

The Effect of Staphylococcal Enterotoxin upon the Frog, (*Rana pipiens*). (17401)

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Significant advances in the study of staphylococcal food poisoning have been extremely difficult because of the lack of a reliable diagnostic test. Bioassay methods have been described using human volunteers, monkeys, suckling pigs, and kittens and cats,¹⁻⁴ none of which has proved to be entirely satisfactory. The ability of an enterotoxin produced from a strain of *Micrococcus pyogenes* var. *aureus* to elicit a vomit reaction in a test animal has been considered evidence of a positive test. Animals such as ruminants, horses, and rodents lack the appropriate coordinating vomit mechanism⁵ and hence the usual laboratory animal (rabbit, guinea pig, rat, and mouse) has been of little value. The common frog, *Rana pipiens*, was chosen for this study because it has the ability to exhibit reverse peristalsis of the stomach,⁶ is readily available, is inexpensive, and is easily maintained under laboratory conditions.

Procedures and results. Preliminary experiments were carried out, using a filtrate prepared from a known enterotoxigenic strain of *Micrococcus pyogenes* var. *aureus*.^{*} Dolman's⁷ technique was followed in detail in the preparation of this and all subsequent filtrates. Only healthy, well-nourished frogs were used.

Filtrates were first administered either parenterally (into the dorsal lymph sac) or orally in No. 00 gelatin capsules. Satisfactory results were obtained, however, by the simpler procedure of direct feeding with a medicine dropper. This was accomplished by opening the frog's mouth with large, blunt-ended forceps, and then, while holding the mouth open by a thumb inserted near the angle of the jaw,⁸ slowly inserting the dropper through the esophageal sphincter. Thus, the filtrate was placed directly into the frog's stomach. The frog under test was placed in an aquarium jar containing a small amount of water and was observed over a period of hours. A positive test for enterotoxin is shown by the production of a series of reactions termed for the purpose of this discussion a "spasm", for although reverse peristalsis of the stomach could be demonstrated, vomiting of the stomach contents never occurred.

A spasm is initiated by a series of swallowing motions (retraction of the eyeballs), a slowly repeated gaping of the mouth, a gradual change in the tone of the skin color, a distension of the abdominal wall, a distinctive sitting position (the forelegs at full height and the back raised so that it parallels the floor of the aquarium). This takes place within 3 to 10 minutes and reaches a climax when the spasm is completed by a slow-motion rubbing of the abdominal wall with a hind-foot followed by a wiping of the face and mouth with a fore limb. This occurs on both sides of the body and may last from 3 to 15 minutes. Gradually, mucous is expelled from the mouth, abdominal distension is decreased and the frog assumes a natural sitting position. Gaping of the mouth may continue for some time after the other characteristic body motions have stopped. The rapidity with which the spasm follows the

¹ Jordan, E. O., *J.A.M.A.*, 1931, **97**, 1704.

² Jordan, E. O., *Proc. Soc. Exp. Biol. and Med.*, 1931, **29**, 161.

³ Hopkins, E. W., and Poland, E. F., *Food Res.*, 1942, **7**, 414.

⁴ Dolman, C. E., *Canad. J. Pub. Health*, 1926, **27**, 489.

⁵ Meyer, H. H., and Gottlieb, R., *Experimental Pharmacology*, J. B. Lippincott, Philadelphia, Pa., 1926, 2nd ed.

⁶ Alvarez, W. C., *An Introduction to Gastroenterology*, P. B. Hoeber, N. Y., 1940, 3rd ed.

⁷ Dolman, C. E., *Canad. J. Pub. Health*, 1940, **51**, 68.

^{*} Strain No. 8 obtained through the kindness of Dr. Gail Dack, Department of Bacteriology and Parasitology, University of Chicago.

⁸ Rose, S. Meryl, personal communication, 1949, Department of Zoology, Smith College.

feeding of the filtrate is apparently dependent upon individual susceptibility and enterotoxin potency. Reactions have been observed within a range of 45 minutes to 7 hours. After the spasm has subsided, further reactions may be elicited, for at least a 10-day period, by feeding the frog. Therefore, it is possible to feed and then leave the animal until the next morning when a meal is given. A typical spasm occurs within 3 to 4 hours.

Such typical reactions have been obtained with other known enterotoxigenic strains of *Micrococcus pyogenes* var. *aureus*,[†] while no such reaction has ever been observed in frogs fed with filtrates prepared from known negative strains of *Micrococcus pyogenes* var. *aureus* No. 902 and 64, *Escherichia coli*, *Proteus vulgaris*, and sterile culture medium. The results of these tests are summarized in Table I.

A series of frogs was fed known enterotoxigenic filtrates in an effort to show reverse peristalsis of the stomach. At the height of a spasm the animal was decapitated. In each frog studied, the esophageal sphincter was relaxed and the stomach was observed in reverse motion. Controls included unfed frogs, those fed hamburger, and those fed sterile

[†] Enterotoxigenic strains Nos. 432 and 422 and non-enterotoxigenic strains Nos. 902 and 64 were obtained through the kindness of Dr. Glenn Slocum, Food and Drug Administration, Washington, D.C.

TABLE I.
Effect of Enterotoxigenic and Non-Enterotoxigenic Filtrates Upon the Frog (*Rana pipiens*).

Organism used in filtrate preparation	Total No. of frogs tested	Reaction observed
<i>Micrococcus pyogenes</i> var. <i>aureus</i>		
No. 161	28	Spasms
No. 8	8	"
No. 432	21	"
No. 422	5	"
No. 64	18	None
No. 902	5	"
<i>Micrococcus pyogenes</i> var. <i>albus</i>	9	"
<i>Escherichia coli</i>	6	"
<i>Proteus vulgaris</i>	3	"
Sterile culture medium	29	"

culture medium. One each of these was sacrificed with each test frog. In no control was any reverse motion of the stomach observed.

Summary. Staphylococcal enterotoxins which gave positive results with the kitten test were capable of producing spasms when fed to frogs. It would appear from the results summarized in Table I, that the frog test was specific for no false positive reactions were noted. It is not possible to use a frog more than once when a positive reaction has been obtained and further study is in progress to determine the nature of the injury to the digestive mechanism.

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Epilation in the Non-Irradiated Member of Parabiotically United Rats.* (17402)

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Despite the lack of our ability to explain this phenomenon—the loss of hair in the non-irradiated members of parabiotically united

rats—it was thought worthy of record as an observation.

The relatively few known fundamental reactions involved when either particulate or electromagnetic radiation reacts with matter are described in the standard textbooks of Atomic Physics. These explanations are fully

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