Brit. J. Pharmacol. (1965), **25**, 781–789.

SOME EFFECTS OF STAPHYLOCOCCAL α-TOXIN ON ISOLATED MAMMALIAN SMOOTH MUSCLE PREPARATIONS

BY

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(*Received March* 23, 1965)

Staphylococcal α-toxin has been reported to produce a contraction of several isolated mammalian smooth muscle preparations (Feldberg & Kellaway, 1937a ; Richmond, Reed, Shaughnessy & Michael, 1942 ; Anderson, James & Marks, 1954 ; Kelsey & Hobbs, 1954 ; Dworetzky, Baldwin & Smart, 1956 ; Brown Prichard & Quilliam, 1959 ; Wiegershausen, 1959, 1962 ; Thal & Egner, 1961). Some of these investigators (Feldberg & Kellaway, 1937a ; Wiegershausen, 1962) have suggested that the contraction might arise not from a direct action on the smooth muscle, but from the local liberation by the toxin of some spasmogenic substance such as histamine or 5-hydroxytryptamine. This paper reports the results of some experiments undertaken to examine this possibility, using the guinea-pig isolated ileum and rat uterus preparations.

METHODS

Contractions of the longitudinal muscle of segments of guinea-pig isolated mid-ileum and of rat isolated uterus were recorded using a frontal lever writing on smoked paper. Guinea-pig ileal segments were suspended in 15 ml. of Tyrode solution at 30° C, or occasionally 37° C, and bubbled with air. Rat isolated uteri were suspended in 10 ml. of de Jalon solution at 30° C bubbled with air (Burn, 1952). Stilboestrol (100 μg in olive oil, subcutaneously) had been administered to the rats 36 to 48 hr before removal of the uteri.

Wellcome staphylococcal α-haemolysin (α-toxin) and anti-α-haemolysin (anti-α-toxin) were products supplied by the Wellcome Laboratories, the doses used being in haemolytic units (H.U.) and international units (I.U.) respectively. The preparation and standardization of these products have been outlined previously (Brown *et al.*, 1959).

Doses of acetylcholine chloride, histamine acid phosphate, 5-hydroxytryptamine creatinine phosphate, atropine sulphate, mepyramine maleate, morphine sulphate, phenoxybenzamine hydrochloride and methysergide hydrogen maleate (Deseril, Sandoz) refer to the weight of salt.

RESULTS

Guinea-pig isolated ileum preparation

The α-toxin in a concentration of 0.2 H.U./ml. or more elicited, after a delay of 1.5 to 3 min, a slow contraction of an ileal segment (Fig. 1, upper trace). The preparation

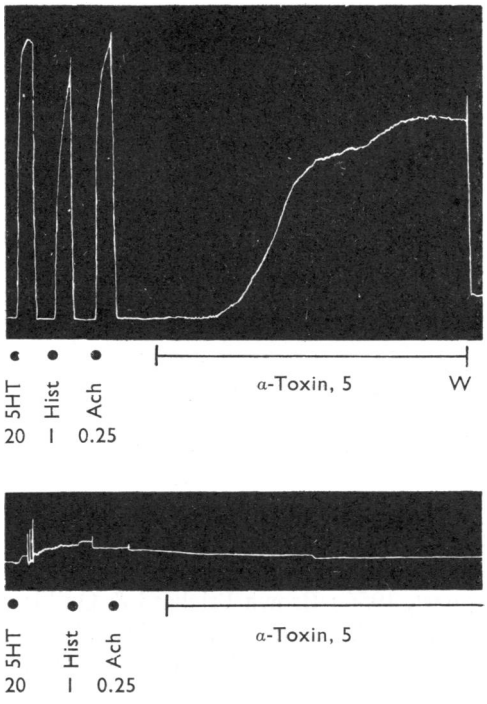

Fig. 1. Effect of staphylococcal α-toxin on the guinea-pig isolated ileum preparation. Records of contraction of the longitudinal muscle of a mid-ileal segment suspended in 15 ml. of Tyrode solutions at 30° C bubbled with air. The lower trace is a continuation of the upper trace. Contractions were elicited by 5-hydroxytryptamine (5HT), histamine (Hist) and acetylcholine (Ach) (doses are in μg added to the bath) and by α-toxin (dose in haemolytic units) left in contact with the ileum for the periods indicated by the horizontal lines. At W, the tissue was washed repeatedly for 15 min with the kymograph stationary.

relaxed only slowly, and often incompletely, with repeated washing. Subsequently (Fig. 1, lower trace) the ileum did not respond to test doses of acetylcholine, histamine or 5-hydroxytryptamine, nor to a second application of α-toxin.

A qualitatively similar response was obtained using segments of other regions of the guinea-pig intestine (terminal ileum, duodenum and colon), of rabbit intestine, and also with guinea-pig and rat isolated uteri. The administration of the anti-α-toxin 5 min before the addition of α-toxin, in a ratio of 1 I.U. of anti-α-toxin to 1 H.U. of toxin, prevented both the contractile response to the toxin and the subsequent desensitization to stimulant drugs.

Liberation of spasmogenic substances. In previous experiments (Brown *et al.*, 1959) high concentrations of α-toxin were placed inside closed loops of guinea-pig isolated ileum, and the surrounding bath fluid was tested for spasmogenic activity on a second ileal segment. No activity was detected in the fluid which could be attributed to the effect of α-toxin. In these experiments the α-toxin had access only to the mucosal surface of the preparation. Recently, Thal & Egner (1961) have reported that α-toxin failed to produce the normal contraction of the rabbit isolated intestine when applied to

the mucosal surface. For this reason, further experiments have been made using open-ended segments, thereby allowing access of the α-toxin to both mucosal and serosal surfaces.

Two adjacent segments of guinea-pig isolated ileum were suspended in separate baths of Tyrode solution at 37° C and their movements recorded simultaneously. The fluid surrounding one segment (the donor) was collected at 20 min intervals and transferred in its entirety to the bath containing the second (recipient) segment. After repeating this procedure several times, sufficient α-toxin was added to the donor segment to provoke a powerful spasm, and left in contact with the donor segment for 2 to 5 min before washing. Transference of fluid from donor bath to recipient was then repeated at 20 min intervals for the next 1 to 2 hr.

Some results from one such experiment are shown in Fig. 2. The greater spontaneous activity of the ileum than that recorded in other experiments shown in Figs. 1 and 3 can be ascribed to the temperature of 37 instead of 30° C. Fluid was withdrawn from the bath containing the donor segment (Fig. 2, upper trace) at *a*, *b* and *c*, and applied to

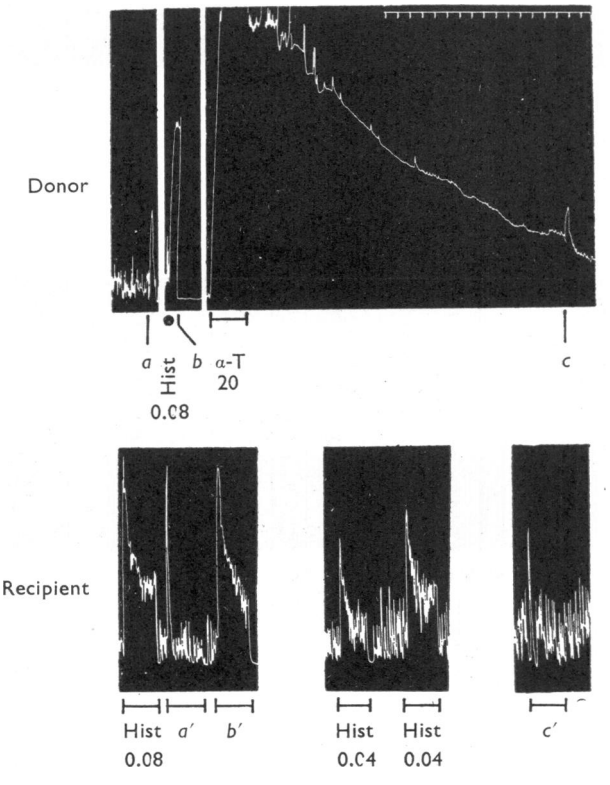

Fig. 2. Records of the responses of two adjacent segments of guinea-pig isolated ileum suspended in separate baths at 37° C. At *a*, *b* and *c*, the bath fluid surrounding the donor segment (upper trace) was withdrawn and added to the bath containing the recipient segment (lower trace, at *a′*, *b′* and *c′* respectively). At Hist, histamine was added (doses in μg added to the bath). α-Toxin (α-T, doses in haemolytic units) was added to the bath containing the donor segment for the duration indicated by the horizontal line beneath the upper trace. Time marks, 1 min.

the recipient segment (lower trace) at *a'*, *b'*, and *c'* respectively. The control sample of fluid (*a*), withdrawn from the donor bath after 20 min contact with the donor segment, produced, when added to the recipient segment (*a'*), a brief initial stimulation of the recipient associated with the change of bath fluid, but no sustained contraction. Fluid withdrawn from the donor bath 1 min after the addition to it of 0.08 μg of histamine (*b*) provoked a sustained contraction of the recipient segment (*b'*) similar to that elicited

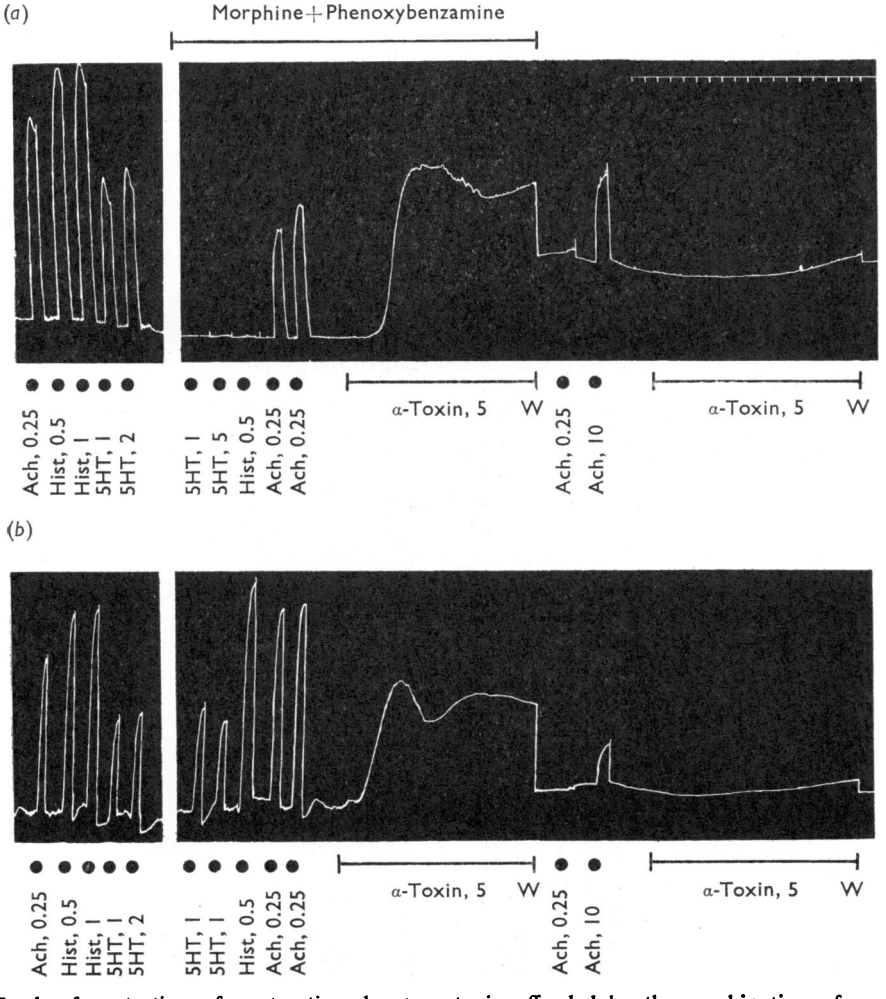

Fig. 3. Lack of protection of contraction due to α-toxin afforded by the combination of morphine and phenoxybenzamine on the guinea-pig isolated ileum. Two adjacent segments of mid-ileum were suspended in separate 15 ml. organ-baths at 30° C and their responses to acetylcholine (Ach), histamine (Hist), 5-hydroxytryptamine (5HT) (doses are in μg added to the bath) and α-toxin (doses in haemolytic units) were recorded simultaneously. Ileal segment (*a*) was treated with morphine (1 μg/ml.) and phenoxybenzamine (0.1 μg/ml.), added to the bath for 20 min at the break in the record, and subsequently left in contact with the tissue for the duration indicated by the horizontal line above the trace. Ileal segment (*b*) was left untreated. At W, each segment was washed repeatedly for 15 min with the kymograph stationary. Time, 1 min.

by the direct addition to the recipient of 0.08 μg of histamine. Thus, the recovery of added histamine from the donor bath was largely complete. In contrast, fluid obtained from the donor bath 20 min after treatment of the donor segment for 3 min with α-toxin (*c*) did not produce a sustained contraction of the recipient segment (*c'*) but only a brief stimulation like that provoked by the control sample of fluid from untreated gut (*a'*). With both samples, the maximum height and the duration of the response of the recipient segment were less than those elicited by direct addition to the recipient bath of 0.04 μg of histamine. In no experiments of this type were detectable amounts of any spasmogenic substance liberated into the surrounding fluid following application of α-toxin to open-ended isolated segments of guinea-pig ileum.

Antagonism to the α-toxin contraction. The effects of antagonists to acetylcholine, histamine and 5-hydroxytryptamine on the response of the guinea-pig isolated ileum to α-toxin have been investigated. Since it was not possible to elicit multiple responses of a single segment of ileum to repeated doses of toxin (see Fig. 1), the procedure adopted was to suspend two adjacent segments of ileum in separate baths under conditions such that the sensitivities of one segment to acetylcholine, histamine, and 5-hydroxytryptamine were closely similar to those of the adjacent segment when recorded simultaneously. One of the segments was then treated with the antagonist under study, while the other was left untreated to serve as a control. The effects of a standard dose of α-toxin on the two segments were then compared.

In the experiment illustrated in Fig. 3, the test segment (*a*) was treated for 20 min

TABLE 1

EFFECTS OF SOME DRUGS ON THE SPASMOGENIC ACTION OF STAPHYLOCOCCAL α-TOXIN ON THE GUINEA-PIG ISOLATED ILEUM PREPARATION

In the control (no drug) experiments, the values were derived by comparison of contraction heights between matched pairs of fresh ileal segments and indicate the variability of the response to α-toxin

Drug	Concn. (μg/ml.)	Expt. no.	Change (%) in contraction produced by: Acetylcholine	Histamine	5-Hydroxytryptamine	α-Toxin	Mean change in contraction due to α-toxin (%)
Control (no drug)		1				−32	
		2				−18	−27
		3				−30	
Atropine	0·02	4	−100	−63	−50	−23	
	0·01	5	−100	−14	0	−36	−36
	0·01	6	−95	−28	−58	−49	
Mepyramine	0·1	7	−17	−100	−15	−73	
	0·1	8	0	−97	0	+43	−1
	0·1	9	0	−100		+29	
5-Hydroxytryptamine (excess)	10	10	0	0	−100	−47	
	50	11	−4	−69	−100	−19	−26
	10	12	0	−5	−100	−12	
Morphine (M) and phenoxybenzamine (P)	M 2, P 0·2	13	−35	−100	−100	0	−12
	M 1, P 0·1	14	−66	−100	−100	−24	
Morphine (M), phenoxybenzamine (P) and atropine (A)	M 1, P 0·1, A 0·01	15	−100	−100	−100	−11	−1
	M 1, P 0·1, A 0·01	16	−100	−100	−100	+9	

with a combination of morphine (1 μg/ml.) and phenoxybenzamine (0.1 μg/ml.), which blocked the responses of the segment to 5-hydroxytryptamine and histamine and reduced those to acetylcholine. Nevertheless, the α-toxin produced a contraction of this segment similar to that obtained in the untreated control segment (*b*). Further, treatment with phenoxybenzamine and morphine did not prevent the subsequent loss of sensitivity of the preparation to acetylcholine or to a second dose of toxin.

To obtain some quantitative estimate of effects of antagonists on the α-toxin spasm, contraction heights in treated and untreated segments were denoted in terms of those initially elicited by test doses of acetylcholine and that in the treated segment was then expressed as a percentage of that in the untreated control segment. This method permitted comparison between different ileal segments. Table 1 gives the percentage difference between contraction heights in treated and untreated segments. Even in adjacent, ileal segments, matched as closely as possible, the magnitude of the contraction in response to the same dose of α-toxin varied considerably, as is shown by the control mean value of —27% (Table 1) obtained in the absence of any drugs.

None of the blocking agents tested exerted any marked antagonism to the spasmogenic

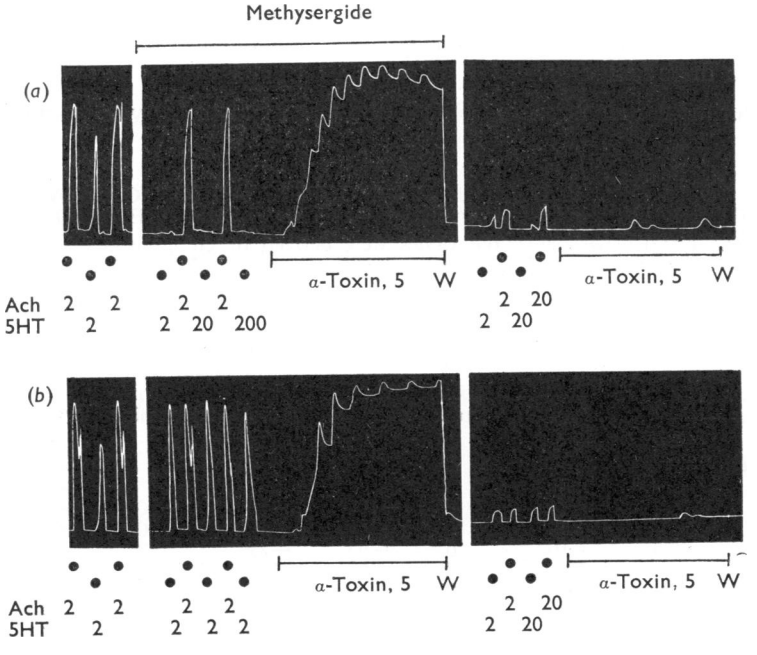

Fig. 4. Lack of effect of methysergide on the response of the rat isolated uterus to α-toxin. Each of the two uteri from a single rat was suspended in a separate organ-bath containing 10 ml. of de Jalon solution at 30° C bubbled with air. The records show the contractions of the two uteri (recorded simultaneously) elicited by acetylcholine (Ach) or 5-hydroxytryptamine (5HT) (doses are in μg added to the bath) and by α-toxin (doses in haemolytic units). Uterus (*a*) was treated with methysergide (0.02 μg/ml.), added for 10 min at the first break in the record, and subsequently left in contact with the tissue for the duration indicated by the horizontal line above the trace. Uterus (*b*) was left untreated. At W, the tissues were washed repeatedly for 15 min with the kymograph stationary.

action of the α-toxin, even when used in combination so that the responses of the ileum to acetylcholine, histamine and 5-hydroxytryptamine were abolished simultaneously, there being no greater difference between the contraction heights elicited in treated and untreated segments under these conditions than those seen in control experiments. Atropine tended to lengthen the latent period preceding the onset of the contraction, as noted previously by Brown *et al.* (1959), but otherwise the nature of the response to the α-toxin was unaltered by the agents tested (see Fig. 3).

Heparin has been reported to impair the release of histamine from tissues by trypsin and peptone (Dragstedt, Wells & Roche e Silva, 1942) and by licheniformin (MacIntosh & Paton, 1949). However, concentrations of heparin up to 10 mg/ml. (one experiment) did not modify the spasmogenic action of the α-toxin on the guinea-pig ileum.

North & Doery (1958a, b) have described a protective action of the venoms of the Australian Tiger Snake (*Notechis scutatus scutatus*) and Black Snake (*Notechis scutatus niger*) against the lethal and dermonecrotic actions of staphylococcal α-toxin in mice. When tested on the guinea-pig isolated ileum, Black Snake venom (10 to 100 μg) produced a slow contraction, followed on washing by desensitization of the tissue to further doses of venom but not to histamine—an effect similar to that of other snake venoms studied by Feldberg & Kellaway (1937b). Prior administration of these doses of the venom did not affect the spasmogenic action of the α-toxin.

Rat isolated uterus preparation

The effects of antagonists to acetylcholine and 5-hydroxytryptamine on the spasmogenic action of the α-toxin on the rat isolated uterus were studied using paired uteri (Fig. 4). The effect of antihistamine drugs was not investigated since this tissue was unresponsive

TABLE 2
EFFECTS OF SOME DRUGS ON THE SPASMOGENIC ACTION OF STAPHYLOCOCCAL α-TOXIN ON THE RAT ISOLATED UTERUS PREPARATION

Drug	Concn. (μg/ml.)	Expt. no.	Change (%) in contraction produced by: Acetylcholine	5-Hydroxytryptamine	α-Toxin	Mean change in contraction due to α-toxin (%)
Control (no drug)		1			−26	−11
		2			+ 4	
Phenoxybenzamine	0·05	3	− 32	−100	+12	− 4
	0·05	4	0	−100	−21	
Methysergide	0·02	5	− 4	−100	+ 2	+ 6
	0·02	6	0	−100	+11	
Atropine	0·05	7	−100	0	−13	+ 3
	0·05	8	−100	− 14	+19	

to histamine. Atropine, phenoxybenzamine or methysergide (Fig. 4), in concentrations which abolished the responses to acetylcholine or 5-hydroxytryptamine, did not materially affect the action of the α-toxin. The results of these experiments, expressed in the manner used for guinea-pig ileum, are given in Table 2.

DISCUSSION

The contraction of the guinea-pig isolated ileum elicited by staphylococcal α-toxin does not appear to be due to the local release of spasmogenic substances. Firstly, antagonists

to likely mediators (acetylcholine, histamine and 5-hydroxytryptamine) did not materially affect the action of the toxin. Secondly, no spasmogenic activity has been detected in the bath fluid surrounding the ileal segments following exposure to the toxin, either in these or previous experiments (Brown *et al.*, 1959; Brown, 1965). It is unlikely that these negative findings are due to the inability of released spasmogens to diffuse from the ileum into the bath fluid because, using an identical technique, Feldberg & Smith (1954) were able to detect the release of histamine from isolated ileum by Compound 48/80. In view of this latter observation, the fact that Compound 48/80 has a far less pronounced stimulant action on the ileum than staphylococcal α-toxin further argues against the view that the spasm produced by the α-toxin is due to histamine release.

Wiegershausen (1962) has reported that certain drugs capable of antagonizing the action of 5-hydroxytryptamine on the isolated rat uterus (lysergide, (+)-lysergic acid cycloacylamide, reserpine and chlorpromazine) reduced the response of this tissue to α-toxin. In contrast, we have found that concentrations of phenoxybenzamine or methysergide which completely and selectively blocked the response of the rat uterus to 5-hydroxytryptamine failed to exert any marked antagonism to the spasmogenic action of the toxin. Since Wiegershausen (1962) did not establish whether the antagonism to 5-hydroxytrytamine was specific, the impaired response to the α-toxin which he observed might reflect an unspecific muscle depressant action of the agents used.

Brown *et al.* (1959) found that the action of α-toxin on the isolated intestine was not impaired by local anaesthetic drugs or ganglion-blocking agents, but could be opposed only by non-specific spasmolytic agents such as papaverine. They concluded that the α-toxin probably acted directly on the smooth muscle. The experimental findings reported in this paper do not conflict with this view.

SUMMARY

1. The α-toxin of *Staphylococcus pyogenes* produced a delayed, slow contraction of the guinea-pig isolated ileum and rat isolated uterus preparations. The contraction was followed on washing by loss of sensitivity to stimulant drugs and to the α-toxin.

2. Treatment of a segment of guinea-pig ileum with toxin did not lead to the appearance in the surrounding bath fluid of any material capable of stimulating a second ileal segment.

3. Antagonists to acetylcholine (atropine), histamine (mepyramine), or 5-hydroxytryptamine (phenoxybenzamine with morphine, or excess 5-hydroxytryptamine), either separately or in combination, did not modify the response of the guinea-pig ileum to α-toxin.

4. Atropine, phenoxybenzamine or methysergide did not affect the spasmogenic action of the α-toxin on the rat isolated uterus.

5. It was concluded that the contraction of these tissues was not due to the release of spasmogenic substances from the tissues by the toxin.

The authors would like to thank Dr Hazel M. Doery, of the Commonwealth Serum Laboratories, Australia, for the gift of Black Snake venom, and Messrs Sandoz for a sample of pure methysergide (Deseril).

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