NEUROMUSCULAR BLOCKING AGENTS

PART II. THE PREPARATION AND PROPERTIES OF A SERIES OF *NSN*- AND *NNN*-TRIS-ETHONIUM COMPOUNDS

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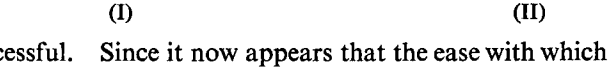
The preparation of two further *NSN*-tris-ethonium compounds, 9-ethyl-9-thioniaheptadecylenebis(triethylammonium) triiodide (dioctasulphonium triethiodide; DOSE) and 11-ethyl-11-thioniaheneicosylenebis(triethylammonium) triiodide (didecasulphonium triethiodide; DDSE) is described. The bis-quaternary compound 7-dioxothiatridecylenebis(triethylammonium iodide), and the *NNN*-tris-quaternary compounds 7:7-diethyl-7-azoniatridecylenebis (triethylammonium) triiodide (dihexazonium triethiodide; DHAE), 9:9-diethyl-9-azoniaheptadecylenebis(triethylammonium triiodide (dioctazonium triethiodide; DOAE) and 11:11-diethyl-11-azoniaheneicosylenebis(triethylammonium) triiodide (didecazonium triethiodide; DDAE) have also been synthesised. All the compounds possess neuromuscular blocking activity in the gastrocnemius muscle-sciatic nerve preparation of the cat, the phrenic nerve-diaphragm preparation of the rat and kitten and as measured by the rabbit head drop and mouse paralysis methods. Dihexazonium triethiodide and the sulphone 7-dioxathiatridecylenebis(triethylammonium iodide) (dihexone) show tubocurarine-like activity; dioctasulphonium triethiodide and dioctazonium triethiodide were predominantly tubocurarine-like but had some transitional properties, whilst didecasulphonium triethiodide and didecazonium triethiodide resembled decamethonium. Dihexazonium triethiodide was about equipotent with tubocurarine on the cat. Marked species variations in potency were observed. Theoretical implications are discussed.

CHEMICAL

IN a recent communication[1] we described the preparation and properties of two compounds, 6-ethyl-6-thioniaundecylenebis(triethylammonium) triiodide (I, $n = 5$, R = Et; DPSE) and 7-ethyl-7-thioniatridecylenebis (triethylammonium) triiodide (I, $n = 6$, R = Et; DHSE), which were found to have neuromuscular blocking activity similar to that of gallamine and tubocurarine. An attempt to prepare the compound I ($n = 4$, R = Et) from the bis-quaternary compound II ($n = 4$, R = Et) was not



(I) (II)

successful. Since it now appears that the ease with which these tris-onium salts can be formed and the neuromuscular blocking activity of onium compounds can both be related not only to the inter-nitrogen distance

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but also in some measure to the nature of the alkyl group, the factors responsible for the failure of this reaction merit discussion at this juncture.

There can be no direct steric interference with the approach of an alkyl halide to the sulphur link. Failure to react is, therefore, the result of direct (electrostatic) interaction between the quaternary nitrogens, and the sulphur electrons, an effect which should increase rapidly as the distance separating the interacting centres decreases. The further observations[1] that the reaction of ethyl iodide with the sulphide (III, $n = 5$, R = Et) can be carried out stepwise to give first the bis-quaternary compound (II, $n = 5$, R = Et) and then the tris-onium compound (I, $n = 5$, R = Et), whilst only one product, the tris-onium compound (I, $n = 6$, R = Et), can be obtained from the sulphide (III, $n = 6$, R = Et), shows that introduction of the third onium group becomes progressively easier as the distance separating the *N*- and *S*- groups increases.

$$R_2N\cdot(CH_2)_n\cdot S\cdot(CH_2)_n\cdot NR_2 \qquad \left[Et_3\overset{+}{N}\cdot(CH_2)_6\cdot SO_2\cdot(CH_2)_6\cdot\overset{+}{N}Et_3\right] \quad 2I^-$$

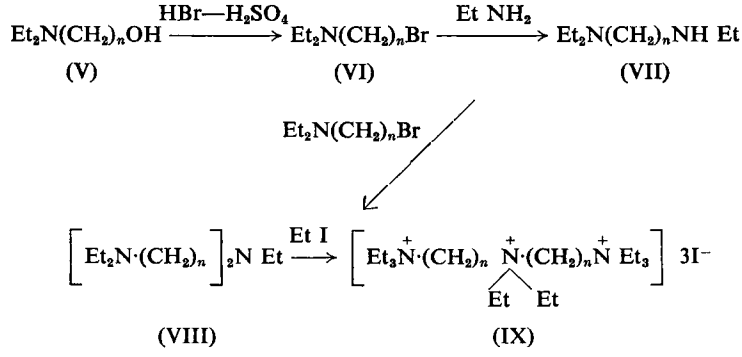
(III) (IV)

That reaction of the sulphide (III, $n = 6$, R = Me) with methyl iodide can also be brought about as a two stage reaction, with the isolation of the intermediate (II, $n = 6$, R = Me), shows that reaction at the sulphide link is also dependent on the nature of the *N*-alkyl substituent. This too can be related to the influence of the residual positive charge on the quaternary ammonium groups; an influence which would be diminished by the greater + I effect of ethyl compared to methyl substituents[2]. The possible significance of these effects in determining the level of neuromuscular blocking activity of quaternary salts will be discussed in later communications.

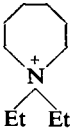
We have now prepared the longer chain *NSN*-tris-onium compounds (I, $n = 8$, R = Et) and (I, $n = 10$, R = Et) to study the effect of increasing the distance between quaternary centres on neuromuscular blocking activity in this series. 11-Ethyl-11-thioniaheneicosylenebis (triethylammonium) triiodide (I, $n = 10$, R = Et; didecasulphonium triethiodide; DDSE) was obtained by reaction of the known bis-10-diethylaminodecyl sulphide[3] with excess ethyl iodide. The octamethylene compound, 9-ethyl-9-thioniaheptadecylenebis (triethylammonium) triiodide (I, $n = 8$, R = Et; dioctasulphonium triethiodide; DOSE) was obtained similarly from bis-8-diethylaminooctyl sulphide. The latter was prepared by chain extension from 6-chlorohexyldiethylamine by the method used in the preparation of bis-5-diethylaminopentyl sulphide from 3-chloropropyldiethylamine[3]. The bis-quaternary sulphone (IV), was also prepared for comparison of its neuromuscular blocking properties with those of dihexasulphonium triethiodide (DHSE)[1]. It was obtained by treating bis-6-diethylaminohexyl sulphone[3] with ethyl iodide.

The function of the tertiary sulphur group in determining the properties of the *NSN*-tris-onium compounds has been further investigated by the

preparation and pharmacological examination of a parallel series of *NNN*-tris-quaternary compounds (IX) which were obtained by the following reaction sequence:



6-Bromohexyldiethylamine (IV, $n = 6$), obtained from 6-hydroxyhexyldiethylamine (V, $n = 6$), reacted with excess ethylamine to yield 6-ethylaminohexyldiethylamine (VII, $n = 6$) together with a small amount of bis-6-diethylaminohexylethylamine (VIII, $n = 6$). 6-Chlorohexyldiethylamine failed to react with ethylamine. Reaction of 6-bromohexyldiethylamine with 6-ethylaminohexyldiethylamine gave only poor yields of bis-6-diethylaminohexylethylamine (VIII, $n = 6$). This is due to the instability of 6-bromohexyldiethylamine, which readily cyclises on heating to yield 1:1-diethyl-1-aza*cyclo*heptylinium bromide (X) (compare with the cyclisation of 5-chloropentyldiethylamine[4]). On the other hand, 6-chlorohexyldiethylamine, although less reactive than the corresponding bromo compound, was much more stable, and could be condensed under more vigorous conditions with 6-ethylaminohexyldiethylamine to give improved yields of the base (VIII, $n = 6$). The latter, when treated with excess ethyl iodide, as before, gave 7:7-diethyl-7-azoniatridecylenebis (triethylammonium) triiodide (IX, $n = 6$; dihexazonium triethiodide; DHAE). 9:9-Diethyl-9-azoniaheptadecylenebis (triethylammonium) triiodide (IX, $n = 8$; dioctazonium triethiodide; DOAE) was obtained by an analagous series of reactions.



(X)

In the preparation of the *NNN*-decamethylene compound 10-bromodecyldiethylamine (VI, $n = 10$) was found to be sufficiently stable for it to be purified by distillation, and the required base, bis-10-diethylaminodecylethylamine (VIII, $n = 10$) was obtained by reaction of this bromo compound with 10-ethylaminodecyldiethylamine (VII, $n = 10$). Reaction of the latter with ethyl iodide as before gave 11:11-diethyl-11-azoniaheneicosylenebis (triethylammonium) triiodide (IX, $n = 10$; didecazonium triethiodide; DDAE).

Experimental

Melting points are uncorrected. We are indebted to Dr. A. C. Syme and Mr. W. McCorkindale for the microanalyses.

6-*Ethylaminohexyldiethylamine.* 6-Hydroxyhexyldiethylamine[3] (35·2 g.) in hydrobromic acid (48 per cent; 95 ml.) and sulphuric acid (33 ml.) was refluxed for 4 hours, cooled, and poured into water (1 l.). The solution was basified with sodium carbonate and extracted with chloroform. The chloroform extract was dried (Na_2SO_4) and the bulk of the chloroform evaporated under reduced pressure to yield a reddish-brown oil containing crystalline material. Excess ethylamine (40 ml.) was added to the crude 6-bromohexyldiethylamine and the mixture refluxed for 2 hours. Evaporation of the ethylamine and chloroform yielded a damp crystalline mass, which was basified and extracted with ether. Evaporation of the ether gave an oil (24·8 g.) which was distilled to yield 6-*ethylaminohexyldiethylamine*, b.p. 86–89°/0·55 mm., n_D^{17} 1·4493 (21·5 g.; 53 per cent). *Dihydrochloride* (from ethanol-ether), m.p. 172–173°. Found: C, 51·8; H, 10·6; Cl, 25·8 per cent. $C_{12}H_{30}N_2Cl_2$ requires C, 52·7; H, 11·1; Cl, 25·9 per cent.

Some of the crystalline material was filtered from the crude 6-bromohexyldiethylamine and re-crystallised from ethanol-ether to give 1:1-*diethyl*-1-*aza*cyclo*heptylinium bromide*, m.p. 250° (decomp.). Found: N, 5·9; Br, 33·9 per cent. $C_{10}H_{22}N\,Br$ requires N, 5·9; Br, 33·8 per cent.

Bis-(6-*diethylaminohexyl*)*ethylamine* (*VIII*, n = 6). 6-Ethylaminohexyldiethylamine (6·5 g.) and 6-chlorohexyldiethylamine[3] (6·3 g.) were refluxed gently in xylene (20 ml.) for 5 hours. On cooling the reaction mixture was extracted with dilute hydrochloric acid (10 per cent) and the latter basified, and extracted with benzene. Evaporation of the solvent yielded an oil (7·7 g.), which on distillation gave after a forerun of starting materials, bis-(6-diethylaminohexyl) ethylamine, as a pale yellow oil, b.p. 165–168°/0·7 mm., n_D^{18} 1·4610 (2·2 g.; 19 per cent). Found: C, 74·7; H, 13·1 per cent. $C_{22}H_{47}N_3$ requires C, 74·3; H, 13·1 per cent.

7:7-*Diethyl*-7-*azoniatridecylenebis* (*triethylammonium*) *triiodide* (IX, $n = 6$). Bis-(6-diethylaminohexyl) ethylamine (0·57 g.) was refluxed with ethyl iodide (3 ml.) for 10 minutes. Evaporation of excess ethyl iodide yielded 7:7-*diethyl*-7-*azoniatridecylenebis* (*triethylammonium*) *triiodide* as colourless needles (from ethanol), m.p. 261–262° (1·02 g.; 77 per cent). Found: N, 4·95; I, 46·2 per cent. $C_{28}H_{64}N_3I_3$ requires N, 5·1; I, 46·2 per cent.

11-*Ethyl*-11-*thioniaheneicosylenebis* (*triethylammonium*) *triiodide* (I, $n = 10$). Bis-10-diethylaminodecyl sulphide[3] (1·1 g.) was refluxed with ethyl iodide (3 to 4 ml.) for 45 minutes. Evaporation of the excess ethyl iodide and recrystallisation of the residual solid from acetone-ether yielded 11-*ethyl*-11-*thioniaheneicosylenebis* (*triethylammonium*) *triiodide* as almost colourless needles, m.p. 123·5–124° (0·6 g.; 27 per cent). Found: N, 3·0; I, 42·0 per cent. $C_{34}H_{75}N_2SI_3$ requires N, 3·0; I, 42·0 per cent.

10-*Bromodecyldiethylamine* (VI, $n = 10$) was prepared from 10-hydroxydecyldiethylamine[3] (20·1 g.) by the method described for 6-bromohexyldiethylamine. 10-*Bromodecyldiethylamine* was obtained as a colourless oil, b.p. 130°/0·5 mm. (21·4 g.; 84 per cent), n_D^{14} 1·4717. Found: equiv. (titration) 294·3; Br, 27·25 per cent. $C_{14}H_{30}NBr$ requires equiv. 292·3; Br, 27·3 per cent.

10-*Ethylaminodecyldiethylamine* (VII, $n = 10$) was prepared from 10-bromodecyldiethylamine (20·9 g.) by the method described for 6-ethylaminohexyldiethylamine. 10-*Ethylaminodecyldiethylamine* was obtained as a colourless oil, b.p. 133–135°/0·8 mm., n_D^{19} 1·4535 (14 g.; 76 per cent). *Dihydrochloride* (from ethanol-ether), m.p. 147–148°. Found: C, 58·1; H, 11·4; Cl, 20·3 per cent. $C_{16}H_{38}N_2Cl_2$ requires C, 58·3; H, 11·6; Cl, 21·5 per cent.

Bis-(10-*diethylaminodecyl*) *ethylamine* (VIII, $n = 10$). 10-Bromodecyldiethylamine (9 g.) in chloroform (10 ml.) was added slowly (40 minutes) to a refluxing solution of 10-ethylaminodecyldiethylamine (7·9 g.) in chloroform (15 ml.), and the mixture refluxed for a further 30 minutes. On evaporation the residue was basified and extracted with benzene. After removal of the solvent, and fractional distillation of the residual oil, *bis*-(10-*diethylaminodecyl*) *ethylamine* was obtained as a pale yellow oil, b.p. 212–216°/0·25 mm., n_D^{14} 1·4660 (3·8 g.; 26·5 per cent). *Trihydrochloride* (from acetone-ether), m.p. 118°. Found: N, 7·1; Cl, 17·8 per cent. $C_{30}H_{68}N_3Cl_3$ requires N, 7·3; Cl, 18·4 per cent.

11 : 11-*Diethyl*-11-*azoniaheneicosylenebis* (*triethylammonium*) *triiodide* (IX, $n = 10$). Bis-(10-diethylaminodecyl) ethylamine (1 g.) was refluxed with ethyl iodide (4 ml.) and ethanol (1 ml.) for 1 hour. Evaporation under reduced pressure gave 11 : 11-*diethyl*-11-*azoniaheneicosylenebis* (*triethylammonium*) *triiodide* (from acetone-ether) as almost colourless crystals, m.p. 202·5–203·5° (1·8 g.; 90 per cent). Found: C, 46·1; H, 8·2; N, 4·4; I, 40·5 per cent. $C_{36}H_{80}N_3I_3$ requires C. 46·2; H, 8·7; N, 4·5; I, 40·7 per cent.

1 : 1-*Bisethoxycarbonyl*-7-*diethylaminoheptane* was prepared from 6-chlorohexyldiethylamine (44 g.) by the method used for the preparation of 1 : 1-bisethoxycarbonyl-4-diethylaminobutane, with the modification that 10 per cent excess sodiomalonic ester was used, and reflux time was increased to 4 hours. 1 : 1-*Bisethoxycarbonyl*-7-*diethylaminoheptane* was obtained as a pale yellow oil, b.p. 147–155°/0·8 mm., $n_D^{15·5}$ 1·4472 (34·5 g.; 47·65 per cent) and used without characterisation in the next stage of the reaction.

Ethyl 8-*diethylaminocaprylate* was prepared from 1 : 1-bisethoxycarbonyl-7-diethylaminoheptane (45·2 g.) by the method used for the preparation of ethyl 5-diethylaminovalerate[1], with the modification that the initial reflux time with hydrochloric acid was increased to 4 hours. *Ethyl* 8-*diethylaminocaprylate*, obtained as a colourless oil, b.p. 111–114°/0·65 mm., n_D^{18} 1·4428 (21·5 g., 62 per cent), was characterised as 7-*ethoxycarbonylheptyl triethylammonium iodide* (prepared by the action of ethyl iodide), m.p. 64·5–65·5° (from acetone-ether). Found: N, 3·6; I, 32·1 per cent $C_{16}H_{34}NO_2I$ requires N, 3·5; I, 32·25 per cent.

8-*Hydroxyoctyldiethylamine*. Ethyl 8-diethylaminocaprylate (35·4 g.) was reduced with lithium aluminium hydride by the method used for the preparation of 5-hydroxypentyldiethylamine[1] to yield 8-*hydroxyoctyldiethylamine* as a colourless oil, b.p. 114–117°/0·7 mm., $n_D^{16·5}$ 1·4590 (26·2 g.; 90 per cent). *Hydrochloride* (from ethanol-ether), m.p. 90–91°.

Found: C, 60·4; H, 11·3; Cl, 15·0 per cent. $C_{12}H_{28}ONCl$ requires C, 60·6; H, 11·9; Cl, 14·9 per cent.

8-*Chlorooctyldiethylamine* was prepared from 8-hydroxyoctyldiethylamine (8·6 g.) by the method described for the preparation of 6-chlorohexyldiethylamine[3]. 8-Chlorooctyldiethylamine was obtained as a colourless oil, b.p. 94–96°/0·55 mm., n_D^{17} 1·4550 (9 g.; 96 per cent). Altman[5] reports b.p. 130·5°/ 11 mm., n_D^{18} 1·4535.

Bis-8-*diethylaminooctyl sulphide* was prepared from 8-chlorooctyldiethylamine (9 g.) by the method described for the preparation of bis-6-diethylaminohexyl sulphide[3]. *Bis*-8-*diethylaminooctyl sulphide* was obtained as a straw-coloured liquid, b.p. 210–212°/0·65 mm., $n_D^{16·5}$ 1·4768 (5·9 g.; 72 per cent). Found: equiv. (titration) 203·5. $C_{24}H_{52}N_2S$ requires equiv. 200·4. *Dihydrochloride* (from ethanol), m.p. 145°. Found: C, 60·85; H, 11·0 per cent. $C_{24}H_{54}N_2SCl_2$ requires C, 60·85; H, 11·5 per cent.

9-*Ethyl*-9-*thioniaheptadecylenebis* (*triethylammonium*) *triiodide*. Bis-8-diethylaminooctyl sulphide (0·63 g.) was refluxed with ethyl iodide (4 ml.) for 15 minutes. Removal of excess reagent under reduced pressure yielded 9-*ethyl*-9-*thioniaheptadecylenebis* (*triethylammonium*) *triiodide* (0·64 g.; 47 per cent) (from ethanol-ether), m.p. 159–160° (decomp.). Found: N, 3·2; S, 3·85, I, 43·0 per cent. $C_{30}H_{67}N_2SI_3$ requires N, 3·2; S, 3·7; I, 43·8 per cent.

8-*Ethylaminooctyldiethylamine* was prepared from 8-hydroxyoctyldiethylamine (8·6 g.) by the method described for the preparation of 6-ethylaminohexyldiethylamine. 8-*Ethylaminooctyldiethylamine* was obtained as a colourless oil, b.p. 104–106°/0·7 mm., $n_D^{17·5}$ 1·4530 (7·4 g.; 76 per cent). *Dihydrochloride* (from ethanol-ether), m.p. 159·5–160·5° (hygroscopic). Found: N, 9·2; Cl, 23·0 per cent. $C_{14}H_{34}N_2Cl_2$ requires N, 9·3; Cl, 23·5 per cent.

Bis-(8-*diethylaminooctyl*) *ethylamine* was prepared from 8-ethylaminooctyldiethylamine (6·95 g.) and 8-chlorooctyldiethylamine (6·7 g.) by the method described for the preparation of bis-(6-diethylaminohexyl) ethylamine. *Bis*-(8-*diethylaminooctyl*) *ethylamine* was obtained as a yellow oil, b.p. 230–250° (bath)/0·8 mm., n_D^{17} 1·4642 (2·3 g.; 18 per cent). *Trihydrochloride* (from acetone-ether), m.p. 165–166° (decomp.). Found: Cl, 20·9 per cent. $C_{26}H_{60}N_3Cl_3$ requires Cl, 20·4 per cent.

9 : 9-*Diethyl*-9-*azoniaheptadecylenebis* (*triethylammonium*) *triiodide*. Bis-(8-diethylaminooctyl) ethylamine (0·56 g.) was refluxed with ethyl iodide (2 ml.) and ethanol (2 ml.) for 15 minutes. On evaporation under reduced pressure 9 : 9-*diethyl*-9-*azoniaheptadecylenebis* (*triethylammonium*) *triiodide* was obtained (from ethanol), m.p. 251–252° (decomp.). Found: C, 43·2; H, 8·1; I, 43·0 per cent. $C_{32}H_{72}N_3I_3$ requires C, 43·7; H, 8·25; I, 43·3 per cent.

7-*Dioxothiatridecylenebis* (*triethylammonium iodide*). Bis-6-diethylaminohexyl sulphone[3] (0·99 g.) was refluxed with ethyl iodide (3 ml.) for 2 hours, when a brownish oil separated from the reaction mixture. After evaporation of excess ethyl iodide, the oil was treated in water with charcoal, and the solution evaporated to dryness. The oily residue

crystallised from acetone-ether gave *7-dioxothiatridecylenebis (triethylammonium iodide)* as a pale buff solid, m.p. 144–145° (0·85 g.; 47 per cent). Found: C, 41·5; H, 7·9; N, 3·9 per cent. $C_{24}H_{54}O_2N_2SI_2$ requires C, 41·9; H, 7·9; N, 4·1 per cent.

PHARMACOLOGICAL

Materials. The following were used: tris-onium derivatives, dihexazonium triethiodide (DHAE), dioctazonium triethiodide (DOAE) and didecazonium triethiodide (DDAE); tris-sulphonium derivatives, dioctasulphonium triethiodide (DOSE) and didecasulphonium triethiodide (DDSE); sulphone (bis-onium) dihexone. Drugs used were: decamethonium iodide (C 10), tubocurarine chloride (TC), edrophonium chloride (edrophonium), eserine salicylate (eserine), neostigmine methylsulphate (neostigmine), adrenaline hydrochloride, (Ad), potassium chloride (KCl), ether, acetylcholine chloride (ACh), sodium pentobarbitone and atropine sulphate (atropine).

Methods and results. The experimental methods used in this investigation were similar to those described by us in an earlier publication[1].

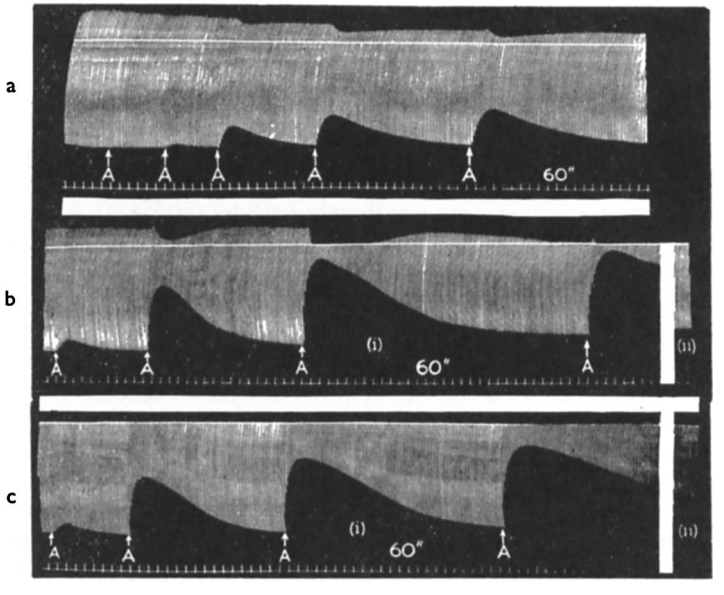


FIG. 1. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a) At A, tubocurarine 0·075 mg./kg.
(b) At A, dioctazonium 0·30 mg./kg.
There was an interval of 40 minutes between (i) and (ii).
(c) At A, dihexazonium 0·20 mg./kg.
There was an interval of 25 minutes between (i) and (ii).

Neuromuscular blocking activity. Cats of either sex, weighing between 2 and 4 kg. were anaesthetised by intraperitoneal injection of sodium pentobarbitone (about 60 mg./kg.). The preparation was set up for recording the contractions of the gastrocnemius muscle in response to indirect stimulation *via* the sciatic nerve. A Dobbie-McInnes stimulator was used to deliver supra-maximal square impulses at a frequency of 4 to 8 per minute, pulse width 1·5 to 3·0 msec., voltage 12 to 20 volts. In any one experiment, frequency, pulse width and voltage were constant except when indirectly tetanising the muscle when the frequency was 1,500 per minute, or when stimulating the muscle directly when the voltage was raised to 40 volts.

Dihexazonium triethiodide, dioctazonium triethiodide, dioctasulphonium triethiodide and dihexone in the dose range 0·10 to 1·0 mg./kg. caused a reduction of the amplitude of the twitch but no initial potentiation of twitch height, muscular twitching or fibrillation were seen. In the case of dihexazonium triethiodide a dose of 0·2 to 0·4 mg./kg. was adequate to give an approximately 95 per cent reduction in twitch height. Smaller doses produced graded but reduced effects. The effect of the initial dose was usually small but that of the second, third and often of the fourth dose was progressively greater. The effect upon twitch height then became approximately constant although the duration of effect increased with successive doses. Similar effects were seen when TC was used at similar

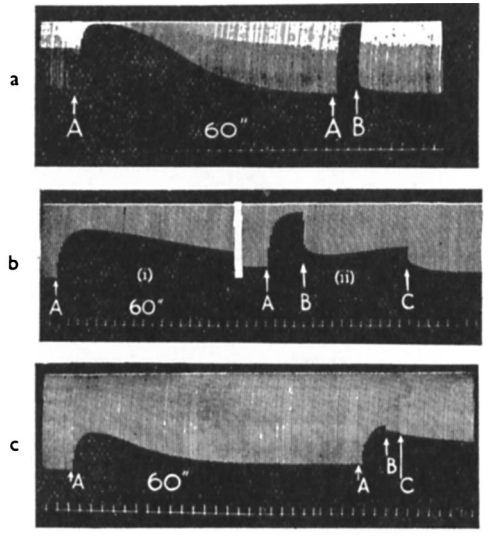


FIG. 2. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a) At A, dihexazonium 0·40 mg./kg.
At B, edrophonium 0·40 "
(b) At A, dioctazonium 0·20 "
At B, edrophonium 0·50 "
At C, edrophonium 1·0 "
(c) At A, dihexone 1·50 mg./kg.
At B, edrophonium 0·50 "
At C, edrophonium 0·75 "

dose levels (Fig. 1). Dihexazonium triethiodide and TC appeared to be about equipotent whilst dioctazonium triethiodide, dioctasulphonium triethiodide and dihexone had properties qualitatively idential with those of dihexazonium but were less potent. Dioctazonium triethiodide had about two-thirds of the potency of TC, dioctasulphonium triethiodide one-half and dihexone one-eighth. The neuromuscular blocking activity of dihexazonium triethiodide, dioctazonium triethiodide, dioctasulphonium triethiodide and dihexone was antagonised by edrophonium (0·5 to 1·0 mg./kg.). In the case of dioctazonium triethiodide and dihexazonium triethiodide recovery of the twitch height was prompt and complete, but after dioctasulphonium triethiodide and dihexone edrophonium did not produce complete recovery (Fig. 2). Neostigmine (0·05 to 0·10 mg./kg.) had similar effects, but eserine (0·5 to 1·0 mg./kg.) was much less effective. The effects of these four blocking agents were partially antagonised by Ad (0·05 to 0·1 mg./kg.), KCl (15 to 20 mg./kg.) and C 10 (0·10 to 0·40 mg./kg.). Some of these effects are shown in

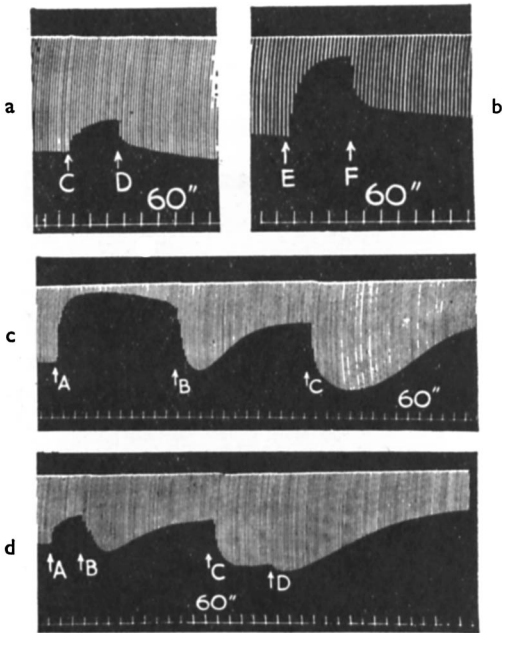


FIG. 3. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a)	At C, dioctasulphonium	0·15 mg./kg.	(d)	At A, dihexazonium	0·40 mg./kg.
	At D, decamethonium	0·07 "		At B, potassium chloride	20 "
(b)	At E, dihexone	0·40 "		At C, adrenaline	0·10 "
	At F, decamethonium	0·06 "		At D, adrenaline	0·05 "
(c)	At A, dihexone	0·40 "			
	At B, potassium chloride	20 "			
	At C, adrenaline	0·08 "			

Figure 3. C 10 (0·10 to 0·40 mg./kg.) rapidly reversed the neuromuscular block caused by dihexazonium triethiodide (0·10 to 0·30 mg./kg.), dioctazonium triethiodide (0·20 to 0·80 mg./kg.), dioctasulphonium triethiodide (0·20 to 0·80 mg./kg.) or dihexone (1·0 to 2·0 mg./kg.). The effects of all four compounds were potentiated by ether and it was found that if the partially blocked muscle was indirectly tetanised the tetanic response was poorly sustained following dihexone, dioctazonium triethiodide and dihexazonium triethiodide but after dioctasulphonium triethiodide it was well maintained (Fig. 4). When the muscle had become unresponsive to indirect stimulation, direct stimulation caused it to contract, but the response did not attain the same amplitude as the control. It was usually possible to obtain a response of 60 to 80 per cent of the control level, but not more. Partial, but temporary decurarisation was seen to follow indirect tetanisation of the muscle in preparations partly blocked by dihexazonium triethiodide, dioctazonium triethiodide, dioctasulphonium triethiodide or dihexone. The influence of tetanisation upon the effects of these drugs is compared in Figure 4 with its effect upon TC blockade which is influenced in a similar fashion.

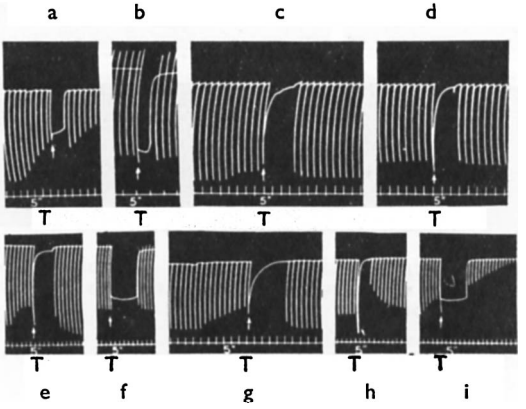


FIG. 4. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. At T, indirect tetanization via sciatic nerve during partial block by (a) dioctasulphonium, (b) didecazonium, (c) dioctazonium, (d) didecasulphonium, (e) dihexazonium, (g) tubocurarine, (h) dihexone, (i) decamethonium and tetanization of the normal muscle (f).

Didecasulphonium triethiodide and didecazonium triethiodide had properties different from those of the drugs already described. Didecazonium triethiodide in the dose range of 0·01 mg./kg. to 0·20 mg./kg. caused initial potentiation of twitch height (Fig. 5) but didecasulphonium triethiodide did not share this property. Potentiation of the twitch height with didecazonium triethiodide was followed by a progressive decline and this was accompanied in its early stages by a generalised, intermittent muscular twitching and fasciculation. Didecasulphonium triethiodide (0·20 to 0·50 mg./kg.) also caused neuromuscular block but this was accompanied by generalised muscular twitching. Both didecasulphonium triethiodide and didecazonium triethiodide caused a very

prolonged neuromuscular block (Fig. 5); during complete neuromuscular paralysis with didecasulphonium triethiodide the response to direct stimulation was very poor, but with didecazonium triethiodide it was as much as 40 per cent of the control height. After didecazonium triethiodide (0·20 mg./kg.) the response of the partially blockaded muscle to indirect tetanization was well maintained but following didecasulphonium triethiodide (0·25 mg./kg.) it waned rapidly and decurarisation did not follow indirect tetanisation (Fig. 4). Neuromuscular blockade with didecasulphonium triethiodide (0·2 to 0·4 mg./kg.) was slightly reversed by neostigmine (0·10 to 0·20 mg./kg.) and edrophonium (1·0 to 2·0 mg./kg.), but eserine (0·5 to 2·0 mg./kg.) had no antagonistic effect. Ad (0·05 to 0·1 mg./kg.) antagonised the effects of didecasulphonium as did KCl (20 mg./kg.).

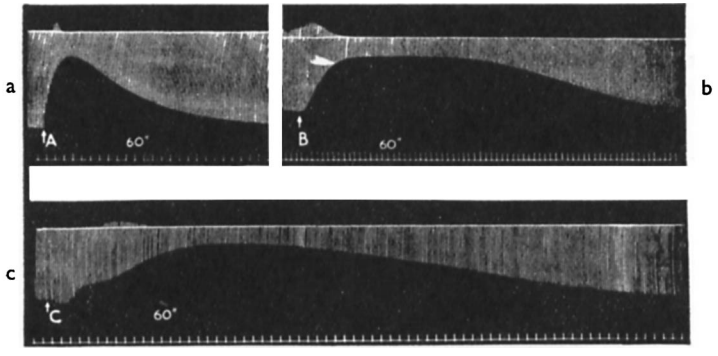


FIG. 5. Cat gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve. Contraction downwards. Drugs administered intravenously.

(a) At A, tubocurarine 0·25 mg./kg.
(b) At B, didecasulphonium 0·30 "
(c) At C, didecazonium 0·25 "

Antagonism to the actions of didecazonium triethiodide was not shown by eserine (0·5 to 2·0 mg./kg.) or by neostigmine (0·10 to 0·20 mg./kg.) and its effects were potentiated by edrophonium (1·0 to 2·0 mg./kg.) and antagonised by Ad (0·05 mg./kg.) and KCl (20 mg./kg.). Ad had a temporary, but well marked antagonism to the neuromuscular blocking effects of all of the six compounds which we have investigated as well as to the effects of TC and C 10. The effects of didecasulphonium triethiodide or didecazonium triethiodide were not potentiated by ether anaesthesia. The neuromuscular blocking effects of didecasulphonium triethiodide and didecazonium triethiodide were additive with those of C 10 and these compounds antagonised the neuromuscular blocking effects of TC.

Rabbit head drop. Comparisons of potency were made with TC using a slightly modified rabbit head drop method and the experiments were repeated in rabbits which had been given neostigmine (0·1 mg./kg.) by

subcutaneous injection 15 minutes before. The results are shown in columns 1 and 2 of Table I. Column 3 shows the ratio of the two head drop doses. This ratio is some indication of the mode of drug action; if it is greater than unity it indicates drug antagonism and hence a TC-like effect; if less than unity it suggests synergism or no antagonism which indicates a C 10-like mode of action.

TABLE I

RABBIT HEAD DROP DOSES (H.D.D.)

Compound	H.D.D. ± s.e. (mg./kg.)	H.D.D. after neostigmine ± s.e. (mg./kg.)	Ratio H.D.D. after neostigmine/ H.D.D.
Dihexazonium	0·51 ± 0·057	0·81 ± 0·057	1·56 (P = <0·01)
Dioctasulphonium	0·24 ± 0·013	0·25 ± 0·013	1·04 (P = >0·90)
Dioctazonium	0·26 ± 0·014	0·33 ± 0·014	1·27 (P = <0·01)
Didecasulphonium	0·21 ± 0·023	0·28 ± 0·023	1·30 (P = >0·05)
Didecazonium	0·28 ± 0·017	0·28 ± 0·017	1·00
Dihexone	0·39 ± 0·017	0·44 ± 0·017	1·12 (P = >0·40)
Decamethonium	0·16 ± 0·008	0·15 ± 0·008	0·93
Tubocurarine	0·11 ± 0·01	0·30 ± 0·01	2·72 (P = <0·01)

Acute toxicity in mice. Using groups of ten mice the approximate LD50 for the six compounds listed was found and compared with that found for TC and C 10. The results are summarised in Table II.

TABLE II

ACUTE TOXICITY (LD50) AND PARALYSING ACTIVITY (PD50) IN MICE

Compound	Approximate LD50 Groups of 10 mice (mg./kg.)	Approximate PD50 Groups of 10 mice (mg./kg.)	Paralysing potency TC = 100
Dihexazonium	2·25	1·20	17
Dioctasulphonium	0·40	0·08	250
Dioctazonium	0·40	0·10	200
Didecasulphonium	0·20	0·10	200
Didecazonium	0·18	0·16	125
Dihexone	2·30	0·60	33
Decamethonium	4·10	3·65	5·4
Tubocurarine	0·28	0·20	100

Paralysing activity in mice. Mice were given an intraperitoneal injection of the drug and placed upon the upper part of a fine wire mesh screen which was inclined at an angle of 60° to the horizontal. This method was used by Thompson[6] for an insulin assay in mice. Groups of ten mice were used at each dose level and four dose levels were used for each compound. The number of mice in each group which were unable to maintain their position on the screen at each dose level was counted and from this an approximate 50 per cent paralysing dose (PD50) was calculated. The results obtained are summarised in Table II.

Effects upon blood pressure and respiration. The effects of didecazonium triethiodide, dioctazonium triethiodide, dihexazonium triethiodide, dioctasulphonium triethiodide, didecasulphonium triethiodide and dihexone on the blood pressure of cats anaesthetised with sodium pentobarbitone were compared with the effects of TC (0·5 to 1·0 mg./kg.). Dose levels of up

to 2 mg./kg. had no significant effects upon the level of the blood pressure but 1 mg./kg. of TC caused a typical prolonged fall. The average doses required to paralyse respiration in the pentobarbitone-anaesthetised cats are shown in Table III. In these experiments, a solution of the drug which contained 0·2 mg./ml. was infused into the external jugular vein using a Palmer's constant rate infusion apparatus at a rate of 0·8 ml./minute.

TABLE III

RESPIRATORY PARALYSING ACTIVITY IN THE ANAESTHETISED CAT

Compound	Mean respiratory paralysing dose (mg./kg.)	Potency (TC = 100)
Dihexazonium	0·85	56
Dioctasulphonium	0·93	51
Dioctazonium	1·24	38
Didecasulphonium	1·19	40
Didecazonium	0·57	84
Dihexone	1·70	28
Decamethonium	0·15	320
Tubocurarine	0·48	100

Ganglion blocking activity. Ganglion blocking activity was investigated by noting the effects of the intravenous injection of 0·5 to 2·0 mg./kg. of the drug upon the response of the nictitating membrane of the pentobarbitone anaesthetised cat to stimulation of the pre-ganglionic fibres of the cervical sympathetic. A Dobbie-McInnes stimulator was used to deliver 15-second bursts of square impulses at a frequency of 800 to 1,200 per minute and at 10 volts. In any one experiment frequency was kept constant. TC (0·5 mg./kg.) caused a typical well marked depression of the amplitude of response but dihexazonium triethiodide, didecasulphonium triethiodide, dioctazonium triethiodide, dioctasulphonium triethiodide and dihexone (up to 2·0 mg./kg.) did not reduce the height of the contraction unless given at very high dose levels. Didecazonium triethiodide appeared to be a ganglion stimulant since after an injection

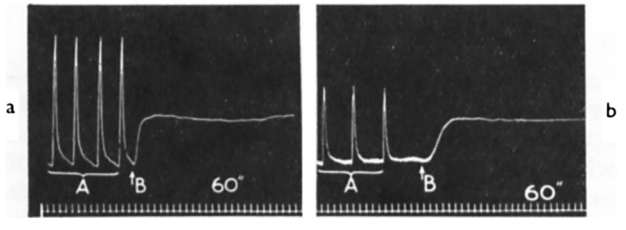


FIG. 6. Cat. Pentobarbitone anaesthesia.

(a) At A, contractions of nictitating membrane elicited by pre-ganglionic stimulation of the cervical sympathetic at 12 volts, 1,000 impulses per minute, pulse width 1·0 msec. bursts of 15 seconds.
At B, didecazonium 0·50 mg./kg. intravenously.

(b) At A, contractions of nictitating membrane elicited by pre-ganglionic stimulation of the cervical sympathetic at 15 volts, 800 impulses per minute, pulse width 1·5 msec. bursts of 15 seconds.
At B, infusion of didecazonium (0·20 mg./ml.) at the rate of 0·80 ml./minute for a period of 10 minutes.

of 0·4 mg./kg. of this drug there was a well marked contraction of the nictitating membrane (Fig. 6).

Frog rectus abdominis muscle. The muscle was set up in a 20 ml. bath containing oxygenated frog Ringer's solution at room temperature[1]. No direct stimulant actions were seen when any of the six compounds were added to the bath at dose levels of up to 25 μg./ml. All of the compounds (1·0 to 5 μg./ml.) antagonised contractures due to ACh (0·10 to 0·25 μg./ml.) and C 10 (1·5 to 2·5 μg./ml.), but in one experiment only, didecazonium triethiodide (2·0 to 5 μg./ml.) caused a slow contracture-like response of the rectus.

Isolated guinea pig ileum. The ileum was set up in a 2 ml. bath of oxygenated Tyrode's solution at 29°[1]. Slight antagonism was shown to contractions induced by ACh (0·10 to 0·5 μg./ml.) and ACh-induced contractions were not potentiated. All the compounds showed direct stimulant properties when given at large dose levels (0·1 to 0·2 mg./ml.) and the contractions of the ileum were blocked by atropine (0·05 μg./ml.).

Isolated rabbit duodenum. The duodenum was set up in a 50 ml. bath of oxygenated Locke's solution at 37°[1]. All of the compounds caused a contraction of the duodenum (10 to 40 μg./ml.). C 10 caused similar contractions whilst TC had no direct effect. The stimulant effects were blocked by atropine (0·004 μg./ml.).

Isolated rat hindquarters. The rat hindquarters were set up and perfused with oxygenated Locke's solution at room temperature according to the method described by Burn[7]. Variations in outflow were recorded by means of a Gaddum drop recorder. None of the compounds in the dose range of 0·25 to 1·0 mg. caused any significant alteration in the outflow of the perfusion fluid, but in this dose range tubocurarine usually caused constriction of the blood vessels.

Rat and kitten phrenic nerve-diaphragm preparations. Comparisons of potency were made on both the rat and kitten diaphragm. The rat diaphragm was set up according to the method of Bülbring[8] and a similar technique was used to set up the kitten diaphragm. A 100 ml. bath of oxygenated "double glucose" Tyrode's solution at 29° was used. The nerve was stimulated by means of a Dobbie-McInnes stimulator, using a Collison's fluid electrode. The frequency of stimulation was 8 per minute, pulse width 3 msec. at 15 volts. The approximate potency ratios (TC = 100) in the rat diaphragm were as follows:

Dihexazonium triethiodide 2; dihexone 0·67; didecasulphonium triethiodide 2; dioctazonium triethiodide 1·4; dioctasulphonium triethiodide 3; dioctazonium triethiodide 2. Using the kitten diaphragm these became (TC = 100): dihexazonium triethiodide 400; dihexone 6; didecasulphonium triethiodide 14; didecazonium triethiodide 14; dioctasulphonium triethiodide 200; dioctazonium triethiodide 150.

We can offer no explanation for this marked species difference.

Discussion

All the compounds tested possess neuromuscular blocking activity. The *NSN*-tris-onium compound dioctasulphonium triethiodide (DOSE;

I, $n = 8$, R = Et), the *NNN*-tris-onium compounds dihexazonium triethiodide (DHAE; IX, $n = 6$) and dioctazonium triethiodide (DOAE; IX, $n = 8$), and the bis-quaternary sulphone (IV) have TC-like properties in almost every respect, although when dioctasulphonium is used to induce partial neuromuscular blockade in the cat gastrocnemius muscle-sciatic nerve preparation and the muscle is then indirectly tetanised, the tetanus is well maintained. This is a property shared with C 10 and with the *NNN*-tris-quaternary didecazonium triethiodide (DDAE; IX, $n = 10$), but not with the *NSN*-tris-onium compound didecasulphonium triethiodide (DDSE; I, $n = 10$, R = Et). Both didecasulphonium and didecazonium show predominantly C 10-like activity.

If the properties of dipentasulphonium and dihexasulphonium are taken into account it is clear that as the interquaternary chain-length increases, members of the *NSN*-series change from being TC-like (non-depolarising) in those compounds with five and six-membered polymethylene chains, becoming first transitional in compounds with eight methylene units, and then predominantly C 10-like (depolarising) when the methylene chain is extended to ten units. Within the series of compounds tested the nature of the third (central) onium group in the tris-onium neuromuscular blocking agents appears to be relatively unimportant in determining the type of activity produced. Replacement of sulphur by nitrogen gives a series of *NNN*-tris-quaternary compounds which show the same broad gradation of properties observed with the analogous *NSN*-compounds, although small differences are evident. Thus the neuromuscular blocking activity of didecasulphonium is not always increased by edrophonium and the tension of an indirect tetanus in the partly blockaded muscle is not well sustained. Didecazonium, on the other hand, possesses an almost classic type of depolarising activity although it does not usually stimulate the frog rectus muscle.

If we exclude from discussion all question of the influence of *N*- or *S*-alkyl group size, it is evident from the results of our experiments that chain extension with the interpolation of a third onium group leads to a pronounced enhancement of the TC-like properties compared with that observed in the bis-quaternary series. Thus, in contrast to dihexasulphonium and dihexazonium, which are approximately equipotent with TC, hexamethonium only causes a TC-like neuromuscular blockade when given at very high dose levels. No such distinction, however, can be drawn between the bis- and tris-quaternary series in regard to depolarising properties which in both only reach significant proportions when the number of methylene groups separating the charged centres is of the order of ten units.

The importance of the third (central) onium group, and the number of units in the polymethylene chain separating the individual quaternary centres, rather than overall molecular chain length, as factors in determining the level of TC-like activity, follows from the much higher potency of dihexasulphonium and dihexazonium as compared with that of the bis-quaternary sulphone (IV) and bis-quaternary compound decaethonium. It is important, however, to distinguish between the number of units in

the polymethylene chain as a measure of interonium group distance within a molecule and the actual distance which separates these centres at the moment of combination with receptors at the site of action. The essential linearity of the individual polymethylene chains of the *NNN*-compounds, both in the solid state and in solution will be favoured by the natural repulsion of the individual positively charged heads within each molecule, and by the tetrahedral nature of quaternary nitrogen. Substitution of one quaternary nitrogen by tertiary sulphur (which is pyramidal) in the *NSN*-compounds should not appreciably affect the shape of the molecules since the C–$\overset{+}{\underset{|}{S}}$–C valency angle differs but little from that of C–$\overset{+}{\underset{/\backslash}{N}}$–C group. In consequence no appreciable difference between the properties of comparable members of the two series is expected or found. Although we are unable at present to ascertain whether or not these molecules retain their linearity at the site of action there is some evidence from studies of the bis-quaternary compounds that this may be so. Thus, for example, TC, in which the molecule is more or less completely rigid, and decaethonium have the same interquaternary distance only if the latter molecule is assumed to be linear, and both show the same *type* of activity (note that our results link *type* of activity strongly with inter-onium group spacing) albeit of significantly differing potency.

The size of the *N*- or *S*-alkyl groups as a factor in determining the level and type of neuromuscular blocking activity is not clear. In gallamine, a tris-ethonium derivative, replacement of *N*-ethyl by *N*-methyl groups reduces the activity but does not alter its character[9]. With decamethonium on the other hand, replacement of methyl by ethyl groups not only reduces activity but changes its character[10–11]. The effect of *N*- or *S*-alkyl group size has not yet been fully investigated in the present series. All the compounds tested have been ethonium salts, although we have previously reported[1] that dihexasulphonium trimethiodide is almost devoid of neuromuscular blocking activity, an observation which suggests that these compounds fall into the same category as gallamine.

The reason for this reduction in activity is not clear, but we hope to investigate the properties of the methonium analogues of the entire series at a later date.

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