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BLOCKING ACTION OF SOME QUATERNARY DERIVATIVES OF 2-(2,6-XYLYLOXY)ETHYLAMINE ON ADRENERGIC NERVES

BY

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Of the many analogues of N,N,N-trimethyl-2-(2,6-xylyloxy)ethylammonium bromide (xylocholine ; TM 10) reported in the literature (Copp, 1964), few involve alteration of the trimethylammonium group, probably because it was demonstrated in 1957 (Exley, 1957) that replacement of this group by triethylammonium completely removed adrenergic neurone blocking activity. However, the trimethylammonium group is not vital for the presence of adrenergic neurone blocking acivity in molecules of this type since N^1-2-(2,6-xylyloxy)ethyl guanidine (Boura, Copp, Green, Hodson, Ruffell, Sim & Walton, 1961 ; Barron, Natoff & Vallance, 1965) and N-o-bromobenzyl-N,N-dimethyl-2-(2,6-xylyloxy)ethylammonium bromide (Abbs, 1964) both possess this type of activity, although higher doses of this latter compound produce marked depression of the response to electrical stimulation of cholinergic as well as adrenergic neurones.

In this paper we have examined the pharmacology of a number of analogues of xylocholine in which the substitution on the quaternary nitrogen has been altered to include allyl, benzyl and higher alkyl substituents. The five series of compounds prepared and examined are shown in Table 1. Details of the general method of preparation and analytical data are given in the appendix.

For ease of reference the following code has been adopted. The quaternary ammonium compounds are derived from four parent tertiary amines—namely N,N-dimethyl-N,N-diethyl, N,N-di-n-propyl-, and N,N-di-n-butyl-2-(2,6-xylyloxy)ethylamine. These amines have been coded as DM 10, DE 10, DP 10 and DB 10 respectively. Normal alkyl quaternary derivatives of these amines are indicated by numerals, prefixed to this code, indicating the length of the carbon chain of the quaternizing group. Thus, N-n-propyl-N,N-dimethyl-2-(2,6-xylyloxy)ethylammonium bromide is coded as 3 DM 10. Allyl or benzyl derivatives are indicated by the prefixed letter A or B respectively. Thus, N-allyl-N,N-diethyl-2-(2,6-xylyloxy)ethylammonium bromide is coded as A DE 10.

The potency of these compounds in blocking the response to electrical stimulation of adrenergic nerves has been determined and their mode of action analysed.

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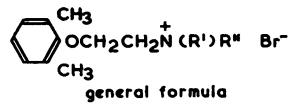
TABLE 1

CHEMICAL FORMULAE OF FIVE SERIES OF COMPOUNDS PREPARED



general formula

Series	R'	R"
I	CH_3	$(CH_2)_n CH_3$, n = 0-7
II	C_2H_5	$(CH_2)_n CH_3$, n = 0-3
III	n-C_3H_7	$(CH_2)_n CH_3$, n = 0-3
IV	CH_3, C_2H_5,	$CH_2{=}CHCH_2$
V	n-C_3H_7 or n-C_4H_9	CH_2 

METHODS

"Surface activity." Solutions of the quaternary ammonium compounds of known concentrations were prepared and their surface tensions determined with a Du Noüy Tensiometer. Graphs of surface tension against $\log_{10}$ concentration were constructed and the strength of a solution having a surface tension of 60 dyn/cm was found for each compound.

Vas deferens preparation. A "stripped" vas deferens (Birmingham & Wilson, 1963) was set up in modified Tyrode's solution (sodium chloride 8, potassium chloride 0.20, calcium chloride (anhydrous) 0.15, magnesium chloride hexahydrate 0.20, sodium bicarbonate 1, sodium dihydrogen phosphate dihydrate 0.05, glucose 2 g/l.) gassed with 95% oxygen, 5% carbon dioxide at 34.1±0.3° C and allowed to settle down for 1 hr. Longitudinal contractions of the preparation were recorded on smoked paper using a lightly loaded isotonic frontal writing lever. The effect of a known concentration of the compound under test dissolved in Tyrode's solution on the submaximal response of the preparation to standard doses of noradrenaline administered at 5-min intervals was determined. The doses of noradrenaline were allowed to act for 60 sec before wash out and the response to the standard dose of noradrenaline in the presence of the compound under test was determined at least five times. The preparation was then washed by overflow with compound-free Tyrode's solution until the response to the standard dose returned to the control level. The effect of the compound on maximal responses of the tissue to noradrenaline elicited at 10-min intervals was then determined.

Transmurally stimulated vas deferens preparation. Vasa deferentia from 6 to 8-month-old guinea-pigs were "stripped," set up in modified Krebs solution (sodium chloride 6.92, potassium chloride 0.35, calcium chloride (anhydrous) 0.21, magnesium sulphate heptahydrate 0.29, potassium dihydrogen phosphate 0.162, sodium bicarbonate 2.1, glucose 2 g/l.) at 34.8±0.3° C and stimulated transmurally according to the method described by Birmingham & Wilson (1963). Longitudinal contractions of the preparation were recorded on smoked paper by means of a lightly loaded isotonic frontal writing lever and the preparation was stimulated transmurally for 12.5 sec in every 3 min 37 sec using rectilinear pulses of 0.3 msec duration and supramaximal voltage delivered at a rate of 50/sec from a Multitone "Ten Pulse" stimulator. The preparation was allowed to settle down for 30 min and, after five or six regular maximal contractions had been recorded, the compound under test was added to the bath and the concentration gradually increased until the response to stimulation had been reduced to approximately 50% of the control responses obtained before the addition of the compound. Measurement of the height of the response in the presence of the compound was made only at equilibrium, and five or six regular responses in the presence of the compound were taken as a criterion of this. The concentration of the compound required to reduce the response of the preparation to approximately 50% of the control value is referred to as the "50% blocking concentration". The tissue was washed by overflow until the effect of the compound was reversed and the

actions of hexamethonium bromide (1×10^{-4} g/ml.) and cinchocaine hydrochloride (1×10^{-5} g/ml.) were investigated as a routine procedure at the end of each estimation.

Each compound was tested on vasa deferentia taken from at least three guinea-pigs and each vas deferens was used for one estimation only.

Rabbit duodenum. Short (2 to 3 cm) pieces of ileum or duodenum taken from rabbits of either sex were prepared according to the method of Finkleman (1930). The preparation was set up in Tyrode's solution (36° C), gassed with a mixture of 95% oxygen, 5% carbon dioxide and allowed to settle down for 2 hr. Longitudinal contractions of the preparation were recorded on smoked paper with a frontal writing lever (load 2 to 3 g). The periarterial nerves leading to the preparation were stimulated electrically for 15 sec in every 10 min with rectilinear pulses of 0.5 msec duration and supramaximal voltage delivered by a Newton Victor Diagnostic stimulator at a rate of 50/sec. The effect of the compounds on the response of the preparation to electrical stimulation was determined.

Cat cervical sympathetic nerve—nictitating membrane preparation. Anaesthesia was induced in cats of either sex with ether and maintained with chloralose (80 to 100 mg/kg) injected through a polythene cannula inserted in the right femoral vein. The trachea was cannulated and respiration maintained artificially. The right post-ganglionic and left preganglionic cervical sympathetic nerves were exposed and stimulated electrically for 15 sec in every min using rectilinear pulses of 0.5 msec duration and supramaximal voltage delivered via shielded platinum hook electrodes from a Newton Victor Diagnostic stimulator at a rate of 50/sec. Both nerves were sectioned proximal to the electrodes. Contractions of the nicitating membranes were recorded on smoked paper with frontal writing levers. In some preparations the right vagus was cut and the peripheral end stimulated electrically for 10 sec with rectilinear pulses of 0.5 msec duration and supramaximal voltage at a rate of 10/sec as required. In these experiments blood pressure was recorded with a mercury manometer from the left femoral artery. All exposed nerves were covered in cotton wool soaked in saline-equilibrated liquid paraffin.

Drugs were dissolved in saline, injected through the polyethylene cannula and washed in with 1 ml. of saline. All animals were given 1,000 u./kg of heparin when the operative procedure was complete.

Guinea-pig intradermal weal test. At least 12 hr before the experiment was carried out, six fully grown male guinea-pigs were clipped as closely as possible over the whole width of the back, posterior to the shoulder blades. Six dose positions were marked out on the back of each animal, positioned alternately left and right of the midline and numbered consecutively. Intradermal injections (0.25 ml.) of a high and a low dose of two of the quaternary ammonium compounds under test and procaine hydrochloride were administered to each animal and the degree of anaesthesia was assessed every 6 min for 36 min by the method described by Bülbring & Wajda (1945). The order of injection was so arranged that each of the six guinea-pigs received the injection of a given strength of a given compound in a dose position bearing a different number.

Isolated vagus nerve preparation. Rabbits of either sex were killed by an injection of air into the marginal ear vein and an approximately 8 cm length of cervical vagus removed and placed in a dish of cold Krebs solution (sodium chloride 6.92, potassium chloride 0.35, calcium chloride (anhydrous) 0.28, magnesium sulphate heptahydrate 0.29, sodium bicarbonate 2.1, potassium dihydrogen phosphate 0.162, glucose 2 g/l.) gassed with 95% oxygen, 5% carbon dioxide. The connective tissue sheath was removed and the nerve transferred to seven platinum electrodes, being secured to the two end electrodes under slight tension. The first two electrodes served as stimulating electrodes, the third as an earth electrode and the remainder served to record evoked potentials. The electrodes were incorporated into the lid of a moist chamber (Eccles, 1952) and the chamber was mounted in a bath at 30° C. Warm, moist 95% oxygen, 5% carbon dioxide was constantly passed through the chamber and the nerve was always bathed in Krebs solution, except when recordings were actually being made when the chamber was drained for periods of up to 2 min. Single shocks of 0.5 msec duration and supramaximal voltage were applied to the nerve as required from a Palmer stimulator (H 44) via an isolation transformer. Evoked potentials were amplified by condenser coupled Telequipment PA 2 and 43 B amplifiers used as single ended inputs and the evoked potentials were displayed on a Telequipment D 43 oscilloscope.

Compounds under test were dissolved in saline and added to the Krebs solution bathing the nerve. Care was taken not to apply any of the added solution directly to the nerve.

Drugs. The following substances were used. Acetylcholine chloride, (−)adrenaline hydrogen tartrate, atropine sulphate monohydrate, bretylium tosylate, 1,5-bis(4-allyldimethylammonium-phenyl)pentan-3-one dibromide (BW284C51k), cinchocaine hydrochloride, guanethidine monosulphate, heparin injection B.P., hexamethonium bromide, lignocaine hydrochloride monohydrate, (−)noradrenaline bitartrate, piperoxan hydrochloride, papaverine hydrochloride, procaine hydrochloride, tolazoline hydrochloride, tetraethylammonium iodide. All concentrations are expressed as final bath concentrations in terms of these salts.

RESULTS

"Surface activity." The concentration of each compound required to give a solution with a surface tension of 60 dyn/cm was taken as an estimate of the "surface activity" of the compound. The values obtained, representing the relative "surface activities" of the compounds under test, are shown in Table 2, and a typical graph from which these values were derived is shown in Fig. 1.

TABLE 2

CONCENTRATIONS OF QUATERNARY AMMONIUM COMPOUNDS REQUIRED TO REDUCE SURFACE TENSION OF WATER TO 60 DYN/CM AND INDICATING RELATIVE "SURFACE ACTIVITY" OF COMPOUNDS

Compound	moles/l.	Compound	moles/l.
1 DM 10	0·144	1 DP 10	0·061
2 DM 10	0·130	2 DP 10	0·0419
3 DM 10	0·079	3 DP 10	0·0171
4 DM 10	0·0315	4 DP 10	0·0132
5 DM 10	0·013		
6 DM 10	0·007	B DM10	0·0385
7 DM 10	0·0037	B DE 10	0·0265
8 DM 10	0·0015	B DP 10	0·0132
		B DB 10	0·0031
1 DE 10	0·140		
2 DE 10	0·080	A DM 10	0·0781
3 DE 10	0·0595	A DE 10	0·051
4 DE 10	0·034	A DP 10	0·027
		A DB 10	0·0145

Transmurally stimulated vas deferens. It was found that the resistance across the stimulating electrodes was so small (about 50 Ω) that the internal resistance of the stimulator contributed appreciably to the total resistance in the circuit. For this reason the voltage indicated on the voltage output dial of the stimulator was not the actual voltage drop across the tissue. The actual voltage drop across the electrodes was measured with a cathode ray oscilloscope and was usually about 10 volts.

When stimulated transmurally the vas deferens responded with regular standard contractions for at least 4 hr. These responses were unaffected by atropine sulphate (1×10^{-6} g/ml.), and by the true anticholinesterase 1,5-bis(4-allyldimethylammonium-phenyl)pentan-3-one dibromide (BW 284C51k) (5×10^{-7} g/ml.) but were completely abolished by the three local anaesthetics, procaine hydrochloride (2×10^{-4} g/ml.), lignocaine hydrochloride (2×10^{-4} g/ml.) and cinchocaine hydrochloride (1×10^{-5} g/ml.). At this concentration cinchocaine hydrochloride had no effect on the response of the vas deferens to exogenous noradrenaline. The responses were also completely abolished by

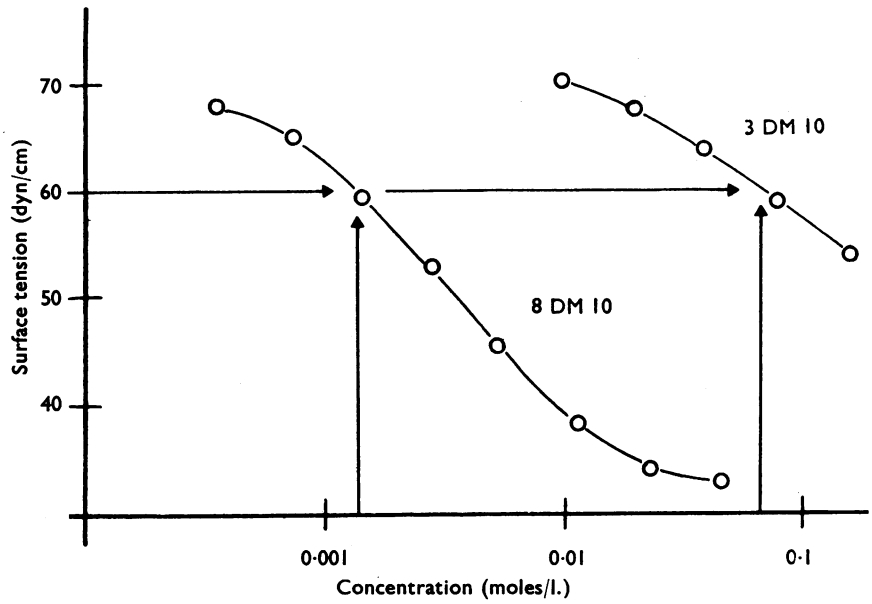


Fig. 1. Variation of surface tension (dyn/cm) with concentration (moles/l.) plotted on $\log_{10}$ scale for N-n-octyl-N,N-dimethyl-2-(2,6-xylyloxy)ethylammonium bromide (8 DM 10) and N-n-propyl-N,N-dimethyl-2-(2,6-xylyloxy)ethylammonium bromide (3 DM 10). From graphs such as these concentration of solution having surface tension of 60 dyn/cm was calculated. Values obtained are given in Table 2.

the known adrenergic neurone blocking agents bretylium tosylate (1×10^{-5} g/ml.) and guanethidine sulphate (1×10^{-5} g/ml.).

The "50% blocking concentration" for procaine hydrochloride was determined nine times. These results were assigned at random to three groups and means and standard errors were calculated for each group. A "t" test revealed that there was no significant difference between the means calculated for each group. These results are shown in Table 3.

The quaternary ammonium compounds produced a blockade of the response of the vas deferens to transmural stimulation which was easily reversed by washing except in the case of xylocholine (1 DM 10). The speed of onset of the blockade varied from compound to compound, the onset usually being faster the larger the quaternary head.

TABLE 3

MEANS AND STANDARD ERRORS CALCULATED FOR GROUPED DETERMINATIONS OF "50% BLOCKING CONCENTRATION" FOR PROCAINE HYDROCHLORIDE

A "t" test was applied to these results, the values of "t" and "P" obtained indicating that there is no significant difference between the mean of each group

Group	Mean	Standard error	Testing between groups	"t"	"P"
A	54·4	±4·4	A and B	0·87	0·4–0·5
B	49·5	±5·3	B and C	0·26	0·8–0·9
C	56·6	±6·8	A and C	0·93	0·4–0·5

The "50% blocking concentration" was measured at least three times for each of the quaternary compounds under test. Since it was impracticable to produce exactly 50% blockade, those concentrations of the test compounds which reduced the response to between the limits 43% to 57% of the control value were taken as valid determinations of "50% blocking concentration". In addition, only those experiments where hexamethonium bromide produced no reduction in the response and cinchocaine hydrochloride produced complete abolition of the response were accepted as valid determinations of "50% blocking concentration". In this way any preparation undergoing preganglionic or "direct" stimulation was eliminated. In practice, hexamethonium bromide never produced a reduction in the size of the standard response and in only one case out of 102 experiments did cinchocaine hydrochloride fail to produce complete abolition of the response. From the valid experiments a "mean 50% blocking concentration" and standard error were calculated for each compound. These results are

TABLE 4

CALCULATED "MEAN 50% BLOCKING CONCENTRATION" WITH STANDARD ERROR AS DETERMINED ON TRANSMURALLY STIMULATED GUINEA-PIG VAS DEFERENS PREPARATION FOR EACH QUATERNARY AMMONIUM COMPOUND

All results are expressed in μ-mole/l. An asterisk indicates that this compound was tested on the rabbit duodenum preparation at its "mean 50% blocking concentration" and a cross (+) that the compound was tested on the rabbit isolated vagus preparation

Compound	"Mean 50% blocking concentration"	Compound	"Mean 50% blocking concentration"
1 DM 10	8·65± 1·46	*A DM 10	31·80± 3·00
*2 DM 10	131·00±17·00	A DE 10	57·10±11·40
3 DM 10	184·00±13·00	+*A DP 10	18·00± 1·80
*4 DM 10	54·20± 7·50	+A DB 10	5·17± 1·23
*5 DM 10	27·80± 4·10		
*6 DM 10	16·60± 2·70	+*B DM 10	7·86± 0·80
7 DM 10	9·40± 0·77	B DE 10	24·10± 3·70
+*8 DM 10	5·43± 0·48	+*B DP 10	3·44± 0·48
		B DB 10	1·13± 0·09
1 DE 10	140·00± 7·00		
+ 2 DE 10	214·00± 7·00		
3 DE 10	58·70± 4·50	Procaine hydrochloride	195·00±11·00
+*4 DE 10	32·50± 5·00		
*1 DP 10	73·60± 6·00		
2 DP 10	44·20± 6·20		
*3 DP 10	24·60± 1·30		
+*4 DP 10	15·10± 1·20		

given in Table 4 and are presented graphically in Figs. 2 and 3. In these two Figures the following parameters have been used as abscissae: Fig. 2, "Carbon Number"—a numerical expression of the number of carbon atoms in the quaternary head of the molecule; Fig. 3, "Surface activity"—the concentration of quaternary ammonium compound required to reduce the surface tension of water to 60 dyn/cm.

A typical example of the record obtained in a determination of "50% blocking concentration" is shown in Fig. 4.

Vas deferens preparation. Concentrations of noradrenaline between 2 and 5×10^{-6} g/ml. elicited standard submaximal responses from the guinea-pig vas deferens for at least 3 hr. Piperoxan hydrochloride (7.5×10^{-6} g/ml.) and tolazoline hydrochloride

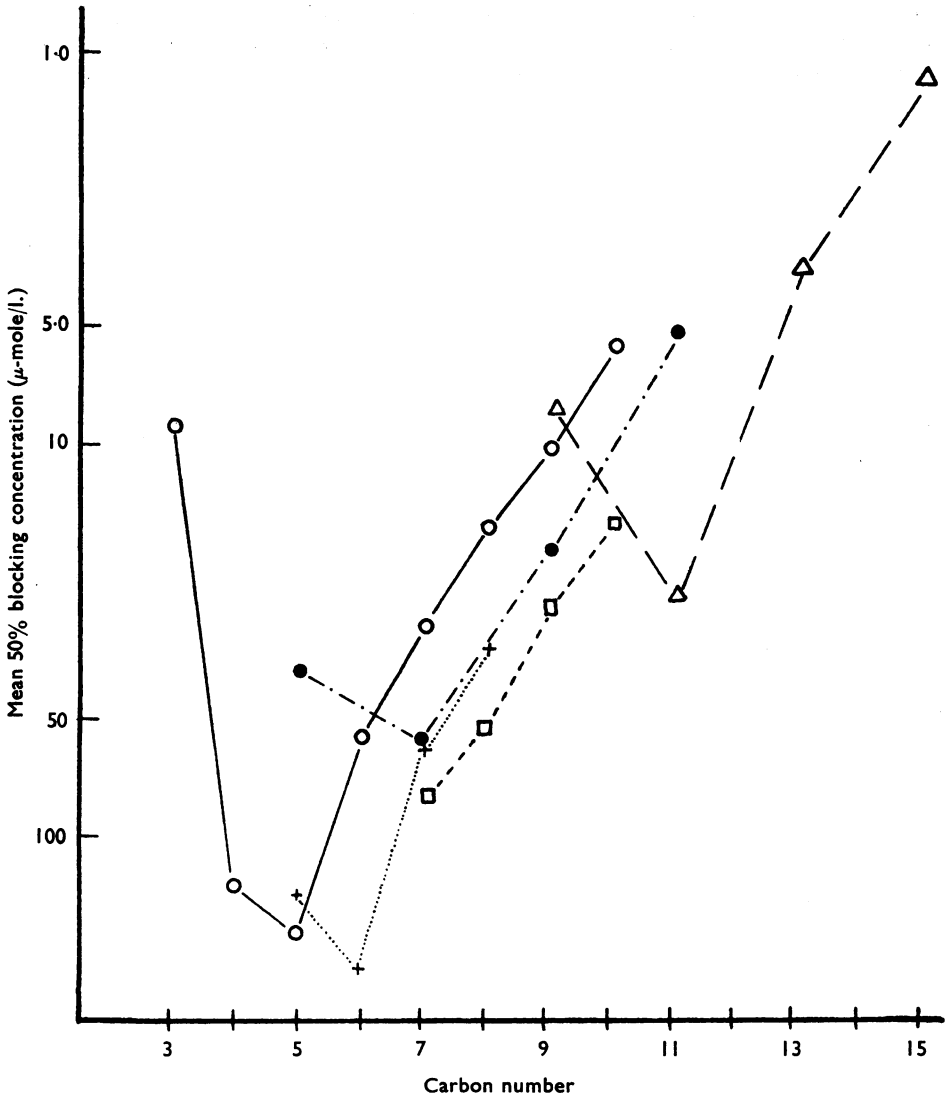


Fig. 2. "Mean 50% blocking concentration" of each quaternary ammonium compound as determined on isolated guinea-pig vas deferens preparation against "carbon number" of compound—i.e., number of carbon atoms in quaternary head of molecule. The five series of compounds investigated are shown as follows: O——O N-n-alkyl-N,N-dimethyl series; +···+ N-n-alkyl-N,N-diethyl series; □ – – – □ N-n-alkyl-N,N-di-n-propyl series; ● — · — · ● N-allyl-N,N-di-n-alkyl series; △— —△ N-benzyl-N,N-di-n-alkyl series. Standard errors have been omitted from this Figure for reasons of simplification.

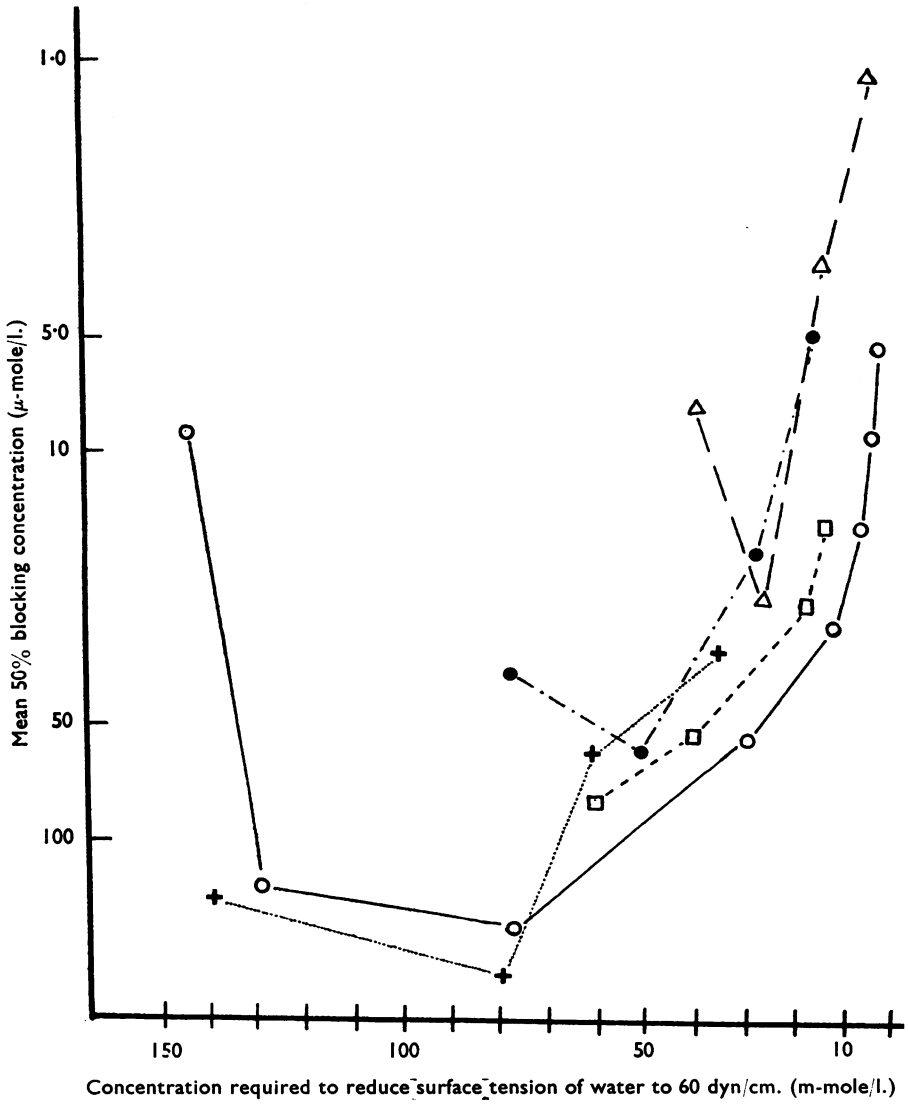


Fig. 3. "Mean 50% blocking concentration" of each quaternary ammonium compound as measured on transmurally stimulated guinea-pig vas deferens preparation plotted against concentration (m-mole/l.) required to lower surface tension of water to 60 dyn/cm. The five series of compounds investigated are shown as follows: O——O N-n-alkyl-N,N-dimethyl series; + · · · + N-n-alkyl-N,N-diethyl series; □ – – – □ N-n-alkyl-N,N-di-n-propyl series; ● — · — · ● N-allyl-N,N-di-n-alkyl series; △— —△ N-benzyl-N,N-di-n-alkyl series. Standard errors have been omitted from this Figure for reasons of simplification. In order to make graph comparable with that obtained plotting "carbon number" on abscissa, concentration required to reduce surface tension of water to 60 dyn/cm has been plotted from right to left.

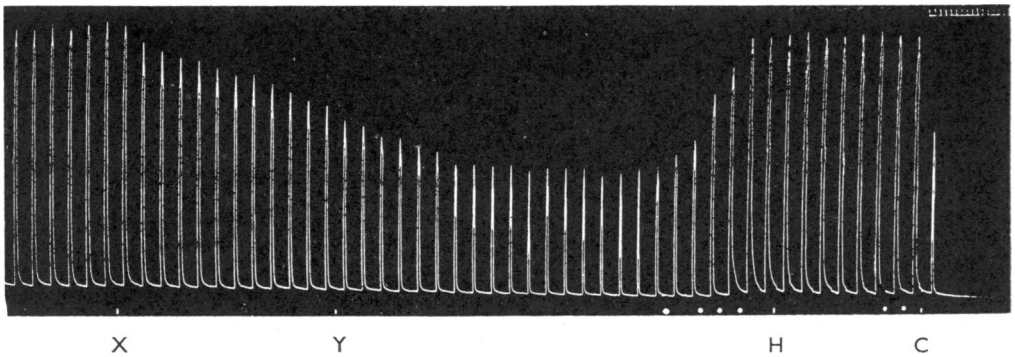


Fig. 4. Example of determination of "50% blocking concentration" on transmurally stimulated (50/sec; 0.3 msec duration) guinea-pig vas deferens preparation. Although stimulator was set to deliver 90 v the actual voltage drop across stimulating electrodes was 9.8 v. At X tissue was exposed to N,N,N-triethyl-2-(2,6-xylyloxy)ethylammonium bromide (2 DE 10) (4.0×10^{-5} g/ml.) and at Y this concentration was raised to 7.5×10^{-5} g/ml. At H tissue was exposed to hexamethonium bromide (1×10^{-4} g/ml.) and at C to cinchocaine hydrochloride (1×10^{-5} g/ml.). Tissue was washed by overflow as indicated by white dots. Time marker = 60 sec.

(7.5×10^{-6} g/ml.) completely abolished and considerably reduced, respectively, the response of the vas deferens to the standard dose of noradrenaline. At this concentration these drugs had no effect on the height of the maximal response which could be elicited from the vas deferens by noradrenaline. Papaverine hydrochloride (2.5×10^{-6} g/ml.) produced a small reduction in the height of the submaximal response to noradrenaline and considerably reduced the height of the maximal response which could be elicited from the tissue.

At their "mean 50% blocking concentrations" most of the quaternary ammonium compounds produced a reduction in the height of the submaximal response of the vas deferens to noradrenaline and, in order to obtain a rough estimate of the magnitude of this effect, the reduction in response was expressed as a percentage of the maximal response of the tissue. Two such estimations were performed with each compound tested by this method and the results are shown in Table 5. The inhibitory effect produced by the compounds was rapid in onset, usually reaching equilibrium blockade within 5 min and was quickly and completely reversed by washing the tissue for 5 min with compound-free Tyrode's solution. One compound, N-benzyl-N,N-di-n-butyl-2-(2,6-xylyloxy)ethylammonium bromide (B DB 10), did not produce any reduction in the submaximal response of the tissue to noradrenaline when tested in the manner described at its "mean 50% blocking concentration".

At their "mean 50% blocking concentrations" none of the compounds tested produced any reduction in the size of the maximal response which could be elicited from the tissue by noradrenaline. A record from a typical experiment is shown in Fig. 5.

Rabbit duodenum. All of the compounds tested by this method produced, within 15 min, a reduction in the inhibitory effect of electrical stimulation of the periarterial nerves. Each of the compounds tested was used at its "mean 50% blocking concentration" and,

TABLE 5

REDUCTION IN SUBMAXIMAL RESPONSE OF GUINEA-PIG ISOLATED VAS DEFERENS PREPARATION TO NORADRENALINE PRODUCED BY SOME OF QUATERNARY AMMONIUM COMPOUNDS

Each compound was used at its "mean 50% blocking concentration" and reduction expressed as a percentage of the maximal response of the tissue. Actual values obtained in two experiments and an average value are given for each compound. *—3 DM 10 produced complete abolition of the submaximal response to noradrenaline

Compound	Percentage Reduction		Average percentage reduction
2 DM 10	12	14	13
3 DM 10	—	—	—*
4 DM 10	49	41	45
5 DM 10	36	42	39
6 DM 10	35	33	34
7 DM 10	22	28	25
8 DM 10	14	19	16·5
A DM 10	33	39	36
A DE 10	22	20	21
A DP 10	29	20	24·5
A DB 10	9	11	10
B DM 10	45	37	41
B DE 10	25	23	24
B DP 10	12	19	15·5
B DB 10	0	0	0

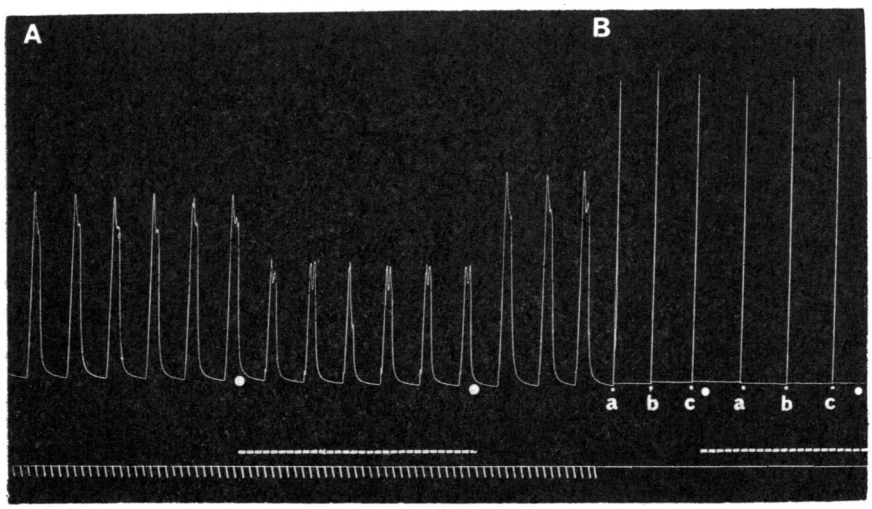


Fig. 5. Effect of N-allyl-N,N-di-n-propyl-2-(2,6-xylyloxy)ethylammonium bromide (A DP 10), present in bath at its "mean 50% blocking concentration" (18.0 μ-mole/l.), as indicated by the white dashes, on response of guinea-pig vas deferens to noradrenaline. Part A shows effect on submaximal responses to noradrenaline (3.2×10^{-6} g/ml.) administered at 5 min intervals and allowed to act for 60 sec before wash out. Time marker=60 sec. Part B shows that maximal response of tissue to noradrenaline (a, b and c 1.3, 2.6 and 5.2×10^{-5} g/ml. respectively administered at 10 min intervals) is not depressed by compound. In this part of record drum was moved on by hand as required.

although the reduction in the effect of electrical stimulation was always marked, it varied in degree from compound to compound. Complete reversal of the blockade was easily accomplished by washing the tissue and in no case was the magnitude of the spontaneous contractions exhibited by the preparation reduced by the presence of the compound. A record from a typical experiment is shown in Fig. 6 and the compounds tested by this method are distinguished by an asterisk in Table 4.

Cat cervical sympathetic nerve—nictitating membrane preparation. In the doses used (see Table 6) none of the compounds tested showed the characteristic action of adrenergic

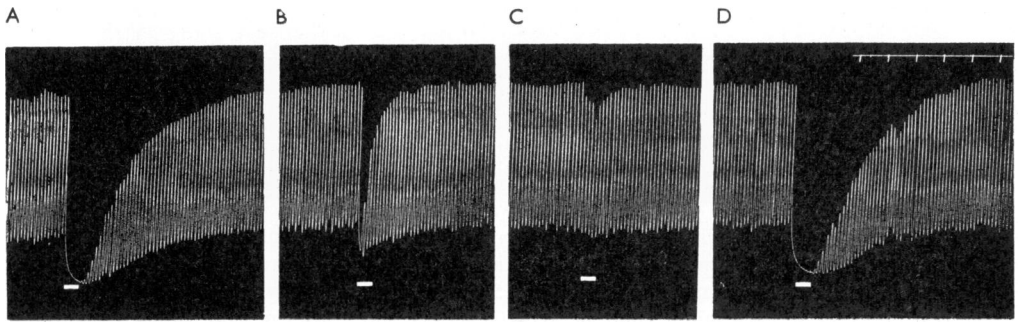


Fig. 6. Effect of N-allyl-N,N-di-n-propyl-2-(2,6-xylyloxy)ethylammonium bromide (A DP 10) at its "mean 50% blocking concentration" (18.0 μ-mole/l.) on response of isolated rabbit duodenum to electrical stimulation (50/sec; 0.5 msec duration; 12 v) of periarterial nerves for 30 sec in every 10 min as shown by white bars. Part A—control response. Parts B and C—response to stimulation 8 min and 18 min respectively after administration of A DP 10. Part D—response to stimulation after A DP 10 had been removed from bath by washing tissue at 5-min intervals for 30 min. Time marker = 60 sec.

TABLE 6

QUATERNARY AMMONIUM COMPOUNDS TESTED FOR ADRENERGIC NEURONE BLOCKING ACTIVITY ON CAT CERVICAL SYMPATHETIC NERVE—NICTITATING MEMBRANE PREPARATION TOGETHER WITH DOSE OF COMPOUND ADMINISTERED AND DURATION OF BLOCKADE OF RESPONSE OF NICTITATING MEMBRANE

Compound	Dose (mg/kg)	Duration of blockade (min)
3 DM 10	1	10
5 DM 10	5	10
8 DM 10	7	5
A DM 10	4·0	12
A DE 10	3·3	10
A DB 10	6·0	100
B DM 10	2·5	6
B DP 10	3·3	30
B DB 10	2	90

neurone blocking agents. The doses administered were low since higher doses produced a precipitate fall in blood pressure which was quickly followed by the death of the animal. All the compounds tested produced an immediate reduction in the response of the nictitating membranes to electrical stimulation of the cervical sympathetic nerves and a fall in blood pressure. The responses to both pre- and post-ganglionic stimulation were affected equally and the duration of the depression varied from compound to

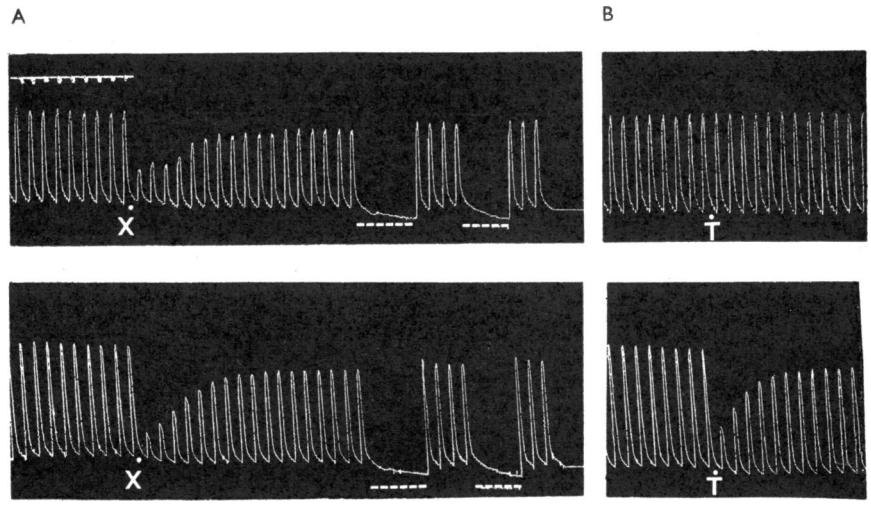


Fig. 7. Effect of N-allyl-N,N-dimethyl-2-(2,6-xylyloxy)ethylammonium bromide (A DM 10) (4 mg/kg I.V. at X) and tetraethylammonium iodide (5 mg/kg I.V. at T) on response of cat nictitating membrane to electrical stimulation (50/sec; 0.5 msec duration; 10 v) of cervical sympathetic nerves for 15 sec in each min. Time marker=60 sec. Upper trace—postganglionic stimulation. Lower trace—preganglionic stimulation. Stimulation was interrupted for periods indicated by white dashes. There was interval of 2 hr between parts A and B of record.

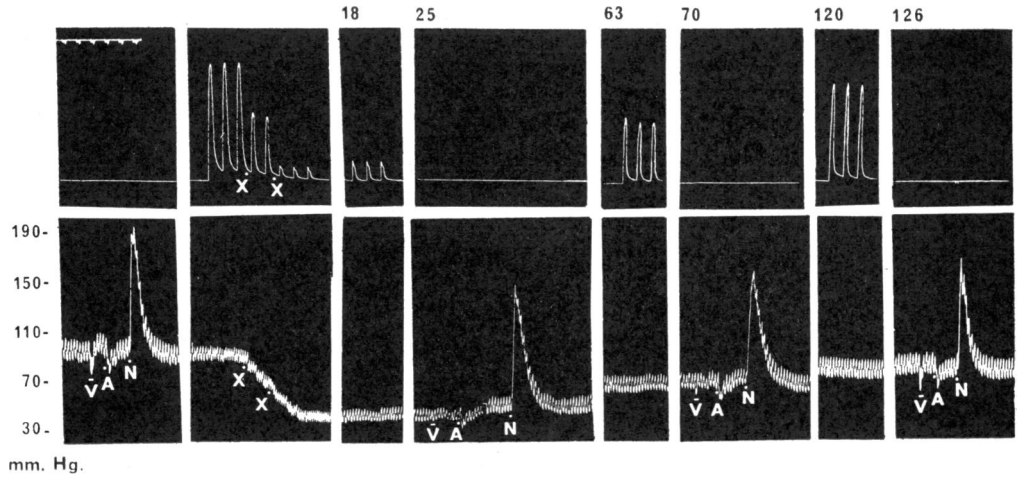


Fig. 8. Response of cat nictitating membrane to postganglionic electrical stimulation (50/sec; 0.5 msec duration; 8 v; for 15 sec in every min) of right cervical sympathetic nerve and response of blood pressure to electrical stimulation of right vagus (10/sec; 0.5 msec duration; 6 v for 10 sec at V) and administration of acetylcholine bromide (1.5 μg/kg I.V. at A) and noradrenaline (6 μg/kg I.V. at N), as modified by N-allyl-N,N-di-n-butyl-2-(2,6-xylyloxy)ethylammonium bromide (A DB 10) (3 mg/kg I.V. at X). Time marker=5 min. Electrical stimulation of cervical sympathetic nerve was stopped while effects of stimulation of vagus and administration of acetylcholine and noradrenaline were observed. Figures along top of record show time elapsed (min) since first injection of A DB 10.

compound. In some cases it was short (Fig. 7) and after complete recovery had occurred could be demonstrated repeatedly in the same animal. In other cases the depression was more prolonged (Fig. 8) and during this prolonged blockade the fall in blood pressure produced by electrical stimulation of the right vagus was considerably reduced or abolished, although the depressor response to injected acetylcholine and the pressor response to a standard dose of noradrenaline remained largely unchanged.

The compounds tested on this preparation, the doses used and the duration of the depression of the response of the nictitating membranes to electrical stimulation of the cervical sympathetic nerves are shown in Table 6.

Guinea-pig intradermal weal test. The total number of negative responses shown by all six guinea-pigs to each of the six treatments was calculated. Since the regression of dose with response is determined in this experiment using two dose levels only, the regression line must go through the mean value of the responses at each dose level and the regression coefficient can be calculated quite simply therefore without the need for complex statistical treatment. Statistical tests for significance (Steel & Torrie, 1960) showed that there was no significant difference between the regression coefficients calculated for each of the quaternary ammonium compounds and that calculated for procaine hydrochloride ($P \leqslant 0.2$). Calculation of a joint regression coefficient for each of the quaternary ammonium compounds with procaine hydrochloride, followed by further standard statistical treatment leads to a value for the potency ratio of each of the compounds to procaine hydrochloride. The values obtained together with fiducial limits ($P=0.05$) are given in Table 7 and the mean values are presented graphically in

TABLE 7

MEAN MOLAR POTENCY RATIO AND FIDUCIAL LIMITS ($P=0{\cdot}05$) FOR EACH QUATERNARY AMMONIUM COMPOUND AND PROCAINE HYDROCHLORIDE AS DETERMINED BY GUINEA-PIG INTRADERMAL WEAL TEST

Compound	Mean	Limits	Compound	Mean	Limits
1 DM 10	2·7	(3·4–2·2)	A DM 10	5·8	(7·0–4·4)
2 DM 10	2·6	(3·1–2·1)	A DE 10	4·5	(5·6–3·6)
3 DM 10	3·0	(3·7–2·5)	A DP 10	19·1	(23·5–15·6)
4 DM 10	4·4	(5·4–3·5)	A DB 10	27·1	(31·6–23·3)
5 DM 10	8·6	(13·1–5·6)			
6 DM 10	12·1	(15·4–9·5)	B DM 10	9·5	(12·5–7·6)
7 DM 10	19·9	(24·2–16·5)	B DE 10	10·9	(14·0–8·5)
8 DM 10	30·8	(38·6–24·5)	B DP 10	45·1	(56·7–36·0)
			B DB 10	97·1	(125·9–74·7)
1 DE 10	2·9	(3·7–2·3)			
2 DE 10	2·9	(3·6–2·4)			
3 DE 10	4·5	(5·5–3·8)			
4 DE 10	10·0	(14·8–6·7)			
1 DP 10	3·6	(4·4–2·9)			
2 DP 10	5·7	(7·4–4·3)			
3 DP 10	9·7	(12·6–7·5)			
4 DP 10	25·6	(30·0–21·7)			

Figs. 9 and 10. In these Figures the same parameters have been used for the abscissae as were used in the presentation of the data from the determination of "mean 50% blocking concentration" on the transmurally stimulated vas deferens preparation (see above).

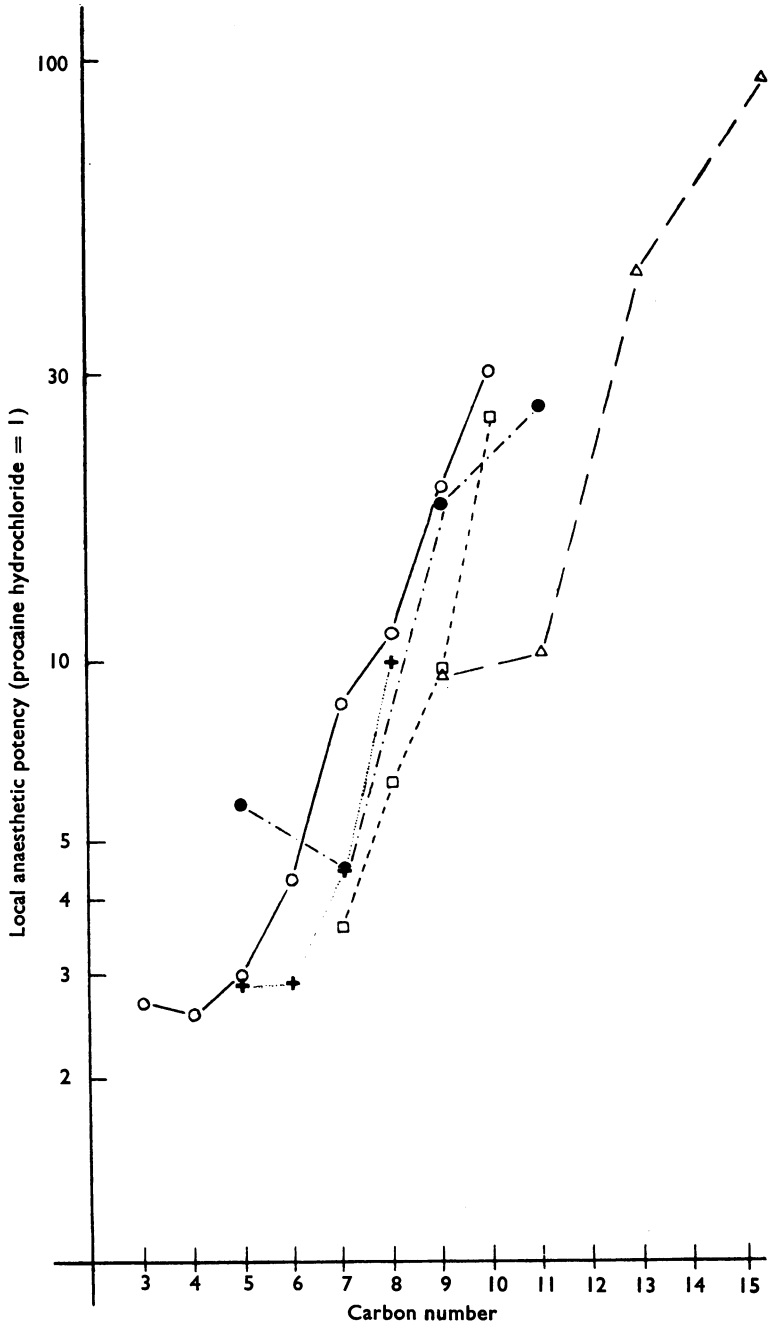


Fig. 9. Variation of local anaesthetic potency with structure. Local anaesthetic potency (ordinate) is expressed as mean molar potency (relative to procaine hydrochloride = 1) as determined in guinea-pig intradermal weal test. Structure (abscissa) is expressed as carbon number—i.e., number of carbon atoms in quaternary head of molecule. The five series of compounds investigated are shown as follows: ○——○ N-n-alkyl-N,N-dimethyl series; + · · · + N-n-alkyl-N,N-diethyl series; □ – – – □ N-n-alkyl-N,N-di-n-propyl series; ● — · — · ● N-allyl-N,N-di-n-alkyl series; △— —△ N-benzyl-N,N-di-n-alkyl series. Fiducial limits of potency estimates have been omitted from Figure for reasons of simplification.

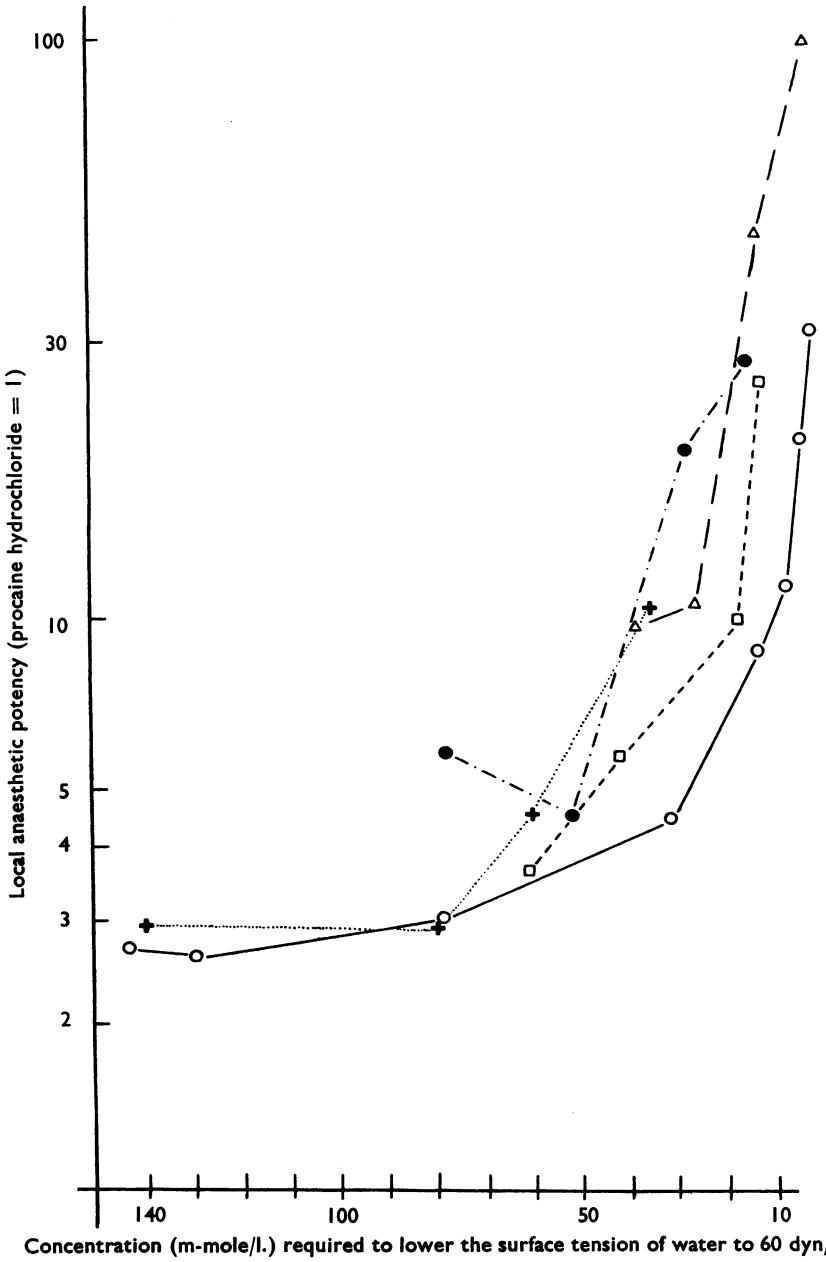


Fig. 10. Variation of local anaesthetic potency with "surface activity" of compounds. Local anaesthetic potency (ordinate) is expressed as mean molar potency (relative to procaine hydrochloride = 1) as determined in guinea-pig intradermal weal test. "Surface activity" (abscissa) is expressed as concentration (m-mole/l.) required to reduce the surface tension of water to 60 dyn/cm. The five series of compound are shown as follows: ○——○ N-n-alkyl-N,N-dimethyl series; + · · · + N-n-alkyl-N,N-diethyl series; □ – – – □ N-n-alkyl-N,N-di-n-propyl series; ● · — · ● N-allyl-N,N-di-n-alkyl series; △— —△ N-benzyl-N,N-di-n-alkyl series. Fiducial limits of potency estimates have been omitted from Figure for reasons of simplification. In order to make graph comparable with Fig. 9, concentration required to reduce surface tension of water to 60 dyn/cm has been plotted from right to left.

Isolated vagus nerve preparation. Control experiments showed that standard potentials could be evoked from the preparation for at least 10 hr and rough measurements of conduction velocity indicated that the evoked potentials travelled at a speed of about 2 m/sec. Repetitive stimulation (10 to 20 shocks/sec) produced a rapid fall in the size of the evoked potentials, which quickly returned to the control value when repetitive stimulation was stopped.

All the quaternary ammonium compounds tested produced partial or complete blockade of the evoked potentials depending upon the concentration and the time of contact. Although no measurements of minimum blocking concentration were made, concentrations needed to produce complete blockade of the evoked potentials were higher (by a factor of about 10) than those needed to produce 50% blockade of the response of the vas deferens to transmural stimulation. They were however, lower than those needed to produce any marked enduring anaesthesia in the guinea-pig intradermal weal test. It was noticeable that compounds with large quaternary heads were more potent as blocking agents than were those with small quaternary heads. Blockade was usually complete within 15 min and could be completely reversed by washing the preparation every 10 min for 2 hr.

Photographs of the action potentials obtained in a typical experiment are shown in Fig. 11 and the compounds tested by this method are indicated by a cross (+) in Table 4.

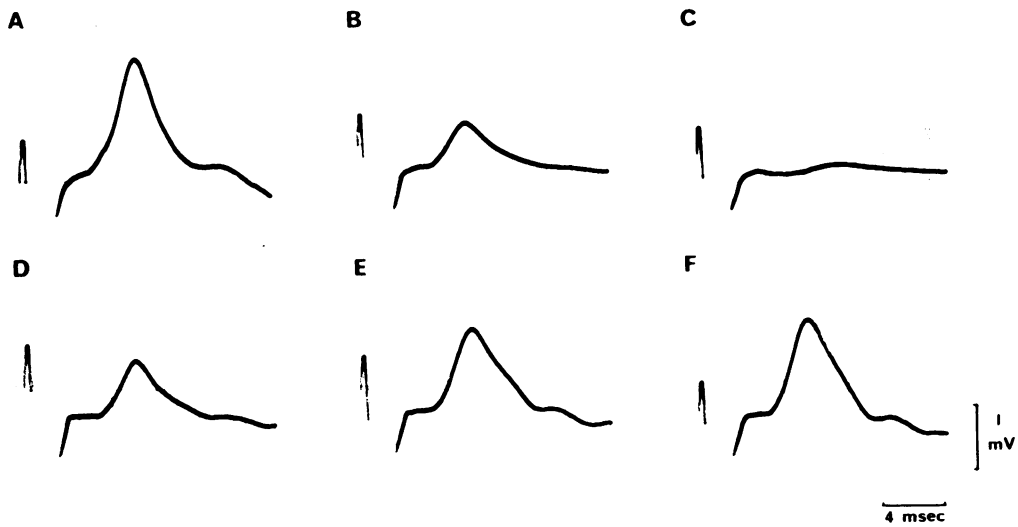


Fig. 11. Effect of N-n-butyl-N,N-di-n-propyl-2-(2,6-xylyloxy)ethylammonium bromide (4 DP 10) (1.7×10^{-4} M) on potentials evoked from isolated rabbit vagus preparation in response to supramaximal stimulation by single shocks (0.5 msec duration; 60 v) applied as required. Stimulating and recording electrodes were 1.4 cm apart. A—control response. B and C—responses after exposure to 4 DP 10 for 5 and 15 min respectively. D, E and F—responses after removing 4 DP 10 from bath by washing preparation every 10 min for 20, 50 and 90 min respectively.

DISCUSSION

The short pulse width (0.3 msec) used to excite the guinea-pig vas deferens together with the total abolition of the response to transmural stimulation produced by the local anaesthetics procaine, lignocaine and cinchocaine suggest that the response is mediated

entirely by nervous elements and that no " direct " component of stimulation contributes to the response. These nervous elements are probably adrenergic in nature since the response is completely abolished by the known adrenergic neurone blocking agents bretylium tosylate and guanethidine sulphate and is unaffected by atropine sulphate and by the anticholinesterase BW 284C51k. These results confirm those of previous workers (Bentley & Sabine, 1963 ; Birmingham & Wilson, 1963) and indicate that when stimulated in this manner the response of the vas deferens to transmural stimulation is mediated entirely by adrenergic neurones and that any cholinergic component contributes insignificantly to the response. Since hexamethonium bromide produces no reduction in the height of the response these adrenergic neurones must be postganglionic in nature.

From Table 3 it can be seen that measurement of the " mean 50% blocking concentration " is a reproducible estimate of the potency of compounds in blocking the response of the guinea-pig isolated vas deferens to transmural stimulation. Since the response is mediated entirely by postganglionic adrenergic neurones this expression of blocking potency is compounded of the potency of the compounds as local anaesthetics, adrenergic neurone blocking agents, α-blocking agents and smooth muscle depressants. This latter type of activity is unlikely to be exhibited by the compounds at their " mean 50% blocking concentrations ", since the experiments with the isolated vas deferens preparation show that, at this concentration, the compounds produce no reduction in the height of the maximal response which can be elicited from the tissue by exogenous noradrenaline, and the spontaneous movements exhibited by the rabbit duodenum preparation are similarly unaffected by the compounds at this concentration.

The graphs of " mean 50% blocking concentration " against " carbon number " and against the concentration of the compounds required to lower the surface tension of water to 60 dyn/cm show that there is a marked change in the direction of the slope of the plots, especially for the N,N-dimethyl series, as most of the series are ascended. This may well indicate that blocking activity on the transmurally stimulated guinea-pig vas deferens preparation is caused by more than one type of pharmacological action. If it is assumed that only one type of action is involved then it is difficult to see how two widely separated members of an homologous series may both fulfil the requirements for high activity, whether the requirements be physical or structural, unless the members of the series in between the two compounds also fulfil these requirements. It is probable therefore that compounds with low " carbon number " showing high blocking potency are producing their effect by a mechanism different from that by which compounds of high potency and high " carbon number " produce blockade.

For compounds with " carbon numbers " above 5 in series I, II, III and IV and above 9 in series V there appears to be a relationship between blocking potency and " carbon number ". The plots for each series are not only approximately linear but are also roughly parallel to those obtained for the other series. This indicates that, for the higher members of each series, the pharmacological action of each series of compounds is probably the same and is dependent upon some physical property of the molecules.

As additional methylene groups are incorporated into the cationic head of the molecule so its lipophilic properties and oil/water partition coefficient will change. Although quaternary ammonium compounds are generally thought of as being freely soluble in water it is of interest to note that in the N,N,N,N-tetra-alkylammonium series of com-

pounds water solubility is gradually reduced as the size of the alkyl groups is increased. Indeed, tetrahexylammonium bromide is only slightly soluble in water and shows appreciable solubility in such solvents as diethyl ether (Clark, unpublished observations). It would be expected that the same trend would be apparent in the series of compounds under investigation. An increase in the lipophilic properties of the molecule will increase the tendency of the molecule to be expelled from the aqueous phase and molecules with larger cationic heads will tend to congregate at the interface in aqueous solution. This will produce a reduction in the surface tension of the solution as can be seen from the results given above.

Although it may be concluded that for the higher members of each series the blocking potency is related to some physical property of the molecule, no indication of the nature of this property can be obtained from these results. In an homologous series each physical property changes in a regular manner and a correlation between blocking potency and a physical property, such as "carbon number" or the concentration of the compound required to lower the surface tension of water to 60 dyn/cm, gives no indication as to what the essential physical property might be.

Tests for anti-adrenaline activity on the isolated vas deferens preparation indicate that at their "mean 50% blocking concentrations" most of the compounds do exhibit a degree of this activity (Table 5). It can be seen however that even though the compounds were tested for anti-adrenaline activity at concentrations which were equi-effective in blocking the response of the transmurally stimulated guinea-pig vas deferens preparation, they showed distinct differences in anti-adrenaline potency. If blockade of the transmurally stimulated guinea-pig vas deferens preparation is due solely to α-blocking activity then these results could be reconciled if the compounds differ in the ratios of their abilities to blockade endogenous and exogenous noradrenaline. However, all known α-blocking agents are less effective in blockading the response to sympathetic nerve stimulation than they are in blocking a similar response produced by the addition of noradrenaline to the bath fluid. It is unlikely, therefore, that the small amount of anti-adrenaline activity possessed by most of the compounds is sufficient to account completely for their blocking activity on the transmurally stimulated vas deferens preparation. N-benzyl-N,N-di-n-butyl-2-(2-6-xylyloxy)ethylammonium bromide (B DB 10) must act by a mechanism not involving α-blockade. Further evidence against a significant contribution by α-blockade to the blockade of the response of the transmurally stimulated guinea-pig vas deferens preparation comes from consideration of the time course of the respective blockades. Blockade of the response of the vas deferens to exogenous noradrenaline appears to reach equilibrium within 5 min, while most of the quaternary ammonium compounds require a considerably longer contact time before equilibrium blockade is attained on the transmurally stimulated guinea-pig vas deferens preparation or on the rabbit duodenum preparation. For these reasons it is concluded that, although the considerable anti-adrenaline activity possessed by compounds such as N-n-propyl-N,N-dimethyl-2-(2,6-xylyloxy)ethylammonium bromide (3 DM 10) at their "mean 50% blocking concentrations" may well contribute appreciably to the blockade produced on the transmurally stimulated vas deferens preparation, this is unlikely to be so for compounds such as N-n-octyl-N,N-dimethyl-2-(2,6-xylyloxy)ethylammonium bromide (8 DM 10) and N-allyl-N,N-di-n-butyl-2(2,6-xylyloxy)ethylammonium bromide

(A DB 10) which show only slight anti-adrenaline activity at their " mean 50% blocking concentrations."

When tested on the cat cervical sympathetic nerve—nictitating membrane preparation none of the compounds tested (Table 6) showed the slowly developing, long lasting selective blockade, which is typical of the adrenergic neurone blocking action of xylocholine. In all cases the responses of the nictitating membranes to both pre- and post-ganglionic stimulation were affected equally, and therefore the fall in blood pressure which accompanied this reduction in response is not attributed to ganglion blockade. The onset of the blockade of the response of the nictitating membrane was immediate and its duration varied from compound to compound (Table 6). In those cases where the depression of the response of the nictitating membrane was sufficiently prolonged to permit investigations to be carried out, it was found that during this period the fall in blood pressure produced by electrical stimulation of the vagus nerve was reduced or abolished, although the depressor response to injected acetylcholine and the pressor response to a standard dose of noradrenaline remained more or less unchanged. These results are not consistent with possession of adrenergic neurone blocking properties or marked α-blocking properties by the compounds and suggest a more generalized depression of conduction in all nerve fibres. It should be noted that the doses used are low, and therefore it can only be concluded that these compounds are not potent adrenergic neurone blocking agents.

Assays for local anaesthetic activity performed by a modification of the guinea-pig intradermal weal method showed that, except for compounds with very low " carbon numbers "—e.g., xylocholine—the order of potency obtained was the same as that obtained on the transmurally stimulated guinea-pig vas deferens preparation. It was found that xylocholine and its triethyl homologue (2 DE 10) were of approximately equal potency as local anaesthetics, thus confirming the result obtained by Hey & Willey (1954). The delay in onset of the anaesthesia produced by xylocholine reported by them was also noted in these experiments but this phenomenon was not a common feature of all the quaternary ammonium compounds tested. Small variations in potency ratio between the quaternary ammonium compounds as determined by the intradermal weal method and by the transmurally stimulated vas deferens method are probably accounted for by variations in the duration of anaesthesia, since measurements of potency on the transmurally stimulated vas deferens preparation were made at equilibrium blockade, while in the intradermal weal test the expression of potency is complicated by a factor of duration of anaesthesia.

The local anaesthetic action of these compounds was confirmed on the rabbit isolated vagus nerve preparation. All the quaternary ammonium compounds tested produced complete abolition or a considerable diminution in the size of the evoked action potentials depending on the concentration of the compound and the period of contact. The concentrations needed to produce complete blockade were intermediate between those producing approximately 50% anaesthesia in the guinea-pig intradermal weal test—i.e., a total score of about 18 negative responses during the 36 min period for each guinea-pig —and the " mean 50% blocking concentration " of the compound. At these concentrations blockade was usually complete within 15 min. In all cases the blockade of the evoked action potential was completely reversed on washing the preparation.

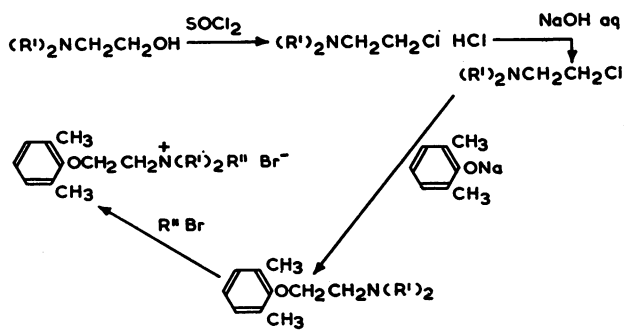
It appears therefore that none of the compounds under investigation (other than xylocholine) possess appreciable adrenergic neurone blocking properties, and for N-ethyl-N,N-dimethyl-2-(2,6-xylyloxy)ethylammonium bromide and N-methyl-N,N-diethyl-2-(2,6-xylyloxy)ethylammonium bromide our conclusions are in accord with those obtained by Fielden, Roe & Willey (1964) using the corresponding iodides. The large reduction in the adrenergic neurone blocking properties of xylocholine produced by the incorporation of only one extra methylene group into the cationic head of the molecule suggests that the parameters to be fulfilled for adrenergic neurone blocking activity are very specific and that combination with a receptor of high structural specificity is involved. If on the other hand it is assumed that adrenergic neurone blocking activity is wholly dependent on a purely physical phenomenon, then it is difficult to see how the small alteration in the physical properties of the molecule produced by the incorporation of one extra methylene group into the cationic head could account for the large reduction in blocking activity which results. The situation appears to be comparable to that encountered with acetylcholine at its muscarinic site of action.

The quaternary ammonium compounds, especially those with large cationic heads, are local anaesthetics in the sense that they produced reversible blockade of nerve conduction in the preparations in which they were tested. The delay in the onset of local anaesthetic action so often encountered with quaternary ammonium compounds (Nádor, Herr, Pataky & Borsy, 1953; Hey & Willey, 1954) was not seen with most of the compounds under investigation. This is probably because of the increased lipophilic properties which must be associated with the large quaternary ammonium heads. The unit positive charge is to some extent shielded from electrostatic interactions by the bulk of the large alkyl groups, the larger size of which enable more cohesive interactions to take place with nonpolar structures.

CHEMICAL APPENDIX

The synthetic route used for the synthesis of the quaternary ammonium compounds is shown on the flow sheet.

Flow Sheet

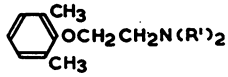


The required dialkylaminoethanols were obtained commercially or, in the case of di-n-propylaminoethanol, from di-n-propylamine and ethylene oxide (Cadogan, 1955). Treatment of these aminoalcohols with excess thionyl chloride yielded the corresponding chloroamine hydrochloride from which the free chloroamine was released by treatment with excess sodium hydroxide solution. The free chloroamines coupled readily with

sodium xylyloxide in t-butanol to give N,N-dialkyl-2-(2,6-xylyloxy)ethylamines the boiling points and analytical data of which are given in Table 8. Gas-liquid chromatography at about 130° C using a column of 15% polyethylene glycol (mol. wt. 1,500) and 2% potassium hydroxide deposited on 100/110 mesh diatomaceous earth demonstrated that these tertiary amines were of high purity. Reaction with the appropriate n-alkyl, allyl or benzyl bromide in dry acetone or ether yielded the required quaternary ammonium

TABLE 8

BOILING POINTS AND ANALYTICAL DATA FOR N,N-di-n-ALKYL-2-(2,6-XYLYLOXY)-ETHYLAMINES OF GENERAL FORMULA

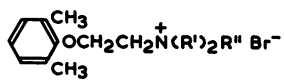


* Hey & Willey (1954) report b.p. of DM 10 and DE 10 as 124°C/10 mm and 131°C/10mm respectively

Compound	R′	b.p. °C/mm	Analysis; found (%) C	H	N	Analysis; calc (%) C	H	N
DM 10	CH_3	123–126°/10*	74·25	10·0	7·15	74·6	9·9	7·25
DE 10	C_2H_5	136–140°/12*	75·9	10·35	6·6	76·0	10·5	6·3
DP 10	n-C_3H_7	153–156°/9	76·8	10·7	5·6	77·1	10·9	5·6
DB 10	n-C_4H_9	145–147°/0·5	77·6	11·1	5·4	77·9	11·3	5·05

TABLE 9

CODE DESIGNATIONS, MELTING POINTS AND ANALYTICAL DATA FOR QUATERNARY AMMONIUM COMPOUNDS OF GENERAL FORMULA



* Hey & Willey (1954) report m.p. of 1 DM 10 and 2 DE 10 as 209° C and 181° C respectively.
(d) indicates decomposition

Compound	R′	R″	m.p. °C	Analysis; found (%) C	H	N	Br	Analysis; calc. (%) C	H	N	Br
1 DM 10	CH_3	CH_3	209·5–210·5*	54·0	7·5	4·6	28·1	54·2	7·7	4·9	27·7
2 DM 10	CH_3	C_2H_5	123–124	55·45	8·0	4·45	26·4	55·6	8·0	4·6	26·4
3 DM 10	CH_3	C_3H_7	159–160	56·65	8·15	4·15	25·5	57·0	8·3	4·4	25·3
4 DM 10	CH_3	C_4H_9	133–134	58·2	8·35	4·3	24·7	58·2	8·5	4·2	24·2
5 DM 10	CH_3	C_5H_{11}	130–131	59·15	8·65	4·1	23·1	59·3	8·8	4·1	23·2
6 DM 10	CH_3	C_6H_{13}	116–118	60·1	8·8	3·7	22·05	60·3	9·0	3·9	22·3
7 DM 10	CH_3	C_7H_{15}	99·5–100·5	61·3	8·95	4·05	21·3	61·3	9·2	3·8	21·5
8 DM 10	CH_3	C_8H_{17}	105–107	62·0	9·3	3·3	20·2	62·2	9·4	3·6	20·7
1 DE 10	C_2H_5	CH_3	140–142	57·0	8·25	4·75	25·5	57·0	8·3	4·4	25·3
2 DE 10	C_2H_5	C_2H_5	178–180*	58·2	8·45	3·95	24·2	58·2	8·5	4·2	24·2
3 DE 10	C_2H_5	C_3H_7	149–151	59·05	8·55	4·1	23·7	59·3	8·8	4·1	23·3
4 DE 10	C_2H_5	C_4H_9	143–145	60·05	8·85	3·8	21·9	60·3	9·0	3·9	22·2
1 DP 10	C_3H_7	CH_3	131–133	58·9	8·5	4·1	23·15	59·3	8·8	4·1	23·2
2 DP 10	C_3H_7	C_2H_5	139–141	60·1	8·75	3·8	22·6	60·3	9·0	3·9	22·3
3 DP 10	C_3H_7	C_3H_7	126–128	61·25	9·15	3·9	21·55	61·3	9·2	3·8	21·5
4 DP 10	C_3H_7	C_4H_9	99–101	62·45	9·4	3·55	20·45	62·2	9·4	3·6	20·7
A DM 10	CH_3	CH_3=$CHCH_2$	147–148	57·5	7·5	4·45	25·4	57·3	7·7	4·5	25·4
A DE 10	C_2H_5	CH_2=$CHCH_2$	149–151	59·8	8·15	4·3	23·2	59·6	8·2	4·1	23·3
A DP 10	C_3H_7	CH_2=$CHCH_2$	107–108	61·4	8·75	3·7	21·9	61·6	8·7	3·8	21·6
A DB 10	C_4H_9	CH_2=$CHCH_2$	121–123	63·4	8·85	3·45	20·25	63·3	9·1	3·5	20·1
B DM 10	CH_3	$C_6H_5CH_2$	148–150	62·65	6·95	3·85	22·35	62·6	7·3	3·8	21·9
B DE 10	C_2H_5	$C_6H_5CH_2$	149–151(d)	64·2	7·65	3·6	20·6	64·3	7·7	3·6	20·4
B DP 10	C_3H_7	$C_6H_5CH_2$	139–141	65·9	8·1	3·45	18·95	65·7	8·15	3·3	19·0
B DB 10	C_4H_9	$C_6H_5CH_2$	—	66·65	8·4	3·0	17·65	66·95	8·5	3·1	17·8

compound which separated from the reaction mixture as white crystals or was precipitated by the addition of excess light petroleum (b.p. 60–80° C) or ether. In some instances the quaternary ammonium compound precipitated as an oil which in the majority of cases crystallized on standing. The reaction times required to obtain reasonable yields from the quaternizations varied considerably, from 1 hr at −80° C for the active bromides such as allyl bromide to 30 days at 60° C for the less active alkyl bromides. In all cases strict precautions to exclude moisture were taken.

Purification was carried out by recrystallization from mixtures of dry acetone, alcohol and light petroleum (b.p. 60–80° C) or, in the case of noncrystalline materials, by dissolving the quaternary ammonium compound in distilled water, extracting the solution with ether and freeze drying the aqueous layer before recrystallization.

All the compounds were obtained as white crystals (needles or needle clusters) with the exception of N-benzyl-N,N-di-n-butyl-2-(2,6-xylyloxy)ethylammonium bromide (B DB 10), which was obtained as a pale yellow glass by the freeze drying procedure. Details of code designation, chemical formula, melting point and analytical data are given in Table 9.

SUMMARY

1. The synthesis of a number of analogues of xylocholine in which the substituents on the quaternary nitrogen have been altered to include allyl, benzyl and higher n-alkyl substituents is reported.

2. The potency of these quaternary ammonium compounds in blocking the response to electrical stimulation of postganglionic adrenergic nerves in the transmurally stimulated guinea-pig vas deferens preparation has been determined.

3. Blocking activity is not ascribed to adrenergic neurone blocking actions, α-blockade or depression of smooth muscle.

4. Assays for local anaesthetic activity by a modification of the guinea-pig intradermal weal method suggest that blockade of the response of the transmurally stimulated guinea-pig vas deferens preparation is probably due to the local anaesthetic properties of the compounds.

5. The local anaesthetic action of the compounds was confirmed on the rabbit isolated vagus preparation.

6. The relationship between structure and activity in the cationic head of the molecule is discussed.

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