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SOME PHARMACOLOGICAL PROPERTIES OF THE α-TOXIN OF *STAPHYLOCOCCUS PYOGENES*

BY

D. A. BROWN, B. N. C. PRICHARD,* AND J. P. QUILLIAM

From the Department of Pharmacology, The Medical College of St. Bartholomew's Hospital, London

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The α-toxin of *Staphylococcus pyogenes* produced a slowly developing contracture of isolated preparations of rabbit jejunum and of guinea-pig ileum which persisted after thorough washing and left the gut unresponsive to further doses of α-toxin or of acetylcholine. After incubation with antitoxin, the α-toxin no longer produced a contracture. Antitoxin only prevented the α-toxin response if added to the bath fluid before but not after the α-toxin. Certain drugs reduced the α-toxin contracture when added to the bath fluid before or after the α-toxin, but the contracture reappeared on washing. Papaverine abolished the contracture and pethidine was only slightly less active. Mepyramine, amyl nitrite, caffeine, aminophylline, adrenaline and ephedrine partly reduced the contracture. Hexamethonium, cocaine, tubocurarine and gallamine had no effect. The effect of atropine was only small. The gut-stimulant activity/haemolytic unit of two α-toxin samples differed greatly; this difference did not appear to be due to activity of impurities. The implications of these observations are discussed.

Certain strains of *Staphylococcus pyogenes*, including those causing food-poisoning outbreaks in man, produce toxins which stimulate gut *in vitro*. Feldberg and Kellaway (1938) first described the action of staphylococcal toxin on isolated guinea-pig ileum, and Richmond, Reed, Shaughnessy, and Michael (1942) described its action on rabbit intestine. Anderson, James, and Marks (1954) concluded that the response of isolated rabbit jejunum to extracts of *S. pyogenes* cultures was determined mainly by their α-toxin content, that is the α-haemolytic fraction.

The present work was mainly undertaken to study further the responses of isolated segments of rabbit jejunum and guinea-pig ileum to the α-toxin of *S. pyogenes*, and to examine how far pharmacological agents could modify these responses.

METHOD

Segments of rabbit jejunum were suspended in Ringer-Locke solution at 37° in a 30 ml. bath and bubbled with pure oxygen. Segments of guinea-pig mid-ileum were usually suspended in Tyrode solution at 30° in a 30 ml. bath and bubbled with air. Contractions were recorded on smoked paper with frontal writing levers giving a 5-fold magnification.

The irreversible nature of the contracture in response to the α-toxin precluded repetitive testing on the same preparation. Therefore, the following methods were used.

Method 1.—Two adjacent lengths of gut were set up in separate baths. Their responses to a reference dose of acetylcholine were recorded. To one bath the α-toxin was added and the height of the contracture was compared with the response to the dose of acetylcholine. 2 min. before adding the same dose of α-toxin to the other bath, the antagonist was administered. The height of the contracture produced by the α-toxin was compared with that following the dose of acetylcholine.

Method 2.—A single preparation was used and, in order to determine the antagonistic effect of drugs on the α-toxin contracture, the antagonists were added to the bath 2 min. before the α-toxin. The ensuing contracture was then recorded in the presence of the antagonist and later after washing the preparation. If the antagonist abolished the contracture, the preparation was washed to see if the contracture developed subsequently.

Method 3.—A single preparation was used, but a contracture was first elicited with the α-toxin and the antagonist was added later.

Materials.—Three batches of α-toxin (K 9634, K 160, and K 782) were obtained from the Wellcome Research Laboratories. They were filtrates from staphylococcal cultures grown on a sloppy nutrient agar medium, clarified by centrifugation. The toxin was precipitated by $(NH_4)_2SO_4$, the precipitate redissolved in M/15-borate buffer and freeze-dried. In this manner the agar and low molecular-weight constituents of the culture fluid were removed, but the

*Present address: Medical Unit, St. George's Hospital, London, S.W.1.

material of high molecular weight remained. The product was dissolved in distilled water immediately before adding to the preparations and had a haemolytic potency of about 3.5 haemolytic units/mg. A haemolytic unit (H.U.) is the smallest amount of α-toxin producing complete haemolysis of 1 ml. of a 1% suspension of fresh washed rabbit erythrocytes in normal saline. Unless otherwise stated, the Wellcome α-toxin was employed.

In a few experiments a purified α-toxin, kindly given us by Dr. L. Butler, was used. This was an $(NH_4)_2SO_4$ precipitate with a haemolytic potency of 75 H.U./mg. of dried powder.

A sample of uninoculated culture medium similar to that used in the preparation of the Wellcome α-toxin samples was used as a control. This medium was treated in the same way as the inoculated medium, with the exception of freeze-drying. The amount of culture medium residue present in 1 ml. of this sample was approximately equivalent to that in 10 H.U. of the Wellcome α-toxin samples.

A staphylococcal antitoxin was used which was a peptic-refined horse serum containing 1,250 units of antitoxin/ml. (Wellcome Research Laboratories, Serial No. RA 318A).

Acetylcholine chloride, histamine acid phosphate, cocaine hydrochloride, amyl nitrite, nicotine hydrogen chloride (Savory and Moore), hexamethonium tartrate, gallamine triethiodide, mepyramine maleate (May and Baker), adrenaline acid tartrate, tubocurarine chloride (Burroughs Wellcome), caffeine sodium salicylate (British Drug Houses), pethidine hydrochloride (Roche), atropine sulphate, ephedrine sulphate, aminophylline, and papaverine hydrochloride were used, and doses are expressed as concentrations of salt added to the bath.

RESULTS

Doses of between 0.03 and 0.3 H.U. of α-toxin/ml. produced a slowly-developing contracture in both rabbit jejunum and guinea-pig ileum (Figs. 1, 2*b*, and 7*b*). In the rabbit jejunum at 37°, there was a delay varying from a few seconds to 2 min. between the addition of α-toxin and the onset of contracture, and a further 1 to 2 min. elapsed before the contracture reached maximum. In the corresponding experiments on the guinea-pig ileum suspended at 37°, the latency was 1 to 2 min., and the maximum of the contracture was reached 2 to 5 min. later. When the guinea-pig ileum was suspended at 30°, the latency was 4 to 5 min., and full development was reached after another 5 to 15 min. Thus the guinea-pig ileum appeared more sluggish in responding to the α-toxin than did the rabbit jejunum.

The spontaneous rhythmic contractions recorded from the rabbit jejunum initially increased on adding the α-toxin, but decreased as the general tone of the gut rose. In the guinea-

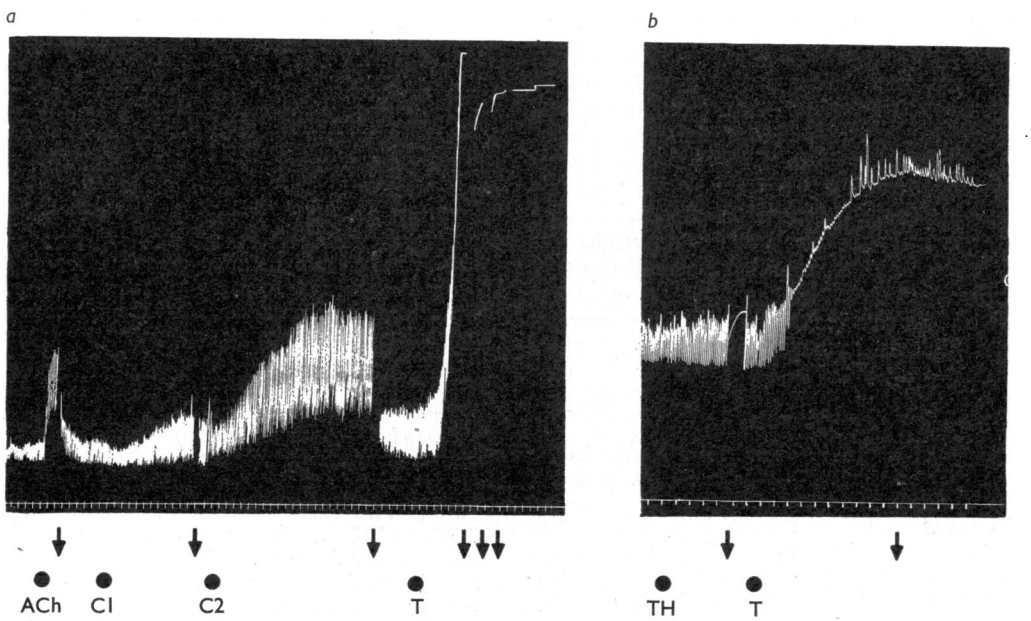

FIG. 1.—Two isolated rabbit jejunum preparations (*a* and *b*) in Ringer-Locke solution. (*a*) Effects of uninoculated culture medium, the amounts being those contained in 10 (at C1) and 20 (at C2) H.U. of α-toxin, and of 10 H.U. of α-toxin (at T). ACh, 0.1 μg. of acetylcholine. (*b*) Effect of 5 H.U. of α-toxin before (at T) and after heating to 80° for 45 min. (at TH). Arrows indicate washing. Time, 30 sec. Bath volume, 30 ml.

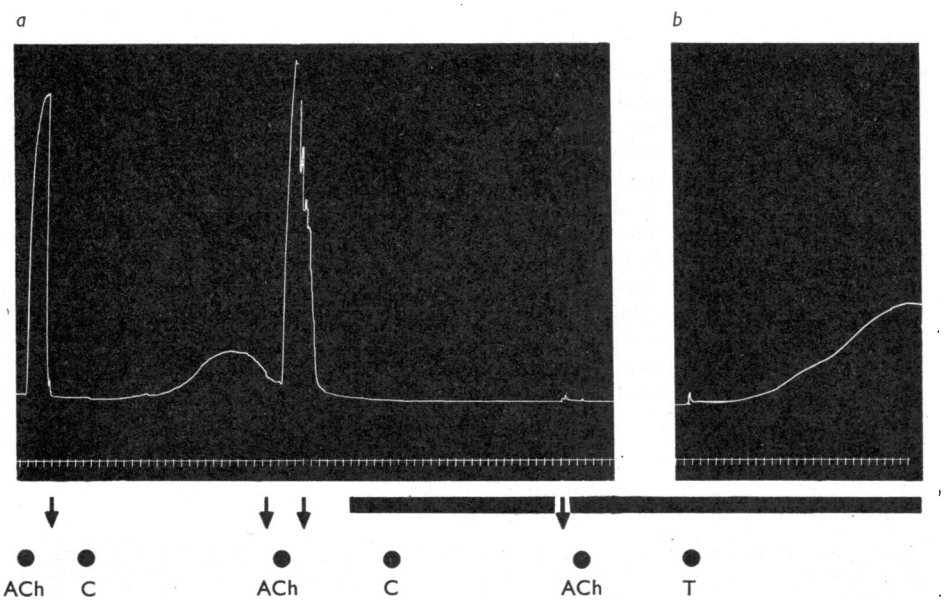

FIG. 2.—Isolated guinea-pig ileum preparation in Tyrode solution. At **C**, uninoculated culture medium, the amount being that contained in 20 H.U. of α-toxin; at **T**, 20 H.U. of α-toxin; at **ACh**, 0.1 μg. of acetylcholine. The horizontal line indicates the presence of 10 μg. of atropine. There was an interval of 10 min. between (*a*) and (*b*). Arrows indicate washing. Time, 30 sec. Bath volume, 30 ml.

pig ileum, spontaneous contractions were absent or small.

After washing, the α-toxin contracture persisted and was frequently increased in both species; occasionally it was reduced but never abolished. When α-toxin or acetylcholine were then added to the bath, there was usually no response. A small response to acetylcholine was sometimes observed particularly in preparations in which washing had produced partial relaxation (Fig. 3). Even in these preparations, the acetylcholine sensitivity, when tested at about 10 min. intervals, decreased progressively.

When α-toxin was heated to 80° for 45 min. or to 100° for 10 min., it no longer caused contracture of the rabbit or guinea-pig intestine. Fig. 1*b* illustrates the ineffectiveness of 5 H.U. of heated α-toxin (at **TH**).

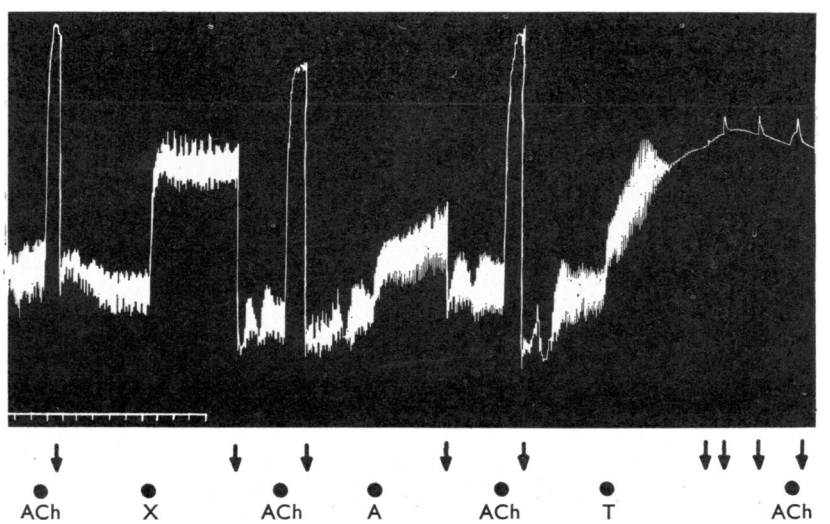

FIG. 3.—Isolated rabbit jejunum preparation in Ringer-Locke solution. At **ACh**, 1 μg. of acetylcholine; at **X**, a mixture of 25 units of antitoxin and 10 H.U. of α-toxin; at **A**, 25 units of antitoxin; at **T**, 10 H.U. of α-toxin. The arrows indicate washing. Time, 1 min. Bath volume, 30 ml.

The action of α-toxin on the intestine may be an indirect effect involving the release of a muscle-stimulating substance (Feldberg and Kellaway, 1938). However, it was not possible to obtain evidence for such a release. For instance, in several experiments, 20 H.U. of α-toxin in 1 ml. of Tyrode solution were placed inside the closed lumen of segments of guinea-pig ileum which were then immersed for 1 hr. in tubes containing 5 ml. glucose-free Tyrode solution at 37°. Control loops containing 1 ml. Tyrode solution alone were set up under identical conditions. The segments were then removed and the fluid was tested on fresh preparations either directly or after 24 hr. dialysis (two experiments). In no experiment was any difference found between control and test fluids. Both produced a contracture which was readily reversed by washing. The contracture was not modified by mepyramine and atropine in doses sufficient to prevent the much larger responses to 33 ng./ml. of histamine and to 33 ng./ml. of acetylcholine. Thus, although dialysable, the substance or substances released in the test or control experiments did not appear to be histamine or acetylcholine and their presence in the fluid was independent of the α-toxin.

Effect of Spasmolytic Agents

Rabbit Jejunum.—Pethidine and papaverine in concentrations of 33 μg./ml. rendered the rabbit intestine insensitive or nearly insensitive to the action of 4 H.U. of α-toxin as long as the spasmolytics were kept in the bath fluid. This is shown for pethidine in the experiment illustrated in Fig. 4 and for papaverine in the experiment in Fig. 5. When the bath fluid was then replaced with fresh solution, the α-toxin contracture

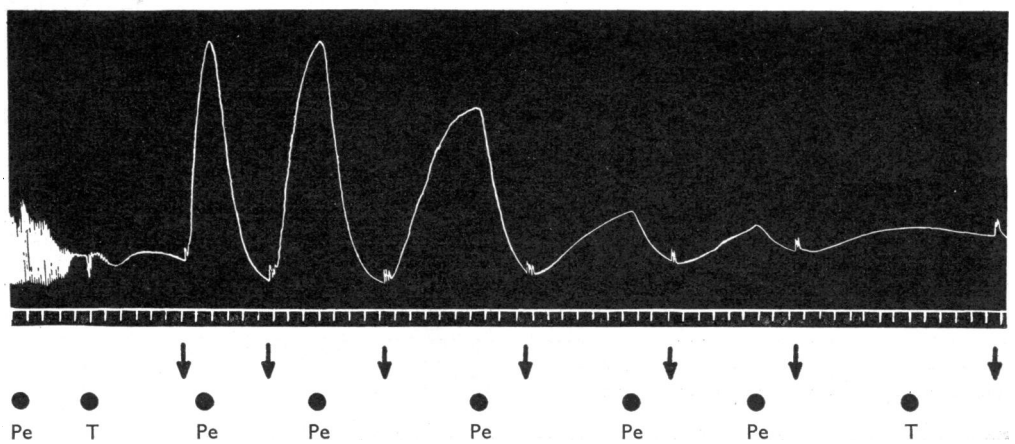

FIG. 4.—Isolated rabbit jejunum preparation in Ringer-Locke solution. At **Pe**, 1 mg. of pethidine; at **T**, 4 H.U. of α-toxin. Arrows indicate washing. Time, 1 min. Bath volume, 30 ml.

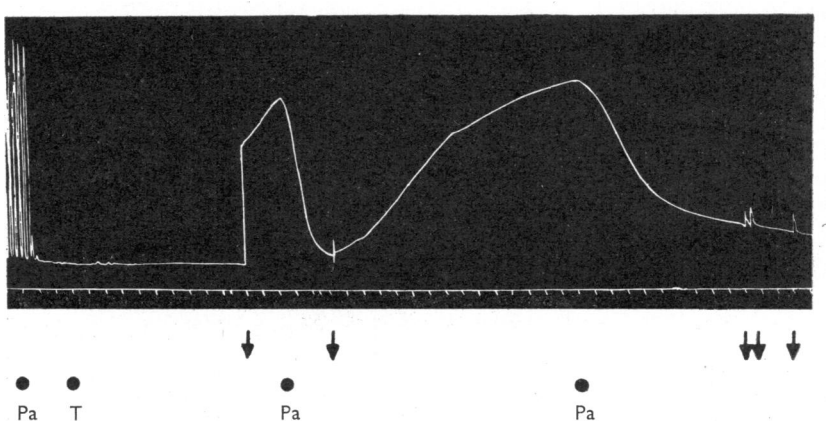

FIG. 5.—Isolated rabbit jejunum preparation in Ringer-Locke solution. At **Pa**, 1 mg. of papaverine; at **T**, 4 H.U. of α-toxin. Arrows indicate washing. After the first washing the drum was stopped for 3 min. Time, 30 sec. Bath volume, 30 ml.

developed but could again be abolished by the renewed addition of the spasmolytic. When this procedure was repeated several times, the contracture on replacing the bath fluid with fresh solution became progressively smaller. The preparation was then insensitive to α-toxin as well as to acetylcholine.

Adrenaline, ephedrine, amyl nitrite, mepyramine, caffeine and aminophylline reduced but rarely abolished the α-toxin spasm when added to the bath fluid in concentrations of 33 μg./ml. The effect lasted only as long as the spasmolytics were kept in the bath, and with adrenaline the effect even disappeared if the adrenaline was kept in the bath fluid and the preparation contracted after 2 to 4 min. Ephedrine produced relaxation of the α-toxin spasm in concentrations 5 to 10 times as large as adrenaline, and the relaxation was maintained as long as the ephedrine was kept in the bath fluid.

Tubocurarine, gallamine, and hexamethonium had no effect on the α-toxin contracture, nor had atropine except in one experiment in which 33 ng./ml. caused a transient relaxation of the α-toxin spasm. When the contracture reappeared the addition of 33 ng./ml. of atropine did not cause a renewed relaxation.

A comparison of the spasmolytic potency of the various drugs on the α-toxin contracture in rabbit intestine is shown in Table I, which gives the approximate % reduction of the α-toxin spasm. Each value is the mean of three to five experiments.

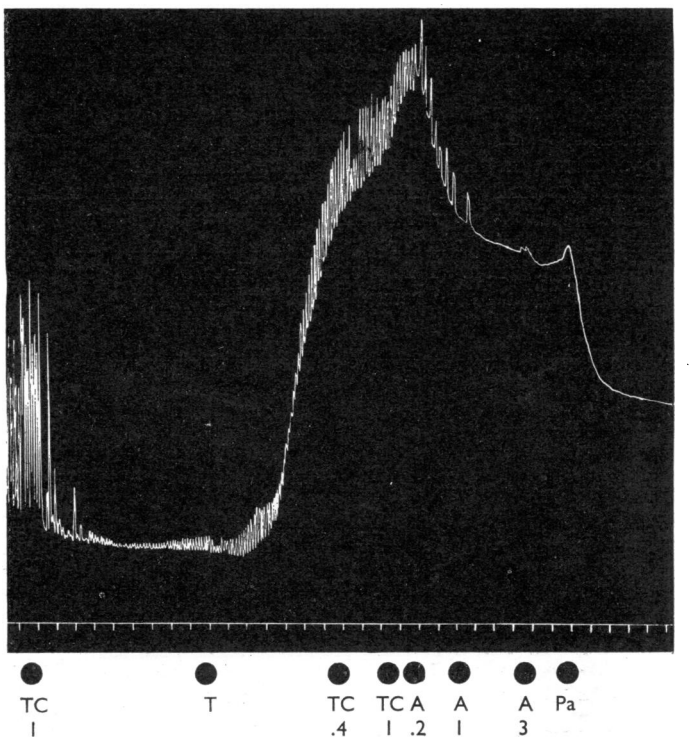

FIG. 6.—Isolated rabbit jejunum preparation in Ringer-Locke solution. At **TC1** and **TC.4**, 10 and 4 mg. respectively of tubocurarine; at **T**, 4 H.U. of α-toxin; **A.2**, **A1** and, **A3**, 0.2, 1, and 3 mg. respectively of adrenaline; at **Pa**, 2 mg. of papaverine. Time, 30 sec. Bath volume, 30 ml.

When the less active spasmolytics had exerted their maximum effect, the more active relaxants produced a further relaxation of the α-toxin spasm. Fig. 6 illustrates an experiment in which 0.33 mg./ml. of tubocurarine (at **TC1**) almost abolished peristalsis but did not oppose the development of a contracture in response to the subsequent addition of 4 H.U. of α-toxin (at **T**). Increases of the tubocurarine concentration, first to 0.47 and then to 0.8 mg./ml. (at **TC.4** and **TC1**), had no effect on the contracture, but the subsequent addition of adrenaline in three concentrations of 6.6, 40, and 140 μg./ml. (at **A.2, A1**, and **A3**) caused partial relaxation; there was some further relaxation with 66 μg./ml. of papaverine (at **Pa**).

Guinea-pig Ileum.—In the presence of pethidine or papaverine in amounts of 6.6 to 8.3 μg./ml. in the bath, α-toxin produced no contracture. This is shown for papaverine in

TABLE I

THE EFFECT OF SPASMOLYTICS ON THE CONTRACTURE OF THE ISOLATED RABBIT JEJUNUM PRODUCED BY 4 H.U. OF α-TOXIN

The numerals give the % reduction of the height of the contracture in the presence of 1 mg. of the spasmolytic in the 30-ml. bath except where otherwise indicated.

Drug	% Reduction	
	Methods 1(*) and 2	Method 3
Tubocurarine, Gallamine, Hexamethonium	0*	0
Atropine (up to 250 μg.)	0*	0
Adrenaline	40	75
Ephedrine (5 mg.)	—	35
Aminophylline	50	60
Caffeine	—	45
Amyl nitrite	80	—
Mepyramine	85	—
Pethidine	95	100
Papaverine	100	100

Fig. 7*a*. As in the rabbit intestine, the antagonistic action of pethidine and of papaverine persisted only as long as these spasmolytics were in the bath fluid, and on replacing the bath fluid with fresh solution a contracture developed. When pethidine or papaverine was given at the height of an α-toxin contracture and kept in the bath, full relaxation ensued within 30 min., and the preparation remained relaxed after replacing the bath fluid with fresh solution. This is shown in Fig. 7*b*. In this condition the relaxed preparation was insensitive to α-toxin, to potassium, and to amounts of acetylcholine which would have usually produced a strong response. As is seen in Fig. 7*b*, even an amount of acetylcholine 200 times greater than that effective on the untreated preparation only caused slight stimulation.

Mepyramine and hexamethonium produced a partial relaxation of the α-toxin spasm for as long as they were kept in the bath. Adrenaline had the same effect, but its action appeared to be as transient as on the rabbit intestine.

The effect of atropine was peculiar. A concentration of 3.3 μg./ml. usually delayed the onset of the α-toxin spasm if added before the α-toxin, although the final height of the contracture did not appear to be influenced. On the other hand, when as small a quantity as 33 ng./ml. was added at the peak of an α-toxin contracture, it reduced the contracture to about half its height, but it was not possible to obtain full relaxation with 3.3 μg./ml. of atropine.

Nicotine, tubocurarine, and cocaine in concentrations up to 33 μg./ml. did not relax the α-toxin spasm in guinea-pig preparations bathed in Tyrode solution at 37°.

A comparison of the spasmolytic potency of the various drugs in relaxing the α-toxin spasm in guinea-pig intestine is given in Table II. Each value is the mean of three to five experiments.

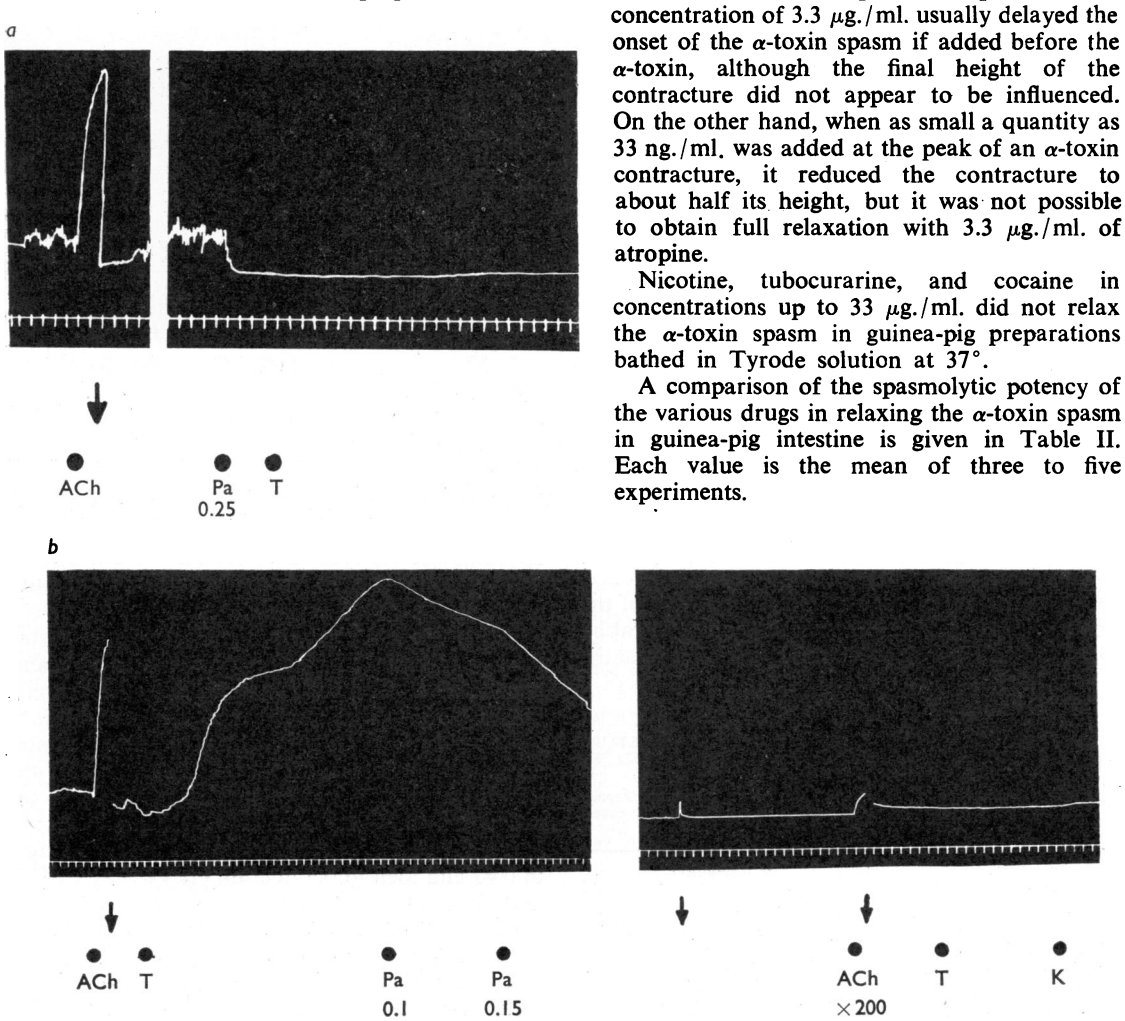

FIG. 7.—Isolated guinea-pig ileum preparation in Tyrode solution. Effects of papaverine added to the bath (*a*) before and (*b*) after the α-toxin. The records in (*a*) and (*b*) are the responses of two adjacent lengths of ileum to each of which α-toxin was added at the same time. The break in the record in (*a*) indicates a time interval of 8 min. The break in the record in (*b*) indicates an interval of 15 min. At **ACh**, 0.025 μg. of acetylcholine; at **ACh** × 200, 5 μg. of acetylcholine; at **T**, 5 H.U. of α-toxin; at **Pa** 0.1, **Pa** 0.15, and **Pa** 0.25, 0.1, 0.15, and 0.25 mg. respectively of papaverine; at **K**, 10 mg. of KCl. Arrows indicate washing. Time, 30 sec. Bath volume, 30 ml.

When the less active spasmolytics had exerted their maximum effect, the more active relaxants such as pethidine, papaverine, mepyramine, and adrenaline could produce, as in the experiments with rabbit intestine, a further relaxation of the α-toxin.

TABLE II

THE EFFECT OF SPASMOLYTICS ON THE CONTRACTURE OF THE ISOLATED GUINEA-PIG ILEUM PRODUCED BY 5 H.U. OF α-TOXIN

The numerals give the % reduction of the height of the contracture in the presence of the spasmolytic in the 30-ml. bath.

Drug	Dose (μg.)	% Reduction	
		Method 1	Method 3
Hexamethonium ..	1,000	0	20
Atropine	100	0	50
Mepyramine	200	15	50
Pethidine	200	30	80
Papaverine	100	80	90
,, ...	250	100	100

Effect of the Antitoxin

Injection of 650 units antitoxin/kg. intraperitoneally into a guinea-pig 4 hr. prior to killing greatly reduced and delayed the contracture produced in an isolated ileal segment of that guinea-pig in response to 5 H.U. of α-toxin. Intraperitoneal injection of 300 units antitoxin/kg. produced little protection against the α-toxin spasm.

In several experiments, mixtures of α-toxin and antitoxin after incubation at 37° for 45 min. were tested on the guinea-pig ileum. Since the antitoxin alone had a strong stimulating effect of its own, it was not possible to use the heights of the contractures to measure its antagonistic effect. For instance, when the heights of the responses to antitoxin (180 units), to α-toxin (5 H.U.) and to a mixture of both were expressed as percentages of responses obtained with 0.025 μg. of acetylcholine added to the bath in different preparations, it was found that the response to antitoxin was 184, that to α-toxin 110 and that to the mixture 177. These were mean values from three experiments. Nevertheless there was an antagonistic effect of the antitoxin. It became evident after washing out the bath fluid. The contracture produced by the α-toxin was more or less irreversible, and the preparation was insensitive to further administration of α-toxin or of acetylcholine. On the other hand, the contracture produced by the antitoxin alone or by the antitoxin incubated with the α-toxin was reversible and the preparation responded to subsequent administrations of α-toxin or of acetylcholine.

The same results were obtained on the rabbit intestine. When a mixture of 10 H.U. of α-toxin and 25 units of antitoxin after incubation for 45 min. at 37° was tested on rabbit jejunum, it caused a contracture greater than that of the antitoxin alone (see Fig. 3 at **X** and **A** respectively). This greater effect did not appear to result from an action of the α-toxin itself since the contracture was fully reversible and the preparation remained sensitive to acetylcholine and to α-toxin which produced an irreversible contracture (at **T**). The greater effect of the mixture can easily be accounted for by the non-specific stimulating effect of the uninoculated culture medium which is shown in Fig. 1*a* at **C1** and **C2**. In fact, if the antitoxin was mixed with uninoculated culture medium, such a mixture produced a larger contracture than that following either alone.

If 50 to 100 units of antitoxin were added to the fluid bathing guinea-pig or rabbit preparations, and allowed to remain in contact with the intestine for 10 min., subsequent addition of α-toxin caused no increase in contracture. After replacing the bath fluid with fresh solution, the intestine responded to acetylcholine and to α-toxin.

When similar doses of antitoxin were added during a contracture in response to α-toxin, the contracture was not reduced and, when the preparation was washed, the contracture remained and the preparation no longer responded to further doses of α-toxin or to acetylcholine. This shows that, once the α-toxin has acted on the preparation, the antitoxin cannot reverse its action.

Relation of Haemolytic and Gut-contracting Activities

In the guinea-pig ileum and rabbit jejunum, 150 to 200 H.U. of purified α-toxin were required to produce a contracture of the same magnitude as 5 H.U. of the Wellcome α-toxin. The nature of the contracture produced by the two toxin samples was similar in that it was not reversed by washing but was opposed by papaverine and mepyramine. The greater stimulant activity/H.U. shown by the Wellcome toxin might have been due to its higher content of impurities derived from the culture medium. To test this possibility, experiments were carried out on the guinea-pig and rabbit intestine comparing the activity of the uninoculated culture medium and the Wellcome α-toxin. Strictly quantitative comparisons could not be made because of the uncertainty regarding

the amount of culture medium present in the Wellcome toxin.

With the guinea-pig ileum, addition of a quantity of uninoculated medium (approximately equivalent to that contained in 20 H.U. α-toxin) produced a much smaller effect than 20 H.U. α-toxin, although similar in time course (Fig. 2); with the rabbit jejunum the difference was even greater (Fig. 1*a* at **C1** and at **T**). In addition, in both preparations the response to the culture medium was readily reversible by washing and the sensitivity of the gut to acetylcholine and α-toxin remained undiminished. In both tissues a dose of atropine, sufficient to abolish the response to a dose of acetylcholine previously producing maximal stimulation, also abolished the effect produced by the culture medium while not affecting the contracture produced by the α-toxin (Fig. 2). Thus the uninoculated medium had not only a weaker action on the intestine, but its action was different from that of the Wellcome α-toxin samples. Therefore the constituents of this medium cannot wholly account for the activity of the impure sample.

From these results, it would appear that the gut-contracting and haemolytic properties of the α-toxin are due to different although perhaps related substances. Otherwise we would have to assume that the impure toxin contains a factor which facilitates the stimulant action of the α-toxin yet is itself inactive. If this were the case the factor would be thermolabile since addition of boiled Wellcome toxin to the purified toxin did not increase the activity of the latter.

Discussion

The contractures of rabbit jejunum and guinea-pig ileum following α-toxin resemble each other, being characterized by (a) a latent period between application of α-toxin and onset of contractures; (b) a slow rise to a maximum over a period of up to several minutes; (c) the persistent nature of the contractures; (d) the desensitization of the gut to further doses of α-toxin and to acetylcholine.

Feldberg and Kellaway (1938) described a contracture of the guinea-pig ileum to a staphylococcal toxin prepared by the method of Burnet and Freeman (1932). They also found that after a single dose the preparation was insensitive to further doses of the toxin. However, their contracture differed in that it developed more rapidly and was readily reversed by washing. The reversibility might be related to the fact that their toxin produced a contracture with a much shorter time of contact, and the differences in the methods of preparation of the toxins might be responsible for these discrepancies. On the other hand, Dworetzky, Baldwin, and Smart (1956) have since described a slow, delayed, irreversible contracture of guinea-pig ileum in response to 0.18 mg. of a dialysed culture filtrate of a pathogenic strain (Wood-46) of *S. aureus*. This finding is more in accord with our observations.

The α-toxin might have two actions, an early stimulant action followed later by a "toxic" action, the development of irreversibility and desensitization. This view is in accord with the findings that the decline in the response of the gut to acetylcholine following application of α-toxin depended on the time elapsed since addition of α-toxin. Also the observation that the prolonged presence in the bath of a spasmolytic, for instance papaverine, prevented the intestine from developing a contracture after the papaverine and the α-toxin were washed out could be explained on a similar basis.

As it has not been possible to separate the stimulant and the "toxic" activities by heat inactivation or antitoxin neutralization, we consider that, if they are not identical, then they must be closely associated and the term "gut-stimulant activity" will be used to cover both of the above hypothetical activities.

In the crude toxin, the ratio of gut-stimulant to α-haemolytic activity was about 40-fold greater than in the purified toxin. As the impurities in the crude toxin contributed but little to the height of the contracture and had effects which were readily reversible, the gut-stimulant and α-haemolytic activities may not be properties of the same principle although evidence of an association of these two activities with different principles in the α-toxin has yet to be forthcoming.

In this connexion, the following observations are of interest. The *in vivo* enterotoxic action, namely that action associated with staphylococcal food-poisoning in man, has been associated with *in vitro* gut-stimulant action on mammalian intestine by Richmond *et al.* (1942) and hence with the α-haemolytic fraction. However, Surgalla and Hite (1945) reported that heating culture filtrates to 100° for 30 to 40 min. did not abolish enterotoxic properties although destroying α- and β-haemolysins. More recently, on the other hand, Dworetzky *et al.* (1956) have claimed that heating to 100° for 15 min. or pre-treatment for 4 to 6 weeks with 0.3% formalin destroyed enterotoxic, *in vitro* gut-stimulant and

α-haemolytic properties of staphylococcal culture filtrates. At present, therefore, the physical relationship between these three properties and such principles in the culture filtrate with which they might be associated is still obscure.

Feldberg and Kellaway (1938) suggested that the stimulant action of staphylococcal toxin on gut is an indirect effect due to release of some stimulant factor. Such an indirect action could account for the latent period and slow rise of the toxin contracture. Feldberg and Keogh (1937) demonstrated the release of histamine by staphylococcal toxin from perfused guinea-pig lung, and Feldberg and Kellaway (1938) showed a similar release of histamine and another stimulant substance producing a delayed contracture of guinea-pig jejunum from perfused dog lung and liver. We have been unable to demonstrate the release of a contracture-producing substance from guinea-pig ileal loops by α-toxin, and a direct stimulating action on the gut wall cannot therefore be excluded. The action could be either on the muscle or through the neuronal network in the intestinal wall. The failure of atropine, cocaine, or hexamethonium to reduce the α-toxin contracture does not suggest an action on the neuronal network. Until convincing evidence for an indirect mode of action of the α-toxin can be found, a direct muscle-stimulating effect seems to be the most likely mechanism of action.

North and Doery (1958) recently described a protective action of a fraction of Australian tiger snake venom against the lethal and dermonecrotic effects of staphylococcal α-toxin in mice. One sixth of the LD50 of the venom fraction protected mice against a lethal dose of α-toxin when administered intravenously simultaneously with or up to 10 min. before the α-toxin, but not if injected after the α-toxin administration. A similar protection was found in the case of the dermonecrotic action of α-toxin. The authors suggested that the protective actions of the venom fractions may have been due to the prior attachment to the sites of action of the toxin. The finding that this protective action of snake venom does not occur if given after the toxin is of interest with regard to the similar relationship observed in the present experiments between α-toxin and antitoxin. This does not, however, imply that the mechanisms underlying the protective action of the venom and of the antitoxin are the same. It would be interesting to know whether the tiger snake venom exerts a protective action against the stimulant activity of α-toxin on mammalian intestine as it does against the lethal effect of α-toxin in mice.

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