Distribution of Mucinolytic Activity in Strains of Shigella. (22368)

SAMUEL B. FORMAL AND JOSEPH P. LOWENTHAL. (Introduced by G. Edsall.)
Division of Immunology, Walter Reed Army Institute of Research, Washington, D.C.

That strains of *Vibrio comma* elaborate mucinolytic enzymes was first noted by Burnet and Stone(1). This finding has since been confirmed and extended by a number of investigators so that it is now established that: a) the mucinolytic enzyme is able to produce a desquamation of the intestinal mucosa of guinea pigs(1) and increase the permeability of the intestinal wall of mice(2); b) intestinal contents of animals experimentally infected with cholera organisms possess demonstrable mucinolytic activity(3); c) sera of patients convalescing from an attack of cholera contain antibodies which neutralize the action of this enzyme(4) and d) that at least two serological types of mucinase are produced by *V. comma*(5). The possibility is therefore recognized that mucinolytic enzymes may be implicated in the pathogenesis of Asiatic cholera.

The characteristics of bacillary dysentery are similar in certain respects to those of cholera. Whether this similarity includes the elaboration of mucinolytic enzymes by members of the genus *Shigella* is the subject of this communication.

Material and methods. Cultures. The cultures employed were obtained from 3 sources: the culture collection of the Walter Reed Army Institute of Research, the 406th Medical General Laboratory in Tokyo, and The Hospital Infantil, in Mexico City.* The strains obtained in the Hospital Infantil were tested for mucinolytic activity immediately after isolation. *Ovomucin.* Ovomucin, as a substrate for the mucinolytic enzymes, was prepared by the method described elsewhere (5,6). *Mucinase.* Cultures to be tested for mucinolytic activity were grown on brain heart infusion soft agar (0.7% agar). After 18 hours incubation at 37°C the liquid was expressed from the soft agar medium and cleared by centrifugation. The supernatant was tested for mucinolytic activity. The mucinase obtained from one culture was concentrated for use in immunization of rabbits. A 10-fold concentration was accomplished by adding cold acetone (60% by volume) to the chilled supernatant and dissolving the resulting precipitate in distilled water at one-tenth the original volume. *Mucinase titrations.* The fact that intact ovomucin but not depolymerized ovomucin is precipitated or clotted by cetyl trimethyl ammonium bromide (CTAB) served as a basis for the mucinase titrations(7). One half ml of ovomucin was added to 0.5 ml of 2-fold serial dilutions (in borate-buffered saline, pH 7.0)† of culture supernatants. After one half hour incubation in a water bath at 40°C, undigested ovomucin was detected by the addition of 1 drop of 0.1% aqueous solution of CTAB. The highest dilution of supernatant in which the addition of CTAB failed to precipitate or clot the ovomucin was recorded as the mucinase titer.

Antimucinase titration. Culture supernatants were diluted in buffered saline to yield a solution with a mucinase titer of 1:8 (8 units per ml). Aliquots of 0.25 ml (2 units) of such preparations were added to 0.25 ml of twofold serial dilutions of the antiserum. Following incubation at room temperature for 1½ hours, 0.5 ml ovomucin was added to each tube and the test incubated at 40°C for one half hour. The test was read in the manner here described except that the titer of the antiserum was taken as the highest dilution in which a clot was observed, indicating neutralization of the mucinolytic enzyme.

Results. Mucinolytic activity by strains

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† .052g $Na_2B_4O_7 \cdot 10H_2O$
1.203g H_3BO_3
1.0 g $CaCl_2$
8.5 g NaCl
1000 ml Distilled H_2O

TABLE I. Distribution of Mucinolytic Activity in Strains of *Shigella*.

Culture type		Source*: Stock collection No. pos.	Stock collection No. neg.†	Tokyo No. pos.	Tokyo No. neg.	Mexico City No. pos.	Mexico City No. neg.	Total tested	Total pos.
Sh. dysenteriae	1	0	7					7	0
	2	0	14					14	0
	3	0	6					6	0
	4	0	7					7	0
	5	0	2					2	0
	6	0	6					6	0
	7	0	5					5	0
Sh. flexneri	1a	0	7	0	1			8	0
	1b	0	15					15	0
	2a	8	3	8	0	8	0	27	24
	2b	0	7	0	5	0	2	14	0
	3	0	13	0	7	0	2	22	0
	4	0	20	0	1	0	1	22	0
	5	0	3			0	1	4	0
	6	0	17					17	0
	X	1	3					4	1
	Y	2	7					9	2
Sh. boydii	1	0	2					2	0
	2	0	2					2	0
	3	0	2					2	0
	4	0	5					5	0
	5	0	6					6	0
	6	0	4					4	0
	7	0	8					8	0
Sh. sonnei	I	0	29	0	6	0	1	36	0

* Cultures from stock culture collection were tested for mucinolytic activity after being maintained on laboratory media for years; cultures obtained in Tokyo were tested approximately 10 months after isolation; strains from Mexico City were tested for mucinolytic activity immediately after isolation.

† Strains with mucinolytic titers which were less than 1:8 were recorded as negative, since on occasion 1:4 dilutions of supernatants of uninoculated medium inhibited the precipitation of ovomucin by CTAB. Thus, it is possible that many strains of *Shigella* produce mucinolytic enzymes in concentrations too small to detect by the method employed.

of *Shigella* was first observed in a culture of *Sh. flexneri* 2a.‡ As a result of this finding a survey of available strains was carried out. Table I gives the results of this study. It will be noted that, under the conditions of growth which were employed, most of the strains of *Sh. flexneri* 2a elaborated the enzyme. A few strains of *Sh. flexneri* X and Y possessed activity, whereas no other strains manifested detectable mucinase. The mucinolytic titer of the crude Shigella supernatants varied from 1:8 to 1:32 thus exhibiting less activity than similarly prepared cholera supernatants.

An immune serum was prepared in rabbits by immunization with a concentrate of the crude supernatant of *Sh. flexneri* 2a, strain 2-2. This antiserum (titer = 0.25 ml of a 1:320 dilution) was capable of neutralizing the mucinolytic activity of 2 units (contained in 0.25 ml) of the homologous mucinase. Serum taken before immunization was inactive in a dilution of 1:20, and antimucinase activity was not observed with an immune serum (agglutinin titer 1:1280) prepared against living cells of strain 2-2. Furthermore, adsorption of the antimucinase serum with viable 2-2 cells caused no reduction in its antimucinase titer indicating that mucinase is antigenically distinct from the somatic antigen. An investigation is now in progress to determine whether this antimucinase serum will neutralize the mucinases produced by other strains of *Sh. flexneri*.

Discussion. Relatively little is known of

‡ While this study was in progress Freter(8) also observed mucinolytic activity in a culture of *Sh. flexneri* 2a.

the pathogenesis of bacillary dysentery. At one time considerable importance was placed on the extreme toxicity of the endotoxin of dysentery bacilli. However, this now seems unwarranted, inasmuch as it has been shown that the somatic antigens of all enteric organisms, nonpathogens as well as pathogens are more or less of the same order of toxicity (9). Thus, while it would be incorrect to assume that the somatic antigen plays no part in pathogenesis, one must look beyond it to determine why organisms of the Shigella group cause disease while most strains of *Escherichia coli,* for instance, do not.

The observation that a strain of *Sh. flexneri* 2a possesses mucinolytic activity suggested that this enzyme may play some part in pathogenesis. This has provided the impetus for the study here reported. Under the conditions of growth described, however, only certain strains of *Sh. flexneri* 2a, X and Y produced the enzyme while all available cultures of the closely related *Sh. flexneri* 2b failed to do so. The length of time the culture had been maintained on laboratory media made little difference, for both stock and freshly isolated strains of *Sh. flexneri* 2a possessed mucinolytic activity while stock and freshly isolated cultures of *Sh. flexneri* 2b, 3, 4, 5, and *Sh. sonnei* were negative.

On the basis of these preliminary studies, therefore, it is not possible to assess the role of mucinase in the pathogenesis of bacillary dysentery. If this particular enzyme is of any great importance in participating in the establishment of infections by the organism, it seems likely that it would be elaborated by most if not all serological types, rather than by only 2 or 3. It is of course possible that the capacity to produce mucinase is rapidly lost by most strains on artificial cultivation, or that types of dysentery bacilli other than *Sh. flexneri* 2a, X and Y will produce mucinolytic enzymes only under conditions of growth different from those employed in this study. Such possibilities remain to be investigated.

Summary. A survey was made to determine the incidence of mucinase production among representative strains of the genus Shigella. With the culture conditions employed, 24 out of 27 strains of *Sh. flexneri* 2a, 1 of 4 cultures of *Sh. flexneri* X and 2 of 9 strains of *Sh. flexneri* Y demonstrated mucinolytic activity while none of the 214 strains of other serological types of dysentery bacilli were found to be active. Immunization of rabbits with a crude enzyme preparation from a strain of *Sh. flexneri* 2a yielded an antiserum which neutralized its mucinolytic activity.

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