Brit. J. Pharmacol. (1961), **17**, 488–506.

ACTION OF CHLOROTHIAZIDE ON THE DISTRIBUTION, EXCRETION AND HYPOTENSIVE EFFECT OF PEMPIDINE IN MAN

BY

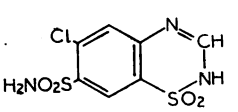
C. T. DOLLERY, D. EMSLIE-SMITH AND D. F. MUGGLETON

From the Department of Medicine, Postgraduate Medical School of London and Hammersmith Hospital, and the Research Laboratories, May & Baker, Dagenham

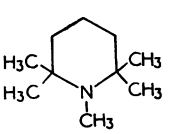
(*Received September* 7, 1961)

When chlorothiazide is given to hypertensive patients who are receiving pempidine a rise in plasma pempidine concentration occurs and this is proportionately greater than the additional fall in blood pressure. After pempidine has been added to human whole blood *in vitro* or *in vivo* the ratio of the pempidine concentration in the red cells to that in the plasma falls in the course of 1 hr from an initial value greater than 2 to about 1.2. If chlorothiazide is present also, however, the ratio remains constant at 0.7. Changes in the plasma pempidine concentration *in vivo* probably result from the binding of pempidine to plasma protein in the presence of chlorothiazide. This has been observed *in vitro* by a dialysis technique.

The principal action of chlorothiazide (6-chloro-7-sulphamoyl-1,2,4-benzothiadiazine 1,1-dioxide, I) is to increase the excretion of sodium, chloride and water. It also enhances the response obtained from a number of drugs used to lower blood pressure and is consequently of value in the treatment of hypertension (Freis & Wilson, 1957; Hollander & Wilkins, 1957). Several mechanisms have been proposed for this second effect although it is now generally considered to result from the reduction of plasma volume following sodium diuresis (Dustan, Cumming, Corcoran & Page, 1959; Dollery, Harington & Kaufmann, 1959; Wilson & Freis, 1960). It is possible, however, that chlorothiazide also affects the excretion of hypotensive drugs because it raises the urinary *p*H, by inhibiting carbonic anhydrase, and may therefore influence excretion by non-ionic diffusion in the renal tubules (Harington & Kincaid-Smith, 1958).



I



II

Pempidine (1,2,2,6,6-pentamethylpiperidine, II) is a ganglion-blocking agent used in the treatment of hypertension. Recently, during studies of hypertensive patients who were receiving the drug orally, we found that (i) the hypotension obtained paralleled the plasma pempidine concentration and (ii) chlorothiazide caused only a slight increase in the response to a dose of pempidine although it

almost trebled the plasma pempidine concentration (Dollery, Emslie-Smith & Muggleton, 1960). This paper describes further work that we have carried out to elucidate the mechanism of the action of chlorothiazide on the distribution, excretion and hypotensive effect of pempidine in man.

METHODS

Plasma pempidine concentrations in hypertensive patients. The effect of chorothiazide on plasma pempidine concentrations was investigated in patients with severe hypertension. Many had retinal haemorrhages and exudates but none had a blood urea level of more than 50 mg/100 ml. and renal function was not seriously impaired.

Patients given oral pempidine and oral chlorothiazide. Five patients were studied before and after chlorothiazide was added to their daily maintenance treatment with pempidine. Both drugs were given orally and each patient was on a stable regime for at least one week before any observations were made. Pempidine (10 to 100 mg daily) was administered as the hydrogen tartrate in four equal doses at 6 a.m., noon, 6 p.m. and 11 p.m.; chlorothiazide (250 to 2,000 mg daily) was given in two equal doses at 6 a.m. and 6 p.m. Blood pressures (lying and standing) were recorded and blood samples were taken just before the mid-day dose of pempidine and again at 1, 2, 4 and 6 p.m. A sixth patient who had received pempidine and chlorothiazide daily for two months was studied before, and again two weeks after, chlorothiazide was withdrawn.

Patients given intravenous pempidine and oral chlorothiazide. Plasma pempidine concentrations and blood pressures were measured in ten patients for 1 hr following an intravenous dose of pempidine. Each patient lay flat in bed and the blood pressure was recorded at intervals until it became steady. After a control sample of blood had been taken the pempidine (usually 2.5 mg) was given intravenously. Further blood samples were taken and the blood pressure measured 5 (or 10), 30 and 60 min after the injection. From a few patients it was possible to obtain extra samples during the first 5 min. Six patients were given a second injection of pempidine on the following day, after having two oral doses of chlorothiazide (500 mg) in the preceding 15 hr. Three days later three of the patients were again given 2.5 mg pempidine intravenously, but no chlorothiazide. Two patients were first of all given 10 mg pempidine intravenously without chlorothiazide and on a subsequent occasion 2.5 mg after premedication with chlorothiazide.

Patients given intravenous pempidine and diuretics other than chlorothiazide. The effect of other diuretics on pempidine was investigated with the same schedule of blood samples and pressure readings as described above for patients given intravenous pempidine and oral chlorothiazide. Three patients were given mersalyl intramuscularly 8 and 3 hr before the test dose of pempidine. A fourth patient had acetazoleamide orally 8 and 2 hr before the pempidine.

Patients given oral pempidine and intravenous chlorothiazide. Nine patients on maintenance treatment with oral pempidine were each given a single intravenous injection of chlorothiazide (100 to 500 mg as sodium salt) 2 to 6 hr after a dose of pempidine. Blood pressures (lying and standing) were recorded and blood samples taken 1 to 2 hr after the injection of chlorothiazide.

Patients given intravenous pempidine and intravenous chlorothiazide. Pempidine and chlorothiazide (as sodium salt) were mixed in a syringe and administered intravenously to two patients. Two patients had intravenous pempidine 1 hr after intravenous chlorothiazide and in a third the drugs were injected simultaneously but separately. In every case blood pressures and blood samples were taken immediately before and 10, 30 and 60 min after injecting the pempidine.

Renal excretion of pempidine. The renal clearance of pempidine was measured in patients during continuous infusion of about 1 mg of pempidine each hour. Blood samples were taken and the entire urine output was collected during periods of 1 hr before and after chlorothiazide

had been added to the infusion at rates varying from 40 to 100 mg/hr. Preliminary experiments showed that an interval of 1 hr between the two collection periods was enough for stable conditions to be re-established after the addition of chlorothiazide. Similar studies were made without chlorothiazide, but sodium bicarbonate (20 to 100 mEq/hr) was then infused to produce a comparable change in the urinary *p*H. The renal clearance of pempidine was also measured over consecutive hourly periods without the addition of either chlorothiazide or sodium bicarbonate. In none of these studies was the bladder catheterized, but the patient's water intake was increased so that relatively large amounts of urine could be obtained and errors of collection thereby minimized.

Distribution of pempidine in the tissues of the rat. The effect of chlorothiazide on the distribution of pempidine in tissues was studied in rats. Five animals, each weighing about 450 g, were deprived of food for 12 hr, and pempidine (1 mg/kg) was then administered in aqueous solution by stomach tube. Thirty minutes later the rats were killed and bled. The various organs and tissues were removed as quickly as possible and weighed. Control experiments without pempidine were performed under identical conditions. Both experiments were also carried out with rats which were given three equal doses of chlorothiazide (5 mg/kg orally) 7, 4 and 1 hr before the pempidine.

Distribution of pempidine between red cells and plasma in vitro. To investigate the distribution of pempidine between red cells and plasma 40 ml. of freshly-drawn human blood was heparinized and kept at 37° C with occasional stirring. Samples were removed 1, 3, 5, 10, 30 and 60 min after the addition of 25 μg of pempidine dissolved in 1 ml. 0.9% sodium chloride solution. Each sample was centrifuged for the same time (1 min) and at the same speed (3,000 rev/min), so that the red cells and the plasma were separated under standard conditions. Similar experiments were made with blood (*a*) which had stood for 1 hr at 37° C; (*b*) to which 1.37 mg of chlorothiazide had been added 1 hr previously; (*c*) after the addition of 0.005 ml. of mersalyl injection *B.P.*; and (*d*) from a patient who had been pretreated with chlorothiazide.

Binding of pempidine to serum albumin in vitro. So far it has not been possible for us to undertake a comprehensive study of the binding of pempidine to proteins, but some exploratory experiments have been carried out using the technique of equilibrium dialysis. Dialysis sacs were prepared from Visking tubing previously boiled for 1 hr in distilled water to remove soluble impurities. Each sac was filled with 5 ml. of solution containing bovine serum albumin (Fraction V) and chlorothiazide, and was placed in a stoppered tube containing 20 ml. of pempidine solution. The system was allowed to equilibrate for 48 hr at 25° C and the concentrations of pempidine and chlorothiazide in the outer compartment were then measured. In some experiments pempidine concentrations inside the sac were also determined, but we could not obtain reliable measurements of chlorothiazide concentration with the protein-containing solution. If pempidine and chlorothiazide were transported across the membrane entirely by diffusion, then their equilibrium concentrations could readily be calculated from the volumes of solution in the outer and inner compartments. On the other hand, binding of either drug to the protein would disturb the equilibrium and the consequent concentration changes could be used to measure the extent of protein-binding. Six series of experiments were performed.

Pempidine (0 to 20 μg/ml.) and chlorothiazide (0 to 50 μg/ml.) were tested separately in the absence of albumin to show that neither pempidine nor chlorothiazide was bound to the dialysis sac. These experiments were repeated with 1% albumin in the dialysis sac to assess the binding of pempidine and chlorothiazide, separately, to serum albumin. Finally, the effect of chlorothiazide added to the albumin (1%) in the sac on pempidine binding was studied. A fixed concentration (50 μg/ml.) of chlorothiazide was tested with varying concentrations (0 to 1 μg/ml.) of pempidine and various concentrations (0 to 50 μg/ml.) of chlorothiazide were tested with a fixed concentration (1 μg/ml.) of pempidine.

Preparation of samples. Samples of blood were heparinized and centrifuged to separate the red cells and plasma. The red cells were lysed by adding an equal volume of distilled water and the debris removed by centrifugation. Urine was centrifuged and, where required,

its *p*H was measured with a glass electrode. Rat tissues were homogenized in distilled water. After material from five rats had been pooled the final volume was adjusted so that 10 ml. of homogenate was equivalent to 1 g of the original tissue.

Estimation of pemipidine. Concentrations of pempidine in plasma, red cells, tissue homogenates and urine were measured by the eosin fluorescence technique. A brief description of this method has already been given (Dollery *et al.*, 1960) and a full account will appear shortly elsewhere (Ivens & Muggleton, to be published).

Estimation of chlorothiazide. Chlorothiazide was estimated by first hydrolysing with sodium hydroxide and then diazotizing and coupling with *N*-(1-naphthyl)ethylenediamine hydrochloride. The intensity of the colour produced was measured at 520 mμ in 1 cm cuvettes (Baer, Leidy, Brooks & Beyer, 1959).

RESULTS

Plasma pempidine concentrations

Oral pempidine and oral chlorothiazide. When doses of chlorothiazide ranging from 250 to 2,000 mg were added to the daily pempidine treatment there was a slightly greater fall in blood pressure than with pempidine alone, as shown in Fig. 1. In every case the plasma pempidine concentration remained at a high level

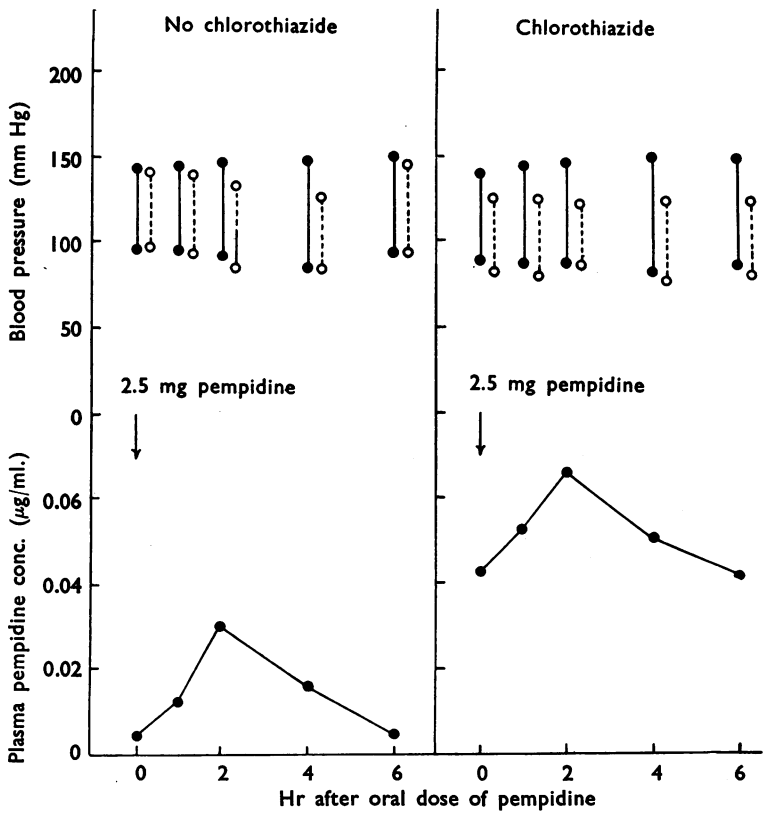


Fig. 1. Effect of chlorothiazide on the plasma pempidine concentration and the hypotensive response after an oral dose of pempidine. Blood pressures (●——● lying and ○- - -○ standing) and plasma pempidine concentrations were measured for 6 hr after one of a series of 6-hourly doses (2.5 mg) of pempidine, with and without 12-hourly doses of chlorothiazide.

throughout the 6 hr period of observation, and the peak value, which occurred about 2 hr after the dose of pempidine, was approximately 2.5 times that found without chlorothiazide. The plasma pempidine concentration in the presence of chlorothiazide was always greater than for the same dose of pempidine given alone. Table 1 contains results for three typical patients. It shows the plasma pempidine concentrations after one of a series of 2.5 mg doses of pempidine, with and without chlorothiazide.

Intravenous pempidine and oral chlorothiazide. After an intravenous injection of pempidine the plasma concentration of the drug fell rapidly during the first 5 min and then more slowly. However, when the patient had previously been given chlorothiazide the plasma pempidine concentration decreased much more slowly and 10 min after the injection it was consistently nearly 7 times greater than that without chlorothiazide. The effect of chlorothiazide on the plasma concentration and hypotensive action of pempidine was very persistent and could be demonstrated 3 days after the last dose of chlorothiazide (Fig. 2).

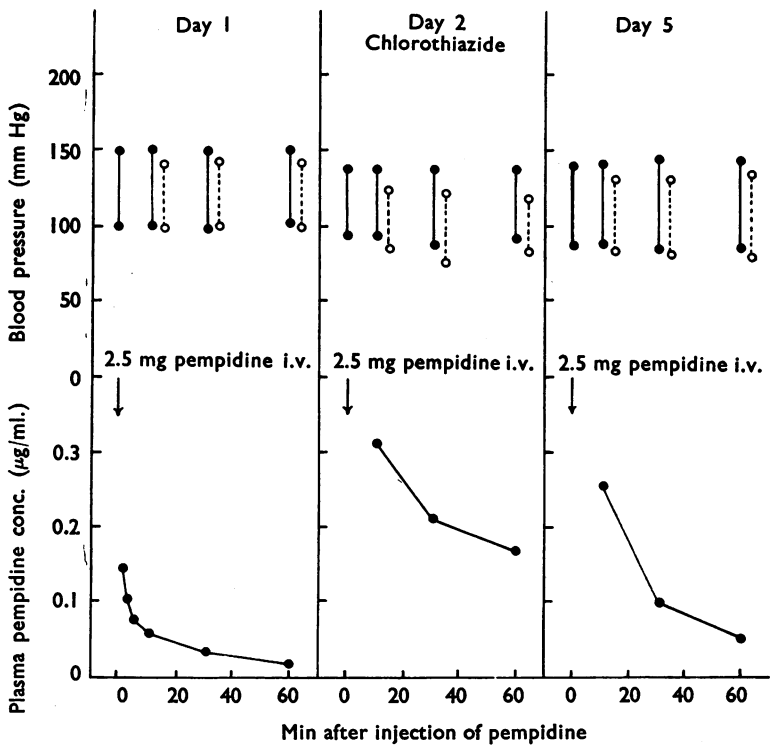


Fig. 2. Effect of chlorothiazide on the plasma pempidine concentration and hypotensive response after an intravenous injection of pempidine. Blood pressures (●——● lying and o- - - o standing) and plasma pempidine concentrations were measured for 1 hr after an intravenous injection of pempidine (2.5 mg) on days 1, 2 and 5. The patient received 2 oral doses of chlorothiazide (500 mg) in the 15 hr preceding observations on Day 2. Note elevated plasma pempidine concentrations on Day 2 and Day 5.

TABLE 1

EFFECT OF CHLOROTHIAZIDE ON PLASMA PEMPIDINE CONCENTRATIONS IN HYPERTENSIVE PATIENTS

Plasma pempidine concentrations (μg/ml.) for 6 hr after one of a series of 6-hourly doses (2·5 mg orally), with and without 12-hourly oral doses of chlorothiazide

Time after dose (hr)	Patient 1. Chlorothiazide (mg/day)		Patient 2. Chlorothiazide (mg/day)		Patient 3. Chlorothiazide (mg/day)	
	0	250	0	500	0	1,000
0	0	0·030	0	0·027	0·003	0·040
1	0·008	0·050	0·009	0·036	0·011	0·055
2	0·030	0·069	0·018	0·045	0·030	0·085
4	0·015	0·055	0·008	0·027	0·013	0·065
6	0·002	0·041	0·002	0·030	0·004	0·043

The effect of chlorothiazide on the distribution of pempidine between red cells and plasma *in vivo* is of great interest. Table 2 shows concentrations of pempidine in plasma and red cells and the corresponding values of the distribution ratio observed in a patient over five days.

TABLE 2

EFFECT OF ORAL CHLOROTHIAZIDE ON CONCENTRATIONS IN PLASMA AND RED CELLS OF PEMPIDINE INJECTED INTRAVENOUSLY

Concentrations of pempidine (μg/ml.) in plasma (P) and red cells (R.C.), and their ratio, for 1 hr after intravenous injection of the drug. Day 1=2·5 mg pempidine; Day 2=2·5 mg pempidine after premedication of the patient orally with chlorothiazide; Day 5=2·5 mg pempidine

Time after pempidine injection (min)	Day 1			Day 2			Day 5		
	P	R.C.	R.C./P	P	R.C.	R.C./P	P	R.C.	R.C./P
1	0·326	0·628	2·09	0·384	0·294	0·765	0·340	0·331	0·974
5	0·269	—	—	0·318	0·221	0·695	0·246	0·244	0·991
10	0·059	0·111	1·88	0·258	0·178	0·669	0·202	0·204	1·01
30	0·034	0·065	1·91	0·199	0·138	0·692	0·160	0·152	0·950
60	0·022	0·028	1·27	0·131	0·094	0·717	0·118	0·105	0·890

On Day 1, 2.5 mg of pempidine was given intravenously and blood samples were taken at intervals for 1 hr. The distribution ratio decreased from 2.09 immediately after the injection to 1.27 1 hr later. The patient next received chlorothiazide orally and another 2.5 mg of pempidine was injected on Day 2. The distribution ratio then had a value which was practically constant (about 0.7). No further treatment was given until a third injection (2.5 mg) of pempidine was administered on Day 5, when an almost constant ratio (about 1) was obtained. On Days 2 and 5, therefore, relatively more of the pempidine was in the plasma than on Day 1, and, although the distribution ratio was slowly returning to its normal value, the effect of chlorothiazide could still be detected even 3 days after the last dose. Measurements more than 3 days after cessation of chlorothiazide were not attempted. Note that the distribution ratio in the presence of chlorothiazide was nearly constant, whereas a steadily decreasing ratio was observed in the absence of chlorothiazide.

Two patients were given 10 mg of pempidine intravenously, and on another occasion 2.5 mg after premedication with chlorothiazide. The larger dose of pempidine gave a greater hypotensive response although the plasma pempidine concentration was less than that following the smaller dose administered after chlorothiazide (Fig. 3).

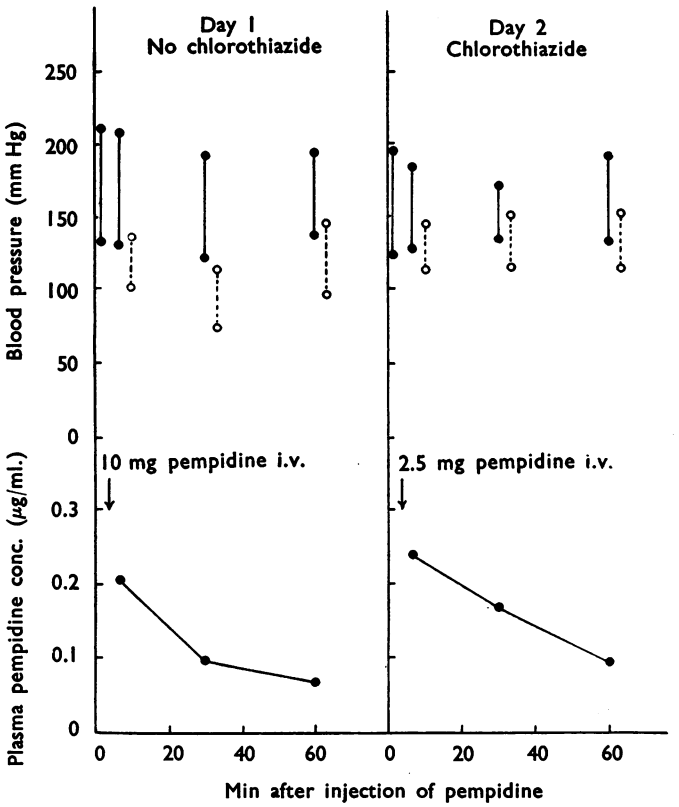


Fig. 3. Effect of chlorothiazide on the plasma pempidine concentration and the hypotensive response after an intravenous injection of pempidine. Blood pressures (●——● lying and o- - - o standing) and plasma pempidine concentrations were measured for 1 hr after injection of pempidine on Day 1 and again on Day 2 when the patient had been premedicated with chlorothiazide. Note that 10 mg of pempidine on Day 1 produced a larger hypotensive response but lower plasma pempidine concentrations than 2.5 mg on Day 2.

Intravenous pempidine and diuretics other than chlorothiazide. Patients given intramuscular mersalyl before intravenous pempidine showed an increase in both plasma pempidine concentration and hypotensive response. However, the effect on the plasma pempidine concentration was neither so great nor so prolonged as with chlorothiazide and was absent 3 days afterwards. The effect of mersalyl on the red cell/plasma distribution of pempidine is indicated in Table 3. Whereas when pempidine was given alone the distribution ratio decreased during the hour following the injection from about 2 to approximately 1.3 (see Table 2), a constant

value approximately 1.0 was obtained after the patient had been premedicated with intramuscular mersalyl (2 ml. mersalyl injection *B.P.* daily).

Acetazoleamide had no effect either on the pempidine concentration in plasma or on the hypotensive response.

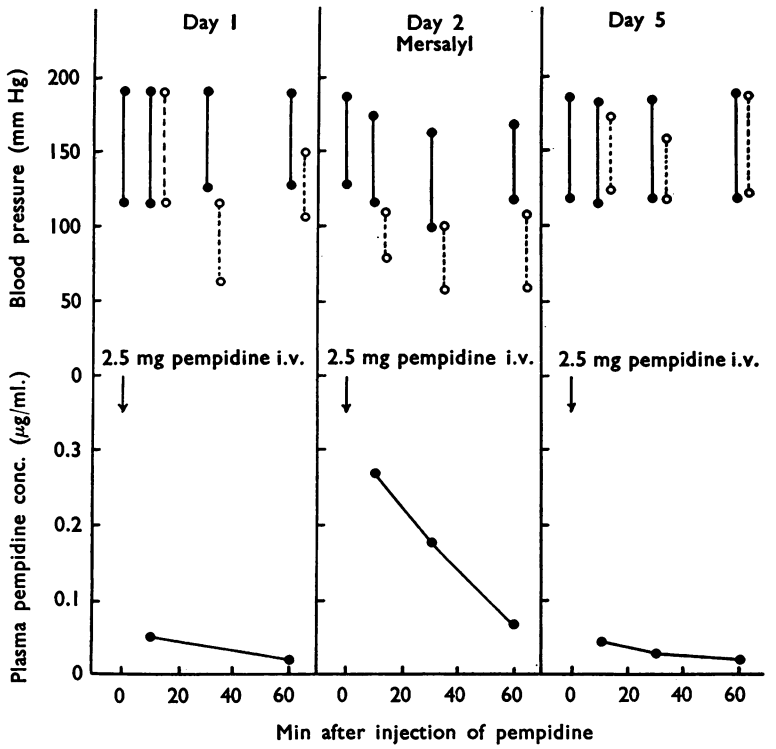


Fig. 4. Effect of mersalyl on the plasma pempidine concentration and the hypotensive response after an intravenous injection of pempidine. Blood pressures (●—— ● lying and o- - - o standing) and plasma pempidine concentrations were measured for 1 hr after an intravenous injection of pempidine (2.5 mg) on Days 1, 2 and 5. The patient received two intravenous injections of mersalyl before observations were made on Day 2. Note high plasma pempidine concentrations on Day 2.

TABLE 3

EFFECT OF INTRAMUSCULAR MERSALYL ON CONCENTRATIONS IN PLASMA AND RED CELLS OF PEMPIDINE INJECTED INTRAVENOUSLY

Concentrations of pempidine (μg/ml.) in plasma (P) and red cells (R.C.), and their ratio, for 1 hr after intravenous injection of the drug (2·5 mg). The patient was premedicated with mersalyl injection *B.P.* intramuscularly (2 ml. daily)

Time after pempidine injection (min)	P	R.C.	R.C./P
10	0·193	0·188	0·98
30	0·148	0·143	0·97
70	0·094	0·094	1·00

Oral pempidine and intravenous chlorothiazide. In each of 9 patients having maintenance treatment with oral pempidine the intravenous injection of 100 to 500 mg chlorothiazide produced within 1 hr a sharp rise in the plasma pempidine concentration. In some patients the increase was as much as 5-fold, without significant change in the postural fall in blood pressure (Fig. 5).

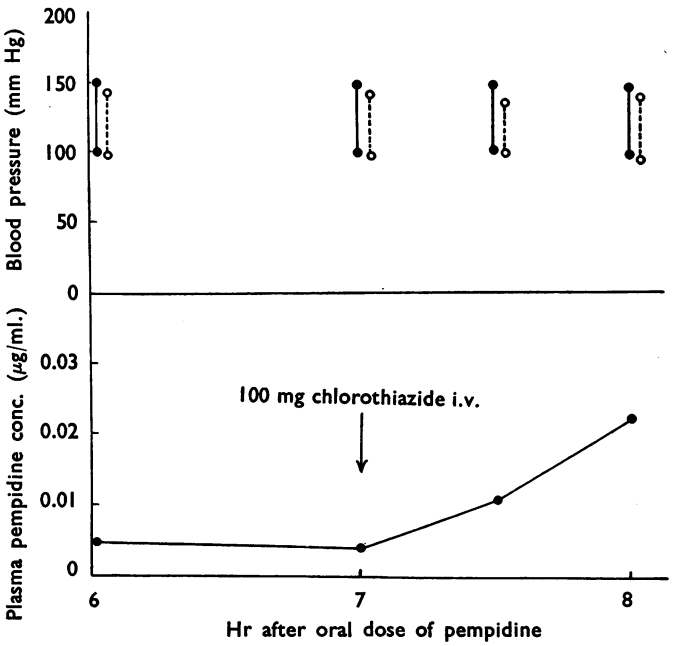


Fig. 5. Effect of intravenous chlorothiazide on the hypotensive response and plasma pempidine concentration after an oral dose of pempidine. 100 mg of chlorothiazide was injected 7 hr after 2.5 mg of pempidine had been given orally. The plasma pempidine concentration increased sharply without significant change in the postural fall in blood pressure (blood pressure standing o - - - o and lying ● —— ●).

TABLE 4

EFFECT OF INTRAVENOUS CHLOROTHIAZIDE ON CONCENTRATIONS IN PLASMA AND RED CELLS OF PEMPIDINE INJECTED INTRAVENOUSLY

Concentrations of pempidine (μg/ml.) in plasma (P) and red cells (R.C.), and their ratio, for 1 hr after intravenous injection of the drug (2·5 mg). Pempidine was given (i) alone, (ii) mixed with chlorothiazide, (iii) simultaneously with intravenous injection of chlorothiazide, (iv) to a patient premedicated with chlorothiazide

Time after pempidine injection (min)	No chlorothiazide			Chlorothiazide + pempidine (mixed)			Chlorothiazide and pempidine (simultaneously)			Premedication with chlorothiazide		
	P	R.C.	R.C./P	P	R.C.	R.C./P	P	R.C.	R.C./P	P	R.C.	R.C./P
5	0·040	—	—	0·060	—	—	—	—	—	0·328	—	—
10	—	—	—	—	—	—	0·044	0·076	1·73	0·258	0·180	0·702
30	0·030	—	—	0·041	—	—	0·035	0·036	1·03	0·202	0·139	0·698
60	0·022	—	—	0·022	—	—	0·028	0·018	0·64	0·134	0·094	0·689

Intravenous pempidine and intravenous chlorothiazide. The results of experiments in which both pempidine and chlorothiazide were administered intravenously are shown in Table 4. Plasma concentrations of pempidine for 1 hr after the injection of 2.5 mg intravenously were not significantly different if (*a*) no chlorothiazide was given, (*b*) chlorothiazide and pempidine were mixed in the syringe, (*c*) chlorothiazide and pempidine were injected simultaneously but separately. However, high plasma pempidine concentrations were obtained when the patient had received chlorothiazide intravenously 1 hr before the injection of pempidine. In this case the plasma pempidine concentrations and red cell/plasma distribution ratios were very similar to those obtained after oral premedication with chlorothiazide (see Table 2). The red cell/plasma ratios observed when chlorothiazide and pempidine were injected simultaneously showed a steady decline from 1.73 to 0.64 during the first hour, although the plasma pempidine concentrations were close to those obtained in similar studies without chlorothiazide. This suggested that the effect of chlorothiazide was not produced at once but developed relatively slowly. In subsequent studies, therefore, premedication with chlorothiazide was always carried out 1 hr before administering pempidine.

Renal excretion of pempidine

Preliminary experiments in which pempidine alone was infused revealed that after the plasma pempidine concentration became constant 2 hr might elapse before a steady rate of excretion was attained. We did not pursue this point further but made measurements of renal clearance only after the plasma pempidine concentration had been steady for at least 2 hr. Little change occurred in the excretion of pempidine when chlorothiazide was infused, but the calculated renal clearance fell to about one-third (Table 5). This decrease in renal clearance, which was not accompanied by a commensurate alteration in the renal excretion, was caused mainly by the 3-fold rise in the plasma pempidine concentration. Infusions of chlorothiazide raised the *p*H of urine, and, although this could be overcome by administering ammonium chloride orally, the renal pempidine clearance decreased as before (Fig. 6).

TABLE 5

EFFECT OF CHLOROTHIAZIDE ON THE RENAL CLEARANCE OF PEMPIDINE

P=plasma concentration (μg/ml.); UV=renal excretion (μg/min)=urine concentration × volume of urine/min; UV/P=renal clearance (ml./min) of pempidine. Pempidine was infused uniformly (about 1 mg/hr) into the patient without chlorothiazide (left-hand columns) and with chlorothiazide, 40 to 100 mg/hr (right-hand columns)

	Control period			Chlorothiazide period		
Patient	P	UV	UV/P	P	UV	UV/P
1	0·057	19·6	336	0·148	19·5	132
2	0·066	8·21	124	0·139	8·1	58
3	0·080	34·66	433	0·145	58·5	403
4	0·055	22·9	418	0·120	12·8	107
5	0·060	12·01	260	0·160	19·07	119
6	0·043	8·25	192	0·091	4·06	45
7	0·036	18·75	521	0·076	9·0	118
8	0·046	14·0	304	0·120	19·8	165

In one patient the urinary *p*H was measured daily for a week, but a definite shift to the alkaline side could be detected only on the first day of chlorothiazide treatment. The effect on renal pempidine clearance of chlorothiazide was different from that of changes in urinary *p*H. The effect of *p*H was studied by infusing sodium bicarbonate and it was generally small and rather variable. A rise in *p*H produced a lower clearance in 6 patients, little change in one and an increase in

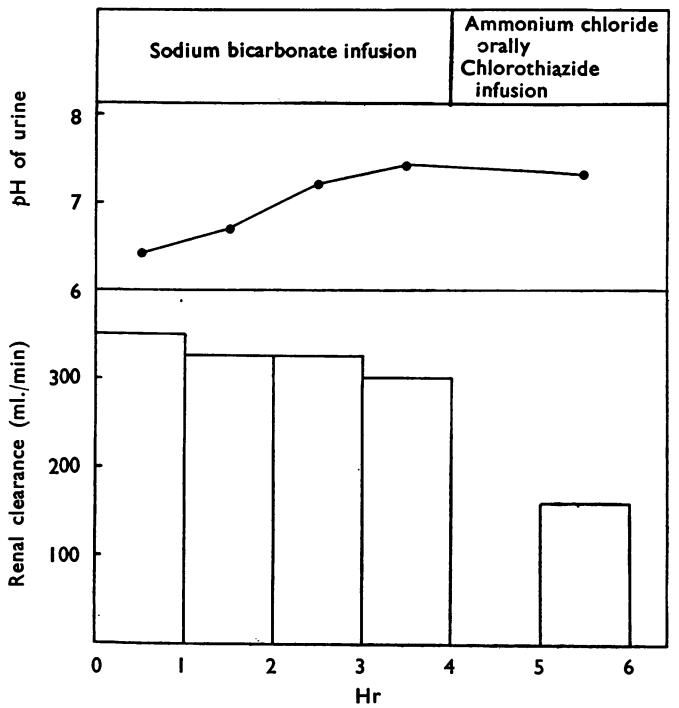


Fig. 6. Effect of chlorothiazide on the renal clearance of pempidine. Pempidine and chlorothiazide were infused at a steady rate (for details see text) and the urinary *p*H was controlled with sodium bicarbonate (intravenous) or ammonium chloride (oral).

another. With one exception the renal excretion of pempidine was less in alkaline urine (Table 6). For a change in urinary *p*H of (say) 6 to 7 the effect on renal pempidine clearance was often quite small; for a similar *p*H change brought about by chlorothiazide, however, a much lower renal clearance was obtained. Chlorothiazide reduced the renal clearance of pempidine by raising the plasma concentration of the drug with only minor changes in the excretion. An alteration in urinary *p*H, however, may reduce the renal clearance by lowering the excretion without affecting the plasma pempidine concentration.

Distribution of pempidine in rat tissues

The effect of chlorothiazide on the distribution of pempidine in rat tissues is shown in Table 7. Results of duplicate experiments in which pempidine was administered orally to rats both without and with chlorothiazide pretreatment are recorded as well as mean values. Chlorothiazide produced a 3-fold increase in the plasma

TABLE 6

EFFECT OF URINARY *p*H ON THE RENAL CLEARANCE OF PEMPIDINE

Urinary *p*H, plasma pempidine concentration (μg/ml.), renal pempidine clearance (ml./min) and renal pempidine excretion (mg/hr) measured during uniform infusion of pempidine (1 mg/hr). Left-hand columns for initial period, right-hand columns after infusing sodium bicarbonate (20 to 100 mEq/hr) to raise *p*H of urine

	Initial period				After infusing sodium bicarbonate			
Patient	Plasma concentration	Urinary *p*H	Renal clearance	Renal excretion	Plasma concentration	Urinary *p*H	Renal clearance	Renal excretion
1	0·043	5·0	337	0·870	0·045	6·8	79	0·213
2		5·6	246			6·6	258	
3	0·043	6·3	332	0·086	0·050	7·1	242	0·073
4		5·2	433			6·4	403	
5		6·2	336			6·5	182	
6	0·046	5·3	279	0·770	0·048	6·7	307	0·884
7	0·060	6·5	288	1·02	0·060	7·9	200	0·721
8	0·048	6·5	330	0·95	0·046	7·4	304	0·840

TABLE 7

PEMPIDINE CONCENTRATIONS IN RAT TISSUES

Concentrations of pempidine (μg/g) in tissues of rats after administration of the drug (1 mg/kg) by stomach tube. The figures on the left of each column represent the results of two experiments on groups of 5 rats, those on the right represent the mean values. Left-hand column: Rats not pretreated with chlorothirazide. Right-hand column: Rats pretreated with chlorothiazide (5 mg/kg) 7, 4 and 1 hr before pempidine

Tissue	μg/g without chlorothiazide	μg/g with chlorothiazide
Plasma	1·38, 1·44 } 1·41	4·31, 4·06 } 4·18
Red cells	2·11, 2·32 } 2·22	3·14, 2·75 } 2·99
Spleen	1·41, 1·46 } 1·44	0·47, 0·51 } 0·49
Kidney	1·95, 2·18 } 2·06	2·54, 1·96 } 2·25
Liver	1·48, 1·38 } 1·43	1·48, 1·58 } 1·53
Lung	0·25, 0·42 } 0·34	0·26, 0·21 } 0·24
Brain	0·25, 0·36 } 0·30	0·24, 0·52 } 0·38
Muscle	0·16, 0·20 } 0·18	0·09, 0·14 } 0·12

pempidine concentration, a smaller rise also being obtained with red cells. The amount of pempidine in spleen was much lower after administration of chlorothiazide, but changes in other tissues were relatively small and are probably not significant because the results represent only 10 animals.

Distribution of pempidine between red cells and plasma in vitro

When the distribution of pempidine between red cells and plasma was studied *in vitro* wholly unexpected results were obtained. A known amount of pempidine was added to fresh whole blood, maintained at 37° C and gently stirred. Aliquots

were withdrawn at intervals and the pempidine content of the plasma and the red cells measured. During the first 3 min of the experiment the plasma pempidine concentration decreased rapidly and thereafter increased steadily. The corresponding concentrations of pempidine in the red cells at first increased rapidly and then decreased so that the pempidine content of the whole blood was constant within

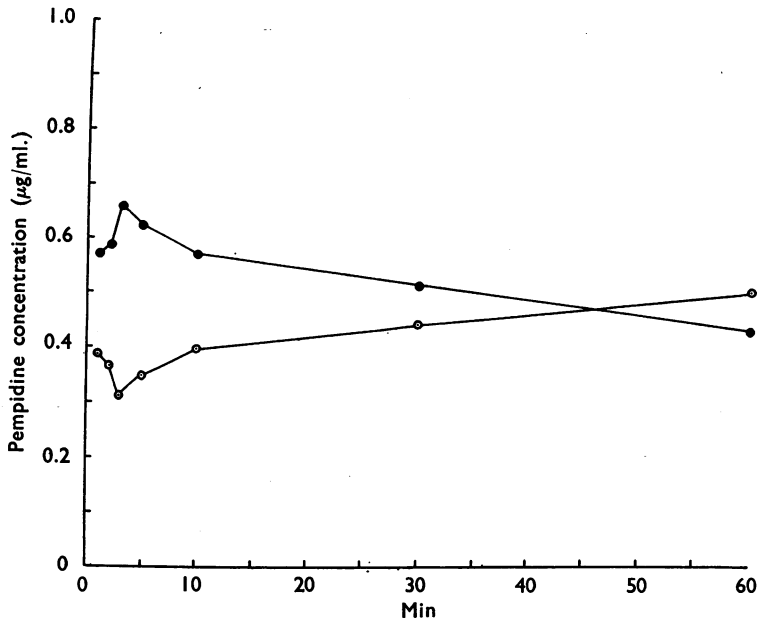


Fig. 7. Concentrations of pempidine in plasma and red cells *in vitro*. Pempidine was added to heparinized whole blood and the amount of pempidine in the plasma (○ —— ○) and red cells (● —— ●) measured at intervals for 1 hr.

the limits of experimental error (Fig. 7). During the first 45 min the concentration of pempidine was higher in red cells than in plasma, but thereafter the plasma concentration became the greater. Almost identical results were obtained with blood which had been incubated for 1 hr at 37° C before the addition of pempidine. In the presence of chlorothiazide, however, an equilibrium was established before the first measurement could be made (1 min) and no change in the pempidine concentration of either red cells or plasma was observed during the course of 1 hr. At all times the plasma pempidine concentration was greater than that in red cells (Fig. 8). Changes with time in the ratio $\frac{\text{red-cell pempidine concentration}}{\text{plasma pempidine concentration}}$ are shown in Fig. 9. Thus, in the absence of chlorothiazide the red-cell/plasma ratio passed through a well-defined maximum, whereas in the presence of chlorothiazide it showed no such variation but attained a steady value almost instantaneously. Within the limits of experimental error the same distribution ratio of pempidine between red cells and plasma was found (*a*) with blood to which chlorothiazide had been added *in vitro* (0.67); (*b*) with blood taken from a patient who had been premedicated with 500 mg of intravenous chlorothiazide 1 hr before (0.69); (*c*) *in vivo*

after intravenous injection of pempidine in patients premedicated with chlorothiazide (0.69). When blood was incubated with mersalyl *in vitro* before the addition of pempidine the distribution ratio of pempidine between red cells and plasma (1.0) did not change with time and, moreover, was the same as that found *in vivo* after intramuscular injections of mersalyl (see Table 3).

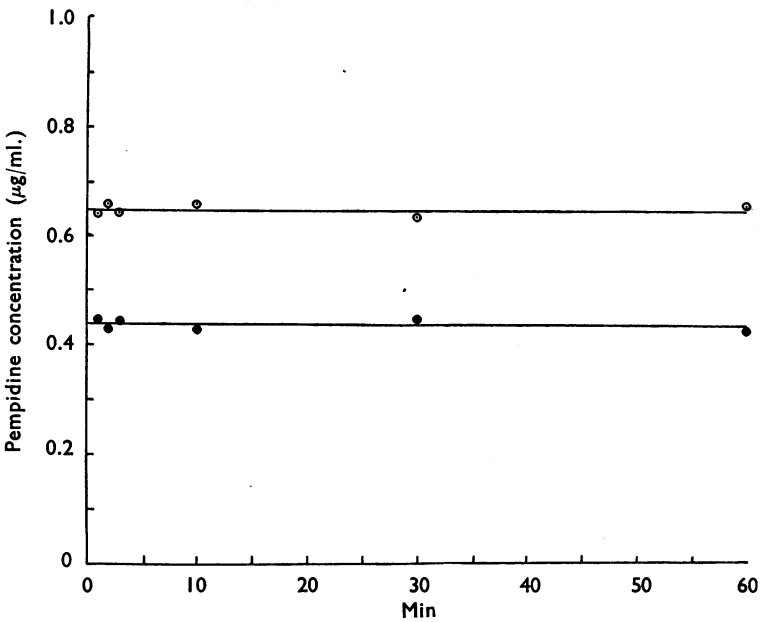


Fig. 8. Effect of chlorothiazide on concentrations of pempidine in plasma and red cells *in vitro*. Pempidine was added to heparinized whole blood containing chlorothiazide added *in vitro* and the concentrations of pempidine in the plasma (○ —— ○) and red cells (● —— ●) measured at intervals for 1 hr.

TABLE 8

THE EFFECT OF CHLOROTHIAZIDE ON THE BINDING OF PEMPIDINE TO SERUM ALBUMIN

Free and protein-bound pempidine measured by equilibrium dialysis. Initial concentration of pempidine (outer compartment) 1 μg/ml. Initial concentration of chlorothiazide (inner compartment) 2·5 to 50·0 μg/ml. Concentration of serum albumin (inner compartment) 1%

Pempidine (μg/ml.)				Chlorothiazide (μg/ml.)			
Total	Free	Bound	Bound/ Total	Total	Free	Bound	Bound/ Total
0·92	0·77	0·15	0·16	1·3	0·3	1·0	0·77
1·04	0·74	0·30	0·29	2·6	0·6	2·0	0·77
1·08	0·73	0·35	0·32	3·1	1·1	2·0	0·65
1·08	0·73	0·35	0·32	5·32	1·17	4·15	0·78
1·16	0·71	0·45	0·39	7·4	1·9	5·5	0·74
1·28	0·68	0·60	0·47	10·6	2·35	8·25	0·78
1·40	0·65	0·75	0·54	14·6	3·85	10·75	0·74
1·52	0·62	0·90	0·59	19·2	5·2	14·0	0·73
1·76	0·56	1·20	0·68	24·6	6·35	18·25	0·74

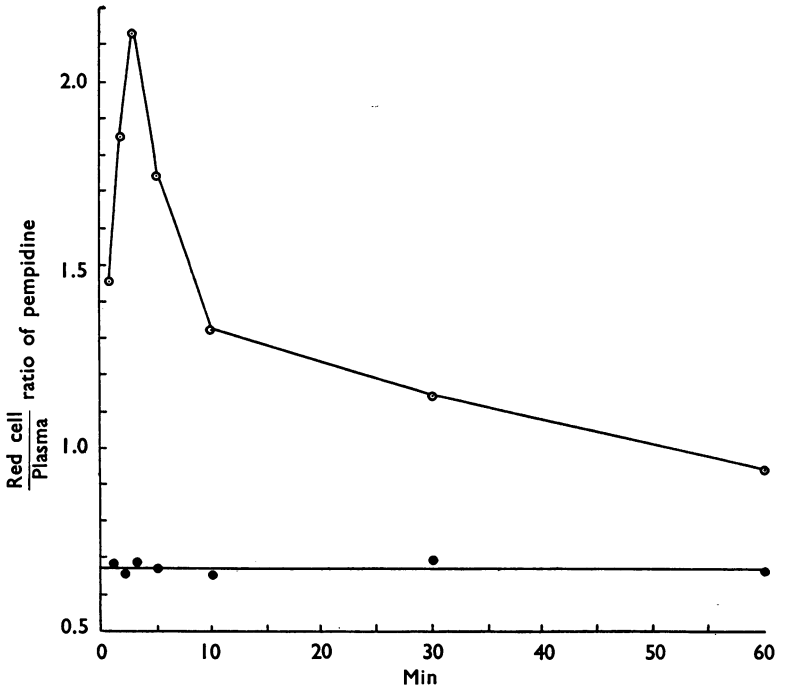


Fig. 9. Distribution ratio of pempidine *in vitro* between red cells and plasma, with (●——●) and without (○——○) chlorothiazide. The chlorothiazide was added *in vitro* to heparinized whole blood.

TABLE 9

THE EFFECT OF CHLOROTHIAZIDE ON THE BINDING OF PEMPIDINE TO SERUM ALBUMIN

Free and protein-bound pempidine measured by equilibrium dialysis. Initial concentration of pempidine (outer compartment) 0 to 1·0 μg/ml. Initial concentration of chlorothiazide (inner compartment) 50 μg/ml. concentration of serum albumin (inner compartment) 1%

Pempidine (μg/ml.)				Chlorothiazide (μg/ml.)			
Total	Free	Bound	Bound/ Total	Total	Free	Bound	Bound/ Total
0·212	0·047	0·165	0·78	23·8	6·55	17·25	0·73
0·400	0·100	0·300	0·75	25·0	6·26	18·74	0·75
0·588	0·153	0·435	0·74	24·0	6·50	17·50	0·73
0·784	0·204	0·580	0·74	23·4	6·65	16·75	0·72
1·000	0·250	0·750	0·75	23·6	6·61	16·99	0·72
1·152	0·312	0·840	0·73	23·9	6·56	17·34	0·73
1·300	0·375	0·925	0·71	24·6	6·35	18·25	0·74
1·408	0·448	0·960	0·68	24·1	6·48	17·62	0·73
1·548	0·513	1·035	0·67	25·8	6·05	19·75	0·77
1·680	0·580	1·100	0·66	26·2	5·95	20·25	0·77

Binding of pempidine to serum albumin in vitro

Equilibrium dialysis experiments carried out in the absence of protein showed that neither pempidine nor chlorothiazide was adsorbed on the dialysis sac. In

every case the observed changes in concentration were due solely to diffusion processes. Pempidine alone (that is, in the absence of chlorothiazide) did not interact with serum albumin to any measurable extent. On the other hand, chlorothiazide alone (that is, in the absence of pempidine) was extensively bound, and the present results agree with the findings of Baer and his colleagues (1959). The results of the two most important series of experiments are shown in Tables 8 and 9.

DISCUSSION

In treating severe hypertension it is often necessary to administer diuretics, and many workers consider that chlorothiazide, when used in this way, potentiates the hypotensive action of ganglion-blocking agents. This is so with pempidine although the effect is quite small. Our previous studies showed that the fall in blood-pressure after giving pempidine orally was related to the plasma concentration of the drug. This correlation no longer holds in the presence of chlorothiazide, which may almost treble the plasma pempidine concentration with only a slight increase in the hypotension.

The increased response to pempidine after the administration of chlorothiazide might be attributed to an inherent hypotensive activity of chlorothiazide itself, but in our experience this cannot be convincingly demonstrated. Chlorothiazide does, however, potentiate the action of ganglion-blocking agents by reducing the plasma volume, although this effect combined with the very high plasma pempidine concentrations now reported should produce a much larger fall in blood-pressure than was actually observed. One must be cautious in comparing results of intravenous studies with those obtained from patients having regular daily doses of pempidine and chlorothiazide by mouth. Sustained plasma pempidine concentrations could conceivably have a different effect from the transient elevation after intravenous injection, while repeated doses of chlorothiazide reduce plasma volume and deplete body sodium. The experiments illustrated in Fig. 3 show that a high plasma pempidine concentration achieved with the aid of chlorothiazide may produce a smaller hypotensive effect than that caused by an equal concentration obtained merely by increasing the dose of pempidine. This implies that chlorothiazide may increase the plasma concentration of pempidine and also modify its pharmacological activity.

High plasma pempidine concentrations obtained by administering chlorothiazide suggest an alteration in renal clearance. Harington & Kincaid-Smith (1958) suggested that chlorothiazide reduced the renal excretion of mecamylamine by inhibiting carbonic anhydrase activity, so that the urine became more alkaline. The hypothesis is not entirely satisfactory because the inhibition of carbonic anhydrase is only transitory and, moreover, acetazoleamide has little effect on the hypotensive effect of mecamylamine although it is a powerful inhibitor of the enzyme (Bayliss, Fleming & Rees, 1958). We do not consider this mechanism to be of importance in the case of pempidine. Nevertheless, chlorothiazide does lower the renal clearance (though not the excretion) of pempidine by more than 50%. This cannot be attributed to an increase in the urinary *p*H because an alkaline urine obtained by infusions of sodium bicarbonate lowered the renal clearance of

pempidine by only about 15%. The observed reduction in renal clearance of pempidine brought about by chlorothiazide must therefore depend on the increased plasma pempidine concentration. During the infusion experiments the renal pempidine clearance increased for some hours after a steady plasma concentration of the drug was established. This probably means that pempidine passed initially into the kidney parenchyma until an equilibrium was set up ; at this point the renal clearance became constant.

If chlorothiazide increased the plasma pempidine concentration by changing the tissue distribution, pempidine would presumably be transferred from intracellular depots to the extracellular fluid. The experiments with rats suggest that this does not happen, because chlorothiazide causes no major redistribution of pempidine among the tissues. The pempidine content of spleen is decreased to some extent, but clearly the amount of drug released is insufficient to account for the very high concentrations of pempidine in the plasma. The most drastic changes in pempidine distribution are those in red cells and plasma.

We consider that a reasonable explanation of our findings in hypertensive patients can be gven in terms of a pempidine/protein interaction induced by chlorothiazide. *In vitro* experiments, using equilibrium dialysis techniques, show that although in solution pempidine does not normally interact with serum albumin to any measurable extent, a pempidine/albumin complex of some kind is formed in the presence of chlorothiazide. The degree of binding can be extensive, depending on the amounts of pempidine and chlorothiazide present. It is reasonable to suppose that similar interactions occur *in vivo* and might involve other proteins as well as albumin.

The high concentrations of pempidine in plasma after the administration of chlorothiazide are due to the presence of a " bound " fraction of the pempidine closely associated with the plasma proteins and producing no hypotensive effect, in addition to the " free " pempidine, which is pharmacologically active. The " bound " pempidine does not cause hypotension because, being bound to protein, it cannot penetrate to the site of action. Moreover, it is not filtered at the glomerulus and the overall renal clearance is therefore reduced.

High plasma pempidine concentrations may be detected chemically although only part of the drug is in a form which can exert a physiological effect. This is consistent with the observation that plasma pempidine concentrations increase rapidly when patients on maintenance treatment are given intravenous injections of chlorothiazide although no change in the blood pressure occurs. The normal passage of drug from intracellular stores to extracellular fluid continues although the pempidine is no longer excreted at the former rate but is instead bound to plasma protein in an inactive form. Consequently, the blood-pressure does not fall significantly although the plasma pempidine concentration may be greatly increased. Protein-binding can account for the high plasma pempidine concentrations and reduced renal clearance which occur after giving chlorothiazide, but other factors also seem to be involved. It is known, for instance, that chlorothiazide is excreted within 18 hr (Ford, Moyer & Spurr, 1957) and it is by no means clear how chlorothiazide continues to affect plasma pempidine concentrations after 3 days.

The striking changes that chlorothiazide produces in the red-cell/plasma distribution ratio of pempidine can also be partly attributed to the formation of a complex by pempidine and plasma proteins. *In vivo* the stable ratio between red cells and plasma is about 1.2:1, but after chlorothiazide it falls to about 0.7:1. The transfer of pempidine from red cells into plasma is presumably a result of binding to plasma proteins and reflects a lower "free" pempidine concentration in plasma. This hypothesis explains neither the abolition by chlorothiazide of the changing red-cell/plasma ratio in the first hour after the addition of pempidine *in vivo* or *in vitro,* nor the red-cell/plasma ratio in patients given intravenous pempidine after premedication with chlorothiazide. It is interesting to note, however, that the red-cell/plasma ratio for pempidine in the presence of chlorothiazide (~ 0.7) is the same as found by Baer, Leidy & Brooks (1957) for the partition of chlorothiazide between red cells and plasma. However, because at present we cannot explain the normal change in the distribution of pempidine between cells and plasma during the first hour after mixing, it is difficult even to speculate about the way in which chlorothiazide modifies it. At first sight the distribution in the pempidine injection studies *in vivo* may seem compatible with the hypothesis of plasma-protein-binding, but a number of difficulties arise. For example, if the "free" fraction of pempidine were cleared from the plasma at the normal rate for pempidine without chlorothiazide, a low "free" concentration would be expected. Furthermore, because the red cells are presumably in equilibrium with the "free" pempidine fraction their pempidine content should also not only be low but should fall rapidly. In fact, this does not happen in practice. It seems probable that just as chlorothiazide promotes interaction of pempidine and plasma protein so it may modify the affinity of red cells for the drug ; however, we have no direct experimental evidence on this point.

The interactions of pempidine and chlorothiazide with proteins and with organized structures such as red cells are of great interest. The results reported in this paper raise a number of important matters which, however, cannot be discussed at length here. For example, the nature of the pempidine/protein complex is quite unknown and merits further study. Again, chlorothiazide and pempidine are distributed between red cells and plasma in almost the same ratio, but the significance of this is not clear at present. The delay in onset of the chlorothiazide effect (see Table 4) is curious. When pempidine and chlorothiazide were injected simultaneously normal plasma pempidine concentrations were obtained, but the red-cell/plasma distribution ratio of pempidine decreased during the first hour to the value observed in patients premedicated with chlorothiazide. This suggests that pempidine had already left the blood before it could be bound to plasma protein by the action of chlorothiazide. This delay is unexplained. Without doubt, however, the most perplexing observation is the rapid transport of pempidine into and out of red cells that occurs immediately after mixing. It is difficult to formulate a satisfactory mechanism, especially as chlorothiazide appears able either to inhibit the process or to increase its rate to such an extent that it is completed instantaneously. We do not at present know if these phenomena have a common basis, but it is tempting to speculate whether chlorothiazide can modify the surfaces of proteins and hence change their adsorptive properties. This would possibly enable the protein/pempidine complex to persist even after the chlorothiazide had been removed. Unfortunately, we have at present

no experimental evidence to support this hypothesis, but an examination by electrophoresis or ultracentrifugation of various proteins and cells before and after treatment with chlorothiazide might be rewarding.

We gratefully acknowledge the valuable technical assistance given by Miss M. I. Tinson and Mr A. F. Ivens who carried out the dialysis experiments and fluorescence measurements respectively. Thanks are due also to Dr H. W. Reading for performing the rat experiments and to Dr H. Campbell and Professor J. McMichael, F.R.S., for their continued interest and support.

REFERENCES

BAER, J. E., LEIDY, L. & BROOKS, A. V. (1957). Physiological disposition of chlorothiazide, a saluretic-diuretic agent. *Fed. Proc.*, **16**, 278–279.

BAER, J. E., LEIDY, L., BROOKS, A. V. & BEYER, K. H. (1959). The physiological disposition of chlorothiazide (diuril) in the dog. *J. Pharmacol. exp. Ther.*, **125**, 295–302.

BAYLISS, R. I. S., FLEMING, P. R. & REES, J. R. (1958). Chlorothiazide and hypotensive action of mecamylamine. *Lancet*, **i**, 529.

DOLLERY, C. T., EMSLIE-SMITH, D. & MUGGLETON, D. F. (1960). Plasma pempidine concentrations in hypertensives. *Brit. med. J.*, **i**, 521–523.

DOLLERY, C. T., HARINGTON, M. & KAUFMANN, G. (1959). The mode of action of chlorothiazide in hypertension: with special reference to potentiation of ganglion-blocking agents. *Lancet*, **i**, 1215–1218.

DUSTAN, H. P., CUMMING, G. R., CORCORAN, A. C. & PAGE, I. H. (1959). A mechanism of chlorothiazide-enhanced effectiveness of antihypertensive ganglioplegic drugs. *Circulation*, **19**, 360–365.

FORD, R. V., MOYER, J. H. & SPURR, C. L. (1957). Clinical and laboratory observations on chlorothiazide (diuril). *Arch. intern. Med.*, **100**, 582–596.

FREIS, E. D. & WILSON, I. M. (1957). Potentiating effect of chlorothiazide (diuril) in combination with antihypertensive agents; preliminary report. *Med. Ann. D.C.*, **26**, 468.

HARINGTON, M. & KINCAID-SMITH, P. (1958). Effect of chlorothiazide on the hypotensive action of mecamylamine and on its urinary excretion. *Lancet*, **i**, 403–404.

HOLLANDER, W. & WILKINS, R. W. (1957). Chlorothiazide: a new type of drug for the treatment of arterial hypertension. *Boston med. quart.*, **8**, 69–75.

WILSON, I. M. & FREIS, E. D. (1960). Relationship between plasma and extracellular fluid volume depletion and the antihypertensive effect of chlorothiazide. *Circulation*, **20**, 1028–1036.