Metabolic Reduction of 1-(*p*-Acetylbenzenesulfonyl)-3-cyclohexylurea (Acetohexamide) in Different Species. (26694)

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The metabolism of tolbutamide(1-4) and chlorpropamide(5) has been studied in man and various laboratory animals. This report is concerned with metabolism of another hypoglycemic agent, acetohexamide [1-(*p*-acetylbenzenesulfonyl)-3-cyclohexylurea]. A metabolic pathway was found that differs from those observed with the other 2 drugs.

Methods. Urine specimens were acidified to pH 4 and extracted twice with equal volumes of chloroform. The combined acid-washed chloroform was reduced to 1/100 volume of urine extracted. This concentrate was spotted on Whatman No. 1 filter paper and developed by ascending chromatography with butanol saturated with 5N ammonium hydroxide. Spots were located by immersing the dried sheets in saturated mercurous nitrate, rinsing, and treating with an acetone solution of *p*-dimethylaminobenzalrhodanine, as described previously(5). For isolation of the metabolite, sheets bearing a series of urine extract spots were subjected to the above chromatographic process and a horizontal strip corresponding to the observed zone was removed. One end of each strip was placed in the solvent trough of a chromatography jar designed for descending development, and elution was achieved with water over a 24-hour period. Recrystallizations were made from aqueous alcohol. Ultraviolet absorption maxima were obtained from acidic methanol solutions (50 λ 2N hydrochloric acid to 3.3 ml methanol). Quantitative determination of metabolite was made by removal of a 1.5 inch square of the above mentioned zone from a chromatogram of a known quantity of urine extract. The shredded paper was tamped into a 10-ml serological pipette and elution, performed with 10 ml of absolute methanol, was allowed to proceed at a rate of approximately 0.5 ml per minute. Ultraviolet absorption of eluates was determined on a recording spectrophotometer and concentration computed by

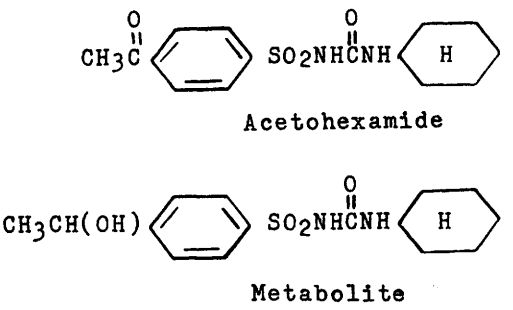

FIG. 1. Acetohexamide and metabolic product.

comparison with spectra obtained from chromatograms of known amounts of the metabolite model. For investigation of possible glucuronide formation, urine samples were buffered at pH 7 and 2 mg/ml of beta-glucuronidase (100,000 units) was added. After a 24-hour incubation period at 37.5°C, samples were acidified and extracted.

Acetohexamide and metabolite model (Fig. 1) were prepared by Drs. F. J. Marshall and M. V. Sigal.

Results. Chloroform extracts from urine of 3 dogs, 2 rabbits, 6 rats, and 4 human subjects that had received oral acetohexamide produced a spot on chromatograms with an R_f 0.56-0.58 in contrast to R_f 0.62-0.64 of acetohexamide. During water elution of the spot white crystals appeared in the eluant. Recrystallization yielded a compound that gave an ultraviolet absorption maximum at 227 mμ contrasted with an acetohexamide maximum at 246 mμ. Since phenyl alkyl ketones are known to undergo biological reduction to the corresponding alcohols(6), 1-(*p*-α-hydroxyethylbenzenesulfonyl)-3-cyclohexylurea was used as a model. A mixed melting point of the metabolite and the model gave no depression. A chromatogram showed a spot of the same mobility as the metabolite. Ultraviolet and infrared absorption spectra as well as the x-ray powder diffraction pattern were identical with the recrystallized metabolite from dog and man.

TABLE I. Twenty-Four-Hour Urinary Excretion of 1-(*p*-α-hydroxyethylbenzenesulfonyl)-3-cyclohexylurea Following Oral Administration of Acetohexamide.

Species	No.	Oral dose acetohexamide	24 hr metabolite excretion, % of dose
Dog	1	50 mg/kg	59
Rabbit	1	50 "	53
Rat	6	50 "	18
Man	2	2 g	49

Also, the rat and rabbit metabolites were identified as being the alcoholic derivative on the basis of chromatography and absorption spectra.

Some dog and human chromatograms showed a very slight spot at R_f 0.62, indicating presence of acetohexamide. Eluates of this area from chromatograms of all 4 species failed to produce an absorption maximum at 246 mμ so that excretion of unchanged drug was not further substantiated.

After oral administration of acetohexamide an estimation of the 24-hour urinary recovery of the alcoholic metabolite was made in a dog, a rabbit, 2 patients, and the pooled sample from 6 rats (Table I). Forty-eight hour recoveries for the 2 diabetic patients were 63 and 50%. Metabolite recoveries from 2 other diabetic patients, who received daily doses of 1 g acetohexamide, ranged from 20 to 59%.

Synthetically prepared metabolite was administered to dog and man. In 2 female patients 24-hour urine recoveries were 41% and 61% for doses of 300 mg and 1 g, respectively. Recovery in a dog was 60% of a 50 mg/kg dose. Urine from one of the patients was incubated with beta glucuronidase for 24 hours. No increased recovery of metabolite was noted.

Discussion. Tolbutamide [1-(*p*-methylbenzenesulfonyl)-3-butylurea], acetohexamide [1-(*p*-acetylbenzenesulfonyl)-3-cyclohexylurea], and chlorpropamide [1-(*p*-chlorobenzenesulfonyl)-3-propylurea] possess para-substituted phenyl groups. Comparison of the metabolism of these drugs points up a marked influence of the phenyl substituent. In man, tolbutamide (*p*-methyl) is oxidized to a paracarboxylic derivative(1), whereas acetohexamide (*p*-acetyl) is reduced to a para-hydroxyethyl derivative, and chlorpropamide (*p*-chloro) is excreted unchanged or hydrolyzed to *p*-chlorobenzenesulfonamide (5).

Other species have exhibited different metabolic treatment of tolbutamide and chlorpropamide. Mohnike *et al.*(2,3) found that the dog, unlike man, did not oxidize tolbutamide but hydrolyzed it to *p*-methylbenzenesulfonamide or *p*-methylbenzenesulfonylurea. A prior publication from this laboratory(5) showed that the dog excreted chlorpropamide and *p*-chlorobenzenesulfonamide as did man, but in addition excreted 35 to 40% of the dose as *p*-chlorobenzenesulfonylurea. The rabbit was shown to excrete more than 80% of chlorpropamide unchanged with no other detectable metabolites.

Acetohexamide was found to be metabolized alike by dog, rabbit, rat, and man. Recovery of the metabolite from the rat was markedly lower than from the other species, but it was not accompanied by additional metabolites. Since total excretion of drug was not determined, low urinary excretion may be due to poor gastrointestinal absorption and/or biliary excretion of drug or metabolite in this species.

Summary. Metabolism of acetohexamide 1-(*p*-acetylbenzenesulfonyl)-3-cyclohexylurea was investigated in dogs, rabbits, rats, and man. Trace amounts of unchanged drug were excreted by dogs and man. All species excreted the reduced form 1-(*p*-α-hydroxyethylbenzenesulfonyl)-3-cyclohexylurea. In a dog, a rabbit and 2 human subjects, 49 to 59% was recovered as metabolite in the urine, but only 18% in the pooled urine of 6 rats. Chromatograms of rat urine revealed no other metabolites.

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Oxidative Effects of 1,1,3-Tricyano-2-Amino-1-Propene. (26695)

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In 1948 it was reported that a solution of malononitrile increased nucleic acid concentration of large nerve cells of the central nervous system of rabbits(1). Since other investigators were unable to reproduce this finding, a series of spectral analyses of fresh and aged solutions of the chemical was undertaken, indicating that active material was probably a decomposition product which formed on standing(2). Isolation and characterization(3) of 1,1,3-tricyano-2-amino-1-propene (U-9189) from similar aged solutions was reported to give the "nucleic acid effect" mentioned above and to uncouple oxidative phosphorylation *in vitro*(4). The compound was found to have antithyroid activity in humans and inhibit thyroxine formation in rats(5) as well as cause pyrexia in rats.* Because this suggested alteration in gross metabolism of treated animals, it was decided to quantitate effects on oxidation rates of various C^{14}-labeled compounds in rats.

Materials and methods. Adult male albino rats of Upjohn colony (Sprague-Dawley ancestry), weighing 175-180 g and fasted 18 hr (overnight), were used for respiratory studies. Fasted young male rats weighing 60-70 g were used for age comparison studies. Intraperitoneal doses of 50 mg/kg of U-9189 or 15 mg/kg of 2,4-dinitrophenol† (DNP) used in these studies produced slight fevers of 1 to 1.5°F for 1-3 hr in intact and thyroidectomized rats. Doses of U-9189 and DNP were made fresh daily in deionized water or 1/10 N sodium bicarbonate respectively; 1 ml volumes were injected. Some radioactive compounds were injected intraperitoneally as 0.5 ml aqueous solutions of: C^{14}-2-acetate, sodium (3.3×10^6 cpm, 0.45 mg), C^{14}-2-glycine (1×10^6 cpm, 0.17 mg), C^{14}-glucose (uniformly labeled, 1×10^6 cpm, 0.19 mg), and C^{14}-1-DL-leucine (8.8×10^5 cpm, 0.27 mg). C^{14}-tripalmitin carboxyl labeled) was incorporated into an emulsion similar to Lipomul‡ and 0.6 ml (2.4×10^5 cpm, 0.43 mg of C^{14}-1-tripalmitin) injected into a femoral vein.

In a typical experiment, rats were injected with either U-9189 or DNP and ½ hr later with the C^{14} compound and placed in glass metabolic units from which the respired CO_2 was collected. Exhaled $C^{14}O_2$ was counted as $BaC^{14}O_3$ by a procedure previously reported (6). In one series of rats, total respiratory CO_2 was evaluated by $BaCO_3$ collection, after which thyroid glands were removed. After 10 days of recovery, respiratory CO_2 was measured again and rats with 24-30% depression in CO_2 output were considered thyroidectomized and used in isotope experiments (Table IV).

Results and discussion. Oxidation rates by intact adult rats. Results (Table I) show that, ½ hr after injection of radioactivity, U-9189 increased oxidation rates of all C^{14}-labeled compounds except glucose. Compared to their respective controls, peak stimulation of acetate oxidation was 83% at ½ hr, tripalmitin 33% at 1 hr, DL-leucine 61% at 1 hr, and glycine 110% at ½ hr. In view of the fact that U-9189 caused an increased output of total CO_2 of about 34% during 5 hr

* Seay, P. H., McNeil Labs., Philadelphia, Pa., Unpublished data.

† Eastman Organic Chemicals, Rochester, N. Y.

‡ Upjohn trademark for intravenous cottonseed oil emulsion.