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SHORT COMMUNICATION

INTERACTION OF O-ANTIGEN WITH HEATED BACTERIAL CELLS¹

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During investigations on O-antigen of *Vibrio parahaemolyticus* it was observed that O-antigen itself had a fairly weak inhibitory action against O-agglutination of the organisms. It was considered that this was probably due to an interaction between O-antigen and the heated cells, and experiments were conducted on this possibility.

Purified O-antigens and O-antisera were prepared as previously described (Torii et al. 1969). All experiments were done in 3 per cent sodium chloride medium because the bacteria were halophilic. The occurrence of an interaction was first confirmed by estimation of free O-antigen in the supernatant of a mixture of O-antigen and cells heated at 100 C for 1 hour. The estimation was performed as follows: aliquots of 1.0 ml of heated cell suspension of *Vibrio parahaemolyticus* O3:K4 (O.D., 0.3 at 550 m μ in a Coleman Junior spectrophotometer with a 16 mm diameter cuvette) were mixed with 1.0 ml volumes of O3-antigen solutions of various concentrations and incubated at 50 C overnight. Controls were set up without cells or antigen. The mixtures were centrifuged at 15,000 rev/min and the supernatants were analyzed for O-antigen by sugar determination and the precipitin reaction. As shown in Figs.

1 and 2, free O-antigen in the supernatants of the reaction mixtures decreased.

Actual evidence for the adsorption of O-antigen on heated cells was obtained by agglutination. Ten ml of heated O3-cells were treated with 10 ml of 0.005 per cent O5-antigen at 50 C overnight, washed three times and resuspended in medium. Aliquots of 0.5 ml of the suspension were mixed with 0.5 ml volumes of serial dilutions of anti-O3 or anti-O5 sera and incubated at 50 C overnight. The O3-cells treated with O5-antigen were agglutinated with anti O5-serum as well as with homologous anti-O3 serum, as shown in Table 1. These results show that O-antigen interacts with heated cells of *Vibrio parahaemolyticus* and is adsorbed on the surface of these cells. The unadsorbed O-antigen, found in the supernatant, as shown in Figs. 1 and 2, could have inhibited the O-agglutination, so the adsorption of O-antigen on heated cells can not fully explain the weak inhibitory activity against O-agglutination of homologous bacteria.

Several recent studies showed that bacterial endotoxins interact with erythrocytes (cf. Neter, 1956), serum or plasma proteins (Oroszlan et al., 1966; Skarnes, 1966) and complement systems (Gewurz et al., 1968). Although the erythrocyte surface receptor which can fix endotoxin was extracted and partially charac-

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terized (Springer et al., 1966), the mechanism
 of the interaction between O-antigen and O-

cells is not known yet.

TABLE 1. *Agglutination of O3-cells and O3-cells treated with O5-antigen*

Cells	Antisera	Agglutination				
		× 200	× 400	× 800	× 1600	× 3200
O3	157 V (Anti-O3)	++	++	++	+	—
	80 V (Anti-O5)	—	—	—	—	—
O3 Treated with O5-antigen	157 V (Anti-O3)	++	++	++	+	—
	80 V (Anti-O5)	++	++	++	+	—
O5	80 V (Anti-O5)	++	++	++	+	—

× 200~× 3200 : Serum dilution
 ++ : Complete agglutination with visible aggregates at the bottom of tubes and clear supernatant
 + : Intermediate degree of agglutination between ++ and —
 — : No agglutination

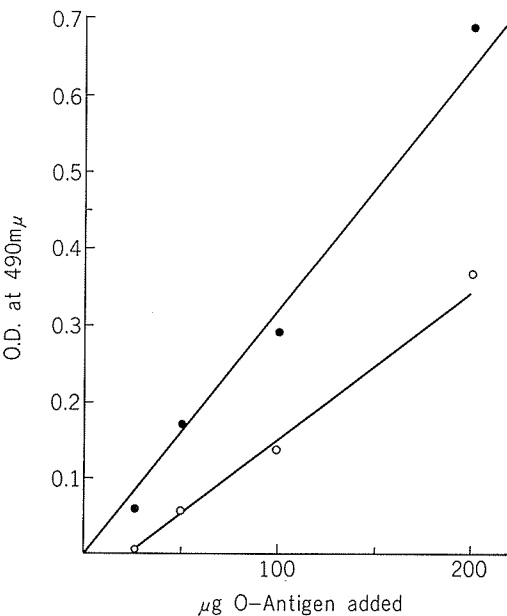


FIGURE 1. O-Antigen remaining in the supernatants of the reaction mixtures (by sugar determination).
 —●—: O-Antigen control
 —○—: Experimental
 Sugar was determined by the phenol-sulfuric acid method (Dubois et al., 1951).

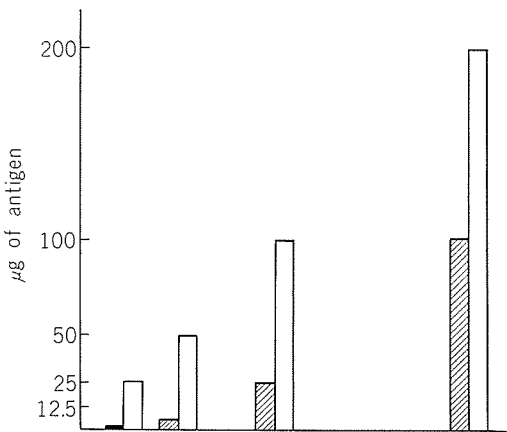


FIGURE 2. O-Antigen remaining in the supernatants of the reaction mixtures (by the precipitin reaction).
 □: Antigen added
 ▨: Antigen found in the supernatant of the reaction mixture
 Determination of antigen was carried out by the ring test with two fold dilutions of antigen solution, the end points of positive precipitation being compared with those of O3 standards.

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