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ABSORPTION, METABOLISM AND ELIMINATION OF PEMPIDINE IN THE RAT

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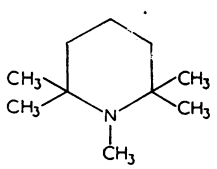
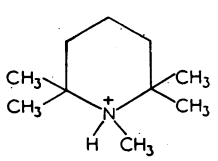
Pempidine (1,2,2,6,6-pentamethylpiperidine) is a ganglion blocking agent introduced recently for the treatment of hypertension by oral administration of its hydrogen tartrate. It can be estimated colorimetrically by coupling with methyl orange, or fluorimetrically by reaction with eosin in xylene, the limits of sensitivity being 0.5 μg./ml. and 0.001 μg./ml. respectively. These methods, combined with appropriate extraction techniques, were suitable for estimating pempidine in aqueous solutions of its salts, in biological fluids and the like, and for investigating the biochemical properties of the drug when given orally to rats in amounts similar to those used clinically.

When administered orally to rats pempidine was rapidly absorbed, the maximum concentration in plasma being attained after 30 min. The drug was preferentially taken up by erythrocytes and a red cell/plasma partition ratio of about 1.2 established with clinical doses. Pempidine was soon distributed throughout the body, including the cerebrospinal fluid, and the highest concentrations were found in kidney, spleen and liver. Pempidine also entered the foetus and passed thence into the amniotic fluid. Protein-binding of the drug occurred only to a very limited extent and there was little evidence that it was metabolized. Pempidine was excreted rapidly in urine during 24 hr. following oral administration.

Pempidine (1,2,2,6,6-pentamethylpiperidine) is well absorbed when given by mouth. It is a tertiary heterocyclic amine (I) and a strong base ($pK_a \sim 11$) which forms a water-soluble hydrochloride and hydrogen tartrate. Under physiological conditions (pH<8) it exists almost entirely (more than 99.9%) in the ionized form (II). The pharmacological properties of this



I



II

compound were first reported by Spinks and Young (1958) and independently by Lee, Wragg, Corne, Edge, and Reading (1958) and by Corne and Edge (1958). The clinical studies by Harington, Kincaid-Smith, and Milne (1958) suggested that pempidine had important advantages in the treatment of hypertension since the drug is freely absorbed after oral administration and is rapidly excreted in the urine.

METHODS

Estimation

Methyl Orange Complex.—Brodie and Udenfriend (1945) estimated various alkaloids and synthetic basic organic compounds in biological material by coupling with methyl orange. This technique was employed by Baer, Paulson, Russo, and Beyer (1956) and again by Allanby and Trounce (1957) in work on mecamylamine, and has now been successfully applied to the estimation of pempidine in biological fluids and tissue extracts.

A master calibration was obtained by adding known amounts of pempidine to ethylene dichloride and treating with the methyl orange reagent. The plot of optical density at 540 mμ against concentration of pempidine was linear up to 50 μg./ml., the lower limit of sensitivity being about 0.5 μg./ml. Standard curves for pempidine in water, urine, plasma, haemolysed red cells, and various tissue homogenates were prepared by adding measured quantities of the hydrogen tartrate to 1 ml. samples of those fluids, making 2 M with respect to sodium hydroxide and extracting with 20 ml. of ethylene dichloride. Methyl orange was added to this extract and the optical density measured at 540 mμ. After appropriate blank corrections, the optical density was a linear function of drug concentration and comparison of these plots with the master

calibration showed that extraction of pempidine from aqueous media into ethylene dichloride was virtually complete (>93%). The technique was rather insensitive but could be used to measure the amount of pempidine in urine from rats which have received the drug orally. However, relatively large doses (>20 mg. base/kg.) were required to produce measurable concentrations of the drug in plasma and tissues.

Eosin Fluorescence.—The methyl orange method could not detect the very low plasma concentrations of pempidine attained after oral administration of the drug in amounts which were effective clinically (0.25 to 0.50 mg. base/kg.) and a more sensitive method of estimation was therefore required. Use has been made of the fact that eosin (tetrabromofluorescein) dissolved in xylene exists in a non-ionized form (HA) which is non-fluorescent. Addition of pempidine (B) produces a proportional change in the reagent to an ionized form which is intensely fluorescent: $HA + B \rightarrow (\overset{+}{B}H)(\overset{-}{A})$. The intensity of fluorescence was proportional to the pempidine concentration, and the technique was extremely sensitive, 0.001 μg./ml. being detectable with the relatively simple equipment shown diagrammatically in Fig. 1. The source of radiation was a 125 watt mercury vapour lamp, **L** (Siemens type MB/D), fed from the constant voltage transformer (**T**). Exciting light of wavelength 545 mμ, isolated by the primary filter F_1 (Ilford type 807), passed through the collimator (**C**) to the fused glass sample cell (**S**). Fluorescence radiation of wavelength 570 mμ emitted by the sample passed through the secondary filter F_2 (Ilford type 808) which absorbs scattered exciting radiation, on to the photomultiplier **P** (RCA type 931 A) connected to the sensitive galvanometer **G** (Cambridge type No. 41127). A stabilized power supply (**V**) was used to operate the photomultiplier. An opaque shutter (**D**) was introduced into the exciting beam when the detector dark current is measured.

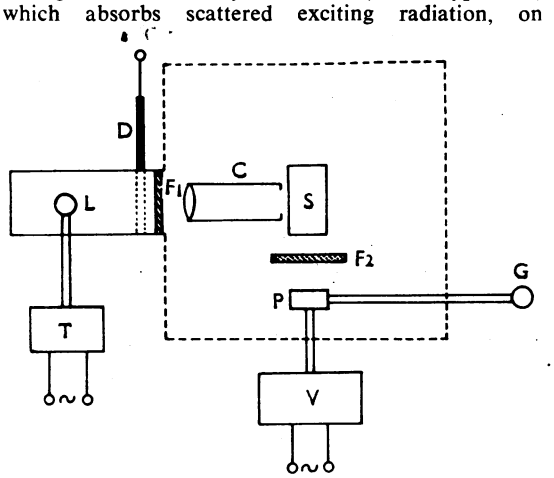


FIG. 1.—Fluorescence apparatus. (L), 125 watt mercury vapour lamp (Siemens type MB/D). (T), Constant voltage transformer. (F_1), Ilford type 807 filter for the selection of exciting light of wavelength 545 mμ. (C), Collimator. (S), Fused glass sample cell. (F_2), Ilford type 808 filter for the absorption of scattered exciting radiation. (P), Photomultiplier (R.C.A. type 931 A). (G), Sensitive galvanometer (Cambridge type No. 41127). (V), Stabilized power-pack to operate the photomultiplier. (D), Opaque shutter.

Eosin was prepared by adding hydrochloric acid to an aqueous solution of the sodium salt (B.D.H.), washing the precipitate until free from acid and drying *in vacuo*. 0.5 g. of the solid was shaken for 1 hr. with 100 ml. of xylene, and the saturated solution filtered. If stored in the dark, the reagent was quite stable for at least a month.

A master calibration was obtained by using suitably diluted standard solutions of pempidine in xylene (15 ml.) and eosin reagent (2 ml.). The plot of fluorescence intensity against concentration of pempidine was linear up to 0.3 μg./ml., the lower limit of sensitivity being approximately 0.001 μg./ml. Standard curves for pempidine in water, plasma, haemolysed red cells, and various tissue homogenates were prepared by adding measured quantities of the hydrochloride to 5 ml. samples of these fluids which were then made 2 M in sodium hydroxide and extracted with ether (15 ml.). The base was taken into M-hydrochloric acid (2 ml.) which in turn was made 2 M in sodium hydroxide, re-extracted with xylene (15 ml.) and finally transferred to the sample cell. Appropriate blank corrections were applied and the fluorescence intensity shown to be a linear function of concentration. Extraction of pempidine from aqueous media by this method was about 75% complete and quite reproducible. The technique cannot at present be employed with urine, which contains interfering substances able to quench fluorescence. Aqueous solutions of pempidine hydrochloride were examined by both methyl orange complex and eosin fluorescence techniques and gave results which agreed within the limits of experimental error. The eosin fluorescence method seemed to offer great possibilities in the field of ultra-micro analysis and full details will be published elsewhere.

Preparation of Samples for Analysis

In most of the experiments described below, pempidine was administered as the hydrogen tartrate, but the hydrochloride was used in experiments designed to investigate its passage into the cerebrospinal fluid. Mecamylamine was used as the hydrochloride. All doses and concentrations of the two drugs are given as base equivalents.

Drug Concentrations in Blood.—Single oral doses of pempidine within the range 0.25 to 20 mg./kg. were administered in aqueous solution by stomach tube to albino rats (100 to 150 g.) which had been deprived of food for 16 hr. The animals were killed at intervals during the ensuing 24 hr. and heparin added to blood obtained by heart puncture. After centrifuging, the volumes of plasma and erythrocytes in pooled samples from two or three rats were

measured and the concentration of pempidine in each estimated by the eosin fluorescence method, the red cells being first diluted with distilled water equal in volume to the plasma removed.

Tissue Distribution.—Pempidine (1 or 10 mg./kg.) was given orally to rats which were killed and exsanguinated at known time intervals after dosage, the various organs then being removed and homogenized in distilled water. Concentrations of the drug in foetus and amniotic fluid were investigated using pregnant rats after oral dosage at 10 mg./kg. Estimations were carried out with eosin (low dose) or methyl orange (high dose) on pooled samples from three or five animals, except with foetal material where only two rats could be used.

Penetration into Cerebrospinal Fluid.—Pempidine (5 mg./kg.) as the hydrochloride in normal saline was injected intravenously into adult rabbits which were killed with pentobarbitone after a measured time interval; samples of cerebrospinal fluid and blood were withdrawn by cisternal and heart puncture respectively. Each of these experiments was done on at least two animals. Similar experiments were carried out with mecamylamine.

Urinary Excretion. — Single oral doses of pempidine (2.5 to 20 mg./kg.) as the hydrogen tartrate were administered to albino rats (100 to 150 g.) which were kept in metabolism cages with free access to water. Twenty-four hour collections of urine were made for three days after dosage, estimations being performed with methyl orange on pooled samples from three animals.

The effect of urinary *p*H on excretion of pempidine and mecamylamine was also studied. Rats in groups of 12 were given N/10-sodium bicarbonate, N/10-ammonium chloride or water to produce alkaline, acid or normal urine before and after administering a single oral dose of pempidine (10 mg./kg.) as hydrogen tartrate, or mecamylamine (10 mg./kg.) as hydrochloride. Thirty-six animals were used for each compound, the urine being collected at hourly intervals.

Methyl orange, however, was not specific for pempidine and any basic metabolites of the drug would also be estimated by this method. All bases present in the ethylene dichloride extract of urine were therefore removed with hydrochloric acid which was then evaporated to dryness for further investigation. That the material extracted from urine was a single substance indistinguishable from pempidine was suggested by (*a*) paper chromatography in the organic phase of the system butanol/acetic acid/water=100/10/100 (v/v), (*b*) paper electrophoresis at *p*H 7, using phosphomolybdic acid to locate the spots according to Chargaff, Levine, and Green (1948), and (*c*) by studies of partition between ethylene dichloride and aqueous buffer solutions at *p*H values between 5.0 and 10.7. Infra-red spectroscopy finally established that the excreted compound was, in fact, pempidine.

In Vitro Experiments

Protein-binding of Pempidine.—The binding of pempidine to plasma proteins was studied by dialysis. Solutions of pempidine (250 μg./ml.) in horse plasma were dialysed in Visking tubing at room temperature against physiological saline. The drug concentrations inside and outside the dialysis sac were estimated with methyl orange until equilibrium was established after 72 hr. Preliminary experiments showed that the membrane was completely permeable to pempidine but retained proteins.

RESULTS

Drug Concentrations in Plasma and Erythrocytes.—Fig. 2 shows the concentrations of pempidine in plasma at various times after a single oral dose of the hydrogen tartrate. The curves are typical of those obtained at all doses within the range 0.25 to 20 mg./kg. At first the drug concentration increased rapidly, and in 30 min. attained a maximum value proportional to the amount of drug administered (Fig. 3). At doses not exceeding 5 mg./kg., the plasma concentration, having passed its maximum, decreased quite quickly but after 2 hr. fell more slowly so that appreciable concentrations of drug were maintained for 6 hr. or more. With larger doses, a second maximum in the plasma concentration appeared at 4 to 6 hr.; this phenomenon occurred regularly and was not attributable to error of measurement, but its significance has not been studied further.

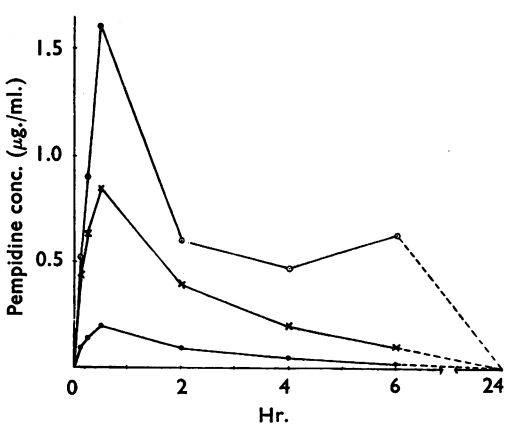


FIG. 2.—Plasma concentrations of pempidine at various times after the administration of single oral doses to rats. The compound was given as an aqueous solution of the hydrogen tartrate. Each point represents an estimation on pooled heart-blood from three animals. The plasma concentrations were measured after 10 mg./kg. (upper curve), 5 mg./kg. (middle curve) and 1 mg./kg. (lower curve).

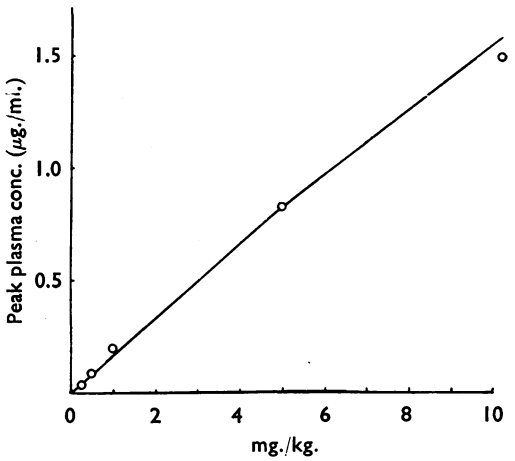


FIG. 3.—Peak plasma concentration of pempidine after various single oral doses to rats. The compound was given as an aqueous solution of the hydrogen tartrate. Each point represents an estimation on pooled heart-blood from three animals 30 min. after receiving the dose.

After a single oral dose of pempidine (1 mg./kg.) as the hydrogen tartrate, the drug concentration in erythrocytes rapidly increased during the first 30 min., and thereafter decreased in a manner closely resembling that observed with plasma (Table I). This indicated that the drug passed rapidly into (or on to) the erythrocytes, a partition ratio of about 1.2 being maintained in favour of the cells. When pempidine was administered in lethal amounts (175 mg./kg. of pempidine base injected intraperitoneally) the partition ratio was lowered to 1.03.

Tissue Distribution of Pempidine.—Concentrations of drug found in tissues from rats dosed orally with pempidine (1 mg./kg. or 10 mg./kg.) as the hydrogen tartrate and killed 1 or 4 hr. later are given in Tables II and III, which also show the corresponding tissue/plasma ratios.

TABLE I

DISTRIBUTION OF PEMPIDINE BETWEEN ERYTHROCYTES AND PLASMA

Pempidine (1 mg./kg.) was administered orally to rats as an aqueous solution of the hydrogen tartrate and each estimation performed on pooled heart-blood from three animals. Estimations by the eosin fluorescence method.

Time After Dose (min.)	Plasma Conc. (μg./ml.)	Erythrocyte Conc. (μg./g.)	Erythrocyte/ Plasma Conc. Ratio
10	0·110	0·128	1·17
20	0·175	0·204	1·17
40	0·165	0·194	1·18
80	0·120	0·138	1·17
160	0·050	0·058	1·21
360	0·020	0·023	1·15

TABLE II

DISTRIBUTION OF PEMPIDINE IN TISSUES (1 MG./KG.)

Pempidine (1 mg./kg.) was administered orally to rats as an aqueous solution of the hydrogen tartrate. Estimations were carried out on tissues obtained from three rats which were killed 1 hr. after receiving the drug. Estimations by eosin fluorescence method.

Tissue	Drug Conc. (μg./g.)	Tissue/Plasma Conc. Ratio
Brain	0·28	2·16
Erythrocytes	0·154	1·19
Kidney	1·77	13·6
Liver	1·25	9·6
Lung	0·36	2·77
Voluntary muscle	0·22	1·54
Plasma	0·130	—
Spleen	1·35	10·4

TABLE III

DISTRIBUTION OF PEMPIDINE IN TISSUES (10 MG./KG.)

Pempidine (10 mg./kg.) was administered orally to rats as an aqueous solution of the hydrogen tartrate. The organs were removed from five rats either 1 or 4 hr. after receiving the drug. Two rats were used for drug estimations in foetal tissue and amniotic fluid. Estimations by methyl orange method, which, when applied to tissues, tends to give high blanks.

Tissue	Drug Conc. (μg./g.)		Tissue/Plasma Conc. Ratio	
	1 hr.	4 hr.	1 hr.	4 hr.
Brain	2·1	Nil	0·5	—
Kidney	85·0	8·1	21·0	4·0
Liver	9·5	7·8	2·0	3·9
Lung	13·5	5·7	3·4	2·9
Voluntary muscle	4·5	Nil	1·1	—
Spleen	16·3	32·3	4·1	16·2
Foetus	24·3	8·8	8·1	4·4
Amniotic fluid	3·0	3·7	0·8	1·9
Plasma	4·0	2·0	—	—

After 1 hr., the highest concentrations of pempidine were found in kidney and spleen with rather smaller amounts in liver. 2 and 4 hr. after administration of the drug, the amount of pempidine in the various tissues had fallen except in the spleen where the drug concentration had increased. Only very small amounts of drug could be detected in both spleen and liver up to 72 hr.

With pregnant rats appreciable concentrations of pempidine were observed in the foetus 1 hr. after giving the drug orally. At the end of 4 hr., however, much of the pempidine had passed into the amniotic fluid.

Penetration into C.S.F.—Fig. 4 shows curves for the concentration of pempidine and mecamylamine in cerebrospinal fluid during the six hours following a single intravenous injection of the hydrochlorides. Each point represents the drug concentration in cerebrospinal fluid expressed as a percentage of the corresponding plasma concentration. Pempidine and mecamylamine entered the cerebrospinal fluid to approximately the same extent, concentrations of

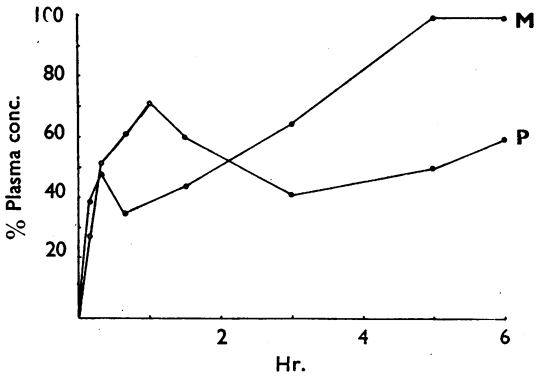


FIG. 4.—Penetration of pempidine and mecamylamine into the cerebrospinal fluid of rabbits following a single intravenous injection of 5·0 mg./kg. of one or other as the hydrochloride in saline.

nearly 50% being attained during the first hour. The concentration of mecamylamine in cerebrospinal fluid relative to plasma remained high for 6 hr., whereas that of pempidine fell during this period. Since the plasma concentrations and the plasma/erythrocyte partition ratios of the two drugs were similar, the absolute rate of elimination of pempidine from the cerebrospinal fluid was more rapid than that of mecamylamine.

Urinary Excretion.—The urinary excretion of pempidine is shown in Fig. 5. Most of the drug was excreted during the first 24 hr., only 2% being found in the second 24 hr. and negligible quantities thereafter. In these experiments the *p*H of the urine (~6.9) was not significantly different from untreated controls. The total recovery of drug varied with the dose, 40 to 45% in the lower ranges, 70 to 80% with larger

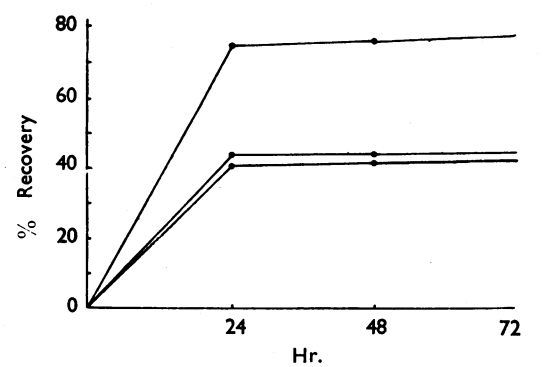


FIG. 5.—Cumulative urinary excretion of pempidine, expressed as % recovery from urine, in rats following single oral doses of aqueous solutions of the hydrogen tartrate. 10 and 20 mg./kg. (upper curve), 2·5 mg./kg. (middle curve), 5·0 mg./kg. lower curve.

amounts. Under all conditions of urinary *p*H. the excretion of pempidine was more rapid and complete than that of mecamylamine. Fig. 6 shows the excretion in acid (*p*H 5.6) and alkaline (*p*H 8.2) urine of the two drugs after a single oral dose. Only very small amounts (less than 0.1% of the dose) of pempidine were found in faeces during the 48 hr. following a single oral dose of the drug.

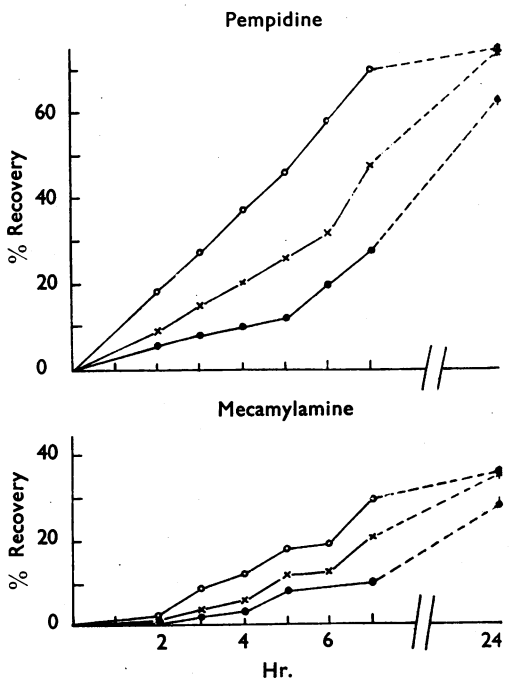


FIG. 6.—Comparison of urinary excretion rates of pempidine and mecamylamine in rats expressed as % recovery from urine. Both compounds were administered as single oral doses of 10 mg./kg. Rats given either N/10-sodium bicarbonate or N/10-ammonium chloride as drinking water to induce excretion of an acid or alkaline urine respectively. Recovery from acid urine, upper curve in each graph; from normal urine, middle curves; and from alkaline urines, lower curves.

Protein-binding of Pempidine.—Binding of pempidine to plasma proteins only occurred to a very limited extent. An average value of 1.2% (limits 0.5 to 2.6%) was obtained in a number of experiments for the proportion of drug bound to protein, the estimations being carried out with varying doses of drug.

DISCUSSION

The methyl orange complex and eosin fluorescence techniques were both satisfactory for estimating pempidine in certain body fluids and may be regarded as complementary. Methyl

orange, with rather limited sensitivity, can be used to study excretion with small doses because the concentration of the drug in urine is relatively high, but measurements of absorption and tissue distribution are possible only if large amounts of pempidine have been administered. If the dose was less than 20 mg./kg. the methyl orange method was unable to detect the very low concentrations of pempidine in plasma and tissues and was too insensitive for use in clinical investigations (Harington *et al.*, 1958). The eosin fluorescence method, on the other hand, is eminently suitable for plasma and tissue samples after oral doses of pempidine in the clinical range but cannot at present be applied to urine because of interfering substances which quench the fluorescence.

When given orally at doses within the range 1 to 10 mg./kg., pempidine was very rapidly absorbed and the peak plasma concentration, attained in about 30 min., was proportional to the dose. The amount in plasma was small when compared with the dose, and this suggested that it was widely distributed. The concentration of pempidine in erythrocytes was significantly higher than in plasma, a partition ratio of $\sim$1.2 being quickly established and then maintained for some hours. After lethal intraperitoneal injections of pempidine (doses of about 200 mg./kg.) we found a lower value (1.03) in agreement with the findings of Harington *et al.* (1958). With these large doses the red cells probably became saturated with pempidine, and the concentration was thus at a constant and maximum level, whilst the amount of drug in the plasma rose and hence lowered the partition ratio. Milne, Rowe, Somers, Muehrcke, and Crawford (1957) reported a similar distribution of mecamylamine between erythrocytes and plasma (partition ratio $\sim$1.15) and showed that it was a function of the *p*H of the extracellular fluid and therefore possibly depended upon the relative concentrations of ionized and unionized drug present. The effect is unlikely to be a consequence of protein binding, for although the partition ratios for mecamylamine and pempidine are similar their protein-binding properties are very different. After adding mecamylamine to plasma *in vitro*, Milne found 25% of the drug was bound to protein, whereas our experiments under similar conditions showed that protein binding of pempidine occurred to only an insignificant extent.

Pempidine, administered orally, rapidly became distributed throughout the body tissues, and, 1 hr. after dosing, the highest concentrations were found in the kidney, spleen, and liver. After 4 hr., the drug was concentrated mainly in the spleen, presumably owing to a rapid turn-over of red cells. The placenta afforded no barrier to pempidine, which readily penetrated into foetal tissue. Four hours after oral administration, the concentration of drug in the foetus had fallen whilst the amount in the amniotic fluid had increased, indicating that the foetus excreted pempidine.

Pempidine and mecamylamine were both found in the cerebrospinal fluid, as was inferred by Corne and Edge (1958), who noted the appearance of tremors when large doses of pempidine or mecamylamine were given orally or parenterally. Brodie and Hogben (1957) postulated that the rate at which drugs pass into or out of the central nervous system depends on the lipoid solubility of the uncharged molecules. But other factors must also be involved, such as the extent to which protein binding occurs and particularly the proportion of base cations and uncharged molecules present at physiological *p*H. Thus mecamylamine and pempidine are almost completely ionized at *p*H 7.2 and have similar low partition coefficients between ethylene dichloride and aqueous buffer at that *p*H [Brodie and Hogben (1957) used partition between chloroform and water as the criterion of lipoid solubility in this context]. The fact that neither drug attained a concentration in cerebrospinal fluid equal to the plasma level even 3 hr. after administration is in accord with the views of Brodie and Hogben (1957). However, while the two drugs entered the cerebrospinal fluid of rabbits at about the same rate, pempidine disappeared more rapidly than mecamylamine. Whatever the reason, it might be expected that any central nervous effects which pempidine might have in man may be of shorter duration and less severe than those seen with mecamylamine.

Pempidine was excreted rapidly in urine during the 24 hr. following oral administration, the rate of excretion in the first 7 hr. being almost twice that of mecamylamine. At doses approaching those used in clinical practice, 45% of the drug appeared in the urine within 24 hr. (70 to 80% with larger doses), but very little of the drug was recovered subsequently. We have no evidence of the fate of the remainder of the dose administered. Renal excretion of both pempidine and mecamylamine is affected by urinary *p*H. Thus 75% and 63% recoveries of administered drug are obtained with pempidine under acid and alkaline conditions compared with 36% and 28%

respectively for mecamylamine. Mecamylamine was used as a reference compound for these studies because it is, we believe, the only non-quaternary ganglion blocking agent which is in clinical use. Differences in behaviour are, however, not wholly unexpected since mecamylamine is a secondary and pempidine a tertiary amine.

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