INFECTION AND IMMUNITY, Oct. 1981, p. 241-249
0019-9567/81/100241-09$02.00/0

Vol. 34, No. 1

# Role of Chemotaxis in the Association of Motile Bacteria with Intestinal Mucosa: In Vitro Studies

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Received 4 February 1981/Accepted 19 June 1981

Various Sephadex G-15 fractions of pepsin-digested mucosal extract inhibited the in vitro association of cholera vibrios with mucosal slices. Inhibitory activity paralleled the taxin activity of the fractions for these bacteria. This supports the theory that inhibition of mucosal association by pepsin-digested mucosal scrapings was due to the blocking of taxin receptors on the bacterial surface. Nonchemotactic mutants were significantly less efficient than the chemotactic parent or revertant strains in associating with mucosal slices in vitro. Control experiments in which filter paper disks replaced the mucosal slices showed a comparable extent of association of chemotactic and nonchemotactic vibrios with this material. Histological studies indicated that vibrios associated predominantly with the mucus gel of the intestinal slices rather than with the mucosal epithelium or the serosal surface. Intestinal slices attracted chemotactic vibrios even after prolonged washing, suggesting continuous production of the taxin by the tissue. Inert polystyrene particles 1.1 μm in diameter penetrated the mucus gel of intestinal slices very poorly, but nevertheless could be detected in low numbers in the deep intervillous spaces within 15 min. In contrast, chemotactic vibrios reached the deep intervillous spaces in significantly higher numbers, whereas motile, nonchemotactic vibrios reacted like the inert particles. It is concluded that mucus gel represents a partial barrier to the penetration of particles of bacterial size and that this barrier can be invaded efficiently by motile bacteria, but only when their locomotion is guided by chemotactic stimuli.

Bacterial motility has long been suspected to be of importance in the pathogenesis of infections. Indeed, Guentzel and Berry have shown (16) that motility of cholera vibrios correlated with virulence. These authors, as well as Bellamy et al. (3), also suggested that the clumping of bacteria by antibody inhibited motility and thereby resulted in protective immunity. Subsequent studies from this laboratory confirmed the association between motility and adhesive properties in cholera vibrios. However, loss of motility was shown to be correlated with a simultaneous loss of the bacterial surface receptor which mediates adhesion (18). Moreover, antibody in low concentrations such as those which presumably prevail in the intestine inhibited vibrio adhesion to intestinal tissue slices even in the absence of bacterial agglutination (10). It is not clear at the present time whether the demonstrated correlation between loss of motility and loss of virulence in cholera vibrios is actually a consequence of the loss of locomotion as such or whether this is simply related to the activity of some other virulence (adhesive?) factor which is phenotypically linked to the expression of motility.

To our knowledge, the role of chemotaxis in the interaction of bacteria with the human or animal body has never been investigated, in spite of a considerable amount of speculation ever since the phenomenon of chemotaxis was described at the end of the nineteenth century. For example, Gotschlich (15), writing in the encyclopedic *Handbuch der pathogenen Mikroorganismen* which compiled the entire knowledge of medical microbiology existing at the time of its publication (1929), stated: "Chemotaxis probably plays an important role in the life of pathogenic microorganisms in the infected host as, for example, in the colonization of bacteria in certain sites of predilection, in certain tissues, etc." (translated). Similar assertions have been put forth repeatedly by authors of microbiology textbooks up to the present time, e.g., Cruickshank (5) and Doetsch and Cook (6). Most recently, Guentzel et al. speculated on various possible virulence mechanisms of cholera vibrios and listed chemotaxis among these (17). None

of these authors referred to experiments testing such hypotheses, however, and no relevant data are listed in a review by Chet and Mitchell (4) concerning the ecological role of chemotaxis.

We became interested in the subject because it offered a possible resolution of an apparent discrepancy among results published earlier, namely, that L-fucose inhibited the adhesion of a strain of *Vibrio cholerae* to intestinal brush border membranes, but not its adhesion to slices of intact ileum. In contrast, a peptic digest of mucosal scrapings (PMS) inhibited the reaction of this vibrio with both preparations (10).

Our original explanation for these findings was to postulate a second receptor for vibrio adhesion in the intestinal mucus gel, which differs from that on the brush border and which is blocked by a substance or substances in PMS, but not by fucose (10). Upon further reflection it became apparent, however, that an alternative explanation would have the advantage of greater simplicity: vibrios are chemotactically attracted into the mucus gel of intestinal slices by one or several attractive substances (taxins) emanating from the mucosa. In the presence of PMS the chemotactic receptors on the vibrio surface become saturated by substances in PMS which resemble the mucosal taxin(s), thus making it impossible for the vibrios to respond chemotactically to attractive substances specific for the blocked receptors. This hypothesis is consistent with current views of the specificity of chemotactic receptors of *Escherichia coli* and the blocking of these receptors by taxins (1).

As reported in a preliminary paper (2), the new hypothesis could be supported by data showing that nonchemotactic vibrio mutants were less efficient than the parent strain in associating with slices of intact mucosa, and that PMS contains chemotactic attractants (and therefore can block the corresponding chemotactic receptors on the vibrio surface). The present paper reports additional studies on the mucosal slice model. Preliminary accounts of this work have appeared previously (2, 12, 13). An accompanying report is concerned with the in vivo role of chemotaxis in experimental animal models of cholera (14).

(These data have been presented at the 13th Symposium on Cholera of the U.S.-Japan Cooperative Medical Science Program. [Freter et al., Proceedings of the 13th Joint Conference on Cholera, The U.S-Japan Cooperative Medical Science Program, p. 152–181, 1978, U.S. Government Printing Office, Washington, D.C.].)

## MATERIALS AND METHODS

**Vibrio strains.** The parent and mutant strains used in the present study are described in an accompanying paper (11).

**Slice test for bacterial adhesion to mucosal slices.** Slices of rabbit ileum were prepared and processed as described earlier (10), except that incubation was in Krebs-Ringer-Tris buffer (10) in an atmosphere of air for a period of 8 min. Subsequent rinsing (by immersion for 5 s each in two changes of sterile saline) and homogenization of the slices were as described earlier (10). Unless otherwise indicated below, the incubation fluid for the slices contained a total of approximately $5 \times 10^5$ vibrios per ml. Slices intended for histological studies were incubated in a similar manner, except that the vibrio concentration was $2 \times 10^9$ cells per ml. The "adhesion index" of vibrios was calculated as: number of bacteria adherent to slices/number of bacteria per milliliter of incubation fluid. For purposes of comparison, the adhesion indices were then normalized by adjusting that of the parent strain of *V. cholerae* to equal 100.

To test the inhibitory activity of a large number of chromatographic fractions, the slice test was miniaturized by using slices of 5-mm diameter punched out of the intestine with a cork borer. The vibrios used for this test were labeled by growing them in 5 ml of Trypticase soy broth without glucose (BBL Microbiology Systems) in the presence of 1 mCi of [$^3$H]thymidine at 37°C overnight. The labeled vibrios were then washed on a membrane filter (Millipore Corp.) and suspended in Krebs-Ringer-Tris buffer. The slices were placed in 1-ml amounts of the vibrio suspension and incubated for 15 min at 37°C. Each was then rinsed by immersion for 5 s each in two changes of sterile saline and digested in Protosol (New England Nuclear Corp.), and the radioactivity was counted in Omnifluor (New England Nuclear). The proper corrections for self-absorption were calculated by adding known amounts of $^3$H to the scintillation vials. Each chromatographic fraction was analyzed for inhibitory activity in triplicate.

**Penetration of bacteria and inert particles into mucus gel.** Slices of rabbit ileum were prepared and processed as described above for the regular slice test, except as noted below. To minimize the presence of indigenous bacteria, the slices were prepared from an area of the rabbit small intestine located equidistant from stomach and cecum. The incubation fluid contained a mixture of approximately $10^9$ each of polystyrene particles (1.1-μm diameter), *Saccharomyces cerevisiae*, and vibrios of either the parent or the mutant strain per ml. After the final rinse the slices were frozen in liquid nitrogen, mounted in nutrient gelatin (Difco Laboratories), and sectioned to 12-μm thickness in a cryostat. The sections were stained without fixation for 5 s with 1% Giemsa stain (National Aniline) in methanol, washed for about 1 s in tap water, and air dried.

The sections were observed at ×500 magnification. Particles and microorganisms were counted in the mucus gel overlying the villi (counting all microorganisms and particles in 40 areas defined by the partially closed diaphragm) and in the spaces between villi. The latter spaces were divided by inspection into two areas of equal length (lumenal half and basal half), and counts were recorded separately for each. Forty intervillous spaces were scored in this manner for each variable. The sections were coded, and counts were performed "blind" by one of us (M.S.M.).

**Capillary test for bacterial chemotaxis.** The capillary test is described in an accompanying paper (11).

**Adhesion of vibrios to brush border membranes.** The assay for vibrio adhesion to brush border membranes was described in an earlier publication (18).

## RESULTS

In a first attempt to characterize the chemotactically active substances (taxins) in PMS, the crude material was fractionated by passage through a column of Sephadex G-15. The fractions were then analyzed quantitatively for (i) chemotactic activity, (ii) the ability to inhibit the association of the parent strain of *V. cholerae* with slices of rabbit ileum, and (iii) the inhibition of vibrio adhesion to isolated brush border membranes. To permit the analysis of a large number of fractions in a small volume of suspending fluid (to which the fractions were added), the microassay with $^3$H-labeled vibrios was used. The results are shown in Fig. 1. The length of the bars in the upper half of the figure represents the taxin activity of each fraction, normalized such that the number of bacteria accumulating in control capillaries containing no taxin is set to 100% (thus, 100% is the negative control and represents no taxin activity). The length of the bars in the lower half of Fig. 1 depicts the ability of the fraction to inhibit the association of vibrios with intestinal slices. These data are normalized such that 100% indicates the number of bacteria which associate with the slices in the absence of any inhibitor (i.e., in the positive control). Therefore, lower percentages indicate increasing degrees of inhibition. The horizontal bars at the top of Fig. 1 delineate the fractions in which Blue Dextran 2000 (Pharmacia Fine Chemicals), L-fucose, or calcium ions eluted from the column.

The data in Fig. 1 indicate that the active substances in PMS were extremely heterogeneous in that they were distributed over a wide

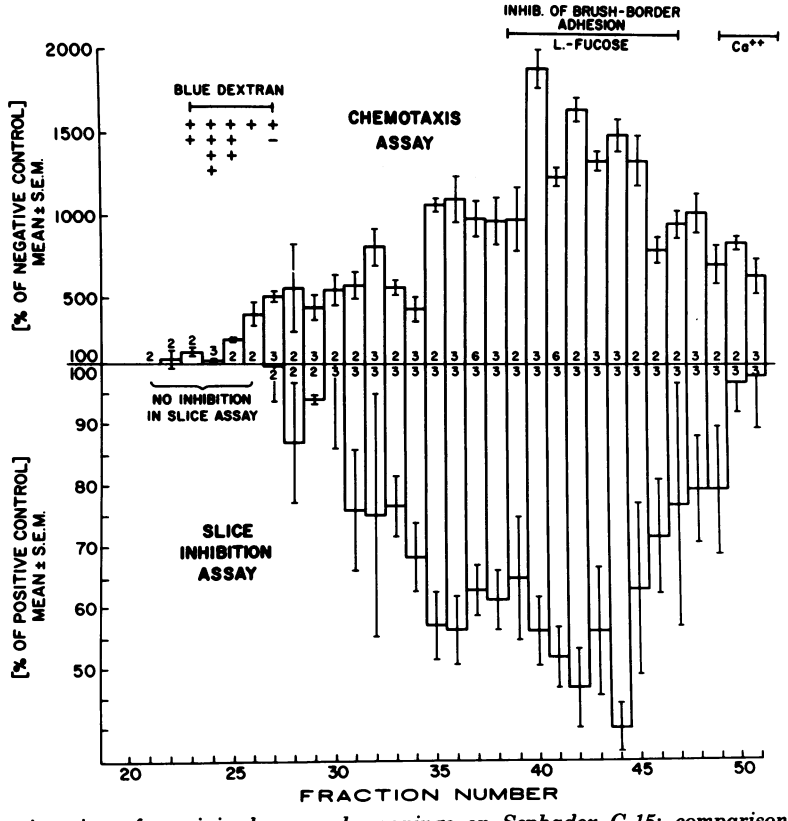


FIG. 1. *Fractionation of pepsinized mucosal scrapings on Sephadex G-15: comparison of individual fractions with respect to chemotactic activity and inhibition of the adhesion of V. cholerae to intestinal slices. Figures within the bars indicate the number of slices or capillary tubes assayed. The horizontal bars at the top of the column delineate those fractions where Blue Dextran, L-fucose, or $Ca^2$ eluted when the column was charged with these substances. Fractions 39 through 47 contained L-fucose when the column was charged with that sugar and had inhibitory activity in the brush border assay when the column was charged with pepsinized mucosal scrapings.*

range of molecular sizes. The chemotactic attractant activities of the various fractions paralleled their ability to inhibit the association of vibrios with mucosal slices, thus giving the juxtaposed bar graphs in Fig. 1 the appearance of mirror images. This is consistent with the working hypothesis that inhibition by PMS of the association of vibrios with the mucosa is due to taxins in the PMS. The figure shows further that vibrio adhesion to brush border membranes was inhibited only by fractions 38 through 47. These were the same fractions in which L-fucose was recovered when the column was charged with a pure preparation of this sugar, indicating that inhibition of vibrio adhesion in this assay is more specific, i.e., is restricted to one or a few substances (probably L-fucose itself), in the PMS. In contrast, there appear to be a large number of chemotactic substances in PMS which are capable of inhibiting the association of vibrios with mucosal slices. The finding that the taxin activity of these fractions consistently paralleled their inhibitory activity in the slice test is in agreement with and supports our original hypothesis (2) that this inhibition is a consequence of the saturation of taxin receptors on the bacterial surfaces by taxins in the PMS. Such saturation is known to abolish the chemotactic reactivity of the bacteria towards the taxin(s) involved (1).

Table 1 illustrates three representative experiments testing the reaction of (i) the chemotactic parent or chemotactic revertant, (ii) motile, nonchemotactic mutants, and (iii) a nonmotile mutant in the mucosal slice test. The changes in proportion of the tested strains in the incubation fluid as compared to their proportions on the intestinal slices is taken as a measure of their relative ability to associate with the mucosa. Table 1 indicates that the nonchemotactic mutants were considerably less efficient in associating with mucosal slices, as evidenced by the fact that their proportions on the slices were much lower than in the suspending fluid. The nonmotile mutant, however, was still significantly less active in that respect than nonchemotactic mutant 31.

The association of vibrios with slices of rabbit intestinal mucosa was also studied histologically. For this purpose, the mucosal slice test was used as described above, except that the concentration of parent strain vibrios in the suspension was increased to $10^9$ cells per ml. The results are shown in Fig. 2 and 3. It appears that vibrios which had associated with ileal tissue slices in the standard assay were found predominantly in the mucus gel. During the 8-min incubation time of the standard assay, the vibrios penetrated deeply into the intervillous spaces. Very few, i.e., never more than 1%, of the vibrios that had associated with a given slice were found adherent to the serosal side of the tissue. Very few vibrios were seen in a position which suggested close association with the epithelial surfaces, a situation which one would perhaps expect to find after the relatively short incubation time of the standard slice test. No bacteria could be detected in sections of control slices incubated in sterile buffer.

The reliability of the slice test in demonstrating the importance of chemotaxis in the association of vibrios with mucosal slices was verified by substituting chemotactically inactive material, namely, small disks of filter paper, for the intestinal slices in the standard test. The results (Table 2) indicate that the parent and the nonchemotactic mutant associated with this material to the same extent. This rules out the possibility that the superior ability of the parent over the nonchemotactic mutant strain to associate with mucosal slices could have been due to a number of possible differences among the

TABLE 1. *Association with the mucosal surface by various mutants of V. cholerae*

| Bacterial mixture | No. of vibrios per slice[a] | Proportion of motile, nonchemotactic mutants | | $P^b$ |
|---|---|---|---|---|
| | | In incubation fluid | On slices | |
| Mutant 31[c] + parent[d] | 6.74 (1.33)[e] | 0.44 (0.37–0.50)[f] | 0.15 (0.11–0.20)[f] | <0.001 |
| Mutant 40[c] + revertant 40R[d] | 3.84 (1.41)[e] | 0.46 (0.45–0.46)[f] | 0.12 (0.12–0.12)[f] | <0.001 |
| Mutant 31[c] + mutant NM31[g] | 4.16 (1.49)[e] | 0.41 (0.41–0.42)[f] | 0.93 (0.87–0.97)[f] | <0.001 |

[a] Mean total number of vibrios $\times 10^{-4}$.
[b] Significance of the difference in proportions of the two vibrio strains on slices versus in incubation fluid.
[c] Motile, nonchemotactic.
[d] Motile, chemotactic.
[e] Number within parentheses indicates the standard deviation.
[f] Numbers within parentheses indicate the 95% confidence interval.
[g] Nonmotile.

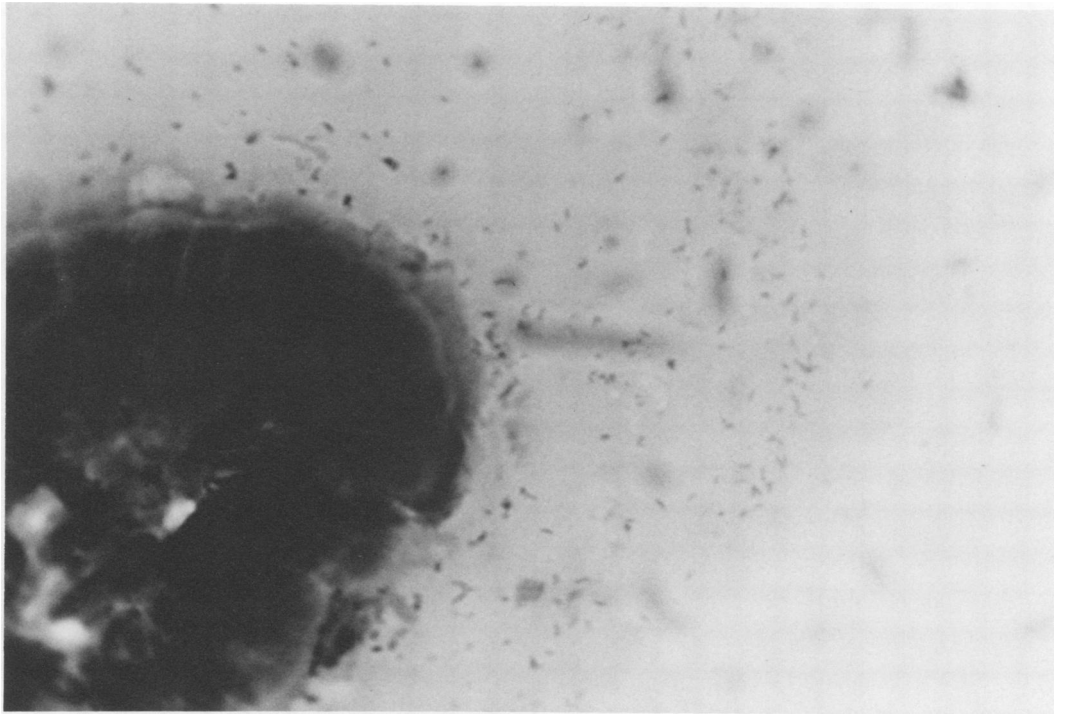

FIG. 2. *Invasion by V. cholerae (parent strain) of mucus gel in intestinal slices after incubation for 8 min at 37°C in a suspension of this bacterium.*

strains that are not directly related to chemotaxis, such as different swimming patterns which, conceivably, might result in different rates of contact with a surface, different sensitivity to damage by blending, etc.

If chemotaxis facilitates the approach of motile bacteria to the surface of the mucus gel, then the advantage of the parent strain over the nonchemotactic mutant should be abolished when the bacteria are deposited directly against the mucosal surface. This was accomplished experimentally by placing the mucosal surface of ileal tissue slices (or disks of filter paper as controls) directly against a thick paste of vibrios which had been spread on a flat glass surface and incubating this preparation at 37°C in a moist chamber for 8 min. The slices were subsequently rinsed by immersion (without agitation) in three changes of buffer at 37°C and five changes at 0°C for a total period of 15 min. They were then homogenized, and cultures were prepared in the standard manner. The data obtained (Table 3) indicate that, indeed, the parent and the nonchemotactic mutant associated with the intestinal slices in the same proportions. Under these conditions, then, intestinal slices reacted with the vibrios in the same nonselective manner as pieces of chemotactically inactive filter paper. Even a nonmotile mutant associated with the mucosal slices to the same extent as did the parent (Table 3). To test the possibility that these results might be artifacts caused by the impaction of bacterial paste on the surface of the mucus gel rather than by invasion of the mucus, Giemsa-stained frozen sections were prepared from tissue slices that had been exposed to bacterial paste and washed as described above. These showed vibrios only in the mucus gel including the intervillous spaces, in a manner similar to that illustrated in Fig. 2 and 3. Apparently, then, the procedure of pressing intestinal mucosa against a thick bacterial paste forces the bacteria into the mucus gel in a manner similar to that normally accomplished by chemotaxis and motility. This experiment rules out the possibility that the differential association of the parent and the nonchemotactic mutants with intestinal slices may be an artifact caused by differential survival or retention in the mucus.

To test the degree of reversibility of mucosal association, 12 slices of rabbit ileum were incubated in a mixed vibrio suspension for 8 min in

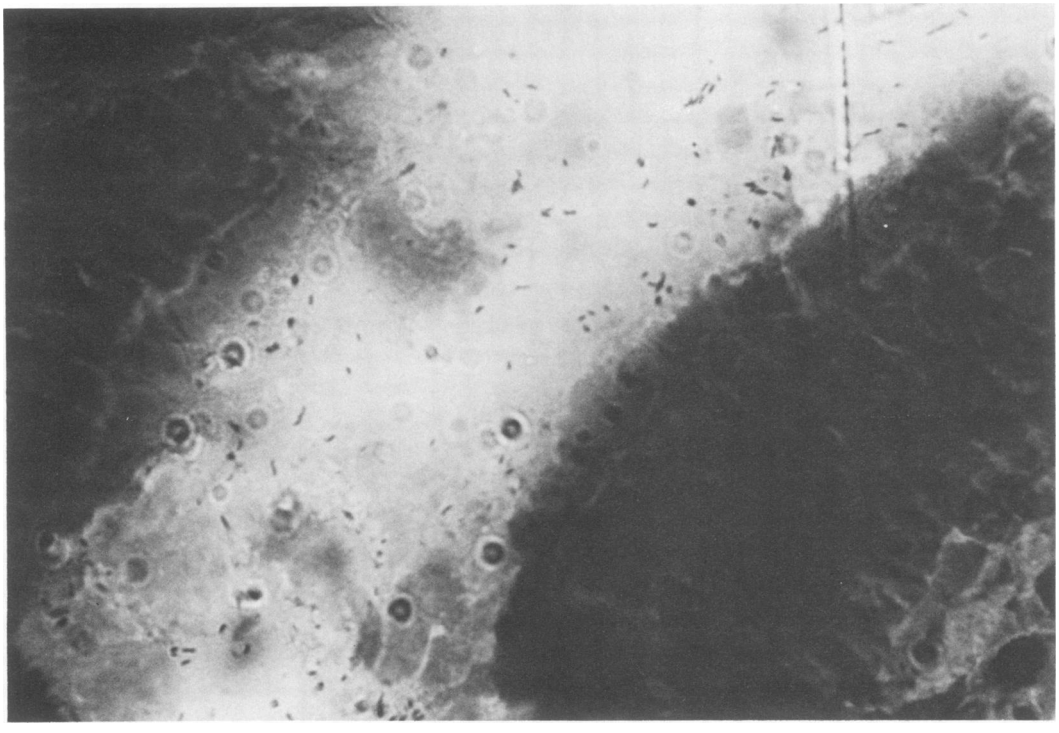

FIG. 3. *Invasion by V. cholerae (parent strain) of intervillous spaces in intestinal slices after incubation for 8 min at 37°C in a suspension of this bacterium.*

TABLE 2. *Association with intestinal and filter paper slices by nonchemotactic mutant 40 and the parent strain of V. cholerae in dilute bacterial suspensions*[a]

| Slice | No. of vibrios per slice[b] | Proportion of mutants | | $P$[c] |
|---|---|---|---|---|
| | | In fluid | On slices | |
| Intestinal | 3.52 (0.66)[d] | 0.31 (0.31–0.31)[e] | 0.10 (0.10–0.11)[e] | 0.001 |
| Filter paper | 2.43 (0.86)[d] | 0.41 (0.41–0.41)[e] | 0.42 (0.42–0.43)[e] | NS |

[a] The total number of vibrios in the suspending fluids was $5 \times 10^5$ cells per ml.

[b] Mean total number of vibrios $\times 10^{-5}$.

[c] Significance of the difference in proportions of mutant and parent strains on slices versus in incubation fluid. NS, Not significant.

[d] Number within parentheses indicates the standard deviation.

[e] Numbers within parentheses indicate the 95% confidence interval.

the standard manner. Six of the slices were then rinsed and cultured as usual. The remaining slices were incubated without agitation for an additional 11 min in two changes of sterile buffer and subsequently rinsed and cultured as usual. The chemotactic parent strain, as expected, was significantly more efficient in associating with intestinal slices (Table 4), such that the proportion of mutants on the slices was considerably lower (0.124) than in the incubating fluid (0.49). Subsequent incubation in two changes of sterile buffer removed 64.4% of the bacteria from the slices. Parent and mutant strains were removed at similar rates by the extended incubation as evidenced by the fact that their proportion in the wash fluid was only slightly different from that remaining on the washed slices.

In view of the strong chemotactic attraction of the parent vibrio to the mucosa, it was of interest to estimate the duration of taxin production by the mucosa. For this purpose intestinal slices were first incubated in five changes of sterile buffer for a total of 34 min. The tissues were then used in the standard slice test. The proportion of nonchemotactic mutants associating with the preincubated slices was not significantly higher than that found on control slices (Table 5). This indicates that preincuba-

TABLE 3. *Association with intestinal or filter paper slices in contact with a concentrated bacterial paste*[a]

| Slice | Bacteria | No. of vibrios per slice[b] | Proportion of mutants | |
|---|---|---|---|---|
| | | | In paste | On slices |
| Intestinal | Mutant NM31[c] + parent[d] | 2.58 (1.35)[e] | 0.58 (0.58–0.59)[f] | 0.49 (0.49–0.49)[f] |
| Paper | Mutant NM31[c] + parent[d] | 2.62 (1.10)[e] | 0.58 (0.58–0.59)[f] | 0.43 (0.43–0.43)[f] |
| Intestinal | Mutant 31[g] + parent[d] | 2.89 (0.58)[e] | 0.46 (0.46–0.47)[f] | 0.49 (0.49–0.49)[f] |
| Paper | Mutant 31[g] + parent[d] | 3.31 (1.09)[e] | 0.46 (0.46–0.47)[f] | 0.54 (0.54–0.54)[f] |

[a] The pastes contained a mixture of parent and mutant resulting in a total of approximately $10^{12}$ viable vibrios per ml of paste.
[b] Mean total number of vibrios $\times 10^{-8}$.
[c] Nonmotile.
[d] Motile, chemotactic.
[e] Number within parentheses indicates the standard deviation.
[f] Numbers within parentheses indicate the 95% confidence interval.
[g] Motile, nonchemotactic.

TABLE 4. *Retention of nonchemotactic mutant 31 and chemotactic parent strain of V. cholerae on the mucosal surface*

| Material tested | No. of vibrios[a] | Proportion of mutant 31 |
|---|---|---|
| Incubation fluid | | 0.49 (0.492–0.497)[b] |
| Slices after 8 min of incubation with vibrios | 2.03 (0.54)[c] | 0.124 (0.094–0.175)[b] |
| Slices after 8 min of incubation with vibrios + 11 minutes in sterile buffer | 0.72 (0.21)[d] | 0.102 (0.064–0.150)[b] |
| Wash fluid | | 0.124 (0.094–0.175)[b] |

[a] Mean total number of vibrios $\times 10^{-6}$.
[b] Numbers within parentheses indicate the 95% confidence interval.
[c] Number within parentheses indicates the standard deviation.
[d] In sterile buffer, 64.4% of the bacteria were lost from six slices.

tion of the slices did not exhaust the supply of the taxin which is responsible for the preferential attraction of chemotactic vibrios to the mucosal surface. To attract bacteria, a taxin must form a concentration gradient extending through the mucus gel and into the surrounding medium. Washing of the slices must consequently be expected to continuously remove considerable amounts of taxin. Therefore, the data in Table 5 indicate that taxin was produced by the mucosa over a prolonged period of time.

The question whether the taxin gradient which attracts the chemotactic parent strain to the mucosal slices also extends deeply into the mucus of intervillous spaces has been answered in the affirmative by experiments reported earlier (13). An extension of these experiments which includes a nonmotile mutant is shown in Table 6. Briefly, slices of rabbit small intestine were suspended in a mixture of vibrios and polystyrene particles. The slices were then washed, sectioned, and stained, and the ratio of vibrios to polystyrene particles in various areas of the mucus gel was compared with that in the incubation fluid, as described above. The chemotactic parent strain penetrated deeply into the intervillous spaces, as evidenced by a vibrio/polystyrene particle ratio which rose from 1.03 in the incubation fluid to 11.03 in the basal half of the intervillous spaces (Table 6). In contrast, the nonchemotactic mutant reacted more like the nonmotile mutant, with only minor increases in the vibrio/particle ratios from incubation fluid to mucus gel, indicating that these two strains were not significantly more active than the inert particles in their capacity to penetrate mucus gel. Control slices, incubated without vibrios (Table 6), showed relatively few indigenous bacteria that could be mistaken for vibrios. Nevertheless, the data in Table 6 have been corrected by subtracting the ratios of vibrio-like bacteria to particles in the control group from those of the other groups. It is interesting to note that the nonmotile mutant was still less efficient in penetrating mucus gel than was the motile, nonchemotactic strain (Table 6). These latter differences were not observed consistently, however, and require further study.

## DISCUSSION

The data presented provide strong evidence that chemotactic attraction constitutes a potentially important factor in the association of bacteria with mucus gel. Together with earlier similar data (13), the experiment described in Table 6 shows conclusively that the chemotactic gradient which the vibrios follow extends deeply into the intervillous spaces. These are in vitro data obtained with surviving slices of intestine, and one may wonder about their general applicability. Similar in vivo results are reported, however, in an accompanying paper; experiments are described in which rabbit and mouse intestinal loops are exposed to mixtures of vi-

TABLE 5. *Prior incubation and washing fail to abolish the chemotactic attraction of rabbit intestinal slices for vibrios*

| Treatment of slice | No. of vibrios per slice[a] | Mean % of nonchemotactic mutant vibrios | | No. of slices | $P^b$ |
|---|---|---|---|---|---|
| | | In fluid | On slices | | |
| Standard preparation | 4.2 (0.81)[c] | 51.6 (51.4–51.7)[d] | 9.63 (9.62–9.69)[d] | 7 | <0.001 |
| Preincubated and washed | 3.3 (0.28)[c] | 49.6 (49.4–49.7)[d] | 11.59 (11.58–11.68)[d] | 7 | <0.001 |

[a] Mean total number of vibrios $\times 10^{-5}$.
[b] Significance of the proportions of the mutants in the fluid versus on slices.
[c] Number within parentheses indicates the standard deviation.
[d] Numbers within parentheses indicate the 95% confidence interval.

TABLE 6. *Penetration of vibrios into the mucus gel of rabbit intestinal slices incubated in a suspension of the bacteria and indicator particles*

| Vibrio strain | Vibrio/particle ratio[a] in: | | | |
|---|---|---|---|---|
| | Incubation fluid | Mucus gel | | |
| | | Above villi | Between villi (lumenal half) | Between villi (basal half) |
| Parent | 1.03 (105) | 1.15 (622) | 3.80[b] (1,919) | 11.03[b] (2,869) |
| Nonchemotactic | 1.19 (110) | 0.98 (460) | 2.18 (975) | 1.67 (371) |
| Nonmotile | 1.06 (103) | 0.56 (308) | 0.56 (258) | 0.41 (86) |
| Control[c] | | 0.007 (24) | 0.20 (35) | 0.08 (7) |

[a] Corrected for ratio of control slices. Total number of vibrios is shown within parentheses.
[b] Difference from ratio of nonchemotactic strains is highly significant ($P \ll 0.001$).
[c] The incubation fluid of the control slices contained polystyrene particles and yeasts, but no vibrios.

brios and polystyrene particles (14). One must conclude, therefore, that the slice test represents a useful tool for the study of the early interaction between bacteria and mucus gel.

Nelson and Finkelstein (Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, B25, p. 19) reported that vibrios adhere predominantly to the serosal side of intestinal slices incubated in a suspension of these bacteria. This observation is probably artifactual, caused by the fact that scanning electron microscopy, as used by these workers, does not demonstrate mucus gel and that, for this reason, they failed to observe large numbers of bacteria on the mucosal side of their preparations. Most conventional staining methods fail to demonstrate mucus gel, presumably because it is washed away during the staining procedure. Our method of staining frozen sections very briefly with highly concentrated dye was developed after fairly extensive testing of other techniques published in the literature and was found to give the most consistent results.

The finding that the parent and the nonchemotactic mutants penetrate the mucus gel equally well when intestinal slices are placed against a bacterial paste (Table 3) might possibly be interpreted to suggest that penetration of the mucus gel is not affected by chemotaxis, once the bacteria have been attracted to (or placed against) the surface of the gel. As discussed above, the results of penetration studies of vibrios and inert particles into the mucus gel show conclusively, however, that chemotactic attraction does indeed extend deeply into the mucus gel itself. The data obtained with bacterial paste may merely mean, therefore, that the pressure involved in the procedure of settling the intestinal slices against the bacterial paste forces the bacteria into the mucus gel. The finding that parent and nonchemotactic mutant vibrios eluted during prolonged incubation in sterile buffer (Table 4) indicates that bacterial association with the mucosa is reversible to some extent. The fact that the parent and the nonchemotactic mutant eluted at nearly equal rates might be due to the extrusion of large areas of the mucus gel (rather than elution of only the superficial mucus layer which should contain a higher porportion of mutants). Even after prolonged incubation the slices still showed the presence of mucus gel. Frozen sections prepared from such slices did not differ in appearance from those of standard slices that were rinsed only briefly (Fig. 2) or from sections of whole intestine that had not been washed at all (e.g., experiments in Tables 1 through 4 of reference 14). Consequently, the mucus gel had not been simply washed away. It is possible that the rate of extrusion of mucus gel by goblet cells was enhanced by the insult of prolonged incubation in buffer. Unfortunately, there appear to be no quantitative data available on the in vitro or in

vivo rates of mucus extrusion into the small intestine, other than the fact that extrusion can be rapid enough to be observed directly (8), and that it can be triggered by a variety of stimuli, including antigen-antibody reactions (19). Consequently, the experiment under discussion demonstrates the fact that bacterial association with the mucosa can be reversible, and one must assume that the magnitude and importance of this effect in vivo will differ on various mucosae and with different bacteria involved.

The function of mucus gel as a barrier to the penetration of microorganisms has been a question which has received various contradictory answers. For example, Edwards (7) recently described mucus as a "particle and macromolecule-proof coating for cell surfaces," whereas Florey (8) observed mucus to be a vehicle for the removal of particles. Shrank and Verwey, in contrast, observed vibrios but not inert particles to penetrate mucus gel slowly, i.e., requiring several hours to become demonstrable histologically (20; for a more detailed review see reference 9). The present data indicate that mucus gel does indeed represent a barrier, but that this barrier can be penetrated, though inefficiently, by inert particles or nonmotile bacteria. However when motile bacteria are guided by chemotactic gradients, they may penetrate efficiently into the deep layers of intervillous spaces within minutes. This association is reversible to some extent, and one can imagine that motile bacteria, like Alice, must "keep running fast just to stay in place" against the natural flow of mucus.

## ACKNOWLEDGMENT

This work was supported by Public Health Service grants AI 07631 and AI 15279 from the National Institute of Allergy and Infectious Diseases.

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