Enhancement of Bacterial Infection by Homologous Gastric Mucin.* (19276)

DAVID GALE AND SANFORD ELBERG. (Introduced by A. P. Krueger.)
From the Department of Bacteriology, University of California, Berkeley.

Although most of the work on enhancement of infection by mucin has been done with hog gastric mucin, mucins from other sources have been used. These include human salivary mucin(1,2), human gastric mucin(3), hog intestinal mucin(4), and guinea pig intestinal mucin(5). In more recent work, mucins from predominantly human sources have also been prepared and assessed for their enhancing activity. These include respiratory tract mucin(6), and mucins prepared from nasal mucus, bronchiectatic fluid, pseudomucinous cyst fluid, stomach, small and large intestine, and umbilical cord(7). However, no work has been reported on the effect of mouse gastric mucin or from mucins of sources other than those mentioned above. In addition, although the guinea pig intestinal extract used by Cantacuzene and Marie(5) was used in guinea pigs with the cholera vibrio as test organism, no other work has been done with a mucin homologous to the animal in which enhancement was being tested. Since mice have been used in work reported elsewhere(8) the preparation of mouse gastric mucin and its enhancing activity was investigated.

Methods. Preparation of the mouse gastric mucin. The method for the preparation of mouse gastric mucin was that described by Bendich, Kabat, and Bezer(9) for the preparation of mucin from individual hog stomach mucosae. From this material Bendich *et al.* (9) extracted "pure" group A substance by the method of Morgan and King(10). The method consisted of peptic digestion of the hog stomach mucosae at pH 2.3, and precipitation of the mucin from the aqueous peptic digest by 4 volumes of 95% ethanol. The stomachs of mice were removed, opened and their contents expressed. They were then placed in 70% ethanol for 10-14 days until enough were available for the next step. Five hundred stomachs, per batch, were washed with water, chopped into small pieces, washed twice with 95% ethanol and once with ether, and dried over P_2O_5 *in vacuo*. The dried stomachs were weighed, and 8.0 ml per g of HCl-citrate buffer at pH 2.3 added. To the suspension was added 2.0 mg crystalline pepsin and 0.2 ml toluene and the mixture was incubated at 37°C for 4-5 days with occasional shaking. The pH was then carefully readjusted to 2.3 with concentrated HCl and 1.0 mg pepsin was added to the mixture. After incubation for 2 more days, the peptic digest was separated by centrifugation. The sediment was suspended in HCl-citrate buffer (4.0 ml per g dried stomachs), 1.5 mg pepsin and 0.2 ml toluene added, and the mixture was incubated for 2 days. It was then centrifuged and the supernate was added to the previous digest. The sediment was washed twice in 60 ml citrate buffer. The combined supernates and washings were clarified by filtration through absorbent cotton. Four volumes of 95% ethanol were added to the clarified, viscous digests, and a heavy, very viscous, white precipitate settled out. This was collected by filtration upon a Buchner filter and after being washed twice with 95% ethanol and once with ether, the precipitate was dried over P_2O_5 *in vacuo*. The resulting grayish-white material was ground in a mortar and pestle, and weighed. This material was the mouse gastric mucin. It was not soluble in either saline or water but would make a stable suspension in either fluid when shaken for 24 hours on a Kahn shaker. Two thousand mouse stomachs were extracted in 4 batches to give a total yield of 2.5 g of the mouse mucin, which represented 4.1% of the weight of the dried stomachs.

"Virulence enhancing" activity of mouse gastric mucin. A stable suspension of the mouse mucin was made by adding 780 mg to 36.0 ml distilled water and shaking on a

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TABLE I. Enhancing Activity of Mouse Gastric Mucin on *Salmonella typhi* Infection in Mice.

No. of organisms	2% mouse mucin*	2% hog mucin	Saline	No. of organisms	5% mouse mucin†	5% hog mucin	Saline
9.3×10^7	—‡	—	4/5	1×10^8	—	—	4/5
10^6	4/5§	—	0/5	10^7	—	—	0/5
10^5	3/5	—	—	10^6	5/5	—	
10^4	0/5	—	—	10^5	5/5	—	
10^3	0/5	4/5	—	10^4	4/5	—	
10^2	0/5	1/5	—	10^3	4/5	4/5	
93	0/5	0/5	—	10^2	4/5	3/5	
0	0/5	0/5	—	10	—	1/5	
				0	0/5	0/5	

* Heat-sterilized. † Not heat-sterilized. ‡ Not done. § Deaths/total, 36 hr after inoculation. ‖ 28 hr after inoculation.

All mice were observed 5 days before being listed as survivors.

Kahn shaker for 24 hours until homogenization occurred. The resulting suspension (approximately 2% w/v) was a grayish, translucent, viscous material which bore little resemblance to a white, opaque, viscous 2% hog gastric mucin suspension. Both mucin suspensions were sterilized at 121°C for 15 minutes. The mouse mucin precipitated with the heat treatment, but was resuspended easily when it was cooled and neutralized with N/10 NaOH. The hog mucin remained stable through the heating and the neutralization thereafter.

The materials were titrated for activity as shown in Table I. Mouse mucin enhanced infection but was not as effective as the 2% hog mucin suspension.

Since the mouse mucin seemed more heat-labile than the hog mucin and since it was possible that the heat had destroyed some of the biological activity, a 5% suspension of the mouse mucin was prepared aseptically in water at pH 7.3. The preparation was sterile. A titration of its activity in comparison to 5% hog mucin is also shown in Table I. This preparation of mouse mucin was as effective as the hog mucin in enhancing infection by *S. typhi*.

Summary. Homologous mucin will induce the phenomenon known as enhancement of infection in mice as well as the more routinely used hog gastric mucin.

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