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Mechanism of Action of Intestinal Antibody in Experimental Cholera

II. Antibody-Mediated Antibacterial Reaction at the Mucosal Surface

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Earlier studies have shown that intestinal antibody (coproantibody) decreases the adsorption of vibrios to the intestinal wall of rabbits. The mechanism underlying this phenomenon was studied by means of an in vitro model in which vibrios were grown on slices of rabbit ileum in a moist chamber. Antibody prevented the adsorption of vibrios onto such slices in the same manner as it did in vivo. Studies of the in vitro growth rate of vibrios on slices of ileum indicated an antibody-dependent antibacterial mechanism on the mucosal surface. This mechanism appeared to require the presence of viable mucosal cells, as it was not present when filter paper was substituted for the tissue slices and it could not be demonstrated in fresh scrapings from the intestinal mucosa. Antigen-antibody mixtures were added to the surface of slices of rabbit ileum or were introduced into the lumen of intestinal loops. Presence of these mixtures did not inhibit the antibody-dependent antibacterial mechanism on the mucosa in either the in vivo or the in vitro system. The amount of antigen-antibody mixture used in vivo was at least 12 times that required to neutralize the complement released by homogenization from an entire intestinal loop. The results obtained support the hypothesis that coproantibody protects by decreasing the adsorption of vibrios on the intestinal mucosa. The mechanism responsible appears to be an antibacterial effect on the mucosal surface which requires antibody plus some additional factor(s) supplied by viable mucosal cells. The postulated factor does not appear to be complement.

Earlier studies from this laboratory (3) have shown that intestinal antibody (coproantibody) prevents the physiological manifestation of experimental cholera (accumulation of fluid in the intestine) without, however, affecting the growth of vibrios in the intestinal lumen. When confronted with such data, one would, as a first reaction, consider an antitoxic protective mechanism of coproantibody. However, the antiserum used in these studies was prepared with heated vibrio antigen and did not neutralize the vibrio enterotoxin in vitro or in vivo. A possible explanation for this seemingly paradoxical finding was provided in a recent study from this laboratory (4) showing that the adherence of vibrios to the wall of in vivo-isolated intestinal loops of rabbits was greatly reduced in the presence of coproantibody. In the same study (4), a model was described involving the adsorption of vibrios to slices of rabbit ileum suspended in Krebs-Ringer solution. This system also showed a reduction of vibrio adsorption when anti-vibrio serum was added to the suspending medium.

In the studies to be described here, the above model system has been used to investigate the mechanism by which antibody prevents the adsorption of vibrios to the intestinal surface. As discussed earlier (4), there are at least two possible explanations for this phenomenon. (i) Antibody may affect the adsorption of bacteria directly, or (ii) adsorption in the presence of antibody may be normal although the adsorbed bacteria (but not those in the lumen) are inhibited in their growth or killed when antibody is present. Experiments will be reported in detail below which provide evidence for the latter hypothesis.

It should be noted that the term "adsorbed" is used in this paper to denote vibrios which are not removed from the intestinal surface by gentle rinsing. This term was chosen for convenience of expression and should not be taken as defining the nature of the association between vibrios and intestinal surface.

MATERIALS AND METHODS

Bacterial strains. The streptomycin-resistant *Vibrio cholerae* strain was the same Ogawa type used in earlier studies (3, 4). *Salmonella senftenberg* (strepto-

mycin-sensitive) was obtained from the stocks of this department. Inocula were prepared by dilutions of Trypticase Soy Broth (BBL) cultures grown for 6 to 8 hr at 37 C.

Antisera. Hyperimmune rabbit serum was used, prepared with the same strain of *V. cholerae* as was used for the infections. The immunizing antigen for these sera was a washed saline suspension of agar-grown vibrios heated in flowing steam for 2 hr. Six intravenous injections were given to the rabbits at 5-day intervals. The rabbits were bled 7 days after the last injection. The sera were sterilized by Seitz filtration and inactivated by heating at 56 C for 45 min. Antisera to *S. senftenberg* were prepared in the same manner with the same strain of *S. senftenberg* as was used in mixed-challenge infections.

Infection of intestinal loops in vivo. Standard procedure was used for infecting and culturing, as described earlier (4).

Adsorption and growth of vibrios on slices of rabbit ileum. A length of ileum was removed from a rabbit. The ileum was opened by a longitudinal incision, washed quickly by dipping it successively for a few seconds into two cylinders of saline, and cut into eight rectangular sections (approx. 1 by 2 cm). These were then dipped into Krebs-Ringer solution containing antiserum or bacteria, as described for the individual experiments. After the final immersion, the slices were dried lightly with filter paper (Whatman no. 1) and placed on stainless-steel wire screens suspended in Pyrex jars kept at 37 C. The bottom of the jars below the screens was covered with Krebs-Ringer solution to insure a high humidity in the jars. A piece of filter paper soaked in bromothymol blue in Krebs-Ringer solution was placed on top of the wire screen next to the tissue slices. A gas mixture of approximately 5% CO_2 in O_2 (humidified by passage through a wash bottle) was circulated through the jars. The *p*H of the paper strip was kept at neutrality (bromothymol blue = dark green) by varying the proportion of CO_2 in the gas phase. The tissue slices and the filter paper were inspected at 10-min intervals and, if necessary, were moistened with distilled water, taking care that no layer of fluid developed on the surface of the tissues. To make quantitative cultures, the slices were placed into Virtis homogenizer jars kept at 13 C. The slices were homogenized in broth and suitable dilutions of the homogenates were plated in parallel on SS agar and on Veal Infusion Agar containing 1 mg of streptomycin per ml. These two media allowed the separate enumeration of *Salmonella* or *Vibrio*, respectively.

Statistical analysis. The tables for Wilcoxon's rank test (2) were used to determine the significance of differences between two series of experimental data.

RESULTS

In the first type of experiments, slices of ileal tissue were incubated as usual in Krebs-Ringer solution containing antiserum or normal serum (1:1,000), respectively, plus a mixed suspension of 10^6 each of *S. senftenberg* and *V. cholerae* per ml. After 1 hr, the tissues were removed and washed, twice in sterile saline and once in sterile Krebs-Ringer solution containing either normal or antiserum (1:1,000). They were then dried lightly on filter paper, and each piece was cut in half. One half was homogenized immediately and plated to determine viable counts. The other half was placed into empty jars in a moist atmosphere of 5% CO_2 in O_2 at 37 C for 1 hr. Viable counts were then determined after homogenization.

TABLE 1. *Multiplication of Vibrio cholerae and Salmonella senftenberg in the presence of normal or vibrio antiserum on slices of rabbit ileum during 1 hr of incubation in a moist chamber*

V. cholerae				*S. senftenberg*			
Normal serum		Antiserum		Normal serum		Antiserum	
Before incubation	After incubation	Before incubation	After incubation	Before incubation	After incubation	Before incubation	After incubation
780[a]	750	90	60	30	450	30	660
1,050	2,310	90	90	60	690	30	1,170
1,110	3,810	90	90	60	720	60	1,200
1,320	4,710	120	120	90	1,050	60	1,260
1,500	4,950	120	120	90	1,110	60	1,560
1,770	5,250	210	330	90	1,500	90	1,650
1,800	5,400	210	390	90	1,770	120	1,800
1,890	5,670	390	420	90	2,070	120	1,860
2,160	5,850	420	480	120	2,100	150	2,130
2,400	6,570	450	780	120	2,220	150	2,250
2,400	8,550	1,290	1,080	210	2,410	180	2,670
4,380	8,700	4,170	2,250	270	2,460	270	3,090
1,785[b]	5,325	210	360	90	1,635	105	1,725

[a] Figures indicate the number of bacteria ($\times 10^3$) on one slice of tissue.

[b] Median count.

Table 1 presents the results obtained in three such experiments. As may be seen, the number of vibrios adsorbed initially in the presence of antiserum was much lower than that in normal serum. This is in line with the results described earlier (4). However, after incubation in a moist atmosphere for 1 hr, the vibrios in the presence of normal serum had multiplied, as evidenced by a three-fold increase in the median count from 1,785 $\times$ 10^3 vibrios per tissue slice (before) to 5,325 $\times$ 10^3 (after). In contrast, the median count rose only very slightly (from 210 $\times$ 10^3 before to 360 $\times$ 10^3 after) in the presence of homologous antiserum. In the case of *Salmonella*, the initial adsorption (before incubation) and multiplication (before versus after incubation) were not affected by the presence of *Vibrio* antiserum, thus serving as a control of the validity of the viable counts.

Although the results shown in Table 1 may be taken to suggest a reduction in the growth rate of vibrios, one must realize that the experimental design had several imperfections. (i) The slices

TABLE 2. *Effect of antiserum on the multiplication of a mixed inoculum containing Vibrio cholerae and Salmonella enteritidis on the surface of slices of rabbit ileum (illustration of a single experiment)*

Determination	Normal serum					Anti-*V. cholerae* serum				
	Individual slices				Avg	Individual slices				Avg
Vibrio										
Before incubation	167[a]	114	87	131	125	92	87	211	90	120
After incubation	541	449	841	757	647	645	249	113	269	319
Vibrio multiplication[b]					5.2					2.7
Salmonella										
Before incubation	125[a]	66	85	116	98	106	60	159	119	111
After incubation	136	157	401	155	212	316	283	45	251	224
Salmonella multiplication[b]					2.2					2.0

[a] Values indicate the number of viable bacteria on one tissue slice; all values to be multiplied by 10^4.
[b] Multiplication = average count after incubation/average count before incubation. For *V. cholerae*, adjusted multiplication = *Vibrio* multiplication/*Salmonella* multiplication (for normal serum, 5.2/2.2 = 2.36; for anti-*V. cholerae* serum, 2.7/2.0 = 1.35). Effect of antiserum on multiplication of *V. cholerae* = (adjusted multiplication of *V. cholerae* in antiserum/adjusted multiplication of *V. cholerae* in normal serum) × 100, i.e., (1.35/2.36) × 100 = 57%.

were kept in vitro for over 2 hr, which might result in the release of substances from the tissues (such as complement) which are not normally present in the lumen of the intestine. (ii) The number of bacteria adsorbed on slices suspended in antiserum was considerably lower than that adsorbed on slices in normal serum. Differences in the number of bacteria present initially may thus have affected their subsequent multiplication during the secondary period of incubation, a phenomenon which is well known to occur during the lag phase of bacterial growth in broth cultures. For these reasons, the experimental system was changed as follows. Slices of rabbit ileum were prepared and washed in Krebs-Ringer solution in the usual manner. They were then immersed in a bath of 25 ml of Krebs-Ringer solution containing a 1:500 dilution of normal or antiserum, respectively. The slices were incubated in this solution for 3 min in a shaker-water bath at 37 C under an atmosphere of 5% CO_2 in O_2. After drying the slices lightly with filter paper, they were placed on stainless-steel wire screens suspended in Pyrex jars, as described above. The top of the slices, i.e., the mucosal side, was then inoculated with 0.01 ml of Krebs-Ringer solution containing a mixed inoculum of *V. cholerae* and *S. enteritidis*.

In a single experiment of this type, eight slices with antiserum and eight slices with normal serum were incubated in two separate jars. Four slices from each jar were cultured before incubation on selective media as described above. The remaining slices were cultured after 1 hr at 37 C. One such experiment is shown in detail in Table 2. As may be seen, there was a decrease in the multiplication of *V. cholerae* in the presence of antiserum (2.7-fold versus 5.2-fold in normal serum). To correct these figures for variations in the size of the small inoculum (0.01 ml) applied to the slices and for variations in processing and diluting the homogenates, use was made of the *Salmonella* counts. It was assumed on the basis of earlier experiments (Table 1) that growth of *Salmonella* was not affected by the presence of vibrio antiserum. Therefore, any variations in the *Salmonella* counts must have been due to variables such as those mentioned above, which would affect the *Vibrio* and *Salmonella* counts to the same degree. The effect of these variables may then be cancelled out by dividing the figure for *Vibrio* multiplication by that of *Salmonella* multiplication on the same tissue slices to give the "adjusted multiplication of *V. cholerae*" (Table 2). If there had been no effect of antiserum on *Vibrio* multiplication, identical figures would be expected for the adjusted multiplication of *V. cholerae* in normal and antiserum. Consequently, the per cent difference between these two figures has been used here as a measure of the effect of antiserum on the multiplication of *V. cholerae*, as illustrated in Table 2 (where this value was 57%).

Table 3 lists the result of 15 replicate experiments of this type. As may be seen, multiplication of vibrios on the mucosal surface of slices of rabbit ileum was reduced in the presence of antiserum. This was true in every experiment of this series.

The above results are subject to criticism on the basis that the lower *Vibrio* counts observed on slices containing antiserum may have been due to agglutination rather than to an antibacterial mechanism. A control experiment was, therefore, designed to test this possibility. Strips of filter

TABLE 3. *Reduction by antiserum of the multiplication of V. cholerae on the surface of slices of rabbit ileum (summary of 15 experiments of the type illustrated in Table 2)*

Per cent				
90[a]	75	59	57	27
89	75	59	55	19
79	63	59	43	11

[a] Each value is based on *Vibrio* and *Salmonella* counts on 16 slices of ileum. Each corresponds therefore to the last value (57%) shown in Table 2 (footnote *b*). A value below 100% indicates that the average multiplication of vibrios was reduced by this factor in the presence of antiserum, as compared to multiplication in the presence of normal serum. A value higher than 100% would indicate increased multiplication in the presence of antiserum.

TABLE 4. *Effect of antiserum on the multiplication of V. cholerae on the surface of filter paper strips (summary of 18 experiments[a])*

Per cent					
330[b]	220	178	125	100	63
240	213	168	116	79	56
240	190	143	108	65	36

[a] Same type of experiment as illustrated in Table 2, except that paper strips were substituted for the tissue slices.

[b] *See* footnote *a*, Table 3.

paper (Whatman no. 1) were substituted for tissue slices. In all other respects, the experiments were carried out in a manner identical to those illustrated in Tables 2 and 3. The results of 18 such experiments, each involving 16 paper strips, are shown in Table 4. The data indicate that there was no reduction of the growth of vibrios in the presence of antiserum. The median of all experiments is 137%, which indicates a slightly higher multiplication of vibrios in the presence of antiserum. Therefore, one must conclude that, if agglutination of vibrios had occurred at all in the presence of antiserum, the resulting clumps of bacteria must have been broken up during the subsequent homogenization of the specimens.

The experiments and controls presented so far indicate the presence of an antibody-dependent antibacterial mechanism on the mucosal surface. Earlier results (4) had shown that this mechanism was not active in fresh mucus scrapings. Consequently, the mechanism appears to be dependent on the presence of viable mucosal cells. This, in turn, would mean that the mucosal cells supply an accessory factor which, in conjunction with antibody, forms an antibacterial system. The following experiments were designed to test whether this accessory factor has the properties of complement.

Slices of rabbit ileum were prepared and washed in the usual manner. They were then preincubated, again as usual, in Krebs-Ringer solution containing 1:500 normal serum or vibrio antiserum for 3 min at 37 C. After light drying with filter paper, six sections each were placed on wire screens in three separate Pyrex jars and inoculated with 0.01 ml of vibrio suspension to which had been added: (i) controls (preincubated with normal serum), 0.00125 ml of bovine serum albumin (BSA, 333 μg/ml) plus 0.00125 ml of saline; (ii) antiserum (preincubated with antiserum), same as for controls; and (iii) antiserum plus BSA–anti-BSA (preincubated with antiserum), 0.00125 ml of BSA (333 μg/ml) plus 0.0025 ml of undiluted rabbit anti-BSA serum

Three slices from each group were homogenized and cultured in the usual manner before incubation. The remaining three slices in each group were incubated for 1 hr at 37 C in 5% CO_2 in O_2, and were homogenized and cultured after this period. Briefly, then, these experiments were identical to those shown in Tables 2 and 3, except that a third experimental group was added in which any complement given off by the mucosal slices during incubation should have been bound by the BSA plus anti-BSA mixture. It is unlikely that the surface of the intestinal slices contained any complement initially because they had been

TABLE 5. *Multiplication of V. cholerae on the surface of slices of rabbit ileum: effect of antiserum on normal slices and on slices "decomplemented" by bovine serum albumin (BSA) + anti-BSA (summary of 13 experiments)[a]*

Slices with antiserum (%)		Slices with antiserum and BSA + anti-BSA (%)	
105[b]	50	154	55
83	43	133	46
82	40	117	43
75	33	84	40
73	27	83	33
64	21	70	33
60		57	

[a] Same type of experiment as illustrated in Table 2, except that a third group was added in which the slices had been overlaid with BSA + anti-BSA, as well as with antiserum.

[b] See footnote *a*, Table 3. A value below 100% indicates that the multiplication of vibrios was reduced by this factor in the presence of antiserum, as compared to multiplication in the presence of normal serum. A value larger than 100% indicates increased multiplication in the presence of antiserum.

TABLE 6. *Effect of anti-vibrio serum on the adsorption of vibrios on in vivo-isolated ileal loops containing human serum plus anti-human gamma globulin*

Rabbit no.	Loops with antiserum		Loops with normal serum	
1	443[a]	2.7	550	321
2	117	177	2031	3600
3	1.83	0.12	120	429
4	99	93	297	639
5	48	78	192	135
6	1.2	78	387	678

[a] Each value indicates the number of vibrios (to be multiplied by 10^4) adsorbed to one intestinal loop.

washed twice and, in addition, had been preincubated in normal or antiserum immediately before application of the inoculum. The proportions of BSA and anti-BSA used were determined in preliminary tests to be optimal for precipitation.

The results of 13 experiments of this type, each involving 18 tissue slices, are shown in Table 5. As many be seen, there was a reduction of the multiplication of vibrios in the presence of vibrio antiserum in all but four instances. Analysis by Wilcoxon's rank test showed that this reduction was significant at the 2% level in both the "antiserum" group and the antiserum plus BSA-anti-BSA group (comparing each group separately to the control group with normal serum). However, the two groups did not differ significantly from each other. Therefore, the presence of BSA plus anti-BSA had no appreciable effect on the reduction of vibrio multiplication in the presence of antiserum.

Similar experiments were also carried out in vivo. Four isolated loops were prepared in a rabbit and infected with vibrios ranging from 7×10^5 to 6×10^6 per loop in different experiments, as described above. Alternate loops received 0.02 ml of vibrio antiserum or 0.02 ml of normal rabbit serum, respectively. In addition, all loops received 0.4 ml of normal human serum (HS) plus 3.3 ml of rabbit anti-human gamma globulin (anti-HGG). The proportions of HS and anti-HGG used were optimal for precipitation. The inoculated loops remained in vivo for 3 hr and were then washed, homogenized, and cultured in the usual manner. Table 6 shows the number of vibrios adsorbed on the washed loops. As may be seen, the number of adsorbed vibrios was consistently reduced in loops containing antiserum. In other words, the presence of HS plus anti-HGG did not prevent the usual decrease in the adsorption of vibrios in loops with antiserum.

The above attempts to remove complement from the reaction of vibrios with the intestinal mucosa would not be significant without some data on the amount of HS plus anti-HGG necessary to remove all the complement present in a given intestinal loop. For this purpose, intestinal loops were prepared in the usual manner in normal rabbits but were not inoculated. The loops were then removed and washed with 10 ml of broth, as is normally practiced prior to culture of infected loops. The loops were placed in Virtis homogenizer jars, containing 4×10^6 to 6×10^6 per ml of *V. cholerae* serum or 0.02 ml of normal rabbit serum, respectively. The contents of the jars were then homogenized and incubated for 3 hr at 37 C. At the end of this period, the homogenates were cultured in the usual manner. The results obtained in three experiments (each involving eight loops obtained from two rabbits) are shown in Table 7. As may be seen, there had been extensive killing of the vibrios in the homogenates containing antiserum. Presumably, this killing was mediated by complement liberated from the homogenized loops. These experiments were then repeated in exactly the same manner except that each batch of homogenate received 0.03 ml of HS plus 0.25 ml of anti-HGG prior to introducing the vibrios plus normal or anti-vibrio serum. The results of this experiment are shown in Table 8. As may be seen, the presence

TABLE 7. *Effect of antiserum on the viable counts of vibrios after incubation in homogenates of normal intestinal loops of the rabbit*

Rabbit no.	Antiserum				Normal serum			
1	0.008[a]	0.002	0.003	0.006	50	52	86	90
2	0.002	0.007	0.003	0.0001	6.2	7.8	6.7	6.6
3	0.005	0.002	0.0004	0.008	15	11	9	17

[a] Each value represents the viable count of vibrios (to be multiplied by 10^6) per milliliter of one batch of homogenate.

TABLE 8. *Effect of antiserum on the viable counts of vibrios after incubation in homogenates of normal intestinal loops containing human serum plus anti-human globulin*

Expt no.	Antiserum				Normal serum			
1	20[a]	16	2	2	15	16	11	13
2	14	13	11	14	12	11	15	14

[a] Each value represents the viable count of vibrios (to be multiplied by 10^6) per milliliter of one batch of homogenate.

of HS plus anti-HGG prevented the antibody-dependent killing of vibrios which had been observed earlier (Table 7) in the absence of HS plus anti-HGG.

The amounts of HS plus anti-HGG added in vitro to one homogenized loop were 0.03 and 0.25 ml, respectively. As demonstrated in Table 8, these amounts were sufficient to neutralize the complement contained in one intestinal loop. In the in vivo experiments shown in Table 6, the amounts of HS plus anti-HGG introduced into the lumen of one loop had been 12 times higher (0.4 and 3.3 ml per loop, respectively). Consequently, the amounts used in vivo would have been sufficient to neutralize at least 12 times the amount of complement present at any given time in one intestinal loop.

The above in vivo experiments involved a 3-hr period during which the HS plus anti-HGG mixture remained in the lumen of isolated loops. It was necessary, therefore, to obtain some estimate of the stability of globulins under these conditions, to rule out the possibility that intestinal enzymes might have destroyed these reagents before the end of the experiment. To obtain such an estimate, in vivo-isolated loops were prepared in the usual manner. Alternate loops were injected with 0.02 ml of normal or anti-*V. cholerae* serum, respectively, but were not infected. After the inoculated loops had remained in vivo for 3 hr, they were removed, washed, and homogenized in the usual manner. The homogenates were then inoculated with 4.7 × 10^6 vibrios (per 30 ml homogenate representing one loop). After 3 hr of incubation at 37 C, the vibrios in the homogenates were cultured in the usual manner. The results are shown in Table 9. As may be seen, sufficient antibody had remained in the loops after 3 hr of in vivo incubation (and after subsequent washing) to cause a strong bactericidal effect in homogenates of these loops.

The amount of antiserum used in this experiment was very small (0.2 ml). It seems unlikely therefore that the relatively large amounts of HS and anti-HGG used in the experiment (Table 6) had been completely hydrolyzed by intestinal enzymes.

TABLE 9. *Stability of rabbit antibody during in vivo incubation in the lumen of intestinal loops: multiplication of vibrios in homogenates of loops previously injected with normal or anti-V. cholerae serum*

Rabbit no.	Antiserum		Normal serum	
1	5.3[a]	2.2	15,000	11,000
2	0.4	8.5	8,800	17,000

[a] Values indicate the number of viable vibrios (to be multiplied by 10^3) per milliliter of intestinal homogenate after 3 hr of incubation at 37 C.

DISCUSSION

The present study was initiated to investigate the mechanism by which intestinal antibody reduced that fraction of the intestinal vibrio population which is adsorbed to the mucosa. Specifically, data were sought to distinguish between two hypotheses: (i) that antibody may affect adsorption directly, or (ii) that adsorption may be normal but that adsorbed vibrios are affected by an antibody-dependent antibacterial mechanism on the mucosa.

The experiments presented in Tables 1 to 3 provide evidence for a bacteriostatic or bactericidal mechanism, as postulated in the second hypothesis. This mechanism could be shown with vibrios adsorbed to tissue slices "normally," i.e., by spontaneous adsorption from a suspension (Table 1), and with vibrios deposited in a minimal amount of liquid on the mucosal surface of slices incubated in a moist chamber (Tables 2 and 3).

Two lines of evidence indicate that the observed reduction in viable vibrio counts was real rather than an artifact caused by agglutination. The first of these is based on the finding that there was no antibacterial effect when filter paper was substituted for tissue slices (Table 4). Unfortunately, filter paper is a very imperfect substitute for tissue, and one could still suspect that agglutination could have taken place on or in the mucus layer of intestinal tissue slices where the viscous material may have prevented the disruption of bacterial clumps. However, when in an earlier study vibrios were grown in batches of intestinal mucus scraped from the small intestine of normal rabbits, there was also no effect of antiserum on the bacterial growth rate (4).

It must therefore be concluded (i) that the observed antibacterial effect was not an artifact and (ii) that this effect could be demonstrated only on the surface of intact or surviving intestine. This in turn implies that the viable mucosa supplied some factor which, in conjunction with antibody, formed an antibacterial system. The only well-known accessory factor which has these properties is, of course, the complement system, and the possibility of its involvement was therefore studied in some detail. However, the experiments and controls reported in Tables 5 to 9 make it highly unlikely that this was the case. In discussing the possible identity of the postulated accessory factor, one must, therefore, look to other systems described in the literature.

The first substance which might be considered in this connection is lactoferrin, an iron-binding protein of secretions which is synthesized in the intestinal mucosa and which coats the mucosal surfaces (7). It is known that such iron-chelating proteins are antibacterial. Recent evidence with other bacteria (1) suggests that the bactericidal effect of antiserum may be reversed in the presence of excess iron, thus suggesting interference by serum transferrin and antibody with the bacterial iron metabolism.

Other accessory substances which might very well be involved in an antibacterial antibody-dependent mechanism on the mucosal surface are enzymes which affect the bacterial cell wall. As reviewed by Strominger (9), a great variety of carbohydrases and peptidases are able to degrade the cell wall peptiodoglycan networks. In the case of lysozyme, it is known that it enhances the bactericidal activity of very small amounts of antibody and complement (8). Thus one, or a combination of mucosal enzymes, may conceivably become bactericidal in the presence of specific antibody. Li and Mudd (6) found a similar system in leukocyte extracts active against staphylococci in the presence of heated antiserum. (These authors emphasize the role of complement in their discussion. It is, however, apparent from their data that heated serum also enhanced the antibacterial effect of leukocytic extracts.) Thus, either lactoferrin or one or several mucosal enzymes may conceivably become antibacterial in the presence of specific antibody. These enzymes may be labile or may be present in minimally effective concentrations on the epithelial cell surface, thus accounting for the lack of an antibacterial effect in the lumen and in scrapings of intestinal mucus. Environmental factors, such as *p*H and O_2-tension may also affect the reaction, as they do in the transferrin-antibody bactericidal system (1). The present studies are being continued in this direction.

All experiments described above were carried out by means of passive immunization with rabbit antiserum. Therefore, one may wonder to what extent serum antibody simulates that found naturally in the intestinal tract. As demonstrated in an earlier paper (4), the shift in the vibrio population in immune animals could be demonstrated in actively immunized rabbits as well as after passive administration of serum antibody. Consequently, intestinal and serum antibody appear to function in a similar manner with respect to the phenomenon under study. It seemed therefore justifiable to conduct the present work with the easily available serum antibody. We plan, nevertheless, to repeat some key experiments with secretory immunoglobulin A antibody as soon as sufficient quantities of this material have been prepared.

The present study demonstrates an antibody-dependent antibacterial mechanism operating on the mucosal surface. This mechanism could explain our original observation of a shift in the distribution of vibrios in intestinal loops containing antibody. It should be emphasized that this finding does not rule out the possibility that antibody may have an additional inhibitory effect directly on the adsorption of vibrios similar to the one observed by Lankford and Legsomburana (5) in studies of adsorption of vibrios to the surface of red blood cells. In fact, a subsequent publication will present evidence indicating that such a mechanism is also operating on the mucosal surface.

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