*Brit. J. Pharmacol.* (1958), 13, 501.

# THE PHARMACOLOGICAL ACTIONS OF PEMPIDINE AND ITS ETHYL HOMOLOGUE

BY

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(RECEIVED AUGUST 29, 1958)

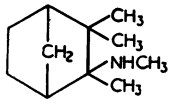
Pempidine, and other highly active ganglion blocking agents of the polyalkylpiperidine series, were developed from tertiary alkylamines, themselves weakly active, on the hypothesis that high activity was conferred by the presence in the molecule of a sterically hindered secondary or tertiary nitrogen atom. Pempidine and its N-ethyl homologue (26539) resembled mecamylamine qualitatively. All three drugs blocked sympathetic and parasympathetic ganglia; this action was slow in onset and protracted. They blocked neuromuscular transmission, but only about one hundredth as powerfully as ganglionic transmission. They caused a fall in amplitude and rate of the isolated heart, and reduced coronary flow. They had local anaesthetic properties in one of four tests used. They caused tremor. All were well absorbed when administered orally. Pempidine was about twice as active as mecamylamine on ganglia, but only about one half to one quarter as toxic as judged by death, growth, induction of tremor, or cardiotoxicity. Compound 26539 was also quantitatively superior to mecamylamine in respect of these safety margins, but unlike pempidine or mecamylamine damaged the pituitary gland and testis when administered daily for several months. The mode of action of the three drugs is discussed: the results give tentative support for the hypothesis that their action is intracellular.

We have recently reported the discovery of powerful ganglion-blocking and hypotensive properties in a series of polyalkylpiperidines (Spinks and Young, 1958). The most promising of these compounds were 1:2:2:6:6-pentamethylpiperidine (pempidine; Tenormal (I.C.I.) is the hydrogen tartrate) and 1-ethyl-2:2:6:6-tetramethylpiperidine (referred to subsequently by our code number, 26539). Lee, Wragg, Corne, Edge, and Reading (1958) have since reported their discovery independently of activity in the same series, and an account of the action of pempidine hydrogen tartrate in man has been given by Harrington, Kincaid-Smith, and Milne (1958).

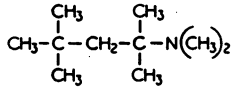
The object of this report is to describe our development of the piperidines, which was different from that of Lee *et al.* (1958), and to give a complete account of our pharmacological and toxicological studies of pempidine and 26539.

During the past five years we have been searching for ganglion-blocking activity among a wide variety of novel compounds; about 2,400 have been tested. Most of them were non-quaternary bases, since it was considered that a highly promising drug should be completely and regularly absorbed when given by mouth, and would almost certainly prove to be non-quaternary. The discovery of the secondary amine, mecamylamine, or 3-methylaminoisocamphane, by Stone, Torchiana, Navarro, and Beyer (1956) first showed that powerful ganglion-blocking activity could indeed be displayed by a non-quaternary base, and that it could be combined with excellent absorption from the gastrointestinal tract. On the other hand, it has become clear that the non-quaternary character of mecamylamine also enables it to enter the central nervous system and elicit side-effects such as tremor, hyper-reflexia, and, possibly, anxiety (Schneckloth, Corcoran, Dustan, and Page, 1956; Perry and Schroeder, 1957; Milne, Rowe, Somers, Muehrcke, and Crawford, 1957; Harrington and Kincaid-Smith, 1958) which, hitherto, have not been reported with quaternary ganglion-blocking agents. Like all ganglion-blocking drugs, mecamylamine also displays side-effects arising from its action on parasympathetic ganglia, and perhaps also from direct actions on smooth muscle (Bennett, Tyler, and Zaimis, 1957). Paralytic ileus has been observed in man (Grant and Boyd, 1957). In the hope of

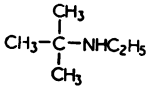
securing improvement in one or both of these respects we prepared and studied the ganglion-blocking actions of a variety of secondary and tertiary bases in which the nitrogen atom was sterically hindered by being closely surrounded by alkyl groups.



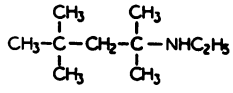
Mecamylamine



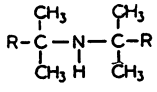
24605



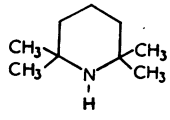
25645



25637



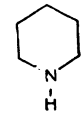
X



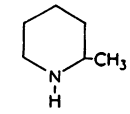
25636

Modest activity as a ganglion-blocking agent, about 10% of that of mecamylamine, was first seen in *NN*-dimethyl-*tert.*-octylamine (24605). A large number of similar tertiary alkylamines showed ganglion-blocking properties: the two best were *N*-ethyl-*tert.*-octylamine (25637) and *N*-ethyl-*tert.*-butylamine (25645), having activities of 20% and 30%, respectively, of that of mecamylamine.

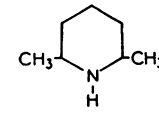
It was thought possible that increase in activity might be obtained by preparing substances in which the nitrogen atom was linked to two tertiary carbon atoms (**X**). The preparation of the simplest compound of this type, di*tert.*-butylamine (X, R = $CH_3$), is extremely difficult (Klages, Nober, Kircher, and Bock, 1941), and the yield is low (Brown, Barbaras, Berneis, Bonner, Johannesen, Grayson, and Nelson, 1953). Compounds containing two tertiary butyl groups are not only characterized by difficulty of formation but also possess relatively low stability (Brown and Barbaras, 1946). However, one cyclic analogue of di*tert.*-butylamine, 2:2:6:6-tetramethylpiperidine (25636), is readily accessible and is a very stable compound. We found it to be about twice as active as mecamylamine and to have other desirable properties, such as adequate persistence. We then prepared a wide variety of related heterocyclic compounds and have so far examined about 160. The results of this examination have supported our original theory that the essential feature of the molecule that confers activity is the close juxtaposition of several alkyl groups to a secondary or tertiary nitrogen atom. The influence of a number of alkyl groups in the 2- and 6-positions of the piperidine nucleus is well illustrated by the following series of secondary amines. Activities are shown as % of mecamylamine activity estimated on the nictitating membrane preparation.



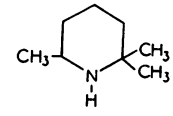
7387
Activity = 0



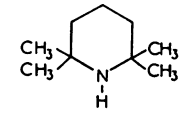
26539
Activity = 0



26388
Activity = 0



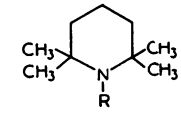
26725
Activity = 40



25636
Activity = 200

It is clear that activity increases as the number of methyl groups in the 2- and 6-positions increases. Again, if the 6-methyl group in 2:2:6-trimethylpiperidine (26725) is replaced by a higher alkyl group, activity increases along the series methyl, ethyl, propyl, *iso*propyl.

Tertiary amines were also studied: a wide variety of groups in the 1-position conferred high activity. Some examples are given below:



| *Compound* | *R.* | *Activity* |
|---|---|---|
| 25636 | H | 200 |
| 25745 (pempidine) | $CH_3$ | 250 |
| 26539 | $C_2H_5$ | 300 |
| 29867 | $n$-$C_3H_7$ | 200 |
| 26857 | $n$-$C_4H_9$ | 150 |
| 28921 | $CH_2.CH{=}CH_2$ | 120 |
| 26687 | $CH_2.C_6H_5$ | 15 |

In other series, for instance, those having additional substituents in the 3- or 4-positions, there has also been a tendency for the 1-methyl or 1-ethyl homologues to show the highest activity. Quaternization of the tertiary amines caused loss of persistence: some quaternary compounds were highly active, but their action was rapid in onset and transient.

It is clear from the limited number of compounds shown that very many polyalkylpiperidines are highly active ganglion-blocking agents. Out of the total of 160 about 50 are as active as hexamethonium, the activity of which is equivalent to about 70 on the scale used, and about 20 are at least three times as active as hexamethonium. These potency ratios are of course likely to apply only in the particular conditions we used.

The examination of such a large number of highly active compounds was a formidable task. Our method was to select the ten compounds that, in the nictitating membrane test, had the highest potency coupled with a persistent action. We then examined each of these compounds by a second test: its effect on the responses of cat nictitating membrane, blood pressure and bladder to serial doses of 1:1-dimethyl-4-phenylpiperazinium (see Chen, Portman, and Wickel, 1954). Our object was to choose any compound that seemed to have specific anti-sympathomimetic actions, particularly on blood pressure. We observed no absolute specificity of this kind, but the results of these tests, and of quantitative studies of toxicity, showed that the most promising drugs of the whole group were pempidine and 26539, the 1-methyl and 1-ethyl derivatives of the first piperidine tested, 2:2:6:6-tetramethylpiperidine. The remainder of this report is concerned with the detailed study of these two compounds.

## METHODS

*Compounds Used.*—Some experiments were carried out with a sample of Inversine (mecamylamine hydrochloride) which was kindly given to us by Dr. H. Molitor. Most, however, were made with a sample of mecamylamine hydrochloride made by ourselves which proved identical with the sample provided by Dr. Molitor by infra-red analysis, and determination of melting point, mixed melting point, and C, H, and N contents. Ecolid (chlorisondamine chloride) and Ansolysen (pentolinium hydrogen tartrate) were kindly provided by Dr. H. J. Bein and Dr. R. Wien respectively. Hexamethonium bromide and tetraethylammonium bromide were made in our laboratories. Pempidine and 26539 were used either as hydrochlorides or hydrogen tartrates; the hydrochloride of the former was somewhat hygroscopic; that of the latter was very hygroscopic and was prepared in solution as required from the base. The physical properties of the bases and salts of pempidine and 26539 are given in Table I. Throughout this paper doses and concentrations of non-quaternary compounds refer to free base, and those of quaternary compounds to cation, unless otherwise stated.

TABLE I
PHYSICAL PROPERTIES

| Salt | Pempidine | 26539 |
|---|---|---|
| Base .. .. | B.p. 184–186°. Slightly soluble in water. Miscible with common organic solvents | B.p. 196–199°. Slightly soluble in water. Miscible with common organic solvents |
| Picrate .. .. | M.p. 276° (dec.) | M.p. 245–247° (dec.) |
| Hydrochloride | M.p. 249° (dec.) | Hygroscopic |
| Hydriodide .. | M.p. 294–295° (dec.). Moderately soluble in water | M.p. 269–279° (dec.). Moderately soluble in water |
| Hydrogen tartrate | M.p. 160–160·2°. Very soluble in water | M.p. 158·5–160°. Very soluble in water |
| Hydrogen succinate | M.p. 118–120°. Very soluble in water | M.p. 118–120°. Very soluble in water |

*Nictitating Membrane.*—Adult cats of either sex were anaesthetized by injection of chloralose, 80 mg./kg., into the saphenous vein. If this amount was inadequate it was supplemented with a second injection of 5 to 15 mg./kg. The right femoral vein and left femoral artery were cannulated for injection and blood pressure recording purposes, respectively, and a tracheal cannula was inserted. The right preganglionic cervical sympathetic nerve was severed and the rostral stump stimulated with rectangular pulses of approximately 8 V. and of 2.5 msec. duration, at a frequency of 4 to 5 sec. The contraction of the right nictitating membrane was recorded continuously, and drugs were injected intravenously during the sustained contraction. After the maximal effect of a dose had been observed, the nerve and membrane were usually rested for a time depending on the known or expected persistence of the drug used.

*Potency Determination on Nictitating Membrane.*—The high persistence of 26539, pempidine, and mecamylamine made determination of their potency difficult and time-consuming. It was found that a single intravenous injection of as little as 0.1 mg./kg. of 26539 or of pempidine, or of 0.2 mg./kg. of mecamylamine, markedly increased the sensitivity of the preparation for several hours. Two techniques were used. In the first a fresh cat was used for each dose of each of the compounds. In the second, a dose $x$, expected to be ineffective, or only slightly effective, was given (0.025 mg./kg. of pempidine or of 26539; 0.05 mg./kg. of mecamylamine), and when sufficient time had passed for the maximal effect to appear (usually 5 to 10 min.) another dose $x$ was given, and followed in its turn by $2x$ and $4x$ in that order. It was assumed

that the successive cumulative effects produced by the four doses were those of $x$, $2x$, $4x$, and $8x$. This assumption seemed valid in view of the known persistence of the compounds and was supported by the finding that the results thus obtained did not differ significantly from those of the first technique. Whichever technique was used injections of one of two standard drugs, tetraethylammonium or hexamethonium, were first given to the same cat. When tetraethylammonium was to be studied three doses, 0.5, 1.0, and 2.0 mg./kg. (expressed as bromide), were given in random order. When hexamethonium was to be studied it was necessary to avoid delay and error due to persistence of large effects. A single dose of 0.5 mg./kg. (expressed as bromide) was therefore given and followed by a second dose of 0.25 or 1.0 mg./kg., according to whether the relaxation caused by 0.5 mg./kg. was greater or less, respectively, than 50%. Slope of effect against log. dose was determined when possible for each compound in each cat. Since the mean slopes thus determined for the five compounds did not differ significantly from the overall mean slope (32% relaxation for a two-fold increase of dose) this mean slope was used for all experiments and the dose causing 50% relaxation (ID50) was estimated for each compound in each cat. The mean ID50 and 95% confidence limits were then calculated in each case. The main source of error was the variation between cats.

*Site of Action on Nictitating Membrane Preparation.*—Two techniques were used in cats anaesthetized with chloralose. First, electrodes were applied to the rostral preganglionic stump of the cervical sympathetic nerve and to the unsevered postganglionic nerve. The two nerves were then stimulated alternately through a rotor with rectangular pulses (4 V., 2.8 msec., 4/sec.). Each nerve was stimulated for 12 sec. every 2 min., so that the membrane gave a sharp response every 1 min. The responses to pre- and postganglionic stimulation were readily made equal by small adjustments of voltage, width or frequency (the values given are those of Fig. 2). Pempidine hydrogen tartrate was injected intravenously and its effect was observed until it declined or until the unaffected response to postganglionic stimulation deteriorated. Second, bipolar silver electrodes were applied to the rostral stump of the severed preganglionic cervical sympathetic nerve and to the caudal stump of the severed postganglionic nerve. The former nerve was stimulated with single rectangular pulses (1.5 V., 50 $\mu$sec.), using a Fleming Radio stimulator provided with an additional RF coupled output stage isolated from earth; the latter was built by our colleague, Mr. G. B. Horsfall, to the design of Schmitt and Dubbert (1949) and was found to be very satisfactory. The postganglionic action potential was amplified by a high gain, high discrimination resistance-capacity-coupled amplifier designed and built by Mr. Horsfall (time constant 1.0 sec., h.f. loss 15% at 200 c./sec.) and displayed for photography on a Cossor 1049 oscilloscope (time base, 150 msec.). The effects of an intravenous injection of pempidine were observed until maximal.

*Pressor Responses.*—Mean blood pressure was recorded from the femoral artery in cats anaesthetized with chloralose. Pressor responses were induced by five methods: (1) intravenous injection of acetylcholine after atropine; (2) injection of dimethylphenylpiperazinium; (3) injection of noradrenaline or adrenaline; (4) occlusion of both carotid arteries; (5) efferent stimulation of the right splanchnic nerve with rectangular pulses (10 V., 3 msec., 10/sec.). In each type of experiment, transient responses of the desired intensity, usually 60 to 80 mm. rise of pressure, were induced at constant intervals (usually 5 min.) and the effects of intravenous injections of drugs observed. As in experiments on the nictitating membrane mecamylamine, pempidine, and 26539 all had very persistent effects, and it was rarely possible to study the effect of more than one dose of each in each experiment unless the divided dose method was used.

*Potency Determination by Pressor Response to Dimethylphenylpiperazinium.* — As in nictitating membrane experiments two methods were used. In the first, a single dose of a single persistent compound was given intravenously to each cat. In the second, blocking doses in a series of $x$, $x$, $2x$, $4x$, $8x$, $16x$, etc., were injected, and two dimethylphenylpiperazinium injections given between each two blocking doses. Each cumulative effect was recorded as the mean of the two appropriate responses, expressed as a % of the mean of three control responses at the beginning of the experiment. This description applies only to mecamylamine, pempidine, and 26539: before any of these was studied effects of three doses of hexamethonium or tetraethylammonium were determined, and allowed completely to disappear.

A third method of assay was tried in which doses $x_1$, $x_2$, $x_3$, $x_4$, $x_5$ of a single persistent drug were calculated so that each new dose increased the cumulative total then given to $\sqrt{2}$ times the cumulative total at the previous dose. Alternate doses were then substituted by approximately equivalent amounts of another persistent drug, thus: $x_1$, $y_2$, $x_3$, $y_4$, $x_5$, $y_6$. The cumulative effect at each dose was recorded and analysis of the results was attempted. The technique appeared practicable, but analysis was exceptionally difficult, and the results of potency determinations by this method are not included, though they appeared not to differ greatly from results by the other two methods.

*Heart Rate.*—Heart rate was recorded in cats anaesthetized with chloralose by causing the chopped QRS complex of the electrocardiogram (lead II or, better, a precordial lead) to trigger a pulse generating circuit. The pulses were fed to a capacitor the charge on which was leaked off through a milliammeter from which the heart rate could be read directly, or were amplified to operate a Thorp impulse counter for kymographic recording. Changes in heart rate were induced by stimulating the distal stump of the severed

right vagus (30 V., 3 msec., 7/sec.) and their effects observed on the meter or on kymograph record of the Thorp impulse counter, and also by recording the mean femoral arterial pressure.

*Gastric Secretion.*—Male rats, weighing 90 to 110 g., were starved for 24 hr. and anaesthetized with ether. The pylorus was ligated, and doses of drugs or saline given subcutaneously or intra-duodenally just before or just after ligation. The rats were allowed to recover from the anaesthesia, and 6 hr. later they were killed and the gastric juices collected. Volume was measured, and the total and free acid were determined by titration using Topfer's reagent as indicator.

*Actions on Gastrointestinal Movement and Saline Diuresis in Mice.*—Groups of six male albino mice, weighing 18 to 22 g., were selected at random from a single stock cage in which the mice had food and water available *ad libitum* up to the time of experiment. They were treated by stomach tube with isotonic saline or solutions of salts of chlorisondamine, hexamethonium, pentolinium, mecamylamine, pempidine, or 26539 in isotonic saline. The concentrations were adjusted so that the dose for a 20 g. mouse was contained in 0.5 ml. All mice therefore received the same volume of fluid of approximately constant tonicity. Each group of mice was placed on 6 mm. mesh in a circular metabolism cage of 13.5 cm. diameter, above a funnel and a disc, 5 cm. in diameter, perforated with 1.5 mm. holes: this disc trapped faeces but allowed urine to pass. Urine volumes were measured at 30 min. intervals and faeces were collected after 5 hr., allowed to dry at room temperature overnight and weighed. In each experiment, two control groups received saline and six received drugs. The experiments were usually repeated six times for doses which seemed likely to contribute to calculation of the ID50, the dose that reduced the weight of the faeces to half. The very large numbers of mice used are shown in Table III. The plot of mean log. faecal weight against log. dose was linear, and ID50 values and confidence limits were calculated in the usual manner. Effects on saline diuresis were analysed graphically.

*Actions on Bladder Tension.*—An indirect indication of actions on bladder tension may have been provided by the "antidiuretic" effects observed in mice when high doses of ganglion-blocking agents were given. This action was also observed in the cat anaesthetized with chloralose by cannulating the bladder, filling it with warm saline, adjusting the pressure of the latter until the bladder became quiescent and recording the pressure with a float recorder. Usually blood pressure and contraction of the right nictitating membrane were also recorded. Responses of the three types were induced by serial injections of dimethylphenylpiperazinium as described for the dog by Chen *et al.* (1954). Between two such injections, a dose of ganglion-blocking agent was given, and injections of dimethylphenylpiperazinium continued until all responses returned to normal or until the preparation appeared obviously to have deteriorated. Often the latter occurred first. It was not possible to obtain satisfactory records of all three responses in all cats, the bladder response being the least reliable.

*Antagonism* in vitro.—Responses of the guinea-pig ileum to dimethylphenylpiperazinium, histamine, acetylcholine, and 5-hydroxytryptamine, and of the rat seminal vesicle to noradrenaline and adrenaline were established in the usual manner, and their antagonism by ganglion-blocking agents was studied. Effects of ganglion-blocking agents on spontaneous movements of rabbit duodenum were also observed.

*Actions on the Neuromuscular Junction.*—Twitch responses of the gastrocnemius muscle to electrical stimulation of sciatic nerve were observed in cats anaesthetized with chloralose. *In vitro* actions were studied on the rat phrenic nerve-diaphragm preparation of Bülbring (1946).

*Cause of Death.*—The lethal actions of large doses of pempidine were studied during recording of respiration with a Gaddum recorder, blood pressure, and electrocardiogram (lead II) of cats anaesthetized with chloralose.

*Cardiotoxicity.*—Effects of drugs were observed on the rate, amplitude of beat, and coronary flow (with a Thorp impulse counter) of the rabbit heart perfused with Ringer-Locke solution.

*Central Nervous System Actions.*—Central nervous system actions have been studied by five methods: (1) Observation in mice, rats, and dogs. (2) Measurement of the convulsant threshold. Convulsions were elicited in rats by application through ear electrodes of a 50 c./sec. alternating current of 7.5 mA and approximately 20 V. r.m.s.; the time taken for this current to elicit a maximal extension of the hind limb was measured and used to calculate the threshold in milliwatt seconds before and after treatment (Bogue and Carrington, 1953). Groups of ten rats (5 male and 5 female) were used, the individual animals being allocated before treatment so that all groups had the same mean threshold. (3) Antagonism to nicotine convulsions (see Stone, Mecklenburg, and Torchiana, 1956) was studied by giving drugs intraperitoneally 30 min. before intravenous injection of nicotine hydrogen tartrate (0.85 mg. base/kg.). (4) The sleeping times of groups of five mice previously treated with ganglion-blocking agents orally, and then given 100 mg./kg. of hexobarbitone intraperitoneally 2 hr. later, were compared with those of controls. The mice were maintained at a constant temperature of 34 to 36° by immersing their metal cages in a bath through which water was circulated by pump from a thermostat. Sleeping time was measured in each mouse as the duration of disappearance of the righting reflex. (5) Since tremor was the main central nervous system action observed, a method of measuring its intensity was designed. Groups of three mice received ganglion-blocking drugs or saline orally. At intervals of 20, 30, 40, and 60 min., and 1½, 2, and 2½ hr. the intensity of tremor in each group was

assessed by an observer, who did not know what drug any group had received and allotted a score from the following scale: violent tremor, 5; excessive, 4; medium, 3; slight, 2; very slight, 1. The total score for the seven observation periods was recorded as a measure of the liability of the drug to cause tremor. Every experiment but the first was carried out at a constant environmental temperature of 34 to 36°, to avoid possibility of error caused by hypothermia and shivering. The results were not obviously changed by warmth, and all have been analysed jointly.

*Local Anaesthetic Tests.*—Four methods were used. (1) Inhibition of the corneal reflex of rabbits by topical application to the eye. (2) The tail-root method of Bianchi (1956). (3) The guinea-pig weal method of Bülbring and Wajda (1945). (4) By recording the compound action potential of isolated frog sciatic nerve before and after application of solutions between the stimulating and recording electrodes.

*Acute Toxicity.*—Acute toxicity was determined in male albino mice weighing 19 to 21 g. Solutions of the salts were given by stomach tube (" oral " administration) or by injection, the dose for a 20 g. mouse being contained in 0.5 ml., except for an intravenous injection when this dose was contained in 0.2 ml. and given in 20 sec.

*Chronic Toxicity.*—Chronic toxicity has been studied in rats of both sexes by administration in three ways. (1) Solutions of hydrochlorides by stomach tube. (2) Solutions of hydrogen tartrates of pempidine and 26539, and of mecamylamine hydrochloride in the drinking water. (3) Mixtures of the two hydrogen tartrates or mecamylamine hydrochloride in the diet. Rats were weighed at least twice weekly and food intakes were measured at intervals. At autopsy, heart blood was withdrawn to estimate the haemoglobin concentration by the method of Sahli and serum cholesterol, in view of possible actions which the drugs might have on the coronary vessels. The following tissues were withdrawn, fixed in Zenker-acetic, sectioned and stained with haematoxylin-eosin: kidneys, adrenals, stomach, small intestine, large intestine, mesenteric lymph node, bladder, gonads, pancreas, spleen, liver, thymus, lungs, heart, thyroid, and pituitary.

Chronic toxicity has also been studied in male and female beagle hounds bred in our laboratories. Hydrogen tartrates of pempidine and 26539, and mecamylamine hydrochloride were prepared as scored, uncoated tablets containing 50 or 100 mg. These were shown to have short breakdown times. The dose for each dog was adjusted to the nearest half tablet, and given by hand once daily. Dogs were weighed weekly, and haematological studies were performed fortnightly. Dogs were observed carefully for toxic signs, which were sometimes recorded by colour cinematography on 16 mm. film. Occasionally it was necessary to treat eye infections (due to inhibition of lachrymation) topically with penicillin cream; no other drugs were given. At the end of each experiment dogs were killed by an injection of thialbarbitone intravenously and the tissues named above were taken for histological examination, and, in addition, the whole brain, the cervical cord, and the eyes. All but the last three were fixed in Zenker-acetic. Brain and cord were fixed in formol-saline and eyes in Szent-Györgi solution.

## RESULTS

*Actions on Nictitating Membrane, Preganglionic Stimulation.*—The effects of mecamylamine, pempidine, and 26539 were qualitatively similar. Fig. 1 shows typical responses with 26539. Intravenous doses of 0.1 to 0.2 mg./kg. of pempidine or 26539, or of 0.2 to 0.4 mg./kg. of mecamylamine, caused a large fall of blood pressure (when the initial pressure was high), and a large relaxation of the nictitating membrane. These effects were slow in onset and persistent. In Fig. 1, the relaxation of the membrane with 26539 was maximal after 15 to 20 min. The retraction of the membrane was still only 65% of normal after 95 min. and about 85% of normal after 125 min., and had apparently returned to normal after 160 min. Even when the membrane was again contracting normally, it was possible to show an increased sensitivity to a further dose of the same or another ganglion-blocking agent, particularly when the original dose had produced an effect greater than 50%. Determination of potencies of any of the three agents was therefore very difficult. Even matching of very small responses to obtain approximate potencies appeared highly unreliable, and the values given above for compounds other than pempidine, mecamylamine, and 26539 were obtained by first estimating the likely range of potency in an experiment in which several compounds were injected in infrequent, just active doses, and then, in another cat, matching the maximal response of a single dose of one compound to one of the responses to several different doses of a compound having a transient action (such as tetraethylammonium) given earlier in the experiment. Such tests on interesting compounds were repeated as often as was necessary to give approximate potencies.

Accurate potency determination required many cats for each compound and the use of either the single dose, or the divided dose, technique described above. Analysis of the combined results of these two techniques gave the following values of ID50, the dose causing 50% relaxation, with 95% confidence limits, in mg./kg.: mecamylamine, 0.22 (0.16 to 0.31; 8 cats); pempidine, 0.092 (0.069 to 0.12; 11 cats); 26539, 0.072 (0.053 to 0.097; 10 cats); tetraethylammonium, 0.56

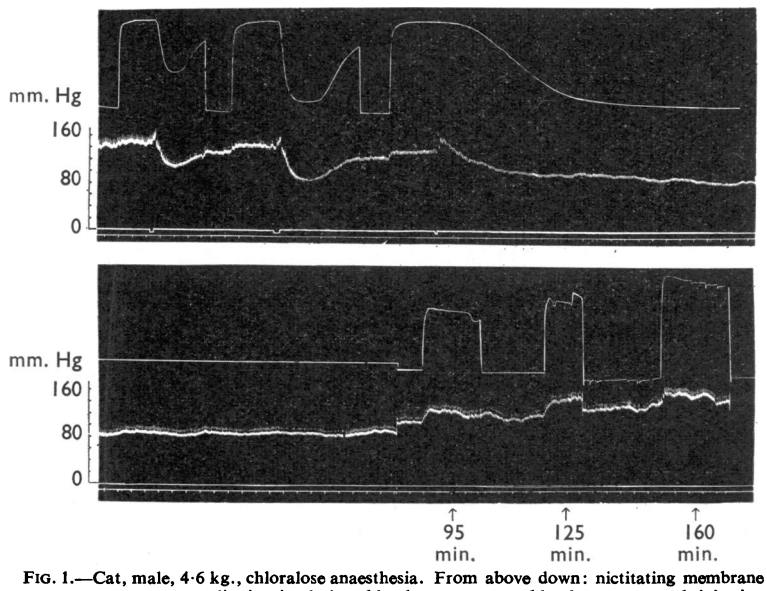



FIG. 1.—Cat, male, 4·6 kg., chloralose anaesthesia. From above down: nictitating membrane response to preganglionic stimulation; blood pressure; zero blood pressure and injection signal; time, min. Effects of tetraethylammonium, 1·0 (first signal) and 2·0 mg./kg. (second signal), and of 26539 (third signal), 0·2 mg./kg.

(0.43 to 0.71 ; 13 cats) ; hexamethonium, 0.30 (0.21 to 0.42 ; 8 cats). The five compounds were administered as hydrochloride, hydrogen tartrate, hydrogen tartrate, bromide and bromide, respectively. Here and subsequently all doses and concentrations are expressed as mg. base or cation.

These estimates of potency agree moderately well with those of Stone, Torchiana, Navarro, and Beyer (1956) and of Lee *et al.* (1958), when the likely differences in conditions of measurement in three different laboratories are taken into account.

*Sites of Action on Nictitating Membrane.*—Fig. 2 shows the results of a typical experiment in which pempidine was administered intravenously during repetitive brief responses of the membrane to alternating pre- and postganglionic stimulation. Although the amount of pempidine used, 0.5 mg./kg., was high in comparison with that causing a 50% relaxation during continuous stimulation, the response to postganglionic stimulation remained completely unimpaired; that to preganglionic stimulation was much reduced. The baseline dropped slightly, perhaps indicating a continuance of ganglionic action throughout the experiment before pempidine was given. This might be attributed to the rather wide rectangular pulse used.

Fig. 3 shows the complete obliteration of the action potential in postganglionic nerve caused, in another experiment, by the intravenous injection of 0.5 mg. of pempidine/kg. The time course of this obliteration coincided approximately with that of the nictitating membrane response. The two records taken at 5 (*b*) and 10 min. (*c*) show an apparent increase in delay at the ganglion.

These two experiments demonstrate that pempidine acts at the ganglion, though its mode of action there requires study. The interesting

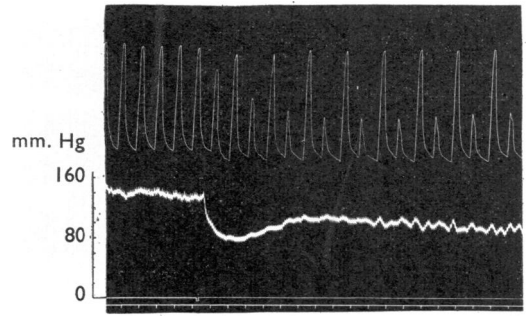



FIG. 2.—Cat, male, 3·2 kg., chloralose. From above down: responses of nictitating membrane to alternate pre- and post-ganglionic stimulation; blood pressure; zero blood pressure and injection signal; time, min. At signal, pempidine, 0·5 mg./kg.

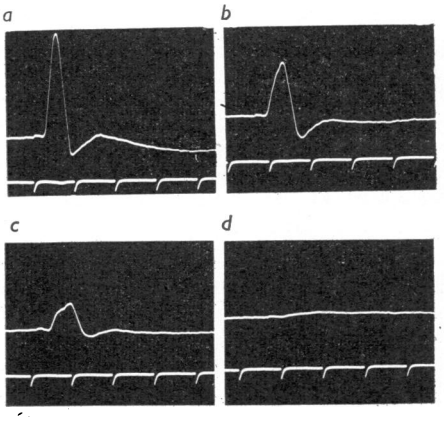



FIG. 3.—Cat, male, 2·6 kg., chloralose. Postganglionic action potentia l elicited by supramaximal preganglionic stimulation of the cervical nerve; time trace, 20 msec. (*a*) before; (*b*) 5 min., (*c*) 10 min., (*d*) 20 min. after pempidine, 0·5 mg./kg., given intravenously.

suggestion of Bennett *et al.* (1957) concerning the type of action of mecamylamine at the ganglionic synapse will be discussed later.

*Actions on Blood Pressure.*—Pempidine and 26539 caused a fall of blood pressure, the time course of which was closely similar to that of the action on the nictitating membrane (Fig. 1). Experiments studying the duration of action in various species have been carried out and particular attention has been given to: (1) measurement of the blood pressure of conscious normotensive rabbits, by cannulation of the central artery of the ear and capacitance manometry; (2) measurement of the blood pressure of conscious normotensive dogs, by passing an occluding cuff round the thigh and a detector cuff round the foreleg; (3) measurement in conscious normotensive and hypertensive rats by a cuff and photoelectric method; (4) measurement by cannulation in dogs anaesthetized with pentobarbitone.

The hypotensive actions recorded under all conditions were extremely variable. In general, pempidine 26539 and mecamylamine had most hypotensive action when the blood pressure was high. When the mean pressure was below 70 to 80 mm. the effect was often very small or absent. This prompted study of the effects of the three drugs on pressor responses to dimethylphenylpiperazinium, acetylcholine, carotid occlusion and efferent splanchnic stimulation. In some dimethylphenylpiperazinium experiments responses of nictitating membrane and bladder were also recorded.

*Effects on Responses of Blood Pressure, Nictitating Membrane and Bladder to Dimethylphenylpiperazinium.*—This test was used as one of the preliminary screening tests. % Inhibitions of response of blood pressure, nictitating membrane, and bladder, respectively, caused by the three drugs (each given as hydrochloride intravenously) were as follows: mecamylamine (0.1 mg./kg.; 2 cats) 63, 85, 44; 26539 (0.1 mg./kg.; 6 cats) 72, 100, 60; pempidine (0.05 mg./kg.; 2 cats) 61, 82, 59.

The nictitating membrane response was the most sensitive to all three drugs, and this finding was confirmed for each of the eleven drugs examined by this technique. Mecamylamine, pempidine and 26539 had similar potencies on bladder and blood pressure, but some other piperidines were found to have less hypotensive action and were discarded. Others were discarded because they were less active, or had brief or more toxic actions.

Repetitive pressor responses to dimethylphenylpiperazinium seemed to us to be more easily reproducible than those to nicotine or to acetylcholine. We did not observe tachyphylaxis when 10 to 30 μg. of dimethylphenylpiperazinium was given at intervals of 5 min. With nicotine tachyphylaxis was prominent, and with acetylcholine the response changed considerably during long experiments in control cats, probably because of declining atropine concentration. We therefore determined the potencies of five ganglion blocking agents as antagonists of the pressor response to dimethylphenylpiperazinium, using for mecamylamine, 26539 and pempidine both one dose/cat and divided dose methods, and for hexamethonium and tetraethylammonium the usual multi-dose methods. The divided dose method using pempidine is illustrated in Fig. 4. Pempidine and the other drugs inhibited the variable depressor (presumably the cardiac) response to dimethylphenylpiperazinium, as well as the pressor response. No attempt was made to measure the former inhibition. The results with both methods used for the three persistent drugs were analysed jointly. The following mean estimates of the ID50 with 95% confidence limits (mg./kg.) were obtained: mecamylamine, 0.081 (0.055 to 0.120; 9 cats); pempidine, 0.035 (0.023

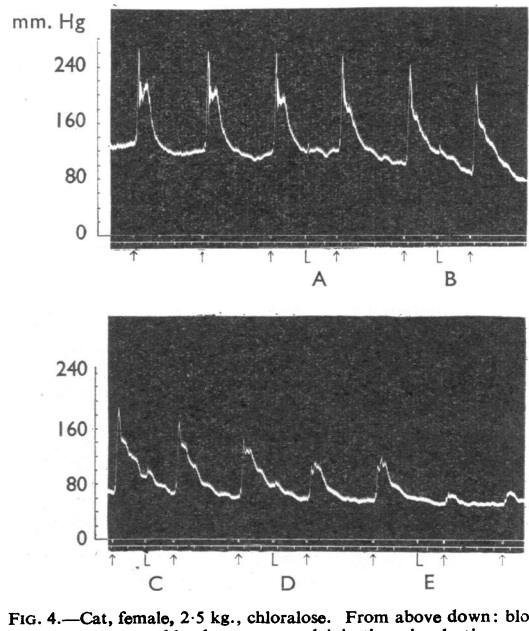



FIG. 4.—Cat, female, 2·5 kg., chloralose. From above down: blood pressure; zero blood pressure and injection signal; time, min. At arrows dimethylphenylpiperazinium, 10 μg./kg. At A, pempidine, 10 μg./kg.; B, 10 μg./kg.; C, 20 μg./kg.; D, 40 μg./kg.; E, 80 μg./kg.

to 0.055 ; 9 cats) ; 26539, 0.059 (0.042 to 0.083 ; 13 cats) ; hexamethonium, 0.037 (0.018 to 0.063 ; 4 cats) ; tetraethylammonium, 0.156 (0.093 to 0.262 ; 4 cats). The salts were those used in determinations of potency on nictitating membrane responses.

These results obtained with pempidine, 26539 and mecamylamine agree quite well with those obtained on the nictitating membrane, when allowance is made for the fact that the ID50 of pempidine was not significantly different from that of 26539 in either test. It seems possible that hexamethonium was more active than mecamylamine in the dimethylphenylpiperazinium test, and less active than mecamylamine in the nictitating membrane test. The number of cats used for the dimethylphenylpiperazinium test with hexamethonium was insufficient to justify great confidence in this finding, but we have certainly found no evidence for the opinion of Bennett *et al.* (1957) that the effect of mecamylamine on blood pressure is greater than would be expected from a comparison of its ganglion-blocking action with that of hexamethonium. However, they suggested that mecamylamine might have a central hypotensive action ; such an action would not have been observed under our experimental conditions.

*Effects on Pressor Responses to Acetylcholine, Carotid Occlusion, Efferent Splanchnic Stimulation, and Noradrenaline.*—Fig. 5 shows the effect of pempidine (0.1 mg./kg.) on the pressor response to acetylcholine in the atropinized cat, Fig. 6 that of pempidine (0.2 mg./kg.) on the response to carotid occlusion, and Fig. 7 that of 26539 (0.2 mg./kg.) on the response to efferent splanchnic

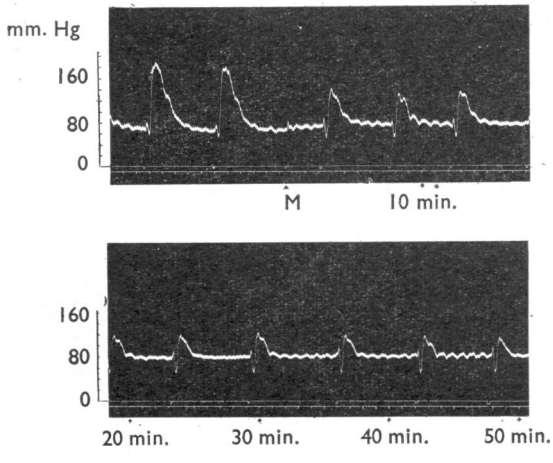



FIG. 5.—Cat, male, 2·8 kg., chloralose. Atropine sulphate, 4 mg. total. Records as Fig. 4. Effect of pempidine (M), 0·1 mg./kg., on responses to acetylcholine, 0·4 mg., at each signal.

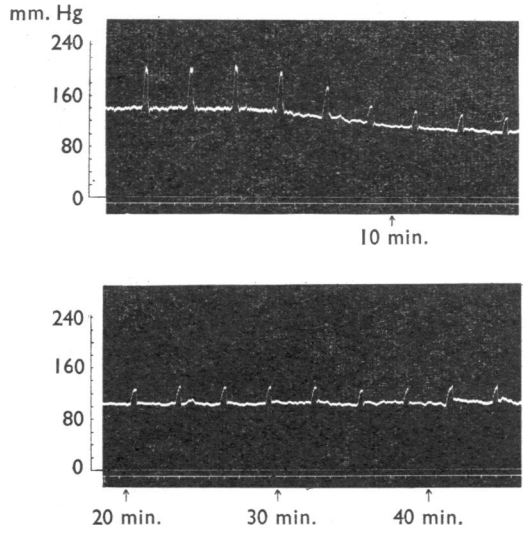



FIG. 6.—Cat, male, 2·7 kg., chloralose. Response of blood pressure to bilateral carotid occlusion (30 sec.). Records as Fig. 4. At signal, effect of pempidine, 0·2 mg./kg.

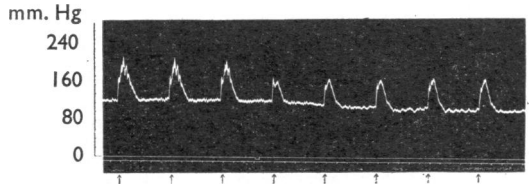



FIG. 7.—Cat, female, 2·7 kg., chloralose. Records as Fig. 4. At arrows, response of blood pressure to efferent stimulation of the right splanchnic nerve for 30 sec. At signal, effect of 26539, 0·2 mg./kg. The brisk vasoconstrictor response was unimpaired by 26539.

stimulation. Our results suggested that doses of 0.1 to 0.2 mg. of pempidine or of 26539 or of 0.2 to 0.4 mg. of mecamylamine/kg. markedly antagonized pressor responses to carotid occlusion and acetylcholine. The duration of the antagonism with each drug seemed similar to that observed in experiments on the nictitating membrane or on responses to dimethylphenylpiperazinium. The brisk response to splanchnic stimulation (partly mediated by postganglionic fibres) was very much less affected than was any other pressor response, and, upon evidence derived from experiments with four cats given pempidine and 26539, we considered that the slow response was probably somewhat less affected than responses to dimethylphenylpiperazinium, acetylcholine or carotid occlusion. Further experiments are required to clarify this problem.

Fig. 8 shows that mecamylamine and 26539, each given in high dosage (5 mg./kg.), failed com-

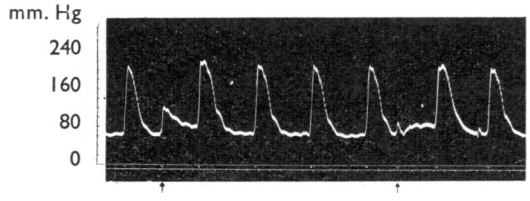



FIG. 8.—Cat, chloralose. Records as Fig. 4. Response of blood pressure to serial doses of noradrenaline, 2 μg., at signal. Effects of mecamylamine (first arrow), 5 mg./kg., and 26539 (second arrow), 5 mg./kg.

pletely to affect the pressor response to noradrenaline. In another experiment pempidine also had no effect.

These experiments support the view that the peripheral actions, at least, of the three compounds occur at the ganglion. Our experiments neither demonstrate nor disprove the existence of a central hypotensive action, but we are inclined to think that a powerful action of this kind is unlikely because of the similarity of effects on the carotid occlusion reflex to those on responses to hypertensive drugs.

*Heart Rate.*—Fig. 9 shows typical effects of pempidine (0.2 mg./kg.) given intravenously on the resting heart rate, and on the bradycardia and hypotension elicited by efferent vagal stimulation. In other experiments, mecamylamine (0.4 mg./kg.) by the same route had similar effects. The resting heart rate fell; the bradycardia and hypotension elicited by vagal stimulation were completely blocked. The latter action could be attributed to block of parasympathetic ganglion cells in the heart; the cause of the fall of the resting heart rate is less certain. A ganglion-blocking drug would be expected to cause bradycardia when control of the pacemaker is predominantly sympathetic, and tachycardia when control is predominantly parasympathetic, and it can be assumed that the bradycardia invariably observed in the cat is partly caused by block of the stellate

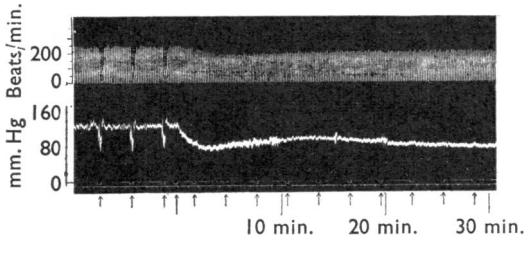



FIG. 9.—Cat, male, 2·6 kg., chloralose. From above down: heart rate (beats/min.); blood pressure record; zero blood pressure and signal; time, min. At small arrows efferent vagal stimulation (20 sec.); at large arrow, pempidine, 0·2 mg./kg.

ganglia, though we have not demonstrated this directly. Doubt whether this is a complete explanation arises from the observation (discussed below) that pempidine, mecamylamine and 26539 all cause a fall in rate of beat of the isolated, perfused rabbit heart. This directly-caused bradycardia may possibly contribute to that observed in the anaesthetized cat, though the concentrations that elicit it *in vitro* are high.

*Effects on Gastric Secretion.*—Mecamylamine, pempidine and 26539 as hydrochlorides powerfully inhibited spontaneous gastric secretion of the rat. The results are given in Table II. When the

TABLE II

DOSES OF GANGLION BLOCKING AGENTS THAT HALVE THE VOLUME, OR FREE ACIDITY OF THE SPONTANEOUS GASTRIC SECRETION OF THE RAT

s.c.=subcutaneous; i.d.=intraduodenal. 95% confidence limits may be calculated by multiplying and dividing the mean by 1·3 (vol.) or 1·5 (acid).

| Comp. | Route | No. of Rats | ID50 (mg./kg.) | |
|---|---|---|---|---|
| | | | Vol. | Free Acid Conc. |
| Mecamylamine hydrochloride | s.c. | 16 | 4·9 | 19 |
| | i.d. | 12 | 9·7 | 22 |
| Pempidine hydrochloride | s.c. | 12 | 2·9 | 12 |
| | i.d. | 12 | 10 | 22 |
| Pempidine hydrogen tartrate | s.c. | 22 | 4·8 | 15 |
| | i.d. | 12 | 7·6 | 23 |
| 26539 hydrochloride | s.c. | 22 | 1·9 | — |
| 26539 hydrogen tartrate | s.c. | 12 | 2·8 | 6 |
| | i.d. | 12 | 3·7 | 12 |

effects of the subcutaneous injection of the drugs on the volume of secretion are compared the two piperidines appear between one and a half and two and a half times as active as mecamylamine. Doses which had effects by intraduodenal administration were only slightly higher than those active by subcutaneous administration, suggesting that oral absorption should be excellent (Lee *et al.*, 1958). The tartrates of pempidine and 26539 usually appeared to be less effective than the hydrochlorides, by either route; pempidine given intraduodenally, however, seems to be an exception.

The three drugs also reduced the concentration of free acid, though the doses required were between two and a half and four times those that reduced the volume. Doses that reduced the concentration of free acid usually raised the concentration of total acid by 15 to 20%.

*Effects on Excretion of Faeces by Mice.*—Weights of faeces excreted by mice during the 5-hr. observation period varied widely, though all mice in a single experiment had a common food

and water supply during the preceding 24 hr. It was necessary to use over one thousand mice for the series of tests on six compounds. The results are shown in Table III. In spite of the large numbers used, the accuracy of each estimate of ID50 was low.

TABLE III

EFFECTS OF COMPOUNDS ON FAECAL WEIGHTS

ID50 is the dose reducing weight of faeces excreted in five hours to half and is recorded with 95% confidence limits.

| Expt. Series | Comp. | No. of Mice | ID50 |
|---|---|---|---|
| 1 | Mecamylamine hydrochloride .. | 336 | 1·4 (0·9–2·3) |
| | Chlorisondamine chloride .. | 180 | 10 (5·8–17) |
| | Pentolinium bromide .. .. | 156 | 6·5 (4·7–10) |
| | Hexamethonium bromide .. | 96 | 40 (32–49) |
| | Pempidine hydrochloride .. | 354 | 1·5 (0·9–2·3) |
| 2 | Mecamylamine hydrochloride .. | 72 | 0·8 (0·5–1·3) |
| | 26539 hydrogen tartrate.. .. | 72 | 0·4 (0·2–0·9) |

Small oral doses of mecamylamine, pempidine, and 26539 reduced gastrointestinal movement as judged by the weight of faeces excreted within 5 hr. All three drugs were much more active than chlorisondamine, pentolinium or hexamethonium. The low activity of these quaternary compounds given orally might be ascribed either to poor absorption and failure of unabsorbed compound to act directly, or to low intrinsic activity. Further information on this important point could probably be obtained by estimating ID50 by our method for a number of compounds given subcutaneously, intramuscularly or intravenously as well as orally. We consider on the basis of the present observations, and of values of oral and parenteral LD50, that the most probable reason for the high activity of pempidine and mecamylamine is their excellent absorption, rather than exceptionally high intrinsic potency on the gastrointestinal tract.

*Effects on Saline Diuresis in the Mouse.*— During each of the experiments on faecal excretion, urine volumes were measured at 30 min. intervals. The total excreted in 5 hr. has been used to compare effects of drugs. The main findings were as follows.

Mecamylamine had little effect on urine volume when doses smaller than about 8 mg./kg. were given. Higher doses had "antidiuretic" effects. The urine volume was halved by approximately 25 mg./kg. and very little urine was excreted after 40 mg./kg. Pempidine seemed to have less "antidiuretic" action and doses as high as 40 mg./kg. reduced the urine volume by only 30%. The ID50 calculated by extrapolation was about 80 mg./kg.

Chlorisondamine chloride had no effect in doses up to 9 mg./kg. Very little urine was excreted after doses of 45 mg./kg. The ID50 was about 25 mg./kg. Pentolinium hydrogen tartrate had no effect in doses up to 5 mg./kg. Very little urine was excreted after doses of 50 mg./kg. The ID50 was about 25 mg./kg. Hexamethonium had no effect in doses of 55 mg./kg.; nor had 26539 in doses of 5 mg./kg.

The cause of the reduction in urine volume has not been investigated, but is presumed to be difficulty of micturition. Pempidine and mecamylamine strongly inhibit the bladder contraction elicited by dimethylphenylpiperazinium and probably reflex contraction. It seems possible that mecamylamine and pempidine have some advantage over pentolinium and chlorisondamine in this respect, if allowance is made for the supposed poor absorption of the last two drugs when they are given orally.

Pempidine, though more potent than mecamylamine by most tests hitherto discussed, caused much less urinary retention.

*Actions on Responses to Agonists* in vitro.— Each result is expressed as the negative logarithm ($pA_2$) of the molar concentration of antagonist that reduced the effect of $2x$ $\mu$g. of agonist to that shown by $x$ $\mu$g. of agonist in the absence of antagonist (Schild, 1947). The results are given in Table IV.

TABLE IV

ANTAGONISM OF VARIOUS AGONISTS BY GANGLION BLOCKING DRUGS

Adrenaline and noradrenaline were tested on the rat seminal vesicle preparation, other agonists on the guinea-pig ileum preparation. NE indicates no effect at stated molar concentration. The number of experiments is given in parentheses.

| Agonist | $pA_2$ | | |
|---|---|---|---|
| | Mecamylamine | Pempidine | 26539 |
| Dimethylphenylpiperazinium .. .. .. | 5·75 (3) | 6·15 (4) | 6·00 (9) |
| Acetylcholine .. .. | 3·55 (5) | 3·35 (3) | 3·40 (4) |
| Noradrenaline .. .. | NE 3·30 (3) | NE 3·30 (3) | 3·60 (2) |
| Adrenaline .. .. | NE 3·30 (3) | NE 3·30 (3) | 3·85 (4) |
| 5-Hydroxytryptamine .. | NE 3·30 (2) | NE 3·30 (2) | NE 3·30 (2) |
| Histamine .. .. | 3·60 (1) | NE 4·20 (1) | NE 4·20 (1) |

All three compounds were powerful antagonists of dimethylphenylpiperazinium; mecamylamine was the least active, as in every other test of ganglion blocking activity used. All three were very feeble antagonists of acetylcholine; the concentrations recorded can have no relevance *in vivo*. Compound 26539 was a feeble antagonist of noradrenaline and adrenaline; mecamylamine and pempidine were not. No compound was an antagonist of 5-hydroxytryptamine. Mecamylamine was a very feeble antagonist of histamine.

*Actions on Isolated Rabbit Duodenum.*—Mecamylamine in a concentration of 40 mg./l. reduced the tone of spontaneously contracting rabbit duodenum, while 80 mg./l. reduced both tone and amplitude. Compound 26539 (80 mg./l.) had only small effects on tone.

Though these experiments might have been taken to suggest an advantage of piperidines over mecamylamine, we thought that the concentrations used would be unlikely to be achieved *in vivo*, and discontinued the experiments.

*Actions at the Neuromuscular Junction.*—Pempidine and 26539 had only very feeble blocking actions at the neuromuscular junction, being 100 times less potent than at the autonomic ganglion. Intravenous doses of 10 mg./kg. caused a protracted reduction in the twitch of cat gastrocnemius muscle elicited by sciatic stimulation by 20 to 30%. Doses of between 2.5 and 5 mg./kg. of either drug had little or no effect alone but seemed to potentiate the effect of tubocurarine and had some effect when given during the action of a dose of tubocurarine (Fig. 10). In an extensive study of the mode of action of mecamylamine at the neuromuscular junction, Bennett *et al.* (1957) obtained similar results (see also Stone, Torchiana, Navarro, and Beyer, 1956).

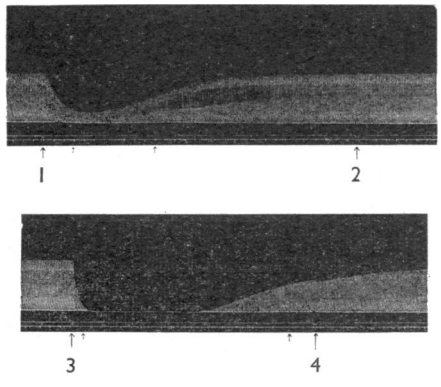



FIG. 10.—Cat, male, 3·9 kg., chloralose. From above down: twitch of gastrocnemius muscle elicited by stimulation of the sciatic nerve; injection signal; time, min. Injections at signals: (1) tubocurarine, 0·2 mg./kg.; (2) pempidine, 5 mg./kg.; (3) tubocurarine, 0·2 mg./kg.; (4) pempidine, 2·5 mg. kg. The two records were continuous. Artificial respiration was given between small arrows.

The actions of mecamylamine and 26539 on the rat phrenic nerve-diaphragm preparation of Bülbring (1946) were also studied. Mecamylamine (40 mg./l.) reduced the twitch height to 8% of its initial value within 6 min. Nine washes each lasting 30 min. were necessary to restore the twitches to their initial height. Compound 26539 had no effect at 40 mg./l., but at 80 mg./l. caused a very slow reduction of contraction, ending in complete block after 40 min. Though positive effects were obtained, the concentrations used were very large, and the experiments were therefore discontinued.

*Cause of Death.*—Large intravenous doses of 26539 and pempidine hydrogen tartrates were given to one and three anaesthetized cats, respectively. As their effects were qualitatively identical, only those of pempidine will be described. Serial doses of 10 mg./kg. were administered intravenously at intervals of 5 min. until death occurred (Figs. 11 and 12). In each of the three experiments irregular gasping respiration occurred after 70 to 80 mg./kg. had been injected and was succeeded by apnoea after about 100 mg./kg. had been injected in two cats to whom artificial respiration was not given (Fig. 11). The mean blood pressure fell after the first dose to about 100 mm. and no further fall occurred: indeed, the pressure tended to rise slowly. In those two cats in which apnoea was allowed to occur, there was a more pronounced increase in blood pressure during the last 10 to 15 min. of dyspnoea (Fig. 11). The circulation deteriorated rapidly after the respiration stopped. Before this the condition of the heart, as judged from the electrocardiogram, lead II, remained normal except for bradycardia, a

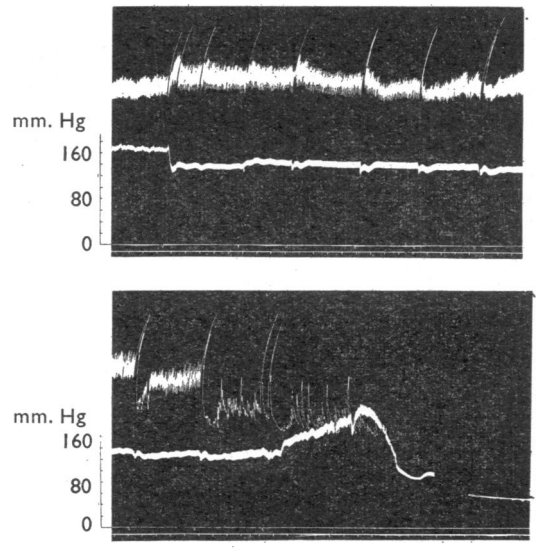



FIG. 11.—Cat, female, 3·2 kg., chloralose. From above down: respiration; blood pressure; zero blood pressure and injection signal; time, min. At signals 10 mg. pempidine/kg., intravenously. No artificial respiration.

lengthening of the PR interval and slight depression of the T wave. In another cat in which artificial respiration was given as soon as dyspnoea was marked, a large dose of pempidine (in all 120 mg./kg.) had no effect on heart or blood pressure other than that seen during the earlier stages of the experiment (Fig. 12). There was no late hypertensive phase. Artificial respiration was then stopped, and the cat died in the same manner as the other two.

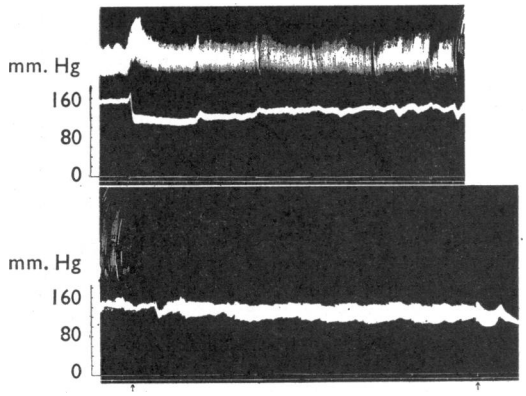



FIG. 12.—Cat, male, 3·5 kg., chloralose. Records as Fig. 11. At signals pempidine, 10 mg./kg., intravenously (total dose 120 mg./kg.). Artificial respiration was given between the arrows.

The cause of the respiratory changes is not known. Obvious possibilities are a central action, or an action at the neuromuscular junction. The experiments with the gastrocnemius muscle suggest that part, at least, of the respiratory paralysis may be due to neuromuscular block.

The cause of the late hypertensive phase is also unknown. As it only occurred in the two cats not given artificial respiration, it may be asphyxial in origin. Whatever its cause it presumably involves some activity of the sympathetic system, which must therefore be incompletely blocked in spite of a dose of pempidine one thousand times greater than the minimal doses causing a moderate hypotension. Payne and Rowe (1957) gave mecamylamine to cats in serial doses of 10 mg./kg., and observed transient but marked hypotensive responses to each new dose, although, as in our own experiments, the general level of pressure tended to rise slowly. We did not see these transient responses after pempidine and consider that, under these conditions, the actions of mecamylamine and pempidine are different.

*Actions on Perfused Rabbit Hearts.*—Bennett *et al.* (1957) showed that mecamylamine reduces the rate and amplitude of beat of the isolated hearts of cat, rabbit and guinea-pig. We have confirmed these observations on the rabbit heart and have also found that mecamylamine reduces the coronary flow (Fig. 13). Pempidine and 26539 have qualitatively similar actions, though pempidine is less active.

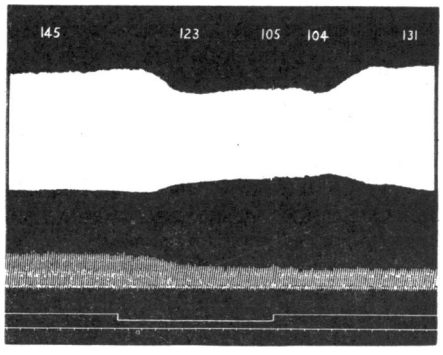



FIG. 13.—Isolated rabbit heart preparation perfused with Ringer-Locke solution. From above down: rate; amplitude; coronary flow; perfusion signal; time, min. During signal, heart perfused with mecamylamine, 20 mg./l.

Twelve isolated rabbit hearts were perfused with Ringer-Locke solution. A fresh preparation was used for each experiment with each concentration of each drug. During perfusions with 10 mg./l. the following maximal % effects were observed on amplitude, rate and coronary flow respectively: mecamylamine, −20, −30, −30 (2 hearts); 26539, −14, −16, −33 (4 hearts); pempidine, 0, −6, +10 (1 heart). At 20 mg./l. the effects were: mecamylamine, −54, −10, −26 (2 hearts, no rate determination in one); 26539, +14, −16, −46 (1 heart); pempidine, −19, −4, −8 (1 heart). The effects of pempidine at 40 mg./litre were: −37, −16, −20 (1 heart).

On the basis of these few observations, it seems that pempidine is about one quarter to one half as toxic as mecamylamine to the isolated heart and that compound 26539 is not much less toxic than mecamylamine.

*Tremor and Other Central Actions.*—Tremor is one of the most important clinical side effects of mecamylamine, though the reported incidence is not high. It is also interesting because it might be caused by interference with central transmission by acetylcholine. We have studied the comparative liability of mecamylamine, pempidine and 26539 to cause tremor and have been able to induce tremor with high doses of all three agents in mice, rats and dogs. We have done no more than record its occurrence in rats, but have carried out comparative experiments in mice and dogs.

The scoring method of assessing tremor in mice, when all tests were carried out by a single, highly skilled observer who never knew which drug had been given to any group, gave surprisingly consistent results. For instance in three experiments, each of which included three doses of each drug, the three doses in each experiment were always scored in the expected order and the means from the three experiments provided excellent plots of effect against log dose. It was often found easier to award scores if the mouse was placed in the palm of the hand, but care had to be taken to distinguish tremor from respiratory movements.

The highest possible hypothetical score was 35, which would have represented the occurrence at each time of observation of a continuous violent tremor. A score of 20 was selected for the calculation of active doses, and usually represented a protracted, readily perceptible but rather fine, rapid tremor. The amounts of the drugs (expressed as mg./kg. by mouth) calculated to give this score with their 95% confidence limits were: mecamylamine hydrochloride, 38 (30 to 48); pempidine hydrogen tartrate, 100 (79 to 126); 26539 hydrogen tartrate, 81 (64 to 102).

The higher toxicity of mecamylamine in this respect was confirmed by studies during chronic toxicity tests in beagle hounds, described in detail later. Two groups, each of two bitches and one male, received 26539 and pempidine, 25 mg./kg., and two males received mecamylamine, 25 mg./kg. Other males and bitches received 5 mg./kg. of pempidine or 26539 and at no time did these dogs exhibit tremor. Both dogs receiving mecamylamine showed tremor from about the fourth day onwards. In one (981), it had become severe by the fourth week and was of a very coarse, flapping type (see Perry and Schroeder, 1957) and was associated with a marked abnormality of stance and gait; though the drug was withdrawn on the fifth day of the fourth week, the dog became comatose and died two days later. The other dog receiving mecamylamine (980) showed moderate tremor throughout the eight weeks during which it received the drug. Although this tremor was inconstant, it could always be seen on occasions during any period of observation longer than a few minutes and often seemed to simulate what has been described clinically as intention tremor, since it almost invariably occurred just before the dog stood or moved. The tremor was of the moderately coarse but never the flapping type.

Neither of the bitches which received 25 mg./kg. of pempidine showed tremor at any time. On rare occasions a very mild tremor, barely perceptible, was observed in one of the two bitches which received 25 mg./kg. of 26539. The two dogs receiving 25 mg./kg. of 26539 or pempidine usually showed tremor during any short period of observation, but the tremor was much less marked than that seen in dog 980: episodes of tremor were invariably very mild and very brief. Cinematograph records were made of the signs in these two dogs and in the two dogs receiving mecamylamine.

We did not attempt to induce more pronounced tremor by giving larger doses of pempidine or 26539, since a preliminary experiment had shown that higher doses caused ocular discomfort and since our experiments seem to have established that pempidine and 26539 were both capable of inducing tremor but were much less active than mecamylamine in this important respect.

With the central effects of hyoscine and benactyzine in mind, it is of interest to enquire whether pempidine, 26539 and mecamylamine, which may interfere with the activity of central cholinergic neurones, might give rise to signs of central effects other than tremor, particularly in view of the clinical reports of the central actions of mecamylamine—for example, Perry and Schroeder (1957). We have not seen, however, striking behavioural changes in mice, rats or dogs receiving any of the three ganglion-blocking drugs. All dogs receiving these drugs, except 980 and 981, remained bright, alert and friendly throughout the chronic toxicity tests. In dog 981 listlessness and withdrawal were observed, but could be considered secondary to the motor disturbance and to the deterioration in general condition. The same signs were also seen to a lesser extent in dog 980.

Rats and mice displayed what might be described as stupor after single doses of 25 mg./kg. of pempidine or 10 mg./kg. of mecamylamine intraperitoneally. The animals were readily aroused, and might well be displaying no more than a response to the discomfort which such doses might be expected to cause. Oral doses of 25 mg./kg. of the three drugs had no effect on the mean sleeping time of mice given hexobarbitone (100 mg./kg. intraperitoneally) 2 hr. later. Benactyzine (5 mg./kg.) caused a large increase in sleeping time under these conditions.

All three drugs raised slightly the threshold to electrical convulsions when given to rats in doses of 10 to 50 mg./kg. Mecamylamine (50 mg./kg.) appeared to protect 3 rats out of 10, the protection being that defined by Bogue and Carrington (1953) in their description of this test. All three drugs also had a curious effect not previously

observed in these laboratories during studies of the action of several hundred potential anticonvulsant drugs. Consciousness was lost by rats during and for a short time after electrical convulsions and respiration was briefly depressed. Rats receiving non-lethal doses of 5 to 25 mg./kg. of 26539 or 5 to 50 mg./kg. of mecamylamine or pempidine often failed to breathe again after their convulsions. The approximate LD50 was 25, 17 and 25 mg./kg. for the three drugs. A possible theoretical explanation is that the cause of these deaths was respiratory depression, not lethal by itself but made so by the addition of post-convulsant depression.

We have confirmed the finding of Stone, Mecklenburg and Torchiana (1956) that mecamylamine prevented convulsions induced by nicotine, an action which we have also found with pempidine and 26539. Tremor was not prevented. Though 470 mice were used there were considerable differences between the ED50 values obtained in two different experiments. Pempidine and 26539 seemed to be somewhat more active than mecamylamine; hexamethonium was about one-fifth to one-tenth as active as mecamylamine. We are continuing other studies of the central actions of these drugs.

*Local Anaesthetic Tests.* — Mecamylamine hydrochloride, pempidine and 26539 (as hydrogen tartrates) were used for all local anaesthetic tests. All concentrations given are w/v.

1% solutions of the three compounds applied to the rabbit eye did not affect the corneal reflex. Stone, Torchiana, Navarro and Beyer (1956) obtained a similar result with 1% mecamylamine.

Injection of 0.05 ml. of a 2% solution of each of the three drugs subcutaneously into the tail root (Bianchi, 1956) had no local anaesthetic effect as judged by the aversive response of the mice to tail pinching and produced no toxic signs. Injection of the same volume of a 4% solution of each drug had apparent small, brief local anaesthetic effects, but this concentration caused severe prostration. We concluded that local anaesthetic action was not demonstrated by these experiments. Lignocaine 2% gave complete local anaesthesia with this method.

The method of Bülbring and Wajda (1945) gave evidence of local anaesthetic activity. Solutions of the three ganglion blocking agents or of procaine hydrochloride were injected intradermally into guinea-pigs in volumes of 0.25 ml. Four positions on each of 16 guinea-pigs were used and doses were allotted from a Latin square design. The reflex responses to a total of 48 pricks in each weal area, given in 8 blocks of 6 during 40 min., were halved by the following concentrations of bases (derived graphically): procaine, 0.15%; mecamylamine, 0.30%; pempidine, 0.35%; 26539, 0.45%. In some experiments tests were continued at intervals from 40 min. to 4 hr. All effects were reversible. That of procaine (0.25%) faded between 1 and 2 hr., that of mecamylamine (0.5%) between 40 min. and 1.5 hr., and those of 26539 and pempidine (0.5%) between 30 and 80 min.

Compound 26539 (0.25% or 2.5%) when applied to isolated frog sciatic nerve between stimulating and recording electrodes caused an increase of about 10% in the height of the action potential elicited by supramaximal stimulation. Mecamylamine (1 or 2.5%) caused a slow fall of action potential to about 80% of the control height after 10, 20% after 20, and 0% after 30 min.; 0.25% solutions had little effect. This change was irreversible though nerve was left to soak in fresh frog Ringer solution for as long as 2 hr. Consequently it is uncertain whether the action of mecamylamine is to be regarded as toxic or anaesthetic and further study is desirable.

The likeliest explanation of our results is that the three compounds potentially have local anaesthetic action but are unable readily to penetrate nerve trunks. In the Bülbring and Wajda (1945) test, the drugs are brought into intimate contact with nerve endings, with single fibres and with collections of a few fibres. Their powerful action in this test presumably reflected their ability to penetrate one, some, or all of these anatomical entities.

*Acute Toxicity.*—Results of acute toxicity determinations are given in Table V. Some of the values given earlier by Spinks and Young (1958) have been modified by additional experiments. The values for mecamylamine are nearly identical with those given by Stone, Torchiana, Navarro, and Beyer (1956), but those for pempidine are higher than the values recently published by Lee *et al.* (1958).

Mecamylamine was more toxic than 26539 and 26539 was more toxic than pempidine. The hydrogen tartrates and hydrochlorides were equally toxic. All compounds were well absorbed orally, as judged by the ratio of oral to intravenous LD50. In this respect, the results agree well with those on gastric secretion and with those of biochemical studies of Lee *et al.* (1958). However, the ratio of oral to intravenous toxicity was somewhat lower for mecamylamine than for

TABLE V

ACUTE TOXICITY OF COMPOUNDS

All doses expressed as base. The number of separate experiments contributing to each mean was 3 unless otherwise shown in parentheses after the number of mice. 95% confidence limits follow LD50 values in parentheses.

| Comp. | Salt | Intravenous | | Intraperitoneal | | Oral | |
|---|---|---|---|---|---|---|---|
| | | Total No. of Mice | LD50 (mg./kg.) | Total No. of Mice | LD50 (mg./kg.) | Total No. of Mice | LD50 (mg./kg.) |
| Pempidine .. .. .. | Hydrogen tartrate | 57 | 64 (50–83) | 55 | 93 (65–134) | 110 (5) | 412 (292–581) |
| | HCl | 39 | 74 (57–96) | 59 | 125 (87–179) | 99 (5) | 413 (293–582) |
| 26539 .. .. .. | Hydrogen tartrate | 54 | 37 (28–48) | 80 | 43 (30–62) | 85 (5) | 139 (89–218) |
| | HCl | 53 | 43 (33–56) | 56 | 48 (33–69) | 81 (4) | 148 (101–218) |
| Mecamylamine .. .. | HCl | 45 | 21 (17–28) | 54 | 37 (25–53) | 84 | 96 (62–151) |

pempidine, which may therefore be less rapidly absorbed or more rapidly eliminated.

*Chronic Toxicity to Rats.*—Preliminary tests in which pempidine, 26539, and mecamylamine were given by catheter once on each of 5 days a week for 2 weeks to groups of 6 rats, showed that the maximum tolerated daily dose of each was of the order of 50 mg./kg. This dose caused loss of weight (see Lee *et al.*, 1958) and condition, but did not kill any animals. Liver and kidney were unaffected by the drugs.

The most convenient method of administering soluble drugs for long periods is to dissolve them in the drinking water, and we next carried out a pilot test in which each of the drugs was so administered at a concentration of 0.025% to groups of 5 rats. The control water intake of 208 ml./kg. (giving an expected daily dose of 52 mg./kg.) fell immediately to 65 ml. in the mecamylamine group, 72 ml. in the pempidine group, and to 48 ml. in the 26539 group. After 8 days these intakes had risen to 190, 168, and 170 ml. respectively, but by this time in the respective groups 1, 2, and 3 rats had died. We thought that dehydration probably contributed to the cause of these deaths, and the experiment was abandoned.

The full scale test on rats was conducted in the following manner. Each group of 10 male and 10 female rats (90 to 100 g.) received drug in the diet. Three groups received 26539 in concentrations of 0.05, 0.015, and 0.005%, three received pempidine in the same concentrations, and two received mecamylamine in concentrations of 0.05 and 0.15%. Individual weights and the food intakes of each half group were recorded daily for the first 2 weeks. Thereafter, food intakes were recorded at weekly, and weights at 3 to 4 day intervals. Half the rats were killed and submitted to autopsy at 8 weeks; the remainder were maintained on the drugs for fertility and carcinogenic tests. Fertility tests were commenced at 17 weeks, experimental females in oestrus (as indicated by daily vaginal smears) being mated with normal males, and experimental males with normal females in oestrus. Females of all groups have given litters. The results of tests on males are incomplete. These experiments are still in progress. Body weights and food intakes become unreliable after 17 weeks and have not been reported. Deaths occurred only in the groups receiving 0.05% of drugs. In the 26539 group one male died on the 3rd day; a second developed paralytic ileus in the 11th week and was killed; a third died in the 18th week. One female died in the 7th week. In the pempidine group one male developed paralytic ileus in the 14th week and was killed. In the mecamylamine group one male was in very bad condition in the 9th week and was killed. This experimental induction of paralytic ileus is, as far as we are aware, the first to be reported. It is interesting that it was observed only after treatment for between three and four months with these relatively enormous doses of drugs, being about 50 times those causing considerable faecal retention in mice.

The food consumption of all experimental groups dropped sharply during the first few days of drug administration. The averages of the experimental groups for the first 3 days ranged between 53 and 72 g./kg. against 100 g./kg. for the control group. Thus, concentrations as low as 0.005% were readily detected by the rats and disliked. By the end of the first week intakes in g./kg. were again similar to those of the control groups and they remained similar up to the 17th week. This means that the animals that grew least ate least.

Control intakes fell steadily from between 100 g./kg. and 92 g./kg. in the 3rd week, to 82 in the 6th, 77 in the 9th, and 62 in the 14th week. Drug intakes therefore fell correspondingly, for example, from 50 mg./kg. at the beginning to about 30 mg./kg. in the 14th week in the groups receiving the highest doses. Generally, females ate about 10% less (g./kg.) than males in all

groups and therefore received slightly lower doses. The values given are means of the results in the two sexes.

The effects of the drugs on growth are shown in Table VI. The effects of mecamylamine were much the most severe; those of pempidine were very slight. We estimate that the concentrations in food causing a 15% reduction in weight gain at the 8th week were: mecamylamine, 0.0125%; 26539, 0.029%; pempidine, 0.06%. The plots of effect against log concentration were far from parallel and comparative toxicities in this respect would therefore be different if a different weight reduction were selected.

TABLE VI

GROWTH OF GROUPS OF 20 RATS RECEIVING DRUGS IN THE DIET

Values for males and females are averaged. Half the rats were killed after 8 weeks.

| Drug | % Conc. in Food (w/w) | Mean Weights | | | Weight Gain as % of Control Weight Gain | |
|---|---|---|---|---|---|---|
| | | Day 0 | 8 Weeks | 17 Weeks | 8 Weeks | 17 Weeks |
| Control .. | — | 94 | 200 | 218 | (100) | (100) |
| Mecamylamine | 0·05 | 102 | 157 | 187 | 52 | 69 |
| | 0·015 | 103 | 187 | 206 | 79 | 86 |
| Pempidine .. | 0·05 | 92 | 183 | 210 | 86 | 96 |
| | 0·015 | 94 | 192 | 225 | 92 | 103 |
| | 0·005 | 93 | 195 | 222 | 98 | 104 |
| 26539 .. .. | 0·05 | 92 | 176 | 180 | 79 | 71 |
| | 0·015 | 96 | 192 | 212 | 91 | 94 |
| | 0·005 | 96 | 213 | 234 | 110 | 112 |

Compound 26539 caused an apparent increase in growth at a concentration of 0.005%.

No macroscopic changes were observed at autopsies at the eighth week, and the mean blood cholesterol concentration and the mean haemoglobin concentration then were approximately the same in all groups.

Histological studies by our colleague Dr. Paget of the tissues named in the Method showed no changes attributable to mecamylamine (compare Stone, Torchiana, Navarro, and Beyer, 1956) or pempidine, but compound 26539 caused changes in pituitary and testis which are still under investigation and which have led us to defer clinical trial of this compound.

*Chronic Toxicity to Dogs.*—A preliminary experiment in which mecamylamine, 26539, or pempidine was each given to one beagle hound daily for 4 weeks showed that the highest tolerated dose was determined by the ocular rather than the lethal or gastrointestinal effects. We began by giving 25 mg./kg. daily as tablets. Though some abdominal distension and ineffective straining to defaecate were occasionally observed in each dog, these effects were never more than slight, and appetite appeared to be unimpaired. The ocular effects were pronounced. We observed a dilated pupil in strong light, a relaxation of the nictitating membrane, a block of lachrymal secretion, and signs of what we considered to be a difficulty of accommodation. Secondary to these effects blinking and a mild suppurative conjunctivitis developed, the latter readily controlled by topical treatment with penicillin cream. After 2 weeks it appeared that 25 mg./kg. caused no, or only trifling, discomfort. We then raised the dose to 50 mg./kg., but the ocular signs immediately worsened and began to cause discomfort. The dose was therefore reduced to 25 mg./kg. after 3 days. The only other severe effect seen was dryness of mouth and tongue.

The dogs were killed after 4 weeks and examined *post mortem*.

The large scale chronic test in dogs involved the daily administration, for 5 days a week, of 25 mg./kg. pempidine and 26539, each to two bitches and one male dog, and of 5 mg./kg. of each drug to one bitch and two male dogs. Mecamylamine, 25 mg./kg., was administered to two male dogs. The experiment lasted 8 weeks.

The autonomic effects were closely similar to those observed during the preliminary experiment. The central nervous signs have already been described. We observed less dependence of the autonomic effects on dose than we expected. The ocular effects in particular were readily seen after doses of 5 mg./kg. pempidine or 26539, though the majority of the animals most affected received 25 mg./kg. The three drugs did not differ greatly in severity of action on the eye or on mouth or tongue.

Tolerance to the autonomic though not to the central nervous effects developed. The effects worsened during the first week, remained approximately similar during the second week, and began to decline during the third week. This decline was obvious during the fourth week, but thereafter little further change occurred. All signs could still be seen at any time during the experiment, particularly 1½ to 3 hr. after the time of dosing. The effect that seemed to decline most was dryness of the mouth and tongue.

Two dogs died. One was dog 981. The other, a bitch receiving pempidine, 25 mg./kg., developed what we think was a sterile pneumonia, and died 20 hr. after accidental tracheal dosing.

The initial weight range of the young adults used was 9.5 to 11.5 kg., and the groups were approximately matched. The following weight changes

were observed at 3 and 8 weeks respectively: pempidine, 25 mg./kg., −6.8%, −7.8% ; pempidine, 5 mg./kg., −0.4%, +5.6% ; 26539, 25 mg./kg., −7.7%, −5.6% ; 26539, 5 mg./kg., +8.7%, +12.8% ; mecamylamine, 25 mg./kg., −16.4% (two dogs), −5.5% (one dog) ; controls, +4.6%, +10.8%.

All drugs in doses of 25 mg./kg. caused a moderately severe loss of weight. Compound 26539, at 5 mg./kg., appeared to have promoted growth, and a similar effect was observed in rats given very small doses of 26539. This response might be associated with the pituitary changes caused by 26539, but it requires further study.

Haematological studies were carried out at fortnightly intervals. We could discern no effect of any drug on red or white cell count, or on haemoglobin concentration. The concentrations of cholesterol in blood specimens obtained at the time of autopsy were normal.

No gross changes were seen at autopsy in the dogs which were killed after 4 or 8 weeks, and our colleague Dr. G. E. Paget reported that no histopathological changes were seen in tissues (listed in the Method) of dogs receiving mecamylamine (see also Stone *et al.*, 1956) or pempidine. Histopathological studies of brains, cords, and eyes are still incomplete, and the pituitary glands were damaged slightly at autopsy and their assessment was difficult.

Compound 26539, 25 mg./kg., arrested spermatogenesis at the late spermatid stage in the single male dog that received this dose. This finding has strengthened our opinion that 26539 is unsuitable for trial in man.

## Discussion

Mecamylamine, pempidine, and 1-ethyl-2:2:6:6-tetramethylpiperidine (26539) are qualitatively very similar. All the actions studied, except one discussed below, were common to all three. They are highly potent non-quaternary blocking agents active on sympathetic and on parasympathetic ganglia ; this action was slow in onset but was protracted. They were well absorbed from the gastrointestinal tract. They displayed central nervous actions of which the most obvious and probably the most important sign was tremor. They had feeble actions at the neuromuscular junction ; they reduced the amplitude and rate of beat of the isolated heart and reduced the coronary flow ; they had local anaesthetic activity if penetration was facilitated by intimate contact with nerve fibres or nerve endings. They lacked more than a trifling ability to inhibit excitatory responses elicited *in vitro by* acetylcholine, adrenaline, histamine, or 5-hydroxytryptamine. This novel pattern of action suggests that they may have a novel type of action. We support the mode of action proposed by Bennett *et al.* (1957) for mecamylamine, but are doubtful of the validity of some of their evidence, which was of two kinds, relating on the one hand to ganglia, on the other to the neuromuscular junction. The potency of all three compounds at the latter site was little more than one hundredth of that at the former, and we prefer not to assume that the two actions are allied, though they may be. The ganglionic evidence adduced by Bennett *et al.* (1957) is of two kinds: first, the duration of action of mecamylamine ; second, the maintenance of a sustained, unfatigued, response to preganglionic stimulation during partial mecamylamine blockade. On occasions we have observed a poorly sustained contraction during partial blockade with mecamylamine, and have been inclined to attribute its occurrence to the condition of the cat rather than to a specific ganglionic event. Whether such an effect during blockade by a conventional quaternary compound could be explained on similar lines is doubtful and discussion will be restricted to the evidence relating to the time course.

We have been more impressed by the slow onset of action than by the duration of action. A quaternary or other compound with conventional competitive blocking actions but which was not readily excreted or degraded could have prolonged action. Perhaps chlorisondamine might be such a compound and, if so, the duration of action need not be necessarily related to the mode of action. On the other hand competitive interference with synaptic transmission is almost certainly extracellular (Paton and Zaimis, 1952) and an exceptionally slow onset of interference is unlikely though not impossible. We were struck by the similarity in time-course between the ganglionic-blocking action of pempidine (Fig. 3) and the likely time-course of entry of substances of this $pK_a$ into cells. This view was reinforced by the properties of the closely related compound 1:1:2:2:6:6-hexamethylpiperidinium, the action of which was rapid in onset and brief in duration, and which we presumed acted extracellularly. The interesting hypothesis of Bennett *et al.* (1957), that the postganglionic cell is altered by the entry of mecamylamine so that its responses to acetylcholine, etc., are modified, consequently seems very attractive. There is, however, some evidence against it. Payne and Rowe (1947) have shown that carbon dioxide, administered after very large doses of mecamylamine, caused an increase in

the plasma concentration of mecamylamine, a brisk hypotension, and an increased neuromuscular block. The simplest explanation of these findings, put forward by Payne and Rowe (1957), is that mecamylamine is active in the extracellular phase, and that carbon dioxide, by lowering the plasma *p*H, causes its passage from the cells into the extracellular phase, where increased activity is then displayed. The theory of extracellular action on ganglia could also explain the large transient hypotensive responses which Payne and Rowe (1957) observed after each of the large serial doses of mecamylamine. However, Payne and Rowe (1957) suggested an alternative explanation which is not against the intracellular hypothesis, namely, that there is a direct vasodilator action of $CO_2$ during complete sympathetic blockade, the transient hypotensive responses to each new large dose of mecamylamine being attributed to myocardial depression. We did not see large transient hypotensive responses to pempidine given in large serial doses (Fig. 11). Since pempidine is so similar to mecamylamine in ganglionic actions we think, therefore, that the large hypotensive responses to successive doses of mecamylamine were not ganglionic in origin. The possibility that they were due to an effect on the heart receives some support from our evidence of the lower toxicity to the heart of pempidine. In our opinion, the balance of the present evidence, so far as hypotensive and ganglionic actions are concerned, favours an intracellular action of mecamylamine and pempidine.

Let us turn next to consider the comparative values of the three drugs. Our results in this connexion have been summarized in Table VII, in which comparative activities and toxicities of pempidine and 26539 are expressed as % of those of mecamylamine. Consider first pempidine and mecamylamine. Desirable ganglionic actions, type A, are those relating to the hypotensive action. By the intravenous route, the results of the four tests are in accord, making pempidine about twice as active as mecamylamine. Unwanted ganglionic actions, type B, are those likely to cause clinical side-effects such as constipation, paralytic ileus, tachycardia, urinary retention, etc. Most of our tests appeared to endow pempidine with some advantage over mecamylamine in that it was less than twice as active, but these particular tests involved absorption. Since other results, notably ratios of parenteral to oral toxicity, suggested that pempidine is somewhat less rapidly absorbed (or more rapidly eliminated) than mecamylamine, we think that we have obtained no conclusive evidence of superiority of the former in respect of

TABLE VII

ACTIVITY AND TOXICITY OF DRUGS EXPRESSED AS % EFFECT OF MECAMYLAMINE

Estimates given within brackets are approximate. Where two salts of pempidine and 26539 were examined, only values for hydrogen tartrates are given. Type A actions are those regarded as desirable ganglionic actions; Type B, unwanted ganglionic actions; Type C, unwanted non-ganglionic actions.

| Type of Action | Preparation or Test Used | Activity or Toxicity | |
|---|---|---|---|
| | | Pempidine | 26539 |
| A | Cat nictitating membrane | 240 | 310 |
| | Pressor response to phenyldimethylpiperazinium | 230 | 135 |
| | Pressor response to acetylcholine | (200) | (200) |
| | Carotid occlusion | (200) | (200) |
| B | Vagal bradycardia | (200) | — |
| | Phenyldimethylpiperazinium on guinea-pig ileum *in vitro* | 250 | 180 |
| | Gastric secretion, s.c. | 100 | 260 |
| | Gastric secretion, intraduodenal | 130 | 260 |
| | Faecal weights | 110 | 200 |
| | Urinary retention, mouse | 30 | — |
| C | Tremor, mouse | 40 | 50 |
| | Acute toxicity: LD50, i.v. | 35 | 55 |
| | ,, ,, i.p. | 40 | 85 |
| | ,, ,, oral | 25 | 70 |
| | Rat growth | 20 | 45 |
| | Local anaesthesia | 85 | 65 |
| | Cardiotoxicity *in vitro* | (40) | — |

actions of type B. This view is supported by the results of the vagal bradycardia and guinea-pig ileum tests, both of which gave potency ratios of about two and neither involved absorption. A possible exception is that in the intact mouse, pempidine caused much less urinary retention than did mecamylamine. Nevertheless, it had as large an effect on the response of cat bladder to dimethylphenylpiperazinium as it had on the pressor response to this drug, and it is possible that the lower potency in the intact animal is due to a non-ganglionic action of one of the two drugs. We conclude that pempidine has no specificity for sympathetic as against parasympathetic ganglia, and that clinical side-effects caused by anti-parasympathetic actions are to be expected.

Unwanted non-ganglionic actions of type C show that pempidine has several important advantages over mecamylamine. For instance, the lethal dose was much higher; the dose causing tremor was higher; the dose depressing rat growth was much higher. If the % potencies in Table VII type C are compared with those derived from ganglionic actions under circumstances involving oral absorption, that is to say, actions on gastric secretion and on faecal weights, then pempidine has a safety margin about three times that of mecamylamine in respect of tremor, five times in respect of death, and six times in respect of general systemic toxicity as indicated by rat

growth. This applies equally to compound 26539. The results in Table VII type B suggest that this drug is probably more rapidly absorbed than pempidine and that the safety margins named above are about 5, 3½, and 5 times respectively. On the one hand, the quantitative findings considered in isolation therefore support clinical test of 26539 equally with that of pempidine. On the other hand, the qualitative histopathological observations of our colleague, Dr. Paget, plainly indicate that such tests on 26539 would be potentially hazardous. Pempidine and mecamylamine were remarkable in that they lacked toxic actions that are demonstrable histopathologically whereas 26539 caused changes in the pituitary gland and in the testis which entirely precluded clinical trial until these effects and their causes have been elucidated. This evidence has strengthened our opinion that clinical tests based on quantitative findings alone may be dangerous and should not be undertaken. The minimal precautions that we consider proper were summarized recently by Paget and Spinks (1958).

The authors gratefully acknowledge their indebtedness to Miss J. M. Peters, Miss S. Bentley, and Dr. J. W. Black for their help with some experiments, to Mrs. J. Slater, Miss M. Rogers, Mr. B. D. Haydock, Mr. N. Harrison, Mr. J. Dee, and Mr. G. B. Horsfall for technical assistance, and to Dr. O. L. Davies and Mr. M. Gent for advice on experimental design, and for statistical analyses. They gratefully acknowledge the gift of Inversine from Dr. H. Molitor, of Merck, Sharp, and Dohme, Inc.; of Ecolid from Dr. H. J. Bein, of Ciba Laboratories; and of Ansolysen from Dr. R. Wien, of May and Baker, Ltd.

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