THE EFFECT OF STAPHYLOCOCCUS ENTEROTOXIN ON ISOLATED RABBIT GUT SEGMENTS

JULIUS J. RICHMOND[1] AND C. I. REED

Department of Physiology, University of Illinois, Chicago Colleges

AND

H. J. SHAUGHNESSY AND V. MICHAEL

Illinois State Department of Health, Chicago, Illinois

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It is the purpose of this paper to report some observations on the response of rabbit intestinal muscle to the enterotoxin extracted from strains of staphylococcus cultured from food known to have produced attacks of food poisoning. The cultural, epidemiological and other aspects of this subject have been discussed by several authors (Barber, 1914; Dack, Cary, Woolpert and Wiggers, 1930; Jordan, 1931; Jordan and Burrows, 1934) and the literature has been reviewed by Richmond (1939).

Except for descriptions of the clinical symptoms, indicating general gastrointestinal irritation, little attention has been given to the physiological mechanisms involved in this train of sequelae. The rapid onset suggested muscular disturbance, consequently the first approach was made in experiments on segments of rabbit intestine.

More recently Bayliss (1940) has published an experimental study of enterotoxin emesis in kittens. A brief paragraph in this paper refers to experiments similar to ours on cat and rabbit intestinal strips, from which the conclusion was drawn that the enterotoxin has no direct effect on smooth muscle. No detailed data were given. However, in the description of the gross responses of the animals to injections of enterotoxin, Bayliss mentions defecation and loss of appetite. The postmortem examinations showed "excessive mucus" in the tract. These observations certainly suggest local irritation. Bayliss finally concluded that the main action of enterotoxin is on peripheral sensory structures, secondly on the vomiting center, with no important influence on the musculature. In view of the paucity of data on intestinal strips we do not feel that our observations are controverted. Since we did no experiments on intact animals we have no data on emesis. The two studies, therefore, deal mainly with different mechanisms, both of which are involved in the response to enterotoxin by the intact organism.

EXPERIMENTAL TECHNICQUE

Contiguous segments of jejunum, about 4 cm. long, were isolated immediately after the rabbit was killed by a blow on the neck, and suspended in oxygenated Ringer solution at pH 7.3 and 38°C. To determine the rôle of the

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mucosa, one segment was closed by ligatures and the everted rosettes of mucosa trimmed off completely. A fine hypodermic needle was used to relieve internal air pressure when necessary. The other segment was suspended so that the fluid circulated freely through the lumen.

A constant volume of 60 ml. of solution was maintained in the bath for each segment. Before the test solution was added, an equivalent volume of Ringer solution was siphoned off.

The enterotoxin was prepared by the technique of Dack and Woolpert (1933). Cultures were grown on semisolid veal infusion medium in an atmosphere of high CO_2 content, the toxin separated by Berkefeld filter, and adjusted to pH 7.3 in a constant volume of Ringer solution, and enclosed in serum bottles until required.

Two types of control material were used. One consisted of extracts made by the same technique from cultures of air-borne strains of staphylococcus. The other was prepared in an identical manner from uninoculated medium.

TABLE 1

TOXIN	INCREASED ACTIVITY		NO CHANGE IN ACTIVITY		DECREASED ACTIVITY		TOTAL NO. TRIALS
	Number	Per cent	Number	Per cent	Number	Per cent	
Mucosa exposed	39.0	72.3	14.0	26.0	1.0	1.8	54
Mucosa not exposed	42.0	77.8	11.0	20.3	1.0	1.8	54

EXPERIMENTAL RESULTS

In 54 trials with the enterotoxin applied to the strip with the mucosa not exposed to the toxin, 77.8 per cent of the trials showed an increase in the activity of the gut segment, 20.3 per cent showed no change, and 1.8 per cent showed a decrease, while in the same number of trials with the mucosa exposed to the toxin, 72.2 per cent of the trials showed an increase in activity, 26.0 per cent showed no change, and 1.8 per cent showed a decrease (table 1). The criteria adopted as a measure of increased activity were primarily increased tonicity, as measured by an elevation of the base line, and increased amplitude. The rate of the contractions was not altered significantly. In almost all instances increased activity meant an increase in tonicity (fig. 1), in a very few an increase in amplitude only, and in many an increase in both tonicity and amplitude. Some strips showed persistently increased tonus after addition of the toxin, while others showed evidence of rhythmic spasms as a characteristic response.

Control material was added to the intestinal strips in the same concentration and under the same conditions. Generally, the control material was applied to strips of gut which had also been subjected to the enterotoxin. Of 40 trials with the control broth extract on the strips with the mucosa not exposed, 55.0 per cent showed no alteration of activity, 27.5 per cent showed a decreased activity (fig. 2), and 17.5 per cent showed increased activity, while in a similar number of trials on the strips with the mucosa exposed, 60.0 per cent showed

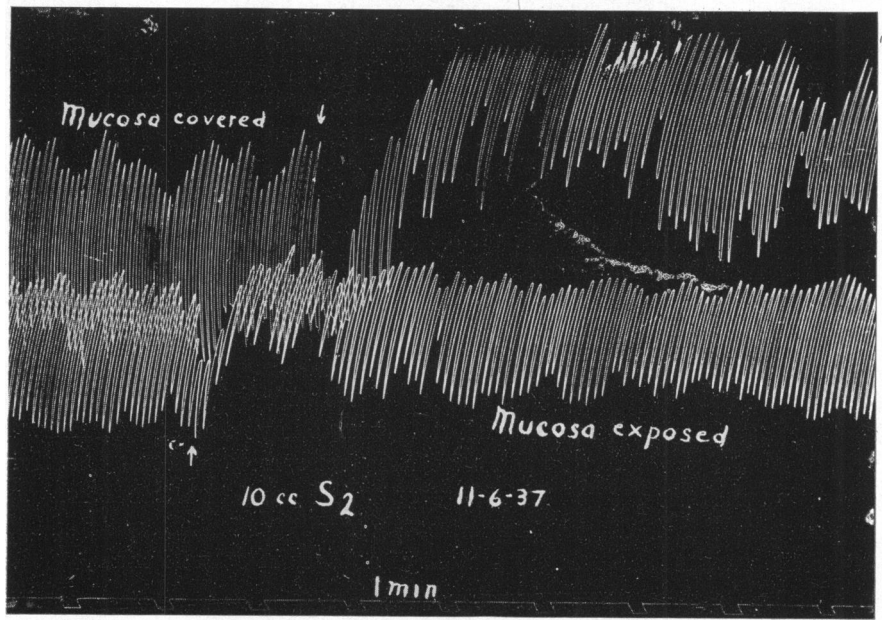

FIG. 1. UPPER TRACING, GUT SEGMENT CLOSED; LOWER, MUCOSA EXPOSED
The response is similar in both. At arrows 10 ml. of enterotoxin in broth filtrate S_2 was added. The culture was isolated from a strain of milk-borne Staphylococcus.

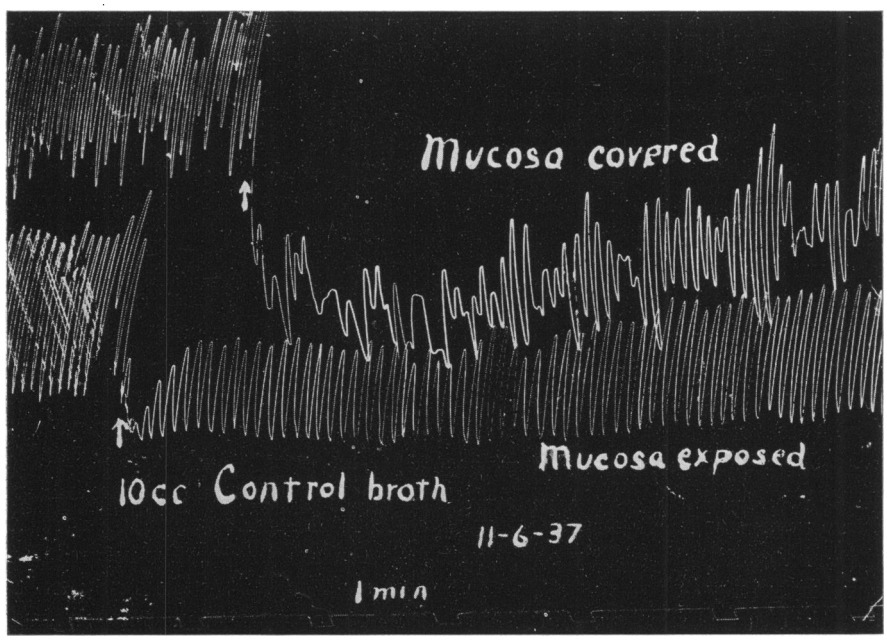

FIG. 2. SAME SEGMENTS AS IN FIGURE 1
Ten milliliter of control were added to each. The response in each is comparable, characterized by decreased tonus and amplitude.

no change in activity, 22.5 per cent showed a decrease, and 17.5 per cent showed an increase (table 2). A preparation obtained from a strain of *Staphylococcus* isolated from the air, as far as could be determined, acted in the same manner as the control broth extract. From our data, the observation of Dolman and his collaborators that the toxin need not be absorbed through the mucosa of the intestine to produce its effect appears to be borne out, since the responses of the strips with the mucosa exposed were practically the same, both quantitatively and qualitatively. The slight quantitative differences (never exceeding 6 per cent) in the number of responses of the two strips of gut subjected to the same conditions are entirely within the range of experimental error. Hence, we feel that the effect of the toxin on the smooth muscle of the gut is probably that of a non-specific irritant.

In addition to these results there were 102 trials with single open segments, of extracts cultured from 18 samples of food known to have caused poisoning. Of these, 80, or 78.4 per cent, responded with definitely increased motor activity. The remainder either showed no change or so little as to be inconclusive. A contiguous segment of gut was used as a control indicator for uninoculated broth extract. There was a mild augmentation of activity in only 15 trials.

TABLE 2

CONTROL	INCREASED ACTIVITY		NO CHANGE IN ACTIVITY		DECREASED ACTIVITY		TOTAL NO. TRIALS
	Number	Per cent	Number	Per cent	Number	Per cent	
Mucosa exposed	7.0	17.5	24.0	60.0	9.0	22.5	40
Mucosa not exposed	7.0	17.5	22.0	55.0	11.0	27.5	40

DISCUSSION

That many investigators have concerned themselves with the development of cultural characteristics specific for enterotoxin-producing strains of staphylococci with little success, is apparent from review of the literature. Therefore, it is readily understood that for the present, it would be decidedly advantageous to have some other method for the identification of food-poisoning strains. Ultimately it may be determined that no uniformity of cultural characteristics for these strains exists and that they have only one common feature—the ability to produce enterotoxin. Dolman *et al.* (1936) have already developed an index for the presence of the enterotoxin by observing symptoms of food poisoning in kittens injected intraperitoneally with the enterotoxin. This method is dependent upon individual differences in the animals and upon subjective interpretations of what constitute food poisoning symptoms in kittens. Therefore, it is suggested that the possibility of the utilization of a technique for recording the increased tonicity of the smooth muscle of the rabbit gut by the enterotoxin as observed in these experiments, in the absence of any extraneous smooth muscle irritant, be investigated further as a more objective method of demonstrating the presence of the enterotoxin.

Although one cannot interpret clinical facts directly in the light of *in vitro* experiments, these experiments tend to explain many of the features of the clinical syndrome of *Staphylococcus* food poisoning. Thus, the nausea and epigastric distress seen early in the clinical syndrome perhaps reflect the early tonic effects of the enterotoxin upon the small intestine. Further, if the marked increase in the tonicity of the smooth muscle in response to the enterotoxin *in vitro* be considered analogous to clinical enterospasm, much of the severe abdominal pain experienced at the height of an attack can be accounted for, since it is well known that enterospasm is an important factor in the production of gastro-intestinal pain. Although the mechanical factor of increased activity of the gastro-intestinal tract undoubtedly plays a significant rôle in the production of the diarrhea observed in the syndrome, the physico-chemical effects of the enterotoxin must undoubtedly play the major rôle in this response as well as in the genesis of the type of shock observed in severe cases. Further pharmacological experimentation will be necessary to explain these more generalized effects of the enterotoxin.

CONCLUSIONS

1. Experiments indicate that the *Staphylococcus* enterotoxin produces predominantly an increase in tonicity of the smooth muscle of the rabbit gut *in vitro*.

2. The increased tonicity of the smooth muscle of the rabbit gut produced by the *Staphylococcus* enterotoxin may be comparable to clinical enterospasm, to which much of the gastro-intestinal pain experienced in food poisoning may be attributed.

3. The observation of Dolman and his collaborators (1936) that the *Staphylococcus* enterotoxin need not be absorbed through the intestinal mucosa to produce its effects on the smooth muscle of the gastro-intestinal tract appears to be confirmed by our experiments. Also, the effect of the enterotoxin on the smooth muscle of the gut is probably that of a non-specific irritant.

4. The possibility of the utilization of the technique of recording the effect of the enterotoxin on smooth muscle as an index of the enterotoxin producing ability of various strains of staphylococci should be investigated further.

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