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Aggressin-Like Character of Gastric Mucin.

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Since Nungester, Wolf, and Jourdonais[1] reported that gastric mucin increases the virulence of meningococci, streptococci, and staphylococci, when injected simultaneously into the abdominal cavity, this technique has been used to reinforce the virulence of gonococci,[2] *H. influenzae*,[3] typhoid organisms,[4] and *H. pertussis*.[5] Mucin suspensions of such microorganisms, otherwise not suited for experimental infection, facilitate their use for determining on a quantitative basis the potency of chemotherapeutic agents, vaccines, and antisera. Staphylococcus[6] and meningococcus infections with mucin are used in our laboratory routinely for studying the activity of chemotherapeutic agents. More recently, we succeeded by the use of the same technique in obtaining a lethal infection with *C. diphtheriae* in mice. This successful infection gave us the opportunity to study the antibacterial activity of certain chemotherapeutic agents against *C. diphtheriae*.[7]

The mode of action of mucin has found various interpretations. It seems, though, that the question is far from settled.

Miller and Castles[8] consider that mucin may interfere locally with the defense mechanism of the host, thereby making the barrier between peritoneal cavity and blood stream permeable for meningococci. According to Nungester, Jourdonais, and Wolf,[9] mucin inhibits the bactericidal properties of phagocytic cells without influencing phagocytosis. Keefer and Spink[10] reported that mucin depresses the bacteriolytic power of serum for gonococci (without lowering the complement titer) by somehow protecting the organisms against the action of natural antibodies. McLeod[11] suggests that mucin acts mainly as a favorable medium for the propagation of meningococci in the peritoneal cavity from where the blood stream is then progressively invaded. The function of mucin as a medium favoring growth is in contrast with previous observations made by Miller and Castles for meningococci[8] and by Keefer and Spink for gonococci.[10] The literature on this subject has been reviewed by McLeod.

In the course of our studies on staphylococcal and diphtherial infections, a number of observations have been made which may contribute towards the elucidation of the mechanism of mucin activity.

Material and Method. The preparation and standardization of the inocula and the technique used for infection have been described before.[6,7] Granular mucin Type 1701-W, batches No. 48555 and 52012 of the Wilson Laboratories, was used throughout the investigation. *Staphylococcus aureus* 6340, a strain originally obtained from an infected patient and *C. diphtheriae* No. 9060, type gravis, used in these experiments were received from the American Type Culture Col-

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8 Miller, C. P., and Castles, R., *J. Inf. Dis.*, 1936, **58**, 263.

9 Nungester, W. J., Jourdonais, L. F., and Wolf, A. H., *J. Inf. Dis.*, 1936, **59**, 11.

10 Keefer, C. S., and Spink, W. W., *J. Clin. Invest.*, 1938, **17**, 23.

11 McLeod, Charlotte, *Am. J. Hygiene*, 1941, **34**, 41, 51.

TABLE I.
Leucocytosis and Destruction of the Staphylococci Injected in Mucin and in Saline.

Infection		Staphylococci in the culture of the peritoneal exudate after hours					Leucocytosis in peritoneal smear after hours	
No. of staphylococci	Vehicle	2½	3½	5	7½	25	7½	25
71,600	saline	±	±	±	0	0	xxx	xxx
71,600	"	0	±	±	±	0		
71,600	mucin	++	+	++	+++	+++*	*x*	x
7,160	"	++	+	+	+	++	*x*	xxx

0 No growth. * Died 4 hours later.
± 2-20 colonies. *x* A few cells.
+, ++, +++ Different intensity of growth. x, xxx Different intensity of leucocytosis.

lection. The meningococcus Group I strain was received from Dr. E. A. Kabat, College of Physicians and Surgeons, Columbia University.

(1) *Fate of injected cocci.* (*a*) *In the peritoneal cavity.* Cultures of peritoneal fluid made on agar plates revealed that staphylococci injected alone in saline readily disappear from the peritoneal cavity. During the first 2½ to 5 hours following inoculation, only occasional colonies grew from the exudate. No growth was obtained in the cultures made from the exudate 10 to 24 hours after inoculation.

Staphylococci injected into the peritoneal cavity with mucin remained viable. Cultures of peritoneal fluid made 2½ to 5 hours after inoculation gave on the agar plate a more or less confluent growth of staphylococcus. The number of colonies increased with the time elapsed after inoculation. The experiment reported in Table I shows that 7000 staphylococci injected in mucin gave a confluent growth from peritoneal liquid 25 hours after inoculation, while 10-fold heavier inocula injected without mucin could not be detected in cultures made after 7½ hours.

(*b*) *Spreading of staphylococci with and without mucin from the peritoneal cavity.* The spreading of cocci from the site of infection after injection in mucin was traced by culturing blood from the tail vein of living mice and by culturing heart and lung tissue of animals killed after different intervals. Heart and lung cultures showed staphylococci ½ to 1 hour after intraperitoneal inoculation with 30,000 to 50,000 cells; growth became particularly abundant after 2 to 3 hours. (Peripheral blood cultures showed the presence of occasional staphylococci during the first 10 hours after inoculation, but the number of organisms definitely increased only after 18 to 24 hours when the mice were beginning to die.) No bacteriemia was observed if the same dose of staphylococci (30,000 to 50,000) was suspended in broth and injected intraperitoneally. Blood-agar plate cultures from lungs and heart, taken at frequent intervals during the first 24 hours, were negative. The cultures from organs remained negative even after increasing the number of injected staphylococci to one million. A typical experiment is given in Table II.

These results suggest that the addition of mucin was responsible for the spreading of the organisms from the site of infection into the organs.

2. *Reactions in the Peritoneal Cavity.* Peritoneal fluid of mice injected with 30,000 to 70,000 staphylococci (with and without mucin) was collected at various intervals after inoculation by puncturing the abdominal wall with a capillary pipette. The fate of the injected cocci and the resulting inflammatory reaction were followed by making smears stained with methylene blue.

Mice injected with 50,000 staphylococci suspended in saline showed a strong leucocytic reaction 2½ to 5 hours after inoculation. On microscopic observations of the stained smears neither extra- nor intracellular cocci could be seen. *A similar inoculum injected with mucin showed a strikingly different picture.* Only isolated leucocytes, if any, appeared and, in many instances, their shape was irregular. While the microscopic observations of stained

TABLE II.
Invasiveness of Staphylococci Injected Intraperitoneally in Mucin and in Broth.

No. of staphylococci injected, × 1000	Interval after inoculation, hr	Infection with mucin		Infection without mucin	
		Heart Culture	Lung Culture	Heart Culture	Lung Culture
26 to 135	½ to 1½	0	0		
		4 colonies	±		
		1 colony	3 colonies	0	0
		+	+	0	0
		+	—		
		±	±		
	3½ to 7½	+*	+*	0	0
	12½	+	+		
		++	++	0	0
	24	+++†	+++	0	0
1,350	4			0	0
	6			0	0

0 No growth.
± Non-confluent growth.
+ Confluent growth.

* 8 mice.
† 6 mice died.

smears taken between 3 to 8 hours after inoculation failed to reveal the presence of cocci in the peritoneal exudate, we know from the results obtained by culturing this exudate that viable cocci were present.

It seems that the effect of mucin on the appearance of peritoneal fluid is two-fold: (1) inhibition of leucocytosis; (2) lack of destruction of cocci. The most plausible interpretation is that staphylococci in mucin survive in the peritoneal fluid, because they are not destroyed by leucocytes which normally would have appeared in large number.

3. Heated Staphylococci. For a further analysis of the effects of mucin, heat-killed staphylococci were used. A heavy suspension of cells was heated for 2 to 3 hours at 60°C. (In some experiments, this treatment failed to kill all the cocci.)

One hundred million of dead staphylococci injected intraäbdominally in 0.5 cc saline caused a strong leucocytic reaction 4 hours after inoculation. As shown by smears, the leucocyte count remained high for 24 hours. With the same amount of vaccine injected in 0.5 cc of mucin, only a few leucocytes were found on the slide 4 hours after inoculation. After 6½ hours, again only a few leucocytes, most of them containing intracellular cocci, were visible.

Thus, mucin exerted the same influence on the disposal of heat-killed as on that of live staphylococci. Death from bacteriemia resulted when mice were given mucin with the vaccine which still contained some live cocci, since as stated earlier, exposure of the bacterial suspensions to heat at 60°C for 2 to 3 hours did not result in killing all the organisms. Staphylococci could actually be cultured from the heart and other organs of these animals.

4. Other Infections. (*a*) *Meningococcus Group I.* A limited number of experiments gave results similar to those described for staphylococcus. A characteristic experiment is given in Table III. It would appear that during the first stage following inoculation, mucin inhibits leucocytosis and, consequently, the destruction of meningococci.

(b) *C. diphtheriae.* The fate of the bacillus was followed in the peritoneal cavity and in the organs of mice infected with and without mucin by making cultures on solid media containing tellurite. The results are given in Table IV and indicate that (1) mucin inhibits local leucocytosis after intra-abdominal injection of *C. diphtheriae;* (2) the presence of mucin interferes with the destruction of diphtheriae bacilli in the peritoneal cavity, while in the absence of mucin, such destruction takes place within 2½ to 5 hours; (3) mucin enhances the spreading of the diphtheriae infection from the peritoneal cavity.

TABLE III.
Influence of Mucin on the Intraäbdominal Infection of Mice with Meningococci (Group 1).

Mouse No.	Slant suspension diluted	Suspended in (1 cc)	1½ hr after infection			3 hr after infection		
			Culture of†			Culture of		
			peritoneal fluid	heart	Local leucocytosis	peritoneal fluid	heart	Local leucocytosis
15647	10-4	mucin*	++	1 colony	0			
648	,,	,,			0	++	1 colony	0
673	,,	saline	±	0	+			
674	,,	,,				+	0	++
670	10-5	mucin	+	1 colony	0			
671	,,	,,			0	++	2 colonies	±
676	,,	saline	0	0	++±			
677	,,	,,				4 colonies	0	++

* 100 MLD.
† On tryptose phosphate agar.

5. Effect of Mucin on Aleuronat Injected Intra-Abdominally. In order to investigate whether the leucocytosis-inhibiting activity of mucin is limited to bacteria, analogous experiments were made with aleuronat.

Two sets of experiments were carried out. In the first, aleuronat suspended in mucin was injected intra-abdominally; in the second, the effect of mucin was studied on mice previously treated with aleuronat.

Mice injected with a 1% aleuronat suspension in 1 cc mucin failed to show the usual leucocytic reaction within the first 2 to 6 hours; after 18 hours, no difference was observed between the effect of aleuronat in mucin and in saline.

One cc mucin injected into the peritoneal cavity of mice treated 18 hours before with aleuronat did not produce any noticeable destruction or diminution of leucocytes.

6. Mucin Antagonists: Aleuronat and staphylococcus infection with mucin.

From the previous experiments, one could draw the conclusion that mucin exerts its claimed virulence-increasing effect by inhibiting leucocytosis. However, since an already existing leucocytosis in the peritoneal cavity is not influenced by mucin, we were able to study the mucin effect in the presence of leucocytes.

Local leucocytosis was induced by aleuronat. Subsequently, a staphylococcus infection with mucin was given. It was expected that the mucin effect in the presence of a leucocyte infiltration would not take place. The data given in Table V confirmed this expectation.

Cultures made of peritoneal fluid 3 and 5½ hours after inoculation gave a confluent growth for the untreated mice and only a small number of colonies (35-55) for mice previously treated with aleuronat. Thus, in the presence of leucocytes, the destruction of staphylococci takes place rapidly even if they had been injected with mucin. In other words, the activity of mucin is neutralized by leucocytes accumulating after a previous aleuronat injection.

No protection against bacteriemia could be obtained with aleuronat if a more strongly invasive coccus, such as *Streptococcus hemo-*

TABLE IV.

Influence of Mucin on the Intraäbdominal Infection of Mice with *C. diphtheriæ*, Type Gravis, No. 9060.

Mouse No.	Broth diluted	in (1 cc)	2 hr after infection: Cultures peritoneal fluid	2 hr after infection: Local leucocytosis	3 hr after infection: Cultures of peritoneal fluid	3 hr after infection: Cultures of heart	3 hr after infection: Local leucocytosis	5 hr after infection: Cultures of peritoneal fluid	5 hr after infection: Cultures of heart	5 hr after infection: Local leucocytosis
11,189	10-2	mucin	+		++	±	±			
190	,,	,,	±							
191	,,	,,	+					+	±	±
195	,,	saline	15 col.	±	10 col.	0	+			
196	,,	,,	3 ,,							
197	,,	,,	15 ,,					+	0	+
192	10-3	mucin		±	+	0	0			+
193	,,	,,	±	±						
194	,,	,,	+	±				+	15 col.	±
10,894	,,	,,	+	±	++		0	++	0	+
895	,,	,,	+	0	++		±	++	6 col.	0
933	,,	saline	0	±	0			0	0	+
934	,,	,,	0	±	0		+	10 col.	0	

± = 1-2 leucocytes per oil immersion field.

lyticus, was injected. The virulence of the β-hemolytic streptococcus 4 (Group A, Type 3) was the same in the controls as in aleuronat pre-treated mice. This may have been due to the fact that even an increased leucocytosis is not sufficient to destroy completely such a virulent organism. The observation would be in agreement with the data reported by Menkin,[12] by Dennis and Berberian,[13] and by Schnitzer and Goddard.[14]

Discussion. The results obtained with live and heated staphylococci, with *C. diphtheriae*, meningococci, and aleuronat injected intra-abdominally suspended in mucin showed marked similarities. In every instance, it appeared that mucin inhibits local leucocytosis in the peritoneal cavity, increasing by this inhibition the invasive power of the injected organism. This observation is in agreement with the findings of Miller and Castles, indicating that cellular elements did not begin to accumulate in the peritoneal cavity until 3 hours after inoculation of meningococci and did not become abundant until the 9th hour.

When leucocytosis was already present, mucin did not seem to interfere with it: this means that leucocytes were not destroyed by mucin; consequently, the increase of virulence was slight when staphylococci plus mucin were injected into the already affected (by aleuronat) peritoneal cavity.

Thus, mucin may be considered an agent with negative chemotaxis and a direct antagonist to substances with positive chemotaxis, such as aleuronat, peptone, etc. Due to its property of negative chemotaxis, mucin decreases the resistance of the host towards bacteria even when their invasive power is low. In terms of classical immunology, one might say that mucin takes the place of the aggressin which is lacking in such microorganisms. As "aggressin substitute," mucin lowers the defensive powers of the host by preventing leuco- and phagocytosis, thus facilitating the spreading of the organisms and the invasion of the blood stream. In the case of diphtheria, for instance, the local defensive mechanism of the peritoneal cavity, which according to Seligmann and Jungeblut[15] "functions perfectly to ward off bacterial invasion and to maintain sterility," is abolished by the aggressin action of injected mucin.

[12] Menkin, V., *J. Exp. Med.*, 1933, **57**, 977.

[13] Dennis, E. W., and Berberian, D., *Ibid.*, 1934, **60**, 581.

[14] Schnitzer, R. J., and Goddard, I. G., *J. Immunol.*, 1943, **46**, 133.

[15] Seligmann, E., and Jungeblut, C. W., *J. Immunol.*, 1941, **40**, 119.

TABLE V.
Antagonistic Effect of Aleuronat on Virulence of Staphylococci Induced by Mucin.

Date of exp.	No. of staphylococci injected × 100	Staphylococcus Infection + Mucin			
		Without aleuronat		With aleuronat	
		No. of mice	Survivors	No. of mice	Survivors
3/3/44	61	10	0	4	2
	610	10	0	5	4
5/10/44	65	5	1		
	650	10	4	5	4

Summary. 1. Virulent staphylococci injected in mucin do not produce a characteristic leucocytic reaction in the peritoneal cavity. 2. Staphylococci injected intraperitoneally in mucin remain viable and spread from the abdominal cavity; without mucin, they are rapidly destroyed. 3. Staphylococcus vaccine suspended in mucin does not produce leucocytosis during the first hours after injection. 4. *C. diphtheriae* and meningococcus injected intra-abdominally are influenced in the same manner by the presence of mucin. 5. The leucocytic reaction of aleuronat is inhibited by simultaneous injection with mucin. A later injection of mucin does not destroy leucocytes already present in the peritoneal fluid. 6. The virulence-enhancing effect of mucin can be neutralized by leucocytosis previously induced by aleuronat. 7. All these observations suggest that mucin decreases the resistance of the host to bacterial invasion and imparts to microörganisms of otherwise low virulence the potentialities of highly invasive microbes.

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Size of Histamine Wheal in Relation to Dose and to Animal Species.*

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Since the first description of the effects of histamine on the skin, there have been many studies and applications of this local reaction. This investigation concerns the variations in response to intradermal injections of histamine in different species, and also the relation of wheal diameter to the concentration of histamine used.

Eppinger[1] first described histamine urticaria produced by scarification. Sollman and Pilcher[2] obtained wheals with histamine by intradermal injection in man, but not in rabbits or cats. They used semi-quantitative methods to measure this skin reaction, finding that the size of the wheal produced by local administration appeared to be a function of the concentration of histamine used. A minimum reaction was obtained with a concentration of 1:100,000. Since this work, there have been numerous methods devised for the determination and assay of histamine, such as the uterine strip, patch test and chemical methods. However, for rapid

* This investigation was undertaken with the aid of the staff of the Department of Pharmacology of the University of Southern California School of Medicine, Los Angeles, California.

1 Eppinger, H., *Wien. med. Wchnschr.*, 1914, **113**, 1913.

2 Sollman, T., and Pilcher, J. D., *J. Pharm. and Exp. Therap.*, 1917, **9**, 309.