METABOLISM OF CEPHALEXIN-^{14}C* IN MICE AND IN RATS

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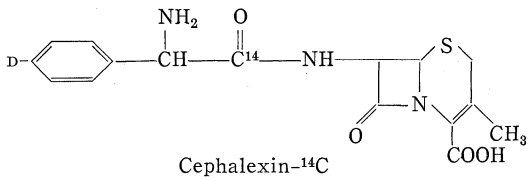
The metabolic fate of the orally effective antibiotic, cephalexin, has been studied in the rat and the mouse. Cephalexin is efficiently absorbed from the gastrointestinal tract as the intact antibiotic. Cephalexin is not metabolized in the body and is eliminated as unaltered antibiotic primarily via the urine. Cephalexin is more rapidly absorbed and eliminated by the mouse than by the rat.

Cephalexin is a broad spectrum antibiotic, active against gram positive cocci, including penicillin-resistant strains, as well as against many gram-negative organisms[1]. Cephalexin, a cephalosporin antibiotic, structurally related to D-cephaloglycin[2]*, appears to be efficiently absorbed from the gastrointestinal tract in both laboratory animals[1,3] and in humans[4]. This antibiotic also appears to be unusually resistant to metabolic alteration[3,4]. As in earlier studies on the fate of cephalosporin antibiotics *in vivo* from these laboratories[5,6,7], radiocarbon labeling was used to facilitate the investigation.



Cephalexin-^{14}C

Materials and Methods

D-(−)-2-Phenylglycine-^{14}C. The procedure used for the preparation of racemic 2-phenylglycine-^{14}C and the enzymatic resolution into the enantiomorphic forms has been described by BILLINGS and SULLIVAN[8].

Cephalexin-^{14}C. D-Phenylglycine-^{14}C (3.05 m-moles, 11.56 μc/mg) was allowed to react with 3.1 m-moles of methyl acetoacetate in a solution consisting of 3.1 m-moles of sodium hydroxide, 0.15 ml water and 1.2 ml of methanol. After heating for 30 minutes at 60°C, the clear reaction solution was evaporated to dryness *in vacuo*. The residual oil was dried for 24 hours *in vacuo* over concentrated sulfuric acid to yield semi-solid methyl 3-(D-β-carboxybenzylamino)crotonate-^{14}C sodium salt.

Ethyl chloroformate (3.7 m-moles) was allowed to react with a solution consisting of 3.1 m-moles of methyl 3-(D-α-carboxybenzylamino)crotonate-^{14}C sodium salt in 9.2 ml of

* Cephalexin is the generic name given to 7-(D-2-amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid. D-Cephaloglycin is the generic name given to 7-(D-2-amino-2-phenylacetamido)-cephalosporanic acid. D-α-Aminophenyl[1-^{14}C]acetic acid will be designated as D-2-phenylglycine-^{14}C.

acetonitrile containing one drop of N, N-dimethylbenzylamine at −10°C. After strring the solution for 15 minutes at this temperature, a solution of 670 mg (3.0 m-moles) of 7-amino-desacetoxycephalosporanic acid (7-ADCA)[9] in 8 ml of acetonitrile-water (1 : 1 v/v) containing 3.0 m-moles of triethylamine was added rapidly with stirring. After 30 minutes 1.0 g of sodium chloride was added to the reaction mixture and stirring was continued for 15 minutes at 0°C. The organic phase was separated and to it was added a solution of 0.35 ml of 100 % formic acid and 0.11 ml of water. The resulting solution was stirred at 0°C for 1 hour to facilitate crystallization of cephalexin-^{14}C. The product was collected by filtration, washed with 5.0 ml of acetonitrile and dried *in vacuo*. The yield was 165 mg (16 %) of pure cephalexin-^{14}C with a specific radioactivity of 4.90 μc/mg. Paper chromatography followed by biological and radiological assay showed the product to be pure.

Chromatographic Methods. A combination of paper and thin-layer chromatography was employed for the detection of radioactive metabolites. Paper chromatography was carried out on Whatman # 1 paper and thin-layer chromatography (TLC) was carried out on Eastman Cellulose Chromagram sheets. Areas of biological activity were located by the bioautographic method described by MILLER[10] using *Sarcina lutea*. The radioactive zones were located by means of a scanner (Vanguard 880 Automatic Chromatogram Scanner) and by radioautography using Kodak Medical X-Ray film.

Paper chromatograms, developed with butan-1-ol - acetic acid - water (3 : 1 : 1 by volume) were used for the identification and quantitation of radioactive metabolites. Radiocarbon quantitation was accomplished by the uniform sectioning of the paper chromatogram and subsequent liquid scintillation counting. Results obtained with the above system were confirmed by the use of one or more of the following systems:

1. TLC employing a butan-1-ol - acetic acid - water (3 : 1 : 1) solvent system.
2. Paper chromatography employing an ethyl acetate - acetic acid - water (3 : 1 : 1) solvent system.
3. TLC employing an ethyl acetate - acetic acid - water (3 : 1 : 1) solvent system.
4. TLC employing an acetonitrile - ethyl acetate - water (3 : 1 : 1) solvent system.

Urinary, Fecal and Biliary Excretion Studies. An aqueous solution of cephalexin-^{14}C, 1.6 mg/ml, was administered by the oral and intraperitoneal routes (46 μmoles/kg) to groups of 200 g male Purdue-Wistar rats and 30 g I. C. R. (Cox) mice. The test animals were kept in stainless steel metabolism cages. Urine samples were collected at regular intervals (2 hours) and feces samples were collected after 24 hours.

The radioactivity contained in each urine sample was determined by liquid scintillation counting. The nature and quantitation of the radioactive metabolites in urine was determined by paper and thin-layer chromatographic analysis as described above. The biologically active metabolite was identified by comparison chromatography using an authentic sample of cephalexin-^{14}C.

Feces samples were dried, ground and analyzed for radiocarbon content by the wet tissue digestion method described by MAHIN and LOFBERG[11].

For the bile study, 200 g male rats were anesthetized with ether and a cannula placed into the common bile duct. The cephalexin-^{14}C was administered orally and the bile fluid collected for 24 hours. Radiocarbon content was determined by liquid scintillation counting of an aliquot of the bile fluid.

Blood and Tissue Level Studies. An aqueous solution of cephalexin-^{14}C (46 μmoles/kg) was administered orally and intraperitoneally to separate groups of three 200 g 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[11].

Eighteen, 30 g male I. C. R. (Cox) mice were administered orally an aqueous solution of cephalexin-^{14}C (46 μmoles/kg). At six time intervals, 3 mice were sacrificed by decapitation and the blood was collected. The blood of each mouse was sampled and was

processed for liquid scintillation counting by the wet tissue digestion method.

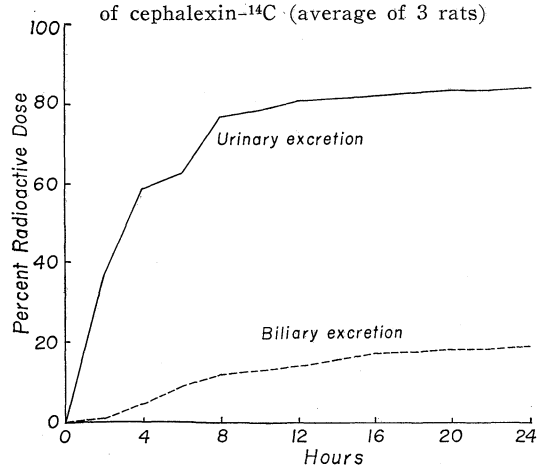
For the tissue level determinations, cephalexin-^{14}C (46 μmoles/kg) was administered orally to 6 male Harlan rats and 6 I. C. R. (Cox) mice. One and four hours after administration, a group of three animals was sacificed by decapitation. The appropriate tissues and organs were removed and their wet weights recorded. A weighed sample of each tissue and organ was removed and was prepared for liquid scintillation counting employing the wet tissue digestion method.

Results and Discussion

Excretion Studies. Rat

Fig. 1 shows the recovery of total urinary radioactivity as well as the distribution of radioactive metabolites in the urine of rats following the oral administration of a single dose of cephalexin-^{14}C. Sixty percent of the administered radiocarbon was recovered in the urine after 4 hours, 77 % after 8 hours and 84 % after 24 hours. The feces, collected over this period of time, were found to contain 15 % of the administered radiocarbon. Cannulation of the common bile duct of a rat immediately prior to the oral administration of a single dose of cephalexin-^{14}C and subsequent collection of a 24-hour sample of the bile fluid, revealed that 18.2 % of the administered radiocarbon was eliminated via biliary excretion (Fig. 1). This result suggests that the greater part of the radioactivity found in the feces of the normal rat was due to biliary excretion and not to the incomplete adsorption of the cephalexin.

Fig. 1. Total urinary and biliary radioactivity recoveries following oral administration of cephalexin-^{14}C (average of 3 rats)



The individual urine and bile samples were examined for the presence of biologically active and radioactive metabolites using paper and thin-layer chromatographic techniques. The results of these examinations revealed the presence of only one metabolite. It was both biologically active and radioactive. This biologically active ^{14}C-labeled metabolite was identified as unchanged cephalexin-^{14}C by chromatographic comparison with authentic cephalexin-^{14}C. The presence of any possible metabolite arising from the hydrolytic cleavage of the side chain amide bond[7], *i.e.* 2-phenylglycine-^{14}C, benzoylformic acid-^{14}C, could not be demonstrated.

Execretion Studies. Mice

The oral administration of cephalexin-^{14}C to a group of I. C. R. (Cox) mice resulted in the rapid elimination of a large percentage of the administered radiocarbon via the kidney. Sixty-two percent of the administered radioactivity appeared in the urine in 3 hours, 80 % in 7 hours and 89 % in 24 hours. Chromatographic examination of

these urine fractions again showed that the only radioactive entity present was unchanged cephalexin.

These results, obtained from excretion studies in rats and mice, show cephalexin to be completely absorbed from the gastrointestinal tract following oral administration. The recovery of unaltered ^{14}C-labeled antibiotic as the single radioactive component present in the urine and the bile fluid after oral administration demonstrates the *in vivo* stability of cephalexin. Although cephalexin is structurally related to cephaloglycin, it is more efficiently absorbed in the gastrointestinal tract than cephaloglycin. Cephalexin is resistant to enzymatic hydrolysis of the side-chain amide moiety, an important metabolic pathway in the biodegradation of *d*-cephaloglycin.

Whole Blood Radiocarbon Levels

The radiocarbon blood level curves obtained after intraperitoneal and oral administration of 46 μmoles/kg of cephalexin-^{14}C to rats are shown in Fig. 2. A maximum radiocarbon level equivalent to 9.0 μg of cephalexin-^{14}C per ml of blood was obtained 30 minutes after intraperitoneal administration. Radiocarbon levels declined with the half-life of about 1.5 hours. Because of *in vivo* stability of cephalexin it is reasonable to assume that this figure represents the half-life of cephalexin in rats. The peak blood level of cephalexin-^{14}C following oral administration was 3.8 μg/ml of blood. This was attained 1 hour after administration. The half-life of cephalexin after oral administration calculated from these results was also near 1.5 hours. The long term blood level (24 hours) curves in each instance did not appear to be bimodal in character.

The radiocarbon blood level curve obtained after oral administration of 46 μmoles/kg of cephalexin-^{14}C in mice is shown in Fig. 3. A peak level of 6 μg/ml of blood

Fig. 2. Whole blood radiocarbon levels in rats after oral and intraperitoneal administration of cephalexin-^{14}C (average of 3 rats)

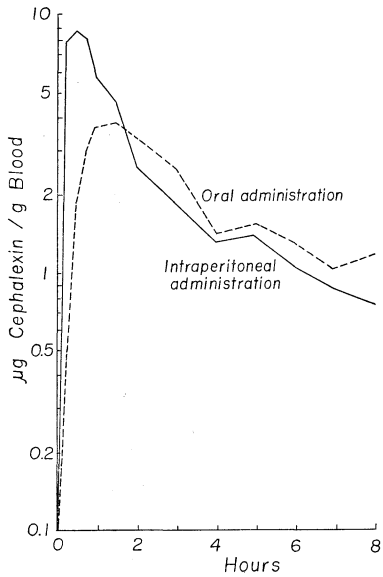
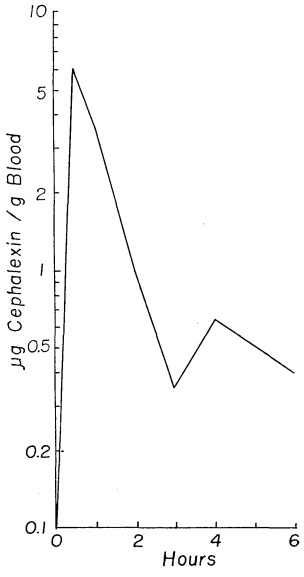


Fig. 3. Whole blood radiocarbon levels in mice following intraperitoneal adminirtration of cephalexin-^{14}C (average of 3 mice)



was attained 30 minutes after administration. The half-life of cephalexin-^{14}C in mice estimated from this data is 45 minutes.

Tissue Level Studies

Cephalexin-^{14}C was administered orally to rats and mice. The radiocarbon levels in tissue and organs were determined 1 and 4 hours after administration. Since the urinary and biliary excretion studies showed that all of the administered cephalexin-^{14}C was eliminated unchanged via the bile duct and the kidney, the level of radiocarbon in the tissues can be expressed as μg of cephalexin per g of body tissue. Table 1 shows the various tissue levels of cephalexin-^{14}C at 1 and 4 hours after oral administration of 46 μmoles/kg of the labeled antibiotic.

Table 1. Cephalexin-^{14}C tissue levels in rats and in mice after a single oral dose of cephalexin-^{14}C (46 μmoles/kg)

Tissue	μg Cephalexin/g tissue			
	Rat		Mouse	
	1 hr.	4 hrs.	1 hr.	4 hrs.
Blood	3.71	2.09	3.59	0.53
Liver	17.11	7.25	12.96	1.93
Spleen	2.21	1.45	1.45	0.40
Kidney	39.93	23.69	27.23	3.53
Lung	3.38	2.58	1.63	0.30
Heart	1.52	1.09	3.31	1.07
Fat	1.54	0.80	1.41	0.34
Muscle	1.16	0.76	1.11	0.32
Brain	0.53	0.24	0.30	0.11

The results of this study show that while all tissues examined contained some cephalexin-^{14}C, the only tissues possessing higher levels than that of the blood were the kidney and the liver, organs responsible for its elimination. It is of interest to note that the mouse eliminates the antibiotic at a rate faster than that of the rat.

Summary

The study of the metabolic fate of cephalexin-^{14}C in the rat and the mouse has shown this antibiotic to be quantitatively absorbed in the gastrointestinal tract as the intact antibiotic. Cephalexin is not metabolized in the body and is eliminated as the unchanged antibiotic via the kidney. Cephalexin is more rapidly absorbed and eliminated by the mouse than by the rat.

These results are of particular interest when compared to those obtained from the study of the metabolism of *d*-cephaloglycin[7]. Cephaloglycin is metabolized in the rat by two pathways: hydrolysis of the amide linkage to form D-2-phenylglycine and by deacetylation to form desacetylcephaloglycin. Thus the replacement of the acetoxymethyl moiety of cephaloglycin by a methyl group produces an antibiotic, cephalexin, which is remarkably more resistant to metabolism and which is more readily absorbed from the gut of the rat and the mouse.

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