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Metabolism of [^{14}C]Cefaclor, a Cephalosporin Antibiotic, in Three Species of Laboratory Animals

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The metabolic fate of the orally effective cephalosporin antibiotic cefaclor (Lilly 99638) has been studied in rats, mice, and dogs. Cefaclor is efficiently absorbed from the gastrointestinal tract as the intact antibiotic. In rats and mice, cefaclor, for the most part, escapes metabolism in the body and is eliminated unchanged as unaltered antibiotic, primarily by renal excretion. In dogs, however, cefaclor is more labile to metabolism and only a portion of the administered antibiotic is eliminated unchanged via the kidney.

Cefaclor (see Fig. 1), 3-chloro-7-D-(2-phenylglycinamido)-3-cephem-4-carboxylic acid (Lilly 99638), is an orally effective, broad-spectrum antibiotic active against gram-positive cocci, including penicillin-resistant strains, as well as against many gram-negative organisms. Cefaclor is a cephalosporin antibiotic structurally related to orally active cephalexin [generic name given to 7-(D-2-amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid] (6). The study described below, which is patterned after earlier studies (1, 7-9), shows that cefaclor is well absorbed after oral administration and that this antibiotic is, like cephalexin, resistant to metabolic degradation or alteration. As in earlier studies on the in vivo fate of cephalosporin antibiotics from these laboratories, radiocarbon labeling was used to facilitate the present investigation.

MATERIALS AND METHODS

[^{14}C]cefaclor. D-[1-^{14}C]phenylglycine (1.59 mmol, 20.86 μCi/mg) was allowed to react with 2.06 mmol of methyl acetoacetate in a solution of 1.62 mmol of NaOH in 15 ml of methanol. After heating the reaction mixture for 1.5 h at reflux temperature, 15 ml of acetonitrile was added and heating was continued for 30 min. Methanol was removed by distillation and was replaced with an appropriate amount of acetonitrile. After cooling to room temperature, the precipitated methyl [1-^{14}C]3-(D-3-β-carboxybenzyl amino)crotonate sodium salt was collected by filtration, washed with acetonitrile, and dried in vacuo. The yield of product was 1.50 mmol.

Methyl chloroformate (1.58 mmol) was allowed to react with 1.50 mmol of methyl [1-^{14}C]3-(D-3-β-carboxybenzylamino)crotonate sodium salt in solution with 25 ml of dry dimethylformamide containing 1 drop of *N,N*-dibenzylamine at −40°C. After stirring the resulting solution for 30 min at this temperature, a solution of 1.40 mmol of *p*-nitrobenzyl-3-chloro-3-cephem-4-carboxylate in 5 ml of dimethylformamide was added rapidly with stirring. The resulting mixture was stirred at −40°C for 2.5 h and then was allowed to stand overnight at room temperature. Upon cooling the mixture to 0°C, water (0.5 ml) and then 0.9 ml of concentrated HCl was added dropwise. Zinc dust (5.46 mmol) was added to the reaction mixture over a period of 30 min, keeping the temperature between 0 and 5°C. After complete addition, the reaction mixture was stirred at 0°C for 2.5 h. Concentrated HCl (0.56 ml) was then added, and stirring was continued for 1 h. The pH of the reaction mixture was then adjusted from 1.6 to 3.5 with triethylamine and filtered. Semicarbazide·hydrochloride (1.44 mmol) was added portionwise to the filtrate over a period of 30 min, maintaining the pH at 3.5 with triethylamine. After complete addition, the pH of reaction solution was adjusted to 6.8 with stirring in an ice bath to facilitate precipitation. The crude product was collected by filtration, washed with dimethylformamide, and then dissolved in a solution containing 9.3 ml of water, 0.12 ml of concentrated HCl, and tetrasodium ethylenediaminetetraacetate dihydrate (0.022 mmol). After adding 0.02 g of decolorizing carbon, the solution was filtered, and the filtrate was adjusted to pH 4.6 with diluted NH_4OH. Acetonitrile (10 ml) was added, and the filtrate was cooled to induce precipitation. The purified product was collected by filtration, washed with 5 ml of water, and dried in vacuo at 40°C for 8 h. The yield of pure [^{14}C]cefaclor was 0.50 mmol (40%), with a specific radioactivity of 7.35 μCi/mg. Paper chromatography, followed by microbiological and radiological assay, showed the product to be pure.

Chromatographic methods. Paper chromatography was carried out on Whatman no. 1 paper and a butan-2-ol-acetic acid-water (3:1:1) solvent system was used for development. Areas of biological activity were located by a bioautographic technique described by Miller (5), using *Sarcina lutea*. The radioactive zones were located by means of a scanner (Vanguard 880 automatic chromatogram scanner).

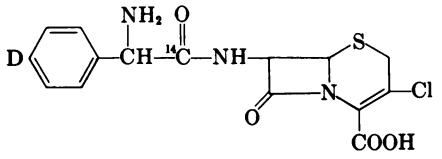

FIG. 1. *Structure of [14C]cefaclor.*

Sample preparation: microbiological assay. Urine and bile samples were acidified with glacial acetic acid immediately upon collection. Samples were diluted as required with pH 4.5 aqueous buffer containing, in millimoles per liter: Na^+, 160; PO_4^{3-}, 5; acetate, 12; Cl^-, 150. Standard solutions of cefaclor containing 0.025 to 1 μg/ml were prepared in the buffer on the day of the animal test.

Whole-blood samples were frozen and thawed to effect hemolysis. One part, by volume, of hemolyzed blood was diluted with 2 volumes of pH 6.0 buffer containing the ionic constituents listed above. Standard solutions of cefaclor were prepared on the day of animal test in a compound diluent composed of 1 volume of whole hemolyzed control blood and 2 volumes of buffer. When further dilutions of blood samples were required, the same compound diluent was used.

Samples of synovial fluid, cerebrospinal fluid, aqueous humor, and serum were diluted 1 part to 3, by volume, with pH 4.5 buffer. Standard solutions of cefaclor were prepared in control samples of these biological fluids diluted appropriately with buffer. All standard solutions were stored at 4°C until assayed.

Microbiological assay method. Antibiotic activity in unknown samples was compared to that in previously prepared standard solutions using a disk-plate agar diffusion test (*S. lutea* X-186: inoculum, 2.5%, by volume, of 53% light transmission stock culture; agar [BBL no. 6] at 20 g/liter, pH 6.6; pour volume, 6 ml/plate; disks, Schleicher & Schuell no. 740E, 6.35 mm). Internal variation in the assay was minimized by measuring 12 zone diameters for each sample and by controlling the significant factors identified by Davis and Stout (2, 3).

Elimination studies: rodents. A solution of [14C]cefaclor (4.25 mg/ml in 0.05 M sodium acetate buffer, pH 4.5) was administered orally (16.9 mg/kg, 46 μmol/kg) to groups of three fasted (24 h), 250-g, male Purdue-Wistar rats and eight male, 30-g, ICR (Cox) mice. Prior to antibiotic administration isotonic saline (20 ml/kg) was administered intraperitoneally to each animal. After antibiotic administration, animals were placed in stainless-steel metabolism cages. Urine samples were collected at 2, 4, 6, 8, and 24 h after administration, and feces samples were collected after 24 h. Immediately after each urine collection, isotonic saline was again administered intraperitoneally to each animal.

The radiocarbon content of each urine sample was determined by liquid scintillation counting. The nature of the radioactive urinary components was determined by paper chromatography. The biologically active metabolite was identified by comparison chromatography using an authentic sample of [14C]cefaclor. Quantitative determination of unaltered [14C]cefaclor in urine samples was accomplished by microbiological assay. Feces samples were dried, ground, and analyzed for radiocarbon content by combustion using a Packard Tri-Carb sample oxidizer.

For biliary elimination studies, three 200-g fasted male rats were anesthetized with ether and a cannula was placed into the common bile duct of each animal. After collection of a 2-h sample of normal bile fluid, [14C]cefaclor (16.9 mg/kg) in buffer solution was administered orally and bile fluid was collected for 24 h. Radiocarbon content was determined by liquid scintillation counting of a sample of the bile fluid. Unaltered antibiotic was again identified by comparison chromatography using an authentic sample of [14C]cefaclor. The levels of unaltered antibiotic present in the bile were quantitated by the microbiological assay method described above.

To determine whole body radiocarbon residues, each animal was sacrificed 24 h after administration of [14C]cefaclor, and the carcasses were dissolved in a solution of 56 g of KOH in 300 ml of ethanol (2 h). The resulting solution was diluted to 500 ml with ethanol. Three 1-ml samples of each solution were removed and dissolved in 10 ml of liquid scintillation counting medium (Isolab Inc.) for radiocarbon assay.

Rodent blood and tissue level studies. A solution of [14C]cefaclor (46 μmol/kg) in 0.05 M sodium acetate buffer (pH 4.5) was administered orally to a group of three 250-g fasted Purdue-Wistar rats. At given time intervals blood samples were removed from the tail vein. The samples, averaging 200 mg in weight, were processed for liquid scintillation counting using the wet-tissue digestion method of Mahin and Lofberg (4).

[14C]cefaclor, 46 μmol/kg, in sodium acetate buffer solution was administered by the oral route to 24 fasted male ICR (Cox) mice. At eight time intervals, three mice were sacrificed by decapitation, and total available blood was collected. Blood from each animal was sampled and processed for liquid scintillation counting by the wet-tissue digestion method.

For tissue level determinations, [14C]cefaclor (46 μmol/kg) in sodium acetate buffer solution (pH 4.5) was administered to 12 fasted male Purdue-Wistar rats (250 g) and to 12 fasted ICR (Cox) mice (30 g). One, four, six, and twenty-four hours after administration, a group of three animals was sacrificed by decapitation. The appropriate tissues and organs were removed and wet weights were recorded. Duplicate weighed samples of each tissue and organ were removed and prepared for liquid scintillation counting using the wet-tissue digestion method.

Metabolism studies in dogs. A solution of [14C]cefaclor (10.6 mg/ml) in pH 4.2 isotonic saline was administered orally (46 μmol/kg) to one fasted female mongrel dog, 15 kg, fitted with a urinary catheter. At specific times after administration, blood samples were removed from the front leg vein and urine samples were collected. Two portions of each blood and urine sample were taken and processed for liquid scintillation counting by the wet-tissue digestion method. Blood samples were then

centrifuged at 1,000 × *g* for 10 min in heparinized tubes to separate the plasma and cellular fractions. Two portions (200 mg) of each plasma sample were processed for liquid scintillation counting in the same manner. The nature of the radioactivity in all plasma and urine samples was determined by comparison paper chromatographic analysis using an authentic sample of [^{14}C]cefaclor. The quantitative determination of unaltered [^{14}C]cefaclor concentrations in plasma and urine was accomplished using the microbiological method described above.

In another study, one female mongrel dog, 14.0 kg, was anesthetized with Seconal and the bile duct was cannulated. A solution of [^{14}C]cefaclor (10.6 mg/ml) in pH 4.2 isotonic saline was administered intravenously via the femoral vein. At specified time intervals, urine, bile fluid, and blood samples were taken, with the latter being obtained from the femoral artery. Radioactivity in the blood, plasma, urine, and bile samples was determined by liquid scintillation counting. The nature of the radioactivity was determined by comparison paper chromatography, using an authentic sample of [^{14}C]cefaclor.

For tissue level studies a solution of [^{14}C]cefaclor (10.6 mg/ml) in pH 4.2 isotonic saline was administered by the oral route (46 μmol/kg) to one fasted female mongrel dog (10 kg), fitted with a urinary catheter. At a given time (90 min) after administration, the dog was anesthetized with Seconal and a sample of cerebral spinal fluid was drawn. The dog was sacrificed with a lethal dose of Seconal, and appropriate tissues and fluids were removed. A weighed sample each tissue and fluid was prepared for liquid scintillation counting using the wet-tissue digestion method. Samples of all fluids were quantitatively analyzed for unaltered [^{14}C]cefaclor using the microbiological assay described above.

RESULTS

Elimination studies: rats. The recoveries of total radioactivity and of unaltered antibiotic in the urine of rats after oral administration of a single dose of [^{14}C]cefaclor are shown in Fig. 2. Forty-four percent of the administered radiocarbon appeared in the urine after 2 h, 69% after 6 h, and 80.8% after 24 h. The recovery of unaltered [^{14}C]cefaclor, determined by microbiological assay, was 38.6% after 2 h, 53.5% after 6 h, and 54.5% after 24 h. The feces collected during this 24-h period were found to contain no detectable quantity of radioactivity. Residual radiocarbon in the rats was found by whole body assay to account for 19.2% of the administered radioactive dose. Cannulation of the common duct of rats immediately prior to oral administration of a single dose of [^{14}C]cefaclor showed that 32.8% of the administered radioactive dose was eliminated via biliary excretion (Fig. 2). The recovery of microbiologically active material in bile fluid was, however, quite low, 6.8%. This microbiological activity was found by radio- and bio-autography to be equally divided between unchanged [^{14}C]cefaclor and a more polar unknown metabolite. No detectable quantity of this biologically active metabolite of [^{14}C]cefaclor could be found in the urine of normal animals. Isolation studies indicated this metabolite to be relatively unstable when compared to the parent antibiotic.

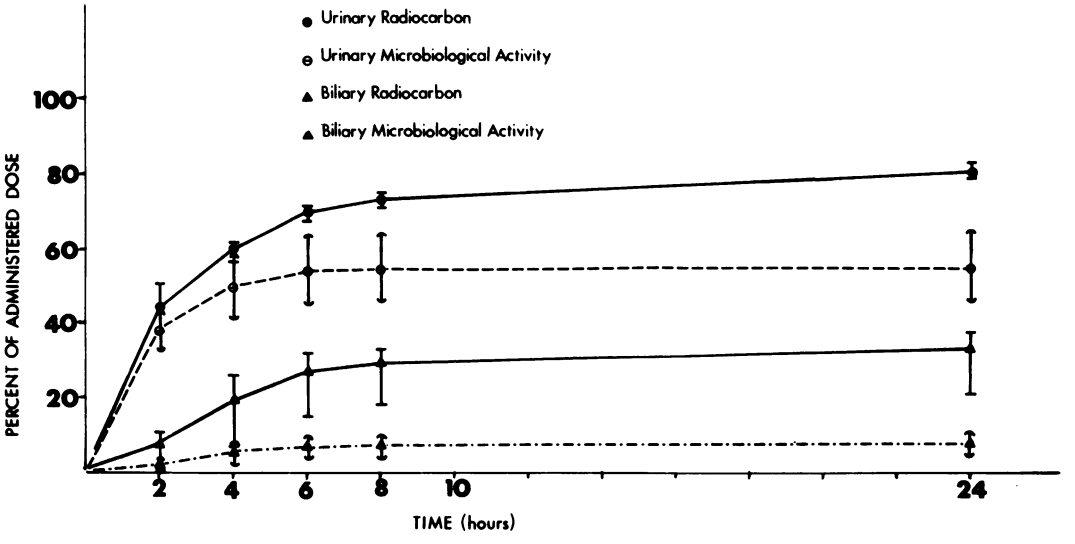

FIG. 2. *Renal and biliary elimination of radiocarbon and microbiological activity in rats (3) after oral administration of 46 μmol of [^{14}C]cefaclor per kg.*

Elimination studies: mice. The oral administration of [14C]cefaclor to a group of ICR (Cox) mice resulted in elimination of a large percentage of administered radiocarbon via the kidney. Twenty-one percent of the administered radiocarbon appeared in the urine in 2 h, 49% in 4 h, 58% in 6 h, and 72% in 24 h. Microbiological assay of these urine samples showed the initial 2-h sample to contain essentially unchanged antibiotic. The recovery of unaltered [14C]cefaclor was 19% of the administered dose in 2 h, 37% in 4 h, 41% in 6 h, and 43% in 24 h. These results, like those with rats, show that initially unaltered antibiotic accounted for a major portion of eliminated radiocarbon. Later samples, however, were found to contain, for the most part, biologically inactive metabolic products. Unlike results found in rat studies, mouse fecal material was found to contain 15% of administered radiocarbon, thus implying biliary elimination and the lack of enterohepatic recirculation in mice. Residual radiocarbon in the mice 24 h after administration was found to represent 13% of the radioactive dose.

Elimination studies: dogs. Figure 3 shows the recoveries of total urinary radioactivity and of unaltered antibiotic in the urine of a dog after oral and intravenous administration of a single dose of [14C]cefaclor. After oral administration, 30% of the radioactive dose was recovered in the urine after 2 h, 57% after 6 h, and 65% after 24 h. The recovery of unaltered [14C]cefaclor after oral administration was considerably below these figures, with 15% being recovered unchanged in 2 h, 21% in 6 h, and 21.5% in 24 h. Six hours after intravenous administration, the recovery of total radiocarbon in the urine was 79%, whereas that in the bile fluid was 3.5%. Microbiological assays showed the urine to contain 47% unchanged antibiotic and the bile to contain 0.4%. It is apparent from these results that cefaclor, after oral administration, was considerably more labile to metabolic degradation in dogs than in the other species studied. The site of this degradation may well be the gastrointestinal tract as opposed to the liver.

Blood level studies. The radiocarbon blood level curve obtained after oral administration of 16.9 mg/kg (46 μmol/kg) of [14C]cefaclor to rats is shown in Fig. 4. A maximum level equivalent to 4.5 μg/ml was obtained 1 h after administration. The initial declining phase of the blood level curve indicated the apparent half-life of [14C]cefaclor in rats to be 2.5 h. These results can be seen to compare quite favorably to similar data obtained after oral administration of 46 μmol of [14C]cephalexin per kg to rats. A peak concentration of 3.8-μg equivalents was obtained in 30 min, and the blood level curve decayed with an apparent half-life of 1.5 h.

The radiocarbon blood level curve obtained after oral administration of 46 μmol of [14C]cefaclor per kg to mice showed that a peak level of 9.4-μg equivalents of [14C]cefaclor per ml was obtained at the first sample time, 15 min after administration. The blood level curve

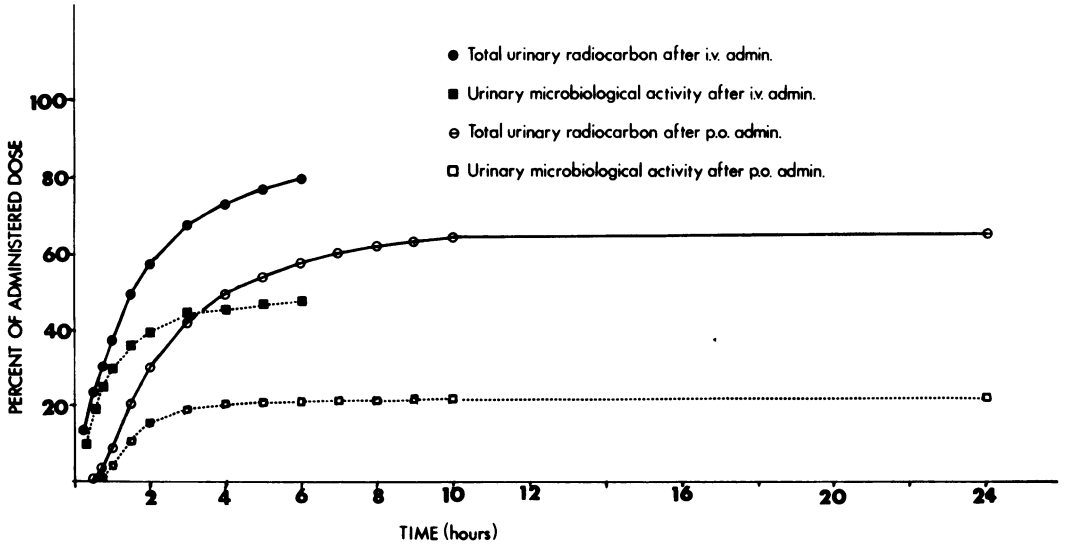

FIG. 3. *Renal elimination of radiocarbon and microbiological activity in a dog after intravenous and oral administration of 46 μmol of [14C]cefaclor per kg.*

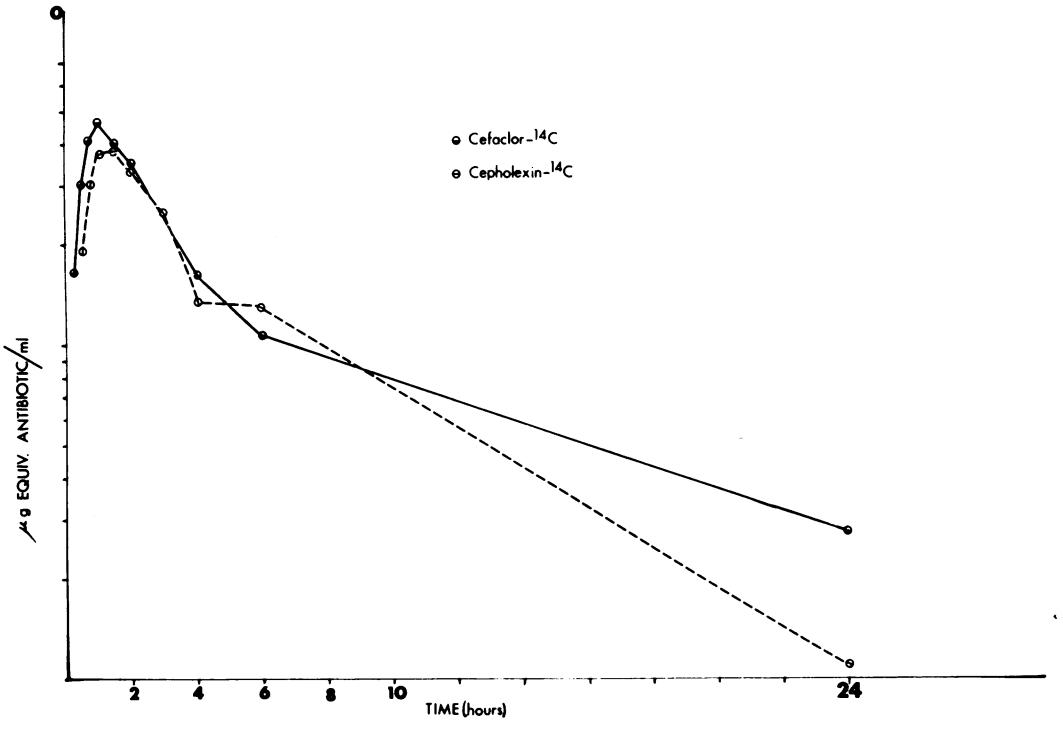

FIG. 4. *Average total radiocarbon blood levels in three rats after oral administration of 46 μmol of [14C]cefaclor and [14C]cephalexin per kg.*

itself was bimodal in character, with the apparent half-life of the initial phase being of the order of 1.5 h.

Blood samples were taken from dogs after oral and intravenous administration of 46 μmol of [14C]cefaclor per kg. Radiocarbon and unaltered [14C]cefaclor concentrations in each sample were determined. Results obtained from analysis of samples taken after oral administration are shown in Fig. 5. A maximum radiocarbon blood level equivalent to 17 μg of [14C]cefaclor per ml was obtained 2 h after administration. The maximum blood level of unaltered antibiotic, determined by microbiological analysis, was 8 μg/ml at the same time. The initial decay phase of the radiocarbon blood level curve declined with an apparent half-life of 2.4 h, whereas the apparent half-life of [14C]cefaclor was 1.9 h. Separation of blood samples into their cellular and plasma fraction, with subsequent radiocarbon and microbiological analysis, revealed that [14C]cefaclor and its metabolites were preferentially concentrated in the plasma fraction by a factor of 1.5 to 1.6 over the cellular fraction.

The total radiocarbon and unaltered antibiotic blood level curves obtained after intravenous administration of 46 μmol of [14C]cefaclor to a dog are shown in Fig. 6. Utilizing that portion of the curves between 30 and 360 min for computerized linear regression analysis, the half-life of total radiocarbon was 106 min, whereas that of unaltered antibiotic was 76 min. By mathematical comparison of the areas under the total radiocarbon blood level curves after oral and intravenous administration, a figure representing the degree of absorption of [14C]cefaclor in dogs after oral administration was calculated to be 75%. By similar treatment of the areas under the unaltered antibiotic curves, the degree of bioavailability of [14C]cefaclor in dogs after oral administration was calculated to be 60%.

Tissue level studies. [14C]cefaclor (46 μmol/kg) was administered orally to rats and mice. The radiocarbon levels in tissue and organs were determined 1, 4, 6, and 24 h after administration. The levels of radiocarbon in these tissues are expressed as microgram equivalents of [14C]cefaclor per gram of body tissue. The levels of radiocarbon found in various tissues of the rat are shown in Fig. 7. These results show that although all tissues examined contained concentrations of antibiotic, the only tissues possessing significantly higher levels than that in the blood were the kidney and the liver, organs

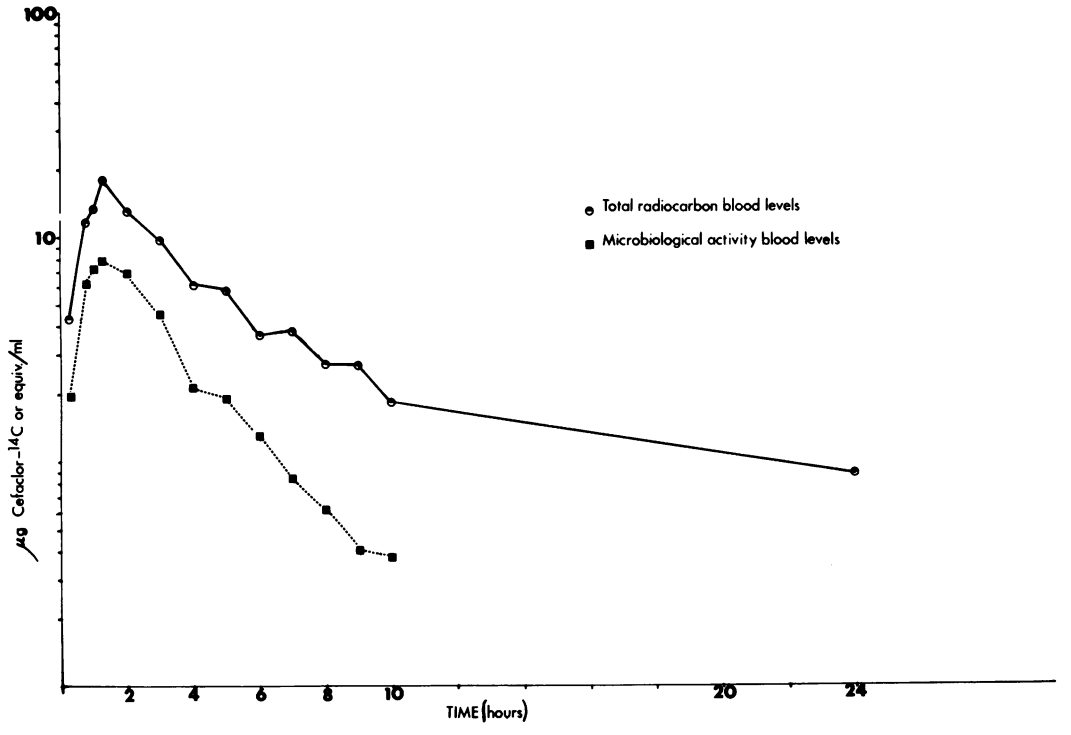

FIG. 5. *Total radiocarbon and microbiological activity blood levels in a dog after oral administration of 46 μmol of [14C]cefaclor per kg.*

responsible for metabolism and elimination. Levels of radiocarbon in the brain were considerably lower than those found in other tissues. In general, the apparent half-life of the disappearance of radiocarbon from all tissues was of the same order and approximated that of the blood.

Radiocarbon tissue levels found in mice 1, 4, 6, and 24 h after oral administration of 46 μmol of [14C]cefaclor per kg are shown in Fig. 8. Curves constructed from these data appeared to be bimodal in character. Tissues possessing levels significantly higher than those found in blood were, again, the kidney and liver. There appeared to be no abnormal deposition of radiocarbon in any one particular tissue. The half-life of radiocarbon in all tissues was quite similar to that determined for the blood level.

Radiocarbon levels and unaltered antibiotic levels were determined in various tissues and fluids of dogs 90 min after a single oral administration of 46 μmol of [14C]cefaclor per kg. Table 1 shows the levels of total radiocarbon and unchanged [14C]cefaclor in the various tissues and fluids. The only tissues having significantly higher radiocarbon levels than those found in blood were the liver and kidney. Hard bone was found, however, to possess radiocarbon levels comparable to the blood concentration. Appreciable levels were also found in bone marrow. High levels of radiocarbon and of unchanged antibiotic were also found in urine. Biliary elimination in dogs, like that in rats, was not an important route of elimination of radiocarbon or of unchanged antibiotic. Of the fluids examined other than the urine and bile, only the synovial fluid possessed levels of radiocarbon and of unchanged antibiotic comparable to blood levels.

DISCUSSION

Rats. Results, obtained from radiocarbon elimination studies in rats, showed cefaclor to be completely absorbed from the gastrointestinal tract after oral administration. The major, if not only, route of elimination of parent antibiotic and metabolites is via renal excretion. The finding that the major portion, 67%, of the urinary radiocarbon represented unchanged [14C]cefaclor implied that the antibiotic is, for the most part, absorbed intact and argues for its in vivo stability to metabolism in this species. These results compare very favora-

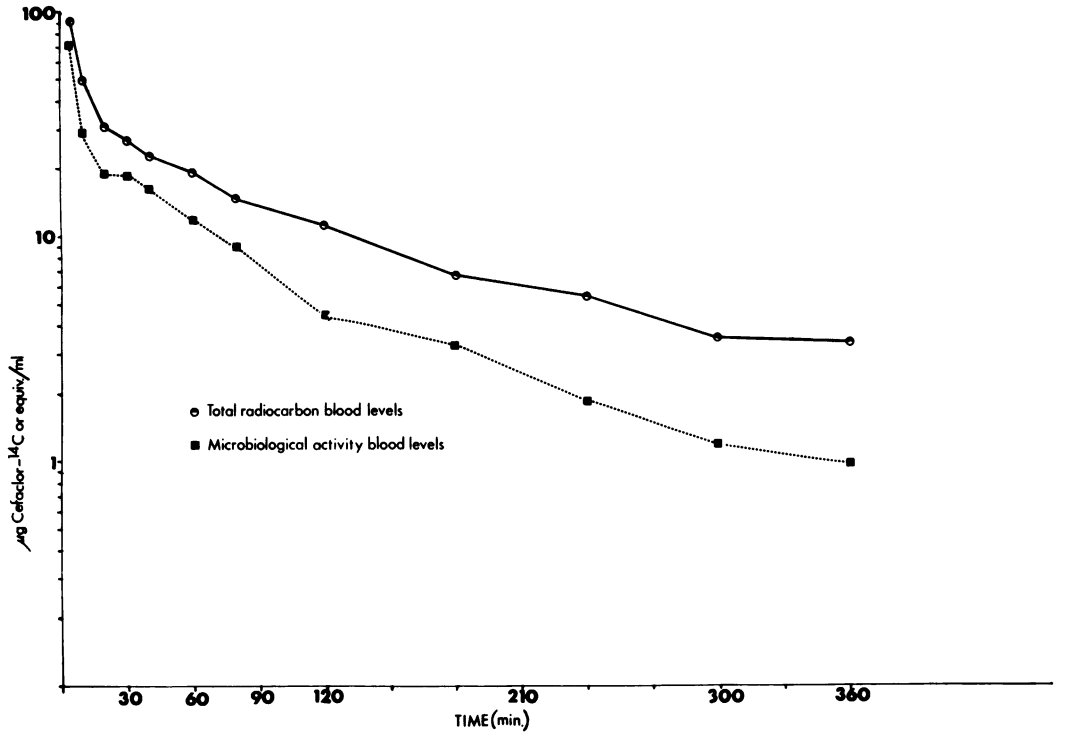

FIG. 6. *Total radiocarbon and microbiological activity blood levels in a dog after intravenous administration of 46 μmol of [14C]cefaclor per kg.*

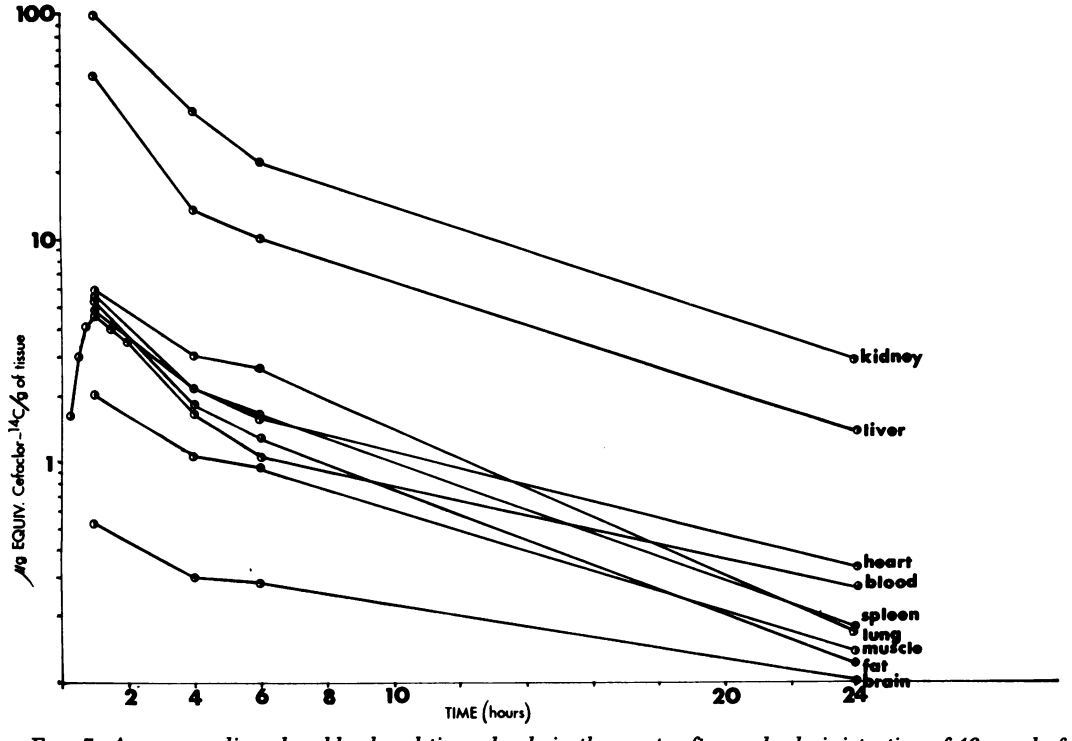

FIG. 7. *Average radiocarbon blood and tissue levels in three rats after oral administration of 46 μmol of [14C]cefaclor per kg.*

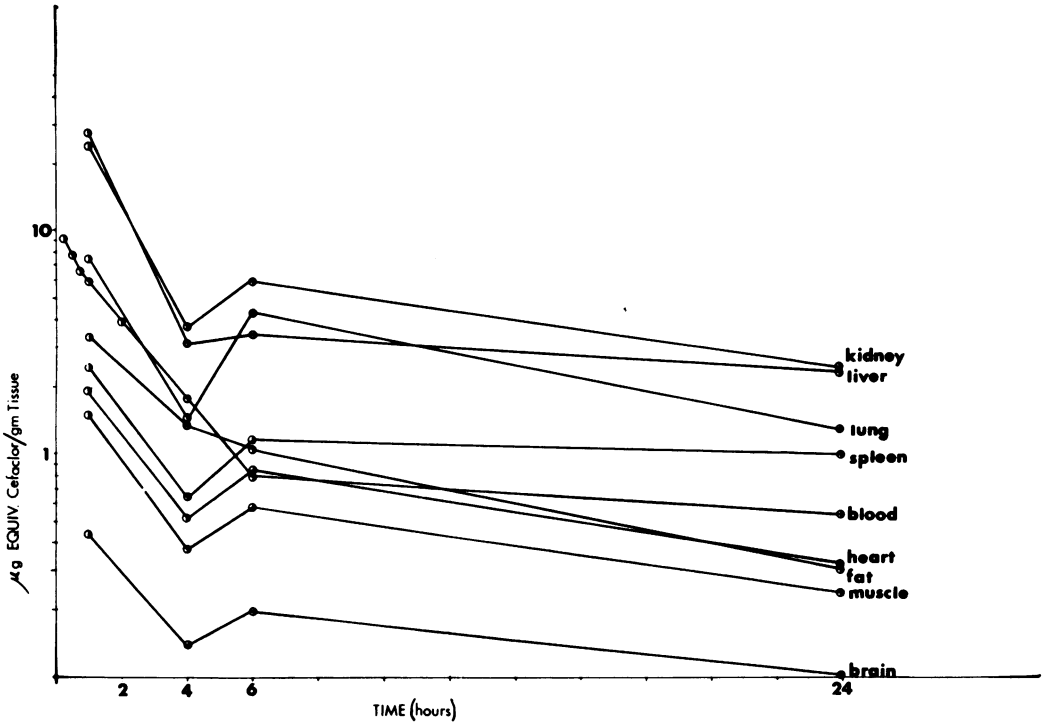

FIG. 8. *Average radiocarbon blood and tissue levels in three mice after oral administration of 46 μmol of [14C]cefaclor per kg.*

TABLE 1. *Total radiocarbon and unaltered [14C]-cefaclor tissue and fluid levels in dogs after oral administration of a single dose of [14C]-cefaclor (46 μmol/kg)*

Tissue or fluid	Radiocarbon levels (μg equivalents of cefaclor/g)	Unaltered antibiotic (μg/ml)
Blood	9.1	10.0
Liver	31.9	ND
Kidney	138.7	ND
Spleen	4.4	ND
Adrenal	5.3	ND
Bone marrow	4.1	ND
Hard bone	9.3	ND
Urine	2,082.0	1,275.0
Bile	127.3	25.0
Synovial	14.9	8.9
Cerebrospinal	0.2	0.3
Aqueous humor	0.7	0.8

[a] ND, Not determined.

bly with those obtained in a similar study after oral administration of [14C]cephalexin to rats (8). Similar studies in bile-cannulated animals show that biliary excretion was indeed involved in the metabolism of cefaclor. Therefore, in normal rats, enterohepatic recirculation is involved in the metabolism and ultimate renal elimination of cefaclor and its metabolites. The isolation of a microbiologically active metabolite from the bile and failure to confirm its presence in urine supports this supposition. The residual radiocarbon in rats 24 h after administration undoubtedly represents phenylglycine, produced by the enzymatic or hydrolytic cleavage of the amide bond of the parent antibiotic and its incorporation into the normal amino acid pools in this species.

Blood and tissue level studies only serve to confirm data obtained in the above studies. Peak radiocarbon levels were obtained in 1 h, indicating rapid absorption in rats. The total radiocarbon blood level curve obtained for [14C]cefaclor can be seen (Fig. 4) to be very similar to that obtained from an identical dose of [14C]cephalexin (9). Results of radiocarbon tissue level studies also show that the absorption of [14C]cefaclor after oral administration is quite rapid. No abnormal deposition of radiocarbon in any tissue was observed.

Mice. Metabolism studies in mice show [14C]cefaclor to be rapidly and completely absorbed from the gastrointestinal tract after oral administration. The major route of elimination of antibiotic and metabolite is by renal excretion. Unlike results observed in rats, however, biliary excretion does play a minor role in the

elimination scheme, as implied by the recovery of 15% of administered radiocarbon in the feces. The recovery again of relatively large portions of administered antibiotic unchanged in the urine argues for its in vivo stability to metabolism. These results again closely parallel results obtained in a similar study of the metabolic fate of the structurally related antibiotic, cephalexin (8). In addition, the unknown microbiologically active metabolite, found only in the bile of cannulated rats, was found in the urine of mice. Its presence, although representing less than 2% of the dose, suggests that enterohepatic recirculation of the antibiotic and its metabolites does not occur in mice. Blood and tissue total radiocarbon level studies serve to confirm the rapid absorption of [^{14}C]cefaclor in mice after oral administration. No abnormal deposition of radioactivity in any tissue or organ was observed.

Dogs. Elimination and blood level studies in dogs after oral administration of [^{14}C]cefaclor indicate that the antibiotic was absorbed from the gastrointestinal tract but that it was considerably more labile to metabolism in dogs than in the other species investigated. Radiocarbon levels both in blood and urine were considerably higher than levels of unaltered antibiotic. Comparison of blood level data obtained after oral administration to that obtained after intravenous administration permitted the calculation of the degree of absorption and the bioavailability of [^{14}C]cefaclor after oral administration. These calculations show that approximately 75% of the oral dosage of [^{14}C]cefaclor was absorbed from the gastrointestinal tract and that the bioavailability of intact antibiotic was approximately 60%. In comparison with other species studied, a relatively small percentage of eliminated radiocarbon was associated with unchanged [^{14}C]cefaclor. As in other species investigated, biliary excretion plays a very minor to trace role in the elimination of parent antibiotic and metabolites. Tissue level studies in dogs 90 min after oral administration of [^{14}C]cefaclor show only the liver and kidney, tissues directly involved with metabolism and elimination, to possess radiocarbon levels greater than that found in the blood. Of the fluids examined, only synovial fluid was found to contain significant levels, comparable to those found in blood, of total radiocarbon and of unaltered antibiotic.

In summary, these metabolism studies show [^{14}C]cefaclor to be rapidly absorbed as the intact antibiotic from the gastrointestinal tract of dogs, rats, and mice after oral administration. In the latter two species the antibiotic, for the most part, is resistant to metabolism and is eliminated unchanged. In dogs, however, [^{14}C]cefaclor is labile to metabolism and only a minor fraction of the administered dose is eliminated unchanged. These results are of particular interest when compared with those obtained from studies of the metabolic fates of cephaloglycin [generic name given to 7-(D-2-amino-2-phenylacetamido)-3-acetoxymethyl-3-cephem-4-carboxylic acid] (7) and cephalexin (8), two closely related cephalosporin antibiotics, in rats and mice. Cephalexin, like cefaclor, is quantitatively absorbed in the gastrointestinal tract as the intact antibiotic. It is not metabolized and is eliminated unchanged via the kidney. Cephaloglycin, on the other hand, is not as efficiently absorbed and is rapidly metabolized in these species via two pathways: hydrolysis of the amide linkage to form D-2-phenylglycine and by deacetylation to form desacetylcephaloglycin. Thus, the substitution of a chlorine or methyl group for the acetoxymethyl moiety of cephaloglycin produces cephalosporin antibiotics, cefaclor and cephalexin, which are more readily absorbed from the gastrointestinal tract of rats and mice and are remarkably more resistant to metabolism.

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