Brit. J. Pharmacol. (1951), **6**, 611.

SOME ACTIONS OF HEXAMETHONIUM AND CERTAIN HOMOLOGUES

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(Received June 1, 1951)

A great deal of attention is being devoted to the clinical potentialities of the alkamethonium compounds (Paton and Zaimis, 1951 ; Barlow and Ing, 1948), in particular hexamethonium and pentamethonium, on account of their ability to paralyse transmission in autonomic ganglia. Although tetraethylammonium had previously been studied over many years (see review by Moe and Freyburger, 1950), its application in therapeutics has been limited because of its relatively weak action and short duration. Hexamethonium has shown greater possibilities in hypertension, and the work of Kay and Smith (1950) pointed out the possible value of this drug in the treatment of peptic ulcer ; this work prompted some of our initial investigations on gastric motility and secretion. One intriguing aspect was the fact that certain ethyl homologues (Chou and de Elio, 1947) displayed marked differences in ganglionic-blocking potency, which led us to examine the effects of successive replacement in hexamethonium of one or more methyl groups by ethyl groups on each of the two quaternary nitrogen atoms.

METHODS

The methods are described under two headings: (*a*) main actions, and (*b*) gastric motility and secretion.

Main actions.—The effects on sympathetic ganglia were determined on the superior cervical ganglion ; the sustained contractions of the nictitating membrane of the cat under chloralose were recorded, preganglionic stimulation of the cervical sympathetic with maximal square wave stimuli at a frequency of 20 per sec. and a pulse length of 0.1 to 1.0 msec. being used. Effects on parasympathetic ganglia were assessed (1) on the peristaltic reflex of the guinea-pig ileum (Feldberg and Lin, 1949), and (2) by determining the intravenous dose in cats under chloralose required to reduce by about 50 per cent the fall of blood pressure on vagal stimulation, either a faradization current or square wave stimuli being used. Experiments on salivary flow were performed in cats under chloralose (Bülbring and Dawes, 1945). Actions on the cardiovascular system were studied (1) by determining the coronary outflow in the heparinized dog under chloralose with a Morawitz cannula introduced into the coronary sinus, any change in the blood pressure being prevented by a counterbalancing pressure in a reservoir of large volume (50 litres) attached to the femoral artery (Krayer and Verney, 1936) ; (2) on the isolated cat's heart (Langendorff's method), the outflow being recorded by Thorp's impulse counter ; (3) on the vessels of the hind limb of the dog perfused with heparinized blood by a Dale-Schuster pump. Neuromuscular paralysis was examined by means of the phrenic nerve-diaphragm preparation of the rabbit (Wien, 1948) ; by the rabbit head-drop method ; and by the sciatic nerve-gastrocnemius muscle preparation in cats under chloralose (Bülbring and Burn, 1942).

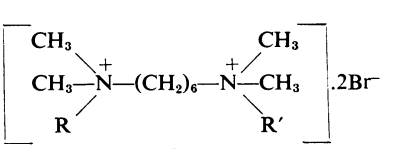
Gastric motility and secretion.—Cats, anaesthetized with chloralose or decerebrate, and rabbits anaesthetized with urethane served to examine the effects of hexamethonium on gastric motility. A rubber balloon filled with water was placed inside the stomach through an incision in the upper end of the duodenum, a levelling bulb was attached, and the pressure within the balloon adjusted to between 8 and 15 cm. of water ; a record of the movements was obtained with a piston volume recorder. Stimulation of the left peripheral vagus with a faradization current afforded a means of observing effects on hypermotility.

Dogs were anaesthetized either with chloralose or with a mixture of chloralose-urethane (1 : 10), and prepared as described by Babkin (1950) for gastric secretion experiments. The duodenum was ligated at the pylorus ; a rubber tube with a non-corrodable metal flange was tied into an opening made in the cardiac end of the stomach on the greater curvature and brought outside the abdominal wall by a stab wound, the fistula being so placed that good drainage resulted. The right vago-sympathetic trunk was stimulated by bursts of induction shocks at a frequency of 20 per second, interrupted 15 times a minute with a Lewis rotary contact. The peripheral end of the nerve trunk was drawn into a fluid electrode consisting of a piece of glass tubing, 2 to 3 mm. bore, into which were inserted two silver plates, and through which passed a slow stream of Ringer's solution ; the nerve could then be stimulated for long periods without fatigue. The blood pressure was recorded from the femoral artery ; the volume of gastric juice was measured every 15 minutes ; free and total acidity were determined by titration using thymol blue as indicator ; and peptic activity was estimated by Hunt's method (1948). In some experiments gastric juice of high acidity but low peptic power was obtained by infusing histamine intravenously at a constant rate of 2.5 μg./kg./min. The compounds examined were prepared by Barber and Gaimster (1951). All injections were intravenous unless otherwise stated.

RESULTS

Hexane-1 : 6-bis-trialkylammonium compounds (homologues of hexamethonium)

Higher homologues of hexamethonium were examined, in which R and R′ in the formula I were ethyl, *n*-propyl, *iso*propyl, *n*-butyl, *iso*butyl, or allyl.



(I)

The results given in Table I show that all the higher homologues were considerably less potent in blocking transmission in autonomic ganglia with the remarkable exception of the bis-ethyldimethylammonium compound which possessed greater activity than hexamethonium on both sympathetic and parasympathetic ganglia (Figs. 1 and 2). We found that reproducible results were easier to obtain with guinea-pig than with rabbit intestine for peristaltic reflex experiments. Even so, the results of an assay might vary during the course of an experiment ; in one experiment the bis-ethyldimethylammonium homologue was initially about twice as potent as hexamethonium, whereas later it was more than four times as potent. An evaluation based on the mean of several experiments showed the homologue to be about twice as potent as hexamethonium.

TABLE I

HOMOLOGUES OF HEXAMETHONIUM

$$R(CH_3)_2\overset{+}{N}—(CH_2)_6—\overset{+}{N}(CH_3)_2R'\}2Br^-$$

Substituent groups R = R′	Relative toxicity (mice) i.v.	Relative potency (paralysing ganglionic transmission)	
		Sympathetic (cat's superior cervical)	Parasympathetic (guinea-pig ileum)
Methyl (hexamethonium)	1.0	100	100
Ethyl	2.3	150	200
n-Propyl	2.9	<5	<5
*iso*Propyl	2.7	15	10
n-Butyl	6.0	<4	<2
*iso*Butyl	2.5	<4	4
Allyl	2.6	4	4

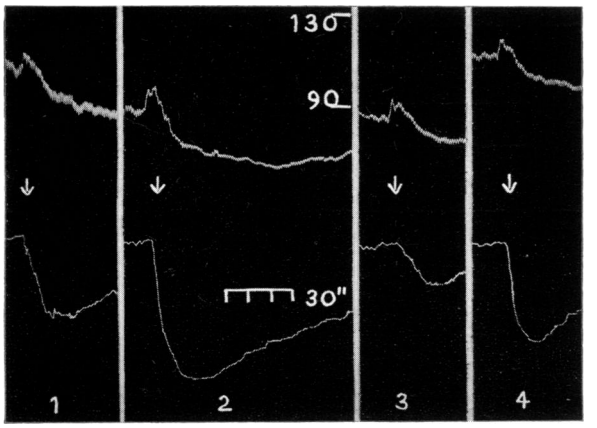


FIG. 1.—Cat, chloralose. Upper record, blood pressure. Lower record, contraction of nictitating membrane on preganglionic stimulation of cervical sympathetic at 20 shocks/sec. Compounds injected intravenously. (1) 0.4 mg., (2) 0.8 mg. bisethyldimethylammonium homologue of hexamethonium bromide, (3) 0.4 mg., (4) 0.8 mg. hexamethonium bromide. The former compound was about 1½ times as potent as hexamethonium.

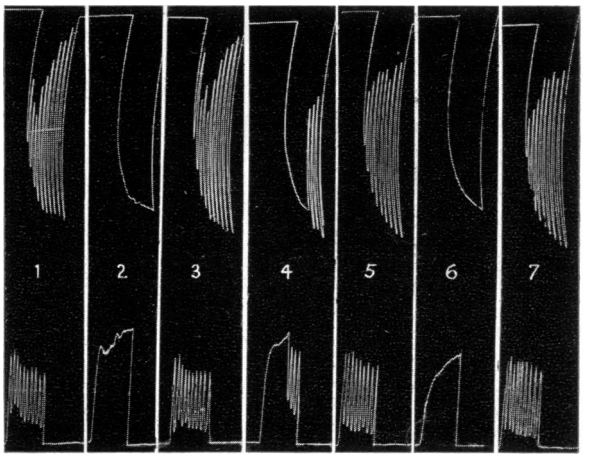


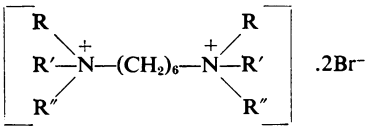
FIG. 2.—Guinea-pig ileum. Circular (upper record) and longitudinal (lower record) movements on stimulation by rise of intra-intestinal pressure of 2.5 cm. of water. (1), (3), (5), and (7) control responses; (2) 50 μg. bisethyldimethylammonium homologue, (4) 200 μg., (6) 300 μg. hexamethonium added to the bath.

The relative toxicity figures are given in terms of the LD50 determined intravenously in mice ; the average lethal doses of hexamethonium dibromide and the bis-ethyldimethylammonium homologue were 0.05 mg./g. and 0.022 mg./g. respectively. The latter compound was more toxic subcutaneously (3.2 times) and orally (1.7 times) as well as intravenously (2.3 times). The cause of death was respiratory failure, and may have been due to a central or neuromuscular-paralysing action rather than to an excessive fall of blood pressure caused by the removal of autonomic vascular control.

The effect of further replacement in hexamethonium of methyl by ethyl substituents was then investigated (Table II).

TABLE II

THE SUCCESSIVE REPLACEMENT OF METHYL BY ETHYL GROUPS IN HEXAMETHONIUM



Substituent groups			Relative toxicity (mice) i.v.	Relative potency (paralysing ganglionic transmission)		
				Sympathetic	Parasympathetic	
R	R′	R″		(Cat's superior cervical)	(Guinea-pig ileum)	(Cat's blood pressure)
Me (hexamethonium)	Me	Me	1.0	100	100	100
Et	Me	Me	2.3	150	200	200
Et	Et	Me	4.0	75	100	100
Et	Et	Et	20.0	<5	<50	<50 (paralysis of respiration)

Similar results were obtained by the substitution of methyl by ethyl groups in pentamethonium ; maximum and minimum potencies for paralysis of transmission in autonomic ganglia were found in the bis-ethyldimethylammonium and bis-triethylammonium homologues respectively. The low ganglionic blocking activity of the bis-triethylammonium homologues is in accord with the findings of Chou and de Elio (1947), who, however, used short periods of stimulation instead of sustained stimulation of the cervical sympathetic in their experiments on the perfused ganglion. In addition, we observed that the bis-triethylammonium homologue of hexamethonium had a much greater effect than the parent drug in paralysing neuromuscular transmission (see Figs. 9 and 10). The implication of these findings is discussed later on.

Mode of action

The increased potency of the bis-ethyldimethylammonium homologue was achieved without any significant alteration in its type of action. Thus on the isolated guinea-pig ileum it prevented the contractions produced by nicotine without modify-

ing those due to acetylcholine, histamine, or pilocarpine (Fig. 3), and in the cat post-ganglionic stimulation of the cervical sympathetic was unaffected during a paralysis of pre-ganglionic stimulation (Fig. 4). It may also be mentioned that in the dog (chloralose) the pressor action of nicotine was blocked and that of adrenaline slightly enhanced by both hexamethonium and the homologue.

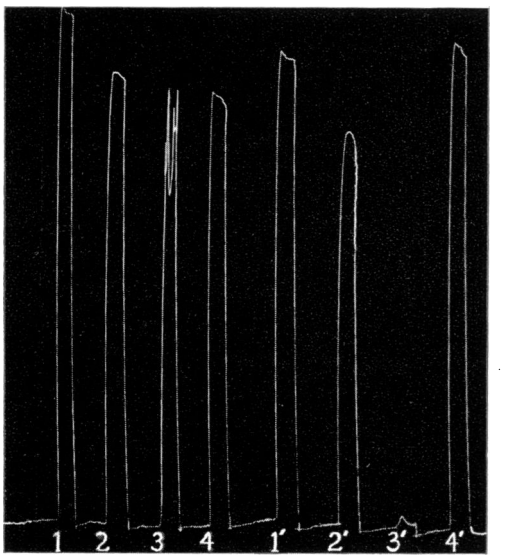


FIG. 3.

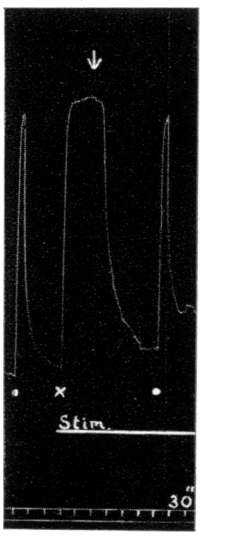


FIG. 4.

FIG. 3.—Guinea-pig ileum. Normal control contractions with (1) 0.4 μg. acetylcholine, (2) 20 μg. pilocarpine, (3) 100 μg. nicotine, (4) 3 μg. histamine; and 1′ to 4′, contractions with same drugs respectively but after the previous addition to the bath on each occasion of 1 mg. bis-ethyl-dimethylammonium homologue of hexamethonium.

FIG. 4.—Cat, chloralose. Contraction of nictitating membrane. At white dot postganglionic stimulation of cervical sympathetic for 5 sec.; at × sustained preganglionic stimulation. Intravenous injection of 0.5 mg. of bis-ethyldimethylammonium homologue of hexamethonium at arrow paralyses preganglionic stimulation, but leaves postganglionic stimulation unaffected.

Action on salivary secretion

Though hexamethonium has a specific paralysing effect on autonomic ganglionic transmission, differing in its mode of action from atropine, evidence has been found of a very weak inhibitory action on salivary secretion evoked by intravenous infusion of carbamylcholine in the cat. A good effect is shown in Fig. 5, No. 1, where it was several thousand times weaker than atropine ; even this response was not consistently obtained, since in some experiments there was very little inhibitory action (Fig. 5, No. 2). Tetraethylammonium had a transient action which was more consistent and about four times greater than that of hexamethonium (Fig. 5, No. 3).

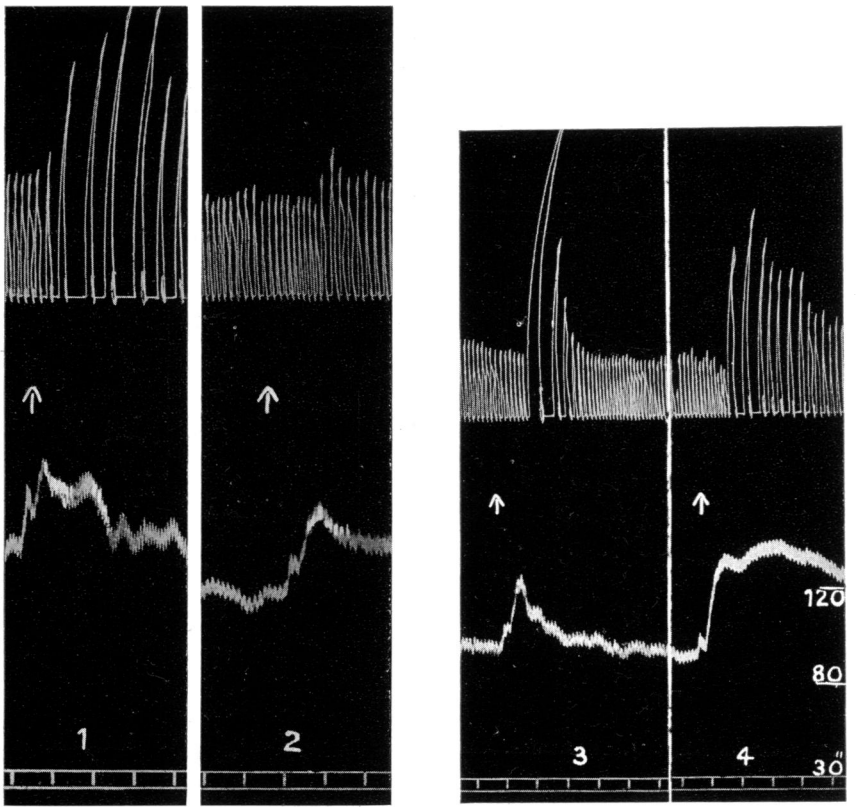


FIG. 5.—Cat, chloralose. Upper record, salivary flow from Wharton's duct on intravenous infusion of carbamylcholine. Lower record, blood pressure. Intravenous injections of (1) 8 mg., (2) 4 mg. hexamethonium bromide, (3) 2 mg. tetraethylammonium bromide, (4) 3 μg. atropine sulphate.

Mydriatic effects

Tetraethylammonium is known to cause mydriasis (Heymans and Hoorens, 1949), and one of the side-effects first noted clinically with hexamethonium was blurring of vision. In view of this we examined the possible mydriatic activity of hexamethonium by the method used by Ing, Dawes, and Wajda (1945), and the results are shown in Table III. Since atropine produced a maximum effect within 20 minutes, and hexamethonium produced its maximum response within 10 minutes, the dose-action curves were plotted with the figures for maximal effects (Fig. 6). Although a linear log dose-response relationship is obtained with atropine, the curve for hexamethonium was non-linear (by analysis of covariance), perhaps on account of the different way in which it exerts its mydriatic action. In another experiment the pupil sizes after 10 minutes were 12.3, 15.3, 18.8, 29.2, and 39.8 with doses of 2.9, 4.4, 6.7, 10.0, and 15.0 μg /g. hexamethonium respectively. A comparison between hexamethonium and tetraethylammonium could not justifiably be made, since the latter compound had a much flatter slope, and with the highest dose, which approximated to the toxic level, the effect was submaximal ; this result might have been due to the stimulant nicotine action of the drug.

TABLE III

THE MYDRIATIC EFFECT OF HEXAMETHONIUM IN MICE

(10 mice on each dose level; compounds injected intraperitoneally)

Compound	Dose μg./g.	Size of pupil (arbitrary divisions of microscope scale)			
		0	10 minutes after injection	20	30
Hexamethonium bromide..	5.0	10.7	12.0	10.1	7.0
	10.0	8.3	23.8	18.7	12.2
	20.0	9.0	28.2	28.1	16.8
	40.0	9.1	52.1	44.0	36.5
Atropine sulphate	0.1	10.5	14.4	16.4	17.7
	0.2	8.5	20.8	31.9	28.7
	0.4	8.7	52.2	45.2	43.3
Tetraethylammonium bromide	20.0	12.3	18.1	15.8	10.7
	40.0	11.4	21.1	12.6	10.1

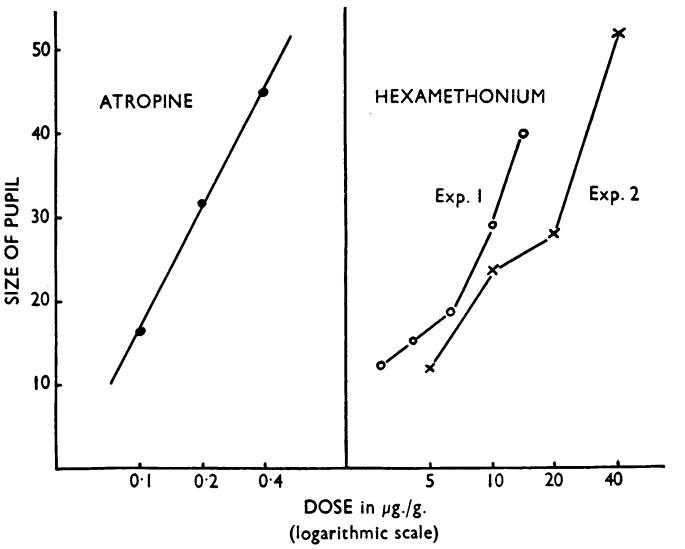


FIG. 6.—Mydriatic action of hexamethonium in mice. Log. dose-response curves for atropine sulphate and hexamethonium bromide (two separate experiments).

Action on the vagus

Hexamethonium reduced or inhibited the fall of blood pressure on stimulation of the right vagus peripherally in the cat, but the fall of blood pressure and slowing of the heart caused by acetylcholine were unaffected. In this way it differed from tetraethylammonium, which did inhibit the bradycardia but not the fall in blood pressure due to acetylcholine.

The bis-ethyldimethylammonium homologue of hexamethonium had an action like that of hexamethonium itself (Fig. 7); it abolished the fall of blood pressure on

vagal stimulation, but had no effect on the bradycardia or fall of pressure due to acetylcholine. The intravenous dose for a 50 per cent reduction in the fall of blood pressure was 1 to 2 mg./kg. for hexamethonium and 0.5 to 1 mg./kg. for the homologue. A vagal block with either compound was demonstrable also in the dog anaesthetized with chloralose-urethane (Fig. 8).

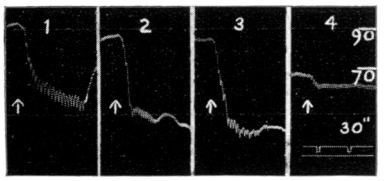


FIG. 7.

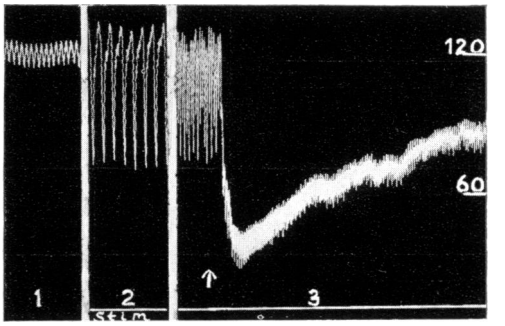


FIG. 8.

FIG. 7.—Cat, chloralose. Record of blood pressure. At (1) and (4) stimulation of peripheral end of right vagus at 20 shocks/sec. for 10 sec. At (2) and (3) intravenous injection of 40 μg. acetylcholine. Between (2) and (3) 0.5 mg./kg. bis-ethyldimethylammonium homologue of hexamethonium.

FIG. 8.—Dog, chloralose-urethane. Record of blood pressure from femoral artery. (1) Before stimulation of vago-sympathetic trunk; (2) and (3) during faradic stimulation of vago-sympathetic trunk, secondary coil at 3 cm.; (3) intravenous injection of 0.5 mg./kg. hexamethonium bromide.

Neuromuscular paralysis

Rabbit phrenic nerve-diaphragm.—The relative potencies for hexamethonium, the bis-ethyldimethyl, bis-diethylmethyl, and bis-triethylammonium compounds were 100:150:200:1600 respectively (Fig. 9). Compared with potent neuromuscular blocking compounds a large dose of hexamethonium is required to produce neuromuscular paralysis, and it is practically free from this action when exerting its main effect—paralysis of autonomic ganglia. The bis-triethylammonium homologue, on the other hand, has appreciable neuromuscular blocking properties.

Rabbit head-drop.—The rabbit head-drop method was used to confirm the results obtained on the isolated nerve-muscle preparation, and the approximate doses required on intravenous injection were 26 mg./kg. for hexamethonium, 21 mg./kg. for the bis-ethyldimethylammonium homologue, 6 mg./kg. for the bis-diethylmethylammonium, and 1 mg./kg. for the bis-triethylammonium compounds. The relative potencies were therefore 100:124:433:2600, which are similar to the values found on the nerve-muscle preparation.

Cat sciatic-gastrocnemius.—These experiments provided additional evidence of the neuromuscular-blocking action of the bis-triethylammonium compound in contrast to the lack of such an effect with either hexamethonium or the bis-ethyldimethylammonium homologue (Fig. 10).

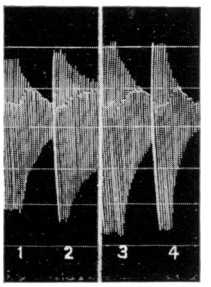


FIG. 9.

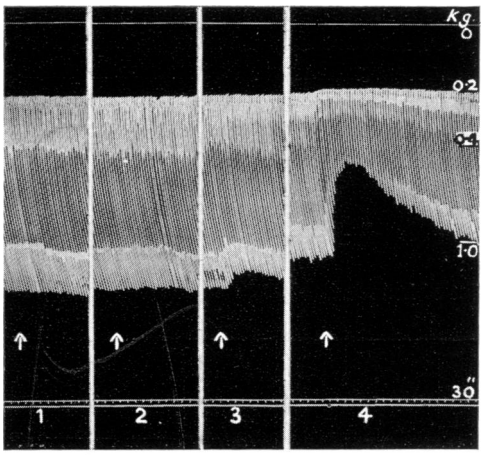


FIG. 10.

FIG. 9.—Rabbit phrenic nerve-diaphragm preparation at 37° C. Record of muscle contractions on maximal stimulation of nerve with 6 shocks/min. (1) and (3) 50 mg. hexamethonium bromide, (2) and (4) 2 and 4 mg. bis-triethylammonium homologue. The bis-triethylammonium homologue was 16 times as potent as hexamethonium in producing neuromuscular block.

FIG. 10.—Cat, chloralose. Record of twitch tensions of gastrocnemius on supramaximal stimulation of sciatic nerve with square wave stimuli 6/min. and 1 m.sec. duration. Intravenous injection of (1) 25 mg. hexamethonium bromide, (2) 25 mg. bis-ethyldimethylammonium homologue, (3) 5 mg. and (4) 10 mg. bis-triethylammonium homologue.

Action on the heart

Isolated cat's heart.—The cat's heart was used, perfused with oxygenated Locke's solution at 37° C., in which the effect of adrenaline was normally dilator on the coronary vessels. Hexamethonium iodide, in a dose of 1 mg. injected into the side-arm leading to the perfused aorta, had no effect on the beat or outflow (Fig. 11, No. 1). Only when the amount was increased to 20 or 40 mg. was an effect produced,

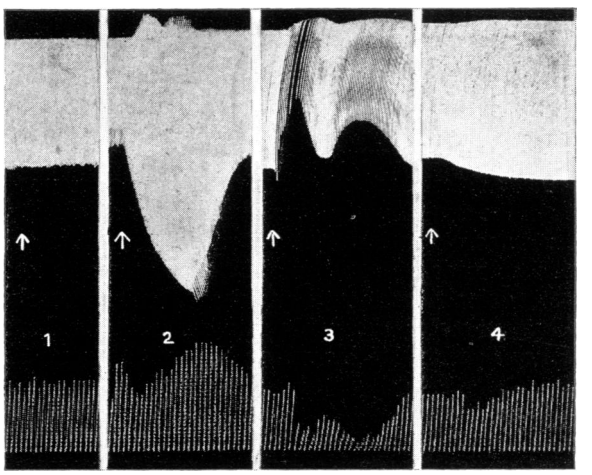


FIG. 11.—Isolated cat's heart, perfused with Locke's solution at 37° C. Upper record, movements of heart; lower record, coronary outflow—an increase in outflow upwards. (1) 1 mg. and (4) 20 mg. hexamethonium iodide, (2) 0.2 μg. adrenaline, (3) 4 μg. acetylcholine.

resulting in an increase in the amplitude and a transient constriction, followed by a dilatation, of the coronary outflow (Fig. 11, No. 4); these concentrations are far in excess of those ever likely to be attained in practice.

Coronary outflow in dog.—Changes in the coronary outflow were observed in the dog (chloralose) by the introduction of a Morawitz cannula, the arterial pressure being maintained constant; the volume of blood flowing from the sinus was measured as required and returned to the animal by the femoral vein. The compounds were given intravenously, and the results are shown in Table IV: the figures

TABLE IV

The effect of hexamethonium bromide (A) and of the bis-ethyldimethylammonium homologue (B) on the coronary flow in dogs

Exp.	Dog kg.	Arterial pressure mm. Hg	Drug	Dose mg./kg.	Coronary outflow* (ml./min.)			Remarks
					Initial	3 min.	10 min.	
1	14	110	A	0.25	76	124	88	Adrenaline, 30 μg., increased the outflow from 92 to 144; hexamethonium (A) increased the heart rate from 148 to 176 a minute
			B	0.25	96	92	92	
2	11	60	A	1.00	54	44	56	
3	16	120	A	0.25	92	90	96	Hexamethonium (A) increased the heart rate from 160 to 180 a minute
			A	1.00	100	93	94	
			B	0.50	78	72	81	
4	12	90	A	1.00	92	78	90	
			B	1.00	80	74	84	
5	11	120	A	1.00	120	140	132	Adrenaline, 25 μg., increased the outflow from 88 to 102
			B	1.00	114	132	126	

* Coronary flow represents 60 per cent of actual output; figures are means of several observations.

are the means of several observations. It was, however, difficult to obtain a clear picture of the effect of hexamethonium on the coronary outflow, since in some dogs there was no change, while in others there was a slight decrease or increase; Wégria (1951) similarly found in several experiments with tetraethylammonium both a decrease and an increase in the coronary flow. The results were further complicated by the fact that once an effect was obtained it was not always possible to repeat it later in the experiment, though adrenaline repeatedly increased the outflow. At least 30 minutes elapsed between each dose in order to allow the effects of each compound to wear off and the outflow to return to normal. On the whole the effects were relatively small and transitory, and no marked constriction of the coronary outflow was ever observed; in some instances hexamethonium might even have a beneficial action by increasing the coronary outflow.

Actions on the vessels

Even though the depressor action of hexamethonium appeared to be mainly accounted for by the blocking of impulses passing through the autonomic ganglia

and consequent removal of sympathetic tone, evidence was required of any possible direct effect on the peripheral vessel walls. To obtain this evidence the vessels of one hind limb of the dog were perfused by a Dale-Schuster pump with heparinized blood, and observations were made on the peripheral resistance and venous outflow. Fig. 12 shows a result, obtained on several occasions, of an experiment where a slight vasodilatation was produced on the injection of hexamethonium into the arterial supply to the limb. The dilatation did not depend on the tone of the vessel walls, since no greater response was obtained when the peripheral resistance was raised by the addition of adrenaline to the reservoir of blood in the system. From these results it was concluded that the vasodilator effects were small and could play no great part in the production of hypotension by hexamethonium.

Gastric motility

In the cat, either decerebrate or under chloralose, hexamethonium given intravenously caused a marked contraction of the stomach. It prevented the cardiac but not the gastric effects of vagal stimulation ; the vagal effects on the stomach were even increased and prolonged (Fig. 13). Increasing the pressure inside the balloon prevented the stimulant action of hexamethonium, but the vagal effects on the stomach were prolonged as before (Fig. 14). Atropine had a purely inhibitory effect. Evidence of an inhibitory action of hexamethonium was, however, obtained in the rabbit, in which the spontaneous movements were reduced (Fig. 15). The different results in the cat and rabbit may depend on differences in the functional autonomic innervation of the stomach in the two species ; Brown, McSwiney, and Wadge (1930) showed that the body of the stomach in cats and dogs receives motor and inhibitor fibres from the sympathetic, contraction or relaxation being produced according to the type of stimulation.

To ascertain whether the stimulant action of hexamethonium in the cat was exerted directly on the stomach, observations were made after nicotine, given in sufficient amount to paralyse sympathetic stimuli completely, since the stimulant action might have been due to the preferential blocking of the sympathetic rather than the parasympathetic system. If this assumption were correct it would result in the removal of any sympathetic inhibitory control of the stomach, allowing any excitatory parasympathetic control to manifest itself. An experimental result in support of this contention is shown in Fig. 16, where the intravenous injection of

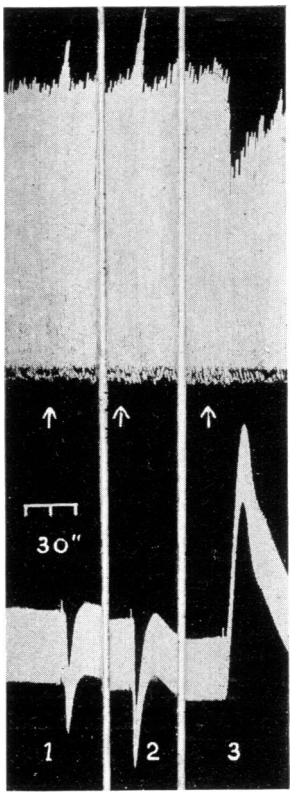


FIG. 12.—Perfusion of vessels of left hind limb of dog with heparinized blood. Upper record, venous outflow; lower record, arterial pressure. Intra-arterial injections of (1) 0.2 mg. hexamethonium bromide, (2) 2 μg. acetylcholine, (3) 1 μg. adrenaline.

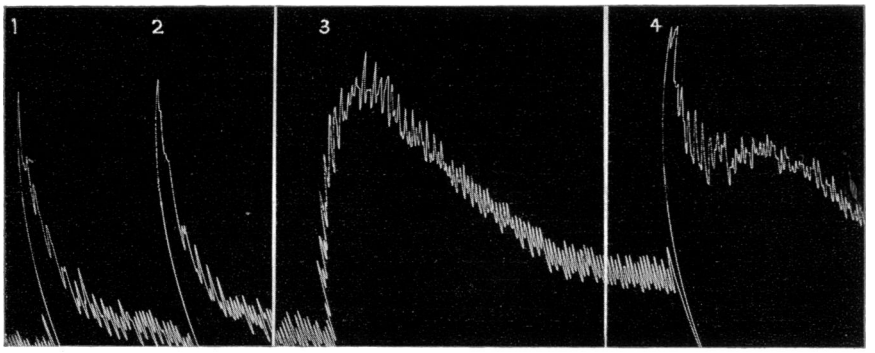


FIG. 13.—Cat, chloralose. Gastric motility, " pressure " in balloon 8–10 cm. water. At (1), (2), and (4) stimulation of left peripheral vagus; at (3) 1.0 mg. hexamethonium bromide intravenously.

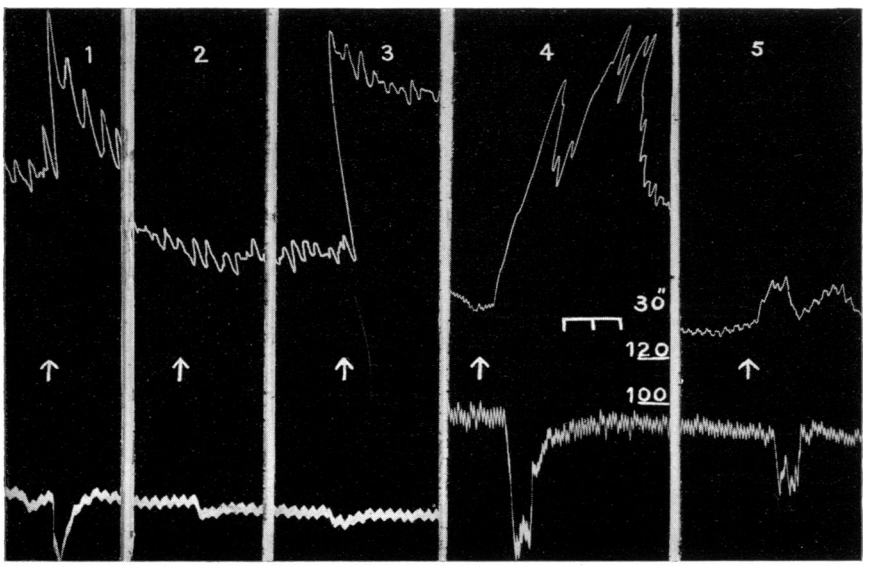


FIG. 14.—Cat, chloralose. Upper record, gastric motility, " pressure " in balloon 15 cm. water; lower record, blood pressure. At (1), (3), (4), and (5) stimulation of left peripheral vagus; at (2) 5.0 mg. hexamethonium bromide. Between (4) and (5) 50 μg. atropine sulphate intravenously.

a dose of hexamethonium which had previously stimulated the stomach movements no longer had this effect after nicotine.

Gastric secretion

On sustained stimulation of the peripheral end of the vago-sympathetic trunk in anaesthetized dogs with bursts of induction shocks every four seconds, a copious flow of gastric juice was produced, the amounts varying from 30 to 180 ml. per hr. A stronger stimulus was required to initiate the flow of gastric juice than that required to slow the heart. The rate of flow varied considerably in different dogs,

but the general trend was the same in all of them. The juice secreted was of high acidity and high peptic power ; after one to two hours the volume and composition remained fairly constant, when it was possible to determine the effect of the intravenous injection of a drug. The cardiac effects of vagal stimulation were inhibited by hexamethonium bromide concurrently with the inhibition of gastric secretion (see Fig. 8), but the effect on the blood pressure usually passed off before the gastric secretion had returned to its original level. There was a graded relationship between the dose of hexamethonium and the extent and duration of the response obtained (Fig. 17) ; the threshold dose for a partial inhibition was 0.06 mg./kg.,

FIG. 15.—Rabbit, urethane. Upper record, gastric motility; lower record, blood pressure. At arrow, 5.0 mg. hexamethonium bromide intravenously.

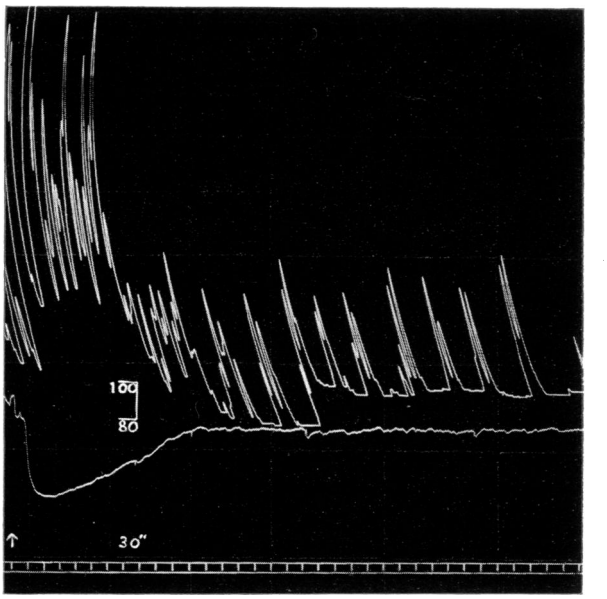


FIG. 15.

FIG. 16.—Cat, chloralose. Record of gastric motility, " pressure " inside balloon 8–10 cm. water. (1) and (2) 0.5 mg./kg. hexamethonium bromide intravenously; between (1) and (2) 12 mg. nicotine.

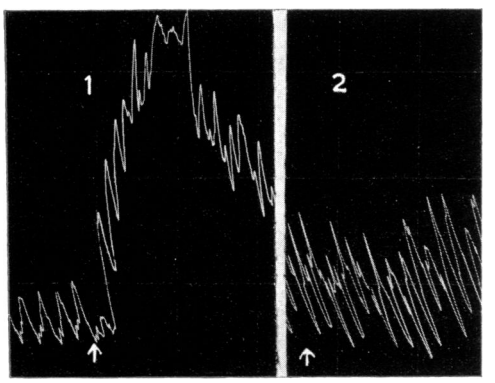


FIG. 16.

FIG. 17.—Effect of intravenous hexamethonium bromide on the free acid in gastric juice evoked by stimulation of vago-sympathetic trunk in dogs. Maximal response 30–60 min. after injection.

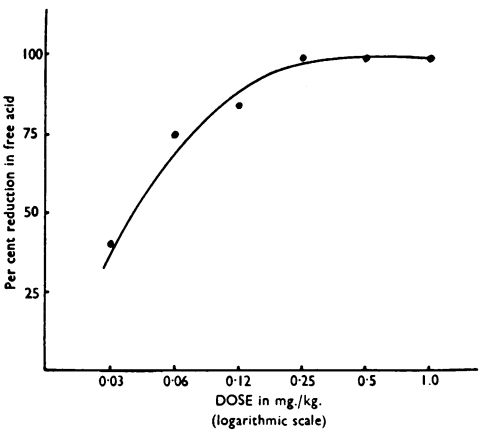


FIG. 17.

while 1 to 2 mg./kg. produced an achlorhydria lasting one or more hours (see Table V). The maximum effect usually occurred within 30 to 60 minutes and the decreases in acidity and in volume closely paralleled one another, but the peptic activity was reduced for a longer period; even three hours after 1 mg./kg. of hexamethonium bromide the peptic power was still only about one half the original figure, although by that time the volume and acidity were restored to their initial values. The bis-ethyldimethylammonium homologue in a comparable dose had a greater effect than hexamethonium itself. In order to produce a comparable effect

TABLE V

GASTRIC SECRETION IN DOGS, EVOKED BY VAGAL STIMULATION

Vol. = Volume of juice in ml. Free A. = Free acid, ml. 0.1N-HCl/100 ml. juice. Total A. = Total acid, ml. 0.1N-HCl/100 ml. juice. Pepsin = Pepsin units (Hunt, 1948). At zero time the mean figures are given for the steady pre-injection level, obtained over several hours

Dog kg.	Dose mg./kg. i.v.		Gastric secretion values							
			0	15	30	45	60	75	90	120
			minutes after injection							
			Hexamethonium bromide							
13	0.03	Vol.	24	15	18	26	21	39		
		Free A.	8	8	5	8	7	8		
		Total A.	140	152	142	156	140	146		
	0.06	Vol.	13	8	9	12	15	17	18	
		Free A.	8	8	7	5	2	7	8	
		Total A.	127	144	130	128	126	140	140	
10	0.12	Vol.	45	10	2	18	28	30	32	
		Free A.	25	20	4	12	18	22	22	
		Total A.	180	184	108	130	155	170	175	
		Pepsin	176		69	114		82	86	
10	0.25	Vol.	8	5	1	0	2	10	8	
		Free A.	8	9	Trace		Trace	6	8	
		Total A.	150	140	74		82	152	166	
10	0.50	Vol.	23	8	2	2	7	8	9	15
		Free A.	17	16	Trace	Trace	5	10	13	16
		Total A.	213	190	72	20	100	120	140	297
		Pepsin	247			95	136		174	
10	1.00	Vol.	10	5	2	1	1	0	0	3
		Free A.	16	16	Trace	Trace	Trace			Trace
		Total A.	140	112	54	30	22			68
		Pepsin	189	102	82					92
			Bis-ethyldimethylammonium homologue							
14	0.06	Vol.	8	9	3	1	4	17	20	
		Free A.	6	5	1	Trace	1	3	6	
		Total A.	150	132	116	130	130	128	156	
			Tetraethylammonium bromide							
14	20.00	Vol.	10	3	1	0	0	1	5	18
		Free A.	38	24	4			Trace	21	34
		Total A.	190	186	98			103	199	187

with tetraethylammonium about 10 to 20 times the dose had to be given. Neither hexamethonium nor the homologue had any influence whatsoever on the secretion evoked by an infusion of histamine; this secretion was of high acidity but of low peptic power, in contrast to the secretion produced by nervous stimulation.

Excretion in urine

We have not made an extensive study of the absorption and excretion of hexamethonium, but in view of the empirical use of the drug by mouth in clinical practice it was of importance to know whether it is well absorbed by this route. Zaimis's method (1950) was employed, which is similar to that described by Rennick *et al.* (1947) for the estimation of the tetraethylammonium ion. It is based on the production of a coloured acetone-soluble precipitate when ammonium reineckate is added to a solution of a soluble salt of hexamethonium. The test is not specific and can be used for choline and other quaternary ions, but these compounds are normally absent from urine. The method is not very sensitive, and when small amounts have to be determined a biological test, for example the nictitating membrane of the cat, is preferable; but a still more sensitive and readily applicable method is desirable in order to investigate more fully the metabolism and distribution of the drug in the body.

Rabbits were used, and they were deprived of food the night before an experiment. Hexamethonium iodide was given in solution by stomach tube or intravenously into the marginal ear vein. When given intravenously up to 84 per cent of the dose administered was excreted in the urine within 24 hours. Intramuscular injection also produced good elimination, but much less was recoverable in the urine after oral administration (Table VI).

TABLE VI

URINARY EXCRETION OF HEXAMETHONIUM IN RABBITS

Route	Dose mg./kg.	Per cent excreted (total) in urine during			
		1	2	3	4 days
Intravenous	10	76.5			
Intravenous	50	84.0			
Intramuscular	50	58.0			
Intramuscular	100	78.4			
Oral	250	2.8			
Oral	500	10.3	11.5	13.8	15.4
Oral	500	23.5	25.6	26.8	27.9
Oral	500	9.8	12.1	14.4	15.9
Oral	1,000	4.2	24.8	28.8	—

We have also had the opportunity, through the courtesy of Dr. S. Locket, at Oldchurch Hospital, of examining the urinary excretion of some patients receiving the bis-ethyldimethylammonium homologue of hexamethonium. Eleven patients received oral doses varying from 500 to 3,500 mg. daily, and the urinary excretions within 24 hours varied from 0.2 to 33.8 per cent of the dose administered. In two patients who received parenteral as well as oral treatment it was possible to

compare the merits of the different routes of administration. The first patient excreted 93 per cent of an intramuscular dose of 120 mg., but only 1 to 6 per cent after oral treatment with doses of 500 to 3,500 mg. daily. The second patient received 1,190 mg. subcutaneously over a period of 14 days, and during that time excreted 1,146 mg. in the urine (96 per cent); only 4 per cent was eliminated after 1,000 mg. by mouth, but 33 per cent of an oral dose of 2,000 mg. (which produced a marked reaction) was excreted within three days. Sufficient evidence was not available to make a significant assessment, but it appeared that there was considerable variation from patient to patient, and that in general the major portion of the drug given by either the intramuscular or the subcutaneous routes could be recovered in the urine, whereas a variable and smaller amount was detected after oral treatment. These results are in agreement with those of Milne and Oleesky (1951).

Discussion

Hexamethonium inhibits the transmission of nervous impulses in the autonomic system. The paralysing effect occurs at the ganglion synapse, since postganglionic stimulation remains effective; its action does not extend to the effector organs. Parasympathetic as well as sympathetic ganglionic transmission is affected, though not necessarily to the same extent at all ganglionic junctions. That hexamethonium affects both parts of the autonomic system might be anticipated in view of the close physiological relationship and interdependence of the two parts, but further information is required on the relative effects on different ganglia throughout the body. Evidence is available that the superior cervical ganglion and the ganglia in the stomach may be affected without there being any concurrent action on the blood pressure.

Having noted the remarkable difference in potency between hexamethonium and the bis-triethylammonium homologue, we examined the effect of successive replacement of methyl by ethyl groups. Optimum activity was found in the bis-ethyldimethylammonium homologue, which was one and a half times to twice as potent as hexamethonium in paralysing transmission in both sympathetic and parasympathetic ganglia. This was achieved without any significant alteration in its type of action. The bis-triethylammonium homologue, by contrast, had a neuromuscular paralysing action which was absent in the bis-trimethyl or bis-ethyldimethylammonium compounds.

Of particular interest in the methonium series was the suggestion that maximum activity depended on an optimum chain length and a " fit " between the groupings at each end of the molecule and the receptor groups. It appears to us now that in hexamethonium not only the distance between the two quaternary nitrogen atoms, or inter-receptor distance, but also the *nature* of the terminal groupings is an important factor in determining optimal activity; the relatively simple substitution of ethyl for methyl groups on the two nitrogen atoms exerted a profound influence. The best fit on the receptors might depend on a certain " effective size " which is optimal when the terminal groupings are bis-ethyldimethyl rather than trimethylammonium. This suggestion might be interpreted on the assumption that the groupings associated with the two nitrogen atoms are able to fit on to the receptors when the " effective size " is that found in the trimethyl or ethyldimethyl groups, but that the triethyl grouping

is too large for a good attachment and consequently activity is lost. But it is curious that corresponding with this diminution of potency in blocking autonomic ganglia there is an increase in neuromuscular-blocking activity ; the problem obviously still requires a more complete explanation.

There are some actions of hexamethonium to which we should like to draw attention. It was found that the drug had a very weak and inconsistent inhibitory effect on salivary secretion induced with carbamylcholine, but since this was produced only with relatively large doses it is not necessarily related to the clinical finding of dry mouth ; but we have not investigated the possibility of an inhibitory effect on transmission through the chorda tympani. Of more significance was the relatively potent mydriatic action, presumably caused by paralysis of the ciliary ganglion: this mydriatic action of hexamethonium might well serve as a method of estimation of the drug in body fluids. No marked deleterious action was observed on the heart ; in some experiments there was even an improvement in the coronary circulation. In dogs, where there is a comparatively high degree of vagal tone, vagal block with hexamethonium was sometimes accompanied by a tachycardia which, however, soon passed off. The dilator action on the vessels was slight: it would hardly account for the large fall in blood pressure caused by hexamethonium which must be mainly due to the removal of sympathetic tone.

The effects of hexamethonium on the motility of the stomach were variable, dependent most probably on the composition of the innervation of the stomach in different species. Ambache (1951) has shown that there are two kinds of functionally distinct ganglion cells in the myenteric plexus ; stimulation of the one causes contraction, of the other inhibition, of the intestine. A similar state of affairs may exist in the stomach, and the results of Paton and Zaimis (1951), who found that hexamethonium caused vigorous contractions of the cat ileum, are in accord with our findings on the stomach. In the rabbit the effect was purely inhibitory, resulting in a decrease in tone and movements, but in the cat the main effect was stimulation. This was not a direct action on the stomach, since it was abolished after a full paralysing dose of nicotine. It was most likely due to an interference of gastric nervous control, by the preferential blocking of sympathetic rather than vagal ganglia, which would result in the removal of sympathetic inhibition of the stomach, allowing any vagal stimulant effect to manifest itself.

Although the state of activity of the sympathetic nervous system influences the motility of the stomach, it is problematical to what extent, if any, the sympathetic exerts control over the secretion of gastric juice. But there is no doubt whatsoever that excitability of the vagus influences secretion, and it was found that both hexamethonium and its bis-ethyldimethylammonium homologue inhibited the formation of acid in the gastric juice of the anaesthetized dog, evoked by vagal stimulation ; the volume of juice and its peptic power were likewise decreased. These compounds were without effect, however, on the acid secretion induced by histamine. This is perhaps no more than might be expected, since the inhibitory action is exerted only indirectly on the mechanism of nervous control, not directly on the acid-secreting cells.

SUMMARY

1. A study has been made of certain actions of hexamethonium and of homologues with ethyl substituents, in which one or more methyl groups on each nitrogen atom were replaced by ethyl groups.

2. The bis-ethyldimethylammonium homologue (hexane-1 : 6-bis-ethyldimethylammonium dibromide dihydrate) was one and a half times to twice as potent as hexamethonium in paralysing autonomic ganglionic transmission in both sympathetic and parasympathetic ganglia, and it had a similar type of action. The bis-triethylammonium homologue was much less potent and possessed neuromuscular-blocking properties absent in the other compounds. The bis-diethylmethylammonium compound was equally as active as hexamethonium on parasympathetic ganglia, but slightly less potent on sympathetic ganglia. These structure-action relationships are discussed, and attention is drawn to the importance of the nature of the terminal groupings as well as the distance between the two quaternary nitrogen atoms as a factor determining optimal activity.

3. Certain actions of hexamethonium, such as inhibition of salivary secretion and vasodilatation, were shown to be very slight, and consequently play little part in the main action of the drug. But hexamethonium has an appreciable mydriatic effect, which is most likely due to paralysis of the ciliary ganglion ; a maximal response was attained more quickly than with atropine, which dilates the pupil by a different—peripheral, not ganglionic—action. Hexamethonium had little effect on the heart ; in some instances it improved the coronary circulation and produced a tachycardia, the latter effect caused by the removal of vagal tone.

4. Gastric motility may be excited or inhibited by hexamethonium dependent on the functional innervation of the stomach in different animals. In the cat stomach movements were increased, owing to the removal of sympathetic inhibitory control, without any changes in blood pressure.

5. Gastric secretion in the dog, evoked by vagal stimulation, was inhibited by both hexamethonium and its bis-ethyldimethylammonium homologue. There were reductions in volume, in free and total acidity, and in peptic power ; the extent and duration of these effects were dependent on the dose. No inhibitory effect was exerted on gastric secretion induced with histamine.

6. Considerably less of these compounds was excreted in the urine after oral than after intravenous administration.

It is a pleasure to acknowledge the interest taken in this investigation by Dr. A. J. Ewins and certain helpful discussions with Professor J. H. Burn.

We have had invaluable assistance, particularly from Mr. G. T. Langston, and also from Mrs. Zwartouw ; Mr. Edge kindly did some experiments on the isolated heart. We wish to thank the directors of May and Baker Ltd. for permission to publish these results.

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