Metabolism of Methocarbamol in the Rat, Dog, and Human

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Abstract □ Methocarbamol-^{14}C has been shown to be metabolized in the dog, rat, and man by dealkylation, hydroxylation, and conjugation with glucuronide and sulfate. Two metabolites have been isolated and identified in the urine of the three species. Blood levels and urinary excretion are also described.

Keyphrases □ Metabolism, methocarbamol, ^{14}C and unlabeled—rat, dog, human □ Methocarbamol-^{14}C—synthesis □ Blood levels—methocarbamol □ Urinary excretion—methocarbamol □ TLC—separation □ Scintillometry—analysis □ IR spectrophotometry—identification □ UV spectrophotometry—identification □ NMR spectroscopy—identification

Methocarbamol was first described by Murphey (1), and its effectiveness as a muscle relaxant was shown by a number of investigators. A study of its distribution and metabolism in dogs and humans was reported by Campbell *et al.* (2). The metabolites were not identified. This study reports the findings of an investigation to determine the metabolic products excreted by the rat, dog, and human.

EXPERIMENTAL

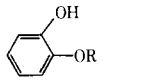
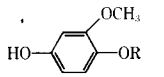
Synthesis—*1,2,3-^{14}C-Glycerol-1,2-isopropylidine (3)*—A 921-mg. sample of uniformly labeled glycerol (10.0 mmoles; 10 mc.) was shaken overnight at room temperature in 30 ml. acetone and 1.5 g. sodium sulfate. Magnesium oxide (750 mg.) was added, and the mixture was shaken until a neutral pH resulted. The solids were removed by filtration, and the concentrated residue was partitioned between chloroform and water, producing 815 mg., 6.18 mmoles, of product; yield 62%. The TLC pattern (10% methanol–chloroform) of the product indicated one major zone of radioactivity.

1,2,3-^{14}C-Glycerol-1,2-isopropylidine Tosylate—The 815 mg. (6.18 mmoles) of isopropylidine derivative was dissolved in 8 ml. of pyridine containing 1.16 g. (6.18 mmoles) of *p*-toluene sulfonyl chloride, and the solution was allowed to stand overnight. After removing the pyridine at reduced pressure, the residue was partitioned between bicarbonate solution and chloroform and then between dilute HCl and water. The chloroform extract crystallized and amounted to 1.37 g. (4.9 mmoles), 81% yield. TLC (5% methanol–benzene) indicated a major zone and one minor radioactive zone.

1,2,3-^{14}C-Glyceryl Guaiacolate (4)—The crystalline tosylate (1.30 g., 4.55 mmoles) was dissolved in 8 ml. of *n*-butanol and refluxed with stirring with 0.209 g. (9.10 mmoles) of sodium and 1.13 g. (9.10 mmoles) guaiacol for 5 hr. The reaction mixture was partitioned between water and ether. After evaporating the solvent, the extract was refluxed 1 hr. in 35 ml. of 1 *N* H_2SO_4. After the solution was made alkaline and extracted several times with chloroform, solvent distillation produced 0.527 g. and 2.2 mc. radioactivity. TLC in 10% methanol–chloroform gave a single spot of radioactive material.

1,2,3-^{14}C-Methocarbamol[1]—The glyceryl guaiacolate sample was refluxed for 1.5 hr. in 15 ml. of diethylcarbonate containing 4 drops of a 30% methanolic solution of sodium methoxide, during which time about two-thirds of the solvent was boiled off. The remaining reaction mixture was taken to dryness on a rotating evaporator. Traces of the reagent were removed from the product by adding two portions of xylene and evaporating on the rotating evaporator. This residue was then dissolved in 3 ml. of toluene, and the solution was shaken in a small vial with 1.4 ml. of concentrated ammonium hydroxide overnight. After removing the reagents under reduced pressure, the crystalline residue was recrystallized from 8 ml. benzene containing a trace of acetic acid. The 408 mg. obtained was recrystallized from 6 ml. of ethyl acetate to give 242 mg., m.p. 94–96.5°. This fraction had a specific activity of 0.995 mc./mmole, and TLC (15% isopropanol–chloroform) indicated complete homogeneity. Unlabeled methocarbamol (432 mg.) was added to the mother liquor from the recrystallization, and a fraction of lower specific activity was crystallized from ethyl acetate, 447 mg., m.p. 93.5–95°, specific activity 0.272 mc./mmole or 2.50×10^6 d.p.m./mg. The overall radioyield from 1,2,3-^{14}C-glycerol was 15%. IR curves of the two samples were identical to authentic samples of methocarbamol.

Table I—Percentages of Total Urinary Radioactivity Excreted by the Rat and Dog after Receiving Methocarbamol-^{14}C Orally

Fraction	^{14}C in Urine, %: Rat[a]	Dog[a]
Extract of fresh urine[b]	8.3	—
Extract of β-glucuronidase-treated urine[b]	28	30
Extract of glusulase-treated urine[b]	78	77
R_f 0.85 component[c]	2	0
Methocarbamol	10	46
 [c,d]	31	10
 [c,d]	24	17

[a] Dose: Rat and dog: 100 mg./kg. orally; 0–24 hr. urine. [b] Extract: three double volumes of ethyl acetate at pH 5.0. [c] Individual component assay obtained by TLC scrape-off, extraction, and counting. [d] R = $-CH_2CHOHCH_2OCONH_2$.

Animal Studies—*Dogs*—The ^{14}C-labeled drug was administered to a mongrel dog as a crystalline solid in a gelatin capsule at a level of 100 mg./kg. Unlabeled methocarbamol was dosed similarly at a level of 200 mg./kg. The urine was collected by means of a runoff tray at 24-hr. intervals.

Rats—Rats were dosed by means of a stomach tube with 100 mg./kg. of methocarbamol in 5 ml. of aqueous solution. They were placed in metabolism cages, and urine and feces were collected at 24-hr. intervals.

Human Studies with Unlabeled Methocarbamol—Two male subjects received oral doses of 2.0 g. of methocarbamol (four 500-mg. tablets).[2] Urine was collected at 2-hr. intervals.

Human Studies with Labeled Methocarbamol—Two normal male subjects were administered the drug.[3] One subject received a single oral dose of 200 mg. of methocarbamol containing 113 μc., and the other subject received 1.0 g. of drug also containing 113 μc. of methocarbamol-^{14}C. Samples of urine and blood were collected at intervals and submitted to these laboratories for analysis.

Analytical—*Radioactivity*—Samples were analyzed for radioactivity using a Packard liquid scintillation spectrometer, series 314E. Aliquots of urine and whole blood were counted directly. Fecal specimens were dried in a vacuum oven prior to grinding and combustion to $^{14}CO_2$, which was trapped in β-phenethylamine for counting. Corrections for quenching were carried out by the internal

[1] AHR-85.

[2] Robaxin.

[3] By Dr. Albert J. Wasserman at the Medical College of Virginia.

Table II—Urinary Excretion of Methocarbamol and Metabolites following Oral Doses of 200 and 1000 mg. Containing 113 μc. of Methocarbamol-^{14}C

Time after Dose, hr.	Total-^{14}C, mg.	Metho-carbamol, mg.	Metabolite 1, mg.	Metabolite 2, mg.
	Subject 1–200 mg.			
2	109.0	14	32	32
4	43.8	3	15	13
6	18.6	1	7	7
8	8.2	0.3	2	3
10	3.8	—	—	—
12	2.4	—	—	—
24	4.8	—	—	—
48	2.2	—	—	—
72	1.2	—	—	—
	Subject 2–1000 mg.			
2	259.0	57	57	57
4	316.0	52	66	66
6	158.0	18	32	37
8	118.0	14	40	52
10	51.0	4	11	14
12	29.0	2	1	10
24	41.0	—	—	—
48	11.0	—	—	—
72	4.0	—	—	—

standard method with addition of a calibrated standard solution of benzoic acid-^{14}C.

Urine Fractionation—Urine samples were incubated at pH 5 and 38° for 48 hr. with glusulase and extracted with three double volumes of ethyl acetate. The extract was concentrated and chromatographed on thin-layer silica gel with isopropanol–chloroform (15:88). Radioactive spots were located by exposing the chromatograms to X-ray film.

RESULTS

TLC of the extract of glusulase-hydrolyzed human, dog, and rat urine showed three major radioactive components at R_f 0.50, 0.35, and 0.27. The rat urine also showed a minor component at R_f 0.85 and several minor components between this and the spot at R_f 0.50.

The residual aqueous phase of unextractable material containing 20–30% of the radioactivity was tested in various ways to obtain additional information. Gel filtration, ion exchange, and TLC were run. Several components were evident from TLC, including what appears to be one major polar component. However, none of these components has been identified.

To identify the three major components, 300 ml. of dog urine was treated with glusulase and extracted with ethyl acetate. The extract was concentrated, and preparative TLC was run on 20 × 20-cm. silica GF plates. The zones were scraped off and extracted with methanol, and the extract was partitioned between water and ethyl acetate. The ethyl acetate was evaporated to dryness, giving 151 mg. at R_f 0.50, 18 mg. at R_f 0.35, and 28 mg. at R_f 0.27. The R_f 0.50 fraction was compared with authentic methocarbamol and proved to be identical according to NMR, IR, and UV. It also cochromatographed with methocarbamol.

The UV, NMR, and IR data of the R_f 0.35 component corresponded exactly with a sample of 3-(2-hydroxyphenoxy)-1,2-propanediol-1-carbamate (5). A small sample of component R_f 0.35 in methanol–ether solution was treated with diazomethane. The R_f of the resulting product by TLC was the same as methocarbamol, further proving its identity.

The spectral data, NMR, IR, and UV, of the component at R_f 0.27 were consistent with the structure: 3-(4- or 5-hydroxy-2-methoxyphenoxy)-1,2-propanediol-1-carbamate. The diacetate was prepared, and its mass spectrum was in complete agreement with the two possible structures. The probable assignment of the hydroxyl group is to position 4 of the guaiacol ring. This assignment is based on a similar metabolic conversion of 4′-fluoro-4-3-(2-methoxyphenoxy)-1-pyrrolidinyl butyrophenone,[4] which is both demethylated and hydroxylated in the 4-position of the guaiacol ring (6). The two possible metabolites of 4′-fluoro-4-3-(2-methoxyphenoxy)-1-pyrrolidinyl butyrophenone were synthesized, and comparison with the metabolite from urine showed definitely that the 4-position was hydroxylated.

[4] AHR-1900.

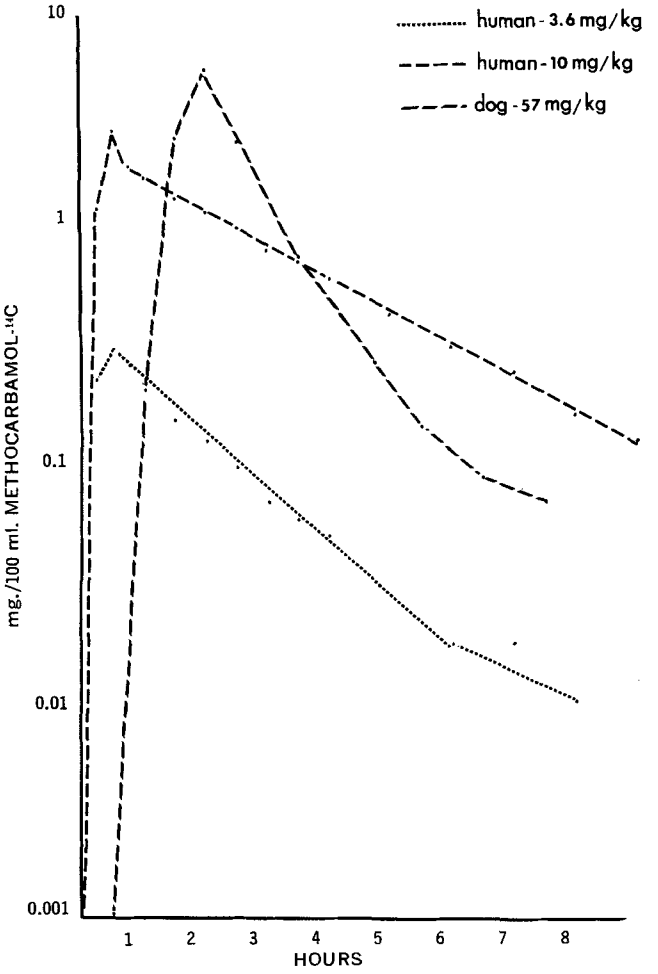
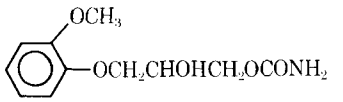
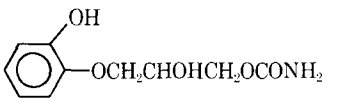


Figure 1—*Blood levels of methocarbamol-^{14}C following its administration to humans and dogs.*



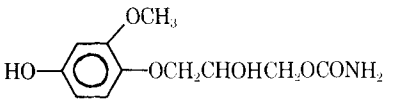
methocarbamol

I



metabolite 1
3-(2-hydroxyphenoxy)-1,2-propanediol-1-carbamate

II



metabolite 2
3-(4-hydroxy-2-methoxyphenoxy)-1,2-propanediol-1-carbamate

III

Table III—Excretion of Methocarbamol-^{14}C by a Female Mongrel Dog following Administration of 57 mg./kg. Orally

Time after Dose, hr.	Methocarbamol-^{14}C, % of Dose
Urinary Excretion	
0–24	85.01
24–51	2.97
51–74	0.80
74–98	0.07
Total	88.85
Fecal Excretion	
0–51	1.64
51–74	0.46
74–98	0.04
Total	2.14

Table IV—Excretion of Methocarbamol-^{14}C by the Rat following an Oral Dose of 100 mg./kg.

Time after Dose, hr.	Rat No. 1	Rat No. 2	Rat No. 3
	% of Dose		
Urinary Excretion			
0–26	90	90	83
26–47	2.0	1.5	1.0
47–71.5	0.5	1.0	0.5
Total	92.5	92.5	84.5
Fecal Excretion			
0–26	—	5.4	13
26–47	—	5.9	0.25
47–71.5	—	0	0
Total	0	11.3	13.25

Blood Levels—The results from the blood level studies in the dog and man are shown as a logarithmic plot in Fig. 1. These results represent the total radioactivity expressed as mg. of methocarbamol per 100 ml. of blood and therefore include both methocarbamol and its metabolites.

DISCUSSION

The blood level curves shown in Fig. 1 indicate that methocarbamol was absorbed slower by the dog than by the human subjects. The maximum concentration in the humans occurred at 0.5 hr., whereas the maximum in the dog occurred at 2.0 hr. The half-lives calculated from these data indicate more rapid elimination by the dog than by the humans. The calculated estimates for half-lives were 2.15 hr. for the 1000-mg. dose, 1.6 hr. for the 200-mg. dose, and 0.6 hr. for the dog. These results agree with those previously found (2), indicating that the drug is readily absorbed by both species.

The results indicate that methocarbamol (I) and its metabolites (II and III) are extensively conjugated before excretion in the urine. The primary paths of metabolism are through dealkylation and hydroxylation.

Conjugation could occur at a number of sites on these compounds and they could be conjugated as glucuronides or as sulfates.

A sample of fresh, unhydrolyzed rat urine was extracted directly at neutral pH. Only 8.3% of the counts present in the urine was extracted, indicating that most of the components were conjugated. A TLC run of this extract indicated that most of the material was unchanged methocarbamol. It is obvious from the studies that the majority of the material excreted is conjugated. Attempts to separate the conjugates were unsuccessful. When a sample of the rat urine was incubated with Sigma bacterial type II β-glucuronidase at pH 6.8 and 37° for 2 days and then extracted at neutrality with ethyl acetate, 28% of the urine counts was extracted. A similar experiment with dog urine gave 30% of urine radioactivity, indicating similar conjugations in both species. The results in Table I indicate that this is, however, less than half the total conjugated material and that the major portion excreted must be as the sulfate, since glusulase contains both glucuronidase and sulfatase.

Chlorphenesin carbamate (7) has been found to be metabolized in the dog to neutral and acidic components. The major metabolite was the glucuronide of chlorphenesin carbamate, and a smaller amount was conjugated as the sulfate. Some 19% of the drug was excreted as free or conjugated *p*-chlorophenol, *p*-chlorophenoxylactic acid, and *p*-chlorophenoxyacetic acid.

Urine from rats given methocarbamol was, therefore, investigated for acid metabolites. A sample of the radioactive glusulase-hydrolyzed rat urine was extracted three times at pH 3.0 with a double volume of ethyl acetate. Back-extraction with sodium bicarbonate solution gave 8% of the original urine counts. After acidifying the bicarbonate fraction, reextracting the radioactivity into ethyl acetate, and removing the solvent under reduced pressure, the residue was chromatographed in a formic acid–ethanol–chloroform system (5:10:75). One major component at R_f 0.20 and several minor components resulted.

None of these components cochromatographed with *o*-methoxyphenoxyacetic acid or β-(*o*-methoxyphenoxy)lactic acid.

Since simple methods were available, it was of interest to study the pharmacodynamics of the urinary excretion of methocarbamol and its metabolites. Following TLC of the extracts, the spots were eluted and the radioactivity in each was determined. The results are shown in Table II. The column headed "Total-^{14}C" shows the total radioactivity excreted and includes not only the compounds shown in the next two columns but also all other radioactive material excreted. This would include one, two, and three carbon fragments resulting from hydrolysis of the glyceryl ether linkage. Such small molecules were not identified. The maximum excretion rates occurred at the 2nd to 4th hours for all the material quantitated, indicating that excretion and metabolism are rapid. The total percentages of radioactivity excreted in the urine was 97% for Subject 1 and 98.7% for Subject 2 in 72 hr. Extremely small amounts were found in the feces.

The excretion of methocarbamol and related materials by the dog and rat is shown in Tables III and IV, respectively. In both cases, excretion was again rapid, with most material being excreted in the first 24 hr. Only 2% was excreted by the dog in the feces, whereas some 11–13% was excreted in the feces by the rat.

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