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METABOLISM OF CHLORMEZANONE IN MAN AND LABORATORY ANIMALS*

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Abstract—The metabolism of chlormezanone has been studied in rats, dogs, and man. After oral administration to the rat, the tissue: plasma concentration ratios are very close to unity, and the mean decrease in concentrations between 2 and 4 hr postmedication is about 30 per cent. In the dog, an oral dose of 7 mg/kg gives a peak plasma level of 5–6 μg/ml, while 200 mg/kg gives a peak level of 140–160 μg/ml. The plasma half-lives for these doses, however, are quite different: about 4 and 12 hr respectively. One hr after the intravenous administration of 25 mg/kg to dogs, the plasma level is about 30 μg/ml, and the regression rate indicates a plasma half-life of 10 hr. In man a single 400-mg dose gives a peak plasma level of 5–6 μg/ml, with an indicated plasma half-life of 24 hr. The use of a simple first-order absorption and elimination model has permitted the calculation of a human dosage schedule designed to maintain the plasma level almost constantly between 5 and 10 μg/ml, and this schedule has been verified experimentally. Chlormezanone is excreted as such in human urine and dog bile. Its ingestion by man results in the excretion of no extra glucuronic acid but does lead to the excretion in the urine of an acid which is evidently 4-chlorohippuric. It appears, therefore, that the degradation of chlormezanone in man may be explained largely on the basis of a nonenzymatic hydrolysis, followed by oxidation and conjugation of the 4-chlorobenzaldehyde formed in the hydrolytic reaction.

CHLORMEZANONE,† or 2-(4-chlorophenyl)-3-methyl-4-metathiazanone-1-dioxide (CM), has proved to be the most useful of a series of central nervous system depressants first described by Surrey *et al.*[1] A study of the profile of its biological activity has been published by Gesler and Surrey,[2] and data on its animal toxicity have been reported by Gesler and Coulston.[3] Recently Rosenberg and Cooke[4] have presented evidence which indicates that one of the sites of action of CM as a muscle relaxant may be the neuromuscular junction. It has been demonstrated[5–8] that CM acts as a muscle-relaxing and tranquilizing agent in man, but no information on its metabolism, or that of other compounds of the 2-aryl-4-metathiazanone series, has been published. For this reason the experiments to be described herein have been performed.

The determination of CM in biological materials is most conveniently based upon its hydrolytic decomposition to give 4-chlorobenzaldehyde, concomitant reaction of the aldehyde with dinitrophenylhydrazine, and conversion of the hydrazone to an alkali salt.[9] This method does not distinguish between CM and 4-chlorobenzaldehyde,

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† Available from Winthrop Laboratories, New York, N.Y., under the trade name of Trancopal.

but the latter has only rarely been detected in the biological materials thus far studied, after the administration of CM.

METHODS OF ANALYSIS

1. *CM in plasma.* To 3 ml plasma in a 50-ml glass-stoppered (g.s.) centrifuge tube are added 12 ml tartrate buffer (0·85 M NaCl, 0·2 M tartaric acid; pH adjusted to 4·5 with sodium hydroxide) and 30 ml reagent grade benzene. The tube is shaken for 20 min, centrifuged, and the aqueous phase removed by aspiration. About 2 g sodium sulfate is added, and the tube is shaken by hand to dry the benzene. Of the latter, 25 ml is transferred to a clean 50-ml g.s. centrifuge tube and the volume is reduced to about 1–2 ml in a boiling water bath. Five ml of DNPH reagent (0·025% dinitrophenylhydrazine, in 15% perchloric acid)* is added, the tube is returned to the boiling bath for 15 min, and it is then cooled to room temperature. Three ml reagent grade benzene is added, the tube is shaken for 1–2 min, after which 5 ml *n*-hexane is added and the tube shaken for another 1–2 min. After centrifugation, 6 ml of the clear solvent phase is transferred to a test tube which contains 2 ml of TEA reagent (2·5% tetraethylammonium hydroxide in 75% methanol). After thorough mixing, the supernatant is aspirated, and the absorbance of the alcoholic phase is read in a suitable colorimeter, at 475 and 650 mμ. In the Coleman Jr. spectrophotometer, with cuvettes of 8 mm internal diameter, the net absorbance (475–650 mμ) per 10-μg increment of CM in the original sample is 0·11–0·13. In the range 5–30 μg, the recovery of CM from plasma is 95 ± 5 per cent; for amounts exceeding 30 μg the analysis is repeated with a smaller aliquot either of the plasma or of the benzene extract.

2. *CM in urine.* The method is the same as for plasma except that the initial extraction time need not exceed 2 min. The blank value on human urine has averaged about 1·3 μg/ml in our hands.

3. *CM in tissues.* Samples of 1–2 g (or the entire organ, if it weighs less than 1 g) are homogenized in 10 ml of the tartrate-NaCl buffer, and the cup is washed twice with 10-ml volumes of the buffer. The homogenate is transferred to a 100-ml g.s. centrifuge tube and is shaken for 30 min with 50 ml reagent grade benzene. After centrifugation, and clarification of the benzene with solid NaCl, the solvent is transferred to a clean tube, in which it is washed successively with 30 ml borate buffer (0·2 M, pH 9·4), 5 ml water, and 30 ml citrate buffer (0·2 M, pH 2·5). After centrifugation, and aspiration of the citrate, the benzene is dried by shaking with about 3 g sodium sulfate. Depending on the amount of CM expected, an aliquot of 5–25 ml of the clear benzene is transferred to a 50-ml g.s. centrifuge tube, after which the procedure is as described for plasma. The results are evaluated against CM standards (10–50 μg) carried through all the above operations. In this laboratory the mean recovery of CM from homogenates of rat liver, at added levels of 20–60 μg/g, has been 92 ± 5 per cent, and the blank values on various rat tissues have ranged from 2 to 8 μg/g (see Table 3).

4. *Glucuronic acid in urine.* This was determined by the method of Ormsby.[10]

5. *Chlorine-containing organic acids in urine.* The sample is acidified to pH 2·5 and is extracted directly with 4 vol. of ether. Alternatively, it may first be adjusted to pH 7 and extracted with 4 vol. of ether (which is discarded) and then acidified to pH 2·5 and

* Prepared from Eastman's dinitrophenylhydrazine after its recrystallization from alcohol. Before the solution in 15% perchloric acid is used it is washed with 2 vol. of solvent mixture (benzene: *n*-hexane, 3:5, v/v).

extracted with 4 vol. of ether, which is evaporated on a steam bath. A suitable aliquot of the residue is burned in an atmosphere of oxygen, as described by Childs *et al.*,[11] the gases so produced are dissolved in water, and Cl^- is determined in this solution by the method of MacLean and Van Slyke,[12] or the equivalent. To eliminate the possibility that traces of NaCl may be carried into the ether extracts, another aliquot of the residue is titrated directly for Cl^-, and the difference between the two determinations is taken as equivalent to the amount of organic Cl in the sample. The recovery of Cl added to urine prior to the ether extraction step(s), in the form of 4-chlorobenzoic acid, 4-chlorohippuric acid, or CM, by this method exceeds 90 per cent.

EXPERIMENTAL

1. *Tissue distribution in the rat.* Ten male albino rats weighing 180–220 g received oral doses of CM (50 mg/kg) as a suspension in 1% gum tragacanth. Two or 4 hr thereafter they were sacrificed by decapitation, and oxalated blood samples were obtained. Representative tissues were immediately removed and were stored in a frozen condition until they could be analyzed. Five male rats of comparable size and age served as controls, to establish the tissue blank values for this species.

2. *Absorption–excretion in the dog.* A mongrel dog received 7 mg CM/kg dissolved in propylene glycol, i.v.; 5 days later it received the same medication orally. Plasma samples were obtained at regular intervals in both these experiments and were analyzed for CM. To study the plasma levels of CM in relation to biliary excretion, three mongrel dogs were prepared (by Dr. F. J. Rosenberg) under "dial-urethane" (a preparation containing diallylbarbituric acid:urethane:monoethyl urea, 1:4:4) anesthesia, for serial sampling of the gall bladder bile. At regular intervals after the i.v. injection of a 25 mg dose of CM/kg in propylene glycol, oxalated plasma samples were obtained, and concomitantly the gall bladder was drained as completely as possible. These materials were analyzed for CM; 4-chlorobenzaldehyde, if present in the bile (see below) would be included in the value found for CM.

Two different groups of five mongrel dogs each received 200 mg CM/kg orally in gelatin capsules, as a 100-mesh powder. (Doses of this magnitude are definitely atoxic, and represent about 0·4 of the LD_{50}[13].) The experiments involved two different time schedules, one covering the 0–4-hr postmedication period and the other the 4–30-hr period. Oxalated plasma samples were obtained at intervals during these periods and were analyzed for CM. However, observations at the 30-hr period were made on only two of the dogs. To determine whether the chromogenic substance in the plasma was CM or 4-chlorobenzaldehyde, five of the premedication and four of the postmedication samples (chosen at random) were extracted with methylene dichloride, which was dried over sodium sulfate and evaporated just to dryness. The residue was taken up in a small volume of the same solvent, which was spotted on a thin layer of silica gel (Camag DF-5), and the chromatograms were first developed with benzene, in which 4-chlorobenzaldehyde has an R_f of 0·2 and CM has an R_f of zero. They were then developed with chloroform:methanol (19:1, v/v), in which CM has an R_f of 0·3.* Either compound was readily detected on these plates as an ultraviolet-absorbing spot.

* By this method 4-chlorobenzaldehyde was not detected in any of the plasma samples studied (see below). However, in view of the subsequent observation that the aldehyde cannot be detected in the plasma after its administration per se, it was not to be expected that the administration of CM would produce detectable levels.

To determine the fate of the metabolically related 4-chlorobenzaldehyde, two mongrel dogs received doses of 25 mg/kg of this compound in gelatin capsules, and were kept in metabolism cages for 24 hr thereafter. Oxalated plasma samples were obtained at 0, 4, and 24 hr postmedication, and urine was collected for the 0–24-hr period. These samples were analyzed for 4-chlorobenzaldehyde (for which the procedure is the same as for CM; see under Methods); the urine samples were also analyzed for organic chlorine. Ether extracts of the urine samples (extractions made at pH 2·5) were analyzed qualitatively by thin-layer chromatography, (TLC), in the solvent system: benzene, 9 vols; methanol, 1 vol; glacial acetic acid, 1 vol. In this system 4-chlorobenzoic acid has an R_f of 0·7 and 4-chlorohippuric acid has an R_f of 0·4.

3. *Absorption–excretion experiments in man.* Four laboratory volunteers served as subjects for three experiments relating to the human metabolism of CM. In the first of these the subjects took single 400-mg doses as tablets, on an empty stomach, after having collected 24-hr control urine samples under toluene. Plasma samples were obtained at five intervals up to 24 hr postmedication, and urine was collected quantitatively for three intervals comprising the first 48 hr postmedication. The plasma samples were analyzed for CM; the urine was analyzed for both CM and extra glucuronic acid. Nine weeks later the same subjects took CM on a multiple-dosage schedule as follows: 150 mg at 8:30 a.m., 12:30 p.m., and 4:30 p.m. on three consecutive days, and at 8:30 a.m. on the fourth day. The morning doses were always taken on an empty stomach, food being provided 1 hr later. Plasma samples were obtained at frequent intervals during this dosage regimen, and were analyzed for CM as were urine samples, which were collected under toluene for the postmedication periods of 0–24 and 48–72 hr. Four weeks later the same subjects took 400-mg doses of CM at 0 and 12 hr, followed by 200 mg at 12-hr intervals for 72 hr. Plasma levels of CM were determined at frequent intervals during and after this dosage regimen.

To measure the excretion of ether-soluble, chlorine-containing organic acids after the ingestion of CM, another subject took 400-mg doses of CM at 0 and 8 hr and collected urine under toluene for periods up to 70 hr after the first dose. As a confirmatory experiment, another subject took a single 400-mg dose of CM and collected urine at intervals for the first 54 hr postmedication. These samples were analyzed for CM and for ether-soluble, chlorine-containing organic acids (i.e. those extracted at pH 2·5 but not at pH 7), as well as for neutral ether-soluble organic chlorine (extractable at pH 7). The former ether extracts were also examined by TLC methods.

RESULTS

The data on the tissue levels of CM in the rat after an oral dose of 50 mg/kg are presented in Table 1. They indicate that this drug, on absorption, becomes quite uniformly distributed throughout the organism. Thus, the only tissues that showed concentrations of CM definitely higher than those existing concomitantly in the plasma were the organs of excretion and the heart; one organ (the spleen) appeared to contain less CM than the plasma at both time intervals studied. However, in view of the individual variations, and the difficulty in obtaining entirely satisfactory blanks for some tissues, it seems unlikely that differences such as that between spleen and plasma, or between heart and plasma, should be considered significant. The mean decrease of CM concentration in tissues between 2 and 4 hr postmedication was 31 per

cent, and that in plasma was 32 per cent. The finding that the concentration in the body fat was no higher than that in the plasma was somewhat unexpected, in view of the prior observation[13] that the distribution coefficient of CM between olive oil and 0·1 M phosphate buffer of pH 7·2 is 2·8, whereas between oleyl alcohol and the buffer it is 4·6.

TABLE 1. TISSUE DISTRIBUTION OF CHLORMEZANONE IN RATS AT TWO INTERVALS AFTER AN ORAL DOSE OF 50 MG/KG

Rat no.	Time after medication (hr)	Tissues analyzed*							
		Plasma	Muscle	Liver	Kidney	Heart	Spleen	Lung	Fat
1†		0·4	4·0	9·9	11·4	2·4	0	0·1	2·4
2		0·6	0·9	4·9	4·7	2·1	3·6	3·1	2·9
3		0·8	1·3	5·7	4·1	4·6	5·5	1·2	3·2
4		0	8·1	13·4	12·0	10·1	6·8	3·1	7·2
5		0	6·2	10·6	5·5	5·8	3·4	3·1	—
Mean		0·4	4·1	8·9	7·5	5·0	3·9	2·1	3·9
S.E.		0·2	1·4	1·6	1·7	1·4	1·2	0·6	1·1
6	2	22·2	27·7	62·6	27·3	39·7	17·7	24·9	17·3
7	2	27·5	28·6	68·2	46·8	42·8	27·4	30·3	24·6
8	2	27·2	24·2	61·5	44·8	36·8	20·6	37·0	23·9
9	2	17·9	20·6	61·0	36·0	34·9	22·2	22·2	20·0
10	2	29·0	40·3	66·0	44·2	54·0	26·4	38·4	29·9
Mean		24·8	27·7	64·9	40·0	41·6	22·9	30·6	23·1
S.E.		2·0	3·4	1·5	3·7	3·4	1·7	4·1	2·2
Tissue/ plasma	Mean‡	—	0·97	2·38	1·33	1·50	0·78	1·17	0·79
11	4	13·0	18·4	35·0	42·8	22·9	10·9	16·9	—
12	4	19·4	26·9	61·0	46·3	32·5	14·1	30·7	—
13	4	23·9	24·6	52·9	50·2	29·1	23·0	30·6	—
14	4	13·7	18·4	29·7	37·5	16·9	25·3	16·3	—
15	4	14·4	18·5	31·5	31·9	17·6	11·6	16·5	—
Mean		16·9	21·0	42·0	41·7	23·8	17·0	22·2	—
S.E.		2·1	1·8	6·2	3·1	3·1	2·9	3·5	—
Tissue/ plasma	Mean‡	—	1·02	2·00	2·07	1·14	0·79	1·22	—

* Values given as mg/kg wet tissue.
† Rats 1–5 were unmedicated controls; rats 4–5 were females.
‡ For the five medicated animals compared to the five control animals.
— Not done.

The dog receiving the CM in propylene glycol (7 mg/kg) gave these results, as μg/ml in the plasma at 0, 1, 2, 4, and 6 hr postmedication, respectively: i.v.: 0·9, 5·3, 5·5, 3·9, 2·8. Oral: 0·6, 5·2, 4·3, 4·1, 3·0.

In the i.v. series the 1-hr value appears definitely to be erroneous; the other three values indicate a half-life of 3·2 hr, a net 0-hr concentration of 7·2 μg/ml, and a volume of distribution equivalent to 97 per cent of the body weight. The oral data indicate that the dose was substantially 100 per cent absorbed when given by this route, and that the half-life was 5·2 hr.

The data on the plasma levels of CM in the dogs which received 200 mg/kg are presented in Fig. 1. They show that absorption of the drug began promptly and proceeded rapidly but, probably as a result of the very massive dose, the peak plasma level was not reached until 8 hr postmedication. Such a delayed peak would hardly be possible unless some of the drug was being excreted in the bile and recycled (see below).

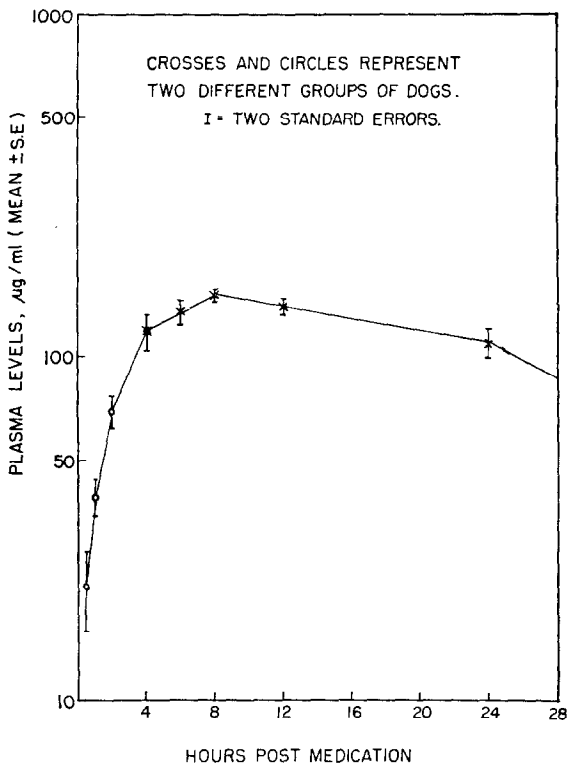

Fig. 1. Plasma levels of chlormezanone in dogs after the oral administration of 200 mg/kg as a 100-mesh powder. One group of 5 dogs was used to study the 0–4-hr period; another group of 5 dogs was used to study the 4–24-hr period. In two of the latter animals observations were also made at 30 hr postmedication, and the mean decline for this interval (31%) was used to establish the slope of the curve for the 24–28-hr period.

The variations among animals were remarkably small, as shown by the standard errors, which averaged only about 10 per cent of the observed means. In fact, in the group of dogs which was followed only to the fourth postmedication hour, the mean level at this time was 118 $\pm$ 4 μg/ml, while in the group which was used to study the 4–30-hr interval, the mean level at the fourth hour was 119 $\pm$ 4 μg/ml. The plasma level regression rate for the 8–24-hr period, when plotted on a semilog scale, was linear, and would correspond to a half-life of 36 hr; however, in the two dogs which were followed over the 24–30-hr period the decline during this time was 31 per cent. In three other dogs which received a different dosage form, the decline over the 24–30-hr period averaged 29 per cent (range, 25–34). A 30 per cent decline in 6 hr is equivalent to a half-life of 11·5 hr; analyses of four of the postmedication samples, by

thin-layer chromatography, indicated the presence in these samples of unchanged CM (and of no 4-chlorobenzaldehyde), in amounts (as estimated by the size of the spots roughly equal to those obtained by colorimetric analysis. The purpose of including some of the premedication bloods in this part of the study was to eliminate artifacts.

The data on concentrations of CM in the plasma and gall bladder bile of the anesthetized dogs which received 25 mg CM/kg i.v. in propylene glycol are presented in Table 2. They show that the biliary concentrations peaked at about 3 hr post-

TABLE 2. PLASMA LEVELS AND BILIARY EXCRETION OF CHLORMEZANONE IN THE DOG AFTER THE INTRAVENOUS ADMINISTRATION OF 25 MG/KG

Time after medication (hr)	Dog A (11·2 kg)			Dog B (7·2 kg)			Dog C (10·0 kg)		
	In plasma (μg/ml)	In bile (μg/ml)	In bile (μg)	In plasma (μg/ml)	In bile (μg/ml)	In bile (μg)	In plasma (μg/ml)	In bile (μg/ml)	In bile (μg)
0	0·8	6		0·8	3		0·7	0·6	
1	24·6	76	140	40·6	73*	133	25·8†	38	73
2	22·9	137	183	37·9	363	432	22·3	116	231
4	17·9	276	162	33·4	454*	590	16·6	102	413
6	16·8	262	256	32·2	114	488	15·3	173	155
								61‡	810
0–6 (total)			741			1643			1682

* Most of the bile samples smelled strongly of 4-chlorobenzaldehyde. It was specifically identified by TLC in these samples.

† Sample lost in processing; value estimated.

‡ Residual bile sample obtained when the dog was sacrificed at 6 hr.

medication, and at levels about eight times those existing concomitantly in the plasma. However, the total amount of CM (and/or 4-chlorobenzaldehyde)* excreted in the bile was small relative to the dose. The plasma level data indicate a half-life of about 10 hr, and a mean 0-hr concentration (by extrapolation) of 33 μg/ml.

The two dogs receiving 25 mg 4-chlorobenzaldehyde/kg showed no measurable amount of this aldehyde in urine or plasma at any of the intervals studied. The 24-h excretion of organic Cl (extracts made at pH 2·5) was equivalent to 68·6 per cent of the dose in one animal, and 65·8 per cent of the dose in the other. Thin-layer chromatographic analysis of the ether extracts indicated the presence of a trace of 4-chlorobenzoic acid, and of a large amount of 4-chlorohippuric acid. Methanolic eluates of the spots corresponding to the latter acid (R_f = 0·38–0·42) gave negative reactions for glucuronic acid, although the original ether extracts contained considerable amounts of this acid.

The data on plasma levels and urinary excretion of CM in man after the single oral 400-mg dose are presented in Table 3. They show that the urinary excretion for the first 48 hr postmedication averaged a little over 1 per cent of the dose, and that the medication resulted in the excretion of no extra glucuronic acid. The mean net peak

* Some of the bile samples from the dogs listed in Table 2 smelled strongly of 4-chlorobenzaldehyde, and its presence was confirmed by TLC methods (see table). As stated above, the colorimetric method for CM measures the aldehyde also. It has been observed by one of us[18] that in aqueous phosphate buffer (pH 7·35) at 37°, CM decomposes to the aldehyde with a half-life of 48 hr.

plasma level for the four subjects studied was 5·5 μg/ml. This peak occurred at 1–2 hr postmedication, and the decline from this peak was a first-order process, with a mean plasma half-life of 24 hr (range 19–30). Assumed that the absorption of CM, as well as its elimination, can be described as a first-order process (as is the case with most drugs) the usual type of model can be applied for a description of the plasma level

TABLE 3. ABSORPTION AND EXCRETION OF CHLORMEZANONE AND OF GLUCURONIC ACID IN MAN AFTER AN ORAL DOSE OF 400 MG OF THE FORMER AT ZERO TIME

Post-medication (hr)	A. Plasma levels, as μg/ml Subjects R.B.	E.C.	L.G.	M.T.	Mean net levels†	Theoretical levels‡
0*	0·4	1·4	2·2	1·7		
1	4·9	3·4	9·9	6·1	4·7	3·9
2	4·8	6·0	8·8	7·3	5·3	5·1
4	4·2	4·8	7·8	6·2	4·3	4·7
7	4·0	4·4	6·8	5·7	3·8	4·4
24	2·1 (est)	3·6	5·2	3·9	2·5	2·7

Post-medication (hr)	B. Urinary excretion, mg/24 hr Item determined	Subjects R.B.	E.C.	L.G.	M.T.
24–0*	CM	2·0	2·0	1·6	2·9
0–8	,,	2·2	5·0	5·0	2·4
8–24	,,	9·7	3·5	4·0	6·9
24–28	,,	8·6	5·1	7·5	3·3
0–48 (net)	,,	16·5	9·6	13·3	6·8
24–0*	Glucuronic	504	662	562	500
0–24	acid	352	675	559	556
24–48	,,	453	607	467	420
0–48 (net)†	,,	−203	−42	−98	−24

* Premedication blank values.

† Premedication blank values subtracted.

‡ Calculated as described herein. The body weights in kg were: R.B., 66·4; E.C., 89·0; L.G., 53·2; M.T., 52·7.

curves. A first-order absorption rate constant of 2·3 hr^{-1} (half-life of 0·3 hr) is required to describe an average peak time of 2 hr (Table 3) for a drug with an elimination rate constant of 0·029 hr^{-1} (half-life of 24 hr), and in order to convert the amount of the drug in the body into terms of concentration, it is necessary to assume a volume of distribution of the order of 110 per cent of the body weight. That this model can actually describe the plasma level curves is apparent from Table 3, in which it is shown that there is a good correlation between the mean plasma level curves and those calculated from the above first-order rate constants. The large volume of distribution which these calculations assume is consistent with the fact that CM is only nominally bound to plasma proteins,* and the finding in the rat that there is uniform distribution of the drug between the body water and fat.

* When 300 mg CM in 10 ml horse serum was dialyzed at 4–5° against 40 ml sodium chloride-phosphate solution (method of Van Dyke *et al.*[14]), the equilibrium concentrations were found to be 9·2 μg/ml in the serum and 5·2 μg/ml in the dialyzing fluid; indicated % protein-binding = 48.

The essential correctness of the tentative model was verified in the experiment in which the same four subjects took 150-mg doses of CM three times daily. The theoretical values were calculated by the general equations for multiple-dose kinetic models as derived by Wiegand *et al.*,[15] and the results of the experiment are presented in Table 4. They show that 2 hr after the initial dose the mean net plasma level was

TABLE 4. PLASMA LEVELS AND URINARY EXCRETION OF CHLORMEZANONE IN SUBJECTS ON A MULTIPLE-DOSAGE SCHEDULE*

After first dose (hr)	A. Plasma levels, μg/ml† Subjects R.B. ♂	E.C. ♂	L.G. ♀	M.T. ♀	Observed, mean ± S.E.	Expected, mean‡
2	1·2	1·3	1·9	2·1	1·6 ± 0·2	1·9
6	2·9	1·2	2·6	1·6	2·1 ± 0·4	3·6
24	2·8	2·3	3·3	2·6	2·8 ± 0·2	3·2
26	4·2	3·8	5·3	2·9	4·1 ± 0·5	5·0
30	4·8	3·6	6·4	3·7	4·6 ± 0·6	6·3
48	3·7	3·7	5·1	4·8	4·3 ± 0·4	4·8
50	5·1	5·4	7·0	6·8	6·1 ± 0·5	6·4
54	5·8	4·8	9·6	6·9	6·8 ± 1·0	7·7
72	5·7	5·3	6·3	3·5	5·2 ± 0·6	5·5
74	7·1	5·8	8·4	5·8	6·8 ± 0·6	7·1
76	6·7	5·8	8·9	5·3	6·7 ± 0·8	6·6
79	6·2	5·4	8·9	4·9	6·4 ± 0·9	6·0
96	4·0	3·2	5·6	3·0	4·0 ± 0·6	3·9
			B. Urinary excretion, mg			
0–24	6·0	5·5	3·6	5·8	5·2 ± 0·6	
48–72	12·8	12·4	16·9	8·3	12·6 ± 1·7	

* Doses of 150 mg given at 0, 4, 8, 24, 28, 32, 48, 52, 56, and 72 hr.

† Zero-hr blank values (mean, 1·2 μg/ml) subtracted from all subsequent readings.

‡ On the basis of the rate-process constants given in the text.

2·2 μg/ml (40 per cent of that observed at the same interval after the single 400-mg dose). However, as expected, equilibrium between intake and output was not achieved within 3 days. Thus, the mean peak plasma level increased to 4 μg/ml after the fourth dose, to 6·1 after the seventh dose, and to 6·8 after the tenth dose. Meanwhile, the daily lows were increasing from 2·8 μg/ml at 24 hr, to 4·3 at 48 hr, and to 5·5 at 72 hr. The rate of decline after the last dose corresponded to a mean half-life of 25 hr (range, 22–27). The urinary excretion of unchanged CM for the first 24 hr averaged 1·2 per cent, and that for 48–72 hr averaged 3 per cent, of the amounts ingested during those intervals.

The correctness of the calculated pharmacokinetic model was further verified by the results of an experiment presented in Fig. 2. The dosage schedule used in this case was designed to raise the plasma levels promptly to a minimum of 5, and to maintain them at values of no higher than 10 μg/ml (for subjects of average body weight) while on the maintenance dose. The data demonstrate that this objective was substantially realized, and in the subjects of a rather wide range of body weights. Thus, in the 26–79-hr period only one plasma level above 10, and five levels below 5, μg/ml were obtained, and of the latter only one was less than 4·3 μg/ml.

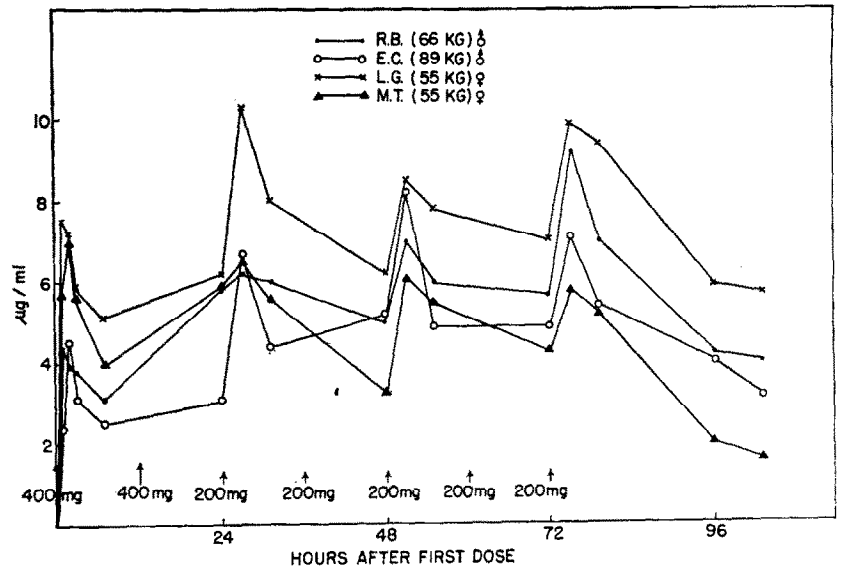

FIG. 2. Plasma levels of chlormezanone in 4 subjects on the schedule indicated. The 0-hr blank values (mean = 0·8 μg/ml) were subtracted from all subsequent readings.

The final human experiment, presented in Table 5, confirmed that the urinary excretion of CM, even when followed over an extended postmedication period, amounts to only 1–2 per cent of the dose. However, it should be noted that, during the third postmedication day, a considerable amount of the unchanged drug was still being

TABLE 5. EXCRETION OF CHLORMEZANONE (CM) AND OF ORGANIC CHLORINE (Cl) IN MAN AFTER TWO ORAL DOSES OF 400 MG OF CM AT ZERO AND 8 HR

Sample interval, days after first dose	Urinary output* (mg)			Urinary output* (% of dose)		
	CM	Cl		CM	Cl	
1	6·9	16·9	(2·4)	0·85	16·3	(2·3)
2	3·1	16·2	(1·0)	0·40	15·7	(1·0)
3	3·3	8·4	(1·5)	0·41	8·1	(1·4)
1–3	13·3	41·5	(4·9)	1·7	40·1†	(4·7)

* Values for CM and Cl corrected for the blanks obtained on a premedication control sample. The numbers in parentheses represent the amounts of Cl extractable with ether at pH 7; the numbers under Cl not in parentheses represent amounts extracted at pH 2·5 but not at pH 7. (No 4-chloro hippuric acid is extractable at pH 7.)

† Another subject who took 400 mg CM excreted in the urine in 54 hr 1·9% of the dose as CM and 36·8% as organic Cl.

excreted. The ether-extractable chlorine-containing organic acids at this point had accounted for about 40 per cent of the dose, but the data suggest that if the urine collections had been continued for two more days, at least another 6 per cent of the intake would have been recovered in this form. There was also suggestive evidence of

the presence in the urine of a neutral chlorine-containing compound. The principal chlorine-containing compound (extractable at pH 2·5, but not at pH 7) was identified as 4-chlorohippuric acid, in three ways. (1) The only ultraviolet-absorbing spot found on TLC analysis of the ether extracts of the urine samples had an R_f corresponding to that of 4-chlorohippuric acid; this spot was not found in an ether extract of the pre-medication sample. (2) A methanolic eluate of this spot gave a negative reaction for glucuronic acid. (3) The ultraviolet spectrum of this eluate had an absorption peak at the proper wavelength for 4-chlorohippuric acid (i.e. 235–240 mμ).

DISCUSSION

Chlormezanone shares with many other compounds the property of becoming widely distributed throughout the available water space of the treated animal, with no evident predilection for any tissue. Evidence for this concept was provided by the distribution study in the rat, but supporting evidence was also obtained from the dog experiments. For example, in the experiment which involved the intravenous administration of 25 mg/kg to three dogs the apparent 0-hr plasma concentrations (by extrapolation) would be 26, 42, and 28 μg/ml, respectively, for animals A, B, and C of Table 2. Uniform distribution of this dose, for these plasma levels, would require volumes of distribution of 97, 60, and 93 per cent of the body weights. Similarly, in the dog which received the 7 mg/kg dose, extrapolation of the plasma level curve to zero time would give a concentration of 7·2 μg/ml, which corresponds to an apparent volume of distribution of 97 per cent of the body weight. In three of the four dogs which received CM intravenously, therefore, the calculated 0-hr plasma levels were in accord with the concept that CM becomes uniformly distributed in an apparent volume which is about 30 per cent greater than the available water space. The space indicated beyond that which is actually available could be accounted for in part by the finding, in the rat, that the compound occupies the available fatty tissue space in about the same concentration as it does the water space.

Chlormezanone shows a variety of elimination half-lives, depending on the species studied and the dose level. In the rat (at 50 mg/kg dose level), although only two post-medication time intervals were studied, the rate of decline indicated a half-life of about 4 hr. In the dog, which was studied at a variety of dose levels, it was clearly shown that the half-life is a function of the dose. For 7 mg/kg, the half-life was 3–5 hr, while at 25 mg/kg in the anesthetized dogs it was about 10 hr. For the very massive dose of 200 mg/kg, the half-life varied from 12 to 36 hr, dependent upon which time intervals were used to make the estimates. It is well known[16, 17] that the half-life of a compound may appear to increase with the size of the dose; therefore, these findings are not unusual. In man there is naturally a definite restriction on the size of the dose that can be used experimentally, but for doses in the usual range the apparent mean half-life of CM in 24 hr.

The pharmacokinetics of the availability and elimination of CM were shown in a preliminary experiment (Table 3) to be describable in terms of a first-order input–output model which permitted a calculation of the results that should be expected from the administration of 150-mg doses three times daily. The close correlation of the calculated and observed plasma levels resulting from this dosage regimen (especially in the interval from 48 to 86 hr after the first dose) suggested the further calculation of the plasma levels that should be obtained by giving 400 mg-doses at 0 and 12 hr,

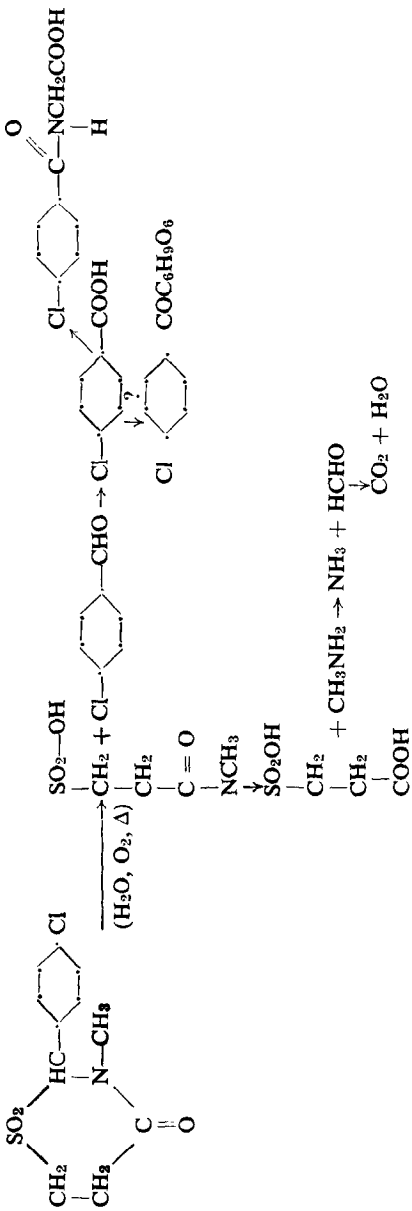

FIG. 3. Tentative scheme for the metabolic degradation of chlormezanone in man.

followed by 200-mg doses at intervals of 12 hr thereafter. The close correlation of the calculated and observed results in this second trial served further to emphasize the essential correctness of the pharmacokinetic model.

The excretion of CM in the urine in unchanged form appears to account for only 1–2 per cent of the dose. The drug is excreted in the bile by the dog, but the amount excreted in 6 hr also accounts for only 1–2 per cent of the dose, and most of this amount would presumably be reabsorbed. The relationship between biliary and plasma concentrations in the dog is such as to suggest the purely mechanical explanation that CM is secreted in the liver bile at a level equal to that in the plasma, and that about an 8-fold concentration of solids is involved in converting liver to gall bladder bile.

It was anticipated, from the ease with which CM decomposes in aqueous media to give 4-chlorobenzaldehyde (see text footnote*), and the apparent complete conversion of this aldehyde to 4-chlorobenzoic acid by the dog, that the principal characteristic urinary excretory product of CM should be 4-chlorobenzoic acid and/or conjugates thereof. The expected conjugates would be 4-chlorohippuric acid and 4-chlorobenzoylglucuronic acid. It is rather surprising that the end product of CM metabolism in both dog and man should actually prove to be the former, since it has been reported[18] that the dog converts very little of ingested benzoic acid to hippuric acid (the principal conjugate in the dog is benzoylglucuronic acid), and that man eliminates appreciable amounts of benzoylglucuronic acid after the ingestion of benzoic acid. However, small doses of benzoic acid may be eliminated by man entirely as hippuric acid.[18]

The fate of the aliphatic portion of the CM molecule is not clear, and would be difficult to establish without the use of radioactive labels at several positions in the molecule. It has been found, however, in a preliminary experiment† that the administration to a rat of CM labeled with ^{14}C in the N-methyl group leads to the excretion of 14 per cent of the label in the expired air within 24 hr. The data at present available, therefore, suggest that CM decomposes *in vivo*, perhaps to a considerable extent non-enzymatically, to give 4-chlorobenzaldehyde, methylamine, and 2-carboxy-ethanesulfonic acid.* The last-named would probably be excreted unchanged. This scheme of degradation is illustrated in Fig. 3, but must be considered tentative. One possible degradation product of CM which would probably be poorly extractable by ether at

* The oral administration to a dog of CM labeled with ^{14}C in the N-methyl group (dose, 5 mg/kg; sp. act. 0·08 μc/mg) resulted in the excretion of 27·3% of the radioactivity in the urine, 12·9% in the bile, and 1·8% in the feces, in the next 24 hr. In the whole blood the peak radioactivity (equivalent to 3·7 μg/ml as CM) was found at 7 hr postmedication, but in the plasma the level at 24 hr (4·3 μg/ml as CM) was slightly higher than at 7 hr. Of the radioactivity remaining in the tissues at 24 hr (27·1% of the dose), the highest concentrations (12·9 and 10·2 μg/g, as CM) were found in the adrenal and liver, followed by kidney, lung, and spleen (4·7, 4·2, and 3·8 respectively). Ether extraction of the urine removed only 17% of the radioactivity; of the extractable portion, TLC showed nearly half to be unchanged CM. An additional 12% of the radioactivity was rendered extractable by gluculase digestion. A rat which received 3·9 mg of the same CM preparation/kg excreted in the next 24 hr 14·1% of the dose in the expired air, 62·3% in the urine, and 3% in the feces. The total recovered from the tissues and digestive tract of this animal at 24 hr postmedication was 9·2% of the dose, with the highest concentration being in liver (2·7 μg/g as CM), followed by lung and kidney (0·9), and then by spleen and skin (0·7 and 0·6). Data courtesy of Dr. W. D. Conway.[13]

† The observed half-life *in vitro* of CM of 48 hr at pH 7·35 (see text footnote on p. 819) would obviously represent the upper limit of the half-life in the various species studied. The values found for the latter would be less, depending on such factors as the rate of biliary and urinary excretion, whether CM is a substrate for some specific enzyme system elaborated by the animal, and whether the compound may be temporarily exposed to a pH higher than that of the *in vitro* buffer system which was used. There is evidence,* for example, that the decomposition of CM to 4-chlorobenzaldehyde proceeds more rapidly in bile than in the phosphate buffer.

pH 2·5 (and which would not, therefore, be measured by the method used herein for the determination of organic chlorine) could be formed by oxidative cleavage of the aliphatic ring at the 1–2 (carbon-sulfur) bond.

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