Brit. J. Pharmacol. (1960), **15**, 71.

THE NEUROMUSCULAR BLOCKING PROPERTIES OF A SERIES OF BIS-QUATERNARY TROPEÏNES

BY

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(RECEIVED AUGUST 11, 1959)

Linkage of two tropine esters through their nitrogen atoms by the chain $-[CH_2]_m-O-CO-[CH_2]_n-CO-O-[CH_2]_m-$, in which m was 2 or 3 and n varied from 0 to 6, gave compounds which produced neuromuscular block without depolarization. Reversibility by neostigmine was confirmed for a few compounds. Potency was found to depend upon the tropine ester employed and upon the values of n and m. Short duration and hypotensive properties were favoured by the higher values of n. The duration of action of the compound based on the phenylacetic acid ester of tropine, in which $n=4$ and $m=2$, varied considerably in different species. Epimerization, in which the relative positions of the methyl group and the linking chain on the quaternary tropane nitrogen atom were reversed, did not produce substances having more favourable properties than those possessed by the unepimerized compounds.

Bis-onium compounds in which the quaternary nitrogen atoms are present as part of substituted tropane nuclei have been examined for neuromuscular blocking properties by Gyermek and Nádor (1957). Some were considerably more active than tubocurarine in laboratory animals, but so far none has shown sufficient advantages over muscle relaxants currently in use to justify its introduction into clinical practice.

Linking of the tropane nuclei in such compounds has been effected in three ways; by means of an alkyl or aralkyl chain between the nitrogen atoms of the tropane nuclei (Kimura and Unna, 1950; Gyermek and Nádor, 1952; Eckfield, 1959), through the tropic acid hydroxyl groups of two atropine molecules with the formation of ether linkages (Kimura, Unna and Pfeiffer, 1949), or through the 3-hydroxyl groups of quaternized tropine molecules to form di-esters of dicarboxylic acids such as isatropic (Hotovy, Jacobi and Kuessner, 1956; Just, 1953), succinic or phthalic acid (Gyermek and Nádor, 1953).

The esterifying acid, the quaternizing group used, and the chain length are all known to be of importance in determining potency and side actions (Gyermek and Nádor, 1957; Haining, Johnston and Smith, unpublished observations), but the effect of chain structure on duration of action has received little attention. In $\alpha\omega$-dicarboxylic esters of choline the presence of ester groups at suitable points within the chain shortens duration of action (Brücke, 1956). However, potent members of this series, such as suxamethonium, have disadvantages (Churchill-Davidson, 1958), one of the most important being that, since they are depolarizing agents, paralysis due to them cannot be reversed by neostigmine. On the other hand, bis-quaternary tropeïne neuromuscular blocking compounds tend to exert a competitive type of action (Haining *et al.*, unpublished observations).

The compounds described here were prepared in the hope that, by linking two quaternary tropeïne moieties by a chain susceptible to attack by esterases, it would be possible to obtain competitive neuromuscular blocking agents of short duration.

METHODS

Neuromuscular Block in Cats, Dogs and Rabbits.—The neuromuscular blocking activity of compounds was investigated in cats anaesthetized with a mixture of chloralose 50 mg./kg. and urethane 500 mg./kg. given by intraperitoneal injection, or in dogs which had received pentobarbitone sodium intraperitoneally. One hind leg was fixed rigidly in a vertical position by means of a pin through the lower end of the femur. Shielded silver electrodes were placed on the sciatic nerve which was then crushed proximal to the electrodes. The gastrocnemius muscle was attached to a flat spring myograph and the contractions recorded on smoked paper. Muscle twitches were elicited by supramaximal rectangular pulses of less than 1 msec. duration.

The standard used throughout these experiments was *NN'*-4,9-dioxo-3,10-dioxadodecamethylenebis(3-phenylacetoxytropanium bromide) (DF596). The relative potency of this compound in different species has been reported previously (Haining, Johnston and Smith, 1959). It was approximately half as potent as tubocurarine in the cat, and the recovery rate was approximately equal to that of suxamethonium (Fig. 1). DF596 has the advantage over compounds such as gallamine triethiodide or tubocurarine commonly used as standards in that it has little or no cumulative properties. This allowed considerably more estimates to be made in any given period of time. The quantities in the text refer to chloride unless otherwise stated. The potency and duration of action of compounds and their effects on blood pressure were determined by comparison with DF596 in the same animal. Whenever possible doses of each compound were chosen which would reduce the height of the gastrocnemius twitch by approximately 25% and 75%. The percentage reduction in twitch height and fall in blood pressure resulting from each dose were plotted against the logarithm of the dose. Since the slopes of the regression lines varied with the rate of recovery, potencies were compared at doses estimated to give a 50% reduction of twitch, and effects on blood pressure were compared at these doses. Estimates of duration of action were made by visual comparison of tracings. Values obtained for long-acting compounds were least reliable since, with compounds of the same duration as gallamine triethiodide, cumulative effects made comparisons difficult.

Blood pressure was recorded from the carotid artery with a mercury manometer. Animals were usually maintained with artificial respiration. Drugs were dissolved in physiological saline and were injected into the femoral vein.

In order to determine the rate of recovery in rabbits, drug solutions were infused into the marginal ear vein by means of a continuous slow injector (Palmer). Head drop was judged to be complete when the rabbit was unable to raise its head if tapped gently on the nose with a finger. The infusion was then stopped. Times required for the following stages in recovery were recorded: (1) The animal was able to raise its head in response to a light tap on the nose. (2) The animal was able to right itself immediately when quickly placed on its back. (3) With the hind limbs lifted clear of the ground, the animal was able to support itself and walk on its forelimbs. The time for complete recovery, when the animal was able to walk without assistance, was also determined. This, however, could not be obtained so precisely as with the other measurements, since animals after recovering from paralysis usually showed little inclination to move. During infusions, animals were allowed to sit on a rubber mat, and were restrained slightly with the hand. They usually remained quite still as long as they were able to get a good grip with their feet.

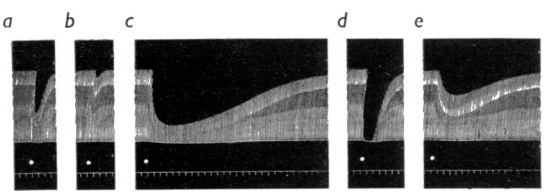


FIG. 1.—Cat. Maximal twitches of the gastrocnemius muscle elicited by indirect stimulation. Time, min. All drugs were given intravenously. (*a*) At time 0 min., 0.5 mg./kg. of DF596 was given. (*b*) At 7 min., 0.3 mg./kg. of DF596. (*c*) At 14 min. 0.3 mg./kg. of tubocurarine chloride. (*d*) At 67 min., 0.8 mg./kg. of DF596. (*e*) At 78 min., 0.15 mg./kg. of tubocurarine chloride.

The effects of large intravenous doses in conscious rabbits were investigated in animals maintained with the aid of a Drinker-type respirator. The rabbit was placed in a Perspex box with head protruding and chin resting on a support. A good seal at the neck was obtained with a collar of tambour rubber. Partial vacuum within the box was obtained by an Edwards two-stage vacuum pump 2SC50, and was broken at intervals by a simple spring-loaded flap valve. An adjustable leak consisting of a plate sliding over a slit in the box was provided to allow for various sizes of rabbits. Initially the valve was operated by a relay and timing circuit (Austin, 1954), but, as this was extremely noisy and gave jerky respiratory movements, it was found more convenient to operate the valve by means of a cam fitted on the shaft of a windscreen wiper motor (Lucas type C.W.1).

The Isolated Frog Rectus Abdominis Muscle Preparation.—Muscle strips were suspended in frog Ringer solution in a 10 ml. bath. Regular contractures to acetylcholine were obtained at 5 min. intervals. Antagonists were added to the bath 1 min. before the agonist, which was allowed to act for 1 min. When compounds were tested for ability to produce a contracture they were allowed to act for 5 min.

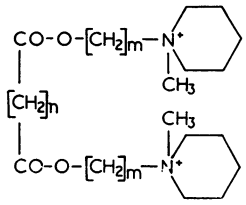
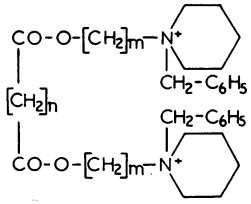
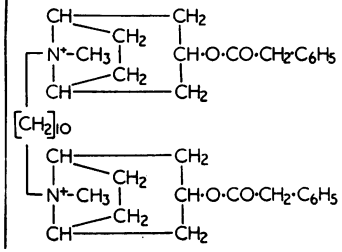
Action in Chicks.—To determine mode of action, compounds were administered subcutaneously to day-old chicks which were then observed until they died (Buttle and Zaimis, 1949). Drugs known to have competitive or depolarizing action were used at the same time for purposes of comparison.

The Isolated Human Foetal Phrenic Nerve-Diaphragm Preparation.—These preparations were obtained from foetuses of between 16 and 24 weeks. Sectors of diaphragm with nerve and ribs attached were removed from the foetus within 1 or 2 hr. of delivery, placed in Tyrode solution (NaCl, 0.9%; KCl, 0.042%; $CaCl_2$, 0.024%; $NaHCO_3$, 0.05%; dextrose, 0.1%) for transport to the laboratory. On arrival they were set up in an organ bath in oxygenated Tyrode solution at 36° to 37° within a further 30 min. Maximal contractions were obtained by stimulating the muscle indirectly with rectangular pulses of less than 1 msec. duration at a rate of about 5/min.

TABLE I

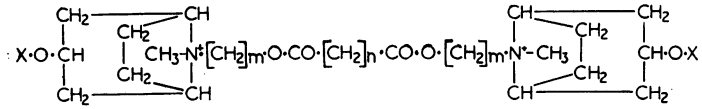
RELATIVE POTENCY, DURATION OF ACTION AND TYPE OF ACTION SHOWN BY BIS-QUATERNARY TROPEÏNE COMPOUNDS IN VARIOUS TESTS

DF596 was used as the standard for comparisons of potency and duration except for DF41. Col. 1, Relative potency in the cat. Number of estimates in parentheses. Col. 2, Relative duration of action in the cat. Col. 3, Degree of hypotension produced in the cat. Shown as $<$, $\equiv$ (equivalent to), $>$ that due to DF596. Col. 4, Anti-acetylcholine activity on the isolated guinea-pig ileum. Atropine sulphate = 1·0. Col. 5, Action on the frog rectus abdominis muscle. C, compound produced a contracture. A, compound antagonized the action of acetylcholine. Col. 6, Type of paralysis produced by injection into chicks. F, flaccid. S, spastic. † Indicates that the compound was also tested in rabbits (Table II). * Indicates that neuromuscular block was reversed by neostigmine. ‡ Comparison with tubocurarine.

Series	DF No.	Name of Compound	Structure	Anion	*m*	*n*	1	2	3	4	5	6	
		Suxamethonium chloride						++					†
		Tubocurarine chloride						+++++					†
		Gallamine triethiodide						++++ to +++++					†
	616	*NN'*-4,9-Dioxo-3,10-dioxadodecamethylenebis(1-methylpiperidinium iodide)		2 I^-	2	4	0·6 (2)	+ to ++	$<$		C	S	
	628	*NN'*-4,9-Dioxo-3,10-dioxadodecamethylenebis(1-benzylpiperidinium bromide)		2Br^-	2	4	$<$0·2 (1)				A	S F	
	41	*NN'*-Decamethylenebis(3-phenylacetoxytropanium bromide)		2Br^-			0·8 (2) ‡	++++ to +++++ ‡		0·02		F	† *

(Continued overleaf)

TABLE I—*continued*



			X	Anion	*m*	*n*	1	2	3	4	5	6	
I	623	*NN'*-4,7-Dioxo-3,8-dioxadecamethylenebis(3-acetoxytropanium bromide)	-CO-CH_3	$2Br^-$	2	2	0·2 (1)	++++	<		A		
	632	*NN'*-4,9-Dioxo-3,10-dioxadodecamethylenebis(3-acetoxytropanium bromide)		$2Br^-$	2	4	0·25 (2)	+++	<		A		
	631	*NN'*-4,11-Dioxo-3,12-dioxatetradecamethylenebis(3-acetoxytropanium bromide)		$2Br^-$	2	6	0·4 (2)	++	≡		A		
II	619	*NN'*-4,6-Dioxo-3,7-dioxanonamethylenebis(3-benzoyloxytropanium bromide)	-CO-C_6H_5	$2Br^-$	2	1	0·5 (1)	++++			A	F	
	618	*NN'*-4,7-Dioxo-3,8-dioxadecamethylenebis(3-benzoyloxytropanium bromide)		$2Br^-$	2	2	0·7 (1)	++++	≡		A	F	†
	620	*NN'*-4,9-Dioxo-3,10-dioxadodecamethylenebis(3-benzoyloxytropanium bromide)		$2Br^-$	2	4	1·2 (2)	+++	<		A	F	†
	622	*NN'*-4,10-Dioxo-3,11-dioxatridecamethylenebis(3-benzoyloxytropanium bromide)		$2Br^-$	2	5	1·3 (3)	++	>		A		†
	634	*NN'*-4,11-Dioxo-3,12-dioxatetradecamethylenebis(3-benzoyloxytropanium bromide)		$2Br^-$	2	6	0·2 (2)	+	≻		A		
III	604	*NN'*-4,5-Dioxo-3,6-dioxaoctamethylenebis(3-phenylacetoxytropanium bromide)	-CO-CH_2-C_6H_5	$2Br^-$	2	0	<0·2 (2)		>	0·01	A	F	
	595	*NN'*-4,6-Dioxo-3,7-dioxononamethylenebis(3-phenylacetoxytropanium bromide)		$2Br^-$	2	1	1·2 (3)	+++ to ++++	<	0·01	A	F	† *
	593	*NN'*-4,7-Dioxo-3,8-dioxadecamethylenebis(3-phenylacetoxytropanium		$2Br^-$	2	2	1·0 (2)	+++ to ++++	<	0·01	A	F	†

		enebis(3-phenylacetoxytropanium bromide)						(1)						
	596	*NN'*-4,9-Dioxo-3,10-dioxadodecamethylenebis(3-phenylacetoxytropanium bromide)		$2Br^-$	2	4	1·0	++		0·1	A	F	† *
	621	*NN'*-4,10-Dioxo-3,11-dioxatridecamethylenebis(3-phenylacetoxytropanium bromide)		$2Br^-$	2	5	0·4 (2)	+	>		A	F	
	635	*NN'*-4,11-Dioxo-3,12-dioxatetradecamethylenebis(3-phenylacetoxytropanium bromide)		$2Br^-$	2	6	<0·1 (3)	+	>		A		
	696	*NN'*-4,9-Dioxo-3,10-dioxadodecamethylenebis(3-β-phenylpropionyloxytropanium bromide)	$-CO-CH_2-CH_2-C_6H_5$	$2Br^-$	2	4	0·9 (2)	++	< ≡		A		†
	767	*NN'*-5,7-Dioxo-4,8-dioxaundecamethylenebis(3-phenylacetoxytropanium bromide)		$2Br^-$	3	1	1·6 (1)	+++	<		A		
	625	*NN'*-5,8-Dioxo-4,9-dioxadodecamethylenebis(3-phenylacetoxytropanium iodide)		$2I^-$	3	2	1·8 (3)	+++	<		A		† *
IV	653	*NN'*-5,9-Dioxo-4,10-dioxatridecamethylenebis(3-phenylacetoxytropanium iodide)	$-CO-CH_2-C_6H_5$	$2I^-$	3	3	1·2 (2)	++	<		A		
	646	*NN'*-5,10-Dioxo-4,11-dioxatetradecamethylenebis(3-phenylacetoxytropanium iodide)		$2I^-$	3	4	0·6 (2)	+	≡		A		
	649	*NN'*-5,12-Dioxo-4,13-dioxahexamethylenebis(3-phenylacetoxytropanium iodide)		$2I^-$	3	6	0·1 (2)	+	>		A		
	723	*NN'*-6,7-Dioxo-5,8-dioxadodecamethylenebis(3-phenylacetoxytropanium bromide)	$-CO-CH_2-C_6H_5$	$2Br^-$	4	0	0·2 (1)	+++ to ++++	>		A		†
	686	Epimer of DF593		$2Br^-$	2	2	0·5 (1)	+++ to ++++	<		A		†
V	668	Epimer of DF596	$-CO-CH_2-C_6H_5$	$2Br^-$	2	4	0·8 (1)	+++			A		†
	719	Epimer of DF635		$2Br^-$	2	6	0·2 (2)	+	>		A		

Anti-acetylcholine Activity.—This was determined on the isolated guinea-pig ileum preparation by the method of superfusion (Adam, Hardwick and Spencer, 1954). Regular submaximal contractions were obtained with acetylcholine at 90 sec. intervals. Antagonists were dissolved in Tyrode solution and applied to the gut for a period of 75 sec. before the application of acetylcholine. The effects of several concentrations of antagonist were compared with those of several of atropine sulphate. The percentage reduction in the height of contraction was plotted against the logarithm of the concentration. Since regression lines were not always parallel, comparisons of concentrations required to reduce the height of contractions by 50% were made.

Results

Table I shows the structure of compounds investigated and the results of tests carried out in cats in which their potency, duration and effect on blood pressure were compared with those of DF596. Relative activity in antagonizing the action of acetylcholine on the isolated guinea-pig ileum, the effect produced on frog rectus abdominis muscle, and the type of paralysis resulting from injection into chicks are also shown.

Table II gives the mean doses of the compounds necessary to cause head drop in rabbits, and the mean recovery times with standard deviations, in each case. The head-drop doses apply only to the particular drug concentrations and infusion rates employed, since with the short-acting compounds variations in either parameter resulted in a marked alteration of estimated potency.

Of the possible variations to the basic structure shown in Table I, the effects due to the following were investigated; changing the tropine ester, reversing the conformation of the methyl group and the linking chain about the nitrogen atom of the tropane nucleus, and altering the values of *n* and *m*.

Derivatives of Phenylacetyltropeïne

Compounds derived from phenylacetyltropeïne in which *m* was 2 (Series III) were investigated in greatest detail. To obtain neuromuscular blocking potency of the same order as DF596, the value of *n* required was not critical in the range 1 to 4. If the chain was lengthened or shortened, there was a drop in potency. In cats, the oxalic acid ester [DF604, *NN'*-4,5-dioxo-3,6-dioxaoctamethylenebis(3-phenylacetoxytropanium bromide)] had less than one sixth the activity of the malonate [DF595, *NN'*-4,6-dioxo-3,7-dioxanonamethylenebis(3-phenylacetoxytropanium bromide)] whilst the suberic acid ester [DF635, *NN'*-4,11-dioxo-3,12-dioxatetradecamethylenebis(3-phenylacetoxytropanium bromide)] was less than one tenth as effective as the adipate (DF596).

Table II

POTENCY AND DURATION OF ACTION OF BIS-QUATERNARY TROPEÏNE COMPOUNDS ESTIMATED BY THE HEAD DROP TEST IN RABBITS

Compound	Infusion Rate (ml./min.)	Conc. (mg./ml.)	Mean Head Drop Dose (mg./kg. with S.D.)	Revovery Time in Min. with S.D.			
				Head Drop	Righting Reflex	Fore Limbs	Walking Unaided
Suxamethonium chloride	1·0	0·3	0·32 (0·07)	2·7 (0·7)	3·9	7·2	7·5 (1·4)
Tubocurarine ,,	0·5	0·2	0·18 (0·04)	8·2 (3·3)	9·9	14·3	17·7 (4·0)
Gallamine triethiodide ..	0·5	0·5	0·43 (0·08)	5·5 (1·3)	7·4	10·4	12·2 (3·4)
DF41	0·5	0·2	0·24 (0·06)	3·6 (2·0)	4·1	4·9	5·4 (1·9)
DF618	0·5	0·3	0·33 (0·07)	1·7 (0·6)	2·3	3·0	3·4 (2·2)
DF620	0·5	0·3	0·21 (0·07)	1·9 (0·7)	2·3	3·2	3·9 (0·9)
DF622	1·0	0·3	0·23 (0·03)	2·9 (1·0)	3·6	5·0	5·3 (1·1)
DF595	0·5	0·3	0·29 (0·05)	2·1 (0·5)	2·2	3·0	3·4 (0·9)
DF593	0·5	0·3	0·32 (0·10)	2·0 (0·5)	2·0	3·2	3·6 (1·1)
DF769	0·5	0·3	0·15 (0·03)	2·5 (1·3)	2·8	3·9	4·6 (1·0)
DF596	1·0	0·3	0·30 (0·13)	1·5 (0·6)	2·2	2·7	3·6 (1·0)
DF696	1·0	0·3	0·33 (0·10)	2·1 (1·2)	2·5	3·7	4·1 (1·3)
DF625	0·5	0·3	0·23 (0·02)	2·5 (1·0)	2·7	4·5	5·7 (1·3)
DF723	1·0	2·0	1·53 (0·26)	1·0 (0·5)	1·1	2·7	3·0 (1·0)
DF686	1·0	0·3	0·33 (0·07)	4·6 (3·6)	5·0	6·9	7·8 (3·1)
DF668	0·5	0·3	0·17 (0·02)	1·4 (0·4)	1·9	2·4	4·6 (1·7)

When the interquaternary distance was altered, by giving n values from 1 to 6, the duration of paralysis was affected. With relatively long chains in which n was 5 (DF621) or 6 (DF635), a duration of action even more transient than that of suxamethonium was obtained as judged by tests in the cat, whilst with short chains as in the malonic (DF595) and succinic (DF593) acid esters the paralysis was intermediate in duration between that of suxamethonium and gallamine triethiodide. In rabbits there was virtually no gradation in recovery time with compounds where n varied between 1 and 4; other values of n were not examined. There was little difference in the times required for recovery from head drop after DF596 ($n=4$) and DF595 ($n=1$) although in the cat the duration of action of DF595 was considerably longer (Fig. 2). The rate of recovery

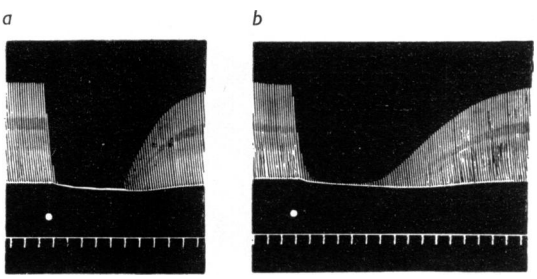


FIG. 2.—Cat. Maximal twitches of the gastrocnemius muscle elicited by indirect stimulation. Time, min. (*a*) 1 mg./kg. of DF596 was given intravenously. (*b*) 0.6 mg./kg. of DF595 intravenously.

following DF596 was comparable with that obtained with suxamethonium in the cat and dog, but this was not so in the rabbit, in which recovery after DF596 took place in about half the time needed after suxamethonium. As with suxamethonium, it was possible to produce a steady neuromuscular block by slow intravenous infusion of DF596 into cats and dogs, and recovery on stopping the infusion was quite rapid (Fig. 3).

In cats, a similar gradation of the effects of compounds upon blood pressure was also observed as the value of n was varied. Least hypotension was observed with the malonic acid ester DF595 ($n=1$), but as the number of methylene groups increased so also did the degree of hypotension produced. DF596 had a considerable hypotensive action in cats; weight for weight approximately 0.05 that of phenactropinium chloride (Robertson, Gillies and Spencer, 1957). In dogs, effects on blood pressure were much less marked. The oxalic acid ester (DF604) behaved anomalously, it was of very low activity in causing paralysis

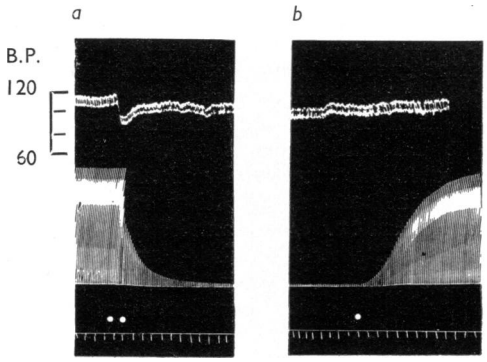


FIG. 3.—Dog anaesthetized with pentobarbitone. Upper tracing, carotid blood pressure in mm. Hg. Lower tracing, maximal twitches of the gastrocnemius muscle elicited by indirect stimulation. Time, min. (*a*) 0.1 mg./kg. of DF596 was injected at first dot. At second dot, an intravenous infusion of 0.015 mg./kg./min. of DF596 was started. (*b*) 45 min. later, the infusion of DF596 was stopped.

and gave a much greater fall in blood pressure than the malonic acid ester (DF595).

Derivatives of Other Esters

With compounds derived from acetyltropeïne (Series I) or benzoyltropeïne (Series II) in which m was 2, changes in duration of neuromuscular blocking action and effect on blood pressure were in the same direction as with the corresponding members of series derived from phenylacetyltropeïne (Series III) as n was increased from 2 to 6.

The most active benzoyltropeïne derivatives were the adipic [DF620, *NN'*-4,9-dioxo-3,10-dioxadodecamethylenebis(3-benzoyloxytropanium bromide)] and pimelic [DF622, *NN'*-4,10-dioxo-3,11-dioxatridecamethylenebis(3-benzoyloxytropanium bromide)] acid esters. These were approximately equal in potency to the most active members of the phenylacetyltropeïne series. In general, however, benzoyltropeïnes were slightly longer acting than their phenylacetyl analogues. If Series II and III are compared, it can be seen that the most active benzoyltropeïnes have higher values of n than in the phenylacetyltropeïnes.

The three acetyltropeïne analogues prepared had low activity in the cat.

The adipic acid ester derived from β-phenylpropionyltropeïne [DF696, *NN'*-4,9-dioxo-3,10-dioxadodecamethylenebis(3-β-phenylpropionyloxytropanium bromide)] closely resembled the corresponding ester derived from phenylacetyltropeïne in potency, duration and effect on blood pressure.

The Effect of Altering the Value of m

The effect of increasing the number of methylene groups in the chain between the ester groups and the quaternary nitrogen atoms from 2 to 3 was investigated only for the phenylacetyltropeïne derivatives (Series IV). This change tended to enhance the activity of the most potent members and at the same time to shorten the duration of action. For example, *NN'*-5,8-dioxo-4,9-dioxadodecamethylenebis(3-phenylacetoxytropanium iodide) (DF625) was considerably more potent and of shorter duration than *NN'*-4,7-dioxo-3,8-dioxadecamethylenebis(3-phenylacetoxytropanium bromide) (DF593). The potency of compounds did not depend only upon interquaternary distance. The positions of the ester groups were of importance in this respect. Compounds DF723 ($n=0$; $m=4$), DF625 ($n=2$; $m=3$) and DF596 ($n=4$; $m=2$), which all contained ten carbon atoms in the chain but differed in the positions of their ester groups, varied greatly in both potency and duration.

The Effects of Alterations at the Quaternary Nitrogen Atoms

When the relative positions of the methyl group and the linking chain on the quaternary tropane nitrogen were reversed (Series V), the properties of phenylacetyltropeïne derivatives were not much altered.

Miscellaneous Compounds

The possibility of substituting simpler groups for tropine esters in these compounds was investigated. Both *NN'*-4,9-dioxo-3,10-dioxadodecamethylenebis(1-methylpiperidinium bromide) (DF616) and *NN'*-4,9-dioxo-3,10-dioxadodecamethylenebis(1-benzylpiperidinium bromide) (DF628), in which the tropeïne moiety was replaced by a piperidine ring, were less potent than DF596, and the mode of action of the compounds differed from that of other members of the series.

NN'-Decamethylenebis(3-phenylacetoxytropanium bromide) (DF41) was included because it enabled a comparison to be made between a bis-quaternary tropeïne compound with only methylene groups in the chain and another (DF593) of about the same interquaternary distance with ester groups also present in the chain.

The Mode of Action of Compounds

The neuromuscular block obtained with several of the more active compounds could be reversed by a subsequent injection of neostigmine or edrophonium, both in the anaesthetized cat and in the isolated mammalian nerve-muscle preparation. Compounds tested in this way are indicated in Table I.

All members of the series described here in which each quaternary nitrogen atom was present in a tropane nucleus produced a flaccid paralysis when injected into chicks. No contracture was observed when one of these compounds was added to the fluid bathing an isolated frog rectus abdominis muscle, but contractures due to acetylcholine were reduced or abolished.

Both DF616 and DF628 containing a piperidine ring in place of the tropeïne moiety showed some of the properties characteristic of depolarizing agents. DF616 gave a spastic paralysis in the chick apparently identical with that given by suxamethonium, tested at the same time. This differed from the paralysis due to DF628, which was spastic initially but subsequently gave way to flaccidity. On the frog rectus muscle, DF616 produced a contracture when added to the bath fluid whereas DF628 itself had no effect on the muscle but antagonized the action of acetylcholine.

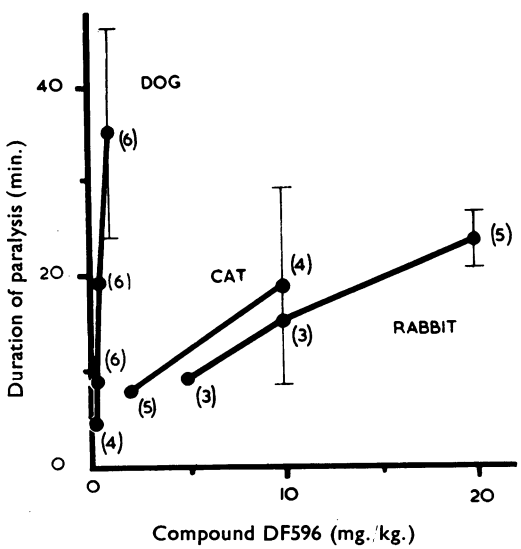


FIG. 4.—Duration of paralysis after an intravenous injection of DF596. Rabbits were maintained in a Drinker type respirator. The time for the first voluntary movement was measured. Dogs were anaesthetized with pentobarbitone and cats with a mixture of chloralose and urethane. Time for recovery of the indirectly-elicited gastrocnemius twitch to 50% of its pre-injection height was measured. Standard deviations are shown by vertical bars. Numbers of animals used are shown in brackets.

Duration of Action of Compounds in Different Species

Different species varied in their ability to inactivate DF596. Fig. 4 shows that rabbits can deal with about 15 mg./kg. and dogs about 0.5 mg./kg. in 20 min. Duration of action of DF596 was compared with that of tubocurarine and suxamethonium in mice by estimating the time elapsing after recovery of the righting reflex until animals were able to retain their hold on an almost vertical wire mesh screen. Recovery after DF596 was more rapid than after suxamethonium or tubocurarine (Table III). Confirmation of these results was obtained by determining the ratio of subcutaneous ED50 (paralysis) to the intravenous ED50 (paralysis) in mice. A high ratio would be expected if considerable metabolism of the compound had taken place before onset of paralysis. Table IV shows that the ratios for the relatively long-acting relaxants tubocurarine and gallamine triethiodide were 2.1 and 1.6 respectively whereas for the much shorter acting suxamethonium chloride the value was 9.7. The values of 32 and 29 obtained for DF595 and DF596 suggest that these compounds are even more rapidly inactivated than suxamethonium.

TABLE III

THE DURATION OF ACTION OF NEUROMUSCULAR BLOCKING AGENTS IN MICE

The values in col. 2 give the time after recovery of righting reflex until the animal was able to support itself on a near-vertical wire mesh screen after an intravenous infusion of the drug in sec. with s.d.

Compound (1)	(2)
Suxamethonium	108±18
Tubocurarine	285±190
DF596	16±18

TABLE IV

RATIO OF SUBCUTANEOUS ED50 TO INTRAVENOUS ED50 OF NEUROMUSCULAR BLOCKING AGENTS IN MICE

ED50, the quantity for paralysis in mice with 95% limits.

Compound	ED50 (mg./kg.)		Subcut. ED50 / I.V. ED50
	Subcutaneous	Intravenous	
Tubocurarine	0·094 (0·087–0·101)	0·045 (0·037–0·056)	2·1
Gallamine triethiodide	1·85 (1·56–2·20)	1·16 (1·02–1·31)	1·6
Suxamethonium	1·60 (1·46–1·74)	0·165 (0·132–0·207)	9·7
DF595	14·3 (13·4–15·3)	0·45 (0·38–0·53)	32
DF596	8·0 (7·1–9·0)	0·28 (0·26–0·31)	29

Effects of Compounds on the Isolated Human Foetal Phrenic Nerve-Diaphragm

This preparation was used to assess the likely potency of compounds in man. It was appreciated that the preparation might lead to erroneous conclusions for compounds destroyed *in vivo* only and that it was unlikely to be a suitable preparation for comparing compounds

TABLE V

RELATIVE POTENCY AND DURATION OF ACTION OF NEUROMUSCULAR BLOCKING AGENTS ON THE ISOLATED HUMAN FOETAL DIAPHRAGM

DF596 was used as the standard of comparison for potency. An asterisk indicates that the compound was antagonized by neostigmine. The relative duration of action is measured arbitrarily by the number of + : the greater the number the greater the duration of action.

Compound	No. of Estimates	Relative Potency	Relative Duration of Action	Approx. Conc. Giving 50% Reduction of Twitch in 5 Min. (μg./ml.)
Suxamethonium	3	>5	++	2–10
Tubocurarine	2		+++++	1–5
Gallamine triethiodide	1	1	++++	
DF595 ..	2	3*	++ to +++	10–20
DF596 ..	4	1*	+ to ++	20–80
DF668 ..	1	<1		

acting competitively with those exerting a depolarizing action. Different preparations were found to vary greatly in their recovery rates after the same compound. Our observations confirmed those of Buller and Young (1949), who found that, generally, recovery was slow so that potency was estimated by bracketing or matching responses. Values shown in Table V are all relative to DF596 compared on the same preparation. DF596 was considerably less potent than suxamethonium on this preparation, and duration of action was also somewhat less. DF595 was approximately 3 times as potent as DF596 and was of longer duration than suxamethonium.

The epimeric form of DF596 (DF668) did not appear to offer any advantages.

Discussion

The results clearly indicate that symmetrical bis-quaternary tropeïne compounds, in which two tropine esters were linked through their nitrogen atoms by a polymethylene chain containing two ester linkages, exhibited a neuromuscular blocking action of short duration which is reversible by neostigmine.

The phenylacetyltropeïne derivatives (Series III) in which n varies between 0 and 5 resembled the dicholine esters of $\alpha\omega$-dicarboxylic acids (I) prepared and tested by Bovet, Bovet-Nitti, Guarino, Longo and Fusco (1951) with regard to the influence which alterations in the value of

$$(CH_3)_3\overset{+}{N}\text{-}[CH_2]_2\text{-O-CO-}[CH_2]_n\text{-CO-O-}[CH_2]_2\text{-}\overset{+}{N}(CH_3)_3$$

I

n have on potency. The two series differed, however, in mode of action since the choline derivatives exerted a depolarizing action while the tropine compounds resembled tubocurarine in their behaviour. The side-effects of the members of the two series were also opposite in nature; increasing the value of n in the choline series increased hypertensive properties whereas similar changes in the tropine series increased hypotension.

Competitive action in neuromuscular blocking agents is usually associated with bulky terminal groups. All symmetrical bis-quaternary tropeïnes tested by us have behaved like competitive agents as judged by tests in chicks and on frog muscle. None has been reported by other workers to exert a depolarizing action. The swing from depolarizing towards a competitive action seen on replacing the *N*-methyl group in the piperidine analogue of DF596 (DF616) by a benzyl group (DF628) supports the idea that the bulk of the terminal cationic head is of importance.

From consideration of structure alone it was expected that the tropeïne derivatives would behave like tubocurarine. Comparison of results obtained by other workers for gallamine triethiodide, benzoquinonium, tubocurarine and laudexium suggests that with competitive neuromuscular blocking agents, the equivalent dose for man on a mg./kg. basis might be intermediate between those required for cat and mouse. With such agents the order of decreasing sensitivity is rabbit>cat>man>mouse. In laboratory animals, DF596 conforms to this pattern. The duration of action of DF596 relative to that of suxamethonium varied considerably in different species; it was approximately the same in cats, slightly shorter in dogs, much shorter in rabbits and mice and also somewhat shorter on the human foetal diaphragm. On this last preparation it was much less potent than suxamethonium, and, assuming that the preparation gives a valid estimate of activity in man, the potency of DF596 would be expected to be less than one fifth that of suxamethonium chloride; possibly only a tenth that of tubocurarine chloride.

It is clear that, with compounds of the type investigated here, marked species differences exist not only in potency, as is known to be so with most neuromuscular blocking agents, but also in duration of action. With DF596, the ratio of doses needed to paralyse in the most and least sensitive species examined was actually less than that found for tubocurarine in the same test, but duration of action in different species varied considerably.

The mechanism controlling the duration of action of members of this series was not investigated, but, by analogy with suxamethonium and other dicholine esters of $\alpha\omega$-dicarboxylic acids, it is reasonable to suppose that splitting of the chain by enzymatic hydrolysis may occur. The possibility of other mechanisms being involved, however, is shown by the failure of Hidalgo, Wilken and Seeberg (1959) to demonstrate hydrolysis of relatively short-acting hypotensive agents under conditions effective with acetylcholine. The relatively brief action of members of Series III in rabbits and the lack of gradation in this effect shown with different values of n leads to the suspicion that esterase activity at the chain is not the sole factor governing duration. DF41, which has only terminal ester links, was long acting in the cat but relatively short acting in rabbits. It is possible that the esterases capable of attacking terminal ester groups in addition to those effective in the chain play a part in the breakdown of these tropine esters; in rabbits this is quite likely

since the presence of an esterase in serum capable of splitting atropine and homatropine is well known (Ambache, 1955).

The authors wish to thank Miss C. Mackay, Miss I. Palmer and Mr. J. Findlay for technical assistance and members of the staff of the Simpson Memorial Maternity Pavilion, Royal Infirmary, Edinburgh, for help in connexion with the foetal diaphragm experiments.

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