was called RP.

5. *R3*

5 mg of zymosan was added to each 1.0 ml of human serum. The mixture was incubated at 37°C for 60 minutes with occasional mixing, and then centrifuged at 3000 rpm for 15 minutes. The supernatant deficient in C'3 was called R3.

6. *RP (B)*

5 mg of bentonite was added to each 1.0 ml of human serum, incubated at 17°C for 60 minutes and centrifuged. The supernatant was designated RP (B).

7. *Quantitative determination of lysozyme activity*

Lysozyme activity was estimated by a modification of Smolelis and Hartsell's method described previously. (Inai *et al.* 1958).

8. *Sensitized sheep cells*

Sheep blood was stored with an equal volume of Alsever's solution at 1°C. Blood cells were then washed twice with physiological saline and once with veronal buffer. A 5 per cent suspension of washed cells was added to an equal volume of veronal buffer containing 5 units of hemolysin, and the mixture was incubated at 37°C for 10 minutes for sensitization.

Sensitized sheep cells were freshly prepared daily.

9. *Acidified saline*

Physiological saline was adjusted to pH 3.0 with 1 N acetic acid.

10. *Diluents*

1/15 M. pH 6.2 phosphate buffer was used for the quantitative determination of lysozyme activity.

The pH 7.4 veronal buffer described by Pillemer et al. (1956a) was used for the hemolytic system.

RESULTS

1. *Adsorption of serum lysozyme by bentonite*

1.0 ml aliquots of human serum were incubated at 17°C for 60 minutes with

5 mg of zymosan or bentonite and then centrifuged for 15 miuutes at 3000 rpm. The lysozyme activity of the supernatants was measured. As shown in Table 1 the lysozyme activity of RP(B) decreased more markedly than that of RP(Z) as compared with that of original serum. Therefore, lysozyme in the serum was almost completely adsorbed by bentonite in comparison with zymosan. However, serum lysozyme could not be eluted from the bentonite-lysozyme complex by acidified saline.

Table 1. Removal of human serum lysozyme by adsorption onto bentonite

Dilution	Extinction		
	Original Serum	RP (Z)	RP (B)
× 4	0.296	0.470	0.570
× 8	0.357	0.570	0.650

2. *Adsorption of properdin by bentonite*

A) 2.0 ml samples of human serum with 20 mg of zymosan or bentonite were centrifuged for 15 minutes at 3000 rpm after incubation at 17°C for 60 minutes with occasional mixing. Supernatants were separated, and 10 mg of zymosan was added to 1.0 mg of the original serum and these supernatents. Then these samples were incubated at 37°C for 60 minutes and centrifuged for 15 minutes at 3000 rpm. A 1.0 ml suspension of sensitized sheep cells was added to 0.5 ml of each supernatant, and hemolysis was measured after incubation at 37°C for 30 minutes. The date are shown in Table 2. Tubes 1 and 2 to which bentonite or zymosan was added before incubation, showed complete hemolysis. But Tube 3 showed no hemolysis at all. From this, it is postulated that inactivation of C'3 did not occur in the serum treated with bentonite even if zymosan was added to the serum. Properdin may make a complex with bentonite at 17°C like zymosan, so that no properdin-zymosan complex formation would result.

Table 2. Removal of properdin in human serum by bentonite at 17°C

Human serum	(ml)	2.0	2.0	1.0
Zymosan	(mg)	20.	—	10.
Bentonite	(mg)	—	20.	—
		Incubated at 17°C for 60 min. Centrifuged		Incubated at 37°C for 60 min.
Supernatant	(ml)	1.0	1.0	
Zymosan	(mg)	10	10	
		Incubated at 37°C for 60 min.		
Supernatant	(ml)	0.5	0.5	0.5
Sensitized sheep cells	(ml)	1.0	1.0	1.0
Hemolysis	(%)	100.	100.	0.

B) Pillemer reported that C'3 was not inactivated by the P-Z complex below 17°C but C'3 was inactivated at 20°C or above. As mentioned above, the properdin-bentonite (P-B) complex like that of zymosan may be formed at 17°C. Attempts were made to whether the P-B complex could inactivate C'3. 10 mg and 5 mg of bentonite were added to 2.0 ml of serum and the mixtures were incubated at 37°C for 60 minutes with occasional mixing. After incubation all mixtures were centrifuged for 15 minutes at 3000 rpm and the supernatants were separated. 1.0 ml of cell suspension was added to 0.5 ml of each supernatant and hemolysis was measured after incubation at 37°C for 30 minutes. The results are presented in Table 3. All tubes showed complete hemolysis. It is clear from these data that, in contrast to the P-Z complex, the P-B complex does not inactivate C'3 even at 37°C.

Table 3. Removal of properdin in human serum by bentonite at 37°C

Tube	1	2	3
Bentonite (mg)	10	5	2.5
Human serum (ml)	1.0	1.0	1.0
	Centrifuged after incubatin at 37°C for 60 min		
Supernatant (ml)	0.5	0.5	0.5
Sensitized sheep cells (ml)	1.0	1.0	1.0
	Incubated at 37°C for 30 min.		
Hemolysis (%)	100	100	100

C) According to Pillemer zymosan does not form any complex with properdin below 10°C. Experiments were performed to see whether bentonite formed a complex with properdin at low temperatures.

As indicated in Table 4, 20 mg, and 5 mg of bentonite respectively were added to test tubes containing 2.0 mg of human serum. Then the tubes were centrifuged for 15 minutes at 3000 rpm. After incubation at 5°C for 60 minutes, 1.0 ml of each supernatant with 5 mg of zymosan was incubated at 37°C for 60 miuntes, supernatants were separated by centrifugation at 3000 rpm for 15 minutes. Finally 1.0 ml of cell suspension was added to 0.5 ml of each supernatant. Hemolysis in the tubes was estimated after incubation at 37°C for 30 minutes. As demonstrated in Table 4, Tube 3 showed 50 per cent hemolysis and Tubes 1 and 2 showed complete hemolysis. These results show that bentonite unlike zymosan, form a complex with properdin at low tempertures.

From the above results it is evident that, in contrast to zymosan, RP could be prepared with bentonite at any temperatre, but R3 which is deficient in C'3 could not be prepared with bentonite.

Table 4. Effect of bentonite on properdin at 5°C

Tube		1	2	3	4
Bentonite	(mg)	20	10	5	—
Human serum	(ml)	2.0	2.0	2.0	—
		Centrifuged after incubation at 5°C for 60 min.			human serum
Supernatant	(ml)	1.0	1.0	1.0	1.0
Zymosan	(mg)	5	5	5	5
		Centrifuged after incubation at 37°C for 60 min.			
Supernatant	(ml)	0.5	0.5	0.5	0.5
Sensitized sheep cells	(ml)	1.0	1.0	1.0	1.0
		Incubated at 37°C for 30 min.			
Hemolysis	(%)	100	100	50	0

3. *Separation of properdin from the properdin-bentonite complex*

As shown above, it was confirmed that there was no properdin actvity in RP (B) which was prepared by addition of bentonite to human serum.

The following two possibilities are considered regarding the mechanism of inactivation of properdin activity by bentonite.

i) Bentonite might inactivate properdin in human serum.

ii) Properdin might be adsorbed by bentonite.

If the latter is the case, properdin could probably be eluted from the P-B complex. The following experiments were done to separate properdin from the P-B complex. As presented in Fig. 1, a modification of the method used by Pillemer (1956a) in purification of properdin from the P-Z complex was employed. The details of the method are as follows: 1.0 mg of bentonite was added to 20 ml of human serum and incubated at 17°C for 60 minutes with occasional mixing. The mixture was centrifuged at 3000 rpm for 30 minutes and the precipitate was separated. 20 ml of pH 6.2 phosphate saline buffer (pH 6.2 phosphate buffer in 19 ml of saline) was added to this precipitate and then thoroughly mixed and centrifuged at 3000 rpm for 30 minutes. The supernatant was discarded. The same procedure was repeated twice. 2.8 ml of 2M saline in 7.2 ml of pH 7.4 phosphate buffer was added to the last precipitate. The reaction mixture was centrifuged at 3000 rpm for 30 minutes after incubation at 37°C for 60 minutes with occasional mixing. The supernatant (properdin eluate) was dialysed at 1°C against 500 ml of distilled water for 3 days. A part of the dialysate was used in the following experiment. The dialysate was adjusted to pH 5.8±0.1 and allowed to stand at 0°C for 60 minutes and the precipitate was separated by centrifugation at 3000 rpm for 30 minutes. This precipitate was extracted by 3.4 ml of pH 7.4 veronal buffer and the supernatant (properdin extract) was separated by centifugation. The second preciptate was similarly extracted by 1.6 ml of pH 7.4 veronal buffer and its supernatant (properdin extract) was separated. These two supernatants (properdin extract) were also used in the following experiment.

Fig. 1. Elution of properdin from P-B complex

1.0 g of bentonite (50 mg/ml) added to 20 ml of human serum
↓ Centrifuged at 3000 rpm for 30 min.
↓ after incubation at 17°C for 60 min.
Precipitate=P-B complex
↓ Washed with 20 ml of pH 6.2 phosphate saline buffer (19 ml of physiological saline plus 1.0 ml of pH 6.2 phosphate buffer) and centrifuged at 3000 rpm for 30 min.
Precipitate=Washed P-B complex
↓ Suspended in mixture of 7.2 ml of pH 7.4 phosphate buffer and 2.0 ml of 2 M saline, then centrifuged at 3000 rpm for 30 min. after incubation at 37°C for 60 min.
Supernatant=Properdin eluate
↓ Dialyzed against 500 ml of distilled water at 1°C for 3 days
Dialysate
↓ Allowed to stand for at least 1 hour at 1°C after adjusting to pH to 5.8± 0.1, then centrifuged at 3000 rpm for 30 min.
Precipitate=Crude properdin
↓ Centrifuged at 3000 rpm for 30 min. following extraction with 3.4 ml of pH 7.4 veronal buffer
Precipitate ; Supernatant=Properdin extract
↓ Centrifuged at 3000 rpm for 30 min. following extraction with 1.6 ml of pH 7.4 veronal buffer
Supernatant=Properdin extract

4. *Recognition of properdin*

A) *Recognition of properdin in the dialysate:*

Reaction mixtures were prepared as indicated in Table 5. 5 mg of zymosan was added to each mixture, and incubated at 37°C for 60 minutes. Then the supernatants was separated by centrifugation at 3000 rpm for 15 minutes. 1.0 ml of cell suspension was added to each supernatant and hemolysis of each tube was estimated after incubation at 37°C for 30 minutes. As shown in Table 5, in Tubes 1 and 2 (dialysate added to RP (B), 5 and 6 (original serum was used as a control) hemolysis was not seen. On the other hand, in Tubes 3 and 4 (RP (B) only) complete hemolysis was shown. It was thought from these results that there might be properdin in the dialysate. The following experiments were made to see if properdin is actually present in this dialysate.

An experiment was carried out as presented in Table 6. All tubes were incubated at 17°C for 60 minutes with 5 mg of zymosan and then the supernatants were separated by centrifugation at 3000 rpm for 30 minutes. 5 mg of zymosan was added again to each supernatant and incubated at 37°C for 60 minutes. Then the cell suspension was added to the separated supernatant and hemolysis of each tube was estimated after incubation at 37°C for 30 minutes. As shown in Table 6, all tubes showed complete hemolysis. From these results, it was

confirmed that the dialysate contained properdin which was removed by zymosan at 17°C and also was able to inactivate complement with zymosan at 37°C.

Table 5. Elution of properdin from properdin-bentonite complex (Dialysate)

Tube		1	2	3	4	5	6
RP (B)	(ml)	0.7	0.5	0.7	0.5	—	—
Original serum	(ml)	—	—	—	—	0.5	1.0
Dialysate	(ml)	0.3	0.5	—	—	—	—
Physiological saline	(ml)	—	—	0.3	0.5	0.5	—
Zymosan	(mg)	5	5	5	5	5	5
		Centrifuged after incubation at 37°C for 60 min.					
Supernatant	(ml)	0.5	0.5	0.5	0.5	0.5	0.5
Sensitized sheep cells	(ml)	1.0	1.0	1.0	1.0	1.0	1.0
		Incubated at 37°C for 60 min.					
Hemolysis	(%)	0	0	100	100	0	0

Table 6. Recognition of properdin in dialysate

Tube		1	2	3	4	5
RP (B)	(ml)	1.4	1.0	1.4	1.0	—
Original serum	(ml)	—	—	—	—	1.0
Dialysate	(ml)	0.6	1.0	—	—	—
Physiological saline	(ml)	—	—	0.6	1.0	1.0
Zymosan	(mg)	10	10	10	10	10
		Centrifuged after incubation at 17°C for 60 min.				
Supernatant	(ml)	1.0	1.0	1.0	1.0	1.0
Zymosan	(mg)	5	5	5	5	5
		Centrifuged after incubation at 37°C for 60 min.				
Supernatant	(ml)	0.5	0.5	0.5	0.5	0.5
Sensitized sheep cells	(ml)	1.0	1.0	1.0	1.0	1.0
		Incubated at 37°C for 30 min.				
Hemolysis	(%)	100	100	100	100	100

B) *Recognition of properdin in the extracts*:

As demonstrated in Table 7, the datails of this experiment were almost identical to that of the above experiment. Slight hemolysis was seen in Tubes 1 and 2, but in Tubes 3, 4 and 5 no hemolysis was seen. Tubes 6 and 7, which contained only RP (B) and zymosan, showed complete hemolysis. Tube 8 and 9, which contained the original serum and zymosan showed no hemolysis.

It was concluded from these results that the extracts contained properdin which could be eluted from the P-B complex.

Table 7. Recognition of properdin in extract

Tube	1	2	3	4	5	6	7	8	9
RP (B) (ml)	0.9	0.85	0.75	0.7	0.5	0.7	0.5	—	—
Original serum (ml)	—	—	—	—	—	—	—	0.7	0.5
Properdin extract (ml)	0.1	0.15	0.25	0.3	0.5	—	—	—	—
Physiological saline (ml)	—	—	—	—	—	0.3	0.5	0.3	0.5
Zymosan (mg)	5	5	5	5	5	5	5	5	5
	Centrifuged after incubation at 37°C for 60 min								
Supernatant (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sensitized sheep cells (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	Incubated at 37°C for 30 min.								
Hemolysis (%)	10	20	0	0	0	100	100	0	0

5. *The influence of bentonite on complement and its componets*

As mentioned above, properdin in human serum was adsorbed by bentonite at any temperature. Therefore RP (B) could be prepared by bentonite at any temperature. The complement titer and C'3 titer of RP (B) prepared by bentonite were estimated. A typical result is presented in Table 8. Decrease of C' titer and C'3 titer in RP (B) was less than that in RP (Z). It is suggested from these results that suitable RP (B) for properdin titration could be prepared by bentonite at any temperature.

Table 8. Complement- cnd C'3 titer in RP (Z) and RP (B)

	C' titer		C'_3 titer
	complete hemolytic unit	half hemolytic unit	
Original serum	12	40	136
RP (Z)	7.5	25	38
RP (B)	12	38	132

6. *Attempts to adsorb various antibodies onto bentonite*

A) *Adsorption of normal anibodies onto various types of red blood cell:*

The following experiment was performed primarily to determine if bentonite could adsorb normal antibody to sheep erythrocyte in human serum. Non-treated human serum and human serum treated with bentonite were diluted 1 in 4, 1 in 8 and 1 in 16, respectively. Human serum was saturated with sheep blood cells at 0°C. As indicated in the control tube of Table 9, the normal antibody to sheep

erythrocyte was completely removed but sufficient complement remained in this serum. 0.2 ml of this serum was added to 0.3 ml aliquots of the diluted test serum as a complement, and 1.0 ml of non-sensitized sheep cell suspension was added to each tube. Hemolysis was measured after incubation at 37°C for 30 minutes. As indicated in Table 9, there was no difference in the intensity of hemolysis between non-treated serum and serum treated with bentonite and there was approximately the same amount of normal antibody left in the treated serum as in the non-treated serum. Similar results were obtained for normal antibody to bovine erythrocyte in human serum.

Table 9. Adsorption of normal antibody by bentonite

Tube	1	2	3	4	5	6	7	8
Originl serum (ml)	×4 0.3	×8 0.3	×16 0.3	—	—	—	—	—
RP (B) (ml)	—	—	—	×4 0.3	×8 0.3	×16 0.3	—	—
Veronal buffer (ml)	—	—	—	—	—	—	0.3	0.3
Human serum after saturation with sheep cells at O°C (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Non-sensitized sheep cells (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	—
Sensitized sheep cells (ml)	—	—	—	—	—	—	—	1.0
	incubated at 37°C for 30 min.							
Hemolysis	C	C	P	C	C	P	(—)	C

C : Complete hemolysis
P : Partial hemolysis

B) *Adsorption of isoagglutinin :*

As shown in Table 10, an experiment was carried out to determine if bentonite could also adsorb isoagglutinin to A and B type human erythrocytes. It was found that bentonite did not have any influence on adsorption of anti-A agglutinin and anti-B agglutinin.

Table 10. Adsorption of isoagglutinin by bentonite (2)

	red cell of A type			red cell of B type		
antiserum dilution	×1	×2	×4	×1	×2	×4
anti-A-serum						
anti-A-serum	+++	++	+	—	—	—
treated with bentonite	+++	++	+	—	—	—
anti-B-serum						
anti-B-serum	—	—	—	+++	++	+
treated with bentonite	—	—	—	+++	++	+

C) *Adsorption o a normal antibody to E. Coli B:*

It is clear from the above results that antibodies to various red cells were not adsorbed by bentonite. Therefore the influence of bentonite on normal antibody to *E. Coli B* was examined. Untreated human serum and various sera which were prepared as shown below were used.

1. OS : Untreated human serum.
2. I S : Inactivated at 56°C for 30 minutes.
3. RP(B) : Human serum with 5 mg/ml of bentonite was incubated at 17°C for 60 minutes. Supernatant was separated by centrifugation.
4. OSC : 1.0 ml aliquots of human serum were incubated at 0°C for 60 minutes with approximately 10 mg of *E. Coli B*. The supernatant was separated by centrifugation at 3000 rpm for 30 minutes.
5. RPC : RP(B) was prepared with *E. Coli B* as described above, instead with orginal serum.

Bacterial suspension: A 16 hour culture of *E. Coli B* was harvested from agar slants and suspended in physiological saline. The concentration of bacterial suspension was 0.1 O. D. at 550 mμ in a Hidachi Electrophotometer.

The original serum and the treated sera were diluted as indicated in Table 11 and 0.1 ml of the bacterial suspension was added to 0.9 ml of the diluted serum. Reaction mixtures were incubated at 37°C for 60 minutes, and 0.1 ml of appropriately diluted mixtures was poured onto agar plate. After incubation at 37°C for 18 hours, the number of bacterial colonies appearing on the agar plate was recorded. The results are presented in Table 11.

Table 11. Adsorption of normal antibody to *E. Coli B.* by bentonite

		Number of colonies				
Dilution of test serums \ Dilution of reaction mixtures		10^{-2}	$\frac{1}{2}10^{-3}$	$\frac{1}{4}10^{-4}$	$\frac{1}{4}10^{-5}$	$\frac{1}{4}10^{-6}$
OS	× 4	0	0	0	0	0
IS	× 4			∞	606	96
RP	× 4	0	0	0		
OSC	× 1	78	0	0		
OSC	× 2	321	15	0		
OSC	× 4	375	78	0		
RPC	× 1	2988	174	15		
RPC	× 2	∞	738	33		
RPC	× 4	∞	1128	99		

The original serum and RP(B) had bectericidal activity to *E. Coli B* and no bacterial colony was seen on the plate of OS and RP(B). On the other hand, bactericidal activity was almost completely eliminated in inactivated serum and 96 colonies were counted even at a 1/4 10^{-6} dilution. Bactericidal activity in

both OSC and RPC was markedly decreased, but there more colonies in RPC than in OSC at the same dilution. As shown in Table 12, the C' titer of OSC and RPC decreased considerably as compared with that of OS and RP(B), but no significant difference in C' titer was found between OSC and RPC.

It is suggested from these results that the bactericidal activity of human serum to *E. Coli B* was inactivated at 56°C for 30 minutes, and this activity was not completely removed by bentonite or bacterial cells only. This activity however, could be removed from human serum by simultaneous treatment with both bentonite and bacterial cells. Properdin and serum lysozyme were removed from RP(B), but the bactericidal activity was not completely eliminated, and this activity was removed by adsorption with bacterial cells at low temperature. It is evident that bactericidal normal antibody removed from serum by bacterial cells can not be adsorbed onto an amount of bentonite sufficient to adsorb properdin in human serum.

Table 12. Complement titer in non-treated- and treated serums

	C' titer	(%)
Non-treated serum	35.2	100
RP (B)	31.8	90.4
OSC	17.0	48.3
RPC	16.0	45.5

D) *Adsorption of other specific antibodies:*

Serum of syphilitic individuals had a positive reaction both in the Wasserman test and flocculation test. It was incubated with 5 mg/ml of bentonite at 37°C for 30 minutes. The treated serum was centrifuged at 3000 rpm for 15 minutes.

The supernatant did not show any difference from the untreated serum in reactivity to the serological tests for syphilis.

DISCUSSION

It was shown that bentonite, which is known to adsorbent for lysozyme, could also adsorb lysozyme in human serum and cause inactivation of properdin. Assuming that the adsorption of properdin by bentonite may be responsible for this inactivation of properdin, led to the successful isolation of properdin from the properdin-bentonite complex. Properdin can be adsobed by bentonite at any temperature. However, the properdin-bentonite complex does not inactivate C'3 even at 37°C, unlike the properdin-zymosan complex.

In the properdin assay proposed by Pillemer (1956a) it is very difficult to obtain adequate R3 and RP for our erperiments. This is one of the reasons why the properdin assay has been thought to be rather unreproducible. The preparation of RP by bentonite does not need so rigid temperature control as that by zymosan. Moreover we can obtain RP rich in complement and C'3 which seems more

suitable for the properdin assay.

Skarnes and Watson (1957) discussed common features of properdin and normal antibody, and wrote as follows:-

"It is concluded from this discussion of the numerous similarities shared by normal antibody and properdin that they are likely the same substance..... It is suggested that the old terminology be discarded unless it can be proved that normal antibody and properdin are distinct substances."

On the other hand, it was postulated by R. A. Nelson (1958) on the basis of his experiments on immune-adherence that properdin might be an antibody against zymosan and C'3 inactivation by zymosan would be a result of complement fixation by an antigen antibody reaction. However, it became evident that the amount of bentonite necessary to adsorb properdin in human serum did not adsorb normal antibodies against various types of red cells and *E. Coli.*

As far as this experiment is concerned, some of the specific antibodies were obviously shown not to be adsorbed by bentonite. In addition, Fujio *et al.* (1959) demonstrated that bentonite does not have any influence on adsorption of an antibody in rabbit against egg white crystalline lysozyme. However from the results of our experiments, it is still impossible to discuss whether properdin may be an antibody aaginst zymosan. But at least, we hesitate to agree with Nelson.

The ambiguos term "normal antibody" may include something consistent with properdin as Skarnes and Watson (1957) pointed out. However, it is suggested that normal antibodies against various types of red cells and some bacteria, which have been thought to be normal antibodies in the classical sense, are obviously different from properdin.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the kind advice and help of Prof. Imasato Donomae (The Third Department of Internal Medicine, Osaka University Medical School) and Prof. Tsunehisa Amano and his co-workers (Department of Bacteriology, Osaka University Medical School).

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