Metabolic Fate of Chlorpropamide in Man and in the Rat

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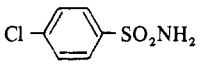
Following oral administration of tritiated chlorpropamide, [(*p*-chlorophenyl)sulfonyl]-3-propylurea (I), to man, 80% of the radioactivity was excreted in urine during a 7-day period. In addn to being excreted in urine unchanged, chlorpropamide was metabolized to *p*-chlorobenzenesulfonamide (II), [(*p*-chlorophenyl)sulfonyl]urea (III), 1-[(*p*-chlorophenyl)sulfonyl]-3-(2-hydroxypropyl)urea (IV), and 1-[(*p*-chlorophenyl)sulfonyl]-3-(3-hydroxypropyl)urea (V) by man. The relative amts of these materials, excreted in urine at 2 time intervals (4–12 hr and 30–36 hr following oral administration of tritiated chlorpropamide), were detd by quant paper, thin-layer, and gas chromatography. In a group of 8 subjects, I, II, III, IV, and V, at the 2 time intervals, averaged 33 and 46, 11 and 8, 8 and 7, 44 and 37, and 3 and 2%, respectively. In the rat, 60% of an orally administered dose of tritiated chlorpropamide was excreted in urine over a 4-day period as I, III, IV, and V. The relative amts of these 4 materials, excreted during a 24-hr period before and after chronic administration of chlorpropamide, were 37 and 37, 33 and 34, 17 and 16, and 13 and 13% for I, III, IV, and V, respectively.

The metabolism of chlorpropamide, 1-[(*p*-chlorophenyl)-sulfonyl]-3-propylurea (I), an orally active hypoglycemic agent, has not been extensively studied. Johnson, *et al.*,[1] and Stowers, *et al.*,[2] reported that the drug is not metab-

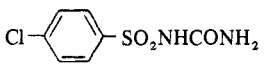
I

olized by man. Welles, *et al.*,[3] however, reported that man excretes *p*-chlorobenzenesulfonamide (II) and an unknown metabolite, in addn to unchanged drug, in urine following oral administration of chlorpropamide. These latter workers



II

also identified I, II, and [(*p*-chlorophenyl)sulfonyl]urea (III) as urinary excretion products of chlorpropamide in the dog. They identified chlorpropamide as the sole drug-related urinary excretion product following its oral administration to the rabbit. During the course of the presently described work, Brotherton, *et al.*,[4] reported I, II, and III as urinary excretion products of chlorpropamide in man. Since these

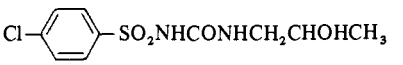


III

3 materials accounted for only 20–61% of the orally administered chlorpropamide, and Johnson, *et al.*,[1] had reported the drug to be almost completely absorbed, they implicated an unknown metabolite to give a material balance. The present work, employing tritium-labeled chlorpropamide, was undertaken to clarify, both qual and quant, the metabolism of this drug in man and in the rat.

Results and Discussion

An av of 80% of the radioactivity of tritiated chlorpropamide, administered orally to a group of 8 normal male subjects, was excreted in urine over a 7-day period. Less than 1% was tritiated water or other volatile radioactive material. It was surprising to find chlorpropamide so extensively metabolized in man (Tables I and II). Only an av of 33 and 46% of the drug-related material in urine, at 2 time intervals following chlorpropamide administration, was unchanged drug. A previously unidentified metabolite, 1-[(*p*-chlorophenyl)sulfonyl]-3-(2-hydroxypropyl)urea (IV), was iso-



IV

lated in cryst form and identified. It accounted for an av of 44 and 37% of drug-related material excreted in the urine at the 2 time intervals. In addition, another previously unidentified metabolite, 1-[(*p*-chlorophenyl)sulfonyl]-3-(3-hydroxypropyl)urea (V), was characterized. It accounted for an av

V

of 3 and 2% of drug-related material excreted in urine at the 2 time intervals. These are probably the chlorpropamide metabolites mentioned, but not isolated or characterized, by Welles, *et al.*,[3] and implicated for material balance by Brotherton, *et al.*[4] Two other metabolites, II and III, identified by thin-layer and paper chromatography, accounted for, in almost equal amts, the remaining chlorpropamide-related material excreted in urine. It is possible that II was not a true metabolite of chlorpropamide in man, but simply a hydrolysis product of IV which arose during storage and work-up of urine. However, the absence of II as a metabolite of chlorpropamide in the rat and the results of stability tests† of I, III, and IV indicate that II was indeed a metabolite in man.

Approx 60% of the radioactivity of an oral dose of tritiated chlorpropamide was excreted in the urine of female rats over a 4-day period following both a single dose and a series of 18 doses of the drug (Table III). Less than 1% of this was tritiated water or other volatile radioactive material. In both cases, this radioactivity was accounted for by compds I, III, IV, and V, the first 2 of which were isolated separately in cryst form and identified. The latter 2 were isolated as a cryst mixt and identified.

As shown in Table IV, 37% of the drug-related material in rat urine was chlorpropamide. Another 33% was accounted for by III. The hydroxypropyl metabolites (IV and V) accounted for the remaining drug-related material, approx 16% IV and 13% V. Thus, chronic administration of chlor-

†Metabolite IV, in contrast to chlorpropamide, was found to be unstable to acid (below pH 4), producing II. During large-scale work-up of urine to isolate the metabolites some hydrolysis of IV to give II occurred. However, when cryst samples of tritiated I, III, and IV were added to urine and the urine was worked up by small-scale extn and thin-layer and paper chromatography as described, no conversion to II or other artifact was noted.

Table I. Relative Distribution of Chlorpropamide and Its Metabolites in Urine of Human Subjects during Period of Peak Excretion of Drug-Related Material

Subject	Collection period, hr	Relative per cent in urine				
		Chlorpropamide[a]	Hydroxypropyl metabolites[b] 3-Hydroxy	Hydroxypropyl metabolites[b] 2-Hydroxy	*p*-Chlorobenzene sulfonamide[a]	[(*p*-Chlorophenyl)-sulfonyl]urea[c]
1	8-12	9	2	62	14	13
2	4-8	41	2	47	5	6
3	4-8	40	2	40	11	6
4	4-8	52	4	32	8	4
5	4-8	40	6	35	10	8
6	4-8	37	4	40	10	8
7	8-12	11	3	63	13	11
8	4-8	37	3	37	15	9
Average ± std dev		33 ± 15	3 ± 1	44 ± 12	11 ± 3	8 ± 3

[a] By tlc. [b] By paper chromatography for the sum of the 2 isomers and glc to differentiate the two. [c] By paper chromatography.

Table II. Relative Distribution of Chlorpropamide and Its Metabolites in Urine of Human Subjects 30-36 Hr Following Drug Administration

Subject	Relative per cent in urine				
	Chlorpropamide[a]	Hydroxypropyl metabolites[b] 3-Hydroxy	Hydroxypropyl metabolites[b] 2-Hydroxy	*p*-Chlorobenzene sulfonamide[a]	[(*p*-Chlorophenyl)-sulfonyl]urea[c]
1	21	3	56	11	9
2	45	2	43	5	6
3	58	2	28	6	7
4	26	3	53	10	8
5	82	1	11	3	3
6	67	1	24	3	4
7	40	2	40	10	8
8	26	4	43	13	15
Average ± std dev	46 ± 22	2 ± 1	37 ± 15	8 ± 4	7 ± 4

[a] By tlc. [b] By paper chromatography for the sum of the 2 isomers and glc to differentiate the two. [c] By paper chromatography.

Table III. Urinary Excretion of Radioactivity Following Oral Administration of Tritiated Chlorpropamide to Rats

Collection period, hr	Per cent of dose in urine[a]							
	First dose				Eighteenth dose			
	Rat no. 1	Rat no. 2	Rat no. 3	Avg ± std dev	Rat no. 1	Rat no. 2	Rat no. 3	Avg ± std dev
0-24	37.4	41.5	41.5	40.1 ± 2.4	38.5	45.0	54.0	46.0 ± 8.0
24-48	8.0	12.2	20.5	13.6 ± 6.4	6.1	7.8	14.9	9.6 ± 4.7
48-72	2.28	4.9	4.15	3.78 ± 1.3	3.26	4.8	4.8	4.3 ± 0.9
72-96	0.58	0.74	1.08	0.80 ± 0.26	0.92	1.57	1.87	1.45 ± 0.49
Total	48.3	59.3	67.2	58.3 ± 9.5	48.8	59.2	76.1	61.4 ± 13.8

[a] Less than 1% of the radioactivity excreted in urine was tritiated water or other volatile radioactive material.

Table IV. Relative Distribution of Chlorpropamide and Its Metabolites in Urine Following Oral Administration of Tritiated Chlorpropamide to Rats

Rat no.	Relative per cent in urine							
	Chlorpropamide[a]		Hydroxypropyl metabolites[b] 3-Hydroxy		Hydroxypropyl metabolites[b] 2-Hydroxy		[(*p*-Chlorophenyl)-sulfonyl]urea[c]	
	1st dose	18th dose	1st dose	18th dose	1st dose	18th dose	1st dose	18th dose
1	34	36	18	13	19	21	28	30
2	33	34	10	12	18	16	39	38
3	44	42	12	13	13	12	31	33
Average ± std dev	37 ± 6	37 ± 4	13 ± 4	13 ± 1	17 ± 3	16 ± 5	33 ± 6	34 ± 4

[a] By tlc. [b] By paper chromatography for the sum of the 2 isomers and glc to differentiate the two. [c] By paper chromatography.

propamide to the rat did not result in a change in either the total amt of drug-related material excreted in urine or the relative amts of these materials.

In a separate study with 2 bile-duct cannulated rats, an average of 13% of an orally administered dose of tritiated chlorpropamide was secreted in bile over a 3-day period. Paper and thin-layer chromatography of the bile revealed the presence of chlorpropamide, II, a small amt of III, IV and/or V, and a very polar radioactive material. Upon prolonged incubation with β-glucuronidase or acid hydrolysis, most of the polar material was converted to II. Although this polar material was not further characterized, it seems likely that it was a conjugate of IV and/or V which hydrolyzed at the urea linkage during prolonged incubation or acid hydrolysis† to give II. It is possible that the II in bile, prior to enzymatic and acid hydrolysis, arose as an artifact from IV and V; as mentioned previously, II was not found in urine.

There was no great difference in the relative amts of unchanged drug excreted in urine by man and the rat, 40% by

man and 37% by the rat. It is interesting that, in contrast to man, the rat did not excrete II in urine. However, the rat produced a great deal more III than did man, 33 *vs.* 8%. The rat produced somewhat less IV than man, 16 *vs.* 40%, but more V than man, 13 *vs.* 3%.

Chronic administration (18 doses over a 35-day period) of chlorpropamide to the rat did not result in adaptation insofar as the distribution of metabolites is concerned; there was no significant change in the relative amts of the 4 metabolites excreted. Although the effect of chronic administration of the drug on its rate of metabolism was not investigated, the urinary excretion results in Table III suggest there was no marked effect. Remmer, *et al.*,[5,6] have reported that tolbutamide (VI), another orally active sulfonylurea hypo-

VI

glycemic agent, does not stimulate its own metabolism, even though it is a strong inducer of microsomal enzymes, in the rat. Tagg, *et al.*,[7] however, using radioactive tolbutamide, have found that this drug is metabolized by a microsomal enzyme system in the rat and does indeed stimulate its own metabolism.

Experimental Section

Radioactivity Measurements. All counting was performed with a Packard Tri-Carb Model 314X or 314EX-2A liquid scintillation spectrometer at −8° under conditions suitable for measuring tritium. Appropriate aliquots of samples were dissolved in 15 ml of diotol scintillation solvent[8] [toluene–dioxane–MeOH (350:350:210 by vol) containing 73 g of naphthalene, 4.6 g of 2,5-diphenyloxazole, and 0.080 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene per l.]. The abs counting efficiency for each sample was detd by recounting after addn of an internal std of tritium-labeled toluene and results were then converted to disintegrations per minute.

Paper and Thin-Layer Chromatography. Paper chromatography was carried out in a 1-butanol–piperidine–water (81:2:17 by vol) system on Whatman No. 1 paper. Dried chromatograms were routinely examined under short-wavelength uv light with a uv scanner[9] to locate stds and, when possible, metabolites by fluorescence quenching. Zones of radioactivity were located and quantified by cutting the developed paper strip into sequential 1.25-cm segments and counting the segments in individual vials in the usual manner.

Thin-layer chromatography was carried out‡ in a $CHCl_3$–MeOH–formic acid (95:4:1 by vol) system on 0.25-mm films of silica gel GF.§ The fluorescence quenching of stds and, when possible, metabolites was detected by viewing the dried chromatograms under short-wavelength uv light. The zones of radioactivity were located and quantified by transferring sequential 1-cm segments of the developed chromatogram into individual vials and counting in the usual manner.

Gas Chromatography. The 2- and 3-hydroxypropyl metabolites (IV and V) of chlorpropamide were quantified by glc of the dibenzoyl derivs of the respective isomeric hydroxypropylamines liberated from the metabolites by acid hydrolysis.

A Hewlett-Packard Model 402 gas chromatograph, equipped with a flame-ionization detector, was used. A 1.2 m × 3 mm I.D. glass column, packed with 5% Lexan on Gaschrom Q, was operated isothermally at 250° with He as carrier gas at a pressure of 40 psi and a flow rate of 50 ml/min. The flash heater and detector were maintained at 320°.

Std samples of the benzoyl derivs were prepared in cryst form by benzoylation of the appropriate amines (Eastman) by the Schotten-Baumann method. Each of the derivs was pure as detd by elemental analysis for C, H, and N. Thus, *N*-[2-(benzoyloxy)propyl]benzamide (VII) and *N*-[3-(benzoyloxy)propyl]benzamide (VIII) served as stds for the derivs of the isomeric hydroxypropylamines, *N*-propylbenzamide (IX) served as a std for the deriv of propylamine, and *N*-[2-(benzoyloxy)ethyl]benzamide (X) served as an internal std. Under the previously described conditions for glc, the 4 compds had retention times of 5.5, 12.0, <1, and 7.5 min, respectively.

An aliquot of an EtOAc ext of urine at pH 4 was evapd to dryness in the bottom of a 15-ml, glass-stoppered, conical centrifuge tube or 1 ml of whole urine was placed in the tube. One ml of 6 *N* HCl (1 ml of 12 *N* HCl in the case of whole urine) was added and tube was stoppered and heated in an oil bath at 120° for 1.5 hr. The tube was cooled to room temp and 1 ml of 10 *N* NaOH (2 ml of 10 *N* NaOH in the case of whole urine) was added. One drop of benzoyl chloride was added and the tube contents were agitated with a Vibramixer at frequent intervals for 15 min at room temp. The tube was then cooled in ice water. The mixt was extd 3 times with 2-ml portions of Et_2O. The Et_2O exts were combined, acidified with 2 drops of ethanolic HCl, and evapd to dryness at 50°, under a stream of nitrogen. The residue was taken up in sufficient Et_2O, containing a known concn of X as internal std, then glc of a 2-μl aliquot gave a suitable response. The peak heights of VII and VIII were normalized to the internal std peak height and converted to concn values by reference to std curves prepd from solns of pure VII and VIII in Et_2O containing the same concn of internal std used for the samples. The std curves, covering the range 0.3–1.5 n*M* of VII and 0.6–3.0 n*M* of VIII per μl, were run each day.

Samples of cryst IV and V (the isomeric mixt isolated from rat urine) and chlorpropamide were hydrolyzed under the conditions described for the assay, and the hydrolysates were subjected to tlc. In each case hydrolysis was complete, as judged by the disappearance of IV, V, and I and the appearance of II.

Recoveries of the isomeric hydroxypropylamines (Eastman, redistilled), carried through the assay procedure including a mock hydrolysis, were 95% over the ranges covered by the std curves.

Normal human and rat urine were found to contain approx 10 μg of 2-hydroxyethylamine per ml, as detd by the gas-chromatographic assay. Neither of the isomeric hydroxypropylamines was found in normal human urine whereas approx 1 μg of 2-hydroxypropylamine per ml and no 3-hydroxypropylamine were found in normal rat urine. The ethyl acetate ext of normal urine at pH 4 did not contain the hydroxyamines in detectable concns, however. When known amts of the isomeric hydroxypropylamines and 2-hydroxyethylamine were added to normal urine, less than 2% of each material was extd at pH 4 with EtOAc. Therefore, the normally occurring hydroxyamines would not have interfered in the assay.

Human Experiments. Finely powdered chloropropamide (0.250 g) containing 96 μCi of tritium# was administered to each of 8 normal male subjects. Urine, feces, and blood samples were taken at appropriate intervals over a period of 7 days. An average of 80% of the administered radioactivity was excreted in the urine of which less than 1% was tritiated water or other volatile radioactive material. Over 37% of the radioactive dose was excreted during the 0–24-hr period and an addnl 29% during the 24–48-hr period. Details of this study and a kinetic interpretation of the dynamics of absorption, metabolism, and excretion will be reported later.

A small aliquot of each subject's urine collection was saved for subsequent analysis and the bulk of the urine was combined into 0–24- and 24–48-hr composite samples. Aliquots of the composite samples were adjusted to pH 4 with 6 *N* HCl and extd 3 times with EtOAc, a process which removed more than 95% of the urinary radioactivity. The appropriate exts were combined and evapd to a small vol with a nitrogen stream. An aliquot of each concd ext was subjected to tlc and the plates were scanned for radioactivity. In both cases, three radioactive zones‡ were detected, corresponding to 39% chlorpropamide, 11% II, and 50% to an unknown compd or compds more polar than the other two.

The 0–24-hr composite sample was entirely used in unsuccessful attempts to isolate the unknown metabolite(s). The 24–48-hr com-

‡During early phases of this study, thin-layer chromatograms were developed to a height of 15 cm (20-cm high plate). Under these conditions, III was not sepd from IV and V. In later phases of the study, development was allowed to continue 1 hr after the solvent had reached the 15-cm height. This resulted in at least partial sepn of III from IV and V and improved the sepns of II and chlorpropamide from each other and from III, IV, and V.

§Brinkmann Instruments, Inc., Great Neck, New York.

#The tritiated chlorpropamide[10] was analyzed and found to be both chemically and radiochemically pure immediately prior to use. Although its intramolecular tritium distribution was not determined, 2 similar compounds, tritiated in an identical manner in these laboratories, contained predominantly aromatic tritium. Tolbutamide (V) contained 85% of its tritium in the aromatic ring (Richard C. Thomas, unpublished) and chlorphenesin carbamate [3-(*p*-chlorophenoxy)-1,2-propanediol-1-carbamate] contained 95% of its tritium in the aromatic ring.[11] Thus, it is likely that the tritium label of chlorpropamide was predominantly in the aromatic ring.

posite sample was adjusted to pH 4 and extd in 1.5-l. batches with five 0.75-l. portions of EtOAc. The exts were combined and evapd *in vacuo* to a damp residue. The residue was taken up in 250 ml of water at pH 4 and extd with three 125-ml portions of $CHCl_3$. Nearly 90% of the initial urinary radioactivity was extd by this procedure. The $CHCl_3$ was removed *in vacuo*, and the residue was subjected to countercurrent distribution in a 200-tube, 10 ml per phase apparatus using the system Me_2CO-methyl ethyl ketone-cyclohexane-0.05 *M* pH 5.3 phosphate buffer (5:3:6:3 by vol) for 200 transfers. An aliquot from the lower phase of every fifth tube was analyzed for radioactivity. Two major zones of radioactivity, each having a leading shoulder, were observed, one peaking in tube 62 and the other in tube 147. Appropriate tube contents, comprising the two major zones were removed and combined. The aqueous phase was adjusted to pH 4, and extd several times with $CHCl_3$. By thin-layer and paper chromatography, the zone peaking at tube 62 was found to be the unknown metabolite contaminated with a little III and the zone peaking at tube 147 was found to be chlorpropamide contaminated with a little II.‡ The chloroform ext containing the chlorpropamide was evapd to dryness *in vacuo*, and the residue was recrystallized from EtOH-water 4 times with the aid of charcoal (Darco G-60) to yield 0.080 g of cryst material. Its uv and ir spectra corresponded to those of authentic chlorpropamide. Paper and thin-layer chromatography revealed a single fluorescence quenching and radioactive zone in each case corresponding to the mobility of chlorpropamide. *Anal.* ($C_{10}H_{13}N_2O_3ClS$) C, H, N.

The $CHCl_3$ soln corresponding to the unknown metabolite was evapd to dryness *in vacuo* to give an oil. Attempts to crystallize the material were not successful, so it was subjected to countercurrent distribution in the same system as previously described. Again, the sample could not be crystd. It was, therefore, subjected to countercurrent distribution in the system $CHCl_3$–0.05 *M* pH 5.5 phosphate buffer. The contents of the tubes corresponding to the metabolite were removed and extd as previously described. The oily residue obtained upon evapn of the solvent was crystd twice from ether–heptane (Nuchar C treatment) and twice from water (Darco G-60 treatment) to obtain 0.027 g of white, cryst material. Paper and thin-layer chromatography revealed a single fluorescence quenching and radioactivity zone in each case corresponding to the mobility of the unknown metabolite. The ir spectrum of the material was very similar to that of chlorpropamide but contained 2 addnl sharp peaks in the OH/NH region at 3510 and 3450 cm^{-1}. The uv spectrum in EtOH was almost identical with that of chlorpropamide. The greater polarity of the metabolite, compared to chlorpropamide, in the paper and thin-layer chromatography systems, together with OH/NH absorption at 3510 and 3450 cm^{-1} in the ir, suggested that the metabolite contained a hydroxyl group. Mass spectrometry of the sample revealed an apparent mol ion peak at 292 mass numbers, corresponding to the addn of an oxygen atom to chlorpropamide. Elemental analysis confirmed this. *Anal.* ($C_{10}H_{13}N_2O_4ClS$) C, H.

Acid hydrolysis of the metabolite produced a product which had the same mobility by paper and thin-layer chromatography as II. This, together with the absence of strong absorption in the 1200-cm^{-1} region of the ir spectrum and lack of shifts in wavelength or increases in absorptivity of the uv absorption peaks (compared to the chlorpropamide), suggested that the hydroxyl group was on the Pr group rather than the aromatic ring. The nmr spectrum of the metabolite was compared to that of chlorpropamide.** Instead of the methyl-hydrogen triplet at δ 0.80 and the methylene absorption at δ 1.45, observed in chlorpropamide, the metabolite gave a Me doublet at δ 1.08 and no methylene absorption in the 1.0–2.0 region. The hydroxyl group of the metabolite was therefore assigned to the 2 position of the Pr group showing that the metabolite was 1-[(*p*-chlorophenyl)sulfonyl]-3-(2-hydroxypropyl)urea (IV). Analysis of the metabolite by glc confirmed the 2-hydroxy assignment although it revealed a trace of the 3-hydroxy isomer. The $[\alpha]^{25}D$ of the metabolite was found to be $-2°$ (*c* 1.715, 95% EtOH).

An aliquot of each subject's urine collection, corresponding to the time of max urinary excretion of radioactivity (see Table I), was adjusted to pH 4 with 6 *N* HCl and extd 3 times with EtOAc. More than 95% of the urinary radioactivity was extd by this procedure. The EtOAc exts corresponding to each subject's urine were combined and evapd to a small vol under a nitrogen stream. Aliquots of each subject's urine ext were subjected to paper and thin-layer chromatography and the developed chromatograms were scanned for radioactivity. In each case tlc revealed 4 radioactivity zones‡ corresponding, in order of increasing migration, to the mobilities of the hydroxypropyl metabolites (IV and V), III, II, and chlorpropamide. Paper chromatography revealed 3 radioactivity zones in each case, corresponding in order of increasing migration to the mobilities of III, the hydroxypropyl metabolites IV and V, and II plus chlorpropamide. Resolution was not always good between the hydroxypropyl metabolites and III by tlc, and II and chlorpropamide were not resolved by paper chromatography. Results from the 2 chromatography systems, however, permitted quantification of I, II, III, and IV plus V. Each urine ext was subjected to gas chromatographic analysis to det the relative amts of IV and V. The results of these analyses are presented in Table I.

A similar analysis was made of each subject's 30–36-hr urine collection and the results are presented in Table II.

Rat Experiments. Each of 3 female Sprague–Dawley rats, weighing approx 250 g, was given 18 successive 50-mg doses of chlorpropamide as a suspension in 0.25% aqueous methylcellulose vehicle by stomach tube every second day. The animals were housed in individual metabolism cages, designed for the sepn and collection of urine and feces, and allowed food, water, and 10% glucose soln *ad libitum*. Each rat received an initial tritium-labeled# dose (300 μCi) followed by 16 nonradioactive doses and finally a terminal radioactive dose. Urine collections were made at daily intervals. Feces were not collected. Radioactivity detns were made on each daily urine collection for 4 days following each of the two radioactive doses. These results are presented in Table III. The 0–24-hr urine collection, immediately following each of the two radioactive doses, was subjected to paper and thin-layer chromatography (without prior extn) and the developed chromatograms were scanned for radioactivity. Radioactivity zones, corresponding to the mobilities of chlorpropamide, III, and the hydroxypropyl metabolites were found and quantified. An EtOAc ext (prepared as described above) of each urine sample was subjected to gas chromatographic analysis to det the relative amts of IV and V. The results for each rat following each radioactive dose are presented in Table IV.

All the rat urine collections were combined and extd as previously described for human urine. The ext was subjected to countercurrent distribution for 200 transfers in the system Me_2CO–methyl ethyl ketone–cyclohexane–0.05 *M* pH 5.3 phosphate buffer (5:3:6:3). Four radioactivity zones, corresponding to III (peaking in tube 40), the hydroxypropyl metabolites (peaking in tube 68), a small amt of II† (peaking in tube 127), and chlorpropamide (peaking in tube 150), were observed. The contents of the tubes containing II and chlorpropamide (poorly resolved from one another) were combined, extd, and evapd to give a dark, cryst residue. This material was dissolved in $CHCl_3$ and extd twice with 0.5 *M* Na_2CO_3. The combined aqueous exts were acidified with HCl, and extd 4 times with $CHCl_3$. The $CHCl_3$ was removed *in vacuo* and the residue was recrystd 5 times from EtOH–H_2O with the aid of charcoal (Nuchar C) to obtain 0.430 g of white crystals. The ir spectrum of the product corresponded to that of chlorpropamide. Paper and thin-layer chromatography revealed a single fluorescence quenching and radioactivity zone in each case corresponding to the mobility of chlorpropamide (I). *Anal.* ($C_{10}H_{13}N_2O_3ClS$) C, H, N.

The contents of the countercurrent distribution tubes containing III and the hydroxypropyl metabolites (poorly resolved) were combined, extd, and evapd to give a dark oil. The residue was subjected to countercurrent distribution for 200 transfers in the system $CHCl_3$–0.05 *M* pH 5.5 phosphate buffer. Two radioactivity zones, corresponding to III and the hydroxypropyl metabolites, were observed. The contents of the tubes containing III were combined, extd, and evapd to dryness to yield a colored cryst residue. The residue was recrystd from MeOH–H_2O with the aid of charcoal (Nuchar C) to yield 0.290 g of white crystals. The ir and uv spectra of the sample corresponded to those of authentic III. Paper and thin-layer chromatography revealed a single fluorescence quenching and radioactivity zone in each case corresponding to the mobility of III. *Anal.* ($C_7H_7N_2O_3ClS$) C, H, N.

The contents of the tubes containing the hydroxypropyl metabolites (IV and V) were combined, extd, and evapd to dryness to yield a colored oil. Attempts to crystallize this material were still not successful so the oil was dissolved in $CHCl_3$–MeOH (9:1 by vol) and applied to a 51 × 4.8 cm diam silica gel (0.05–0.20 mm particle size§) column. The column was eluted with mixts of $CHCl_3$ and MeOH, starting with a 9:1 by vol mixt and terminating with a 1:3 by vol mixt, while collecting 10-ml fractions. A small peak corresponding to *p*-chlorobenzenesulfonamide emerged first and a large, broad peak corresponding to the hydroxypropyl metabolites emerged next, as detd by tlc. The column fractions corresponding to the hydroxypropyl metabolites were combined and evapd *in vacuo* to give a

**Samples (1.25 mg) of the metabolite and chlorpropamide in 0.4 ml of acetone-d_6 were employed. The spectra were an av of 510 scans employing a Varian C-1024 time-averaging computer attached to a Varian A-60 nmr spectrometer.

colored oil. Attempts to crystallize this material were not successful. Paper and thin-layer chromatography of the material, however, revealed a single radioactivity zone in each case corresponding to the mobility of the hydroxypropyl metabolites and several fluorescence-quenching zones which were not radioactive. The oily material was taken up in Et_2O and the soln was subjected to preparative tlc on a 20 × 20 cm film of silica gel§ 2-mm thick. The silica gel corresponding to the zone of radioactivity was removed from the plate and eluted with four 10-ml portions of Me_2CO. The residue obtained upon evapn of the Me_2CO was recrystd once from Et_2O and once from EtOH-H_2O with the aid of charcoal (Darco G-60) to obtain 0.036 g of white solid material. The uv and ir spectra corresponded to those of IV, although there was a weak peak at 1045 cm^{-1} in the ir attributable to a primary alcohol. Paper and thin-layer chromatography revealed a single fluorescence quenching and radioactivity zone in each case corresponding to the mobility of IV. *Anal.* ($C_{10}H_{13}N_2O_4ClS$) C, H, N.

The nmr spectrum of the material was obtained†† and compared to those of chlorpropamide and IV. Instead of the Me-hydrogen triplet at δ 0.80 and the methylene absorption at 1.45, observed for chlorpropamide, this material gave a doublet at 1.06, typical of IV and a quintet at 1.62 attributable to a 3-hydroxypropyl metabolite, 1-[(*p*-chlorophenyl)sulfonyl]-3-(3-hydroxypropyl)urea (V). The ratio of areas under the δ 1.06 and 1.62 peaks indicated the material was a mixt of 75% IV and 25% V.

Analysis of the material by glc confirmed that the product was a mixt of the 2 isomers, 76% IV and 24% V.

††A 2.0-mg sample of the material in 0.4 ml of acetone-d_6 was employed. The spectrum was an average of 233 scans employing a Varian C-1024 time-averaging computer attached to a Varian HA-100 nmr spectrometer.

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References

(1) P. C. Johnson, A. R. Hennes, T. Driscoll, and K. M. West, *Ann. N. Y. Acad. Sci.*, 74, 459 (1959).
(2) J. M. Stowers, L. W. Constable, and R. B. Hunter, *ibid.*, 74, 689 (1959).
(3) J. S. Welles, M. A. Root, and R. C. Anderson, *Proc. Soc. Exp. Biol. Med.*, 101, 668 (1959).
(4) P. M. Brotherton, P. Grieveson, and C. McMartin, *Clin. Pharmacol. Ther.*, 10, 505 (1969).
(5) H. Remmer and H. J. Merker, *Ann. N. Y. Acad. Sci.*, 123, 79 (1965).
(6) H. Remmer, M. Siegert, and H. J. Merker, *Arch. Exp. Pathol. Pharmakol.*, 249, 71 (1964).
(7) J. Tagg, D. M. Yasuda, M. Tanabe, and C. Mitoma, *Biochem. Pharmacol.*, 16, 143 (1967).
(8) R. J. Herberg, *Anal. Chem.*, 32, 42 (1960).
(9) N. A. Drake, W. J. Haines, R. E. Knauff, and E. D. Nielson, *ibid.*, 28, 2036 (1956).
(10) *J. Pharm. Sci.*, 55, 112 (1966).
(11) *Ibid.*, 56, 507 (1967).

Effect of the Sulfur-Covering Group on the Antiradiation Activity of Substituted 2-Aminoethanethiols†

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Highly effective N-substituted derivatives of *S*-2-aminoethyl hydrogen thiosulfate as antiradiation agents have been modified to include the corresponding thiols, disulfides, and thiazolidines. In addition, many new substituents have been included. Based on intraperitoneal administration, Bunte salts had the best activity and thiazolidines the poorest. Thiols and disulfides were intermediate in their action. Of the new Bunte salts, *S*-2-{5-[(2-isopropyl-5-methylcyclohexyl)oxy]pentyl}amino)-ethyl hydrogen thiosulfate (**7**) derived from L-menthol was the most active. The best 3-substituted thiazolidine was 3-[5-(*o*-tolyloxy)pentyl]thiazolidine (**62**). *S*-2-{[4-(*o*-Cumenyloxy)butyl]amino}-ethyl hydrogen thiosulfate (**11**) was highly effective against tapeworm infections in mice, and the corresponding disulfide, *N,N'*-(dithiodiethylene)bis[4-(*o*-cumenyloxy)butylamine] dihydrochloride (**49**), had broad spectrum antibacterial activity in *in vitro* systems. 1-Substituted aziridines served as useful intermediates to Bunte salts and thiols by ring opening with $(NH_4)_2S_2O_3$ and H_2S. The thiols were oxidized to disulfides and treated with sodium formaldehyde bisulfite to give 3-substituted thiazolidines. Alkylation of ethylenimine using alkyl bromides and 7–10 molar excesses of ethylenimine in the presence of powdered K_2CO_3 was found to be a convenient route to 1-substituted aziridines.

Substituted 2-aminoethanethiol remains the most important structural type having antiradiation effectiveness.[1] Extensive work in many laboratories has been devoted to varying the substituents on nitrogen and sulfur. We previously have published several series of N-substituted *S*-2-aminoethyl hydrogen thiosulfates (I) as antiradiation agents.[2] Cycloalkylalkyl, alicyclic ether, aralkyl, and aryloxyalkyl groups were used as nitrogen substituents. Included in those series are some of the most active and best tolerated radioprotectors known. Antiradiation effectiveness was found to be especially sensitive to minor structural modifications of the nitrogen substituent. It remained to be determined what effect changes in the sulfur-covering function would have on radioprotective properties. Sulfur-containing groups of active radioprotectors in the main have included the parent thiol, disulfide, thiosulfates, and phosphorothioates (esters of H_3PO_3S). A summary including other miscellaneous types has been published.[3] Because of the importance of Bunte salts, we now report some additional thiosulfates along with modifications of the thiosulfate ester

$$RNHCH_2CH_2SSO_3H$$
I

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