just before the experiment, with control experiments using only saline injections at a similar time of day twice a week. All drug-treatments were replicated and the data represent the average of two experiments.

TABLE 1. ACTION OF IMIPRAMINE AND AMPHETAMINE ON AVOIDANCE LATENCIES IN AUTOMATIC POLE-CLIMB RESPONSE

	Average group avoidance (sec) for numbers of trials after injection									
	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100
Controls (16 days)	5·3	5·2	5·4	5·7	5·3	5·5	5·4	5·4	5·3	5·3
Imipramine (10 mg/kg) ..	4·8	4·7	5·5	5·3	5·6	5·9	6·0	5·3	5·8	5·9
Amphetamine (0·25 mg/kg)	4·8	3·9*	4·4	5·0	5·4	5·5	5·7	5·4	5·0	5·3
Amphetamine (1 mg/kg) ..	3·3*	2·2*	2·2*	2·4*	3·0*	3·1*	3·3*	3·6*	3·8*	4·0*
Imipramine (10 mg/kg) + amphetamine (0·25 mg/kg)	3·5*	1·6*	1·8*	2·5*	2·8*	3·5*	3·5*	3·6*	3·5*	3·8*

* $P < 0.05$ from controls.

Results, as summarized in the Table, indicate that 1 mg/kg of amphetamine significantly facilitated avoidance response latency and 10 mg/kg of imipramine, a dose which was without effect in this test, potentiated the effects of 0·25 mg/kg of amphetamine into a response obtained when 1 mg/kg of amphetamine was given alone. The method seems suitable for screening imipramine-like agents.

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Non-depolarising neuromuscular blockade by 3α,17α-bis(quaternary ammonium) 5α-androstanes

SIR,—Although many molecular features are known to influence neuromuscular blocking activity and plausible alternative explanations exist (Loewe & Harvey, 1952; Cavallito & Gray, 1960; Waser, 1959, 1962), the potent neuromuscular blocking activity characteristic of certain bisquaternary salts is often envisaged in terms of a "two-point" attachment in which the cationic heads interact simultaneously with separate anionic sites of the acetylcholine receptors on the post-synaptic membrane as first proposed by Barlow & Ing (1948a, b) and Paton & Zaimis (1948, 1949).

Deductions concerning the spatial separation of these anionic sites have been made (Barlow, 1960 and refs cited) in terms of the interonium distance of the decamethonium molecule, since this compound in several species exhibits the highest potency of the homologous polymethylenebis(trimethylammonium) salts, in the belief that maximal potency is a direct reflection of ability to span exactly the gap between two of the sites which are considered to lie in a regular lattice. It was assumed that the decamethonium molecule underwent interaction

in the fully staggered conformation (interonium distance *ca.* 14Å). But doubts about the validity of this assumption arise from conductimetric studies with polymethylene bisquaternary salts (Brody & Fuoss, 1956; Rice, 1956, 1958; Elworthy, 1963, 1964), which indicate that the extended conformation is not favoured in aqueous or ethanolic solution, and from the high potencies present in a number of bisquaternary salts not capable of an interonium distance as large as 14Å (Bovet & others, 1946, 1947; Lüttringhaus & others, 1957; Haining & Johnston, 1962), including the fully rigid toxiferine I (Craig, 1955) with an interonium distance of *ca.* 9·7Å.

In an attempt to reduce the ambiguities inherent in studies employing freely flexible molecules, we examined (Alauddin, 1962) the steroid nucleus as a supporting moiety upon which to append two quaternary ammonium groups in different spatial arrangements. The compounds so prepared were then to be used for an investigation of the importance of interonium distance and cationic head size in neuromuscular blocking activity. Our assumption that such compounds would possess a suitable hydrophilic to lipophilic balance—known to be of importance in neuromuscular blocking activity (Cavallito & Gray, 1960)—was subsequently borne out by the observation that the steroidal alkaloid malouetine possessed activity similar to that shown by (+)-tubocurarine (Janot & others, 1960).

Initially we aimed to synthesise and study pharmacologically a series of 3α, 17α-bis(quaternary ammonium) 5α-androstanes since inspection of models showed the interonium distance in these compounds to fall within the apparently favourable range of 9·2–10·6Å (according to the conformation adopted by ring A), and since the α-configuration of the quaternary ammonium functions was expected to eliminate possible steric hindrance to interaction with the receptors by the β-methyl groups on C-10 and C-13. Recent publications from another laboratory (Biggs, Davis & Wien, 1964; May & Baker, 1963), describing compounds closely related to some prepared by us, has prompted us to report on our work. The relevant compounds studied by us were 3α, 17α-bisdimethylamino-5α-androstane di(methiodide) (I), 3α, 17α-bisethylmethylamino-5α-androstane di(methiodide) (II), 3α,17α-bisdiethylamino-5α-androstane di(methiodide) (III) and 3α,17α-bisdiethylamino-5α-androstane di(ethiodide) (IV), all of which were prepared from 5α-androstane-3,17-dione by reduction to the corresponding 3β,17β-diol, S_N2 displacement of the derived dimethanesulphonate ester by azide ion (*cf.* Henbest & Jackson, 1962), reduction of the resulting 3α,17α-diazido-5α-androstane to the primary diamine and quaternisation with the appropriate alkyl groups.

Table 1 shows the relative potencies of these compounds as tested on different preparations. On the cat and hen gastrocnemius muscle-sciatic nerve preparations, all four compounds showed a duration of action of approximately one

TABLE 1. NEUROMUSCULAR BLOCKING POTENCY

Preparation	Molar potency (+)-Tubocurarine = 100			
	Compound I	Compound II	Compound III	Compound IV
Cat gastrocnemius muscle/sciatic nerve	26·7	83·7	87·4	45·5
Hen gastrocnemius muscle/sciatic nerve	53·4	111·8	116·5	90·9
Frog rectus abdominis muscle ..	45·7	183·5	497·6	211·9
Rat phrenic nerve/diaphragm ..	5·53	8·04	10·7	11·2

half that of (+)-tubocurarine. All exhibited typical non-depolarising activity as shown by the absence of muscular contracture in the hen, potentiation of the block by ether and (+)-tubocurarine in the cat, failure to support a tetanus in the cat, complete rapid reversal by edrophonium in both the hen and cat and by the results obtained with the frog and rat preparations. This is of interest, as decamethonium shows an initial depolarising block in some species, including the cat, and on replacement of *N*-methyl groups by higher alkyl radicals this is converted into a purely non-depolarising block. Only compound III reduced the height of contraction of the nictitating membrane to pre-ganglionic stimulation of the superior cervical sympathetic nerve in the cat, but this was at a dose of 2 mg/kg which is twenty times that required to produce a 50% neuromuscular block in this animal. Although there was no reduction in blood pressure in the anaesthetised cat, all four compounds had a significant inhibitory action on the Trendelenburg preparation and were equipotent with hexamethonium.

Employing enzyme prepared from rat brain (Fenwick & others, 1957) in a modification of the method of Ammon (1933), all four compounds exhibited weak anticholinesterase activity (pI50 values of 4·11 to 4·73; (+)-tubocurarine, 3·05; eserine, 6·68) suggesting that their shorter duration of action in the cat and hen compared to (+)-tubocurarine could be due to a partial inhibition of acetylcholinesterase.

Table 2 shows the ED50, LD50 and therapeutic index of each compound as determined by intraperitoneal injection into mice using the inclined screen method of Thomson (1946) and calculated by the method of Miller & Tainter (1944). Death in all animals resulted from respiratory paralysis.

TABLE 2. ED50 (±S.E.), LD50 (±S.E.) AND THERAPEUTIC INDEX IN MICE

	Compound I	Compound II	Compound III	Compound IV	(+)-Tubocurarine
ED50 mg/kg ..	4·5 ± 0·36	3·58 ± 0·14	2·72 ± 0·32	5·0 ± 0·5	0·3 ± 0·018
LD50 mg/kg ..	6·1 ±0·38	4·25 ± 0·11	3·82 ± 0·3	7·9 ± 1·52	0·51 ± 0·026
Therapeutic Index LD50/ED50 ..	1·36	1·19	1·41	1·58	1·71

An interesting feature of our compounds is, that unlike the polymethylene bisammonium salts (Elworthy, 1964), the interonium distance can be expected to remain virtually constant as *N*-ethyl groups replace *N*-methyl groups. Thus, the observed increase in potency from the *N*-trimethyl compound to the *N*-diethyl methyl compound and subsequent falling off in the *N*-triethyl compound can probably be directly attributed to the variation of the *N*-alkyl substituents. The high potency present in the stereoisomeric 3β,17β-, 3α,17β- and 3β,17α- compounds related to ours (Biggs, Davis, Maxwell & Wien, personal communication, data to be published; May & Baker, 1963), each series having interonium distances which can be expected to differ from the 3α,17α-series, indicates the relative unimportance of a "fixed" interonium distance in determining neuromuscular blocking activity. This not only emphasises the importance that must be attached to other factors such as hydrophilic to lipophilic balance, transport mechanisms and sites of loss, but is of interest in the light of the work of Gill (1959) on ganglion-blocking activity who concluded that potency should fall over a range of interonium distance due to variability in the receptors

(*cf.* also Koshland, 1958). Moreover, the activity of the 3β,17β-, 3α,17β- and 3β,17α-bisquaternary salts makes it clear that there is no steric impedance to receptor interaction by the angular methyl groups on C-10 and C-13 in these compounds and that, as with the malouetine series (Khuong Huu-Lainé & Pinto-Scognamiglio, 1964), stereoisomerism in steroidal bisquaternary salts appears to have little effect on neuromuscular blocking activity. The presence of activity in 3α,17β- and 3β,17α-bis(quaternary ammonium) 5α-androstanes where the quaternary heads lie on opposite sides of the steroid nucleus might perhaps support the adumbration theory (Loewe & Harvey, 1952; *cf.* also Fakstorp & others, 1957) or the receptor pore theory (Waser, 1959, 1962) rather than the "two-point" attachment theory, but the possibility of an edge-on approach of the steroid molecule to the receptor along the staggered chain of nine carbon atoms at positions 3, 2, 1, 10, 9, 11, 12, 13 and 17 cannot be ruled out.

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Determination and identification of amphetamine in urine

SIR,—We report herein an improved and more convenient method than that reported previously, for the determination and identification of amphetamine in urine (cf. Beckett & Rowland, 1964). The determination is based on a modification of the method of Cartoni & Stefano (1963). Gas-liquid chromatography was used with *NN*-dimethylaniline as the internal standard, while amphetamine was identified and separated from related amines as the acetone derivative (cf. Brochmann-Hanssen & Svendsen, 1962).

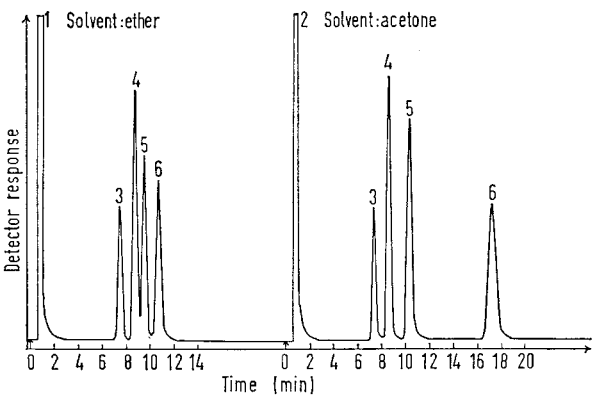

FIG. 1. Chromatograms of amphetamine, methylamphetamine, β-phenylethylamine and *NN*-dimethylaniline in the absence and presence of acetone: **1** ether, **2** acetone, **3** *NN*-dimethylaniline (t_R 7·5 min), **4** methylamphetamine (t_R 8·7 min), **5** amphetamine (t_R 9·5 min in ether, 10·4 min in acetone), **6** β-phenylethylamine (t_R 10·8 min in ether, 17·4 min in acetone).

Procedure. Urine (2-5 ml) was pipetted into a glass-stoppered centrifuge tube, neutralised with dilute hydrochloric acid or sodium hydroxide solution, as appropriate, and then 0·1 ml 5N hydrochloric acid added. The urine was extracted with freshly distilled Analar diethyl ether (3 × 2·5 ml), centrifuged and the ether extract rejected. Sodium hydroxide (0·5 ml, 5N) was added to the urine which was then extracted with ether (3 × 2·5 ml), centrifuging between each extraction. These ethereal extracts were transferred to a 15 ml Quickfit test tube, the base of which was finely tapered. 1 ml of *NN*-dimethylaniline solution in ether (5 μg base/ml) was added and the solution then concentrated on a water bath at 40° to about 50 μl. Approximately 3–5 μl of the concentrate was injected for analysis into the chromatograph. A calibration curve was obtained by measuring the ratio of peak heights of amphetamine to *NN*-dimethylaniline for known concentrations of amphetamine in urine. The curve was found to be linear over the range 0·1–10 μg amphetamine per ml of urine and the method had