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METABOLIC STUDIES OF TOLBUTAMIDE IN THE RAT*

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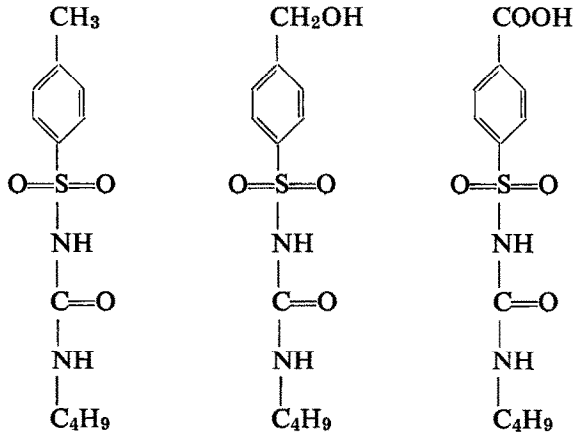
Abstract—Hydroxymethyltolbutamide was found to be the major oxidation product in the urine of rat and rabbit given tolbutamide. The metabolite was identified by its infrared spectrum and by paper and thin-layer chromatography. The enzyme system responsible for the oxidation of tolbutamide is a typical microsomal drug-metabolizing system subject to induction by 3,4-benzpyrene, phenobarbital, and tolbutamide itself. The only product observed after incubating tolbutamide with either the fortified microsomes or with liver homogenate was hydroxymethyltolbutamide. Only a small isotope effect was seen in the oxidation of trideuterated tolbutamide. Hydroxymethyltolbutamide was approximately half as active as tolbutamide in its hypoglycemic activity in mice.

THE ANTIDIABETIC drug, tolbutamide (I), has been shown to be excreted mainly as N-*p*-carboxybenzenesulfonyl-N′-*n*-butylurea (carboxytolbutamide, III) and, to a lesser extent, as N-*p*-hydroxymethylbenzenesulfonyl-N′-*n*-butylurea (hydroxymethyltolbutamide, II) by man, rabbit, guinea pig, and rat.[1–3]



I II III

Wittenhagen *et al.*[3] and Bander and Scholz[4] first demonstrated that tolbutamide was oxidized to these products by slices and homogenates of rat liver and kidney. However, Remmer *et al.*[5] failed to associate this metabolic activity with the microsomal fraction. They also reported that only the microsomes of dog liver catalysed the metabolism of tolbutamide. Furthermore, in species other than the dog,

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tolbutamide did not activate its own breakdown. Tolbutamide was reported to be excreted as *p*-toluenesulfonylurea and *p*-toluenesulfonamide by the dog.[5]

Evidence is presented in this paper to show that tolbutamide is metabolized by the liver microsomes in the rat. This microsomal activity was stimulated by pretreating the rat with tolbutamide. Hydroxymethyltolbutamide was identified as the major metabolite in both the urine and incubation mixtures. In addition, the effect on the rate of tolbutamide catabolism of complete deuteration of the methyl group on the tolyl moiety was examined.

MATERIALS AND METHODS

Synthesis of hydroxymethyltolbutamide

p-Aminomethylbenzenesulfonamide (obtained from Aldrich Chemicals) was converted to *p*-sulfamylbenzylalcohol,[7] which was then acetylated to form *p*-acetoxymethylbenzenesulfonamide.[8] The latter was condensed with *n*-butyl isocyanate (obtained from Aldrich Chemicals) to form 1-*p*-acetoxymethylphenylsulfonyl-3-butylurea. The acetoxy group was cleaved in alkali to obtain the desired product.[9] The product was recrystallized from methanol–water; m.p. 98·5–100°, reported m.p. 101–103°.[9] The infra-red spectrum and chromatographic properties of this compound are presented under Results.

Synthesis of labeled tolbutamide

N-*p*-Toluenesulfonyl-N′-*n*-butylurea-^{14}C, N-*p*-benzotrideuteridesulfonyl-N′-*n*-butylurea and doubly labeled N-*p*-benzotrideuteridesulfonyl-N′-*n*-butylurea-^{14}C were prepared essentially by the same method. The synthesis of the doubly labeled compound is described below.

Valeryl chloride-1-^{14}C. Sodium valerate-1-^{14}C (obtained from New England Nuclear Corp.), sp. act. 1 mc/24·8 mg, was diluted with 3·905 g of unlabeled sodium valerate. Phthaloyl dichloride, 25·7 g (0·126 mole), was slowly added to 3·930 g (0·0316 mole) of sodium valerate-1-^{14}C, and the resulting mixture was heated to 150° for 1 hr. The mixture was distilled at 125° on a micro-Claisen column to collect the product. The yield was 3·21 g (0·0266 mole), 84·3 per cent of theoretical. The infra-red spectrum of the product was identical with that of authentic valeryl chloride.

n-Butyl isocyanate-^{14}C. A mixture of 3·21 g (0·0266 mole) of valeryl chloride-1-^{14}C and 4·15 g (0·0638 mole) of sodium azide (practical grade obtained from Aldrich Chemicals) was stirred for 3 days at room temperature. Vapor-phase chromatography indicated that very little of the starting material remained. The reaction mixture was heated to 100–120° for 3 hr, at which time no more nitrogen was evolved. The product, 1·79 g (0·0181 mole) corresponding to 68·1 per cent yield, was collected in a dry ice-acetone bath at 1-mm pressure with the pot temperature set at 90°. The infrared spectrum of the product was identical with commercially obtained *n*-butyl isocyanate.

N-*p-Benzotrideuteridesulfonyl*-N′-*n-butylurea*-^{14}C. The doubly labeled tolbutamide was synthesized by the method described by Lemieux *et al.*[10] Starting with *p*-benzo trideuteridesulfonamide* and *n*-butyl isocyanate-^{14}C, the product was obtained in 80 per cent yield. The melting point compared favorably with that of unlabeled tolbutamide supplied by the Upjohn Co., the mixture melting point was not depressed.

* The extent of deuteration of this compound as determined by combustion analysis was 93·6 ± 1 per cent. The deuterium analysis was performed by Mr. Josef Nemeth of Urbana, Ill.

The specific activity of the labeled tolbutamide with respect to ^{14}C was 0·024 $\mu c/\mu mole$.

Pretreatment of animals for induction studies

Adult male Sprague–Dawley rats (200–400 g) were used for all studies. Tolbutamide (Orinase diagnostic, Upjohn Co.) was given orally (300 mg/day) for 3 days, and the urine was collected during the period of treatment. 3,4-Benzpyrene (25 mg/kg in sesame oil) was injected i.p. as a single dose 24 hr before sacrifice. Na-Phenobarbital (75 mg/kg) was given i.p. in two equally divided doses daily for 4 days. Control animals received no treatment. The animals treated with tolbutamide or 3,4-benzpyrene were sacrificed 24 hr after, and those treated with phenobarbital 17 hr after, administration of the last dose. The kidneys and livers from each group were removed, pooled, and either used immediately or frozen and stored at −20°.

Tissue preparations

Homogenates of kidney or liver were prepared with a glass tissue homogenizer in 2 volumes of 1·15% KCl. The resulting preparation was centrifuged for 10 min at 10,000 *g* in a refrigerated Servall centrifuge, and the supernatant was used as "10,000-*g* supernatant fraction."

Microsomes were sedimented from the 10,000-*g* supernatant fraction by centrifuging at maximal speed for 0·5 hr in a Spinco model L ultracentrigufe (rotor No. 30). The postmicrosomal supernatant was recentrifuged for 1 to 1·5 hr in the No. 30 rotor to obtain the "microsome-free supernatant" fraction. The microsomes were washed twice with 1·15% KCl and resuspended either in 2 ml KCl or 2 ml of the microsome-free supernatant fraction for each gram of the original liver.

Incubations

Incubation mixtures, in general, consisted of 0·5 μmole^{14}C-tolbutamide in 1 ml of M/15 K_2HPO_4 buffer (pH 7·0), 15 μmoles nicotinamide, 0·25 μmole NADP, 40 μmoles glucose 6-phosphate, 20 μmoles $MgCl_2$, and 1·0 ml of 10,000-*g* supernatant or microsomes in a total volume of 2·5 ml. For the incubations with washed microsomes, 4 units (0·025 mg) of glucose 6-phosphate dehydrogenase (Sigma, type V) were also added. Incubation was for 1–3 hr with shaking in a Dubnoff incubator at 37° in air. The reaction was stopped by the addition of 1 ml of 3 N HCl, and the incubation mixture was extracted as described below.

Extraction methods

To determine the completeness of extraction of tolbutamide and its two known metabolites by an organic solvent, the property of these compounds to absorb maximally at 230 mμ was utilized to measure the amount left in the aqueous phase. All three compounds were found to be quantitatively extracted from acidified aqueous medium after 1 hr of shaking with 3 volumes of ether.

For the quantitation of product, it was necessary to remove unreacted ^{14}C-tolbutamide from the incubation mixture before extracting the product into ether. The extraction of acidified incubation mixture several times with *n*-heptane containing 1·5% isoamyl alcohol removed tolbutamide quantitatively. Under these conditions hydroxymethyltolbutamide was not removed, although about 70 per cent of

carboxytolbutamide was lost into the organic phase. Quantitation of the carboxytolbutamide present in incubation mixtures by chromatography of the ether extracts indicated that this component was not present at all in the incubation mixtures examined. In addition, paper chromatography of the heptane extract of incubation mixtures indicated that only one radioactive peak, corresponding to tolbutamide, was present in these extracts. Thus, the loss of carboxytolbutamide into the heptane phase during extraction was not relevant to the quantitation of products in the incubation mixtures.

Counting and chromatographic techniques

After incubation, unreacted ^{14}C-tolbutamide was quantitatively removed from the incubation mixture with *n*-heptane–1·5% isoamyl alcohol prior to ether extraction. Four 15-min extractions with 15 ml of solvent were required to remove the tolbutamide. Several milligrams of unlabeled tolbutamide were added to the aqueous phase after each extraction. The radioactive product remaining in the incubation mixture after heptane extraction was extracted into ether by two 1-hr extractions with 15 ml each of ether. The ether extracts were combined, evaporated to dryness, and the residue taken up in 0·5 ml ethanol for liquid scintillation counting or for identification by paper chromatography. The extent of reaction of the ^{14}C-tolbutamide was determined by comparing the radioactivity present in the tolbutamide-free ether extracts to the total radioactivity incubated.

Radioactive counting was done in a model 725 Nuclear-Chicago liquid scintillation spectrometer. Bray's liquid scintillator[11] was used as the solvent.

Tolbutamide and its metabolites on paper and thin-layer chromatograms were localized by spraying with a ninhydrin spray and/or by scanning for radioactivity with a Vanguard 880 autoscanner. The paper chromatographic system was that described by Wittenhagen *et al.*[3]

Infrared spectra were obtained from a Perkin-Elmer model 137 spectrometer.

Glucose in mouse serum was determined by the glucose oxidase–peroxidase method.[12]

RESULTS

Studies in vivo

Two male Sprague–Dawley rats were each given a single oral dose of 100 mg (370 μmoles) of ^{14}C-tolbutamide (4000 counts/min/μmole) and the urine collected for 24 hr. In 24 hr, 40 per cent of the administered radioactivity could be recovered in the urine. This was acidified to pH 1 and extracted three times by shaking with 3 volumes of ether for 2 hr each. The ether extracts were combined, evaporated to dryness, and the residue taken up in absolute ethanol. Counting data showed that the ether extracts contained 87 per cent of the radioactivity present in the urine. The ether extract was chromatographed, sprayed with a ninhydrin reagent, and scanned for radioactivity. Three ninhydrin-positive areas which were all radioactive were observed: a major peak R_f 0·69 (0·68–0·75), with a shoulder at R_f 0·79 (0·77–0·80); and a smaller peak R_f 0·50 (0·43–0·57). A typical chromatographic scan is presented in Fig. 1. Authentic tolbutamide and the metabolites II and III had R_f values of 0·87–0·89, 0·79–0·81, and 0·40–0·48 respectively. The compounds present in the ether extracts consistently had a somewhat different mobility than the authentic compounds. This was probably due to the presence of salts in the sample. When the authentic

tolbutamide, II, and III, were co-chromatographed as mixtures with the ether extract, an increase of ninhydrin-positive material was seen only at the R_f values 0·79, 0·69, and 0·50, indicating that these areas corresponded to tolbutamide, the hydroxymethyl, and carboxy derivatives respectively. The intensity of the peak at R_f 0·69 often obscured the smaller peak at 0·79, which consequently appeared only as a shoulder on the larger peak (Fig. 1A). When authentic ^{14}C-tolbutamide was chromatographed with the ether extract, an increase in the radioactivity of the 0·79 peak was observed. The 0·79 peak was thus identified as tolbutamide.

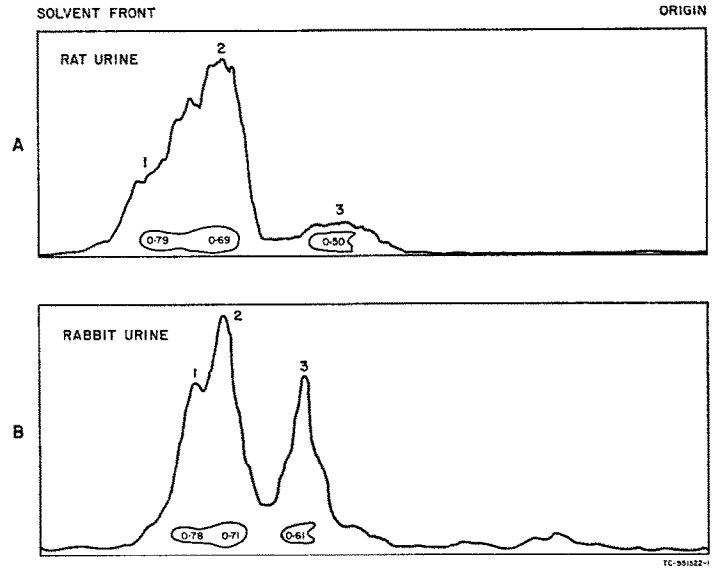

FIG. 1. Scan of radiochromatograms of ether extracts of rat and rabbit urines (24-hr specimen) after the oral administration of ^{14}C-tolbutamide. The R_f values of ninhydrin-positive spots are indicated at the bottom of each scan.

To characterize the major peak, the 72-hr urine sample from tolbutamide-induced rats was acidified and extracted with 2 volumes of heptane–1·5 per cent isoamyl alcohol to remove tolbutamide and then with 2 volumes of ether to remove the other metabolites. The ether extracts were subjected to preparative thin-layer chromatography on silica gel GF with acetone:ether (1:1) as the developing solvent. The metabolites were located under u.v. light. Some material remained at the origin and was not resolved further. A large portion of the applied material had a mobility intermediate to that of authentic tolbutamine and II. When this area was removed from the plate and recrystallized, the i.r. spectrum was found to be identical with that of authentic hydroxymethyltolbutamide (Fig. 2). The recrystallized compound melted at 95–97°; the mixture of the isolated material and the synthetic compound melted at 95–98°. When the purified material was rechromatographed on silica gel GF, the mobility corresponded to that of authentic II. Thus the major urinary product of tolbutamide in the rat was identified as hydroxymethyltolbutamide (peak 2 in Fig. 1A). The compound corresponding to R_f 0·50 was not characterized beyond its

identification as carboxytolbutamide from chromatographic comparison with the authentic compound.

To explore the urinary products in another species, a white male New Zealand rabbit was given a single oral dose of 100 mg (370 μmoles) ^{14}C-tolbutamide (5940 counts/min/μmole), and the urine was collected. In 24 hr, 80 per cent of the administered radioactivity could be recovered in the urine. The urine was acidified and extracted with ether. Chromatography of the ether extracts showed the presence of

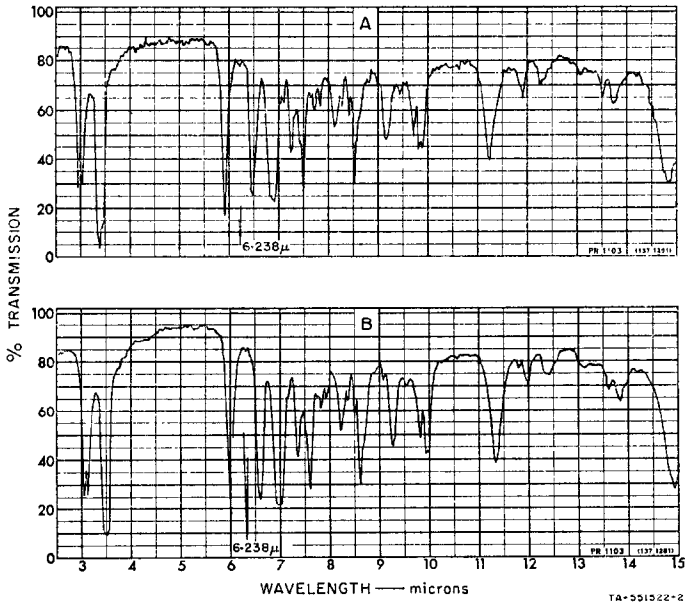

FIG. 2. Infared spectra in Nujol of (A) synthetic hydroxymethyltolbutamide and (B) a metabolite isolated from rat urine.

three radioactive metabolites (Fig. 1B) in a pattern very similar to that seen for the rat (Fig. 1A). There was a major peak at R_f 0·71, with a shoulder at R_f 0·78 and a smaller peak at R_f 0·61. As in the rat, salt effect apparently influenced the mobility of the metabolites, since none of the radioactive areas corresponded to the R_f values of the authentic metabolites. A sample of the ether extracts was taken up in water, acidified, and extracted with heptane-1·5 per cent isoamyl alcohol and then re-extracted with ether. When the ether extracts were chromatographed, the shoulder originally present (peak I, Fig. 1B) was eliminated. The re-extraction appears to have eliminated some of the salt effect seen in the chromatogram of the original ether extracts. A single large peak (R_f 0·78) corresponding to the authentic hydroxymethyltolbutamide (R_f 0·73), and the smaller peak R_f 0·43 corresponding to the carboxy derivative (R_f 0·35) were observed. The addition of authentic hydroxymethyltolbutamide to the extract increased the intensity of the ninhydrin spot at R_f 0·77.

Thus the major product of tolbutamide metabolism to appear in the urine of both the rabbit and the rat is hydroxymethyltolbutamide, with smaller amounts of the carboxy derivative as well as some unchanged tolbutamide.

Studies in vitro

When the 10,000-*g* supernatant fractions of the liver homogenates were incubated with ^{14}C-tolbutamide, and the ether extracts of the incubations were chromatographed, two radioactive compounds (peak 1, R_f 0·79; and peak 2, R_f 0·65) were observed (Fig. 3A). Extraction of the incubation mixture with heptane–1·5% isoamyl alcohol prior to ether extraction eliminated peak 1 (Fig. 3B). This peak could be

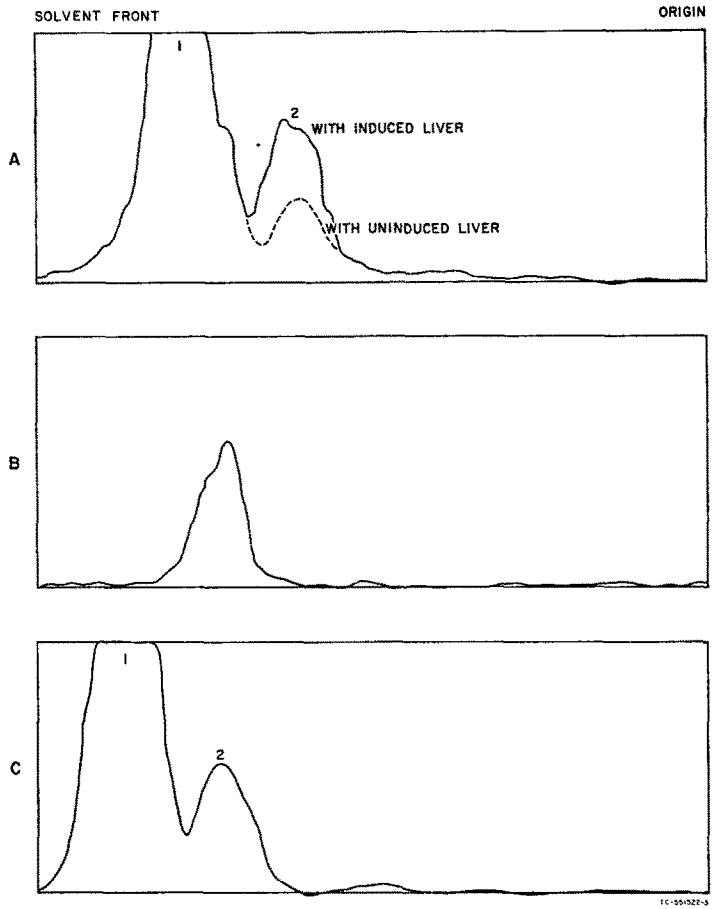

FIG. 3. Radiochromatogram scans of ether extracts of the incubation mixtures. Pattern in A was obtained from the ether extract; in B after heptane extraction of the incubation mixture followed with ether extraction; in C by adding ^{14}C-tolbutamide to an incubation mixture after the extraction with heptane (as in B) but before ether extraction.

restored by rechromatographing the extracted preparation with added ^{14}C-tolbutamide (Fig. 3C). Peak 2 was identified as the alcohol derivative by thin-layer chromatography of the tolbutamide-free ether extracts on silica gel GF plates with acetone:ether (1:1) as the developing solvent. One radioactive peak was identified on the plate. The position of the peak corresponded to that of the authentic hydroxymethyltolbutamide

No evidence for the presence of carboxytolbutamide was obtained in ether extracts of incubations carried on up to 3 hr.

Induction studies

If the animals were pretreated with 3,4-benzpyrene, phenobarbital, or tolbutamide, the catabolic activity of the liver against tolbutamide was stimulated (Table 1). A limited study with the livers of female rats showed no stimulation with tolbutamide, the only inducer tested. Benzpyrene and tolbutamide were not effective in stimulating the activity of the kidney. Counting data indicated that homogenates prepared from the kidneys of tolbutamide-induced rats converted only 1 per cent of the incubated tolbutamide to the product.

TABLE 1. EFFECT OF INDUCTION ON THE METABOLISM OF ^{14}C-TOLBUTAMIDE BY THE 10,000-*g* SUPERNATANT FRACTION OF RAT LIVER HOMOGENATES

Inducer	Conversion to hydroxymethyltolbutamide (%)	
	Liver	Kidney
None	11·6 ± 1·6	
3,4 Benzpyrene (25 mg/kg, once)	25·3 ± 9·5	
Tolbutamide (300 mg/kg, 3×)	41·4 ± 3·5	1·1
Phenobarbital (75 mg/kg, 4×)	50·4 ± 9·9	

Incubation mixtures consisted of 0·5 μmole ^{14}C-tolbutamide (0·024 μc/μmole) in 1 ml of M/15 phosphate buffer, pH 7·0; 15 μmoles nicotinamide; 0·25 μmole NADP; 40 μmoles glucose 6-phosphate; 20 μmoles $MgCl_2$; and 1·0 ml liver supernatant in a final volume of 2·5 ml. Incubation was carried out for 1 hr in air. The protein content (mg/ml) of the liver supernatant was for control 37·5, benzpyrene-treated 36·0. tolbutamide-treated 40·0, and phenobarbital-treated 37·3. The results are expressed as averages ± standard deviations obtained from three incubations.

Site of metabolism

Homogenates prepared from the livers of tolbutamide- and phenobarbital-induced rats were separated into microsomes and microsome-free supernatant fractions in order to determine which fraction contained the tolbutamide-metabolizing activity. Data are given in Table 2 indicating that the activity of the homogenates was associated with the microsomes rather than with the supernatant fraction. However, the activity of the recombined fractions was much lower than would be expected from Table 1. The livers from which these preparations were made had been stored at −20° for 6 days. When homogenates were prepared from the livers after 11 days of storage, the activity of the 10,000-*g* supernatant fraction was only 40 per cent of the activity originally seen. Apparently both the storage of liver and the manipulation involved in the preparation of microsomes were detrimental to the activity of the resulting preparations. Remmer *et al.*[5] also reported a substantial loss in this enzymic activity upon homogenization of liver slices.

Isotope effect

In order to determine the effect of deuterium substitution on the rate of oxidation of tolbutamide, liver supernatants (10,000-*g*) from tolbutamide-induced rats were incubated with ^{14}C-tolbutamide and with that in which the hydrogen atoms of the methyl group of the tolyl moiety were replaced with deuterium. It was expected that if the carbon hydrogen bond-breaking step were rate limiting in the oxidation of tolbutamide, the molecule with the heavy isotope substituted at the site of reaction would have a lower rate of reaction, and a kinetic isotope effect would become evident.

TABLE 2. METABOLISM OF ^{14}C TOLBUTAMIDE BY SUBCELLULAR FRACTIONS OF THE RAT LIVER

Liver fraction	Conversion to hydroxymethyltolbutamide (%)	
	Tolbutamide induced	Phenobarbital induced
(1) Washed micrsomes	9·7 ± 0·3	7·7 ± 0·3
(2) Microsome-free supernatant	0·92 ± 0·06	0·75 ± 0·01
(3) (1) + (2)	10·8 ± 0·3	9·0 ± 0·2
(4) 10,000-*g* supernatant	14·7 ± 4·4	20·2 ± 4·5

Incubations were carried out as described under Table 1 in triplicate or quadriplicate. The protein (mg/ml) in each of the tolbutamide-induced and phenobarbital-induced fractions was: (1) 15·9 and 12·3, (2) 34·6 and 36·9, and (4) 50·1 and 45·2 respectively. Washed microsomes were supplemented with 4 units (0·025 mg) of glucose 6-phosphate dehydrogenase (Sigma Chemical Co.).

A comparison of the rates of oxidation of deuterium-labeled and non-deuterium-labeled tolbutamide is shown in Table 3. The rate of reaction for the deuterated compound is seen to be only slightly lower than that of the control.

TABLE 3. RELATIVE RATES OF METABOLISM OF NONDEUTERATED AND DEUTERIUM-LABELED TOLBUTAMIDE

Expt. No.	Length of incubation (min)	Conversion to hydroxymethyltolbutamide (%)		Ratio C/D
		Control (C)	Deuterium labeled (D)	
1	15	40·5 ± 1·2 (3)	30·3 ± 2·6 (3)	1·34
2	30	33·5 ± 3·1 (3)	32·8 ± 1·5 (3)	1·02
3	30	44·8 ± 5·2 (3)	35·2 ± 0·3 (3)	1·27
4	45	40·0 ± 2·1 (4)	39·1 ± 1·9 (4)	1·02
5	60	47·1 (2)	45·0 ± 2·0 (3)	1·05
6	60	64·2 ± 1·8 (3)	57·3 ± 0·1 (3)	1·12
			Average	1·14

Incubations were carried out on 1·0 ml of 10,000-*g* supernatant fraction equivalent to 0·5 g liver. The incubation conditions were as described under Table 1.

Figures on parentheses refer to the number of incubations conducted on each sample.

Hypoglycemic activity of hydroxymethyltolbutamide

Since hydroxymethyltolbutamide was found to be the major metabolite of tolbutamide, it was of interest to examine the hypoglycemic activity of this product. As shown in Table 4, the metabolite was approximately half as active as tolbutamide in this respect.

TABLE 4. HYPOGLYCEMIC EFFECT OF TOLBUTAMIDE AND HYDROXYMETHYLTOLBUTAMIDE

Compound	Dose (mg/kg)	Blood glucose (mg/100 ml)	Dose (mg/kg)	Blood glucose (mg/100 ml)
Tolbutamide	50	151 ± 33	50	137 ± 12
Hydroxylmethyl-tolbutamide	50	215 ± 20	100	135 ± 14
None		262 ± 13		220 ± 13

The compounds were administered i.v. and the mice were killed 15 min after injection. Six mice were used in each group.

DISCUSSION

Our results from the urine studies confirm the findings of Wittenhagen *et al.*[3] that hydroxymethyltolbutamide as well as carboxytolbutamide are excreted by rats and rabbits. However, our experiments with ^{14}C-labeled tolbutamide indicated the former to be the major metabolite.

In the present study, only hydroxymethyltolbutamide was formed by the rat liver homogenate preparation. Wittenhagen *et al.*[3] and Bänder and Scholz[4] reported the formation of carboxytolbutamide in their *in vitro* systems. The reason for this discrepancy is not clear. Since the formation of carboxytolbutamide was not observed with either liver or kidney homogenates and yet the compound is excreted in the urine, it appears that the oxidation of the hydroxymethyl form to the carboxyl form must occur very slowly, if at all, in these tissues. *p*-Nitrotoluene has been reported to be oxidized to *p*- nitrobenzoic acid in rabbit liver homogenates.[13]

The inability of Remmer *et al.*[5] to localize the site of oxidation of tolbutamide to the liver microsomes of the rat may have been due to the relatively insensitive analytical technique they used. Our method of using ^{14}C-labeled tolbutamide facilitated the quantitative estimation of extent of tolbutamide catabolism. Our data indicated that the enzyme system for tolbutamide metabolism is a typical liver microsome system which can be stimulated by pretreating the rats with well-known microsome inducers such as 3,4-benzpyrene, phenobarbital, and tolbutamide. However, this particular enzyme system appeared somewhat labile, since homogenization of the liver resulted in substantial loss in the activity.

In a previous communication,[14] we reported on the kinetic isotope effects in the metabolism of deuterated *o*-nitroanisole and 5-butyl-5-ethylbarbituric acid (Neonal). The ratio, k_H/k_D, was approximately 2 with these compounds. Although Lemieux *et al.*[10] had reported on the absence of a distinct primary isotope effect with N-*p*-benzotrideuteridesulfonyl-N'-*n*-butylurea, their study was conducted *in vivo* and based on the excretion rate of carboxytolbutamide. We felt it worthwhile to reinvestigate this reaction *in vitro* with our system, which catalysed a one-step oxidation reaction. The

small isotope effect observed in our study indicates that the carbon-hydrogen bond breaking is also not the rate-determining step in this oxidative reaction.

Acknowledgement—We wish to express our gratitude to the Upjohn Co. for their generous gifts of tolbutamide and carboxytolbutamide. We are also indebted to Dr. Myna Theisen and Mrs. Nanci Badger for carrying out the blood glucose analyses.

ADDENDUM

After this manuscript was submitted for publication, a paper by Thomas and Ikeda[15] appeared in which the authors showed 1-butyl-3-(*p*-hydroxymethyl)phenylsulfonylurea to be the major metabolite in the rat urine.

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