NEUROMUSCULAR BLOCKING AGENTS

PART III. SOME LINEAR *NNNN*-TETRA-ETHONIUM COMPOUNDS

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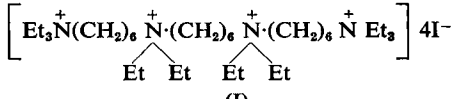
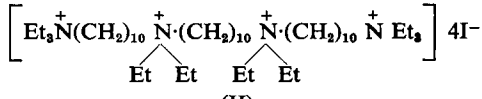
IN extension of our observation of neuromuscular blocking activity in tris-onium compounds reported in Part I[1] and Part II[2] we have now prepared the linear tetra-azonium compounds, 7:7:14:14-tetraethyl-7:14-diazoniaeicosylenebis(triethylammonium) tetraiodide (Trihexatetrazonium tetraethiodide; THAE; I), m.p. 248–248·5° (Found: N, 4·9; I, 45·9 per cent. $C_{38}H_{86}N_4I_4$ requires N, 5·1; I, 45·9 per cent) and 11 : 11 : 22 : 22 - tetraethyl - 11 : 22 - diazoniadotriacontylenebis(triethylammonium) tetraiodide (Tridecatetrazonium tetraethiodide; TDAE; II), m.p. 186–187° (Found: C, 46·8; H, 8·5; I, 39·4 per cent. $C_{50}H_{110}I_4N_4$ requires C, 47·1; H, 8·7; I, 39·8 per cent).



(I)



(II)

The experimental methods and materials have been described in detail in our previous publications[1,2]. The neuromuscular blocking actions of I were similar to those of tubocurarine (TC). For example, I (0·03 to 0·06 mg./kg.) caused a reduction of twitch amplitude of the gastrocnemius muscle of the cat excited indirectly *via* the sciatic nerve. There was no initial potentiation of twitch height and no muscular twitching or fasiculation. On the cat, I was about three times as potent as TC and its neuromuscular blocking activity was antagonised by edrophonium (0·5 to 1·0 mg./kg.), neostigmine (0·6 to 0·1 mg./kg.), eserine (0·5 to 1·0 mg./kg.), adrenaline (0·05 to 0·1 mg./kg.), potassium chloride (20 mg./kg.) and decamethonium (C 10) (0·02 to 0·6 mg./kg.). The effects of I were additive with those of TC and were potentiated by ether. The muscle partially blockaded by I (0·02 mg./kg.) was unable to maintain an indirect tetanus but when the muscle had become unresponsive to indirect stimulation, direct stimulation caused a contraction.

The properties of II were quite different from those of I and TC. It was noticed that 0·2 to 0·4 mg./kg. of II caused marked muscular twitching; the movements were at times almost convulsive with apparent

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involvement of all of the skeletal muscles. Twitch amplitude was reduced but maximum depression of twitch height was not obtained until 10 to 20 minutes after the dose had been given. The effects were prolonged and the response after one dose of 0·2 mg./kg. did not return to control levels for about 1 hour. A second and similar dose caused a more prolonged depression and recovery took from 2 to 3 hours. The neuromuscular blocking actions of II were not antagonised by eserine (1 mg./kg.) or neostigmine (0·1 mg./kg.) whilst 0·6 to 1·0 mg./kg. of edrophonium potentiated it. The effects of II were additive with those of C 10, whilst TC antagonised its actions. When the muscle had become unresponsive to indirect stimulation direct stimulation caused only a very small response. The response to indirect tetanisation of the muscle was fairly well maintained but not comparable with the response of the untreated or C 10-treated preparation (Fig. 1).

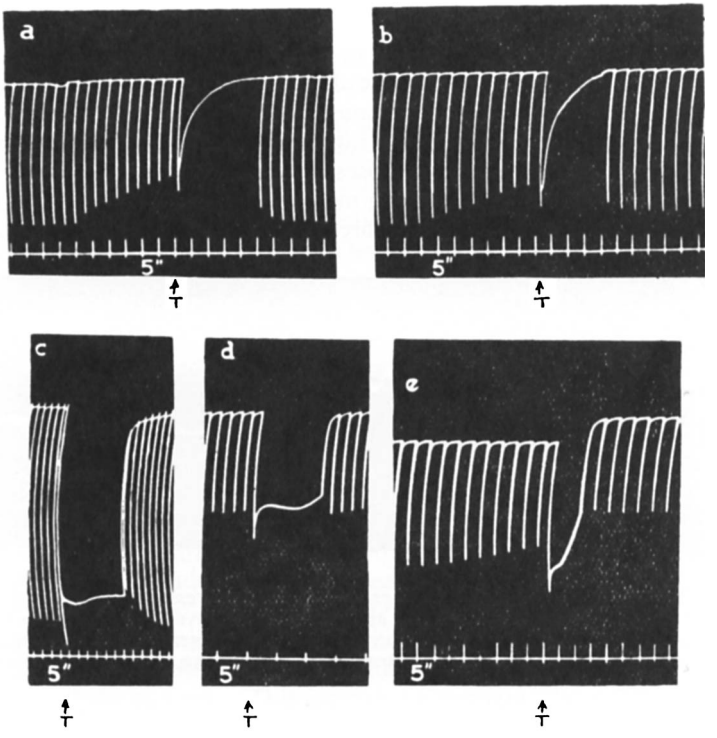

FIG. 1. Cat. Gastrocnemius-sciatic preparation. Pentobarbitone anaesthesia. Contraction downwards. Drugs administered intravenously. At T, indirect tetanisation of the gastrocnemius *via* the sciatic nerve during partial block by (*a*) tubocurarine, (*b*) trishexatetrazonium, (*d*) decamethonium, (*e*) trisdecatetrazonium, and (*c*) normal muscle.

The head drop doses of I and II both before and after treatment with neostigmine have been obtained and compared with TC. The figures are shown in Table I and indicate that I probably acts in a similar manner to

TC, whilst II has a different mechanism of action and appears to resemble C 10.

TABLE I

A COMPARISON OF THE HEAD DROP DOSES OF TRIHEXATETRAZONIUM (I) AND TRIDECATETRAZONIUM (II) WITH TUBOCURARINE IN THE RABBIT BEFORE AND AFTER TREATMENT WITH NEOSTIGMINE

Compound	Mean head drop dose ± s.e. (mg./kg.)		Potency	Ratio: neostigmine treated/control
	Control	After neostigmine (0·10 mg./kg.)		
Trihexatetrazonium tetra-ethiodide ..	0·19 ± 0·014	0·31 ± 0·014	58	1·63
Tridecatetrazonium tetra-ethiodide ..	0·40 ± 0·024	0·33 ± 0·024	28	(P = 0·01) 0·82
Tubocurarine	0·11 ± 0·01	0·30 ± 0·01	100	2·72 (P = 0·01)

I (1·0 to 2·0 mg./kg.) was found to have no significant effect upon the arterial blood pressure level of anaesthetised cats, but II (1·0 to 2·0 mg./kg.) caused a moderate, fairly prolonged fall and its effects were similar to those usually seen when TC (1·0 mg./kg.) is used (Fig. 2). In contrast to TC (0·5 mg./kg.), I and II in doses of up to 2·0 mg./kg. had no effect upon the response of the nictitating membrane of the anaesthetised cat to stimulation of the preganglionic fibres of the cervical sympathetic.

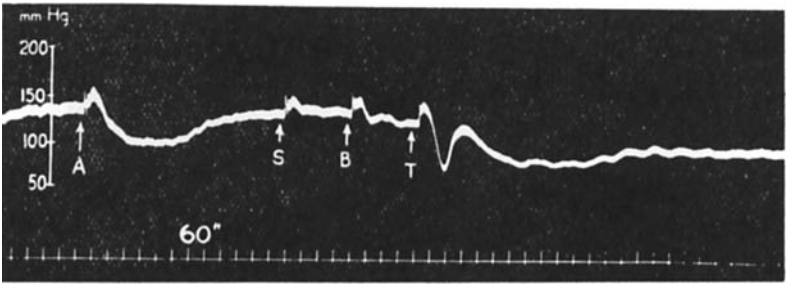

FIG. 2. Cat. Pentobarbitone anaesthesia. Blood pressure record from the common carotid artery. Drugs administered intravenously and in each instance followed by 4 ml. saline. At A, trisdecatetrazonium 1·0 mg./kg. At S, saline 4 ml. At B, trishexatetrazonium 1·0 mg./kg. At T, tubocurarine 1·0 mg./kg.

Our experiments show that the properties of the tetraethonium compounds I and II are similar to those of the corresponding tris-ethonium compounds which we have investigated[1,2], in that inter-onium group chain length is a major factor in determining the type of neuromuscular blocking activity. Thus, when the quaternary centres are separated by polymethylene chains containing five or six units[1,2], activity is virtually purely TC-like. Increase of the inter-quaternary chain length to eight methylene units[2] leads to activity which is predominantly TC-like, but some C 10-like activity appears, whilst compounds with polymethylene chains of ten

units show activity which closely resembles that of C 10. It is of interest that these linear-*NNNN*-compounds are more potent than the corresponding *NNN*- and *NSN*-derivatives, and we are investigating the effect of increasing the number of quaternary centres still further.

REFERENCES

1. Edwards, Lewis, Stenlake and Zoha, *J. Pharm. Pharmacol.*, 1957, **9**, 1004.
2. Edwards, Lewis, Stenlake and Zoha, *ibid.* 1958, **10**, Supplement, *106 T*

DISCUSSION

The paper and short communication were presented by MR. J. J. LEWIS.

THE CHAIRMAN. Had the compounds anticholinesterase activity?

DR. G. F. SOMERS (Liverpool). The use of a mixed muscle, the gastrocnemius, was open to criticism; the tibialis and soleus of the cat, or avian muscle, would have been useful in distinguishing depolarising action from competitive block.

DR. F. HARTLEY (London). Had differences been found according to the concentration use? If potency increased as the chain lengthened, the effect was to double the concentration with the di-onium derivatives. What was the effect of asymmetry in the tris compounds?

DR. A. H. BECKETT (London). The stereochemistry of the compounds should be considered. With a branched chain in the vicinity of the nitrogen the isomers could be examined critically. There was little information about the spatial arrangements of isomers with different biological action. Was work being done on this? If a desired biological effect was present, instead of large molecular changes, there should be a three-dimensional approach, with small molecular changes.

MR. LEWIS replied. In neuromuscular blocking agents there was great species variation in mode and type of action. Other cat muscles had been used, and results on avian muscle were in line with those reported, but the cat was a quantitative rather than qualitative preparation. The type of action was independent of the concentration. With a chain length of eight the action was still predominantly tubocurare-like; only in toxicity was there a close relationship to decamethonium. Although *NNN* with six carbons between the nitrogen was like two hexamethonium molecules it did not behave as such. This indicated a fundamental difference in the type of action between ganglionic and neuromuscular blocking agents. They intended to interest themselves in the more fundamental inter-onium chain lengths before investigating the stereochemical problem. The action of rigid molecules with limited steric changes or rotation was of considerable interest. The *NSN* and *NNN* compounds, dihexasulphonium and dihexazonium, which were equi-potent and had the same activity in the cat and other animals, had considerable differences in man. The change of S to N made a great difference in potency in man. Anticholinesterase activity had not been directly tested. Six different species

of animals had been used, and there was variation. The tris $N(CH_2)_6$ compound was equipotent with gallamine in man but with tubocurare in cat. The analogue with a middle sulphur although equipotent with tubocurare in the cat had only $\frac{1}{6}$ to $\frac{1}{8}$ the potency of gallamine in man.

DR. STENLAKE replied. No work had been done on asymmetric compounds in the tris series, but in the tetra-onium series, compounds had been prepared where the inter-onium units were 6 : 10 : 6 and 10 : 6 : 10. The former was curare-like and the latter predominantly depolarising.