Metabolism of Oral Cephalothin and Related Cephalosporins in the Rat

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The fate of orally administered [^{14}C]cephalothin has been studied in the rat. This antibiotic undergoes degradation in the gut followed by the subsequent absorption of a portion of the degradation products. About 50% of the administered radioactivity appears in the urine as a mixture of thienylacetyl glycine, thienylacetamidoethanol and an unidentified polar metabolite. Evidence is presented indicating that thienylacetamidoethanol arises by the enzymic reduction of a metabolic intermediate, thienylacetamidoacetaldehyde. The metabolic fate of cephalothin is very similar to that of cephaloram reported earlier. The fate of [^{14}C]cephaloridine and 7-phenoxy[1- ^{14}C]acetamidocephalosporin was also investigated. In addition to the expected metabolites, about 5% of the cephaloridine dose is absorbed unchanged. With 7-phenoxy[1- ^{14}C]acetamidocephalosporin, 15% of the dose is recovered in urine as deacetyl-7-phenoxy[1- ^{14}C]acetamidocephalosporin.

One of the most significant of recent discoveries in the antibiotic field has been the isolation of cephalosporin C from *Cephalosporium acremonium* by Newton & Abraham (1956). The structure of this unique antibiotic was established in 1961 (Abraham & Newton, 1961; Hodgkin & Maslen, 1961) and its total synthesis was described by Woodward *et al.* (1966). A large number of semi-synthetic cephalosporin derivatives have been reported (for a recent review see Rollo, 1966). The first clinically important analogue to be described was 7-(thien-2-ylacetamido)cephalosporanic acid, cephalothin (Chauvette *et al.* 1962*a,b*). Cephalothin shows a broad spectrum of antibiotic activity against Gram-positive cocci, including penicillin-resistant staphylococci, and many Gram-negative bacilli (Boniece, Wick, Holmes & Redman, 1962). Cephalothin possesses a low toxicity (Lee, Herr & Anderson, 1963) and has found wide clinical acceptance in the treatment of respiratory, urinary and gastrointestinal infections (Walters, Romansky & Johnson, 1963; Rollo, 1966).

Other cephalosporin derivatives of interest include cephaloridine (Muggleton, O'Callaghan & Stevens, 1964) and cephaloram, 7-phenylacetylcephalosporanic acid (Jago, 1964). The simpler 7-acylcephalosporanic acids do not appear to be absorbed from the gastrointestinal tract. Possibly for this reason, reports on the fate of orally administered cephalosporins have been limited. A study of the metabolism of cephaloram (Culp, Marshall & McMahon, 1963) established, however, that this

antibiotic underwent partial degradation in the gut and that some of the degradation products were absorbed. Metabolites of oral cephaloram found in urine included phenylaceturic acid and an unidentified, relatively non-polar, compound. In the present study the metabolic fate of orally administered cephalothin, cephaloridine and 7-phenoxyacetylcephalosporanic acid was investigated. ^{14}C -labelling was used as a tool to aid the study.

MATERIALS AND METHODS

[^{14}C]Cephalothin. [^{14}C]Cephalothin, 7-(thienyl[1- ^{14}C]acetamido)cephalosporanic acid, was prepared by allowing 1.3 m-moles of thienyl[1- ^{14}C]acetyl chloride to react with 3.8 m-moles of 7-aminocephalosporanic acid (Morin, Jackson, Flynn & Roeske, 1962) in 64 ml. of acetone-water (9:7, v/v) at 5° and maintaining the pH of the solution at 6.0 by the dropwise addition of aq. 5% (w/v) NaHCO_3 . After 4 hr. the reaction mixture was adjusted to pH 3.5 with N-HCl and extracted with six 20 ml. portions of benzene to remove all thienylacetic acid. The aqueous solution was adjusted to pH 2.0 with N-HCl to liberate the [^{14}C]cephalothin. The antibiotic was taken up into 60 ml. of ethyl acetate and the solution was dried over anhydrous MgSO_4 . Addition of 1.25 ml. of 3 M-potassium ethylhexanoate-ethyl acetate solution (Nettleton, Johnson, O'Herron & Hooper, 1962) caused the crystallization of 920 mg. of [^{14}C]cephalothin as the potassium salt, specific radioactivity 2.05 $\mu\text{C}/\text{mg}$. Paper chromatography indicated that the product was essentially pure material, free from any deacetylcephalothin or thienylacetic acid.

[^{14}C]Cephaloridine. [^{14}C]Cephaloridine, *N*-[7-(thien-2-yl- ^{14}C]acetamido)ceph-3-em-3-ylmethyl]pyridinium-4-

carboxylate, was prepared by allowing 2.9 m-moles of [^{14}C]cephalothin (3.2 mc) to react with 3.7 m-moles of pyridine in 1.5 ml. of aq. 0.4 M- H_3PO_4 containing 5.0 g. of KCNS at 60° for 4 hr. The reaction mixture was dissolved in 15 ml. of water and washed with three 5 ml. portions of chloroform. The aqueous solution, chilled to 0°, was acidified to pH 2.0 with 4 N-HCl. The isothiocyanate salt of [^{14}C]cephaloridine crystallized from this solution as fine crystals. The crystals were recovered by filtration, washed with 5 ml. of cold water and dried *in vacuo*. The yield of product was 800 mg.

The [^{14}C]cephaloridine isothiocyanate salt was dissolved in 2.0 ml. of water and 6.0 ml. of a solution consisting of 1.5 g. of Amberlite LA-1 resin in 4.5 ml. of 4-methylpentan-2-one. After stirring for 30 min. the phases were separated and the aqueous phase was washed with three 4 ml. portions of a 25% solution of Amberlite LA-1 resin in 4-methylpentan-2-one, followed by one 5 ml. portion of ethyl acetate. The aqueous phase was chilled to facilitate crystallization of the [^{14}C]cephaloridine. The yield of [^{14}C]cephaloridine was 230 mg. and it possessed a specific radioactivity of 2.24 $\mu\text{C}/\text{mg}$.

7-Phenoxy[^{14}C]acetamidocephalosporanic acid. Ethyl chloroformate (1.84 m-moles) was allowed to react with a solution consisting of 1.84 m-moles of phenoxy[^{14}C]acetic acid (500 μC) in 21 ml. of acetone-dioxan (2:1, v/v) containing 1.84 m-moles of triethylamine at 0°. A solution of 1.84 m-moles of 7-aminocephalosporanic acid in 100 ml. of water containing 1.84 m-moles of triethylamine was added rapidly to the cold reaction mixture. The resulting solution was stirred at room temperature for 2 hr. and concentrated *in vacuo* to 65 ml. volume. The concentrated solution was chilled to 10°, covered with 50 ml. of ethyl acetate and acidified to pH 2.4 with 5 N-HCl with stirring. The ethyl acetate phase was separated, chilled to 10° and mixed with 50 ml. of water. This mixture was neutralized to pH 7.0 with 2 N-KOH with stirring. The aqueous solution was separated and freeze-dried to yield the crystalline antibiotic. The potassium salt of 7-phenoxy[^{14}C]acetamidocephalosporin, after recrystallization from 8 ml. of methanol, amounted to 430 mg. and possessed a specific radioactivity of 2.05 $\mu\text{C}/\text{mg}$. Paper chromatography and subsequent radioautography showed the product to be pure.

Chromatographic methods. Paper chromatography was carried out on Whatman no. 1 paper. Butan-2-one saturated with water was employed for development when chromatography was to be followed by biological assay (Miller, 1962). Separation of the biologically inactive radioactive metabolites was accomplished by using butan-1-ol saturated with 5 N- NH_3 for development. The radioactive spots were located by using a scanner (Vanguard 880 Automatic Chromatogram Scanner). Areas of antibiotic activity were located by bioautographic techniques (*Bacillus subtilis*).

All thin-layer chromatography was carried out on silica gel F (E. Merck A.-G., Darmstadt, Germany) plates. Separation of the neutral radioactive metabolite from the metabolism of [^{14}C]cephalothin and [^{14}C]cephaloridine was achieved by using ethanol-ethyl acetate (1:9, v/v) for development. The separation and identification of phenoxy-[^{14}C]acetic acid and phenoxy[^{14}C]acetyl glycine from the metabolism of 7-phenoxy[^{14}C]acetamidocephalosporin was accomplished by using benzene-acetic acid (9:1, v/v) for development.

Urinary excretion studies. Aqueous solutions of the

potassium salts of the radioactive antibiotics, 46 $\mu\text{moles}/\text{kg}$., were administered orally to 200 g. male Purdue-Wistar rats. The rats were kept in stainless-steel metabolism cages and urine samples were collected at regular intervals. The radioactivity of each sample was determined by liquid-scintillation counting.

The amount present and the nature of the radioactive metabolites in urine were determined by paper-chromatographic analysis of each urine sample. The biologically active metabolites were separated and identified by comparison chromatography.

Isolation of thienylacetamidoethanol from urine. The elucidation of the structure of this metabolite was achieved by investigating material obtained from the urine of six rats given daily dosages of 46 μmoles of the labelled antibiotic/kg. for 10 days. The total urine (400 ml.) collected was neutralized to pH 7.5 with aq. 5% NaHCO_3 and extracted with four 200 ml. portions of dichloromethane. The combined organic phase was evaporated to dryness *in vacuo* to yield a dark oily residue. This material was spotted on a number of preparative thin-layer plates and developed with ethyl acetate-ethanol (9:1, v/v). The radioactive zone was removed from the plates and the material was eluted from the silica with methanol. Removal of the methanol *in vacuo* yielded the neutral metabolite. Thin-layer chromatography and subsequent elution was repeated an additional three times to obtain 4.6 mg. of a low-melting yellow crystalline substance of sufficient purity for structure determination.

Formation of thienylacetamidoacetaldehyde by acid hydrolysis of cephalothin. Cephalothin (500 mg.) was dissolved in 250 ml. of 0.1 N- H_2SO_4 and heated at 100° for 3 hr. with stirring. A saturated solution of HgCl_2 was added dropwise to the cooled colourless solution until precipitation of a mercuric complex was complete. This complex was removed by filtration. A solution of 2,4-dinitrophenylhydrazine (125 mg.) in 0.2 ml. of water and 0.5 ml. of conc. H_2SO_4 was then added to 125 ml. of the filtrate with stirring. The resulting yellow crystalline 2,4-dinitrophenylhydrazone of thienylacetamidoacetaldehyde was collected by filtration; on recrystallization from ethanol it had m.p. 180–182° (decomp.) (Found: C, 46.3; H, 3.6; N, 19.3; S, 8.8. $\text{C}_{14}\text{H}_{13}\text{N}_5\text{O}_5\text{S}$ requires: C, 46.2; H, 3.7; N, 19.1; S, 8.7%). The remaining hydrolysate was neutralized to pH 6.8 with dilute NaHCO_3 and extracted with three portions of dichloromethane. After separation, the organic phase was evaporated to dryness *in vacuo* to yield a light-yellow oil whose i.r. spectrum was consistent with that expected for thienylacetamidoacetaldehyde. This spectrum possessed an aldehyde CO peak at 1760 cm^{-1} , an amide CO peak at 1660 cm^{-1} , a secondary amide CO peak at 1510 cm^{-1} and an NH stretching band at 3400 cm^{-1} .

RESULTS AND DISCUSSION

[^{14}C]Cephalothin. The data in Fig. 1 show the recovery of total urinary radioactivity as well as the distribution of radioactive metabolites in the urine of rats after a single oral dose of [^{14}C]cephalothin. These results show that the appearance of radioactivity in the urine is quite slow. About 5% of the administered radioactivity had appeared in the urine after 4 hr., 25% in 10 hr. and 46% after

Fig. 1. Total radioactivity and radioactive metabolites found in urine of rats after oral administration of [^{14}C]-cephalothin. ■, Total radioactivity in urine; ▲, unidentified ^{14}C -labelled polar metabolite; ○, thienyl[1- ^{14}C]acetyl-glycine; □, deacetyl[^{14}C]cephalothin; ●, thienyl[^{14}C]-acetamidoethanol.

40hr. These data are indicative of little or no absorption of the antibiotic in the stomach or upper gut.

The separation of radioactive metabolites and determination of their relative amounts in each urine sample was accomplished by paper chromatography. Only one biologically active metabolite was found in the urine and this was not present after 8hr. In addition to deacetyl[^{14}C]cephalothin, paper chromatography indicated the presence of three biologically inactive radioactive metabolites. The major metabolite was very polar (R_f 0.05) and, as illustrated in Fig. 1, accounts for 50% of the total urinary radioactivity. The second metabolite represented 32% of the urinary radioactivity and had R_f 0.35. The third metabolite, representing 13% of the urinary radioactivity, was a fast-moving material with R_f 0.85.

The second metabolite possessed an R_f value close to that of thienyl[1- ^{14}C]acetic acid. Comparison chromatography followed by radioautography, however, showed the two materials to be different. It had been reported by Culp *et al.* (1963) that one metabolite resulting from the oral administration of the phenylacetyl analogue of cephalothin was phenylaceturic acid (*N*-phenylacetyl-glycine). As phenylacetyl-glycine is the expected metabolite of phenylacetic acid, it was proposed that the antibiotic had undergone hydrolysis to this acid, which had then undergone conjugation with glycine. It therefore seemed likely that the second metabolite found in the present study was also a glycine conjugate, i.e. thienyl[1- ^{14}C]acetyl-glycine.

This proved to be correct, as shown by comparison chromatography with an authentic sample of thienyl[1- ^{14}C]acetyl-glycine: a mixture of the ^{14}C -labelled second metabolite and this authentic sample failed to separate on paper chromatography.

Further confirmation was obtained by studying the metabolic fate of thienyl[1- ^{14}C]acetic acid in the rat. After 24hr. only thienyl[1- ^{14}C]acetyl-glycine had appeared in the urine and its recovery in the urine amounted to 86% of the administered dose.

The third radioactive metabolite accounted for 13% of the total urinary radioactivity and had R_f 0.85. Its isolation was accomplished by first neutralizing the urine to pH 7.5 with 5% sodium hydrogen carbonate solution followed by extractions with dichloromethane. After subsequent purification by thin-layer chromatography, this biologically inactive metabolite was found to be a neutral material whose i.r. spectrum was consistent with that of a mono-substituted amide of a carboxylic acid. It had a primary amide CO band at 1660cm^{-1} , a secondary amide CO band at 1520cm^{-1} , an NH stretching band at 3280cm^{-1} and an OH stretching band at 3400cm^{-1} . The u.v. spectrum of this metabolite showed a maximum at $235\text{m}\mu$. The molecular ion mass was 185.05257 (Calc. for $\text{C}_8\text{H}_{11}\text{NO}_2\text{S}$: 185.05105). Other mass peaks present were: 70 ($^+\text{CO}\cdot\text{NH}\cdot\text{CH}\cdot\text{CH}_2$); 88 ($^+\text{CO}\cdot\text{NH}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$); 97 (thienylmethylene); 142 ($\text{C}_4\text{H}_5\text{S}\cdot\text{CH}_2\cdot\text{C}(\text{OH})\cdot\text{NH}_2$). These data suggest the structure of this radioactive metabolite to be that of thienyl[1- ^{14}C]acetamidoethanol. Thin-layer chromatographic, i.r. and u.v. comparison of an authentic sample of this material, prepared by the procedure described by Jones, Soper, Behrens & Corse (1948), confirmed this structure. The mass spectrum of an authentic sample of thienylacetamidoethanol possessed a molecular ion peak of 185, with other peaks at 70, 88, 97 and 142, in agreement with the mass spectrum found for the metabolite.

The isolation and identification of thienyl-acetyl-glycine and thienylacetamidoethanol as metabolites in urine from rats after dosages of orally administered cephalothin suggests one possible metabolic pathway that would involve a common metabolic precursor, thienylacetamidoacetaldehyde. Reduction of this aldehyde would yield thienylacetamidoethanol whereas its oxidation

would lead to thienylacetyl-glycine. Thienylacetyl-glycine could also arise, however, from the enzymic hydrolysis of the thienylacetyl moiety of the cephalothin molecule. Numerous workers (Claridge, Luttinger & Lein, 1963; Cole, 1966; Huang, English, Sito, Shull & Sobin, 1960) have reported the presence of penicillin amidase or acylase among fungi, yeasts, actinomycetes and bacteria. This enzyme catalyses the hydrolysis of the side-chain amide moiety of many penicillins to yield the corresponding carboxylic acid and 6-aminopenicillanic acid. This enzyme also shows similar activity towards certain cephalosporin antibiotics other than cephalosporin C (Huang, Sito & Shull, 1963; Holt & Stewart, 1964).

A number of hydrolysis experiments *in vitro* with a variety of mammalian enzymes were carried out on [^{14}C]cephalothin. The enzymes and tissues investigated included: rat bile, intestinal strips, kidney and liver homogenates, carboxypeptidase A and B, pancreatin, trypsin and chymotrypsin. However, in no instance was hydrolysis to thienyl-[^{14}C]acetic acid demonstrable.

Although no evidence for the presence in urine of the proposed metabolic precursor, thienyl[1- ^{14}C]acetamidoacetaldehyde, could be found in the studies *in vivo*, there is chemical evidence to support this concept. The acid hydrolysis of penicillin G yields a neutral product that was identified as phenylacetamidoacetaldehyde (Clarke, Johnson & Robinson, 1949). Further, the acid hydrolysis of cephalosporin C has been reported (Jeffery, Abraham & Newton, 1960) to yield a material that on subsequent oxidation with silver oxide yielded δ -amino- δ -carboxyvaleryl-glycine. This suggests strongly that the corresponding aldehyde is indeed the product isolated after acid hydrolysis of the antibiotic. In the present study [^{14}C]cephalothin, on acid hydrolysis with 0.1N-sulphuric acid, yielded thienyl[1- ^{14}C]acetamidoacetaldehyde, which was isolated and identified both as the free aldehyde and as its 2,4-dinitrophenylhydrazones. This chemical degradation of [^{14}C]cephalothin to the aldehyde lends credence to the suggestion that the aldehyde is a metabolite produced during degradation of this antibiotic. Additional evidence that thienylacetamidoacetaldehyde is a metabolic precursor of thienylacetyl-glycine and thienylacetamidoethanol, both identified metabolites of cephalothin, was obtained by investigation of the metabolic fate of this aldehyde itself in the rat. Thienyl[1- ^{14}C]acetamidoacetaldehyde was administered by intraperitoneal injection to two rats and their urine collected for 24 hr. The total urinary radioactivity recovered accounted for 62% of the dose administered. Paper chromatography established that the major radioactive metabolite in the urine was thienyl[1- ^{14}C]-

acetyl-glycine and that the minor metabolite was thienyl[1- ^{14}C]acetamidoethanol.

These experiments illustrate not only that the formation of the thienylacetamidoacetaldehyde is chemically demonstrable, but also that its metabolism in the rats yields thienylacetyl-glycine and thienylacetamidoethanol. This is strong evidence that one of the metabolic pathways in the degradation of orally administered cephalothin in the rat involves the aldehyde and the products of its subsequent oxidation and reduction.

Preliminary attempts to demonstrate the reduction of thienylacetamidoacetaldehyde *in vitro* with liver alcohol dehydrogenase or with a NADPH-linked carbonyl reductase from kidney (Culp & McMahon, 1965) were not successful. These studies are being continued.

The polar radioactive metabolite (or metabolites) was not identified. It seems likely that the opening of the β -lactam ring may be one step in the formation of this metabolite. Alicino (1961) and Fleming, Goldner & Glass (1963) have shown that the β -lactam ring of cephalosporin C is opened both in alkaline solution and by enzymic hydrolysis. The results on the metabolism of orally administered [^{14}C]cephalothin in the rat are remarkably similar to the results of Culp *et al.* (1963) on 7-phenylacetylcephalosporanic acid (cephaloram). It now seems very likely that the non-polar metabolite described by these workers was in reality phenylacetamidoethanol.

[^{14}C]Cephaloridine. Fig. 2 shows the total urinary radioactivity recovered and the distribution of radioactive metabolites after a single, orally administered, dose of cephaloridine to three rats. These results, like those of cephalothin, show the slow appearance of radioactivity in the urine. About 5% of the administered dose had appeared in the urine after 4 hr., and 50% after 40 hr. As

Fig. 2. Total radioactivity and radioactive metabolites found in the urine of rats after oral administration of [^{14}C]cephaloridine. ■, Total radioactivity in urine; ▲, unidentified ^{14}C -labelled polar metabolite; ○, thienyl-[^{14}C]acetyl-glycine; □, [^{14}C]cephaloridine; ●, thienyl-[^{14}C]acetamidoethanol.

with cephalothin, this is indicative of poor absorption in the stomach and upper gut.

The number and nature of the radioactive metabolites in urine were determined in a manner identical with that described for cephalothin. Unchanged [^{14}C]cephaloridine was the only biologically active metabolite present in the urine. It was found in the urine during the first 14 hr. and its total accumulation accounted for 5% of the administered dose. Three biologically inactive metabolites were found in the urine of these rats. One major radioactive metabolite, representing 30% of the total urinary radioactivity, was identified as thienyl[1- ^{14}C]acetylglycine by chromatographic comparison with an authentic sample. Another metabolite, representing about 17% of the total urinary radioactivity, was identified as thienyl[1- ^{14}C]acetamidoethanol by comparison techniques with the same compound isolated and identified in the cephalothin study. The other major metabolite was very polar (R_f 0.05) and represented 43% of the total urinary radioactivity. As in the cephalothin study the structure of this very polar material was not established.

These results are very similar to those obtained from the cephalothin study, both with respect to the metabolites and their relative amounts. The appearance of unchanged cephaloridine in the urine in small amounts is the only important difference.

7-Phenoxy[1- ^{14}C]acetamidocephalosporin. Fig. 3 shows the recovery of total urinary radioactivity and the distribution of radioactive metabolites after a single orally administered dose of 7-phenoxy-[1- ^{14}C]acetamidocephalosporin to three rats. The curve for the recovery of total urinary radioactivity, unlike those of cephalothin and cephaloridine, shows a more rapid appearance and a greater recovery of radioactivity in the urine. After 4 hr. 13% of the administered dose had appeared in the urine, and 62% after 25 hr. Compared with the other cephalosporin antibiotics studied, 7-phenoxy-[1- ^{14}C]acetamidocephalosporin is more readily absorbed in the stomach and upper gut.

The number and nature of the radioactive metabolites in urine were determined in a manner similar to that described for cephalothin. Deacetyl-7-phenoxy[1- ^{14}C]acetamidocephalosporin was found by comparison chromatography to be the only biologically active metabolite in the urine. Its total accumulation accounted for 15.5% of the administered radioactive dose. Whether 7-phenoxy[1- ^{14}C]acetamidocephalosporin is absorbed unchanged or as the deacetyl derivative is not known. Another major metabolite, representing 50% of the total urinary radioactivity, had R_f 0.38 on paper-chromatographic development. From experience gained in the study of the metabolism

Fig. 3. Total radioactivity and radioactive metabolites found in the urine of rats after oral administration of 7-phenoxy[1- ^{14}C]acetamidocephalosporin. ■, Total radioactivity in urine; ○, phenoxy[1- ^{14}C]acetic acid; □, deacetyl-7-phenoxy[1- ^{14}C]acetamidocephalosporin; ▲, unidentified ^{14}C -labelled polar metabolite; ●, phenoxy[1- ^{14}C]acetamidoethanol.

of cephalothin, it was reasoned that this metabolite was either phenoxy[1- ^{14}C]acetic acid or its glycine conjugate. Phenoxy[1- ^{14}C]acetic acid was administered intraperitoneally to three rats and within 24 hr. 96% of the administered radioactivity had been recovered. The only radioactive metabolite present in the urine was identified by comparison chromatography as unchanged phenoxy[1- ^{14}C]acetic acid. Paper chromatography, in this instance, did not afford good separation of phenoxyacetic acid and phenoxyacetylglycine. Good separation of these acids was achieved, however, by thin-layer chromatography on fluorescent silica-gel plates, with acetic acid-benzene (19:1, v/v) for development and u.v. light for detection.

A mixture of urine from rats given 7-phenoxy-[1- ^{14}C]acetamidocephalosporin and authentic samples of phenoxyacetic acid and phenoxyacetylglycine were chromatographed by the above thin-layer technique. The zones corresponding to phenoxyacetic acid and phenoxyacetylglycine, detected with u.v. light, were isolated and the relative amounts of radioactivity present determined. Phenoxyacetic acid and the phenoxyacetylglycine zone accounted for 47 and 3% of the total urinary radioactivity respectively. Since it has been demonstrated that phenoxyacetic acid is excreted unchanged by the rat, the phenoxyacetylglycine must therefore arise from the oxidation of an intermediate metabolite, phenoxyacetamidoacetaldehyde. The unconjugated phenoxyacetic acid recovered must result from the enzymic hydrolysis of phenoxyacetamido bond.

Another radioactive metabolite was relatively non-polar (R_f 0.85) and represented 5% of the

Table 1. *Distribution of radioactive metabolites in urine of rats after oral administration of cephalosporin derivatives*

The antibiotics have the general structure:

Antibiotic		% of total urinary radioactivity				
R	R'	% of radio- active dose in urine	R- ¹⁴ CO ₂ H	R- ¹⁴ CO-glycine	R- ¹⁴ CO-NH-CH ₂ -CH ₂ -OH	Polar metabolite
	-O-CO-CH ₃	46	—	32	13	5
		50	—	30	17	—
*	-O-CO-CH ₃	50	—	40	36	8
	-O-CO-CH ₃	62	47	3	5	31

* Culp *et al.* (1963).

total urinary radioactivity. Its chemical characteristics were similar to those of the neutral metabolite obtained in the cephalothin and cephaloridine studies, i.e. thienylacetamidoethanol. Although its structure was not characterized, it is undoubtedly phenoxyacetamidoethanol, resulting from the enzymic reduction of the intermediate metabolite, phenoxyacetamidoacetaldehyde.

Another radioactive metabolite representing 14% of the urinary radioactivity or 8% of the administered dose was very polar in nature (R_f 0.05). Although its structure was not established, it is similar to the polar metabolite from the cephalothin and cephaloridine studies. The percentage of this material present as a metabolite in urine is much less than that observed with cephalothin or cephaloridine.

The results of this study indicate that, when cephalosporin derivatives are administered orally, they undergo extensive degradation in the gastrointestinal tract. Some of the resulting degradation products are subsequently adsorbed from the gut. The current knowledge of the nature of the metabolites of orally administered cephalosporin derivatives in urine is summarized in Table 1.

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