SOME *NSN*-TRIS-QUATERNARY NEUROMUSCULAR BLOCKING AGENTS*

BY D. EDWARDS, J. J. LEWIS, J. B. STENLAKE AND M. S. ZOHA†

From the School of Pharmacy, The Royal College of Science and Technology, Glasgow, and the Department of Materia Medica and Therapeutics, University of Glasgow

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The tris-quaternary compounds 7-ethyl-7-thioniatridecylenebis(triethylammonium) triiodide (Dihexasulphonium triethiodide; DHSE), 6-ethyl-6-thioniaundecylenebis(triethylammonium) triiodide (Dipentasulphonium triethiodide; DPSE), and 7-methyl-7-thioniatridecylenebis(trimethylammonium) triiodide (Dihexasulphonium trimethiodide; DHSM) have been synthesised. DPSE and DHSE have been shown to be neuromuscular blocking agents, which act by a mechanism resembling that of tubocurarine and gallamine rather than decamethonium. DPSE and DHSE are about equipotent with tubocurarine on the cat gastrocnemius muscle, but less potent on the frog rectus abdominis muscle and rat diaphragm, and the head drop doses are higher. Neuromuscular block can be reversed by neostigmine, eserine, and edrophonium. DHSE and DPSE do not cause ganglion blockade or histamine release, do not increase heart rate and are less potent than tubocurarine in causing respiratory paralysis.

THE properties and modes of action of neuromuscular blocking agents which act by competition, by depolarisation or by an intermediate type of action have been carefully investigated[1–9]. These studies have led to the synthesis of suxamethonium, benzoquinonium and gallamine. Gallamine, a tris-ethonium compound, possesses properties similar to tubocurarine, and lacks some of the undesirable properties of the latter but it is less potent. On the other hand it sometimes causes tachycardia[10,11]. Thioalkane-$\alpha\omega$-bis-quaternary salts have been shown to possess neuromuscular and ganglion blocking activity[12,13]. 3-Thiapentane-1:5-bis(trimethylammonium iodide) (I, $n = m = 2$, R = Me) was described by Marxer and Miescher[14], whilst Andrews, Bergel and Morrison[12] have prepared a series of thioalkane-bis(quaternary ammonium) salts (I, $n = m = 2$, R = Me; $n = 2$, $m = 3$, R = Me; $n = m = 3$, R = Et; $n = m = 4$, R = Me; $n = 4$, $m = 6$, R = Me) and dithioalkane-bis(quaternary ammonium) salts (II, $n = m = 2$, R = Me; $n = m = 3$, R = Et; $n = m = 4$, R = Me). As with the corresponding polymethylene compounds, those compounds with more than seven atoms between the quaternary nitrogen atoms exhibit predominantly neuromuscular blocking action, whilst the lower homologues exhibit a depressor action on cat blood pressure and block transmission at the cat superior cervical sympathetic ganglion.

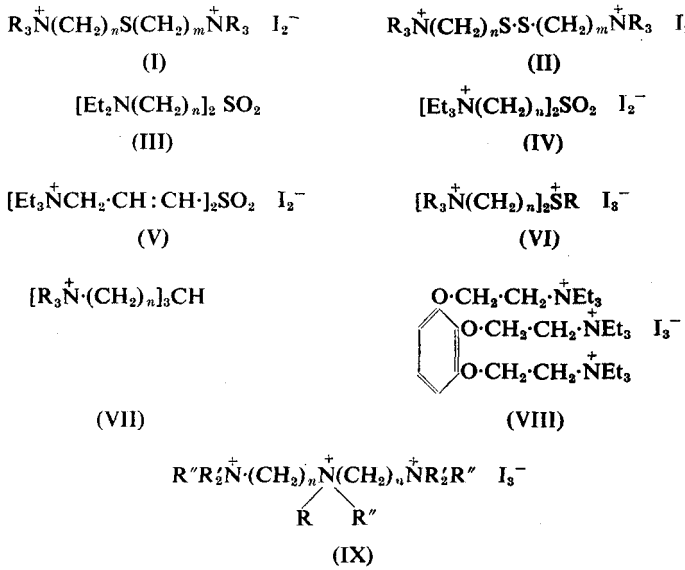
CHEMICAL

The availability of a series of long chain bisdiethylaminoalkyl sulphones[15] (III) prompted us to investigate the corresponding dioxothiaalkane-$\alpha\omega$-bistriethylammonium salts for neuromuscular blocking activity.

* Patent rights pending. † Pakistan Government Scholar.

For this purpose, 4-dioxothiaheptane-1 : 7-bis(triethylammonium iodide) (IV, $n = 3$) and 4-dioxothiahepta-2 : 5-diene-1 : 7-bis(triethylammonium iodide) (V) were readily obtained by direct quaternisation of the corresponding bisdialkylaminoalkyl sulphones[15] with ethyl iodide. Bisdiethylaminohexyl sulphone (III, $n = 6$) and bisdiethylaminodecyl sulphone (III, $n = 10$) however, yielded oily products with ethyl iodide, which crystallised only on long standing *in vacuo*. Fakstopp[16] has recently reported side reactions in the preparation of quaternary salts from amino sulphones.

A chance observation showed that bis-6-diethylaminohexyl sulphide[15] yielded 7-ethyl-7-thioniatridecylenebis(triethylammonium) triiodide) (VI, $n = 6$, R = Et; dihexasulphonium triethiodide; DHSE) when treated with excess ethyl iodide under reflux. The use of limited amounts of ethyl iodide (2 mol.) gave the same product, though in reduced yield and failed to yield the expected 7-thiatridecan-1 : 13-bis(triethylammonium iodide) (I, $n = m = 6$, R = Et).



(I) (II) (III) (IV) (V) (VI) (VII) (VIII) (IX)

Compounds of this type have not previously been examined for neuromuscular blocking activity, though a number of tris-quaternary nitrogen compounds have been described. Kensler, Zirkle, Matallana and Conduris[17] prepared a series of compounds, VII, where $n = 2$, 3, or 4 and R = Me or Et, of which the most active, VII ($n = 4$, R = Et), showed about 40 per cent of the activity of gallamine (VIII) in the rabbit head drop assay. DHSE and DPSE resemble gallamine in possessing three quaternary centres although one is now sulphur. The steric pattern of the three quaternary centres, however, is non-linear in the compounds VII and in gallamine, whilst DHSE and DPSE resemble decamethonium in that the molecules are linear, and of approximately equivalent chain length. On

the other hand DHSE and DPSE resemble gallamine in that all three are ethonium compounds, whilst decamethonium is a methonium compound. The short-chain linear tris-quaternary nitrogen compounds, IX, $n = 2$ or 3, described by Marxer and Miesher[14] and by Delaby, Damiens and Marquist[18], as expected, exhibit ganglion-blocking activity. Longer-chain linear tris-quaternary nitrogen compounds have not been examined for neuromuscular blocking activity. We therefore attempted the preparation of further compounds of the *NSN*-trisquaternary type VI. Bis-dimethylaminohexyl sulphide, prepared by condensation of 6-chlorohexyldimethylamine[12] with sodium sulphide, unlike bisdiethylaminohexyl sulphide, gave two products with methyl iodide, depending upon the reaction conditions. With a limited amount of methyl iodide in the cold 7-thiadecan-1 : 13-bis(triethylammonium iodide) (I, $n = m = 6$, R = Me), was obtained, whilst excess reagent under reflux gave 7-methyl-7-thioniatridecylenebis(trimethylammonium) triiodide, (VI, $n = 6$, R = Me; dihexasulphonium trimethiodide, DHSM).

Bisdiethylaminopentyl sulphide was prepared by the following reaction sequence:

$$Et_2N{\cdot}(CH_2)_3Cl \xrightarrow[NaOEt]{CH_2(COOEt)_2} Et_2N(CH_2)_3CH(COOEt)_2 \xrightarrow[2{\cdot}EtOH{-}H_2SO_4]{1{\cdot}H^+}$$

$$Et_2N(CH_2)_4COOEt \xrightarrow{LiAlH_4} Et_2N{\cdot}(CH_2)_5OH \xrightarrow{SOCl_2} Et_2N{\cdot}(CH_2)_5Cl \xrightarrow{Na_2S}$$

$$[Et_2N{\cdot}(CH_2)_5]_2\ S$$

It underwent a stepwise reaction with ethyl iodide, so that even when the latter is present in excess, 6-thiaundecan-1 : 11-bis(triethylammonium iodide) (I, $n = m = 5$, R = Et), was always deposited as the primary product of the reaction. More prolonged treatment with ethyl iodide gave 6-ethyl-6-thioniaundecylene bis(triethylammonium) triiodide (VI, $n = 5$, R = Et; dipentasulphonium triethiodide; DPSE).

5-Thianonane-1 : 9-bis(triethylammonium iodide) (I, $n = m = 4$, R = Et) obtained from 1-chloro-4-bromobutane by the action of first triethylamine and then sodium sulphide, failed to yield the corresponding tris-quaternary compound with excess ethyl iodide even under forcing conditions.

Experimental

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

4-*Dioxothiaheptane*-1 : 7-*bis*(*triethylammonium iodide*). Bis-3-diethylaminopropyl sulphone[15] (3·87 g.) was refluxed with ethyl iodide (5 ml.) for 30 min. Excess reagent was removed by distillation under reduced pressure and the residue crystallised from ethanol (90 per cent) to yield pale yellow crystals (4·15 g. 51·9 per cent) of 4-*dioxothiaheptane*-1 : 7-*bis*(*triethylammonium iodide*), m.p. 220–222°. Found: C, 35·7; H, 7·0; N, 4·7; I, 42·7 per cent; $C_{18}H_{42}O_2N_2SI_2$ requires: C, 35·8; H, 7·0; N, 4·6; I, 42·0 per cent.

4-Dioxothiahepta-2:5-diene-1:7-bis(triethylammonium iodide). Bis-3-diethylaminoprop-1-enyl sulphone dihydrochloride[15] (0·94 g.) in water (2 ml.) was basified by the addition of sodium hydroxide (N; 5·5 ml.) and extracted with benzene. The benzene extract after drying with Na_2SO_4 was evaporated under reduced pressure and the residual oily base refluxed with ethyl iodide (2 ml.) for 30 minutes. The residue (0·58 g. 37·3 per cent) remaining after removal of excess reagent under reduced pressure was crystallised from ethanol (charcoal) to yield pale yellow needles of *4-dioxothiahepta-2:5-diene-1:7-bis(triethylammonium iodide)*, m.p. 179° (decomp.). Found: C, 35·8; H, 6·6; N, 4·5; I, 41·8 per cent; $C_{18}H_{36}O_2N_2SI_2$ requires: C, 36·0; H, 6·4; N, 4·7; I, 42·3 per cent.

7-Ethyl-7-thioniatridecylene bis(triethylammonium) triiodide. Bis-6-diethylaminohexyl sulphide[15] (1·64 g.) was refluxed with ethyl iodide (3 ml.) for 25 minutes. Removal of excess reagent under reduced pressure and recrystallisation of the product from ethanol gave almost colourless crystals (2·95 g. 76·3 per cent) of *7-ethyl-7-thioniatridecylene bis(triethylammonium) triiodide*, m.p. 142–143°. Found: N, 3·7; I, 46·6 per cent; $C_{26}H_{59}N_2SI_3$ requires N, 3·5; I, 46·9 per cent.

1:1-Bisethoxycarbonyl-4-diethylaminobutane. Diethyl malonate (72 g.) was added slowly (30 min.) to a solution of sodium (10·5 g.) in ethanol (320 ml.) at 50°. 3-Chloropropyldiethylamine (67 g.) was added slowly (30 min.) and the mixture then refluxed for a further 2 hours. The bulk of the ethanol was removed by distillation, water (100 ml.) and dilute hydrochloride acid (10 per cent; 200 ml.) added. The solution was saturated with sodium chloride and extracted with ether to remove unchanged diethyl malonate. The solution was made alkaline with sodium hydroxide (20 per cent; 120 ml.) and again extracted with ether. The ether extract was dried with Na_2SO_4, evaporated, and the residual liquid distilled to yield 1:1-bisethoxycarbonyl-4-diethylaminopropane as a colourless oil (55·4 g.; 45 per cent), b.p. 173–176° at 22 mm., $n_D^{23·5}$ 1·4387. Marvel, Zartman and Bluthardt[19] give b.p. 163–170° at 23 mm., n_D^{25} 1·4380.

Ethyl 5-diethylaminovalerate. 1:1-Bisethoxycarbonyl-4-diethylamino propane (55·4 g.) was refluxed with concentrated hydrochloric acid (260 ml.) for 2 hours, and then evaporated to dryness under reduced pressure. The yellow crystalline residue was refluxed with ethanol (250 ml.) and sulphuric acid (25 ml.) for 4 hours. The bulk of the ethanol was removed by distillation, the remaining liquid basified with sodium hydroxide solution, and extracted with ether. The ethereal solution was dried with Na_2SO_4, evaporated, and the residue distilled to yield ethyl 5-diethylaminovalerate (20 g.; 49 per cent), b.p. 136–155° at 22 mm., $n_D^{19·5}$ 1·4352. Magidson and Strukov[20] give b.p. 130–131° at 25 mm., n_D^{20} 1·4354. Found: Equiv. titration 202·5. Calc. for $C_{11}H_{23}O_2N$. Equiv. 201.

5-Hydroxypentyldiethylamine. Ethyl 5-diethylaminovalerate (12·4 g.) in dry ether (10 ml.) was run slowly into a stirred hot suspension of lithium aluminium hydride (1·5 g.) in dry ether (65 ml.) at a rate sufficient to keep the mixture refluxing (30–40 min.). The reaction mixture was cooled in

ice, and brine added dropwise to decompose the excess lithium aluminium hydride, and then the complex. Sodium hydroxide solution (20 per cent; 50 ml.) was added and the ethereal layer decanted. The residual gel was extracted with ether (2 × 200 ml.), the mixed ethereal solutions dried with Na_2SO_4, the solvent removed, and the residual oil distilled to give 5-hydroxypentyldiethylamine (7·1 g.; 73 per cent), b.p.131–135° at 22 mm., n_D^{23} 1·4512. Synerholm[21] gives b.p. 125–130° at 20 mm., n_D^{20} 1·4544.

Bis-5-diethylaminopentyl sulphide. Excess thionyl chloride (9 ml.) in benzene (20 ml.) was slowly added to a stirred solution of 5-hydroxypentyldiethylamine (14·3 g.) in benzene (100 ml.). The yellow crystalline mass obtained on removal of the solvent and excess reagent was dissolved in water (20 ml.), the solution cooled to 0° and basified with sodium hydroxide solution (30 ml.; 20 per cent). Extraction with ether, drying with Na_2SO_4 and evaporation of the solvent gave crude 5-chloropentyldiethylamine (15·9 g.). The latter in ethanol (8 ml.) was slowly added to a hot solution of anhydrous sodium sulphide (4·4 g.) in water (5 ml.) and ethanol (16 ml.), and the mixture refluxed for 3 hours with continuous stirring. The residual liquid, after removal of the solvent at 100° was poured into brine (50 ml.) and extracted with ether. The ethereal extracts were dried with Na_2SO_4, evaporated, and the residue distilled to yield *bis-5-diethylaminopentyl sulphide*, as a pale yellow oil (3·55 g., 25 per cent), b.p. 200–205° at 1·2 mm., $n_D^{17·5}$ 1·4707. Found: Equiv. titration 157·3. $C_{18}H_{40}N_2S$ requires equivalent 158·3. *Bis-5-diethylaminopentyl sulphide dihydrochloride* from ethanol-ether, m.p. 155–156°. Found: C, 55·8; H, 10·9 per cent; $C_{18}H_{42}N_2SCl_2$ requires C, 55·5; H, 10·9 per cent.

6-Thiaundecane-1:11-bis(triethylammonium iodide). Bis-5-diethylaminopentyl sulphide (0·85 g.) was refluxed with excess ethyl iodide for 15–20 minutes. The crystalline deposit gave from ethanol *6-thiaundecane-1:11-bis(triethylammonium iodide)*, m.p. 199–200°. Found: I, 40·8; N, 4·6 per cent; $C_{22}H_{50}N_2SI_2$ requires I, 40·4; N, 4·5 per cent.

6-Ethyl-6-thioniaundecylene bis(triethylammonium) triiodide. Bis-5-diethylaminopentyl sulphide (0·85 g.) was refluxed with excess ethyl iodide (4 ml.) for not less than 30 minutes. Removal of excess reagent under reduced pressure, and recrystallisation of the product from ethanol gave *6-ethyl-6-thioniaundecylene bis(triethylammonium) triiodide*, m.p. 136·5–137·5°. Found: I, 48·3; N, 3·5 per cent; $C_{24}H_{55}N_2SI_3$ requires I, 48·5; N, 3·6 per cent.

6-Chlorohexyldimethylamine prepared by the method of Andrews, Bergel and Morrison[12] was obtained as a colourless oil b.p. 69° at 3·5 mm., $n_D^{17·5}$ 1·4467.

Bis-6-dimethylaminohexyl sulphide was prepared from 6-chlorohexyldimethylamine (19·2 g.) by the method described under bis-5-diethylaminopentyl sulphide. *Bis-6-dimethylaminohexyl sulphide* was obtained as a colourless oil (12·8 g. 76 per cent), b.p. 164–165° at 0·75 mm., $n_D^{18·5}$ 1·4742. Found: Equiv. titration 145·4; N, 9·6 per cent; $C_{16}H_{36}N_2S$ requires equiv. 144·3; N, 9·7 per cent.

7-Methyl-7-thioniatridecylene bis(trimethylammonium) triiodide. Bis-6-dimethylaminohexyl sulphide (1·14 g.) was refluxed with methyl iodide

(5 ml.) and ethanol (5 ml.) for 50 minutes. Evaporation under reduced pressure, and crystallisation of the product from ethanol gave yellow crystals (2·2 g. 79 per cent) of *7-methyl-7-thioniatridecylene bis(trimethylammonium) triiodide*, m.p. 170–171°. Found: C, 32·0; H, 6·1; I, 52·6; N, 3·9 per cent. $C_{19}H_{45}N_2SI_3$ requires C, 32·0; H, 6·4; I, 53·3; N, 3·9 per cent.

7-Thiatridecane-1 : 13-bis(trimethylammonium iodide). Bis-6-dimethylaminohexyl sulphide (1·29 g.) was mixed with benzene (6 ml.) and methyl iodide (1 ml.). Separation of a crystalline product commenced within 2–3 minutes, and, after standing overnight, this was separated by filtration and recrystallised from ethanol to yield colourless needles (1·3 g. 51·2 per cent) of *7-thiatridecane-1 : 13-bis(trimethylammonium iodide)*, m.p. 179–180·5°. Found: C, 37·7; H, 7·3; N, 5·0; I, 44·3 per cent; $C_{18}H_{42}N_2SI_2$ requires C, 37·8; H, 7·4; N, 4·9; I, 44·3 per cent.

5-Thianonane-1 : 9-bis(triethylammonium iodide). 5-Thianonane-1 : 9-bis(triethylammonium bromide) (4·3 g.), prepared by the method used by Andrews, Bergel and Morrison[12] for the production of the corresponding methyl analogue, in ethanol (30 ml.) was treated with excess silver carbonate (2·5 g.), and the mixture shaken for 3 hours. After filtration, the filtrate was titrated against a solution of hydriodic acid (11 per cent) until just acid to methyl red. The solution was evaporated to dryness, the colour discharged by addition of minimum amount of sodium thiosulphate solution, and the solution again evaporated. Extraction of the residue with ethanol, and addition of ether gave *5-thianonane-1 : 9-bis-triethylammonium iodide)*, m.p. 212–212·5°. Found: I, 41·9; N, 4·7 per cent; $C_{20}H_{46}N_2SI_2$ requires I, 42·3; N, 4·7 per cent.

Attempted preparation of 5-ethyl-5-thionianonylenebis(triethylammonium triiodide. 5-Thianonane-1 : 9-bis(triethylammonium iodide) (0·137 g.) in ethanol (2 ml.) and ethyl iodide (0·5 ml.) was refluxed for 30 minutes. Evaporation under reduced pressure, and crystallisation from ethanol gave unchanged material, m.p. and mixed m.p. 211·5–212°.

PHARMACOLOGICAL

Methods and Materials

Composition of perfusion fluids (g./litre).

Frog Ringer's Solution: NaCl 6·5, $NaHCO_3$ 0·2, KCl 0·138, $CaCl_2$ 0·12, dextrose 1·0. Tyrode's Solution: NaCl 8·0, $NaHCO_3$ 1·0, KCl 0·198, $CaCl_2$ 0·2, $MgCl_2$ 0·1, NaH_2PO_4 0·005, dextrose 1·0. Locke's Solution: NaCl 9·0, $NaHCO_3$ 0·5, KCl 0·42, $CaCl_2$ 0·24, dextrose 1·0.

The following drugs were used: acetylcholine chloride (ACh), (—)-adrenaline hydrochloride (Ad), (—)-noradrenaline hydrochloride (NA), histamine acid phosphate (Hm), tubocurarine chloride (TC), decamethonium iodide (C 10), hexamethonium bromide (C 6), eserine salicylate (eserine), neostigmine methyl sulphate (neostigmine), edrophonium bromide (edrophonium), potassium chloride (KCl), ether, dihexasulphonium triethiodide (DHSE), dipentasulphonium triethiodide (DPSE),

dihexasulphonium trimethiodide (DHSM), 4-dioxothiahepta-2:5-diene-1:7-bis(triethylammonium iodide), 4-dioxothiaheptane-1:7-bis(triethylammonium iodide).

Neuromuscular Blocking Activity

Cat gastrocnemius muscle—sciatic nerve preparation. Cats of either sex weighing between 2·0 to 5·0 kg. were anaesthetised with intraperitoneal pentobarbitone (60 mg./kg.). The gastrocnemius muscle was partially dissected free from the surrounding tissue and the achilles tendon severed at a point near its insertion to the calcaneus. The tendon was attached by means of a strong linen thread to a myograph lever. The sciatic nerve was then partially dissected at a point proximal to the anterior tibial nerve. The nerve was stimulated by means of a Dobbie McInnes square wave generator at a frequency of 4 to 6/minute at 8 to 12 volts, the pulse width being 2 to 3·5 msecs. In any one experiment, frequency, voltage and pulse width were constant except that in some experiments the muscle was also tetanised indirectly with a frequency of 1500/minute and in others the muscle was stimulated directly at 40 volts after it had become unresponsive to indirect stimulation. Drugs were injected into the external jugular vein. In a few experiments contractions of the anterior tibialis muscle or soleus muscle were recorded after sciatic nerve stimulation. The conditions of the experiments were identical with those just described.

Rabbit head drop method. Rabbits of either sex weighing 1·7 to 3·2 kg. were used. Solutions of DPSE, DHSE, and TC, 0·05 mg./ml. in normal saline were administered by infusion into a marginal ear vein at a constant rate of 1·4 ml./minute. Infusion was continued until, following a light tap on the muzzle, the animal was no longer able to raise its head.

Rat diaphragm. The usual method was used[22]. The frequency of indirect stimulation was 6 square pulses per minute at 8 volts and the pulse width was 1 msec. Drugs were left in contact for 3 minutes.

Frog rectus abdominis muscle. Reproducible submaximal contractions of the rectus muscle were induced by ACh (0·1 to 0·2 μg./ml.) or C 10 (2 to 3 μg./ml.) which were left in contact with the tissue for 1·5 minutes. DPSE or DHSE and TC were added 1 minute before the addition of ACh or C 10. In a few experiments rectus muscles from toads were used.

Mouse test. DPSE, DHSE, or TC at different doses were given by intraperitoneal injections to groups of mice weighing 30 to 40 g.

Other Properties

Effects on blood pressure, respiration and nictitating membrane. Cats, chloralosed, 80 to 100 mg./kg., of either sex weighing 1·5 to 3·5 kg. were used. All drugs were administered by injection into the external jugular vein.

Blood pressure was recorded from the common carotid artery, and respiration, by a thread sewn into the skin of the epigastrium, and attached to a recording lever. A solution of 0·2 mg./ml. of DPSE, DHSE or TC was infused into the external jugular vein at a constant rate of 0·8 ml./

minute. Infusion was stopped as soon as respiration failed and artificial respiration begun.

Contractions of the nictitating membrane were elicited by stimulation of the preganglionic fibres of the cervical sympathetic and recorded by conventional methods. Stimulation was by means of square impulses at a frequency of 800 to 1200/minute 8 to 15 volts, pulse width 0·2 to 1·0 msec. In any one experiment frequency, voltage, and pulse width were constant. Two kinds of experiments were made. Intermittent stimulation for 15 to 20 seconds at 3 minute intervals, and continuous tetanisation for an indefinite period. In the former reproducible responses were obtained by stimulating the nerve at 3 minute intervals and drugs were injected 1 minute before stimulation. In the latter, drugs were injected when the recorded response of the nictitating membrane had reached a constant level.

Effects on isolated perfused hearts. Rabbit or kitten hearts were perfused by Langendorff's method[23] with oxygenated Locke's solution at 37°. Outflow was measured by collecting the perfusate into a measuring cylinder at 5 minute intervals. Drugs were administered by injection into the cannula.

Guinea pig ileum. Pieces of the terminal ileum about 4 cm. long were suspended in a 2 ml. bath containing oxygenated Tyrode's solution at $30 \pm 0{\cdot}5°$. Reproducible submaximal contractions were obtained to ACh, 0·2 μg./ml., added at 3 minute intervals and left in contact with the tissue for 30 seconds. Drugs were added 1 minute before ACh.

Rabbit duodenum. Pieces of duodenum, about 4 cm. long, were set up in a 40 ml. bath containing oxygenated Locke's solution at 37°. The spontaneous movements of the duodenum were recorded. Drugs were added to the bath at varying intervals of time.

Rat hind quarters. The hind quarters of rats were perfused with oxygenated Locke's solution at room temperature as described by Burn[24]. Outflow was measured by Gaddum's drop recorder and drugs were administered by injection into the cannula.

RESULTS

Neuromuscular Blocking Activity

DPSE and DHSE, 0·1 to 0·4 mg./kg., caused incomplete reversible neuromuscular block. When the dose was increased, 0·5 to 1·0 mg./kg., neuromuscular block was complete but still reversible. Similar results were obtained using TC at similar doses. For all three compounds the duration of block depended upon the dose. After complete block the muscle still responded to direct stimulation (Fig. 1). Potentiation of twitch height was not seen and there were no muscular fasciculations. When muscles partly blocked by DPSE, 0·1 mg./kg., or DHSE, 0·1 mg./kg., were indirectly tetanised, the tension rapidly waned. Similar effects are seen when TC is used but, as is well known, the normal muscle or the muscle partly blocked by C 10 can maintain a tetanus (Fig. 2).

Partial or complete neuromuscular block produced by DPSE or DHSE is rapidly and completely antagonised by intravenous injection of edrophonium, 0·2 to 0·6 mg./kg. Block is also antagonised by neostigmine, 25 to 75 μg./kg., eserine, 30 to 40 μg./kg., and C 10, 0·05 to 0·1 mg./kg.

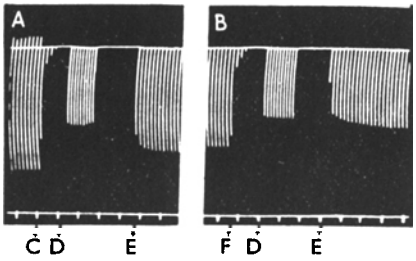


FIG. 1. Direct stimulation of blockaded muscle. Cat, 2·0 kg., female, sciatic nerve-gastrocnemius muscle preparation. Pentobarbitone anaesthesia.

Stimulation of nerve by square impulses, 15 V., frequency 8/min., width 2 msec.

Stimulation of muscle by square impulses, 40 V. frequency, 8/min., width 3·5 msec.

A. DPSE. B. DHSE.

At C, 0·75 mg./kg. of DPSE i.v.

D, Direct stimulation of muscle.

E, 0·5 mg./kg. of edrophonium bromide.

F, 0·75 mg./kg. of DHSE i.v.

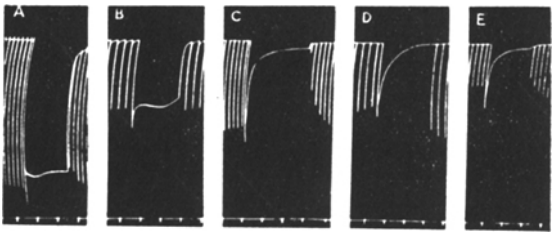


FIG. 2. Comparison of the effects of DHSE, DPSE, TC and C10 on the response of the cat gastrocnemius muscle to indirect tetanisation via the sciatic nerve.

Tetanisation by square impulses, 10 V. frequency 1500/min. during incomplete neuromuscular block, width 3 msec. Time interval 10 sec.

At A, normal response, no drug given.

B, C10 0·02 mg./kg. i.v.

C, DHSE 0·1 ,, ,,

D, TC 0·1 ,, ,,

E, DPSE 0·08 ,, ,,

The edrophonium effect was usually much shorter-lasting than that of neostigmine or eserine (Figs. 3, 4 and 5).

Block produced by DPSE or DHSE, 0·1 to 0·2 mg./kg., is potentiated by ether anaesthesia (Fig. 6). Subsequent doses of the same drug given after complete recovery appeared to produce an increased effect. The first dose of DPSE, 0·4 mg./kg., reduces twitch height by about 50 per cent.

A second and similar dose given after complete recovery of twitch height reduces this by about 80 per cent whilst third and fourth doses reduce it by roughly 95 and 100 per cent. DPSE, DHSE and TC all behave in a similar fashion. DPSE and DHSE do not potentiate one another nor do they potentiate TC. On the other hand the effects of the three compounds are additive. If, for example, DPSE is used to produce neuromuscular

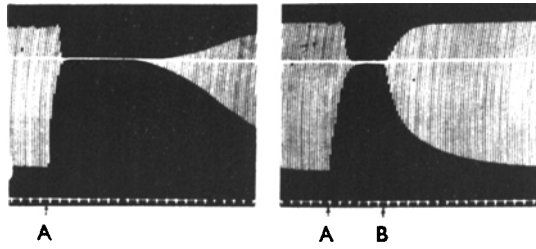


FIG. 3. DHSE-neostigmine antagonism. Cat, 3 kg., female, gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve, 4 square impulses/min. 10 V., width 3 msec. Time interval 1 minute.

At A, 0·5 mg./kg. DHSE i.v.
B, 0·07 ,, neostigmine methyl sulphate i.v.

block, the intensity of effect can be increased by administering DHSE and *vice versa*. In the same way DPSE and DHSE are additive with TC.

Rabbit head drop. In our hands, the average doses to cause head drop in groups of 6 rabbits were, DHSE, 0·36, DPSE, 0·38, and TC, 0·11 mg./

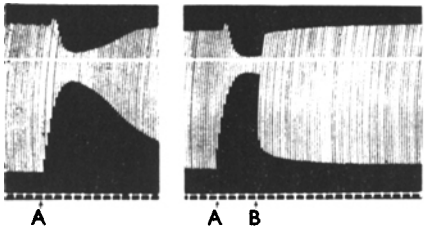


FIG. 4. DPSE-edrophonium antagonism. Cat, 3 kg., female, gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve, 4 square impulses/min., 10 V., width 3 msec. Time interval 1 min.

At A, 0·5 mg./kg. DPSE i.v.
B, 0·35 ,, edrophonium bromide i.v.

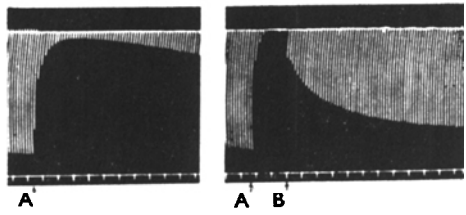


FIG. 5. DPSE-C10 antagonism. Cat, 3·25 kg., female, gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve, 4 square pulses/min. 10 V., width 3 msec. Time interval 1 minute.

At A, DPSE 0·5 mg./kg. i.v.
B, C10 0·15 ,, ,,

kg. When TC was used failure of spontaneous respiration always occurred. With the other compounds it occurred in 2 animals in each group.

Rat diaphragm. DPSE and DHSE, 0·2 mg./ml., reduced the twitch height, but a complete block was not produced, and the effect was always reversible. TC was much more potent on this preparation.

Frog rectus abdominis muscle. Neither DPSE nor DHSE at the doses used had any direct stimulant effect. DPSE and DHSE, 0·5 to 2·5

μg./ml., antagonised contractions induced by 0·1 to 0·2 μg./ml. of ACh or 2 μg./ml. of C 10. TC, 0·1 to 0·5 μg./ml., produced similar effects. Toad muscle was less sensitive to ACh or C 10. On toad muscle preparations DPSE and DHSE, 0·05 to 0·2 mg./ml., antagonised contractions due to ACh, 0·4 μg./ml., or C 10, 3·0 μg./ml.

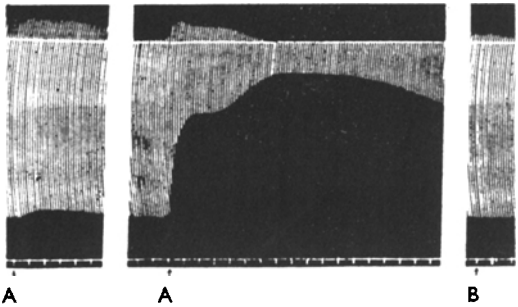


FIG. 6. Ether potentiation of DHSE. Cat, 3·25 kg., male, gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia. Indirect stimulation via sciatic nerve, 4 square impulses/min., 10 V., width 3 msec. Time interval 1 min.

At A, 0·1 mg./kg. DHSE i.v.
B, 0·1 ,, ,, ,, with ether anaesthesia.

Mouse test. Intraperitoneal injection of DPSE and DHSE, 1·0 to 1·35 mg./kg., caused flaccid paralysis. This was followed by failure of respiration which was the apparent cause of death because the heart continued to beat for some time after the respiration had ceased. At low doses, 1·0 mg./kg., there was partial muscular paralysis and some embarrassment of the respiration. DHSE appeared to be slightly more potent in causing respiratory paralysis in mice. The LD50 of DHSE was 1·2 $\pm$ 0·1 mg./kg. and that of DPSE was 1·3 $\pm$ 0·1 mg./kg.

Other Properties

Neither DPSE or DHSE, 0·5 mg. to 1·0 mg./kg., caused a fall or rise in the blood pressure of the anaesthetised cat. In contrast, TC, 0·5 mg./kg., caused the blood pressure to fall. Neither antagonism nor potentiation was shown by DPSE or DHSE to the characteristic effects of ACh, 0·4 to 1·0 μg./kg., Ad, 0·4 to 2·0 μg./kg., NA, 0·4 to 2·0 μg./kg., or Hm, 0·4 to 1·0 μg./kg.

DPSE, 0·1 mg./kg., caused a slight relaxation of sustained contractions of the nictitating membrane induced by electrical stimulation of preganglionic fibres of the cervical sympathetic. After this compound, 0·5 to 0·8 mg./kg., there was also a slight reduction of the height of contraction of the nictitating membrane following indirect intermittent stimulation. DHSE on the other hand caused neither relaxation of the tetanised membrane, 0·1 mg./kg., nor a reduction in the height of contraction after intermittent stimulation, 0·25 to 0·5 mg./kg. TC was much more potent on both of these preparations.

Continuous infusion of both DPSE and DHSE caused respiratory paralysis. If administration of the drug was stopped when respiration had ceased, then in some animals spontaneous respiration began again, but as a rule artificial respiration had to be given. This always produced a complete recovery. The approximate dose of DPSE to paralyse respiration was 0·76 mg./kg.; that of DHSE was 0·92 mg./kg. The dose of TC to cause paralysis of respiration was 0·48 mg./kg.

Neither DPSE or DHSE, 0·2 to 2·0 mg., had any effect on the rate, amplitude or outflow of the isolated perfused heart of the rabbit or kitten. No effect was observed on the peristaltic movements of the isolated rabbit duodenum at doses of 20 μg./ml. Very large doses, 0·25 mg./ml., antagonised ACh induced contractions of the guinea pig ileum.

Both these compounds, 0·2 to 1·0 mg., caused vasoconstriction in the isolated perfused rat hind quarters but they were much less potent vasoconstrictors on this preparation than TC (50 to 250 μg.).

Discussion

Both DPSE and DHSE are neuromuscular blocking agents which appear to act in a similar manner to TC. We were unable to show evidence of a depolarising action such as is shown by C 10. Both compounds are about equipotent with TC on the gastrocnemius muscle of the cat, but are less potent on the rat diaphragm and frog rectus muscle and have a higher head-drop dose. They appear to lack ganglion blocking activity and not to release histamine. They are less potent than TC in paralysing respiration in the cat. Tris-quaternary compounds have been shown to possess curare-like properties[11,17]. The tris-nitrogen compound gallamine has about one fifth of the potency of TC; it lacks ganglion-blocking activity[25] and does not cause significant histamine release[26], and is not potentiated by ether anaesthesia[27]. It has an atropine-like vagolytic action[10,11,27]. The compounds we have studied appear to have a great deal in common with gallamine. They act by a similar mechanism and do not cause ganglion block or histamine release unless given in massive doses. In addition they have the advantage that they do not appear to alter the heart rate. Like TC, their action is potentiated by ether anaesthesia.

We have also studied the ganglion-blocking and neuromuscular blocking properties of three other compounds (IV, $n = 3$), V, VI ($n = 6$, R = Me). None possess neuromuscular-blocking activity. DHSM was occasionally observed to have slight ganglion-blocking activity but the other compounds were almost inert.

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DISCUSSION

The paper was presented by MR. J. J. LEWIS.

The CHAIRMAN. Had the authors any theory for the failure to quaternise in the case of the butyl sulphide, were the sulphonium salts very soluble in water and was there any information regarding stability?

PROFESSOR K. BULLOCK (Manchester). Had the authors tried the *in vitro* anticholeresterase activity of the compounds.

DR. G. E. FOSTER (Dartford). Since sulphonal and trional were sulphones, had the compounds under discussion any soporific value.

In reply MR. LEWIS said that the *in vitro* anticholeresterase activity of the compounds had not been tried and no soporific properties had been observed.

DR. J. B. STENLAKE had not been able to obtain a satisfactory explanation why the third quaternary atom could not be introduced in the butyl derivatives. The compounds were all very soluble and he had experienced no difficulty in preparing any solution required for pharmacological tests. As regards stability, compounds with *NNN* quaternary groups were stable, but he was uncertain about the *NSN* compounds.