Brit. J. Pharmacol. (1953), 8, 147.

THE NICOTINE-LIKE ACTIONS OF THE 3-BROMO- AND 3 : 5-DIBROMO-PHENYL ETHERS OF CHOLINE (MBF AND DBF)

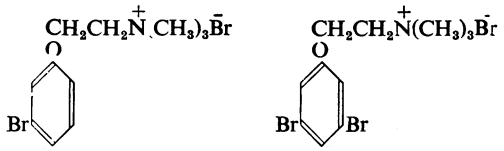
BY

N. AMBACHE AND P. A. ROBERTSON

From the Ophthalmological Research Unit (Medical Research Council), Institute of Ophthalmology, Judd Street, London, W.C.1

(RECEIVED NOVEMBER 12, 1952)

The 3-bromo- and 3 : 5-dibromo-phenyl ethers of choline were first synthesized by Hey (1952); their structures are shown in Fig. 1, and they will be referred to as MBF and DBF respectively. Hey



3-Bromophenyl ether of choline bromide (MBF)

3 : 5-Dibromophenyl ether of choline bromide (DBF)

FIG. 1

tested these new drugs for nicotinic activity by examining their effect on the blood pressure of atropinized cats. By this test the potency of MBF and DBF, on a molar basis, was 2–3 times that of the unsubstituted phenyl ether of choline and 4–5 times that of nicotine. The rise in blood pressure produced by these new derivatives persisted after ligation of the suprarenals, and was abolished by known ganglion-blocking agents. The pressor action found by Hey therefore implies an ability of MBF and DBF to excite autonomic ganglia. In the present series of experiments on the perfused superior cervical ganglion of cats we have confirmed the fact that these compounds are endowed with a powerful ganglionic action. Additional nicotine-like properties on a number of other tissues, including skeletal muscle, are described in this paper. For instance, it is shown that MBF and DBF elicit pilomotor axon reflexes in the human skin, a type of cutaneous reaction due to stimulation of axonic endings, and first noted after intradermal injection of nicotine by Coon and Rothman (1940). This " axonic " action of MBF and DBF (and also that of nicotine) is blocked by hexamethonium. The existence of an action of this type on a set of autonomic nerve-endings gives rise to the theoretical possibility that compounds endowed with nicotinic activity, such as these and possibly others, may exert an effect at both ends of autonomic neurones, a ganglionic action on the cell bodies and an " axonic " action at their terminals.

The concluding section of this paper deals with the action of MBF and DBF on isolated mammalian intestines. Our observations have been confined to the effect on the longitudinal muscle layer, principally in rabbits. It is shown that MBF and DBF elicit contractions which are blocked by hexamethonium and reversed by botulinum toxin. Thus the motor action of MBF resembles that of nicotine in these and other respects, and can likewise be attributed to a stimulation of the motor neurones in the enteric plexus. There is no evidence at present whether this " indirect " action is purely ganglionic or whether it also includes an " axonic " component.

METHODS

Technical details of the various types of preparation used for these experiments can be found in an earlier paper (Ambache, 1949). The following alterations need to be mentioned.

Frog's Rectus Abdominis.—The bath fluid was aerated.

Avian Iris.—Pigeons were used instead of hens. They were anaesthetized with 6–12 mg. of pentobarbitone sodium (nembutal) intramuscularly and with 1–2 drops of 1% amethocaine HCl instilled into the conjunctival sac. MBF or DBF was injected into the anterior chamber in a volume of 0.01–0.02 ml.

Ganglion Perfusion.—Cats were anaesthetized with 38 mg./kg. of pentobarbitone sodium intraperitoneally. The method for the perfusion of the superior cervical ganglion was as before except that (*a*) the use of heparin was omitted, (*b*) the glucose content of the Locke's solution for perfusion was doubled to

2 g./l., and (*c*) the perfusion fluid was warmed, by the animal's own body-heat, during its transit through a length of transparent "Portex" tubing inserted into the stomach and emerging from a lateral incision in the oesophagus at the root of the neck, where connection was made to the perfusion cannula. We are indebted to Professor F. C. MacIntosh for this suggestion. Contractions of the nictitating membrane were recorded with an isometric lever at a basal tension of 10 g.

Pilomotor Reaction.—MBF and DBF were injected intradermally in several human subjects in a volume of 0.05 ml. of 0.9% NaCl.

Isolated Intestine.—13 adult rabbits, 4 guinea-pigs, and 2 kittens were killed by a blow on the back of the neck. A 4–5 cm. portion of ileum was suspended, after emptying its lumen, in a 10-ml. organ-bath in Mg-free Tyrode's solution containing 1.1% $NaHCO_3$, and oxygenated with 95% O_2 and 5% CO_2 (for guinea-pig gut pure O_2 was used). The contractions of the longitudinal muscle were recorded with a frontal writing lever which had a fivefold magnification.

In five other experiments on normal rabbits the required segment of ileum was excised from the live animal under ether anaesthesia. The initial presence of ether in these preparations did not appear to affect the results in any noticeable way. Four other preparations were taken, also during ether anaesthesia, from rabbits in which the anterior mesenteric and both coeliac ganglia had been removed at a previous operation for another purpose. As there did not appear to be any noticeable difference in the pharmacological reactivity of these rabbit intestinal preparations from the three different sources mentioned, the results obtained on them will be presented together without further sub-classification.

Experiments with Botulinum Toxin.—The technique of injecting botulinum toxin subperitoneally into an intestinal segment has been described previously (Ambache, 1951). An important modification in procedure in the present experiments has been that, during the initial ether anaesthesia but before the injection of toxin, a 5–10 cm. segment of ileum was excised between ligatures; the middle 5 cm. of this was suspended at once in an organ-bath for control tests with drugs. An adjoining segment of gut, 10 cm. in length and situated 5–10 cm. above or below the site of excision, was then injected with 2–4.6 mg. of toxin A_2. The rabbits were killed by an injection of air into the marginal ear vein 1½–4¼ hours later, and the injected segment was removed for pharmacological examination in the organ-bath. Thus each experiment was conducted in duplicate on two preparations from the same animal: the first a "control," the other treated with botulinum toxin. The two preparations were made as nearly equal in length as possible (4–5 cm. usually), and the same lever was used for both.

Drugs.—The bromide salts of MBF and DBF were obtained through the courtesy of Dr. P. Hey. All drug doses given in the text refer to the salts: nicotine as the hydrogen tartrate, hexamethonium as the bromide, *d*-tubocurarine as the chloride, atropine as the sulphate, muscarine as the chloride, etc.

Results

Nicotine-like Actions of MBF and DBF

Skeletal Muscle.—Both compounds are able to produce contractions of the frog's rectus abdominis muscle in the absence of eserine. MBF appears to be about twice as active as DBF (Fig. 2); *d*-tubocurarine (10^{-5}) antagonizes these effects, but the antagonism is reversible.

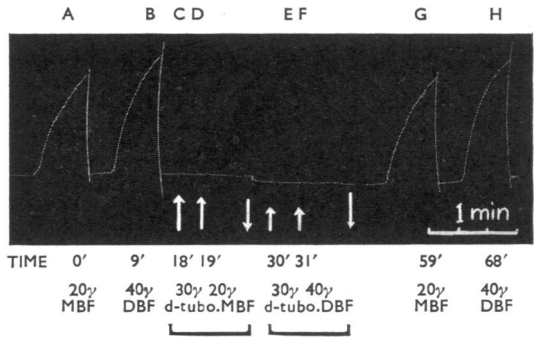


FIG. 2.—Frog's rectus abdominis in a 3 ml. bath. Actions of MBF and DBF, and reversible antagonism by *d*-tubocurarine. The minutes below refer to the time of administration of each drug. Drum stopped between tests. At A, D, and G, 20 μg. of MBF. At B, F, and H, 40 μg. of DBF. At C and E, 30 μg. of *d*-tubocurarine.

The skeletal muscle in the pigeon's iris also responds to these compounds, and 1–2 μg. of either drug, injected into the anterior chamber, promptly constricts the pupil (Fig. 3).

Ganglia.—From his analysis of the nicotine-like effect of these compounds on the cat's blood pressure, Hey (1952) inferred that MBF and DBF were capable of exciting autonomic ganglion-cells. In unpublished experiments Dr. Hey and his colleagues (personal communication) have also

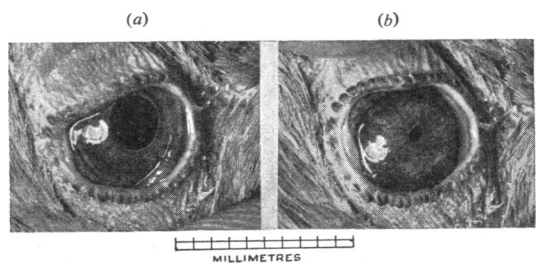


FIG. 3.—Pigeon's iris (*a*) before and (*b*) immediately after injection, into the anterior chamber, of 1.5 μg. of MBF in 0.15 ml. of 0.9% NaCl. Photographs taken with 0.2 msec. flashes.

observed a stimulation of the cat's superior cervical ganglion with these compounds. We have confirmed Hey's conclusions in the present series of ganglionic perfusions. Furthermore, our results show that, like all other substances endowed with nicotinic activity, MBF and DBF in larger doses produce a ganglion-cell paralysis which results in an arrest of synaptic transmission. In seven experiments we have obtained stimulation with doses of 0.05–0.1 μg. of MBF and 0.05–0.2 μg. of DBF. Block occurred after doses of 0.4–10 μg. of MBF or 0.05–1 μg. of DBF, and its intensity appeared to be proportional to the dose.

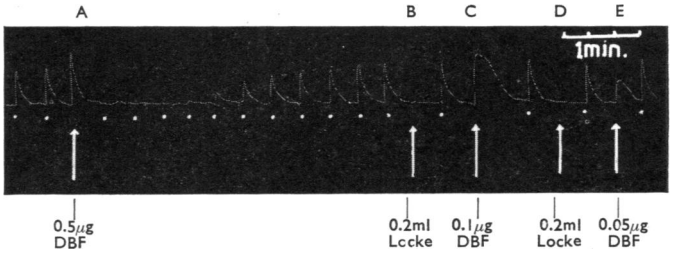


FIG. 5.—Continuation of Fig. 4. Injections of DBF: at A, 0.5 μg.; C, 0.1 μg.; and E, 0.05 μg. At B and D, 0.2 ml. of glucose-free Locke's solution. Following the stimulant action of DBF at A, there is complete block of the response to preganglionic stimulation for 3 min. and slow recovery during the next 7 min.

In a typical experiment injections of 1 μg. of MBF elicited effects which were nearly equal to those of maximal preganglionic stimulation at 20 cycles/sec. applied for 5 sec. The latency of the MBF responses was 2.5–3 sec. from the moment of injection. In order to detect the presence of block, "test" maximal preganglionic stimuli were delivered at minute intervals throughout the experiment. The first sign of block appeared after the second dose of 1 μg. of MBF. It was pronounced after 1.5 μg. and was complete for a period of 1 min. after 2 μg., and for 3 min. after 5 μg. It took 6–7 min. for transmission to recover fully after the 2 and 5 μg. doses of MBF, and also after 1 μg. of DBF.

In another experiment it was found that, during the phase of total block following injections of 1–2 μg. of MBF, maximal contractions of the nictitating membrane could still be elicited by postganglionic stimulation. This observation showed that the ineffectiveness of preganglionic stimuli was due to a block within the ganglion.

The block produced by 2 μg. of MBF and by 0.5 μg. of DBF, and the time-course of subsequent recovery, are shown in Figs. 4 and 5. In this experiment the response to 0.2 μg. of MBF was equated with that to 2 μg. of nicotine. As shown in Fig. 4, 0.5 and 1 μg. of MBF produced effects greater than that of a 5 sec. period of maximal preganglionic stimulation at 20 cycles/sec. Later on (not shown in Fig. 4) the response to 1 μg. of MBF was reversibly abolished by a 250 μg. dose of hexamethonium.

The last two experiments in this series were performed on "denervated" ganglia, the preganglionic nerve supply of which had been cut 9–15 weeks previously. The first of these ganglia responded to 0.5 μg. of MBF. In the second, maximal responses were obtained with 0.1 μg. of MBF; these were equated with 1 μg. of nicotine and were blocked by 500 μg. of hexamethonium or by 200 μg. of *d*-tubocurarine administered 1 min. previously. The ganglion-cell-paralysing properties of MBF were also evident in this experiment, as shown by the observation that the normal response to 5 μg. of acetylcholine was blocked 2 min. after a dose of 0.4 μg. of MBF, but recovered within 5 min. of that dose.

Pilomotor Nerve-endings.—In 1940 and 1941 Coon and Rothman discovered that a number of drugs possessing nicotinic activity could, on intradermal injection into the human skin, initiate axon reflexes in the branching terminals of several categories of autonomic nerve-fibres innervating structures in the skin. The localized pilomotor, sudomotor, and vasoconstrictor responses which they described provided the first examples of nicotine-induced axon reflexes at adrenergic and cholinergic motor nerve-endings respectively. In the introduction we have referred to this type of action as

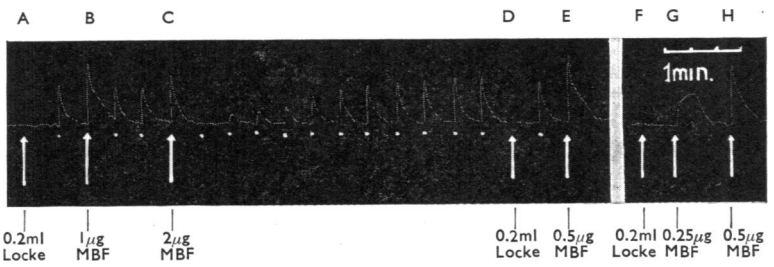


FIG. 4.—Perfusion of cat's superior cervical ganglion. The white dots indicate responses of the nictitating membrane (contraction upwards) to maximal preganglionic stimulation (20 cycles/sec. applied for 5 sec.). At A, D, and F, control injections of 0.2 ml. glucose-free Locke's solution. MBF injections: at B, 1 μg.; C, 2 μg.; E and H, 0.5 μg.; and G, 0.25 μg. Following the stimulant action of 2 μg. at C, there is complete block of the response to preganglionic stimulation for 1 min. and slow recovery during the next 9 min.

"axonic." Brown and Gray (1948) have shown, further, that nicotine can excite yet another kind of nerve-ending in the skin, namely, those subserving the sensation of touch. Later Douglas and Gray (1953) found that this action of nicotine, which may perhaps also be considered as "axonic," was abolished by hexamethonium. Thus it is clear that various types of nerve-ending may possess pharmacological properties hitherto considered as characteristic of autonomic ganglion cells. This fact is perhaps less surprising than would appear at first, since even in the superior cervical ganglion preganglionic fibres may come into synaptic, and therefore possibly into humoral, relation not only with the cell bodies of ganglia but also (Ranson and Billingsley, 1918, Fig. 15) with dendrites, i.e., with a type of nerve-ending.

Coon and Rothman made the suggestion that the reactions which they described might serve as specific pharmacological tests for the detection of nicotinic activity. We have found the pilomotor reflex the easiest to observe, and have subjected the action of MBF and DBF to this single test by injecting them intradermally into four human

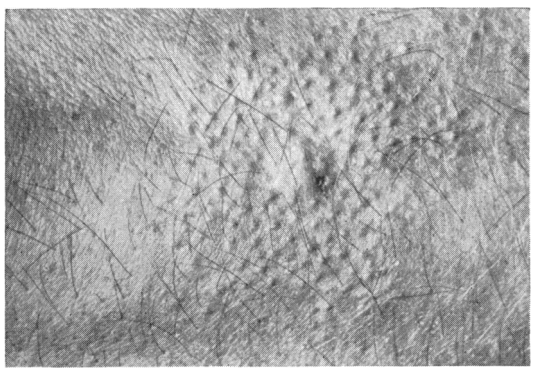


FIG. 6.—Pilomotor axon reflex elicited by an intradermal injection of 0.3 μg. of MBF in 0.05 ml. of 0.9% NaCl.

subjects. Both substances are active in concentrations of $3\text{–}6\times10^{-6}$ for MBF and 5×10^{-7} to 2×10^{-6} for DBF. The effect begins within a few seconds of the intradermal injection and fades away after $\frac{3}{4}$ to $1\frac{1}{4}$ min. As shown in Fig. 6, the reaction consists of a circular area of gooseflesh with characteristic "pseudopodia" due to the erection of isolated groups of satellite hair follicles outside the main area of the response, sometimes at a considerable distance from it. The initiation of this reflex, whether by MBF, DBF, or by nicotine (10^{-5}), is abolished by previous intradermal injection of hexamethonium (10^{-4} or 10^{-3}) into the same site. Blocking properties were observed with higher concentrations of MBF (10^{-4}), which abolished the effect of 10^{-5} of nicotine.

Intestinal Actions of MBF and DBF

With the exception of those experiments below which deal with guinea-pig and kitten gut, the results reported here were obtained on the longitudinal muscle of the rabbit ileum. Although the bulk of this work was carried out with MBF, DBF was used in a few trials. In six of seven experiments 1–15 μg. of DBF elicited contractions, whilst 10–45 μg. produced relaxation; in four of these preparations variable mixtures of contraction and inhibition, or the converse state of affairs where relaxation preceded contraction, were sometimes observed. Therefore, as the results with MBF appeared to be more consistently uniform, DBF was discarded for the purposes of the present investigation, which was then confined to MBF.

Stimulating Action.—Longitudinal muscle contractions were elicited in 22 experiments on different preparations by MBF in doses of 2–50 μg. (final concentration in the organ-bath: 2×10^{-7} to 5×10^{-6}); in two of these experiments relaxation was produced by higher doses of MBF, i.e., by 50 and 100 μg. A noticeable feature of the motor response to MBF, also seen with DBF, was the decrease in the magnitude of the contractions recorded as the dose was increased. This phenomenon is illustrated in Fig. 7; the diminution in the height of the MBF contraction occurred as the dose was increased from 10 μg. at D to 25 μg. at E; 62 min. later the effect of 40 μg. of MBF at I was even smaller. This phenomenon may be related to the onset of a nicotine-like "paralysis" which will be discussed below.

The potency of MBF was compared with that of nicotine in 11 of these experiments. Satisfactory matching between two doses, one of MBF and the other of nicotine, was possible on most of the preparations as, for example, in Fig. 11 B and C. In this particular instance the activity ratio of MBF to nicotine was unity; in the remaining preparations where such matching was possible the ratio varied between 0.5 and 2 (the ratio for DBF was, in two experiments, 2 and 2.5 respectively). But on a few occasions matching was made difficult both by variations in the MBF response and also by the fact that large contractions could be obtained in response to nicotine but not to MBF because of the onset, as the dose of MBF was increased, of the "paralytic" effect described above. "Paralysis" may also account for the fact that the MBF contraction is often not sustained at a plateau and

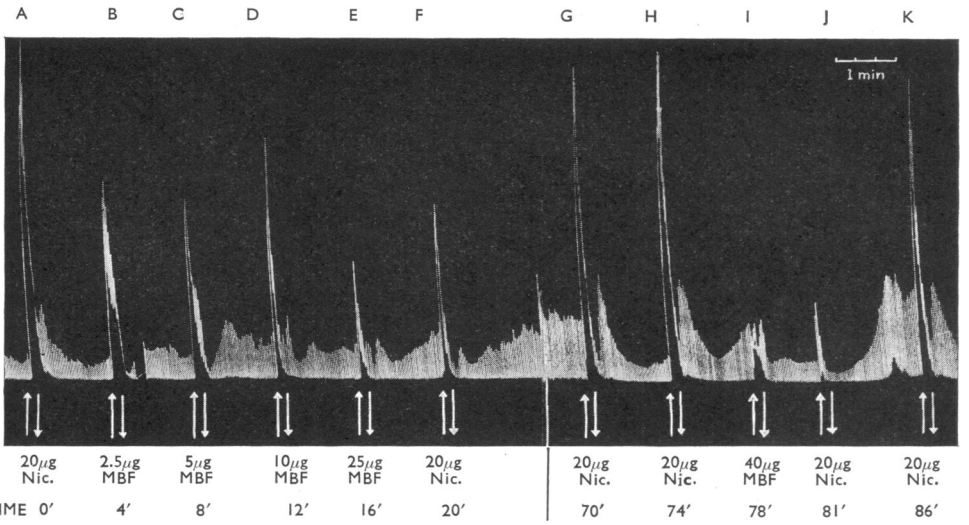


FIG. 7.—Rabbit, 2.2 kg. (pregnant). Ileum preparations 165 cm. below pylorus. All drugs left in the organ-bath (vol. 10 ml.) for 30 sec. only. Time in minutes at the bottom of the tracing. At A, F, G, H, J, and K: 20 μg. of nicotine. MBF at B, 2.5 μg.; C, 5 μg.; D, 10 μg.; E, 25 μg.; and I, 40 μg. Note the decrease in effect with the larger doses of MBF and the subsequent reversible depression of the response to nicotine (at F and J).

begins to subside before the drug is washed out of the organ-bath.

It has been shown by Feldberg (1951) that nicotine-induced intestinal contractions can be abolished by hexamethonium, an effect attributed at the time solely to the ganglion-blocking properties of hexamethonium. In the present series of experiments the motor response to MBF could likewise be abolished, or very greatly reduced, by hexamethonium in concentrations of $1\text{–}4\times10^{-4}$. Fig. 8 illustrates this antagonism; it shows the initial response to 20 μg. of MBF at A and B, its extinction at D in the presence of 4 mg. of hexamethonium, and lastly its recovery 3 min. later at E. The tracing also shows that the tone and rhythmic activity of the gut were affected by the presence of hexamethonium (at C). This was a usual finding, though the direction of the effect was unpredictable; it was sometimes inhibitory, as in this experiment, but in other preparations hexamethonium produced an augmentation of pendulum movements.

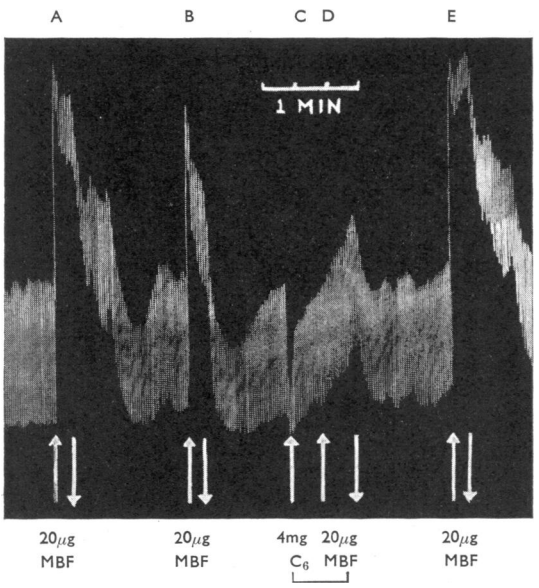


FIG. 8.—Reversible antagonism of MBF by hexamethonium in a rabbit's ileum preparation taken 35–40 cm. below pylorus. A, B, and E: 20 μg. MBF for 30 sec. C: 4 mg. hexamethonium producing a temporary inhibitory effect by itself and antagonizing, 60 sec. later, the effect of D, 20 μg. MBF for 60 sec.

Since, as shown above, MBF acts very much like nicotine at ganglia and nerve-endings, it would be reasonable to suppose that in the intestine, which incorporates a complex plexus of ganglion-cells and nerve-endings, the action of MBF would also resemble that of nicotine in many respects. The evidence presented so far supports this view. In particular the antagonism of MBF contractions by hexamethonium would seem to point to a localization of the motor action at least to the motor neurones within the gut. Further aspects of this "indirect" type of action are described below.

Blocking Action.—Several examples of the blocking properties of MBF have been given above, showing that in larger doses this compound renders nerve-cells and nerve-endings pharmacologically inexcitable. The experiments on the superior cervical ganglion show that this inexcitability may persist

for several minutes. It was to be expected that a similar block would occur in the gut, at least at the motor neurone level. The " paralytic " phenomena referred to in the preceding section are probably an expression of this type of block, as shown by the following considerations.

In the experiment of Fig. 7, and several other experiments of this type, the use of larger doses of MBF (and of DBF) was characterized not only by a diminution of the contraction to MBF itself but also by a subsequent depression of the response to a fixed dose of nicotine. As shown in Fig. 7, the response to a 20 μg. dose of nicotine was constant and repeatable even after a long period of time (see, for instance, A, G, and H). Four minutes after the 25 μg. dose of MBF at E the response to nicotine, at F, was reduced to less than half its original height at A. An interval of 50 min. was allowed for recovery before the next two test doses of nicotine at G and H, which elicited contractions of constant height again. The 40 μg. dose of MBF at I, although itself not causing any appreciable contraction, was followed 3 min. later at J by a reduction of the nicotine response to a quarter of its previous height and by recovery at K 5 min. later. It is necessary to stress the fact that this temporary inexcitability to nicotine is not due to an effect of the type described by Cantoni and Eastman (1946). These authors found that after a vigorous intestinal contraction the guinea-pig gut exhibits a depression of pharmacological responses; it is not clear as yet whether this effect is due to metabolic exhaustion after a powerful contractile effort, or to a state of true inexcitability. In the rabbit's intestine Beznák (1952) has shown that large contractions induced by acetylcholine are followed by temporary arrest of pendulum movements. However, the decreased response to nicotine in Fig. 7 J cannot be due to a phenomenon of this kind, since the administration of MBF at I was neither attended by contraction nor followed by an arrest of pendulum movements.

It is clear from these experiments that MBF can in small doses stimulate, and in excess depress, the particular structure or structures in the gut which normally respond to stimulating doses of nicotine. Although it would be surprising if the autonomic ganglia in the enteric plexus were insensitive to nicotine, it is still uncertain whether nicotine-sensitivity in the gut is restricted to the ganglion-cell bodies, since it is now known that nicotine is also capable of stimulating some types of nerve-endings. It is therefore theoretically possible that the " axonic " actions of nicotine and MBF may contribute partly to the effect of these drugs on the gut. In the absence of evidence on this point, it is perhaps advisable at present to think of the motor action of these drugs as being due to stimulation of the motor neurones in the gut, possibly at both ends of these neurones. Likewise the " paralytic " effect of larger doses of these drugs indicates a state of " block " in those neurones, not necessarily confined entirely to the ganglion-cell end.

Reversal by Botulinum Toxin.—The experiments with botulinum toxin provide further evidence of the " indirect " nature of the motor response to MBF. There are numerous examples of the ease and rapidity with which botulinum toxin is able to paralyse cholinergic nerves at their endings. And as it is commonly supposed that the motor neurones in the intestine are cholinergic, it should be possible, with this toxin, to put these neurones out of action by paralysing their endings. This opens the possibility of eliminating the contractile response to certain drugs whose principal effect on the gut is supposed to be due to stimulation of the motor neurones; it provides another means of examining to what extent the action of these drugs is " indirect." In fact, as was shown previously, when the rabbit's intestine is treated with toxin, the motor action of nicotine is eventually suppressed and then actually undergoes a complete reversal (Ambache, 1951). In the present experiments the motor effect of MBF has likewise been greatly reduced or even abolished by the toxin. In seven of nine experiments the extinction of the motor response to MBF proceeded far enough to allow a reversal to become apparent, as with nicotine. This may be looked upon merely as an unmasking, by the motor neurone paralysis, of an underlying inhibitory action of MBF, resembling the inhibitory action of nicotine which was discussed in the earlier paper. It is necessary to stress the fact that, in all the seven experiments in which reversal occurred, the inhibition was produced by doses of MBF which in the 22 normal preparations had always been motor. In order to emphasize the specificity of this reversal the following procedure was adopted in all but one of these experiments. As shown in Figs. 9 and 10, each experiment was begun by obtaining a match on the control segment between contractions produced by MBF (and sometimes by nicotine) on the one hand, and contractions produced by drugs possessing an intense direct (or " muscarinic ") action on the muscle fibres of the gut, such as 2268F, acetylcholine, or muscarine itself. After these matches were obtained on the control, the poisoned segment from the same animal was suspended in the organ-bath, and the previously matched doses of these various drugs were tried again. Whenever this was done it was

found that a given dose of MBF, which in the control segment of gut had elicited a contraction, would produce relaxation in the botulinized segment from the same animal. Moreover, the reversal produced by the toxin was restricted to the action of MBF (and of nicotine, Fig. 10 F). Thus, the motor effect of muscarine, of 2268F, and of acetylcholine was unaltered on the gut treated with botulinum toxin, as shown in Figs. 9 and 10; in contrast, the previously equiactive doses of MBF not only failed to evoke a contraction but actually inhibited pendulum movements. In three of these experiments the " reversed " or inhibitory action of MBF was abolished by hexamethonium (Fig. 9 I).

Interaction with Atropine.—The observation that the motor response to nicotine of the rabbit's intestine is resistant to small doses of atropine which abolish the action of acetylcholine has been made by numerous workers in the past (for detailed references see Ellis and Rasmussen, 1951, p. 1). Furthermore it has been shown by Vogt (1943), von Euler (1945), and Ellis (1951) that the responses to other substances, likewise believed to be ganglion-cell-stimulating agents (NaCl, Na lactate, and piperidine), are also unaffected by atropine in rabbits. As first suggested by Vogt (1943), this phenomenon, which has been observed so far only in the rabbit's intestine but not in that of guinea-pigs or young kittens (Ambache and Edwards, 1951), appears to bear some resemblance to the atropine-resistance of certain parasympathetic cholinergic nerve-effects.

Ellis (1951) has suggested that the species differences in response to atropine referred to above may be of some use in the classification of drug actions. According to him, additional information as to whether an action is " muscarinic, nicotinic, or musculotropic " may be obtained by duplicate-testing on the atropinized intestines from two species, one of which must be known to exhibit atropine-resistance to nicotine, as in the rabbit, and the other not (guinea-pig or cat). Substances with a muscarine-like action would be antagonized by atropine 10^{-6} (Ellis, 1951) on both preparations; substances with a " musculotropic " action would not be antagonized by the same dose of atropine on either preparation; lastly, nicotine-like substances would be atropine-sensitive in intestinal preparations from guinea-pigs (or cats), but would exhibit some measure of atropine-resistance in rabbit gut. Our present experience with nicotine and MBF and the earlier experiments of Ambache and Edwards (1951) show that atropine-resistance in rabbits and guinea-pigs is itself subject to variation. Nevertheless, if allowance is made for this,

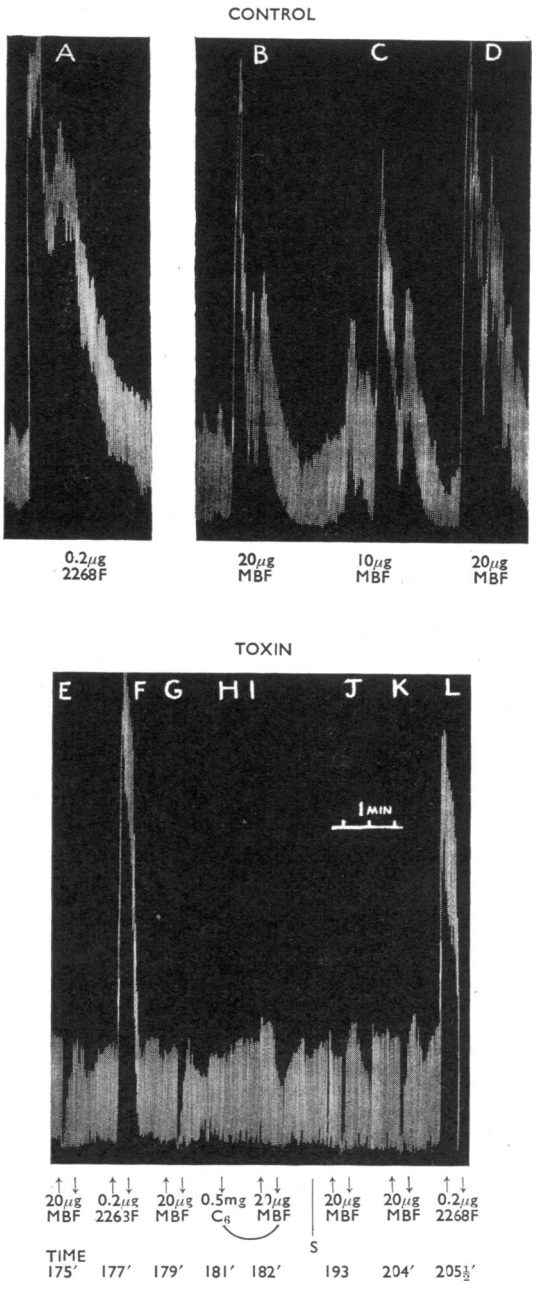


FIG. 9.—Reversal of the response to MBF by botulinum toxin Rabbit, 2.6 kg. *Top record* from a control segment of ileum (85 cm. from pylorus) excised before the injection of toxin. *Bottom record* from a segment 5–10 cm. lower, which was injected with 3 mg. of toxin A_2. The rabbit was killed 190 min. later. Time on the bottom record is in minutes from the moment of death. Drum stopped at S for 8 min. All drugs administered for 30 sec., except H. At A, F, and L, 0.2 μg. 2268F. At B, D, E, G, I, J, and K, 20 μg. of MBF. At C, 10 μg. of MBF. At H, 0.5 mg. of hexamethonium 60 sec. before I.

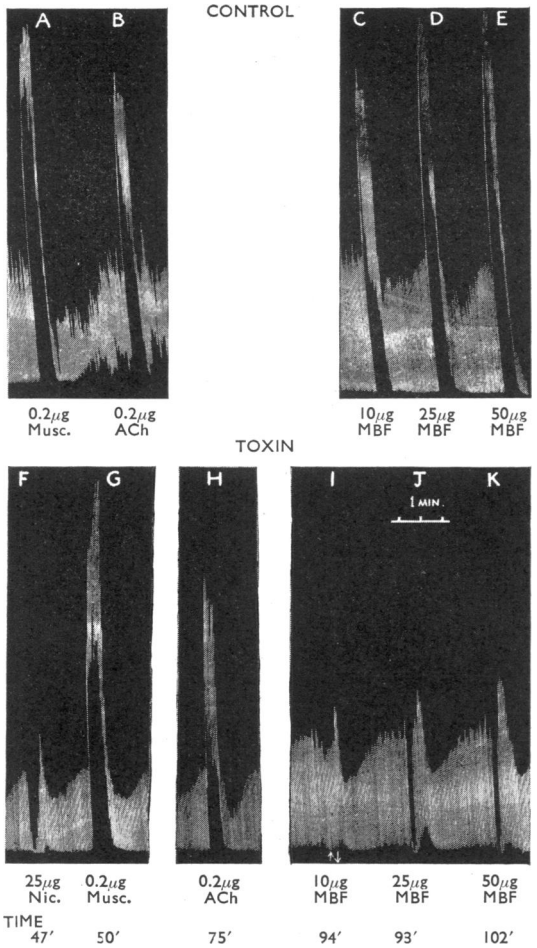


FIG. 10.—Specificity of the MBF-reversal by botulinum toxin. Rabbit, 3.4 kg. (coeliac and superior mesenteric ganglia excised 14 months previously). *Top record* from a control segment of ileum excised before the injection of toxin. *Bottom record* from an adjoining segment which was injected with 2.4 mg. of toxin A_2. Rabbit killed 85–90 min. after injection. Time in the bottom record in minutes from the moment of death. At A and G: 0.2 μg. muscarine. At B and H: 0.2 μg. acetylcholine. MBF at C and I, 10 μg.; at D and J, 25 μg.; at E and K, 50 μg. At F, 25 μg. nicotine.

a certain amount of information can be gleaned from duplicate tests of this kind. The results may be summarized by saying that the nicotine response was not invariably atropine-resistant in rabbits, but that, when it was so (in three of five preparations), the MBF response followed suit and persisted (though slightly reduced) in the presence of atropine 10^{-7} or 10^{-6}. But in guinea-pigs atropine $0.5–2\times10^{-7}$ was sufficient to extinguish nicotine and MBF responses.

Because of the variations mentioned the atropine-resistance of MBF was studied in parallel with that of nicotine, and the two appeared to vary to the same extent. In detail the results on rabbits were as follows. In preparation No. 1 the contraction produced by 10 μg. of MBF persisted in the presence of atropine 10^{-6}, though reduced by 20%; an equivalent dose of muscarine was ineffective. In preparation No. 2, 20 μg. of MBF was matched by 20 μg. of nicotine and 0.4 μg. of muscarine (see Fig. 11). In the presence of atropine 10^{-7} the response to muscarine was abolished; that to nicotine and MBF persisted, though both were reduced to the same extent. In experiment No. 3 a match was obtained between 10 μg. of MBF and 8 μg. of nicotine; both effects were equally reduced, to 50% by atropine 10^{-7} and to 40% by atropine 10^{-6}. In two other experiments both the MBF and the nicotine effects were greatly reduced or abolished by atropine $0.5–2\times10^{-7}$.

In three of the four experiments on guinea-pig's ileum $0.5–1\times10^{-7}$ of atropine completely abolished the response to equipotent doses of MBF and nicotine; this effect was reversible. As in the rabbit gut, the MBF response was blocked by hexamethonium. In the two experiments on kitten's ileum the actions of 2–20 μg. of MBF and of 2.5–10 μg. of nicotine were abolished by $0.5–2\times10^{-7}$ of atropine.

DISCUSSION

It is clear that the mono- and dibromo- derivatives of choline phenyl ether possess intense nicotine-like actions not only on ganglia but also on skeletal muscle and on nicotine-sensitive axons in the skin. The motor action of these compounds on the longitudinal layer of the gut also resembles that of nicotine in the following respects: (1) An increase in dose may produce a decrease in effect, perhaps because of the onset of nicotine-like "paralysis"; (2) the effect is antagonized by hexamethonium; (3) it can be reversed by botulinum toxin; (4) the effect of these compounds is usually atropine-resistant in intestinal preparations from rabbits, but not from guinea-pigs or cats. All these properties would seem to point to an "indirect" action of these compounds on the gut through its motor nerve-supply, but there is no clue at present whether this action is entirely confined to the ganglion-cells or not. The fact that it is antagonized by hexamethonium is no longer decisive, since hexamethonium blocks nicotinic effects not only on ganglia but also at nerve-endings (Douglas and Gray, 1953). Hexamethonium can therefore no longer be used with absolute certainty to decide whether a nicotinic effect is due to stimulation at the ganglion-cell level or at nerve-endings. An action at both levels is theoretically possible with

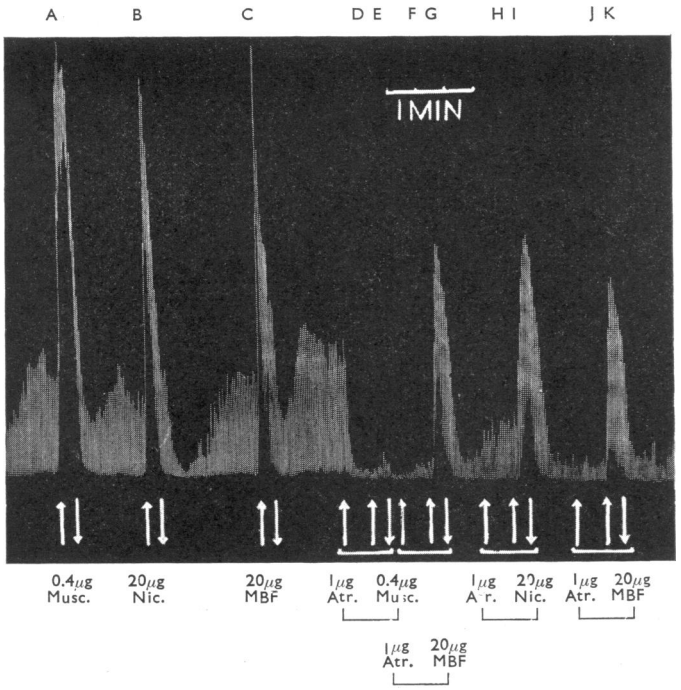


FIG. 11.—Example of atropine-resistance of the MBF response. Rabbit ileum 150 cm. below pylorus. Approximate matches between A, 0.4 μg. of muscarine; B, 20 μg. of nicotine; and C, 20 μg. of MBF. Atropine 1 μg. at D, F, H, and J for 60 sec. before repeating 0.4 μg. of muscarine at E, 20 μg. of nicotine at I, and 20 μg. of MBF at G and K. The action of muscarine is abolished by atropine, but the response to MBF and to nicotine persists, though reduced (both to a similar extent).

MBF and DBF (as with nicotine), since these drugs are capable, apart from stimulating ganglia, of exciting axon reflexes in certain ganglion-cell-free nerve-nets, for instance in the skin. There is still no evidence whether such an "axonic" action of nicotine and its congeners can or does occur at other nerve-endings in smooth muscle, but it is a possibility which cannot be overlooked.

SUMMARY

A number of nicotine-like actions of 3-bromo- and 3:5-dibromo-phenyl ethers of choline (MBF and DBF) are described, notably:

1. Their ability to excite skeletal muscle, as exemplified by the response of the frog's rectus abdominis (antagonized by *d*-tubocurarine) and by constriction of the pigeon's iris.

2. Stimulation and block of autonomic neurones either at the cell-body or at the nerve-endings. The action on ganglia has been examined on the perfused superior cervical ganglion of cats. That on nerve-endings (here termed the "axonic" action) can be conveniently studied in the human skin on the pilomotor endings. Both actions are blocked by hexamethonium.

3. The intestinal motor action, which can be interpreted in terms of nicotine-like stimulation of the motor neurones in the gut. This effect is blocked by hexamethonium and reversed by botulinum toxin. Its interaction with atropine varies in different species in the same way as that of nicotine. There is evidence of block with MBF in larger doses, as shown by the depression of nicotine-responses.

We wish to thank Dr. P. Hey for the gift of these compounds, and Miss Daphne Taylor for her technical assistance.

REFERENCES

Ambache, N. (1949). *J. Physiol.*, **110**, 145.
—— (1951). *Brit. J. Pharmacol.*, **6**, 51.
—— and Edwards, J. (1951). Ibid., **6**, 311.
Beznák, M. (1952). Ibid., **7**, 237.
Brown, G. L., and Gray, J. A. B. (1948). *J. Physiol.*, **107**, 306.
Cantoni, G. L., and Eastman, G. (1946). *J. Pharmacol.*, **87**, 392.
Coon, J. M., and Rothman, S. (1940). Ibid., **68**, 301.
—— —— (1941). Ibid., **73**, 1.
Douglas, W. W., and Gray, J. A. B. (1953). *J. Physiol.*, **119**, 118.
Ellis, S. (1951). *Science*, **114**, 325.
—— and Rasmussen, H. (1951). *J. Pharmacol.*, **103**, 259.
Euler, U. S. von (1945). *Acta Pharmacol. Kbh.*, **1**, 29.
Feldberg, W. (1951). *J. Physiol.*, **113**, 483.
Hey, P. (1952). *Brit. J. Pharmacol.*, **7**, 117.
Ranson, S. W., and Billingsley, P. R. (1918). *J. Comp. Neurol.*, **29**, 313.
Vogt, M. (1943). *J. Physiol.*, **102**, 170.