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Adhesive Properties of *Vibrio cholerae*: Adhesion to Isolated Rabbit Brush Border Membranes and Hemagglutinating Activity

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Adhesion of vibrios to the small intestine may occur (i) by association of the bacteria with secreted mucus gel or (ii) by adherence of the bacteria to the surface of epithelial cells. In the present study, vibrios readily adhered to isolated brush border membranes obtained from rabbit intestinal epithelial cells. Adhesion was temperature dependent and required the presence of divalent cations such as calcium. The agglutination of human O erythrocytes by *Vibrio cholerae* was observed also, and the hemagglutination test appeared to detect the same mechanism that was involved in the adhesion of vibrios to brush borders. When the bacteria were grown in broth they were adhesive and hemagglutinating, but vibrios grown on agar plates or suspended in buffer for 15 min at 37 C lacked these abilities, even though they retained undiminished motility. These two model systems differed, however, in that strontium promoted only adhesion to brush borders. The significance of this difference remains to be determined. Vibrios were observed to penetrate intestinal mucus gel and occasionally to become entrapped in it. However, there was no evidence that vibrios attached to mucus gel.

For some time it has been recognized that *Vibrio cholerae* associates intimately with the intestinal mucosa (9) and that the inability to do so results in a reduction in virulence (8, 14, 25). A similar relationship between mucosal adhesion and virulence has been shown or suspected for other pathogens (3, 17). One may therefore assume that the ability of *V. cholerae* to colonize the intestinal mucosa constitutes an important pathogenic mechanism of this organism. However, neither the host nor the microbial factors involved in colonization have been defined. Several workers demonstrated by fluorescent microscopy (9, 19) or by electron microscopy (5, 22) that in experimental cholera a close association exists between the vibrios and the surface of epithelial cells. In contrast, Schrank and Verwey (25) have presented evidence which they interpreted to show that vibrios cannot reach the epithelial surface of the rabbit intestine at all unless the bacteria first traverse a continuous mucus blanket covering the villi. In fact, Schrank and Verwey's micrographs do not show vibrios in close approximation to epithelium, even in infected rabbit intestinal loops that had progressed to the stage of fluid production. Their findings are in contrast to the classical work of Florey (6) on cat and rabbit intestine, who observed a rather patchy mucus blanket with many areas of free villi. In Florey's studies, carbon particles applied to the intestinal mucosal surface contacted the villi first and were subsequently "rolled up" into secreted mucus by the contractile movements of the villi. Other intestinal pathogens, such as K88-positive *Escherichia coli*, react strongly in vivo with the epithelial surface, rather than the secreted mucus, of the intestine (17). The only well-defined instance where bacteria associate with the mucus layer is in the rodent large intestine (24). One must therefore conclude that the precise microanatomical structure(s) involved in the mucosal adhesion of *V. cholerae* during the natural or experimental disease is uncertain.

In view of the above, we have expanded our earlier work on the adhesion of *V. cholerae* (10) and *E. coli* (17). To achieve a broader approach to the problem, we have studied the adhesion of *V. cholerae* in a variety of model systems that hopefully simulate important aspects of the interaction of these bacteria with epithelial cell surfaces as well as with secreted mucus. The present paper presents data on the direct interaction between the bacteria and two types of cell surfaces, namely, between the brush border membranes of epithelial cells of the rabbit small intestine and human group O erythro-

cytes, and between vibrios and mucus gel extruded from the small intestines of rabbits.

For reasons of clarity and consistency (16), the term "adhesin" will be used to denote the substance on the bacterial surface that is responsible for adhesion. In contrast, the substance with which adhesin reacts on the mucosa or other eukaryotic cell surface will be referred to as the "receptor."

MATERIALS AND METHODS

***V. cholerae* strains.** The streptomycin-resistant strain (designated P) of the Ogawa serotype described in previous publications (9) was used in this study unless otherwise stated. In addition, 12 strains of *V. cholerae* of both Ogawa and Inaba serotypes that had been isolated during various cholera epidemics over a period of some 30 years were kindly supplied by H. L. Smith (Jefferson University, Philadelphia). These are listed in Table 1.

Preparation of brush borders from the small intestines of rabbits. Brush borders were prepared from the epithelial cells of the small intestine with a method (26) adapted from existing procedures (4, 21). An adult rabbit was killed by intravenous injection of pentobarbital. The small intestine was excised, and the lumen was washed free of digesta with several changes of 0.85% saline. The intestine was partially filled with buffer solution (96 mM NaCl; 5.6 mM Na_2HPO_4; 8 mM KH_2PO_4; 1.5 mM KCl; 10 mM EDTA [ethylenediaminetetraacetic acid]; pH 6.8). The intestine was incubated at room temperature for 15 min in a bath of the same buffer in which the EDTA had been replaced with 0.3 M sucrose. It was then drained and filled with the same buffered sucrose solution, and the epithelial cells were released by manipulation of the intestine. Epithelial cells were recovered at 4 C by centrifugation at 1,200 × *g* for 10 min. The cells were suspended in 5 mM EDTA (pH 7.4) and disrupted in a Teflon-tipped Elvejehm tissue grinder (Thomas, Philadelphia). The brush borders were recovered by centrifugation at 1,200 × *g* for 10 min. Brush borders were purified by repeated alternate homogenization in 5 mM EDTA and centrifugation at 300 × *g* for 10 min, until the supernatant fluid, after centrifugation, was free of debris. Remaining debris was removed by filtration through glass wool.

Brush borders were stored at 4 C in 5 mM EDTA (pH 7.4) containing 10% (vol/vol) formalin (40% commercial formaldehyde solution) at a concentration of approximately 10^7 brush borders per ml. Such preparations could be used for at least 3 months after preparation. The extent of the adhesion of *V. cholerae* to formalin-preserved brush borders was similar to that found with freshly prepared brush borders.

Adhesion of *V. cholerae* to brush borders. Cultures of *V. cholerae* were grown in Trypticase soy broth without glucose (TSB; obtained from Baltimore Biological Laboratories) for 16 h at 37 C. Bacteria were harvested by centrifugation at 1,200 × *g* for 20 min and resuspended in a modified Krebs-Ringer solution, which was designated KRT [NaCl, 7.5 g/liter; KCl, 383 mg/liter; $MgSO_4 \cdot 7H_2O$, 318 mg/liter; $CaCl_2$, 305 mg/liter, buffered with 0.01 M tris(hydroxymethyl)aminomethane - hydrochloric acid at pH 7.4]. The total bacterial count was adjusted to 10^9/ml. Brush borders were washed twice in KRT and resuspended in KRT to 1.25 × 10^6/ml. Brush borders (0.4 ml), *V. cholerae* suspension (0.05 ml), and KRT (0.05 ml) were mixed and incubated together at 37 C for 15 min with agitation. Drops of suspension from each of duplicate tests were examined by phase-contrast microscopy. The numbers of vibrios on each of 20 brush borders were recorded for each test mixture. The average number of vibrios per brush border constituted the adhesion index.

Hemagglutination tests. A modification of a technique described previously was used (18). Serial dilutions of vibrio suspension were prepared in KRT with calibrated loops in Microtiter plates (Cooke Laboratory Products, Alexandria, Va.) in 25-μl volumes. Equal volumes of a 1% (packed cell) suspension of human group O erythrocytes were added, and the mixtures were incubated at 22 C for 30 min. For use in the hemagglutination test, vibrios were grown in TSB at 37 C for 16 h. The bacteria were then sedimented at 1,200 × *g* for 20 min and resus-

TABLE 1. *Adhesive and hemagglutinating (HA) activities of various strains of V. cholerae*

Serotype	Designation	Origin	Adhesion index[a]	HA titer (reciprocal)
Ogawa	P	Calcutta, 1951	14.25	64
Ogawa	Og 1	Manila, 1927 (VRL 421)	5.80	16
Ogawa	2	Chungking, 1945 (VRL 490)	1.30	4
Ogawa	3	Chungking, 1945 (VRL 17)	12.60	64
Ogawa	4	Chungking, 1945 (VRL 490)	5.75	32
Ogawa	5	Calcutta, 1953 (VRL 20)	8.50	32
Ogawa	6	Calcutta, 1958 (VRL 59)	10.90	64
Ogawa	7	Calcutta (VRL 8)	8.15	16
Inaba	In 1	El Tor, 1933 (VRL 30)	8.65	8
Inaba	2	Kasauli (VRL 6)	3.65	8
Inaba	3	Kasauli, 1942 (VRL 98)	9.65	128
Inaba	4	Shiba, 1945 (VRL 25)	6.50	8
Inaba	5	Calcutta, 1958 (VRL 58)	10.55	64

[a] Average number of adherent vibrios per brush border.

pended in KRT to approximately 10^{10} bacteria/ml. Unless indicated otherwise in the text, erythrocytes from the same group O donor were used throughout the study. Erythrocytes stored for more than 24 h were discarded.

Interaction of *V. cholerae* with mucus. The lumen of the intestine of an adult rabbit was washed with saline to remove digesta. Mucus material was then stripped from the intestine and stored on ice until used within 2 h of collection. The mucus obtained was a gel in that it maintained its cohesiveness and did not dissolve in buffer.

The reaction of *V. cholerae* with intact mucus gel was observed in Petroff-Hausser chambers. Chambers were partially filled with mucus, and a *V. cholerae* suspension in KRT was introduced into the chambers while observations were made by phase-contrast microscopy. The experiments were carried out in an incubator room at 37 C.

In addition, suspensions of vibrios and mucus gel in KRT were incubated together at different temperatures (22 and 37 C) and for different lengths of time ranging from 5 to 60 min. Microscopic observations were then made to test for aggregation of vibrios.

Scanning electron microscopy. Rabbit brush border membranes incubated in the usual manner with a vibrio suspension were mixed with an equal volume of 10% buffered formalin solution and left standing at room temperature overnight. The brush borders were then collected by centrifugation and dehydrated in ethanol solutions of increasing concentration by suspension and recentrifugation procedures. They were then taken up in a graded series of increasing concentrations of amyl acetate and critical-point freeze-dried in liquid CO_2. The material was finally gold coated. Observations were made with a model JSM-U3 microscope (Japan Electron Optics Co.).

RESULTS

Interaction of vibrios with rabbit intestinal mucus. Experiments in Petroff-Hausser chambers demonstrated that the forward movement of vibrios was impeded by rabbit intestinal mucus gel. Consequently, vibrios accumulated at the interface between the mucus and the aqueous phase. Subsequently, vibrios were observed to be moving randomly along numerous tracks within the mucus gel. Stretching of the gel determined that vibrios moved along tracks that paralleled the lines of strain created within the stretched gel. It is probable, therefore, that vibrios moved along lines of least resistance created by the alignment of the glycoprotein components of mucus (13). The total number of vibrios entering the gel and becoming entrapped, however, was only a minute fraction of the total vibrio population present (in contrast to 10% of the vibrios adhering to brush borders; see below). Attachment of vibrios to the mucus and agglutination of vibrios by mucus was not observed, although occasional vibrios became entrapped within the gel. It should be noted that vibrios attached normally to brush borders from the same rabbits that were used as the source of the mucus.

Adhesion of *V. cholerae* to rabbit brush borders. Isolated brush border membranes folded in a way that exposed the microvilli on the outer surface. Vibrios adhered only to the microvillus surface (Fig. 1). Microscopically, attachment appeared to result from chance collision between brush border and vibrio cell. Attachment occurred mainly at the tips of the microvilli and appeared to follow immediately after collision; attached vibrios showed little or no movement. Adhesion to brush borders was a property exhibited by all the *V. cholerae* cultures examined (Table 1).

In routine tests, the standard concentrations of brush borders and strain P vibrios (10^6/ml and 10^8/ml, respectively) reacted in such a way that an average of about 10 vibrios attached per brush border; i.e., only about 10% of the total number of vibrios present in the test mixture were attached to brush borders after the standard 15-min incubation. When the concentration of vibrios in the test mixture was varied, an approximately linear relationship was found between the average number of attached vibrios per brush border (adhesion index) and the vibrio concentration (Fig. 2). This suggested that the presence of a limited number of adhesion sites on the brush borders was not a major factor in limiting vibrio adhesion to about 10% of the total bacterial population, and a more likely explanation was that only about 10% of vibrios were capable of adhering to brush borders. This explanation was also supported by experiments in which the brush border concentration was varied against a fixed concentration of vibrios; in such experiments, a linear relationship was found between the reciprocal of the brush border concentration and the adhesion index. Thus, when the concentration of brush borders was altered by a given factor, the average number of vibrios adhering to one brush border was altered by the reciprocal of that factor. Consequently, about 90% of the vibrio population appear to be incapable of effecting adherence to brush borders at any given point during the test.

Temperature dependence of adhesion. It is probable that the proportion of vibrios that remained attached after 15 min of incubation at 37 C did not reflect truly the total number of adhesive vibrios. Examination of single test mixtures after different times of incubation at 37 C revealed that adhesion occurred rapidly and reached a maximum after approximately 15 min (Fig. 3). Thereafter there was a marked reduction in the number of vibrios attached to

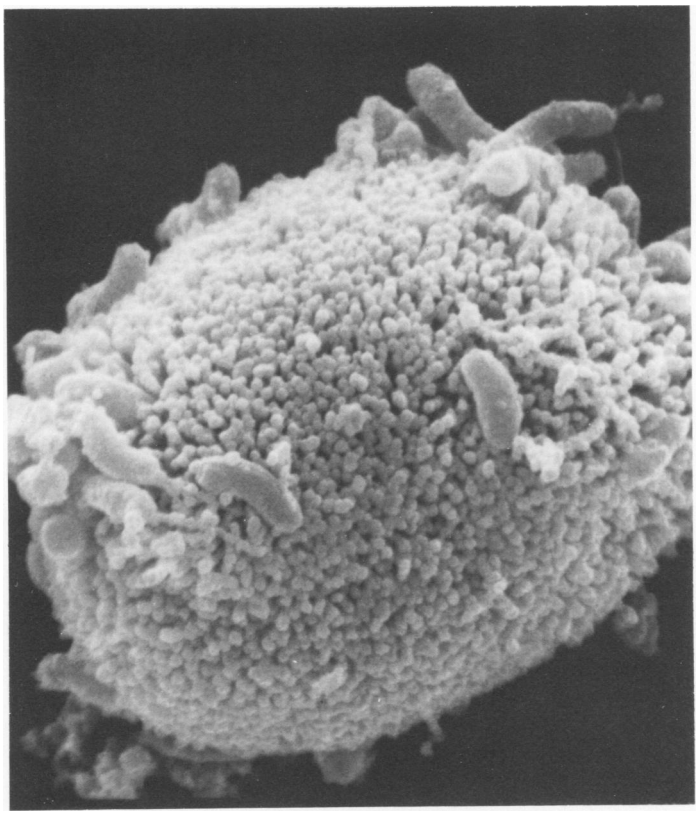

FIG. 1. *Scanning electron micrograph of V. cholerae attached to the microvilli of a brush border membrane isolated from the small intestine of a rabbit (×10,000).*

the brush borders (elution); after 45 min of incubation, few vibrios remained attached. Elution appeared to be due to the spontaneous loss or denaturation of the adhesive component (adhesin) on the vibrio surface because vibrios incubated in KRT in the presence as well as in the absence of brush borders for 45 min (i.e., when maximum elution had occurred) had a strikingly reduced capacity to adhere to fresh brush borders, in spite of the fact that their motility was not diminished. In contrast, brush borders incubated in KRT for 45 min in the presence or absence of *V. cholerae* retained the capacity to react with fresh preparations of adhesive *V. cholerae* (Table 2). Little elution occurred when the incubation temperature was 22 C (Fig. 4), and this probably accounted for the greater adhesion at this temperature compared with adhesion in tests incubated at 37 C. In contrast, little or no adhesion occurred at 0 to 4 C (Fig. 4). However, once attached to the brush border surface, vibrios did not elute readily at 4 C. Thus, the observed adhesion appeared to be the result of an equilibrium between a temperature-dependent phase of adhesion and a second temperature-sensitive step of adhesin inactivation or elution from the bacterial surface.

Influence of ions on adhesion. The adhesion of *V. cholerae* to rabbit brush border membranes required the presence of calcium ions within an optimal concentration range of 1 to 10 mM $CaCl_2$. Vibrio adhesion did not occur when the calcium ion concentration was 0.02 M or less. Strontium ions could replace partially the requirement for calcium ions but at equivalent concentrations strontium supported only half the degree of adhesion supported by calcium (Table 3). In contrast, calcium could not be replaced by barium.

Influence of cultural conditions on adhesiveness. *V. cholerae* grown in TSB elaborated adhesive properties in the logarithmic (up to 6 h) and stationary (until 48 h) phases of growth. Adhesive properties were apparent in cultures grown in flasks that were in contact with the atmosphere and in cultures grown under strictly anaerobic conditions. In marked contrast, vibrios obtained from cultures grown on Trypticase soy agar (TSA) were only feebly ad-

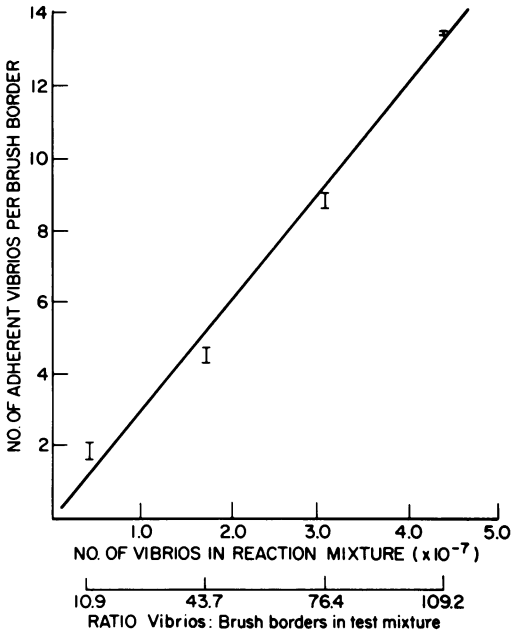

FIG. 2. *Relationship between the average number of adherent vibrios per brush border and the concentration of vibrios in the reaction mixture when the concentration of brush borders was held constant. Each bar represents the average number of vibrios per brush border; averages were obtained from microscopic counts of the vibrios attached to 20 randomly selected brush borders.*

hesive, with an adhesion index of less than one adherent vibrio per brush border. This observation has been made on many occasions over the last 2 years and is unlikely, therefore, to be an artifact. Inspection of suspensions of TSA-grown, nonadhesive vibrios under the phase-contrast microscope revealed no difference as compared with TSB-grown, adhesive vibrios, both in terms of the percentage of the total vibrio population that was moving at any given moment and with respect to the speed of movement of individual vibrio cells. Vibrios grown on TSA under strictly anaerobic conditions were also nonadhesive.

Hemagglutinating activity of *V. cholerae*. All *V. cholerae* cultures examined agglutinated human group O erythrocytes (Table 1). Microscopically, the erythrocytes were agglutinated into aggregates of 10 or more. Although the presence of vibrios within such aggregates could not always be discerned with certainty, the occasional vibrio attached by one pole to the surface of an erythrocyte was observed. Complete hemagglutination (17; i.e., erythrocytes sedimented to form thin, evenly spread layers over the bottom and sides of the cups) occurred in reaction mixtures containing three or more vibrios per erythrocyte. Surprisingly, all detectable hemagglutinin was associated with the bacterial cell. Supernatants of vibrio cultures were inactive. Commercially available erythrocytes pooled from a number of individual animals of the following species were also tested for agglutination by suspensions from TSB cultures of *V. cholerae*: rabbit, guinea pig, horse, chicken, sheep, and cow. All of these gave negative reactions or at best very weak, incomplete hemagglutination with clear evidence that substantial numbers of erythrocytes remained unagglutinated and sedimented freely to form discrete pellets on the bottom of the cup. In contrast, erythrocytes from three additional human donors of blood groups O, A, and B, respectively, agglutinated as strongly as those of the original (group O) donor.

Comparison of hemagglutinating and adhesive activities. The temperature dependence of vibrio hemagglutination was similar to that of vibrio adhesion. Vibrio hemagglutinins were most active when the reaction mixtures were incubated at 22 C (titer of 1:64), less active at

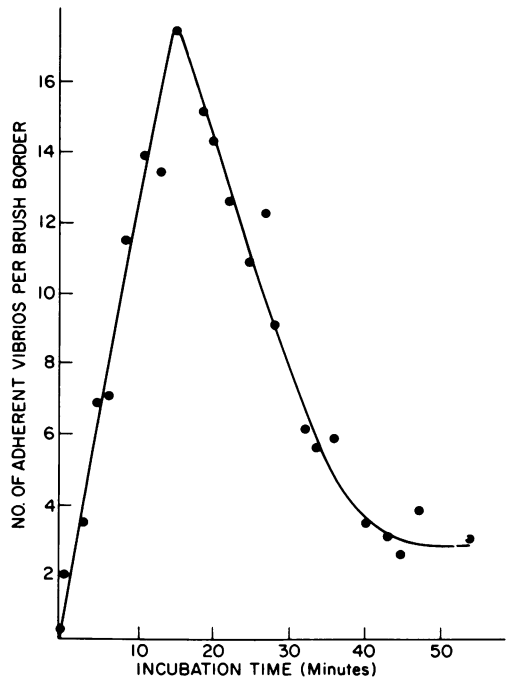

FIG. 3. *Average number of vibrios attached to brush borders at 37 C as a function of time. Each point represents the average number of vibrios per brush border obtained from microscopic counts of the number of vibrios attached to 20 randomly selected brush borders. The figure is composed of values obtained from triplicate tests.*

TABLE 2. *Effect of incubation at 37 C and elution on the receptivity of brush borders and the adhesiveness of V. cholerae*

Treatment of:		Adhesion index[a]			
Brush borders	Vibrios[b]	I	II	Avg	%
Untreated[c]	Untreated[c]	11.90	12.50	12.20	100
Eluted[d]	Untreated	12.10	11.70	11.9	97.5
Untreated	Eluted[d]	2.30	1.90	2.10	17.2
Eluted	Eluted	2.15	2.25	2.20	18.0
Untreated	Untreated	9.25	8.60	8.93	100
Incubated[e]	Untreated	10.05	9.50	9.78	104
Untreated	Incubated[e]	2.70	2.20	2.65	27.5

[a] Average number of adherent vibrios per brush border, duplicate experiments.

[b] Vibrios eluted from brush borders or incubated in KRT retained their original motility as determined by inspection of hanging-drop preparations under the phase-contrast microscope.

[c] Brush borders or vibrios stored in ice until assayed.

[d] Brush borders and *V. cholerae* incubated together at 37 C for 45 min and brush borders and vibrios reisolated before assay.

[e] Incubated alone in KRT at 37 C for 45 min before assay.

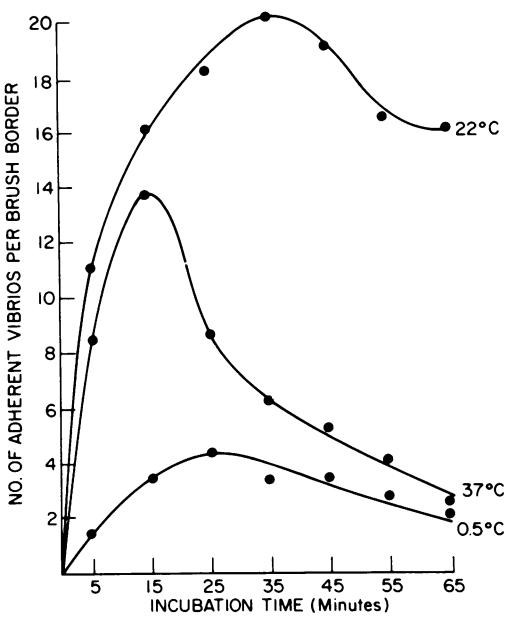

FIG. 4. *Influence of temperature on the adhesion of V. cholerae to rabbit brush borders. Each point represents the average number of vibrios per brush border obtained from microscopic counts of the number of vibrios attached to 20 randomly selected brush borders.*

37 C (titer of 1:16), and least active at 0 to 4 C (titer of 1:4 or less). Prolonged incubation of hemagglutination test mixtures at 37 C reduced the hemagglutination titer markedly (elution). The hemagglutination titer remained relatively unaltered after prolonged incubation of tests at 22 C.

As with adhesion to brush borders, calcium ions were necessary for the expression of hemagglutinating activity. However, strontium ions did not promote hemagglutination. In addition, hemagglutinating activity developed in TSB cultures but not in TSA cultures of strain P. Incubation at 37 C in KRT reduced the hemagglutinating activity of vibrios in the same manner as it decreased their adhesive properties.

DISCUSSION

The surface glycocalyx of the brush border is composed of many heterosaccharide moieties of the membrane-bound glycoprotein and glycolipid (2, 7, 15), and it is this site on the epithelial cell that the cholera vibrio will most probably colonize if in vivo adhesion indeed involves direct contact with the epithelial cell. Alternatively, *V. cholerae* may proliferate within the epithelial mucus layer of the intestinal wall. We addressed ourselves in this initial investigation to the problem of whether *V. cholerae* has some of the more important qualities that a bacterium must possess in order to colonize one or both of these habitats. We have therefore attempted to determine whether *V. cholerae* cells possess the ability to adhere to, or in some other way to associate intimately with, components of the brush border and mucus-gel environments.

Immobilization of vibrios by adherence to mucus was not observed, but some mechanical entrapment of actively motile vibrios within the gel did occur. *V. cholerae* usually penetrated and traveled within the intestinal mucus along well-defined tracks. It is possible that these tracks represent lines of least resistance to movement created by alignment of the glyco-

TABLE 3. *Influence of calcium, strontium, and barium on the adhesion of V. cholerae to brush borders*

Suspending fluid of KRT[b] plus:	Adhesion index[a]			
	I	II	Avg	%
Calcium[c]	7.70	6.70	7.20	100
Strontium	3.30	2.70	3.00	42
Barium	0.40	0.30	0.35	5
Water	0.45	0.40	0.43	6

[a] Average number of adherent vibrios per brush border.

[b] $CaCl_2$ omitted.

[c] Calcium, strontium, and barium added as chlorides at final concentrations of 2.75 mM.

protein moieties. Such alignment can be brought about in certain gels by stretching, in which case alignment occurs parallel to the lines of strain (13). Thus, the observation that vibrios moved through mucus in directions that were parallel to induced lines of strain suggests that the rheological properties of intestinal mucus could be of some importance. Analogy with the role of cervical mucus and sperm migration suggests that vibrios could migrate along tracks that terminate at the tissue surface (13). The relative number of vibrios penetrating mucus gel under the conditions of our experiment was quite small when compared with the number of vibrio adhering to brush borders. It is quite possible, however, that in vivo the rheological properties of mucus may differ considerably and may therefore allow more or less vibrio penetration than has been observed here.

A possibly important characteristic of *V. cholerae* is its ability to adhere to the microvillus surface of brush border membranes. Vibrio attachment appeared to result from chance collision with the brush border; the vibrios attached firmly to the microvilli, showed little or no movement, and were not readily removed by simple manipulations such as pipetting. However, attachment was a temperature-dependent step and required a minimal activation temperature. Adhesion was unstable at 37 C in our test system, and vibrios lost their adhesiveness and eluted from the brush border surface. Once the bacteria were bound to the brush border surface, cooling of the reaction mixture to 4 C stabilized the adhesion and elution did not occur. In contrast to the instability of the adhesive properties of vibrios suspended in KRT at 37 C, adhesiveness was retained by broth cultures incubated at 37 C for up to 48 h. It is possible that the surface adhesin is continually resynthesized in broth cultures, whereas in KRT, which contains no metabolizable components, endogenous reserves limit the extent to which the adhesin can be renewed. This is consistent with an earlier observation that bacteriostatic and bactericidal antibiotics inhibited the in vivo adhesion of vibrios to the mucosal surface (10).

The continual need to replace adhesin may account for the observation that only 10% of vibrios in a culture were adhesive at any one time. This figure is probably an underestimation of adhesiveness because of the continual association and dissociation of vibrios with the brush borders. It is nevertheless probable that the proportion of adhesive vibrios in a broth culture is relatively small because even at a temperature at which little elution occurred (22 C), adhesion seldom involved more than 25% of the total vibrio population present.

Because of its simplicity, the hemagglutinin test would greatly facilitate the study of interactions between vibrios and mucosal surfaces, provided one could be certain that vibrios react on the surface of human O erythrocytes with receptors that are similar to those on rabbit brush borders. It is noteworthy, therefore, that hemagglutination and adhesion to brush borders of *V. cholerae* were similar in all respects except one. Both activities had similar temperature dependence and were present in broth- but not agar-grown cultures. A major difference was that hemagglutination was not promoted by strontium ions. The cationic requirements for adhesion or for hemagglutination have not been investigated in detail, and clearly we are not in a position to explain this difference. However, cations can influence the adhesion of two surfaces, for example by effectively reducing the negative electrostatic charge of a surface (27) and/or by binding to ligands, which possibly results in the "zippering-up" of two surfaces (1). Accordingly, in the case of ion modification of surface potential, one may expect trivalent cations to be more effective promoters of adhesion and hemagglutination than, for example, divalent cations. However, in the case of ligand binding, ionic size and differences in surface properties other than charge density assume some importance. Furthermore, adhesion and hemagglutination differ importantly in that the former is the binding of single vibrio cells to the brush border surface whereas the latter is the attachment of a vibrio to more than one erythrocyte, which results in the binding together and consequently the agglutination of the erythrocytes. Presumably, greater adhesive forces are necessary to agglutinate erythrocytes than to hold the smaller vibrio cell on the brush border surface. Thus, if cations directly or indirectly affect the affinity of the adhesin for its receptor, it is possible that the lower affinity promoted by strontium ions (evidenced by lower numbers of vibrios attached to brush borders) may not necessarily result in a reduction of the hemagglutinin titer but may cause the total loss of stable hemagglutinating activity. Thus, the discrepancy between the influence of strontium on adhesion and on hemagglutination does not necessarily indicate that the two activities are due to different adhesive mechanisms. Although others (20, 28) have reported hemagglutinating activity in *V. cholerae* cultures, the relation between this activity and the ability to interact with components of the mucosa has not been reported previously.

In summary, one can conclude that *V. cholerae* are capable of attachment to brush border surfaces and also possess a certain ability to penetrate mucus gel. Colonization of the mu-

cosa therefore may involve (i) penetration of the mucus gel, followed by entrapment within the gel or migration through the mucus (assisted by certain rheological characteristics of the gel) to the tissue surface, and/or (ii) attachment to the glycocalyx of the epithelial cells. It is interesting to note that the ability of vibrios to attach to brush border membranes was entirely dependent on the environment in which the bacteria found themselves. Thus, vibrios grown in broth were adhesive, whereas those grown on agar plates or suspended in buffer were not so. Further studies are needed, of course, to decide whether and under what circumstances the in vivo intestinal environment is suitable for the development of the adhesive vibrio phenotype. The present study, however, furnishes another example of the complex interactions between bacteria and host that must be unraveled before the mechanisms of bacterial virulence can be understood (11).

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