Journal of Medicinal and Pharmaceutical Chemistry

VOL. 3, No. 2 (1961)

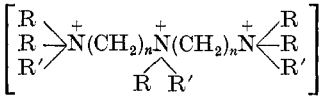
Neuromuscular Blocking Agents—VI. Linear Tetra-, Penta-, and Hexa-onium Compounds

D. EDWARDS and J. B. STENLAKE, *School of Pharmacy, Royal College of Science and Technology, Glasgow*

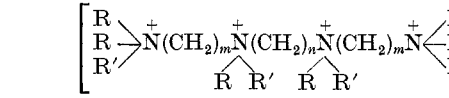
and

J. J. LEWIS and FAY STOTHERS, *Experimental Pharmacology Division, Institute of Physiology, University of Glasgow*

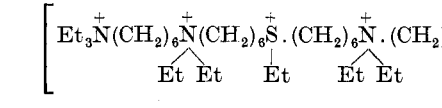
In Parts II[1] and IV[2] in this series we described the preparation and properties of some linear *N*,*N*,*N*-tris-quaternary compounds (I), and in Parts III[3] and V[4] we reported some preliminary observations on the neuromuscular blocking activity of a series of linear tetra-onium compounds (II), and of the penta- and hexa-onium compounds (III) and (IV). The preparation and more detailed pharmacological properties of these compounds are now described.



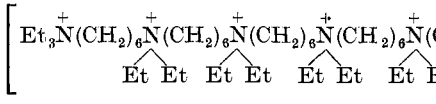
3 I⁻ (I)



4 I⁻ (II)



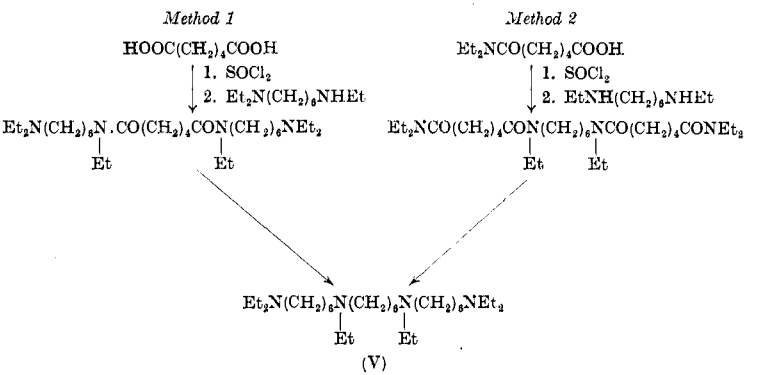
5 I⁻ (III)



6 I⁻ (IV)

The base, 1,20-bisdiethylamino-7,14-diethyl-7,14-diazaeicosane (V), the key intermediate for the formation of the tetra-onium

compounds (II; $m = n = 6$), was prepared by the two routes outlined below.



Initially method 2, from *N*,*N*-diethyladipamic acid, gave a better yield of product than the alternative route from adipic acid. In subsequent runs, however, method 1 was found to be more satisfactory and greatly improved yields of the base (V) were obtained. The following bases (VI) were also obtained by similar routes using the appropriate intermediates:

1,32-bisdiethylamino-11,22-diethyl-11,22-diazadotriacontane
(VI; $m = n = 10$)

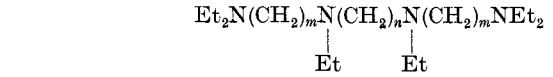
1,28-bisdiethylamino-11,18-diethyl-11,18-diaza-octacosane
(VI; $m = 10$, $n = 6$)

1,24-bisdiethylamino-7,18-diethyl-7,18-diazatetracosane
(VI; $m = 6$, $n = 10$)

1,22-bisdiethylamino-7,16-diethyl-7,16-diazadocosane
(VI; $m = 6$, $n = 8$)

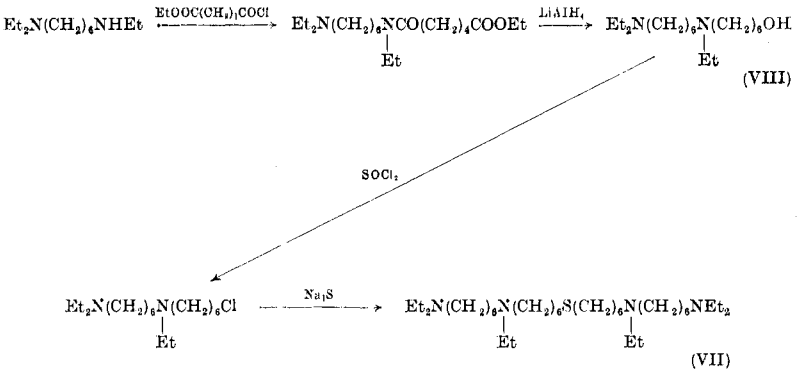
1,26-bisdiethylamino-9,18-diethyl-9,18-diazahexacosane
(VI; $m = n = 8$)

1,24-bisdiethylamino-9,16-diethyl-9,16-diazatetracosane
(VI; $m = 8$, $n = 6$)



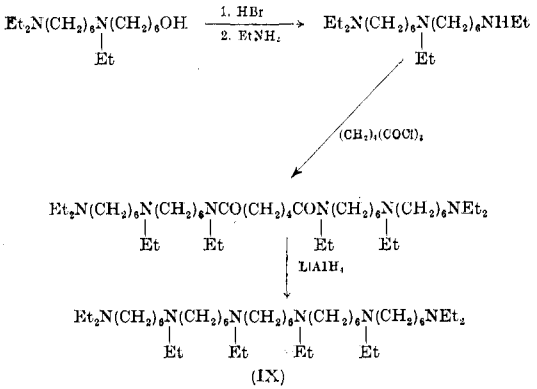
(VI)

The bases were converted to the corresponding quaternary salts (II) shown in Table I by treatment with the appropriate alkyl halides. The penta-onium compound (III) was prepared similarly by quaternization of bis-(13-diethylamino-7-ethyl-7-azatridecyl) sulphide (VII), which was obtained from 6-diethylaminohexyl-ethylamine and ethyl adipoyl chloride as outlined below.



(VIII)

(VII)



(IX)

The intermediate, 13-diethylamino-7-ethyl-7-azatridecan-1-ol (VIII), was also used as the starting point for the following synthesis of 1,34-bisdiethylamino-7,14,21,28,-tetra-ethyl-7,14,21,28-tetrazatetratriacontane (IX), which with ethyl iodide gave the hexa-azonium compound (IV).

Experimental*†

1,20-Bisdiethylamino-7,14-diethyl-7,14-diazaeicosane. (*a*) Adipic acid (2·54 g) was refluxed with excess thionyl chloride (5 ml) for 1·5 h. Excess reagent was removed, the acid chloride heated in benzene (50 ml) over a water bath, and excess 6-diethylaminohexylethylamine[1] (14·2 g) added with stirring (10 min). The mixture was refluxed gently for 10 min, cooled and extracted with 10 per cent hydrochloric acid. The acid solution was basified, extracted with benzene, the solvent evaporated, and unchanged 6-diethylhexylethylamine removed by distillation. Reduction of the crude (non-volatile) *N,N'*-diethyl-*N,N'*-bis-6-diethylaminohexyladipamide with lithium aluminium hydride in ether, and distillation of the product gave *1,20-bisdiethylamino-7,14-diethyl-7,14-diazaeicosane* as a yellow oil (1·15 g, 13 per cent), n_D^{16} 1·4695, b.p. 227–229°/0·6 mm.

(*b*) *N,N*-Diethyladipamic acid[2] (17·4 g) in benzene (7 ml) was refluxed with excess thionyl chloride (8 ml) for 5 min. After removal of the solvent and excess reagent, the acid chloride in benzene (25 ml) was added with stirring (25 min) to a solution of *N,N'*-diethylhexamethylenediamine[5] (7·5 g) in benzene (40 ml), and the mixture refluxed gently for 20 min. The solution was cooled, extracted with 10 per cent hydrochloric acid, and the acid solution basified and extracted with benzene. Evaporation of the solvent gave crude *N,N'*-diethyl-*N,N'*-bis-(*N'',N''*-diethyladipamoyl) hexamethylenediamine, which on reduction with lithium aluminium hydride and distillation of the residual oil yielded *1,20-bisdiethylamino-7,14-diethyl-7,14-diazaeicosane* as a yellow oil (6 g, 28·8 per cent), n_D^{22} 1·4665, b.p. 227–229°/0·6 mm.

The *tetrahydrochloride* (from ethanol–ether) had m.p. 249–250° (d.).

* Analyses by Miss M. Buchanan, Mr. W. McCorkindale and Dr. A. C. Syme of the Royal College of Science and Technology, Glasgow.

† Melting points are uncorrected.

Anal. Calcd. for $C_{30}H_{70}Cl_4N_4$: Cl, 22·5; N, 8·9. Found: Cl, 22·3; N, 9·0.

The following *α,ω-bisdiethylamino-*x,y*-diethyl-*x,y*-diaza-alkanes* were also prepared by the above methods. The appropriate dibasic acid and diethylaminoalkylethylamine used, yield, boiling point and refractive index are stated for each compound in that order.

1,22-Bisdiethylamino-7,16-diethyl-7,16-diazadocosane, from suberic acid (4·43 g) and 6-diethylaminohexylethylamine (20·46 g); 10·7 g (82 per cent), b.p. 238–240°/0·25 mm, $n_D^{20·5}$ 1·4663.

Anal. Calcd. for $C_{32}H_{70}N_4$: N, 10·7; equiv. wt., 128·8. Found: N, 11·0; equiv. wt., 128·0 (titration).

1,24-Bisdiethylamino-7,18-diethyl-7,18-diazatetracosane, from sebacic acid (5·27 g) and 6-diethylaminohexylethylamine (25·4 g); 8·15 g (58 per cent), b.p. 280–290° (bath)/0·6 mm, n_D^{20} 1·4673.

Anal. Calcd. for $C_{34}H_{74}N_4$: N, 10·4; equiv. wt., 134·8. Found: N, 10·2; equiv. wt., 135·9 (titration).

Tetrahydrochloride, m.p. 196–197° (from ethanol–ether).

Anal. Calcd. for $C_{34}H_{78}Cl_4N_4$: Cl, 20·7; N, 8·2. Found: Cl, 20·3; N, 8·1.

1,24-Bisdiethylamino-9,16-diethyl-9,16-diazatetracosane, from adipic acid (2·92 g) and 8-diethylamino-octylethylamine (18·3 g);[1] 7·15 g (66 per cent), b.p. 280–290° (bath)/0·3 mm, $n_D^{22·5}$ 1·4663.

Anal. Calcd. for $C_{34}H_{74}N_4$: N, 10·4; equiv. wt., 134·8. Found: N, 10·2; equiv. wt., 135·4 (titration).

1,26-Bisdiethylamino-9,18-diethyl-9,18-diazahexacosane, from suberic acid (3·48 g) and 8-diethylamino-octylethylamine (19 g); 8·60 g (76·4 per cent), b.p. 300–310° (bath)/0·3 mm, n_D^{21} 1·4670.

Anal. Calcd. for $C_{36}H_{78}N_4$: N, 9·9; equiv. wt., 141·8. Found: N, 9·7; equiv. wt., 143·2 (titration).

1,28-Bisdiethylamino-11,18-diethyl-11,18-diazaoctacosane, from adipic acid (2·61 g) and 10-diethylaminodecylethylamine (18·46 g); 6·7 g (63 per cent), b.p. 300–310° (bath)/0·6 mm, n_D^{10} 1·4679.

Anal. Calcd. for $C_{38}H_{82}N_4$: N, 9·4; equiv. wt., 148·8. Found: N, 9·4; equiv. wt., 150·2.

Tetrahydrochloride, m.p. 161·5–162° (from ethanol–ether).

Anal. Calcd. for $C_{38}H_{86}Cl_4N_4$: Cl, 19·15; N, 7·6. Found: Cl, 19·2; N, 7·5.

1,32-Bisdiethylamino-11,22-diethyl-11,22-diazadotriacontane, from sebacic acid (2·53 g) and 10-diethylaminodecylethylamine[1] (13·1 g); 4·5 g (55 per cent), b.p. 360–380° (bath)/0·7 mm, n_D^{18} 1·4692.

Anal. Calcd. for $C_{42}H_{90}N_4$: equiv. wt., 162·8; N, 8·6. Found: equiv. wt., 165·5 (titration); N, 9·1.

N,N,N,N-Tetra-onium compounds were prepared from the above α,ω-bisdiethylamino-*x,y*-diethyl-*x,y*-diaza-alkanes by refluxing with the appropriate alkyl halide in ethanol, evaporation of the solvent and crystallization. Reflux times, crystallization solvents and yields are indicated for each compound in that order in parenthesis.

7,7,14,14-Tetraethyl-7,14-diazoniaeicosylenebis(triethylammonium) tetraiodide (20 min, ethanol, 43 per cent), m.p. 248·5–249°.

Anal. Calcd. for $C_{38}H_{86}I_4N_4$: I, 45·9; N, 5·1. Found: I, 45·9; N, 4·9.

7,14-Diethyl-7,14-di-n-*propyl-7,14-diazoniaeicosylenebis(diethyl*-n-*propylammonium) tetraiodide* (50 min, ethanol–acetone–ether, 78 per cent), m.p. 197·5–198·5° (d.).

Anal. Calcd. for $C_{42}H_{94}I_4N_4$: I, 43·6; N, 4·8. Found: I, 43·7; N, 4·9.

7,14-Diethyl-7,14-dimethyl-7,14-diazoniaeicosylenebis(diethylmethylammonium) tetraiodide (10 min, methanol–ethanol, 80 per cent), m.p. 230–231°.

Anal. Calcd. for $C_{34}H_{78}I_4N_4$: I, 48·3; N, 5·3. Found: I, 48·0; N, 5·2.

7,7,16,16-Tetraethyl-7,16-diazoniadocosylenebis(triethylammonium) tetraiodide (30 min, ethanol–ether, 69 per cent), m.p. 235–235·5° (d.).

Anal. Calcd. for $C_{40}H_{90}I_4N_4$: I, 44·9; N, 4·9. Found: I, 45·2; N, 4·8.

9,9,16,16-Tetraethyl-9,16-diazoniatetracosylenebis(triethylammonium) tetraiodide (30 min, ethanol–ether, 89 per cent), m.p. 246·5–247° (d.).

Anal. Calcd. for $C_{42}H_{94}I_4N_4$: I, 43·6; N, 4·8. Found: I, 43·7; N, 4·8.

7,7,18,18-Tetraethyl-7,18-diazoniatetracosylenebis(triethylammonium) tetraiodide (30 min, ethanol, 91 per cent), m.p. 167·5–168·5°.

Anal. Calcd. for $C_{42}H_{94}I_4N_4$: I, 43·6; N, 4·8. Found: I, 43·2; N, 4·7.

9,9,18,18-Tetraethyl-9,18-diazoniahexacosylenebis(triethylammonium) tetraiodide (30 min, ethanol–ether, 92 per cent), m.p. 253·5–254°.

Anal. Calcd. for $C_{44}H_{98}I_4N_4$: I, 42·6; N, 4·7. Found: I, 42·5; N, 4·7.

11,11,18,18-Tetraethyl-11,18-diazoniaoctacosylenebis(triethylammonium) tetraiodide (30 min, ethanol, 95 per cent), m.p. 221–222°.

Anal. Calcd. for $C_{46}H_{102}I_4N_4$: I, 41·6; N, 4·6. Found: I, 41·6; N, 4·7.

11,11,22,22-Tetraethyl-11,22-diazoniadotriacontylenebis(triethylammonium) tetraiodide (50 min, acetone–ether, 75 per cent), m.p. 186–187°.

Anal. Calcd. for $C_{50}H_{110}I_4N_4$: C, 47·1; H, 8·7; I, 39·8. Found: C, 46·8; H, 8·5; I, 39·4.

Ethyl N-*ethyl*-N-(*6-diethylaminohexyl*)-*adipamate.* Ethyl adipoyl chloride prepared from ethyl hydrogen adipate (52 g) and thionyl chloride, was dissolved in benzene (100 ml). 6-Diethylaminohexylethylamine (58·9 g) in benzene (25 ml) was added slowly (45 min) with stirring and the mixture refluxed gently for 30 min. After extraction with 10 per cent hydrochloric acid, basifying and extracting with ether, fractional distillation yielded *ethyl*-N-*ethyl*-N-(*6-diethylaminohexyl*)-*adipamate* as a yellow oil, b.p. 199–201/0·6 mm (42·6 g, 43·2 per cent), $n_D^{21·5}$, 1·4648.

Anal. Calcd. for $C_{20}H_{40}N_2O_3$: N, 7·9; equiv. wt., 356·5. Found: N, 7·5; equiv. wt., 354·6.

13-Diethylamino-7-ethyl-7-azatridecan-1-ol. Ethyl-*N*-ethyl-*N*-(6-diethylaminohexyl)-adipamate (35·46 g) in dry ether (150 ml) was added to a stirred and refluxing suspension of lithium aluminium hydride (7 g) in dry ether (600 ml) at a rate sufficient to maintain refluxing. The reaction mixture was cooled, and water added dropwise to decompose the excess lithium aluminium hydride, and then excess water added. The ethereal layer was decanted and the residue washed with ether. The combined ethereal solutions were dried (Na_2SO_4) and the residue distilled to yield *13-diethylamino-7-ethyl-7-azatridecan-1-ol* as a colourless oil, b.p. 170–175°/0·6 mm (21 g, 70 per cent), n_D^{21} 1·4673.

Anal. Calcd. for $C_{18}H_{40}N_2O$: N, 9·3; equiv. wt., 150·3. Found: N, 9·2; equiv. wt., 149·4 (titration).

Dihydrochloride (from ethanol–ether), m.p. *ca.* 155°.

Anal. Calcd. for $C_{18}H_{42}Cl_2N_2O$: Cl, 19·0. Found: Cl, 19·0.

13-Diethylamino-7-ethyl-7-azatridecyl chloride. 13-Diethylamino 7-ethyl-7-azatridecan-1-ol (19 g) in benzene (60 ml) was treated with thionyl chloride (8 ml) in benzene (20 ml). The crystalline mass obtained on removal of the solvent and excess reagent was dissolved in water (10 ml), the solution cooled to 0° and basified with sodium hydroxide solution. Extraction with ether, drying (Na_2SO_4), evaporation of the solvent and distillation gave *13-diethylamino-7-ethyl-7-azatridecyl chloride* as a pale yellow oil, b.p. 167–169°/0·7 mm (13·6 g, 67 per cent), n_D^{21} 1·4648.

Anal. Calcd. for $C_{18}H_{39}ClN_2$: equiv. wt., 159·5. Found: equiv. wt., 158·5 (titration).

Dihydrochloride (from ethanol–ether), m.p. 151·5–152·5°.

Monohydrochloride (from ethanol–acetone–ether), m.p. 224–225° (d.).

Anal. Calcd. for $C_{18}H_{40}Cl_2N_2$: ionized Cl, 10·0. Found: Cl, 10·0.

Bis-(13-diethylamino-7-ethyl-7-azatridecyl) sulphide was prepared from 13-diethylamino-7-ethyl-7-azatridecyl chloride (13·3 g) and anhydrous sodium sulphide as described for bis-5-diethylamino-pentyl sulphide.[6] The *product* was obtained as a yellow oil, b.p. 305–315° (bath)/0·5 mm (8·67 g, 69 per cent), n_D^{24} 1·4780.

Anal. Calcd. for $C_{36}H_{78}N_4S$: equiv. wt., 149·8. Found: equiv. wt., 150·5 (titration).

Tetrahydrochloride (from ethanol–ether), m.p. 173–174° (d.).

Anal. Calcd. for $C_{36}H_{82}Cl_4N_4S$: N, 7·5. Found: N, 7·5.

7,7,14,21,21-Pentaethyl-7,21-diazonia-14-thioniaheptacosylenebis (triethylammonium) pentaiodide. Bis-(13-diethylamino-7-ethyl-7-azatridecyl) sulphide (2·1 g) was refluxed with ethyl iodide (3 ml) and ethanol (3 ml) for 35 min. Evaporation under reduced pressure and recrystallization of the product from acetone–ether yielded *7,7,14,14,21-pentaethyl-7,21-diazonia-14-thioniaheptacosylenebis (triethylammonium) pentaiodide,* m.p. 165·5–166·5° (d.), (3 g, 62 per cent).

Anal. Calcd. for $C_{46}H_{103}I_5N_4S$: I, 46·0; N, 4·1. Found: I, 46·2; N, 4·2.

13-Diethylamino-7-ethyl-7-azatridecylethylamine. 13-Diethylamino-7-ethyl-7-azatridecan-1-ol (34·4 g) was refluxed with 48 per cent hydrobromic acid (140 ml) for 5 h. The mixture was evaporated to dryness over a boiling water-bath, water (50 ml) was added and the product then evaporated under reduced pressure. Ethanol (50 ml) was added and the solution again evaporated under reduced pressure. Repetition of the latter procedure with ethanol gave crude 13-diethylamino-7-ethyl-7-azatridecyl bromide hydrobromide. This was dissolved in hot ethanol (100 ml) and added slowly (30 min) to excess refluxing ethylamine (250 ml), and the mixture refluxed for 1 h. After distilling off excess ethylamine, the residual solution was evaporated to dryness, and the solid residue basified and extracted with ether. The ethereal solution was dried (Na_2SO_4), the solvent evaporated and the residual oil fractionated to yield *13-diethylamino-7-ethyl-7-azatridecylethylamine*, as a colourless oil, b.p. 164–168°/0·3 mm (29·33 g, 75·2 per cent), $n_D^{22\cdot5}$ 1·4602.

Anal. Calcd. for $C_{20}H_{45}N_3$: equiv. wt., 109·2. Found: equiv. wt., 112·5 (titration).

Trihydrochloride. m.p. 200·5–202° (d.).

Anal. Calcd. for $C_{20}H_{48}Cl_3N_3$: Cl, 24·35. Found: Cl, 24·36.

1,34-Bisdiethylamino-7,14,21,28-tetraethyl-7,14,21,28-tetrazatetratriacontane. Adipoyl chloride was prepared in the usual way by refluxing adipic acid (3 g) with excess thionyl chloride for 1½ h. After removal of excess reagent the acid chloride in benzene (75 ml) was refluxed over a water-bath and 13-diethylamino-7-ethyl-7-azatridecylethylamine (19·46 g) in benzene (60 ml) was added with stirring (15 min). The mixture was then refluxed for 20 min, cooled and extracted with 10 per cent hydrochloric acid. The acid solution was basified, extracted with ether and dried (Na_2SO_4). After removal of the solvent the bulk of 13-diethylamino-7-ethyl-7-azatridecylethylamine was distilled, leaving the crude *N,N'*-diethyl-*N,N'*-bis(13-diethylamino-7-ethyl-7-azatridecyl)-adipamide. Lithium aluminium hydride reduction of this amide in ether and distillation of the residual oil gave *1,34-bisdiethylamino-7,14,21,28-tetraethyl-7,14,21,28-tetrazatetratriacontane* as a yellow oil (6·19 g, 40·9 per cent), b.p. 335–350° (bath)/0·25 mm, n_D^{21} 1·4730.

Anal. Calcd. for $C_{46}H_{100}N_6$: N, 11·4; equiv. wt., 122·9. Found: N, 11·0; equiv. wt., 124·7 (titration).

7,7,14,14,21,21,28,28-Octaethyl-7,14,21,28-tetrazoniatetratriacontylenebis (triethylammonium) hexaiodide. 1,34-Bisdiethylamino-7,14,21,28-tetraethyl-7,14,21,28-tetrazatetratriacontane (0·99 g) was refluxed with ethyl iodide (3 ml) and ethanol (4 ml) for 30 min. Evaporation under reduced pressure and recrystallization from ethanol gave *7,7,14,14,21,21,28,28-octaethyl-7,14,21,28-tetrazoniatetratriacontylenebis (triethylammonium) hexaiodide* (1·7 g, 76 per cent), m.p. 248° (d.).

Anal. Calcd. for $C_{58}H_{130}I_6N_6$: I, 45·5; N, 5·0. Found: I, 45·6; N, 5·0.

Pharmacology

Methods and Results

Muscle-relaxant activity in the cat. Cats of either sex weighing from 1·5 to 3·0 kg were anaesthetized by intraperitoneal injection of 50 mg/kg of sodium pentobarbitone. The trachea was cannulated and the cannula made ready for rapid attachment to a Starling-type artificial respiration pump. One external jugular vein was cannulated and the cannula attached by means of rubber tubing to a glass burette filled with normal saline. The gastrocnemius muscle of one leg was partially dissected free from surrounding muscular and connective tissues. The leg was clamped into a position in which its long axis was perpendicular to the operating table and the Achilles tendon severed at a point near to its insertion into the calcaneus and attached by a strong linen thread led over pulleys to a Sherrington myograph lever. The sciatic nerve was exposed between the hamstring muscles on the lateral aspect of the thigh and placed over a pair of shielded platinum electrodes attached to a square wave stimulator. Indirect stimulation was by means of single supra-maximal square impulses, frequency 8–10/min, voltage 10 to 25, pulse width 2 to 3 msec. For indirect tetanisation the frequency was increased to 1,600/min. In any given experiment, frequency, voltage and pulse width were constant. Drugs were administered as aqueous solutions by injection into the jugular vein cannula and washed in with 3 ml of saline. Muscle relaxant activity was compared with that of tubocurarine. A dose of the drug was found which

gave a response approximately equal to that of the dose of tubocurarine which caused a 50 per cent reduction in twitch height. As a rule the preparation was initially sensitized to tubocurarine so that subsequent administration of a given dose no longer produced a sharply increasing depression of twitch height. Where possible the preparation was always allowed to recover fully before giving the next dose.

Additive effects with tubocurarine. A small dose (0·05 to 0·10 mg/kg) of tubocurarine was first given followed, after complete recovery, by a dose of drug producing a response roughly equivalent to that produced by the previous dose of tubocurarine. After recovery the same dose of drug followed by that of tubocurarine was given at an interval of two minutes.

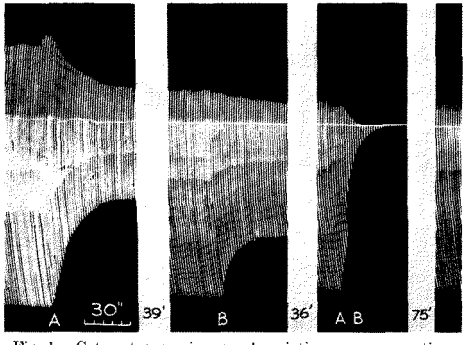


Fig. 1. Cat gastrocnemius muscle–sciatic nerve preparation. At A: III, 0·1 mg/kg. At B: tubocurarine, 0·1 mg/kg.

Decamethonium antagonism. A dose of drug which caused about 50 per cent reduction of twitch height was given. At the point of maximum effect, 0·025–0·050 mg/kg of decamethonium was injected.

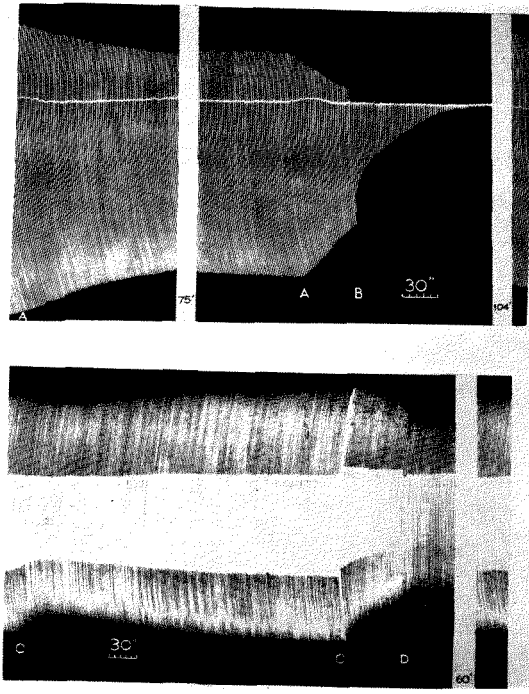


Fig. 2. Cat gastrocnemius muscle–sciatic nerve preparation.
At A: IIH, 0·2 mg/kg. At B: C 10, 0·05 mg/kg. At C: IIH, 0·15 mg/kg.
At D: neostigmine 0·05 mg/kg.
All drugs given by intravenous injection. The muscle stimulated indirectly (25 volts, frequency 9/min, pulse width 2·5 msec).

Effects of neostigmine and edrophonium. A dose of drug which produced about 50 per cent depression of twitch height was given. When the maximum effect was reached, either 0·05–0·10 mg/kg of neostigmine, or 0·20–0·40 mg/kg of edrophonium was given.

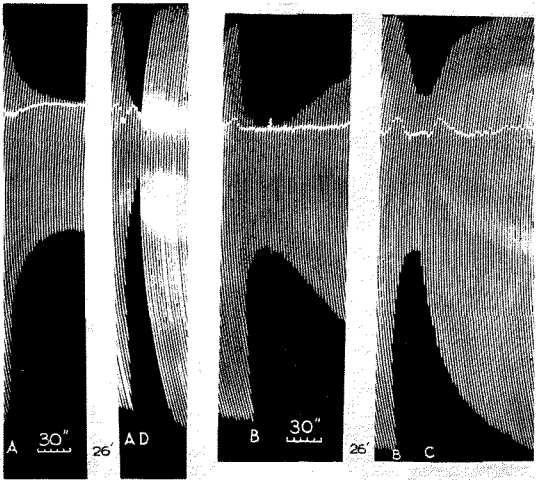


Fig. 3. Cat gastrocnemius muscle–sciatic nerve preparation.
At A: IIA, 0·15 mg/kg. At B: Edrophonium, 0·3 mg/kg. At C: Neostigmine, 0·05 mg/kg.
All drugs given by intravenous injection. The muscle stimulated indirectly (25 volts, frequency 9/min. Pulse width 2·5 msec).

Effects of adrenaline and potassium chloride. A dose of drug which produced about 50 per cent depression of twitch height was given. When the maximum effect was reached, either 10 to 20 μg/kg of adrenaline or 5 mg/kg of potassium chloride was given. Because potassium chloride was very toxic to the preparation, only a few experiments were done using it.

Effects of inhalation of ether. A dose of drug was given which depressed twitch height by about 20 per cent of the original level. The twitch height was then allowed to recover to the original level, the animal allowed to breathe ether vapour and the same dose of drug repeated. Ether was administered for a further 5 to 10 min and respiration maintained artificially when required.

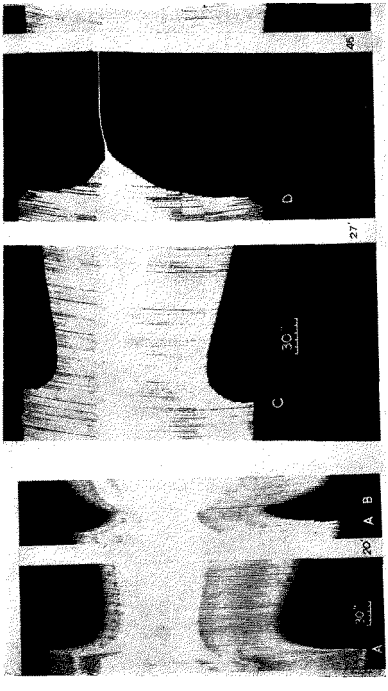


Fig. 4. Cat gastrocnemius muscle–sciatic nerve preparation.
At A: HD, 0·05 mg/kg. At B: C 10, 0·05 mg/kg. At C, HD, 0·1 mg/kg. At D, HD, 0·1 mg/kg plus ether.
The muscle stimulated indirectly (25 volts, frequency 9/min, pulse width 2·5 msec).

Effects upon an indirect tetanus. The nerve of the non-curarized preparation was stimulated at the higher rate (1,600/min) for 20 sec to record a control response. A dose of drug was given which partially blocked neuromuscular transmission and caused from 20 to 60 per cent depression of twitch height. When the muscle was partially curarized tetanization was repeated. In each preparation the drug was compared with tubocurarine.

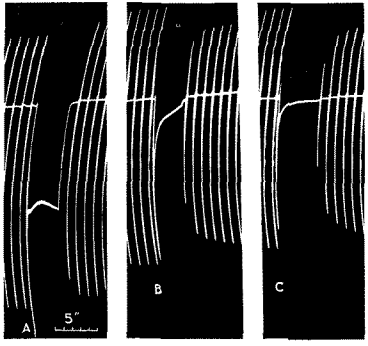


Fig. 5. Cat gastrocnemius muscle–sciatic nerve preparation.
At A: indirect tetanus, normal effect. At B: indirect tetanus on tubocurarine-treated muscle. At C: indirect tetanus after IIA.
All drugs given by intravenous injection. The muscle stimulated indirectly (25 volts, frequency 16/min, pulse 2·5 msec. For tetanus, frequency 1,600/min).

The results are summarized in Tables I–IV. Figs. 1–5 show some typical effects. Of the compounds tested on the cat, IIA was the most typically tubocurarine-like (Figs. 3 and 5). The effects of this compound were antagonized rapidly and effectively by neostigmine, edrophonium, decamethonium and tetanization. Adrenaline caused transient decurarization and ether and tubocurarine increased the intensity of block. III was also predominantly tubocurarine-like; it was more potent than tubocurarine but more toxic. IIH and IIE were less like tubocurarine than

Table I. Relative molar potencies (compared with TC–100) in the cat, rabbit, mouse, frog and chick

Structure	R	R′	*m*	*n*	Cat (gastrocnemius)	Rabbit	Mouse	Frog	Chick	Ref.
IA	Et	Et	—	6	104	22	18	52	593	1
IB	Et	Et	—	10	119	46	149	12		1
IIA	Et	Me	6	6	128	102	134	121	229	
IIB	Et	Et	6	6	269	82	70	70	420	
IIC	Et	*n*-Prop	6	6	187	164	370	22	222	
IID	Et	Et	6	8	174	173	284	53	347	
IIE	Et	Et	6	10	320	228	237	86	290	
IIF	Et	Et	8	6	197	265	423	89	394	
IIG	Et	Et	8	8	284	264	778[a]	120	260	
IIH	Et	Et	10	6	101	220	1340[a]	63	372	
IIJ	Et	Et	10	10	81	46	363	29	827	
III					212	221	274	336	343	
IV					437	356	425	94	511	
TC					100	100	100	100	100	

[a] Probably not solely due to paralysis (see text).

any of the other compounds and appeared to possess some transitional properties. Both were very long acting and very toxic and after one dose it was often impossible to get recovery of the response to control levels. IIH was more toxic and had a longer action than IIE. If IIH was added at the beginning of the experiment it was additive with neostigmine and decamethonium but after six doses had been given it became more tubocurarine-like and then tended to antagonize both neostigmine and decamethonium. Its effects upon an indirect tetanus were variable.

IIE had similar properties but was antagonized by decamethonium. IID and IIF were mainly tubocurarine-like. IIF was very toxic and good responses were difficult to obtain and were not readily reversible. It was more toxic than IID. IIC was very toxic to the preparation; IIG was mainly tubocurarine-like but also very toxic. IV had similar properties but was much more potent and was less toxic than IIG but more toxic than IIA.

The compounds tested on the cat fell into two groups. IIA III and IID were predominantly tubocurarine-like. The other compounds differed from tubocurarine primarily by being much more toxic to cats, longer acting and in two cases (IIE and IIH) showing some transitional properties. Apart from IIH there was no very positive evidence at all of decamethonium-like activity as typified for example by muscular fasciculation, initial potentiation of twitch height and additive effects with neostigmine or edrophonium.

Muscle relaxant activity in the rabbit. The method employed was that of Varney, Linegar and Holaday.[7] The effects of pretreatment with neostigmine (0·05 mg/kg) on the head-drop dose were also investigated. The results are shown in Table II which includes figures for tubocurarine. With the exception of IIA all were toxic and in some cases it was felt unjustifiable to carry on with the series. The most potent muscle relaxants were IIF, IIG, IV and IIE, but all were toxic and apparently caused some central stimulation because all of the animals convulsed violently before dying. IIH, IID and III were also toxic but less potent. It was difficult to obtain a precise assessment of potency or type of effect in the most toxic compounds, IIG and IIH, as convulsant activity sometimes appeared before a proper estimation of the head-drop dose could be made.

Table II. Relative molar potencies (compared with TC–100) based upon the rabbit head drop dose (HDD), the influence of pretreatment with neostigmine upon the HDD, and the mortality in rabbits

Compound	HDD ± S.E.	HDD ± S.E. after neostigmine	Ratio	Molar potency	Mortality	
					Without neostigmine	After neostigmine
III	0·195 ±0·03	0·3412 ±0·04	1·38	221	6/9	6/9
IIE	0·155 ±0·039	Not done due to toxicity	—	228	8/9	
IIH	0·169	Not done due to toxicity	—	220	3/3	
IIC	0·2237 ±0·04	Not done due to toxicity	—	164	9/9	
IID	0·2099 ±0·04	0·2941 ±0·03	1·38	173	5/9	3/9
IIF	0·139	Not done due to toxicity	—	265	6/6	
IIG	0·143	Not done due to toxicity	—	264	5/5	
IV	0·149	Not done due to toxicity	—	356	3/3	
IIA	0·327 ±0·04	0·74 ±0·1	2·26	102	1/9	2/9
TC	0·249 ±0·06	0·437 ±0·06	1·82	100	2/9	5/9

Paralysing activity in mice. The method adopted was that of Thomson[8] using a screen inclined at 45° to the horizontal. The results are set out in Table III, while Table I shows the relative molar potencies in mice alongside those in the other species used.

Table III. Approximate PD_{50} and LD_{50} in mice

Compound	PD_{50} (mg/kg)	LD_{50} (mg/kg)	Paralysing potency (molar)
III	0·25	1·10	274
IIE	0·25	0·7	237
IIH	0·047	0·13	1340
IIC	0·16	0·24	370
IID	0·2	0·63	284
IIF	0·14	0·43	423
IIG	0·078	0·27	778
IV	0·2	0·47	425
IIA	0·4	1·15	134
TC	0·4	0·74	100

In mice, IIH and IIG were apparently much more potent than any of the others but in these cases it was difficult to decide whether this was due to convulsant activity rather than to muscular paralysis. Next in order of potency were IV, IIF, IIC, IIJ, IID, III, IIE, IB, IIA and IA. The toxicity appeared to parallel their potency. This was very clear in the case of IIH, which was the most potent muscle relaxant and also the most toxic compound investigated; it caused violent tonic–clonic convulsions in the test animals. IIC, IID, IIF, IIG and IV were also potent muscle relaxants and muscle relaxant activity was paralleled by the toxic-convulsant properties. The least toxic compounds were III and IIA, but these were still more potent than tubocurarine. The most toxic compounds caused violent tonic–clonic convulsions which were not apparently due to anoxia.

Paralysing activity in chicks. Two-day-old chicks were used. Drug (0·5 mg) was injected intraperitoneally every 30 sec until paralysis occurred. All caused a flaccid paralysis except IIH which caused spastic paralysis which changed to flaccid. The

most potent compounds were IIJ, IA, IV, IIB, IIF, IIH and IID. Next in order came III, IIE, IIG and IIA. The results are shown in Table IV, while Table I shows the relative molar

Table IV. Paralysing potency in the chick

Compound	Chick paralysis dose (mg/kg)	Potency chick (molar)
IIA	3·5	229
IIC	0·4	222
IID	2·5	347
IIE	3	290
IIF	2·25	394
IIG	3·5	260
IIH	2·5	372
III	3	343
IV	2·5	511
TC	6	100

potencies on the chick and compares these with data from the other species used.

Potency in the frog rectus abdominis muscle. Chang and Gaddum's method was used.[9] A 10-ml bath was employed and the bath fluid was oxygenated frog Ringer's solution (NaCl, 6·5 g; $NaHCO_3$, 0·2 g; KCl, 0·14 g; $CaCl_2$, 0·12 g; glucose, 1 g; distilled water to 1 l.) at room temperature. A 90-sec time cycle was employed and drugs were added 30 sec before the next dose of acetylcholine. The results are shown in Table I. None of the compounds caused a direct contraction of the muscle. On the frog, III was the most potent compound but, in general, potency was much lower on this preparation than on cat, rabbit, mouse or chick (see Table I). IIA was next in order of potency (about half that of III). The least potent was IIC.

Sympathetic ganglion-blocking activity in the cat. Cats weighing from 1·5 to 3·0 kg were used. They were anaesthetized by means of intraperitoneal injection of 50 mg/kg of sodium pentobarbitone. Drugs in aqueous solution were injected into the cannulated femoral vein and the preganglionic fibres of the cervical sympathetic were stimulated by means of 30-sec bursts of square

impulses, frequency 1,500/min, voltage 10 to 12, pulse width 1·5 to 2·0 msec. In any given experiment frequency, voltage and pulse width were constant. Three minutes were allowed to elapse between each period of stimulation. 0·4 to 1·0 mg/kg of the drugs had no effect upon the response of the nictitating membrane to preganglionic stimulation of the cervical sympathetic.

Parasympathetic ganglion-blocking activity in the guinea-pig ileum. Trendelenburg's method[10] as modified by Feldberg and Lin[11] was employed. A 50-ml bath was used; it contained Tyrode's solution (NaCl, 8·0 g; KCl, 0·2 g; $CaCl_2$, 0·2 g; $MgCl_2$, 0·01 g; $NaHPO_4$, 0·05 g; $NaHCO_3$, 1·0 g; dextrose, 1·0 g; distilled water to 1 l.) at 37° and was gassed with oxygen. Drugs in aqueous solution were added to the bath 30 sec before the reservoir of Tyrode's solution was raised to initiate peristalsis. Peristaltic movements were recorded for from 1 to 2 min. The time was constant for each piece of gut but varied from gut to gut. Peristaltic movements were recorded by means of a float recorder or a piston recorder. Doses of 15 to 30 μg/ml of drug were used. Compounds III, IIE, IIH, IIF, IIG, IV and IIA caused no inhibition of peristalsis but IIC and IIA caused a slight inhibition of the peristaltic response.

Effects upon the cat blood pressure level. Cats weighing from 1·5 to 3·0 kg were used. They were anaesthetized by means of an intraperitoneal injection of 50 mg/kg of sodium pentobarbitone. Drugs were administered into the cannulated external jugular vein and washed in by 3 ml of saline. Blood pressure was recorded from the common carotid artery using a mercury manometer. Compounds IV and IIE caused a slight fall in blood pressure level at doses of 0·4 mg/kg. This fall in blood pressure was abolished by atropine (0·75 mg/kg) in both cases. The other compounds at this dose level had no pressor or depressor effects.

Effects upon the hen gastrocnemius muscle–sciatic nerve preparation. Hens weighing from 2·0 to 3·0 kg were anaesthetized by injection of either 80 mg/kg sodium pentobarbitone or by urethane (10 ml of the 25 per cent w/v solution). One external jugular vein and the trachea were cannulated. The sciatic nerve and gastrocnemius muscle were dissected and arrangements made for recording twitch height and tension as described for the cat, but

Table V. A qualitative pharmacological analysis of the properties of

Compound	Duration of action and ease of spontaneous recovery	Effect of TC on block	Effect of C 10 on block	Effect of Tetanus on block
IIA	Short acting (duration < TC) 15–20 min, recovers readily	Additive (+ + +)	Marked antagonism (+ + +)	Decurarizat
IIC	Long acting (duration > TC) 20–50 min, may not reverse fully	,,	,,	,,
IID	Short acting (duration =TC) 16–26 min, recovers readily	,,	,,	,,
IIE	Very long acting 16–80 min, may not fully reverse	,,	Antagonism (+ +)	,,
IIF	Long acting (duration > TC) 21–76 min, recovers readily	,,	,,	,,
IIG	Long acting (duration > TC) 20–53 min, recovers readily	,,	,,	,,
IIH	Very long acting (duration > TC) 12–100 min, recovers readily	,,	Addition (+) changing to antagonism (−)	None
III	Long acting (duration > TC) 18–55 min, recovers readily	,,	Antagonism	Decurarizat
IV	Very long acting (duration > TC) 18–62 min, recovers readily	,,	,,	,,

romuscular block produced by compounds, IIA, IIC–IIH, III and IV

fect of block on Tetanus	Effect on block of edrophonium and neostigmine	Effect of ether on block	Action in chick (paralysis)	Remarks
rp fall in sion -like)	Marked antagonism (+ + +)	Marked potentiation (+ + +)	Flaccid	Typically TC-like
sion less well ntained than 0 Intermediate	Antagonism (+ +)	,,	,,	Mixed but more allied to TC than C 10
rp fall in sion -like)	Marked antagonism (+ + +)	,,	,,	Typically TC-like
,,	,,	,,	,,	Mixed but very much more allied to TC than C 10
,,	,,	Potentiation (+ +)	,,	,,
sion less well ntained than 0. Intermediate to like	Antagonism (+ +)	Marked potentiation	,,	Mixed but more allied to TC than C 10
,,	*Edrophonium* None changing to antagonism (+) *Neostigmine* Potentiation (+) changing to antagonism (−)	None	Spastic changing to flaccid	Mixed but more allied to C 10 than TC
rp fall in sion TC-like	Antagonism	Potentiation	Flaccid	Mixed but very much more closely allied to TC than C 10
,,	,,	,,	,,	Typically TC-like

stimulation was by 6 square impulses/min and the pulse width was 6 msec. Drugs were administered into the cannulated external jugular vein. Only compound IIH (0·05 mg/kg) produced a contracture in addition to depressing twitch height.

Discussion

In earlier publications[1-4] we came to the conclusion that the *N,N,N*-tris-ethonium compounds I and the analogous *N,S,N*-tris-ethonium compounds were essentially linear in character both in the solid state and in solution. For similar reasons we believe that compounds II, III and IV are also probably linear, although we have no information upon the configuration which any of these molecules assume when they are adsorbed upon the receptor surface. The present experiments confirm and extend our earlier conclusions[1-4] that in all these polyonium compounds, inter-onium distance is responsible for determining the type of activity whilst the number of onium groups in the molecule influences potency, reversibility and duration of action. Thus tris-, tetra-, penta- and hexa-onium compounds in which the onium groups are separated by 5- or 6-methylene groups, are predominantly tubocurarine-like. Compounds in which the onium groups are separated by 8-methylene units (IIG) have mixed effects, whilst those in which the onium groups are separated by 10-methylene groups are predominantly C 10-like. A gradual shading of properties from one extreme to the other, which parallels inter-onium group separation, can be seen when compounds such as IIE, IIF and IIH with mixed inter-onium spacings are also considered. This classification is based on the properties which are summarized in Table V, and on an assessment of toxicity. IIH had more intermediate or 'mixed' properties than any of the other compounds. On the cat, for example, its effects are initially enhanced by edrophonium, neostigmine, and decamethonium, but after a series of doses had been given some antagonism was shown by edrophonium and neostigmine. The presence of transitional properties showing that neuromuscular block is of a mixed type is of practical importance since it may indicate that compounds which are not entirely tubocurarine-like in the laboratory (Table VI) are unlikely to be so in man.

That inter-onium distance is of overriding importance in determining the type of action and that overall chain length is not important is clear from Table VI. This shows that compounds of almost identical overall chain length, such as IIB, IB, and IID; IIG, III, and IIH; IIJ and IV can have contrasting types of action.

The relative molar potencies (compared with tubocurarine = 100) of the hexamethylene separated ethonium compounds with 2,3,4 and 6 onium groups in the rabbit, cat, mouse and frog are compared graphically in Fig. 6. The *N*,*N*,*N*,*N*,*N*-pentaazonium

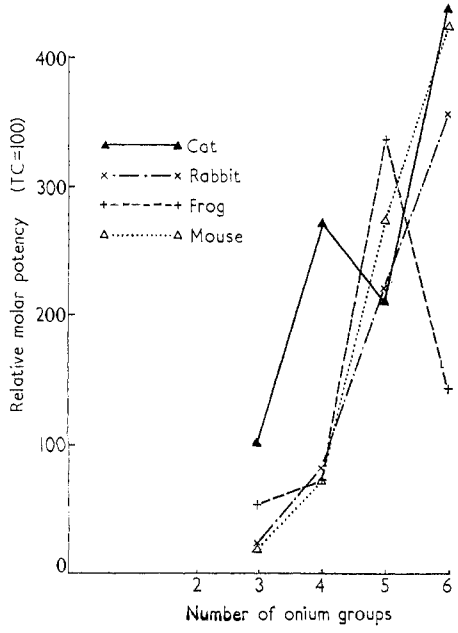
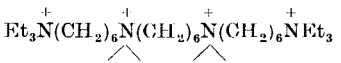
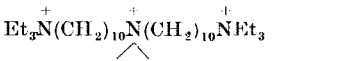
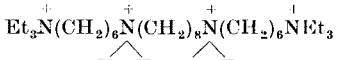
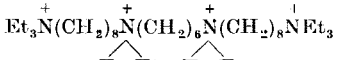
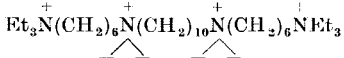
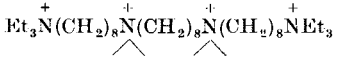
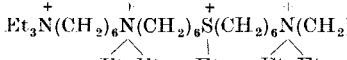
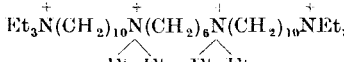
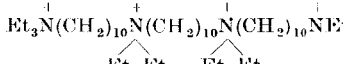
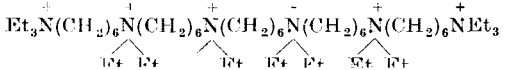


Fig. 6. Relative molar potencies of hexamethylene-separated ethonium compounds.

compound was not available, and the series has been completed by inclusion of the analogous *N*,*N*,*S*,*N*,*N*-pentaethonium compound. Since we have already shown that the potencies of dihexasulphonium and dihexazonium were closely similar in the rabbit and mouse we felt this step was justified. Certain trends in molar potency are obvious irrespective of the species used. For

Table VI. Comparison of chain length of poly-onium compounds with type of neuromuscular block exhibited

Compound	Structure	Type of block	Number of atoms separating terminal onium groups
HB		TC-like	20
IB		C 10-like	21
HD		TC-like	22
HF		TC-like	24
HE		TC-like	24
HG		Transitional	26
HI		TC-like	27
HH		C 10-like	28
HJ		C 10-like	32
IV		TC-like	34

example, in cat, rabbit, mouse, chick and frog, compounds IA, IIB and IV increase in potency in that order with the greatest increment in potency between the first two. It is also of interest that in the rabbit, mouse and frog IIA is more potent than the corresponding ethonium compound. In some compounds increase in the number of ethonium groups seems also to confer convulsant activity.

The increase in potency seen with increase in the number of onium centres may be a reflection of an increase in the number of onium receptor sites occupied by the drug molecule. If it is assumed that there is a 'one point' receptor attachment analogous to that postulated by Fakstorp and his colleagues for ganglion blocking agents,[12] activity may be due to shielding of the receptor surface by the larger poly-onium compound, an effect contributed to by the increased possibility of the formation of ionic and van der Waal's bonds. The idea of a one point attachment, however, seems less probable in view of the much greater influence on potency of an additional onium-alkyl unit as compared with that of an additional alkyl group of comparable size.

The marked differences in the curves in Fig. 6 illustrate species

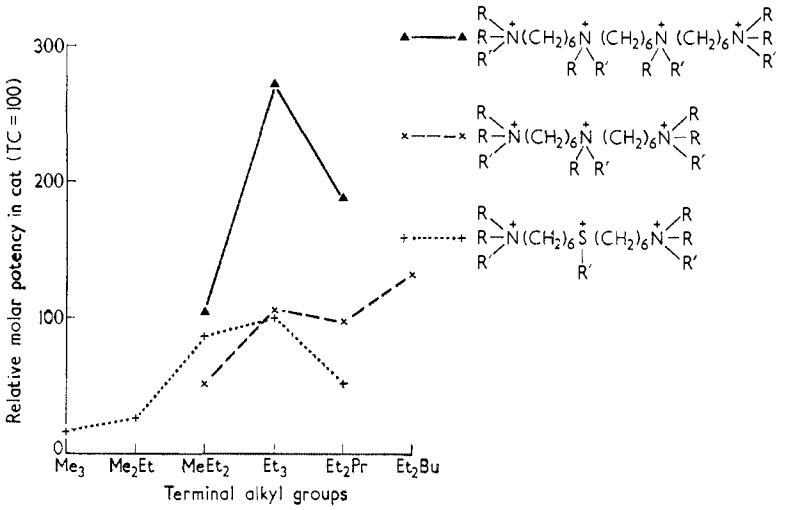


Fig. 7. Effect of onium group substituents on relative molar potencies in cat.

differences already well known. The lower molar potency of the *N,N,S,N,N*-pentaethonium compound (IV) is not an anomaly, but reflects the depressant effect upon potency in the cat of the presence of the larger sulphonium group. This is further illustrated by the comparison in Figs. 7 and 8 of the influence of the

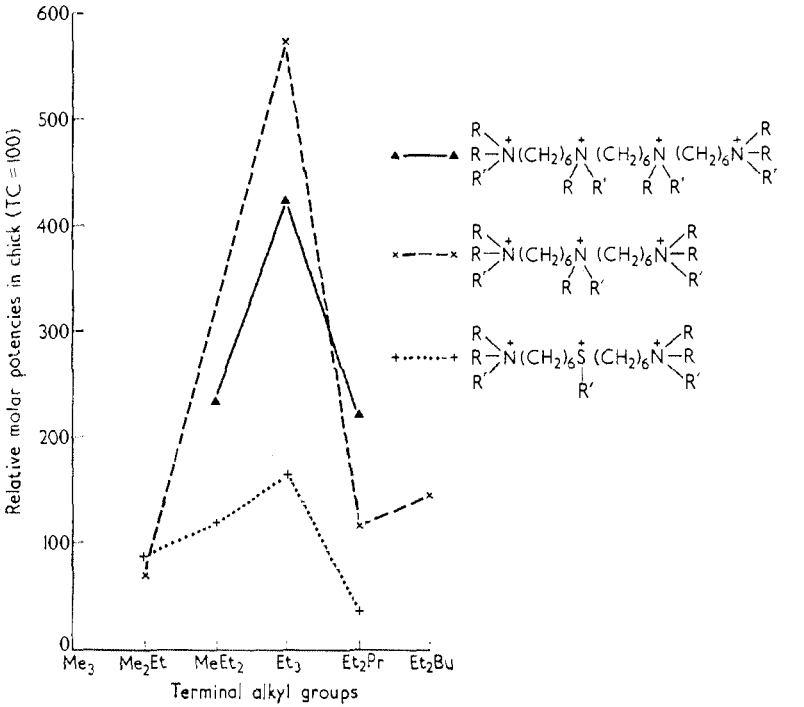


Fig. 8. Effect of onium group substituents on relative molar potencies in chick.

oniumalkyl substituents on molar potency in the cat and chick of the hexamethylene separated *N,N,N*-, *N,S,N*, and *N,N,N,N*-onium compounds. Similar but less well defined effects are seen in the frog (Fig. 9) and these are in contrast to the effects of alkyl group size on potency in the rabbit (Fig. 10) and mouse (Fig. 11).

The effects of neuromuscular blocking agents in the cat probably approximate more closely than those in other species to

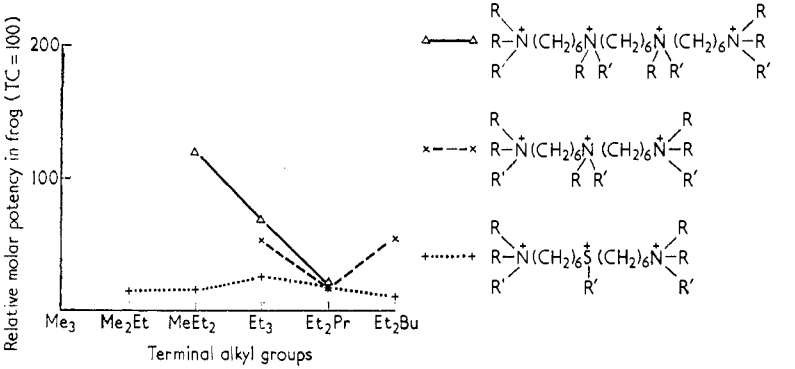


Fig. 9. Effect of onium group substituents on relative molar potencies in frog.

man and it seems significant (Levy, personal communication) that observations in man indicate the following order of potency: dihexazonium (I; R = R′ = Et), dipropazonium (I; R = Et, R′ = Pr), dihexasulphonium ($Et_3N^+(CH_2)_6S^+(Et)(CH_2)_6N^+Et_3$), from which it can be seen that the presence of the larger alkyl groups and the larger sulphur atom lower potency.

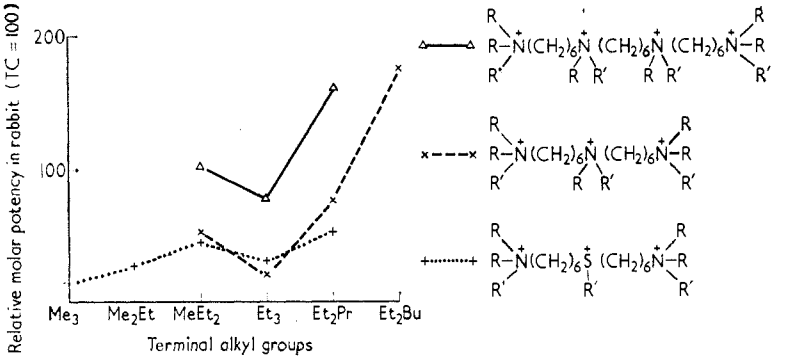


Fig. 10. Effect of onium group substituents on relative molar potencies in rabbit.

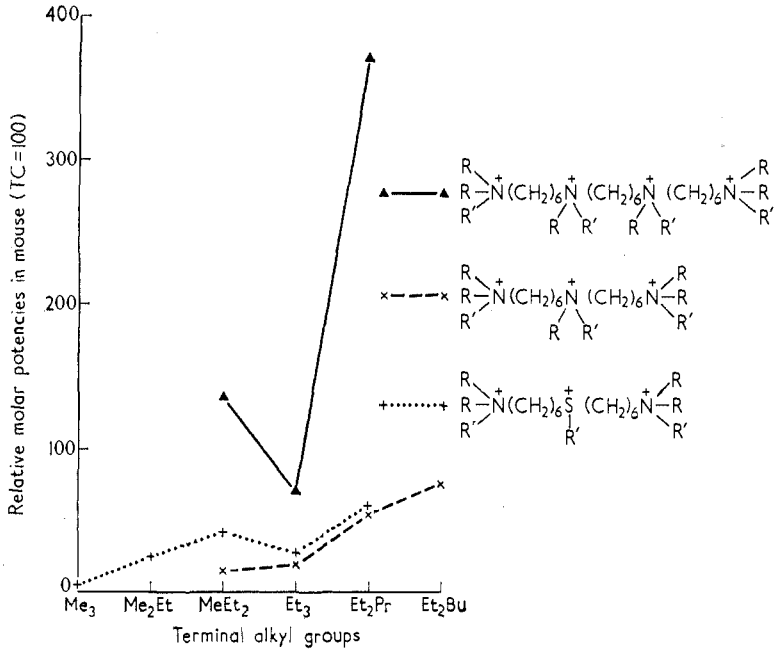


Fig. 11. Effect of onium group substituents on relative molar potencies in mouse.

Summary. The preparation of a series of *N,N,N,N*-tetraonium compounds (II,A to IIJ), an *N,N,S,N,N*-pentaonium compound (III) and an *N,N,N,N,N,N*-hexaonium compound (IV) is described. Inter-onium distance has been shown to be responsible for determining the type of activity, whilst the number of onium groups in the molecule influences potency, reversibility and duration of action. Compounds which are separated by 5 or 6 methylene groups are predominantly tubocurarine-like in their actions. Those in which the first two onium groups are separated by 8 methylene groups have mixed effects, and these are accentuated when the inter-onium chain is increased to 10 methylene units.

Acknowledgements. We wish to acknowledge the technical assistance of Miss Gladys Marren and to thank Mr. R. Callander for the drawings.

(*Received 26 April, 1960*)

References

[1] Edwards, D., Lewis, J. J., Stenlake, J. B. and Zoha, M. S. *J. Pharm., Lond.*, **10**, 106T (1958)

[2] Carey, Fiona M., Edwards, D., Lewis, J. J. and Stenlake, J. B. *J. Pharm., Lond.*, **11**, 70T (1959)

[3] Edwards, D., Lewis, J. J., Stenlake, J. B. and Zoha, M. S. *J. Pharm., Lond.*, **10**, 122T (1958)

[4] Edwards, D., Lewis, J. J., Stenlake, J. B. and Stothers, Fay. *J. Pharm., Lond.*, **11**, 87T (1959)

[5] Edwards, D., Lewis, J. J., Stenlake, J. B. and Zoha, M. S. *J. Pharm., Lond.*, **9**, 1004 (1957)

[6] Wittbecker, E. L., Houtz, R. C. and Watkins, W. W. *J. Amer. chem. Soc.*, **69**, 579 (1947)

[7] Varney, R. F., Linegar, C. R. and Holaday, H. A. *J. Pharmacol.*, **97**, 72 (1949)

[8] Thomson, R. E. *Endocrinology*, **39**, 62 (1946)

[9] Chang, H. C. and Gaddum, J. H. *J. Physiol.*, **79**, 255 (1933)

[10] Trendelenberg, P. *Arch. exp. Path. Pharmak.*, **81**, 55 (1917)

[11] Feldberg, W. and Lin, R. C. Y. *Brit. J. Pharmacol.*, **4**, 33 (1949)

[12] Fakstorp, J., Pedersen, J. G. A., Poulsen, E. and Schilling, M. *Acta pharm. tox., Kbh.*, **13**, 52 (1957)