Buccally, EED is approximately 40 times more effective than progesterone, administered by the same route. EED is estrogenic as measured in both the mouse uterine growth assay and the rat vaginal smear assay yet when administered in conjunction with estrone EED acts as an estrogen-antagonist. These opposing activities can best be explained by the differences in hormonal dominance at low and high dose levels.

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Enhancement of Resistance in Mice to Staphylococcal Infection by Preliminary Treatment with a Staphylococcal Extract.* (26823)

ALFRED L. FLORMAN, HELEN ZEPP, EUGENE AINBENDER AND JEANNE L. SCOMA
(Introduced by H. L. Hodes)
Pediatric Service, Mount Sinai Hospital, New York City, and Division of Pediatrics, North Shore Hospital, Manhasset, N. Y.

Protection against staphylococcal infection has been the subject of extensive clinical and experimental investigations. Among the most provocative experiments are those in which resistance is enhanced by methods which are independent to those involved in specific acquired immunity(1,2).

While investigating the properties of a staphylococcal extract which we were using as an hemagglutinating antigen, it was found that this extract had a definite resistance enhancing effect in mice against experimental staphylococcal disease. This resistance could be demonstrated after 20 hours and held for 2 heterologous strains of staphylococci as well as for the homologous strain and for one strain of *E. coli.* The extract did not visibly disturb mice and in rabbits did not behave like an endotoxin. In this study the details of these experiments are reported.

Materials and methods. Mice—Swiss mice (CFW) weighing 15-20 g were used in all experiments, except where indicated. *Staphylococci*–"Bartlett", an epidemic, 80-81 strain, resistant to penicillin, obtained from Dr. David Rogers, then at the New York Hospital. was used for preparation of the extract and except as indicated in the text for challenging the mice. For the latter purpose the organisms from an overnight culture in trypticase soy broth were obtained by centrifugation and suspended in sterile saline. The concentration of organisms per inoculum was determined by plating out serial dilutions on blood agar plates. Two other strains were used: "H"—a non typable strain sensitive to penicillin which was obtained from Dr. Stanley Schneierson, Mt. Sinai Hospital, and "Giorgio"—a coagulase positive strain which was sent by Dr. Rene Dubos, Rockefeller Institute for Medical Research.

Staphylococcal extract. 40 ml of trypticase soy broth were inoculated with a few drops of a 4 to 6 hour culture of the "Bartlett" strain of staphylococcus. After 18 hours of incubation at 37°C the culture was centrifuged and

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the supernate removed. It was acidified by addition of 0.5 ml of concentrated glacial acetic acid, then boiled over a hot plate until the volume was reduced to approximately 2 ml. This concentrate was passed through a Whatman #5 paper filter and added dropwise to approximately 14 ml of 95% ethyl alcohol. The precipitate was collected by centrifugation and dried under a vacuum for storage. It was reconstituted by addition of 2 ml of distilled water. If the solution was not water clear, it was centrifuged before use.

The extract was usually light brown in color. Preliminary chemical analysis, done by Dr. Anita Opper, Mt. Sinai Hospital Chemistry Dept., indicated that it contained insignificant amounts of polysaccharide and large amounts of protein and lipid. It caused no visible disturbance in mice when as much as 0.3 ml were given intravenously. Repeated subcutaneous injections caused no local reaction and did not interfere with growth of the animals. The peritoneal reaction to intra-abdominal injection was similar to that produced in response to saline injection except for a slight increase in number of polymorphonuclear cells. The extract was non-antigenic for mice since pools of serum obtained from mice 2 weeks after injection did not contain antibodies which could be detected by bacterial agglutination, hemagglutination or latex fixation. It did not behave like an endotoxin in rabbits. When 0.2 ml of twice concentrated extract were injected intradermally there was no visible reaction, nor did any appear at these sites 24 hours after 2 ml of the same material were given intravenously.

Triton X-100. This material produces a mucin-like enhancement of virulence. It is non toxic in 0.5% concentration(3).

Experimental staphylococcal disease in mice. A staphylococcal infection which produced death usually between 8 and 24 hours was produced by injecting the mice by the intra-abdominal and the intra-cerebral routes. Animals dying before 8 hours were not counted, and experiments were usually terminated at 7 days. The intra-abdominal infection was achieved with 0.5 ml of a mixture of equal parts of a saline suspension of staphylococci and 0.5% Triton X-100. The number of organisms required uniformly to kill more than 50% of the mice by this route was in the range of 10^8. The intra-cerebral infection did not require Triton. For this infection, about 10^6 organisms were given in 0.03 ml. It was usually necessary to use several dilutions of organisms in the indicated range in each experiment to attain a critical dilution. This was considered to be the highest dilution, or smallest number of organisms, which killed more than 50% of the control animals.

Results. Protection against intra-abdominal infection. In these experiments the mice were injected intra-abdominally with 0.5 ml of either saline or extract. Twenty hours later they were challenged by the same route with 0.5 ml of a mixture of equal parts of a dilution of staphylococci and 0.5% Triton X-100. Selection of the proper dilution of challenging organisms was critical. If too large a challenge was given the protective effect was obscured. However, if the effect of only the highest dilution of organisms which killed more than 50% of the control mice was studied, it soon became apparent that our extract did indeed protect. This is shown in the upper half of Table I where the results of 8 such experiments are summarized. Under the conditions of these experiments only 32% of control mice survived, as compared with 69% of the extract treated animals. The difference of 37% is highly significant.

However, since both the extract and challenge had been given by the same route, the possibility that this represented only peritoneal blockade had to be excluded. This was done by giving the challenge intra-cerebrally.

Protection against intra-cerebral infection. In these experiments, the mice were given 0.5 ml of either saline or extract intra-abdominally, and 20 hours later they were challenged by giving the organisms intra-cerebrally. In the lower half of Table I are summarized the results of 10 experiments in which critical dilutions were available. Only 24% of the control mice survived as contrasted to 56% of those given the extract. This difference of 32% is also significant, clearly indicating that the protective effect is not local but systemic.

Protective effect unrelated to size and age of mice. All of the above experiments were done with mice weighing 15-20 g. In a few experiments larger mice, mothers, weighing

TABLE I. Protective Effect of Staphylococcal Extract in 15-20 g Mice.

		Preliminary saline (.5 ml intra-abdom.)			Preliminary staph. extract (.5 ml intra-abdom.)		
Challenged:	Exp. #	No. of mice	Deaths	Survivors	No. of mice	Deaths	Survivors
Intra-abdominally (.25 ml staph. organisms + .25 ml Triton X-100)	4-32	14	12	2	13	3	10
	7-29	8	6	2	8	0	8
	8-28	13	7	6	10	4	6
	9-27	16	13	3	15	3	12
	10-26	10	6	4	10	3	7
	12-24	25	14	11	25	8	17
	14-22	15	13	2	15	6	9
	16-20	14	7	7	15	7	8
		115	78	37	111	34	77
		37/115 or 32% survivals			77/111 or 69% survivals		
		Difference of 37% p = <.001					
Intra-cerebrally (.03 ml of staph. organisms)	1-16	10	9	1	10	4	6
	3-13	31	24	7	31	17	14
	4-12	29	23	6	31	14	17
	5-11	31	20	11	31	13	18
	6-10	15	11	4	15	4	11
	13- 3	10	6	4	10	4	6
	14- 2	10	8	2	10	4	6
	15- 1	10	6	4	10	7	3
	16- 0	22	15	7	21	9	12
	17- 0	30	28	2	30	13	17
		198	150	48	199	89	110
		48/198 or 24% survivals			110/199 or 56% survivals		
		Difference of 32% p = <.001					

25-30 g, were used. In one such, after an intra-abdominal challenge, there were only 8 survivors out of 26 in the control group, while 23 of 25 extract-treated animals survived. In 2 experiments suckling mice (1 to 3 days old) were used. The amount of extract and of challenging organisms used with these small animals were different than in the other experiments. The results are summarized in Table 2. Only 8 out of 80 sucklings (10%) in the control group survived, as compared to 29 out of 83 (35%) of those which had previously received the extract.

Non-specificity of enhanced resistance. In all of the above experiments the animals were challenged with the same staphylococcus from which the extract had been prepared. In 3 experiments other organisms were used and the results indicate that this enhancement of resistance or protection is non specific. In one, the heterologous "H" strain was used to challenge the mice intra-abdominally. There were 10 mice in each group. Only 3 of the 10 control mice survived, as contrasted with 8 of 10 extract-treated animals. In a second experiment the heterologous "Giorgio" strain was used. This time the challenge was intracerebral. There were 15 animals in each group. Only 5 of the controls survived in contrast to 14 of the 15 extract-treated mice. In a third experiment an *E. coli* (0111-B4) was used without Triton to challenge the mice intra-abdominally. There were 15 animals in each group. None of the controls survived as compared with 6 of those which had been previously given the staphylococcal extract.

Discussion. The mode of action of this staphylococcal extract is unknown. Since the protective effect occurs within 24 hours, and since antibodies have not been demonstrated after 2 weeks, it is apparently not by antibody production.

North and Pawlyszyn(2) have suggested that this type of resistance is similar to interference between animal viruses. Interference is thought to operate at the level of the individual susceptible cell. The exact mechanism is unknown, but a widely held view is that it is by competition for cellular constituents which are available only in limited supply. However, the staphylococcus is not an obligate intra-cellular parasite, and so it is diffi-

TABLE II. Protective Effect of Staphylococcal Extract in Suckling Mice.

Challenged:	Exp. #	Preliminary saline (.03 ml intra-abdom.) No. of mice	Deaths	Survivors	Preliminary staph. extract (.03 ml intra-abdom.) No. of mice	Deaths	Survivors
Intra-abdominally (.03 ml staph. organisms)	11-25	44	42	2	46	35	11
	12-24	36	30	6	37	19	18
		80	72	8	83	54	29
		8/80 or 10% survivals			29/83 or 35% survivals		
		Difference of 25%			$p = <.001$		

cult to see how the enhanced resistance reported here can be called interference in the sense in which it is used in virology.

The rapid appearance of resistance might also suggest that properdin were involved, were it not for the observation(4) that gram positive cocci are insensitive to the properdin system, at least as measured by the *in vitro* bactericidal test.

A more likely mode of action for the extract is stimulation of the reticuloendothelial system. This is suggested by Bohme and Bouvier's observation(5) that the reticuloendothelial system of the mouse fails to respond in experimental staphylococcal infections, and Rowley's report(6) that extracts of *S. typhi,* which also lead to an early enhanced resistance(1), stimulate the reticuloendothelial system. However, it must be noted that our staphylococcal extract does not have the endotoxic activity of *S. typhi* extracts.

That the protective effect of the staphylococcal extract is obscured if too large a challenge is given, suggests that mice are capable only of a limited increase in this type of resistance.

Since this staphylococcal extract is relatively non toxic it offers interesting possibilities for further investigation. If it can be shown to work in other species, it may prove to be a useful prophylactic agent—not only against staphylococcal infections, but as suggested by our *E. coli* experiment, against a variety of bacterial diseases.

Summary. An extract from the supernate of an epidemic strain of staphylococcus which was not an endotoxin was injected intra-abdominally in mice. Twenty hours later the systemic resistance of these mice was significantly enhanced to lethal infections with homologous and heterologous strains of staphylococci and a toxogenic strain of *E. coli.* This effect was demonstrable in suckling, young and old mice.

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