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SHORT COMMUNICATION

INTERACTION OF O-ANTIGEN WITH HEATED BACTERIAL CELLS[1]

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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 mμ 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*

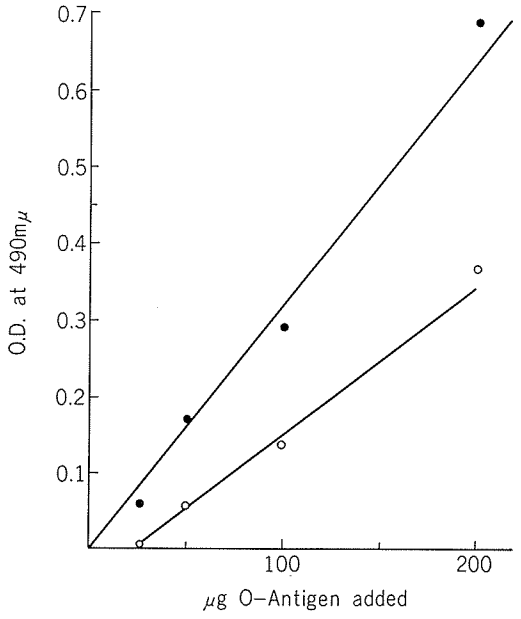

FIGURE 1. *O-Antigen remaining in the supernatants of the reaction mixtures (by sugar determination).*
——●——: *O-Antigen control*
——○——: *Experimental*
Sugar was determined by the phenol-sulfuric acid method (Dubois et al., 1951).

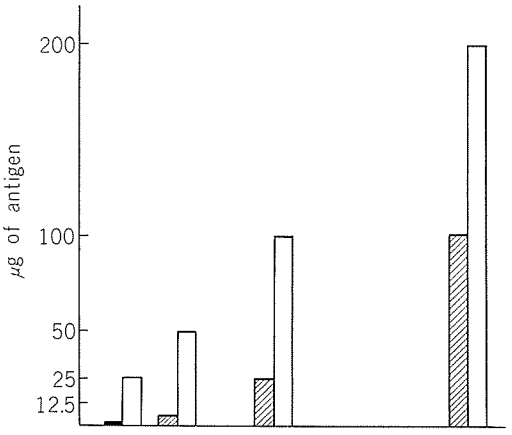

FIGURE 2. *O-Antigen remaining in the supernatants of the reaction mixtures (by the precipitin reaction).*
□: *Antigen added*
▨: *Antigen found in the supernatant of the reaction mixture*
Determination of antigen was carried out by the ring test with two fold dilutions of antigen solution, the end points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

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