Brit. J. Pharmacol. (1963), **20**, 378–387.

THE ACTION OF SUBSTANCES WHICH BLOCK SYMPATHETIC POSTGANGLIONIC NERVOUS TRANSMISSION

BY

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(*Received January* 25, 1963)

The substances which block sympathetic postganglionic transmission, xylocholine, bretylium and guanethidine, also block neuromuscular and sympathetic ganglionic transmission. To see if these last properties were related to the sympathetic blocking property, phenyltrimethylammonium, which blocks the neuromuscular junction (Riker, 1953), was used. It blocked the inhibition of the rabbit ileum produced by stimulating the periarterial nerves in the mesentery, though with higher concentrations the effect of stimulation was initially increased. The action was not modified by the presence of hyoscine. The blocking action was exerted on the response to stimulation of the highest frequency first, and on the response to stimulation of the lowest frequency last. This relation of block to stimulus frequency is similar to that at the neuromuscular junction when tubocurarine is used. Nine compounds have now been shown to block responses to sympathetic postganglionic stimulation, and seven of these are onium compounds. They are, however, mon-onium compounds, and not bis-onium compounds like hexamethonium and decamethonium, so that they can probably enter the postganglionic fibre, which bis-onium compounds (having a charged group at each end of the molecule) may not be able to do. Since these mon-onium compounds have some blocking action at neuromuscular junctions and at sympathetic ganglia, their block of postganglionic transmission may be essentially similar to that by hexamethonium at ganglia and to that by decamethonium at neuromuscular junctions. It is known that acetylcholine releases noradrenaline from sympathetic postganglionic terminations, and xylocholine and bretylium block this release in the vessels of the rabbit ear and in the rabbit isolated atria.

The substance first found to block the responses to stimulation of sympathetic fibres was xylocholine. Because it was a powerful local anaesthetic it was at first thought to block conduction along the fibre (Hey & Willey, 1954). However, Exley (1957) showed that the blocking action of xylocholine on the fibres leaving the superior cervical ganglion was not accompanied by a similar block of parasympathetic nervous transmission. Moreover, even when doses of xylocholine sufficient to cause total adrenergic block were given, there was no depression of the action potentials recorded from the main part of stimulated fibres such as those in the splenic and inferior cardiac nerves of the cat. Later, after the introduction of

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bretylium, Exley (1960) re-examined the action of both xylocholine and bretylium on the splenic nerves using an improved method. He concluded that the block by each drug must be exerted predominantly on the terminal portions of the neurones, preventing the release of noradrenaline.

A third compound, guanethidine, was introduced by Maxwell, Plummer, Schneider, Povalski & Daniel (1960). This had a similar mode of action, blocking the response of the nictitating membrane to postganglionic stimulation, though the membrane remained as sensitive as usual to intravenous noradrenaline.

The three substances, xylocholine, bretylium and guanethidine, have other properties in common. Each has some blocking action at sympathetic ganglia and also at neuromuscular junctions. Edge, Mason & Wyllie (1957) showed that xylocholine acted at the neuromuscular junction (sciatic-tibialis), and Exley (1957) showed that it had a short-lasting ganglion-blocking action. Boura & Green (1959) showed that bretylium possessed both properties. Maxwell *et al.* (1960) showed that guanethidine had a ganglion-blocking action, and Dixit, Gulati & Gokhale (1961) showed that guanethidine and bretylium each has an action like tubocurarine and decamethonium on the phrenic nerve-diaphragm preparation of the rat.

Both xylocholine and bretylium are "onium" compounds and are therefore related to acetylcholine in structure. Guanethidine is not an onium compound but has a guanidine group which is strongly basic and highly ionized like the trimethylammonium group. Since the three compounds have some action in blocking responses to acetylcholine, their action in the sympathetic fibre might be related to this property. To test this possibility a compound was selected which was an onium compound and which was known to block the neuromuscular junction. Riker (1953) studied the actions of neostigmine and of substances related to it. One of these, phenyltrimethylammonium, was obtained by removing the side-chain, and Riker showed that it blocked the neuromuscular junction in all doses. This substance was examined to see if it would cause block at the postganglionic terminations in the rabbit ileum.

METHODS

Pieces of ileum with attached mesentery were removed from a freshly killed rabbit. They were transferred to a beaker containing Locke's solution bubbled with 95% O_2 and 5% CO_2. One of the pieces was set up in an isolated organ-bath at 32° C. A silk thread was tied to the main artery supplying the loop of ileum and was passed through the hole in the end of a pair of electrodes constructed as described by Burn & Rand (1960a). The silk thread was used to draw the end of the artery, with its covering of fat, through the hole in which it was held securely. Within the hole there were two coaxial platinum rings to stimulate the periarterial nerves. The piece of ileum was attached by its upper end to a lever with a frontal writing point. Maximal stimuli (0.5 msec duration) were used, and a fixed number of shocks was given at each stimulation. Three frequencies were chosen, one low, one near the optimal (which varied between 30 and 50 shocks/sec) and one yet higher frequency. In some experiments hyoscine hydrobromide (0.1 μg/ml.) was added to the bath. Solutions of phenyltrimethylammonium chloride and of bretylium tosylate were prepared freshly.

RESULTS

The two effects. The change produced by the addition of phenyltrimethylammonium to the bath is illustrated in Fig. 1. The top left records (Fig. 1 A)

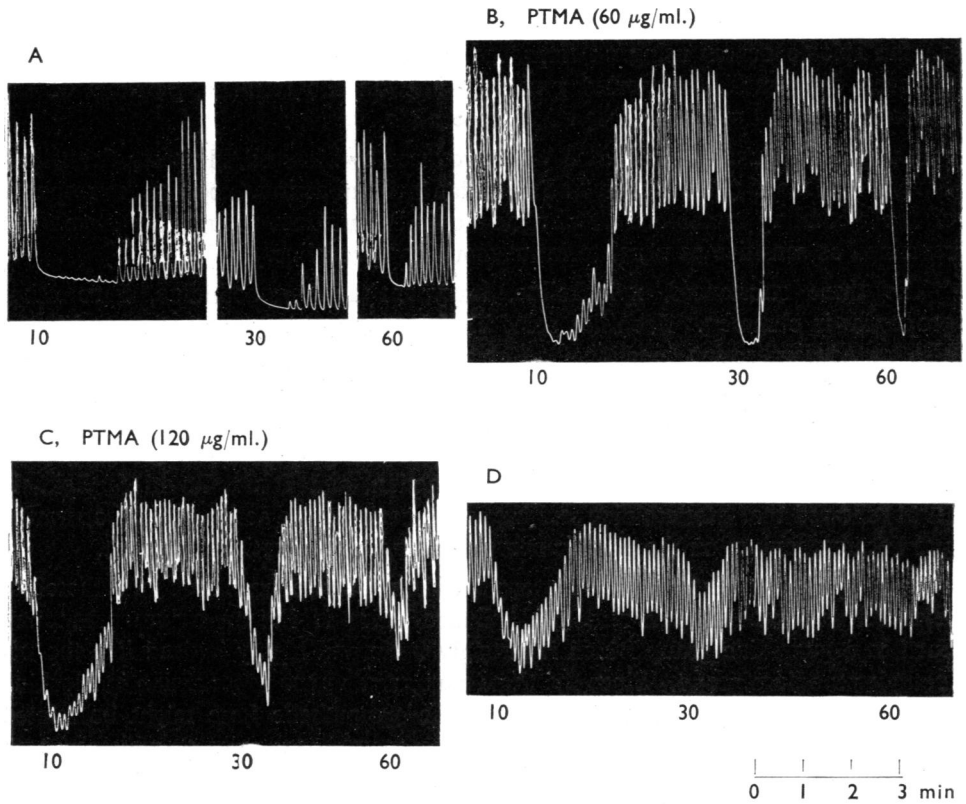


Fig. 1. Preparation of rabbit ileum in organ-bath at 32° C. Periarterial nerves stimulated by 1,000 maximal shocks at frequencies of 10, 30 and 60 shocks/sec. Control observations in A. Observations in B were made in the presence of phenyltrimethylammonium (PTMA), 60 μg/ml. A concentration of 60 μg/ml. was present for 10 min, and 30 μg/ml. for 70 min, before the observations. Note the increase in the inhibitions produced by all frequencies of stimulation. Observations in C were made in the presence of phenyltrimethylammonium, 120 μg/ml., 90 min after B. Observations in D were made 1 hr after those in C. Note that in C and D there was progressive block of the inhibitions, which was greatest for the frequency of 60 shocks/sec and least for the frequency of 10 shocks/sec.

show the effect of giving 1,000 shocks to the periarterial nerves at three different frequencies, 10, 30 and 60 shocks/sec. The drug was added to the bath to make a concentration of 30 μg/ml., and acted for 70 min. At that point the concentration was doubled, and 10 min later the records shown in Fig. 1 B were obtained. The mean tone of the ileum had risen, and the inhibitions obtained at all three frequencies were greatly increased. Such an increase in the early part of the drug-action was seen in several experiments, though it was not usually so large. During the following hour, the concentration of phenyltrimethylammonium was doubled again, so that it became 120 μg/ml. In Fig. 1 C are shown the responses 3 hr after the first addition of phenyltrimethylammonium. The effect of the high frequency stimulation was at this time very much reduced, while that of the middle frequency was reduced rather less, and that of the low frequency was only slightly reduced. After 1 hr

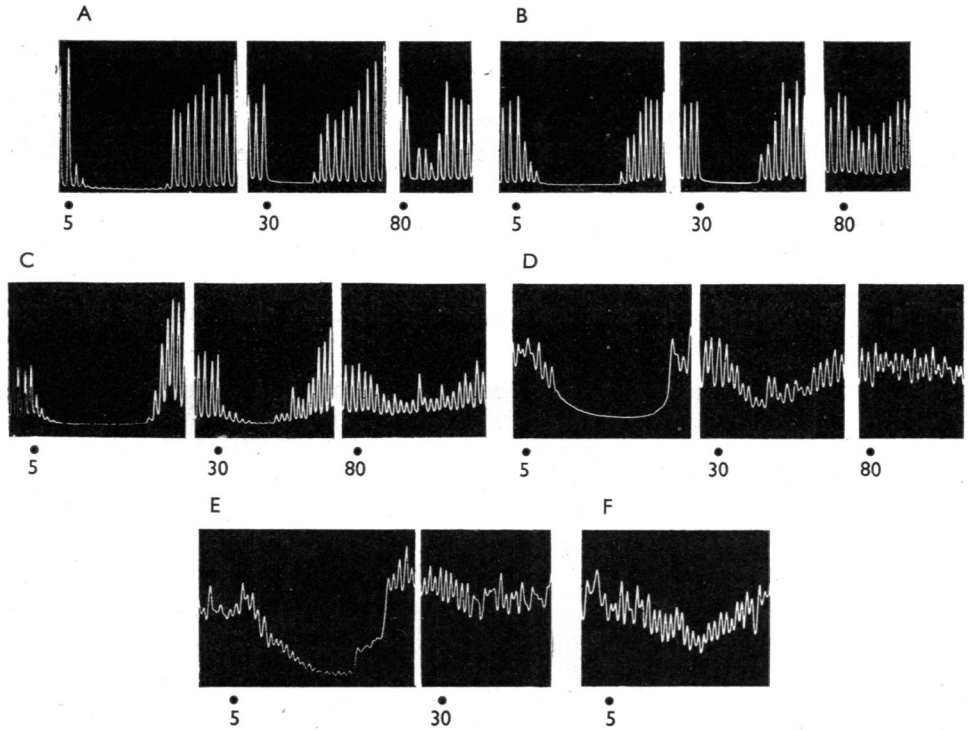


Fig. 2. Observations similar to those in Fig. 1. Hyoscine hydrobromide (0.1 μg/ml.) was present throughout. Periarterial nerves stimulated with 400 shocks at frequencies of 5, 30 and 80 shocks/sec. Between A and B, phenyltrimethylammonium was added in a concentration of 30 μg/ml. There was no further addition. B was 20 min after the addition of the drug; C was 1 hr; D was 2 hr; E was 3 hr; and F was 4 hr. Note that inhibition at 80 shocks/sec was absent at 2 hr, and inhibition at 30 shocks/sec was absent at 3 hr. Inhibition at 5 shocks/sec was almost absent at 4 hr.

more, the effect of the high frequency was abolished (Fig. 1 D) and the effect of the middle frequency was very small. The effect of the low frequency stimulation was, however, still conspicuous.

Thus phenyltrimethylammonium had two effects on the inhibition of intestinal movement caused by stimulation of the periarterial nerves. There was an initial increase in the inhibition which affected stimulation of all frequencies. After the concentration of the drug was raised, the effect of stimulation progressively diminished, the diminution being seen most clearly in the response to stimulation of the highest frequency. This response was finally abolished, and the responses to lower frequencies were also diminished, that to stimulation of the lowest frequency being diminished the least.

Responses in the presence of hyoscine. Some responses in the presence of hyoscine were observed, and one of these is shown in Fig. 2.

The purpose of adding hyoscine (0.1 μg/ml.) to the bath was to ensure that the effect of any acetylcholine released by the stimulation of the periarterial nerves was

excluded so far as its direct action on the intestine was concerned. It has been suggested (Gillespie & Mackenna, 1961) that the periarterial nerves contain parasympathetic fibres.

In the experiment of Fig. 2, each stimulation consisted of 400 shocks applied at the frequencies of 5, 30 and 80 shocks/sec. Phenyltrimethylammonium was added to the bath in a concentration of 30 μg/ml., and no further addition was made throughout the experiment. Since xylocholine has often been used in a concentration of 10 μg/ml., the concentration of phenyltrimethylammonium in this experiment was not very high. Fig. 2 A shows control observations; the responses in Fig. 2 D, obtained 2 hr after the addition of the drug, show that by this time

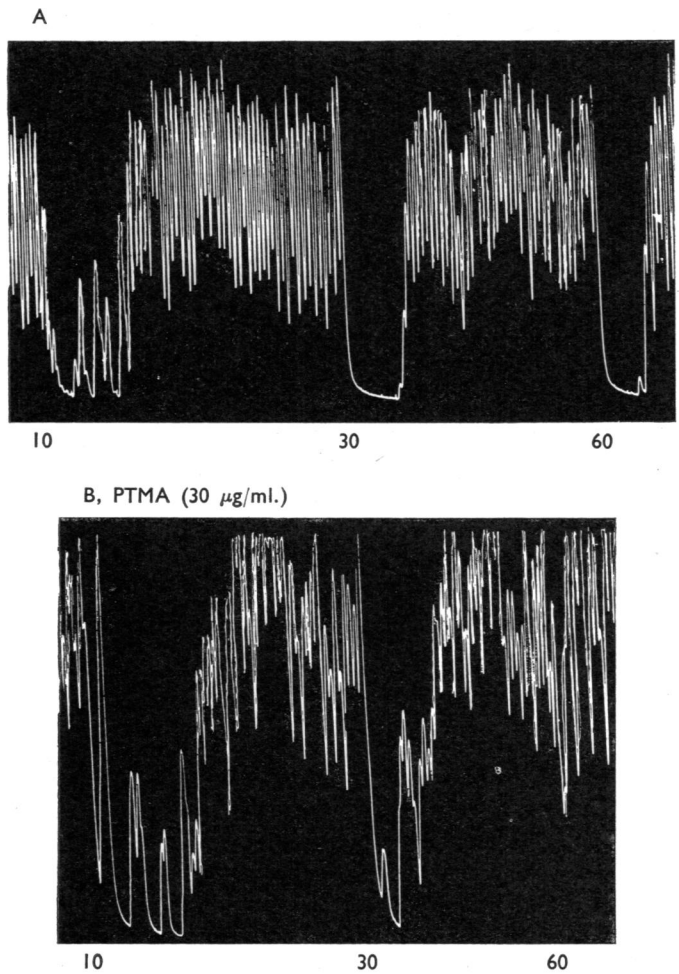


Fig. 3. Observations as in Fig. 1. Stimulation by 1,000 shocks at frequencies of 10, 30 and 60 shocks/sec. Hyoscine was present throughout in a concentration of 0·1 μg/ml. A, control; B, 1 hr after addition of phenyltrimethylammonium (PTMA), 30 μg/ml., which practically abolished the inhibition produced by stimulation at 60 shocks/sec, and reduced the inhibition produced by 30 shocks/sec.

stimulation at 80 shocks/sec had no effect, and that stimulation at 30/sec had much less effect than before. In Fig. 2 E, 3 hr after the addition of phenyltrimethylammonium, the response to stimulation at 30 shocks/sec was abolished, and in Fig. 2 F, 4 hr after the addition of the drug, the response to stimulation at 5 shocks/sec was negligible. In this experiment there was no evidence of the increase in the inhibition seen in the experiment of Fig. 1 ; such an increase was mainly evident in the period soon after the addition of concentrations of phenyltrimethylammonium higher than 30 μg/ml., such as 90 or 120 μg/ml.

The time required to act. In both of the experiments described, the action of phenyltrimethylammonium in depressing the response to stimulation of the periarterial nerves developed very slowly. This, however, was not always true, as shown in Fig. 3. Inhibition was recorded in response to 1,000 shocks at frequencies of 10, 30 and 60 shocks/sec (Fig. 3 A). These responses occurred in the presence of hyoscine (0.1 μg/ml.) in the bath. Phenyltrimethylammonium (30 μg/ml.) was then added and the responses shown in Fig. 3 B were obtained 1 hr later. Stimulation at 60 shocks/sec had a very small effect, and at 30 shocks/sec had a smaller effect than before. However, the response to stimulation at 10 shocks/sec appeared to be increased. There was much variation in different experiments in the time taken for phenyltrimethylammonium to abolish the inhibitory response to stimulation of the periarterial nerves.

The effect of bretylium. In order to compare the effect of bretylium with that of phenyltrimethylammonium, some experiments were carried out with bretylium using different frequencies of stimulation. One of these is illustrated in Fig. 4, which

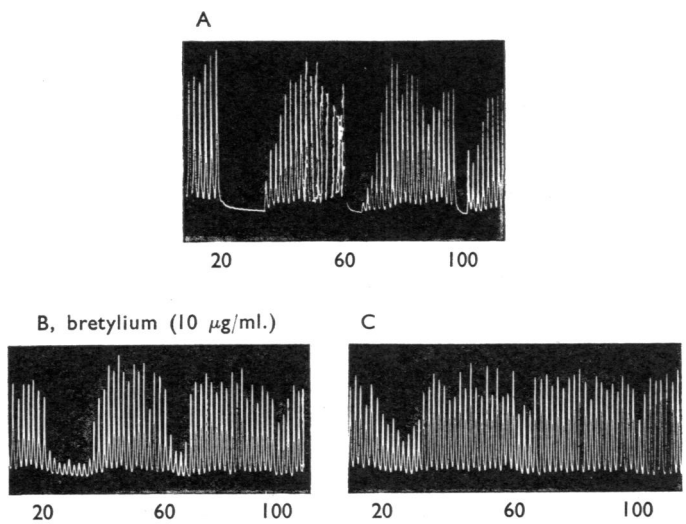


Fig. 4. Observations as in Fig. 1. A shows inhibitions produced by 1,000 shocks at frequencies of 20, 60 and 100 shocks/sec. B shows the effect of the same stimuli 10 min after the addition of bretylium (10 μg/ml.) to the organ-bath. C shows the effect of the same stimuli 20 min after the addition of bretylium. Note that stimulation at the highest frequency was blocked first.

shows control responses to stimulation at 20, 60 and 100 shocks/sec (Fig. 4 A). Bretylium (10 μg/ml.) was then added to the bath and the results in Fig. 4 B were obtained 10 min later. Stimulation produced responses which were smaller for all three frequencies, but the greatest reduction was seen in response to the highest frequency, and the least in response to the lowest. Observations 20 min later showed further reductions in the responses, so that those to 60 and 100 shocks/sec were negligible.

DISCUSSION

Phenyltrimethylammonium was tested in these experiments because it was known to block at the neuromuscular junction (Riker, 1953). According to the hypothesis of Burn & Rand (1959) such a compound might act on the sympathetic postganglionic fibre, in which it would block the action of acetylcholine in releasing noradrenaline. It was found to block the inhibition produced by stimulating the periarterial nerves to the ileum; after an initial augmentation, the inhibition diminished and disappeared. The block affected stimulation at the highest frequency first and stimulation at the lowest frequency last. Thus in this respect the block was similar to that caused by tubocurarine at the neuromuscular junction (Chou, 1947).

Nine compounds have now been shown to block responses to sympathetic postganglionic nerve stimulation (Table 1). Seven of these contain the group $-N^+(CH_3)_3$ or a group closely similar, and are therefore "onium" compounds. They are, however, mon-onium compounds, since they have a charged group at one end of the molecule only, and are to be distinguished in this respect from bis-onium compounds like hexamethonium and decamethonium, which have a charged group at each end. Mon-onium compounds may be expected to penetrate a nerve fibre more easily than bis-onium compounds. The two agents which block postganglionic transmissions and which are not "onium" compounds are guanethidine and nicotine. Guanethidine has a guanidine group at the end of the side-chain; this forms a strongly basic and highly ionized group similar to an onium group.

Acetylcholine blocks the effect of sympathetic stimulation on the pilomotor muscles of the cat's tail (Brücke, 1935). This observation was confirmed by Coon & Rothman (1940) and by Burn & Rand (1960b). Acetylcholine also blocks the vasoconstriction caused by stimulating the postganglionic fibres to the rabbit's ear (Burn & Rand, 1960b), and the contraction of the nictitating membrane caused by stimulating the fibres leaving the superior cervical ganglion (Comer & di Palma, 1961).

Low concentrations of dimethylphenylpiperazinium block the inhibitory effect of stimulating the periarterial nerves of the rabbit ileum (Bentley, 1962; Wilson, 1962). Ling (1959) investigated the action of this drug at the neuromuscular junction and showed that it blocked the sciatic-gastrocnemius preparation in the cat and reduced the contraction of the nictitating membrane when this was maintained by continuous preganglionic stimulation.

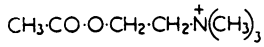
Edrophonium blocks the contraction of the nictitating membrane in response to postganglionic stimulation (Comer & di Palma, 1961). Katz & Thesleff (1957) studied the action of edrophonium at the neuromuscular junction in skeletal muscle, and

TABLE 1

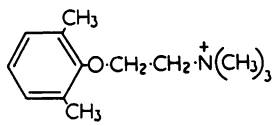
COMPOUNDS WHICH PREVENT THE LIBERATION OF NORADRENALINE FOLLOWING STIMULATION OF THE SYMPATHETIC POSTGANGLIONIC FIBRE

1. *Mon-onium compounds*

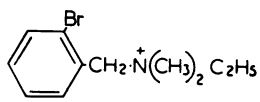
a. Acetylcholine



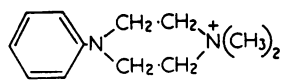
b. Xylocholine



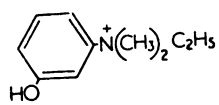
c. Bretylium



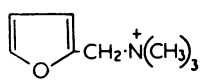
d. Dimethylphenylpiperazinium



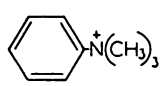
e. Edrophonium



f. Furfuryltrimethylammonium

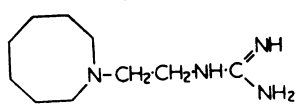


g. Phenyltrimethylammonium

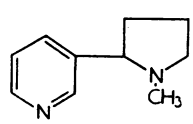


2. *Other compounds*

a. Guanethidine



b. Nicotine



found that large doses exerted a depolarizing action. Edrophonium is closely related in structure to phenyltrimethylammonium, which may explain why both compounds block responses to sympathetic stimulation.

Furfuryltrimethylammonium is a compound with the muscarinic properties of acetylcholine (Fellows & Livingston, 1940), and has recently been found to have a blocking action in the phrenic nerve-diaphragm preparation. Comer & di Palma (1961) showed that it blocks the response of the nictitating membrane to post-ganglionic stimulation.

It is clear that none of the compounds which block sympathetic postganglionic transmission are related in either structure or properties to noradrenaline, and there seems to be no means of explaining their action if the view is held that the nerve impulse releases noradrenaline directly.

All the compounds, however, are related in structure or in action or in both to acetylcholine, and their blocking action is readily understood if the hypothesis is accepted that the nerve impulse liberates acetylcholine which then in turn liberates noradrenaline. The liberation of noradrenaline by acetylcholine may involve a depolarization process, and the blocking agents may cause a competitive or a depolarization block. The blocking agents thus have an action of the same kind as have hexamethonium and decamethonium, but because of their structure are more able to enter the postganglionic fibre and to exert their action within it.

We wish to express our warmest thanks to Professor Walter Riker, Jr for his kindness in sending us a supply of phenyltrimethylammonium.

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