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NEUROMUSCULAR BLOCKING PROPERTIES OF SOME BISTROPINIUM ESTERS

BY

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The neuromuscular blocking, anti-acetylcholine and ganglion blocking properties of two series of bistropinium esters were examined. The neuromuscular blocking activities of the mandelic acid esters of *NN'*-polymethylenebis(tropinium halides) were found to depend upon the number of carbon atoms (*n*) in the linking chain. Potency was enhanced more than 50 times as *n* was increased from 2 to 7. Compounds in which *n* equalled 7, 8, 9, 10 and 12 differed little in activity, but were generally more potent than tubocurarine in cats and rabbits. A peak of ganglion blocking action was obtained at the pentamethylene member. Esterification enhanced the feeble neuromuscular blocking properties of *NN'*-decamethylenebis(tropinium halide), the mandelic acid ester being more effective than the tropic, benzoic or phenylacetic acid esters in cats and rabbits. When two benzoic or mandelic acid esters of tropine were linked through their nitrogen atoms by a phenylenedimethyl grouping (-$CH_2.C_6H_4.CH_2$-), *meta* substitution was more effective than was *ortho* or *para* in producing neuromuscular block. The effectiveness of esterifying acids in *m*-phenylenedimethyl derivatives decreased in the following order, phenylacetic> tropic or mandelic>benzoic>acetic and diphenylacetic.

The linkage of two atropine molecules through their nitrogen atoms by a suitable chain gives compounds with pronounced neuromuscular blocking properties (Kimura, Unna & Pfeiffer, 1949). Kimura & Unna (1950) showed that when the number of methylene groups in *NN'*-polymethylenebis(atropinium iodides) was increased from five to ten the dose required to produce head-drop in rabbits fell from 0.35 mg/kg to 0.06 mg/kg, and since this work was started values obtained for the hexamethylene and octamethylene members have also been reported (Eckfeld, 1959). Nádor, Issekutz & Kovatsits (1950), Issekutz (1952) and Gyermek & Nádor (1952) have also demonstrated that esters of *NN'*-*p*-phenylenedimethylbis-(tropinium bromide) are potent muscle relaxants.

We were interested in exploring further the structural requirements for neuromuscular blocking activity in bistropinium compounds, and this paper deals mainly with the effects of varying the esterifying acid on the pharmacological properties of polymethylene- or phenylenedimethyl-linked bis-quaternary tropeïnes.

METHODS

Determinations of intravenous toxicity in mice, anti-acetylcholine activity on the isolated guinea-pig ileum and the effects of compounds on the blood pressure of anaesthetized cats were carried out as described previously (Haining, Johnston & Smith, 1960).

The ganglion blocking activity of compounds was compared with that of hexamethonium iodide after intravenous injection into cats anaesthetized with chloralose 80 mg/kg intraperitoneally. The central end of the cut vago-sympathetic trunk was stimulated by rectangular pulses of 0.2 msec duration at a rate of 30 to 50/sec for 9 sec in each 72. Relative potencies of compounds were calculated from the doses estimated to reduce the height of the contractions of the nictitating membrane by 50%.

Curare-like activity was determined using several preparations. The mean head-drop dose in rabbits was estimated by a technique similar to that described by Dutta & Macintosh (1949), and activity in mice was determined on a rotating drum after intravenous injection (Collier, Hall & Fieller, 1949) using three groups of ten mice for each compound. Compounds were compared with tubocurarine chloride in cats using the gastrocnemius muscle stimulated indirectly by condenser discharges of less than 1 msec duration, usually at a rate of 8/min (Burn, Finney & Goodwin, 1950). Relative potencies of compounds were determined by plotting either the time taken for recovery of the twitch to a fixed percentage of its pre-injection height or the maximum reduction of twitch height, against the logarithm of the dose on the assumption that regression lines were parallel.

Compounds examined are shown in Table 1. Relative potencies and doses of quaternary compounds are expressed throughout in terms of cation unless the anion is also stated.

TABLE 1

NAMES OF COMPOUNDS TESTED IN THIS INVESTIGATION

No.	
1	*NN'*-Ethylenebis(3-mandeloyloxytropanium bromide)
2	*NN'*-Trimethylenebis(3-mandeloyloxytropanium bromide), ethyl acetate complex
3	*NN'*-Tetramethylenebis(3-mandeloyloxytropanium bromide)
4	*NN'*-Pentamethylenebis(3-mandeloyloxytropanium bromide)
5	*NN'*-Hexamethylenebis(3-mandeloyloxytropanium iodide)
6	*NN'*-Heptamethylenebis(3-mandeloyloxytropanium bromide), ethyl acetate complex
7	*NN'*-Octamethylenebis(3-mandeloyloxytropanium bromide)
8	*NN'*-Nonamethylenebis(3-mandeloyloxytropanium bromide)
9	*NN'*-Decamethylenebis(3-mandeloyloxytropanium bromide)
10	*NN'*-Dodecamethylenebis(3-mandeloyloxytropanium bromide)
11	*NN'*-Decamethylenebis(tropinium bromide)
12	*NN'*-Decamethylenebis(3-phenylacetoxytropanium bromide)
13	*NN'*-Decamethylenebis(3-benzoyloxytropanium bromide)
14	*NN'*-Decamethylenebis(3-tropoyloxytropanium iodide) or *NN'*-Decamethylenebis(atropinium iodide)
15	*NN'*-Tetramethylenebis(3-phenylacetoxytropanium bromide)
16	*NN'*-Pentamethylenebis(3-phenylacetoxytropanium bromide)
17	*NN'-o*-Phenylenedimethylbis(3-mandeloyloxytropanium bromide)
18	*NN'-p*-Phenylenedimethylbis(3-mandeloyloxytropanium bromide)
19	*NN'-o*-Phenylenedimethylbis(3-benzoyloxytropanium bromide)
20	*NN'-p*-Phenylenedimethylbis(3-benzoyloxytropanium bromide)
21	*NN'-m*-Phenylenedimethylbis(3-acetoxytropanium bromide)
22	*NN'-m*-Phenylenedimethylbis(3-phenylacetoxytropanium bromide)
23	*NN'-m*-Phenylenedimethylbis(3-diphenylacetoxytropanium bromide)
24	*NN'-m*-Phenylenedimethylbis(3-benzoyloxytropanium bromide)
25	*NN'-m*-Phenylenedimethylbis(3-mandeloyloxytropanium bromide)
26	*NN'-m*-Phenylenedimethylbis(3-tropoyloxytropanium bromide) or *NN'-m*-Phenylenedimethylbis(atropinium bromide)

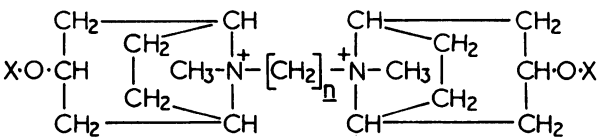
RESULTS

Esters of decamethylenebis(tropinium halides). In order to determine the extent to which the structure of the esterifying acid influenced neuromuscular blocking activity compounds consisting of two identical tropine esters linked through their

TABLE 2

THE NEUROMUSCULAR BLOCKING AND ANTI-ACETYLCHOLINE ACTIVITY OF ESTERS OF POLYMETHYLENEBIS(TROPINIUM HALIDES)

The number of estimates is shown in parentheses. Effects marked with an asterisk were reversible with neostigmine



Cpd. no.	*n*	X	Anti-acetylcholine activity on guinea-pig ileum (atropine=1·0)	Neuromuscular block: Cat relative potency	Neuromuscular block: Rabbit head-drop dose (mg/kg) (s.d.)	Neuromuscular block: Rat diaphragm relative potency	Neuromuscular block: Mouse ED50 and 95% limits (mg/kg)
11	10	-H	0·15	0·02 (1)	>10		
12	10	$-CO\cdot CH_2-C_6H_5$	0·02	0·8 (2)*	0·19 (0·07)	0·05 (3)	0·30 (0·27–0·33)
13	10	$-CO\cdot C_6H_5$	0·04	1·2 (2)	0·20 (0·03)	0·05 (3)	0·12 (0·10–0·14)
9	10	$-CO\cdot CH(OH)\cdot C_6H_5$	0·2	3·7 (3)	0·08 (0·04)	0·03 (3)	0·11 (0·10–0·12)
14	10	$-CO-CH(CH_2OH)\cdot C_6H_5$	0·25	1·8 (2)	0·15 (0·07)	0·03 (3)	0·16 (0·15–0·17)
15	4	$-CO\cdot CH_2\cdot C_6H_5$			0·30 (0·04)		
16	5				0·49 (0·16)		
Tubocurarine				1·0	0·22 (0·10)	1·0	0·039 (0·034–0·045)

nitrogen atoms by a decamethylene chain were examined in the cat, rabbit, rat and mouse (Table 2). The anti-acetylcholine activity of these compounds was also estimated on the isolated guinea-pig ileum in order to determine whether there was any correlation between the structural requirements of the esterifying acid for antagonism at muscarinic and nicotinic sites.

The *NN'*-decamethylenebistropinium ion (11) itself had only about one-fiftieth the neuromuscular blocking potency of tubocurarine in cats and rabbits, but esterification of both hydroxyl groups by the same aromatic acid gave compounds which were highly active in both species. In cats the most potent compound was the mandelic acid ester (9), which was approximately four times as effective as tubocurarine. Tropic (14), benzoic (13) and phenylacetic acid (12) esters were progressively less active although comparable to tubocurarine in potency. The order of effectiveness was similar in rabbits but not in mice or on the isolated phrenic nerve-diaphragm preparation of the rat. The potency of these tropeïnes relative to tubocurarine was considerably less in mice and rats than in cats or rabbits; for the mandelic acid ester (9) there was almost an eightfold reduction of sensitivity in mice and a hundredfold in rats.

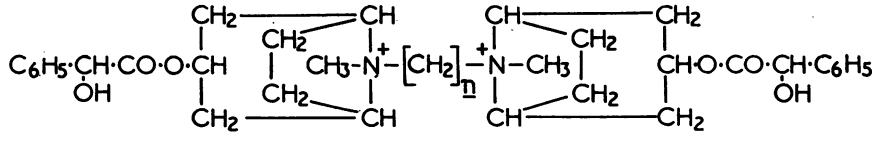
Only the tropic (14) and mandelic acid (9) esters showed marked activity against acetylcholine on the isolated guinea-pig ileum, the tropic acid derivative having a quarter of the activity of atropine.

Mandelic acid esters of polymethylenebis(tropinium halides). The influence of inter-onium distance on neuromuscular and ganglion blocking properties was examined in a series of mandelic acid esters of tropine linked through their nitrogen atoms by a polymethylene chain in which the number of methylene groups was varied between two and twelve (Table 3, Fig. 1).

TABLE 3

EFFECT OF CHAIN LENGTH ON THE NEUROMUSCULAR AND GANGLION BLOCKING ACTIVITY OF MANDELIC ACID ESTERS OF POLYMETHYLENEBIS(TROPINIUM HALIDES)

The number of estimates is shown in parentheses. Effects marked with an asterisk were reversible with neostigmine



No.	*n*	Anti-acetylcholine activity on guinea-pig ileum (atropine=1·0)	Neuromuscular blocking activity in the cat (tubocurarine=1·0)	Ganglion blocking activity in the cat (hexamethonium=1·0)
1	2	0·2	<0·04 (1)	0·3 (1)
2	3	0·1		
3	4	0·2		<0·1 (2)
4	5	<0·01	0·1 (1)	2·2 (4)
5	6	<0·01	1·1 (1)*	<0·1 (1)
6	7	0·02	≃2·0 (1)	
7	8	0·1	1·7 (1)	0·1 (1)
8	9	0·1		
9	10	0·2	3·7 (3)	0·5 (2)
10	12	0·25	1·7 (1)	

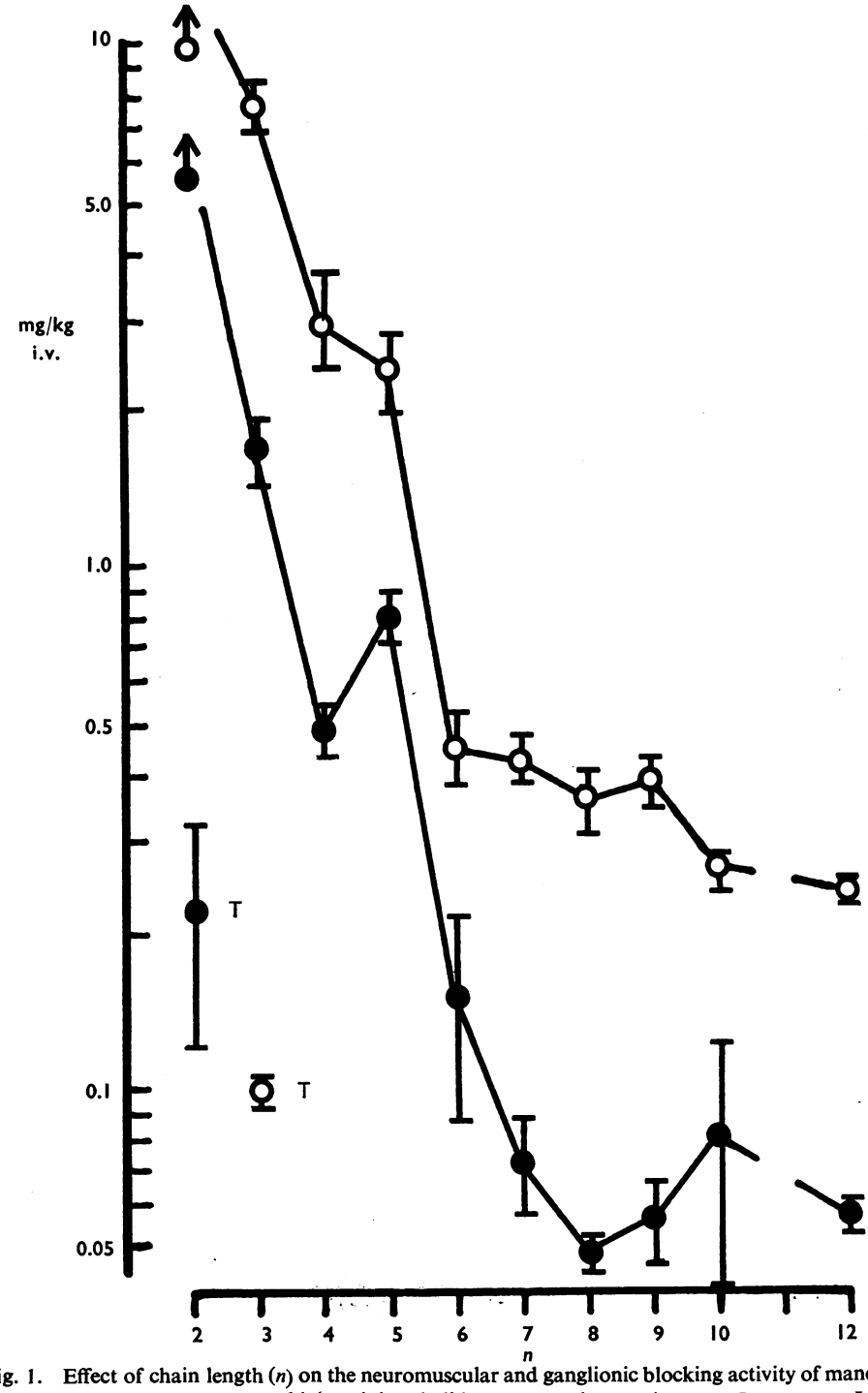


Fig. 1. Effect of chain length (n) on the neuromuscular and ganglionic blocking activity of mandelic acid esters of polymethylenebis(tropinium halides). T = Tubocurarine. ○ = Intravenous LD50 in mice with P 0.95 limits. ● = Mean head-drop dose in rabbits by intravenous infusion with s.d.

Neuromuscular blocking activity was found to depend upon the number of methylene groups in the chain (n). As n was increased from two to seven there was more than a fiftyfold reduction in the dose required to produce head-drop in rabbits from over 5 mg/kg to less than 0.1 mg/kg, and potency in cats and intravenous toxicity in mice increased to a comparable extent. No sharp peak in activity was obtained with compounds containing between seven and twelve methylene groups, all those tested being more active than tubocurarine in cats and rabbits. Visual comparisons of recovery curves obtained after administering these compounds to cats gave no indication that the recovery rate of any of them differed from that of tubocurarine.

The series showed a peak of ganglion blocking activity at the pentamethylene member (4) with the suggestion of a smaller peak at the decamethylene (9) compound. Direct comparisons of the hypotensive properties of these compounds in cats gave the following estimates of relative potency: $n=2$, 0.1 ; $n=5$, 1.0 ; $n=6$, 0.1 ; $n=10$, 0.4.

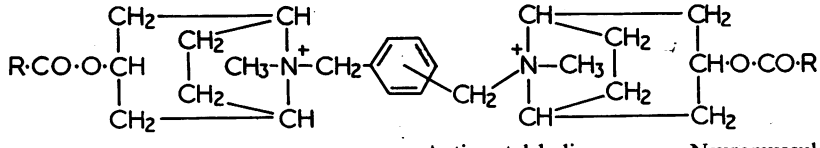
The activities of compounds against acetylcholine on the isolated guinea-pig ileum varied with the length of the polymethylene chain, those in which n was 5, 6 or 7 being considerably less active than the remaining members of the series.

Esters of phenylenedimethylbis(tropinium halides). Comparison of the effects of *ortho*, *meta* and *para* linkages on the neuromuscular blocking properties of phenylenedimethylbistropinium esters were determined in rabbits and cats. Similar results were obtained in both species (Table 4, Fig. 2). Potencies relative to tubo-

TABLE 4

THE NEUROMUSCULAR BLOCKING AND ANTI-ACETYLCHOLINE ACTIVITY OF ESTERS OF PHENYLENEDIMETHYLBIS(TROPINIUM HALIDES)

The number of estimates is shown in parentheses. Effects marked with an asterisk were reversible with neostigmine



No.	Orientation	R	Anti-acetylcholine activity on guinea-pig ileum (atropine=1·0)	Neuromuscular blocking activity in the cat (tubocurarine=1·0)
17	*ortho*	$-CH(OH)\cdot C_6H_5$		0·2 (1)
18	*para*			0·9 (2)
19	*ortho*	$-C_6H_5$		<0·3 (1)
20	*para*			0·9 (2)
21	*meta*	$-CH_3$	<0·01	0·4 (2)
22		$-CH_2\cdot C_6H_5$	0·01	4·9 (2)*
23		$-CH(C_6H_5)_2$	<0·002	0·3 (2)
24		$-C_6H_5$	<0·01	1·2 (2)
25		$-CH(OH)-C_6H_5$	0·05	4·0 (5)*
26		$-CH(CH_2OH)\cdot C_6H_5$	0·35	3·9 (3)

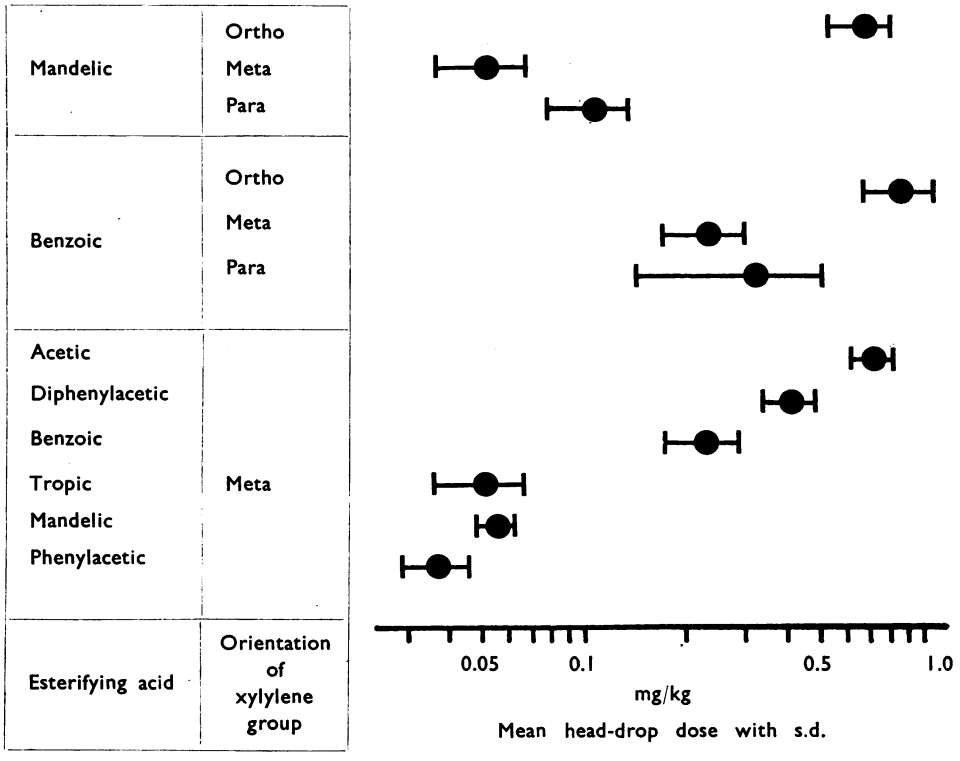


FIG. 2

curarine obtained for the mandelic (18) and benzoic acid (20) esters of *p*-phenylenedimethylbis(tropinium halides) are in agreement with those determined by Nádor, Issekutz & Kovatsits (1950) in frogs.

In the case of mandelic and benzoic acid esters, *ortho* substitution was considerably less effective than was *meta* or *para*. The difference was most marked with *NN'-m*-phenylenedimethylbis(3-mandeloyloxytropanium bromide) (25), which was over fifteen times more effective than its *ortho* isomer (17). Differences between *meta*- and *para*-substituted compounds were less marked, but the *meta* form tended to be more active, *NN'-m*-phenylenedimethylbis (3-mandeloyloxytropanium bromide) (25) being two to four times as potent as the corresponding *para* compound (18). For this reason the effects of altering the esterifying acid were investigated in esters of *m*-phenylenedimethylbis(tropinium halides).

NN'-m-Phenylenedimethylbis(3-acetoxytropanium bromide) (21) was less than half as active as tubocurarine, but replacement of one hydrogen atom of the acetyl group by a phenyl group (22) gave more than a tenfold increase in potency. Replacement of a second hydrogen atom by a hydroxyl group (25) or a hydroxymethyl group (26) did not enhance neuromuscular blocking activity, but a hydroxymethyl group (26) considerably increased atropine-like properties. The effect of a second phenyl group (23) was to reduce neuromuscular blocking potency to the level of the acetic acid ester. At least one methylene group between the phenyl and

carbonyl groups of the esterifying acid appeared to be of advantage in conferring high activity, since the potency of the benzoic acid ester (24) was only between a third and a quarter that of the corresponding phenylacetic acid ester (22).

All compounds shown in Table 1 were administered to chicks by subcutaneous injection and in each case a flaccid paralysis was produced. Reversibility of neuromuscular block in cats by neostigmine was confirmed for compounds 5, 12, 22 and 25.

NN'-m-*Phenylenedimethylbis(3-phenylacetoxytropanium bromide).* This ester (22) was examined in greatest detail, since it was the most active compound tested and preliminary results had suggested that its duration of action in rabbits was somewhat less than that of tubocurarine. Comparisons of its potency and duration of action with tubocurarine were carried out in cats, and, to exclude cumulative effects, the effect of a single dose of either compound was determined in each animal. In seven cats the mean dose of tubocurarine giving between 75% and 100% reduction in the height of the gastrocnemius twitch was 0.28 mg/kg (s.d. 0.037), the mean reduction of twitch height 94% (s.d. 6.3) and the time for recovery of twitch to 70% of its initial height 22.0 min (s.d. 9.6). Corresponding values obtained with *NN'-m*-phenylenedimethylbis(3-phenylacetoxytropanium bromide) (22) in six animals were 0.066 mg/kg (s.d. 0.014), 94% (s.d. 7.9) and 23.4 min (s.d. 14.3). This ester (22) was therefore approximately four to five times as potent as tubocurarine on a weight basis and there was no significant difference in the duration of action of the two compounds.

NN'-m-Phenylenedimethylbis(3-phenylacetoxytropanium bromide) (22) produced considerably less effect on blood pressure than did tubocurarine. The effect of a single dose of each compound was determined in anaesthetized but otherwise untreated cats maintained with the aid of intratracheal oxygen. In three animals tubocurarine 0.32 mg/kg failed to abolish the twitch completely and gave a rapid fall of blood pressure (58 to 87 mm mercury), but in four cats receiving 0.16 mg/kg of the ester (22) and four receiving 0.32 mg/kg complete inhibition of twitch was obtained with a fall of 15 mm in one case only and a slight gradual rise with the others. Anti-acetylcholine activity determined on the cat blood pressure was 0.01 that of atropine.

NN'-m-Phenylenedimethylbis(3-phenylacetoxytropanium bromide) (22) showed cumulative properties in cats which would presumably be attributed to its prolonged action, and behaved in a manner characteristic of non-polarizing compounds. Paralysis was readily reversed by neostigmine and antagonism towards decamethonium could be demonstrated. Responses to a tetanus were poorly maintained and post-tetanic facilitation was obtained. On the isolated frog rectus abdominis muscle no contracture was produced, but the responses to acetylcholine were antagonized. The ganglion blocking activity was approximately half that of hexamethonium when compared on the cat nictitating membrane preparation.

DISCUSSION

The esters of decamethylenebis(tropinium halides) may be considered as derivatives of decamethonium [decamethylenebis(trimethylammonium)] in which each trimethylammonium group is incorporated in a tropinium moiety. Successive replacement

of the methyl groups in decamethonium by bulkier structures usually leads to a transition from depolarizing to non-depolarizing properties (Barlow, Roberts & Reid, 1953 ; Thesleff & Unna, 1954 ; Arïens & de Groot, 1954), generally accompanied by a loss of potency which has been ascribed partially to steric hindrance preventing the nitrogen atoms approaching sufficiently close to the anionic receptors (Cavallito, 1959). The magnitude of the potency change appears to depend upon the sensitivity of the test preparation to depolarizing drugs. Replacement of trimethylammonium grouping by the tropinium moiety gives a non-depolarizing compound with negligible neuromuscular blocking activity, and it is only after esterification that potent blocking compounds are obtained.

Steric hindrance may possibly account in part for the feeble activity of *NN'*-decamethylenebistropinium (11). Such steric effects would be even more prominent on acylation, and the fact that the bis-quaternary acyl tropiniums are much more potent implies that the acyl moieties themselves are contributing to the neuromuscular blocking properties. The provision of additional active centres capable of attachment to complementary sites on the receptor substance could increase the overall binding capacity of the molecule not only directly but also by diminishing the average distance of the positively charged nitrogen from the surface.

Thus, if stimulant properties imply an ability to complex very closely with the receptor (Gill, 1959), by the above argument suitable substitution at position 3 of the tropine molecule may enable the electrostatic attraction between cationic nitrogen and the anionic site to be more strongly exerted. That this is so is suggested by the fact that replacement of the hydroxyl group of α-tropine by a phenyl group confers pressor properties on a depressor compound, whilst, in the case of β-tropine, its pressor action is enhanced (Lands & Archer, 1960). Substitution of halogen at the position 3β of tropane also confers stimulant properties which can be demonstrated on the isolated frog rectus abdominis muscle (unpublished observation).

It seems most likely, however, that the main role of the ester group is to provide active centres which can themselves be attracted by the receptor surface. In this connexion Kimura & Unna (1950) have postulated that there is a relationship between the active centres of bistropinium esters and those for acetylcholine at the motor end-plate as is the case with tropine esters at muscarinic sites. Although the receptors at the motor end-plate are cholinergic, results obtained in the present experiments indicate that with the two types of linking chains employed structural requirements for antagonism at these sites are not necessarily those which are most effective at muscarinic receptors. This, of course, does not imply that structures effective as antagonists at muscarinic sites will not also be active when incorporated in bisonium molecules. The difficulty may be to ensure that all active centres are able to achieve optimal fit at receptor sites in view of the restrictions imposed on molecular orientation by the linking structure. The presence of a phenyl group separated from the carbonyl group of the esterifying acid by one methylene group was sufficient to confer marked neuromuscular blocking properties on esters of *m*-phenylenedimethylbis(tropinium halides), but alpha substitution by hydroxyl or hydroxymethyl groups, so effective in enhancing the atropine-like properties of

phenylacetyltropeïne, had little effect on the neuromuscular blocking properties of *NN'*-decamethylenebis(3-phenylacetoxytropanium bromide) (12) and *NN'*-*m*-phenylenedimethylbis(3-phenylacetoxytropanium bromide) (22).

In the compounds investigated the following factors were found to influence potency: nitrogen–nitrogen distance, constitution of the linking chain and the presence of terminal groups which could influence the attachment of the molecule to receptors. In the mandelic acid esters of polymethylenebis(tropinium halides) the effect of increasing the number of methylene groups in the chain was to enhance potency (Table 3, Fig. 1). There was, however, no sharp peak of neuromuscular blocking activity such as is found in the methonium series (Barlow & Ing, 1948; Paton & Zaimis, 1949), and this contrasted with the sharp rise in ganglion blocking properties where *n* equalled five. In this series there was a considerable enhancement of neuromuscular blocking activity when *n* was increased from five to six, an effect observed in cats, mice and rabbits, but with values between six and twelve no striking changes in potency occurred. Thus the head-drop dose of the pentamethylene compound (4) in rabbits was 0.8 mg/kg, but increasing the chain by one carbon atom reduced it to 0.15 mg/kg, whereas further extension to twelve carbon atoms gave compounds with head-drop doses within the range 0.05 mg/kg to 0.15 mg/kg. In mice, too, when *n* equalled five the intravenous LD50 was 2.4 mg/kg, but between the hexamethylene and the dodecamethylene members the range was only 0.24 mg/kg to 0.45 mg/kg.

The extended lengths of flexible chains such as these give no indication of the optimum requirements for inter-onium distance, as it is fallacious to assume that the energetically most stable conformation of a drug molecule necessarily represents the form involved in complexing with a biological receptor. Considering "two-point contact" of bis-quaternary ganglion blocking compounds containing a flexible chain, Gill (1959) calculated the probability distribution of the nitrogen–nitrogen distances. This is an improvement on the concept that a particular nitrogen–nitrogen interjacence is sharply optimal, but calculations refer to isolated molecules and not to those in a biological environment where they are exposed to attractive and distorting forces from all directions, not least from the receptor surface itself. The focus of positivity conventionally represented as residing entirely on the nitrogen atom can to a certain extent vary according to the electron supply presented by the receptor site, and hence an appreciable electrostatic attraction may be exerted even if fit is sub-optimal. From this point of view no sharp peak of activity would be expected in bisonium compounds in which the linking chain was relatively long and electronically neutral, especially in non-depolarizing compounds, in which, by hypothesis, the nitrogen-surface distance may exceed the critical distance for a depolarizing compound. When, as in the present series, other groupings in the cationic heads, or in the chain itself, are themselves capable of exerting attractive forces towards the receptors, the dependence of activity on the nitrogen–nitrogen distance may be expected to be still further diminished.

In the phenylenedimethyl compounds the relative positions of the nitrogen atoms are much restricted as compared with the corresponding polymethylene compounds. In these more rigid molecules the maximum inter-nitrogen distances obtainable

are *ortho* 6.0 Å, *meta* 7.4 Å and *para* 7.5 Å. The length of a *m*-phenylenedimethyl group is equivalent to a fully extended chain of 4 or 5 methylene groups. If the rabbit head-drop doses of the mandelic acid ester (M) and the phenylacetic acid ester (P) of *NN'-m*-phenylenedimethyl(tropinium bromide) (M = 0.055 mg/kg, P = 0.037 mg/kg (Fig. 2)) are compared with those of the same esters of the tetramethylene (M = 0.49 mg/kg (Fig. 1), P = 0.30 mg/kg (Table 2)), and the pentamethylene series (M = 0.81 mg/kg (Fig. 1), P = 0.49 mg/kg (Table 2)), it is clear that the *m*-phenylenedimethyl compounds have considerably greater activity than have the corresponding straight-chain compounds, and this must be attributed to the presence of the benzene ring between the nitrogen atoms. One must ascribe the fourfold difference in potency in the cat observed on passing from *para* to *meta* orientation with the mandelic acid ester (Table 4) to factors other than alteration to the inter-nitrogen distance, possibly the most important being that in these isomers the benzene ring of the phenylenedimethyl grouping must necessarily occupy different positions relative to a line joining the terminal cationic nitrogens when these are optimally applied to the surface, and hence will differ in the efficiency with which it can contribute to the drug-receptor binding.

It would appear that there is room for doubt regarding the reality of the commonly stated view that in bis-quaternary neuromuscular blocking compounds the optimum interjacence is 14 to 15 Å. It has been indicated that no reliable information on this matter can be expected from examination of single polymethylene series including the tropine derivatives described in this paper, and the early workers in this field were careful to stress the number of atoms in the chain separating the onium groups without any suggestion that the chain should be regarded as maximally extended. Other potent bis-quaternary neuromuscular blocking agents with short inter-nitrogen linkages have been reported ; in particular, Carey, Edwards, Lewis & Stenlake (1959), from an examination of various linear poly-onium series, postulate an optimum distance of approximately 9 Å between anionic centres in the receptor. They also suggest that in tubocurarine, the model of which possesses a moderate flexibility, the most reasonable average inter-onium distance is not far removed from 9 Å, and with this view we are inclined to agree. An even more forcible argument is to be found from an examination of the rigid model of the highly potent substance Toxiferine-I for which we derive an inter-onium distance of 9.7 Å. This molecule can be visualized as a broad shallow arch with the quaternary nitrogen atoms occupying diagonally opposite positions at the extremities so that the structure is capable of preventing the access of other molecules to a relatively large area between the two quaternary nitrogen atoms, and the beneficial effect of the benzene ring in the chain of phenylenedimethylbistropinium compounds may in part be due to a similar shielding action.

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