[Chem. Pharm. Bull. 20(6)1105—1113(1972)]

UDC 547.796.1'73'869.09 : 615.31.03.076.9

Cefazolin, a New Semisynthetic Cephalosporin Antibiotic. V.[1] Distribution of Cefazolin-^{14}C in Mice and Rats after Parenteral Administration[2]

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(Received September 16, 1971)

To investigate the metabolic fate of cefazolin (CEZ), a new semisynthetic cephalosporin analogue, two kinds of ^{14}C-labeled CEZ were synthesized. The distribution of ^{14}C-labeled CEZ in rats and mice after parenteral administration was studied using whole body autoradiography and liquid scintillation counting. The results of both studies agreed well in many aspects. Very rapid distribution throughout the whole body, except for the brain and fetuses, and complete elimination of the radioactivity in a short term were observed.

Cefazolin (CEZ) is a new semisynthetic cephalosporin developed in this laboratories.[4] It is a broad-spectrum antibiotic, active against not only gram-positive bacteria but also many gram-negative bacteria including those of clinically isolated strains. Its laboratory evaluations were already reported by Nishida, *et al.*[5]

Studies on the metabolism of cephalosporin analogues were made by several investigators including the present authors,[6] who used ^{14}C tracers assigned to the amide side chain attached to the 7-position of 7-aminocephalosporanic acid (7-ACA). The present paper deals with the distribution of CEZ in rats and mice, constituting a part of experiments designed to investigate the fate of this antibiotic after parenteral administration.

Experimental

^{14}C-Labeled Cefazolin——Two different preparations of ^{14}C-labeled CEZ were employed: one with ^{14}C-atom in the tetrazolylacetamide side chain at the C-7 position of 7-ACA, and the other, with ^{14}C in the thiadiazole ring side chain at the C-3 position of 7-ACA. Sodium 7-[1-(1H)-tetrazolylacetamido-2-^{14}C]-3-[2-(5-methyl-1,3,4-thiadiazolyl)thiomethyl-Δ^3-cephem-4-carboxylate (I) (hereinafter referred to as CEZ-Na-7-^{14}C) was prepared from glycine-2-^{14}C (25 mCi, 10 mCi/mM; from Daiichi Pure Chemicals Co.) as a starting material, through the five steps shown in Chart 1. Sodium 7-[1-(1H)-tetrazolylacetamido]-3-[2-(5-methyl-1,3,4-thiadiazolyl)thiomethyl-2-^{14}C-Δ^3-cephem-4-carboxylate (II) (hereinafter referred to as CEZ-Na-3-^{14}C) was prepared from acetic-1-^{14}C-anhydride (30 mCi, 49.3 mCi/mM; from Daiichi Pure Chemicals Co.) as a starting material, through the six steps shown in Chart 2. The physicochemical properties of the purified radioactive CEZ-Na preparations were compared with the authentic unlabeled CEZ (free) for the potency. The results (Table I) verified the purity of these labeled antibiotics. They were diluted with the standard preparation of CEZ-Na (antibiotic activity: 927 μg/mg) to give the desired specific radioactivities, then subdivided into suitable vials, stored in a desiccator at −80° over silica gel until use.

1) Part IV: Y. Mine, M. Nishida, S. Goto and S. Kuwahara, *J. Antibiotics* (Tokyo) Ser. A, **23**, 195 (1970).
2) This forms Part III of "Studies on the Metabolism of Cephalosporins."
3) Location: *1, Kashima-cho, Higashiyodogawa-ku, Osaka.*
4) K. Kariyone, H. Harada, M. Kurita and T. Takano, *J. Antibiotics* (Tokyo) Ser. A, **23**, 131 (1970).
5) M. Nishida, T. Matsubara, T. Murakawa, Y. Mine, Y. Yokota, S. Goto and S. Kuwahara, *J. Antibiotics* (Tokyo) Ser. A, **23**, 137 (1970); *idem, ibid.*, **23**, 184 (1970).
6) H.W. Culp, F.J. Marshall and R.E. McMahon, *Antimicrobial Agents Chemoth.*, **1963**, 243 (1964); H.R. Sullivan and R.E. McMahon, *Biochem. J.*, **102**, 976 (1967); M. Okui, K. Hattori and M. Nishida, *J. Antibiotics* (Tokyo) Ser. A, **20**, 287 (1967); H.R. Sullivan, R.E. Billings and R.E. McMahon, *ibid.*, **22**, 27 (1969); *idem, ibid.*, **22**, 195 (1969); H.R. Sullivan, R.E. McMahon and E.R. Heiney, *ibid.*, **24**, 375 (1971).

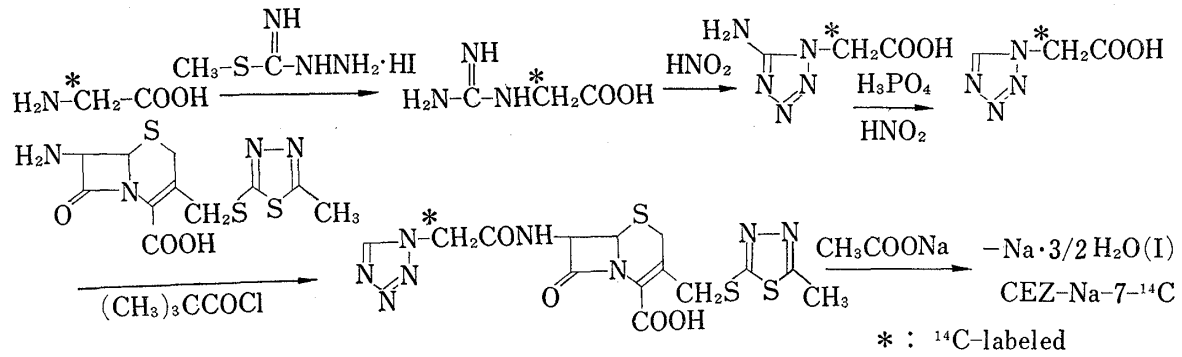


Chart 1. Synthetic Pathway of CEZ-Na-7-^{14}C

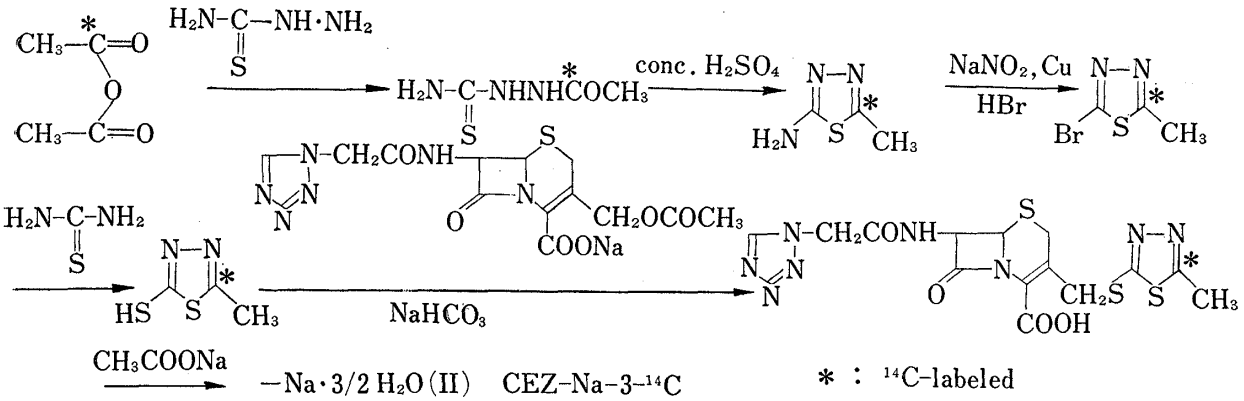


Chart 2. Synthetic Pathway of CEZ-Na-3-^{14}C

TABLE I. Properties of ^{14}C-Labeled CEZ-Na

Compound	Specific radioactivity (μCi/mg)	Radiochemical[a] purity (%)	Chemical[b,c] purity (%)	Antibiotic[c,d] potency (μg/mg)
CEZ-Na-3-^{14}C·3/2 H_2O	3.80	90.2	83.3	830
CEZ-Na-7-^{14}C·3/2 H_2O	4.70	93.0	83.0	864

a) Assayed by TLC (*n*-BuOH: HOAc: H_2O=4: 1: 2 v/v, Silica gel F_{254} by Merck)
b) Assayed by UV absorption, $\lambda_{max}^{buffer\ (pH\ 6.4)}$ 272 mμ
c) CEZ (free) was used as a reference standard.
d) Cup method (*B. subtilis* ATCC-6633)

Whole-body Autoradiography in Mice——Ten male mice of the ICR-JCL strain, weighing 20 to 25 grams, were each injected with 20 mg/kg (1.52—1.88 μCi/mouse) of labeled CEZ into the tail vein. They were killed 5, 20 minutes, 1,4 and 24 hours after administration. Separately, 4 pregnant mice, weighing about 40 g and 1 to 2 days before delivery, were each administered any of the labeled antibiotics in a similar manner and killed 30 minutes or 4 hours afterwards.

The experiment was performed by the following modification of Ullberg's method.[7] The animals were lightly anesthetized with ether and killed by immersion in acetone cooled with solid CO_2. The frozen animals were then transferred to a refrigerated room kept at about −5°. Sagittal sections (40 μ) were taken at various levels of each male animal, and horizontal sections, from each pregnant animal. They were mounted to tapes (Adhesive Plaster No. 5A, Nichiban Co.), and dried at −15° for 7 days. The section-mounted tapes were pressed against industrial X-ray films (Sakura-type N, Konishiroku Photo Ind. Co.) for 28—30 days. The films were then developed in a Konidol-X developer for 5 minutes and fixed in Konifix for 10 minutes.

Tissue Distribution in Rats——Twenty-seven Sprague-Dawley-JCL rats (♂21, ♀6[8]; 170—220 g) were administered 20 mg/kg of CEZ-Na-7-^{14}C (1.5—3 μCi/rat) intramuscularly, and killed at various times by

7) S. Ullberg, *Acta Radiol. Suppl.*, **118**, 1 (1954).
8) Female rats were examined for vaginal smear, and those in the estrus phase were excluded.

exsanguination at the femoral artery and vein. A small volume of blood was collected in heparinized tubes, the residual blood was collected in other tube and, allowed to clot. The brain, lungs, liver, kidneys, heart, spleen, whole blood and serum were examined for radioactivity. Rats killed 30 minutes and 24 hours after administration were examined for radioactivity on organs and tissue. Care was taken to prevent cross-contamination of samples with radioactivity. Immediately after dissection, the samples and organs were weighed wet, and lyophilized. Prior to lyophilization, the digestive tracts and skeletal muscles were each homogenized in water using a Virtis-45 homogenizer.

Separately, two male rats were subcutaneously injected unlabeled CEZ-Na at the priming dose of 4 g/kg daily for 21 days and, on the 22nd day, the same dose of CEZ-Na-7-^{14}C (4.87 μCi/rat). Twenty-four hours afterwards, they were killed and examined for residual radioactivity in various organs and tissue.

Measurement of Radioactivity——One hundred μl of serum, urine, bile, and carcass solution (prepared by the method described later), and whole blood were measured with an Eppendorf micropipette in counting vials, and dissolved by adding 1 ml of hydroxide of Hyamine-10X (Packard Instrument Co.). Wholeblood samples were decolorized by 2 or 3 drops of 30% hydrogen peroxide. Fifteen ml of toluene scintillator contain 0.5% PPO and 0.03% dimethyl-POPOP was added.

Lyophilized feces and organs were each ground in a mortar to give a homogeneous sample. To about 20 mg of each of these samples, accurately weighed, one ml of Soluene-100 (Packard Instrument Co.) was added, and heated overnight in an oven at 60°. After cooling, 15 ml of the scintillator were added.

The carcasses were transferred to a one liter flask, equipped with a Gimroth condenser connected to a train of two traps containing 5% (w/v) of sodium hydroxide in methanol for collecting $^{14}CO_2$. Then 200 ml of 6N HCl was added and refluxed at 160° for 2 hours. When 100 μl of the product and 0.5 ml of trapping reagent were analyzed for radioactivity, the CO_2 radioactivity was negligibly low. All the samples were counted in a Tri-Carb 3375 liquid scintillation spectrometer (Packard Instrument Co.). The external standard channels ratio technique was used to correct quenching.

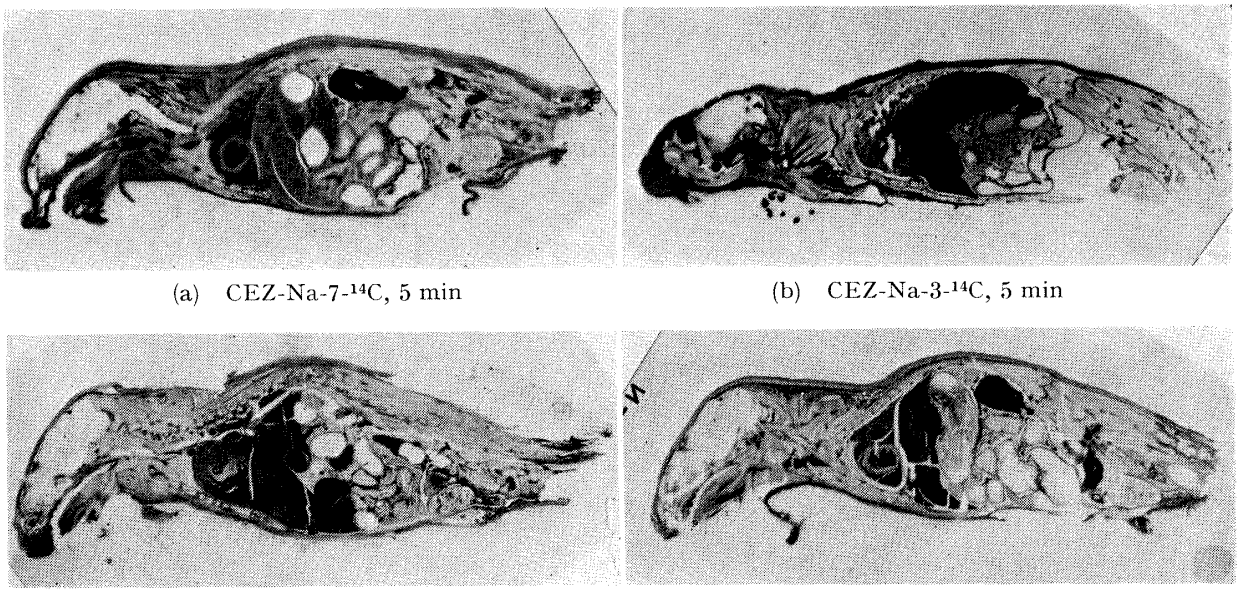
Result and Discussion

Whole-body Autoradiography

Representative autoradiograms of the two labeled cefazolins in male mice, at different intervals after a single intravenous administration, are shown in Fig. 1 and Fig. 2. The blackened areas on the autoradiograms show the presence of radioactivity.

5 min after Administration

The radioactivity left the blood very soon after a ^{14}C-CEZ injection. The highest activity was found in the kidneys, adrenals, lungs, eyes, and skin. Less activity was seen in the liver, oral, and nasal mucosa, salivary glands, stomach and intestinal mucosa. Most organs and tissue with moderate radioactivity showed a broad and rapid distribution of CEZ. The



(a) CEZ-Na-7-^{14}C, 5 min (b) CEZ-Na-3-^{14}C, 5 min

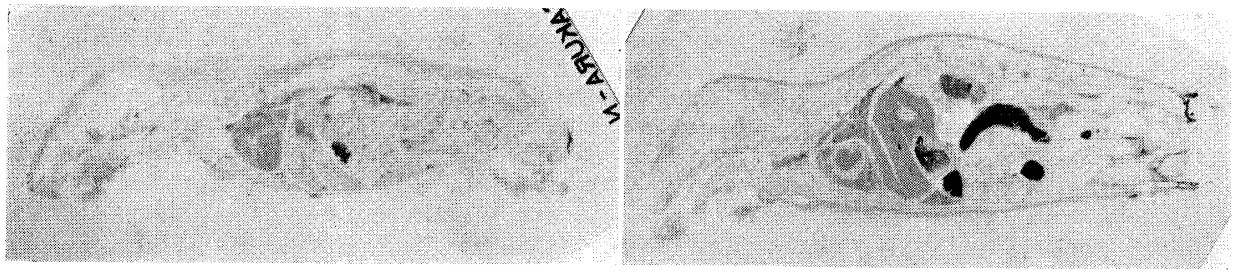
(c) CEZ-Na-7-^{14}C, 20 min (d) CEZ-Na-3-^{14}C, 20 min

Fig. 1. Autoradiograms Showing the Distribution of Radioactivity in Male Mice after *i. v.* Administration (20 mg/kg)

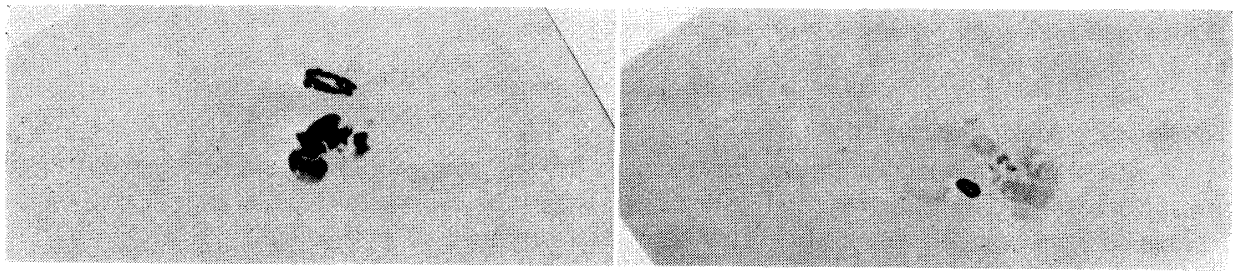
brain and spinal cord, however, did not show the presence of radioactivity, indicating that CEZ does not pass the blood brain barrier (Fig. 1, a, b).

20 min after Administration

The highest levels were found in the liver, gall bladder and intestinal contents, which fact suggesting the promptness of urinary and biliary excretion of CEZ. A rather high activity was recorded in organs or tissue such as the lungs, adrenals, oral and nasal mucosa, skin, penis, intestinal mucosa, and salivary glands (Fig. 1, c, d).

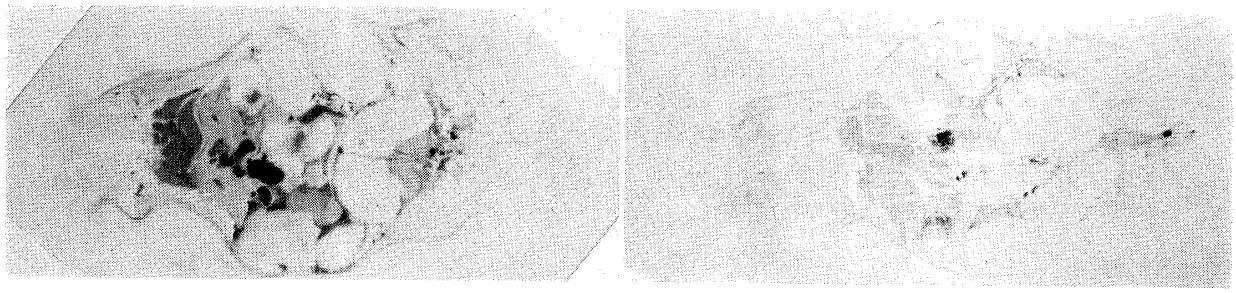


(e) CEZ-Na-7-^{14}C, 1 hr (f) CEZ-Na-3-^{14}C, 1 hr

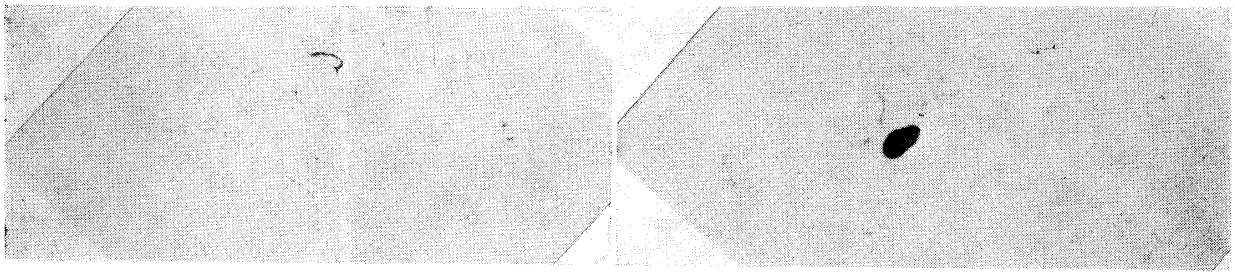


(g) CEZ-Na-7-^{14}C, 4 hr (h) CEZ-Na-3-^{14}C, 4 hr

Fig. 2. Autoradiograms Showing the Distribution of Radioactivity in Male Mice after *i. v.* Administration (20 mg/kg)



(i) CEZ-Na-7-^{14}C, 30 min (j) CEZ-Na-3-^{14}C, 30 min



(k) CEZ-Na-7-^{14}C, 4 hr (l) CEZ-Na-3-^{14}C, 4 hr

Fig. 3. Autoradiograms Showing the Distribution of Radioactivity in Pregnant Mice after *i. v.* Administration (20 mg/kg), (Horizontal Sections)

1 hr after Administration

There was a markedly decreased radioactivity in most organs and tissue except for excretory organs such as the kidneys, urinary-, gall-bladder, and penis. The intestinal contents showed a very high radioactivity (Fig. 2, e,f).

4 hr after Administration

The radioactivity has been nearly completely excreted from all the organs except those responsible for its elimination. The medullar zone of the kidneys shows the highest activity. (Fig. 2, g,h).

Table II. Amount of Radioactivity, determined Semiquantitatively by Visual Evaluation of Autoradiographic Films, in Tissues after *i.v.* Injection of CEZ-Na-7-^{14}C and CEZ-Na-3-^{14}C to Male Mice

Organ	Time after inj.									
	5 min		20 min		1 hr		4 hr		24 hr	
^{14}C-CEZ	7	3	7	3	7	3	7	3	7	3
Brain	0	0	0	0	0	0	0	0	0	0
Hypophysis	2	—	1	—	1	—	—	—	0	0
Spinal cord	0	0	0	0	0	0	0	0	0	0
Eye	—	4	—	—	—	—	0	0	0	0
Sinus hair follicle	4	5	4	2	1	1	0	0	0	0
Tongue	4	2	4	1	2	1	0	0	0	0
Salivary gland	3	3	2	2	1	1	0	0	0	0
Trachea	2	1	0	—	0	1	0	0	0	0
Lymph node	—	2	—	2	—	1	0	0	0	0
Brown fat	—	4	—	—	—	—	0	0	0	0
Thymus	1	2	1	2	0	1	0	0	0	0
Myocardium	2	2	1	1	1	1	0	0	0	1
Heart blood	5	4	4	4	3	3	0	0	0	1
Hepatic blood	5	5	5	5	1	3	0	0	0	0
Blood in vena cava	—	4	—	—	2	2	0	—	0	0
Lung	5	4	4	4	3	3	0	0	0	0
Liver	4	5	4	4	2	2	0	1	0	1
Gall bladder	—	5	5	—	5	—	—	—	—	—
Pancreas	2	2	1	1	1	1	0	0	0	0
Spleen	2	—	1	2	1	1	0	0	—	0
Stomach contents	0	0	0/5	1	0/4	1/3	0	1	0	1
Stomach mucosa	3	2	2	2	1	1	0	0	0	0
Partial contents of small intestine	0	2/3	0/5	1	0	0/5	5	1/4	3	0
Small intestinal mucosa	3	2	2	2	1	1	0	0	0	0
Partial contents of large intestine	0	0	0	0	0	0	0	3	2/3	1
Large intestinal mucosa	3	1	2	2	1	1	0	0	0	0
Adrenal	4	5	4	—	2	1	0	0	0	0
Kidney (medulla)	5	5	—	5	3	5	5	0	—	—
Kidney (cortex)	5	4/5	—	4/5	3	4	1	0	—	0
Urinary bladder	—	—	5	5	5	5	0	0	0	—
Penis	2	—	3	—	5	5	1	—	0	0
Testis	2	1	2	1	1	1	0	0	0	0
Bone marrow	1	1	1	1	1	0	0	0	0	0
Skin	4	4	3	3	2	2	0	0	0	0

The following arbitarary units were used:
5: very high 4: high 3: moderate 2: low 1: extremely low 0: no visible amount
—: not detected in the sections or difficult to diagnose

24 hr after Administration

The excretion of radioactivity was nearly completely excreted, except for intestinal contents, which showed a slight activity (Fig. are not shown).

Whole-body Autoradiography in Pregnant Mice

The distribution of radioactivity in pregnant mice after an intravenous injection of CEZ-Na-7-^{14}C or CEZ-Na-3-^{14}C is shown in Fig. 3. The placenta shows a considerable amount of radioactivity, but the fetuses show much lower activities than the dams. This indicates that the placental passage is almost negligible. This is very likely due to the rapid disap-

Table III. Tissue Levels and % of the Administered Dose of CEZ-Na-7-^{14}C after a Single Dose in Rats (*i. m.*, 20 mg/kg)

Time	Organ							
	Brain		Heart		Lung		Spleen	
	μg/g[a)]	%	μg/g	%	μg/g	%	μg/g	%
5 min	1.28 ±0.19	0.05 ±0.01	17.35 ±2.23	0.30 ±0.03	21.15 ±3.00	0.54 ±0.08	6.32 ±1.00	0.11 ±0.02
10 min	1.42 ±0.12	0.05 ±0.00	16.19 ±0.85	0.28 ±0.03	20.61 ±1.16	0.54 ±0.03	5.53 ±0.14	0.09 ±0.01
15 min	1.26 ±0.03	0.05 ±0.00	20.55 ±6.61	0.44 ±0.22	19.38 ±0.62	0.42 ±0.03	6.05 ±0.20	0.10 ±0.00
30 min	1.40 ±0.42	0.05 ±0.01	12.89 ±2.36	0.23 ±0.06	15.97 ±2.21	0.33 ±0.04	5.50 ±1.25	0.14 ±0.04
1 hr	0.73 ±0.05	0.03 ±0.00	6.62 ±0.53	0.11 ±0.02	9.99 ±0.19	0.26 ±0.03	3.12 ±0.03	0.06 ±0.01
2 hr	0.35 ±0.04	0.01 ±0.00	2.95 ±0.28	0.05 ±0.00	4.23 ±0.47	0.12 ±0.03	1.65 ±1.20	0.02 ±0.01
24 hr	0.14 ±0.02	0.01 ±0.00	0.24 ±0.01	trace	0.30 ±0.02	0.01 ±0.00	0.28 ±0.03	trace
Half-life (min)	56		42		49		58	

Time	Organ					
	Liver		Kindey		Whole blood	Serum
	μg/g	%	μg/g	%	μg/ml	μg/ml
5 min	28.24 ±6.58	5.70 ±1.10	89.40 ±20.28	3.17 ±0.76	63.28 ±11.60	92.97 ±12.98
10 min	29.41 ±3.08	5.59 ±0.61	88.65 ±13.95	3.50 ±0.55	—	86.09 ± 2.40
15 min	36.38 ±5.09	7.02 ±0.94	90.88 ±10.08	3.01 ±0.46	47.60 ± 2.44	79.55 ± 2.42
30 min	28.22 ±4.73	5.65 ±0.75	129.15 ±30.80	5.15 ±1.37	50.49 ± 6.44	74.54 ±10.93
1 hr	9.54 ±1.12	1.80 ±0.21	44.49 ± 2.65	1.52 ±0.06	23.52 ± 1.40	40.64 ± 1.00
2 hr	4.23 ±0.75	0.75 ±0.13	23.16 ± 3.23	0.83 ±0.08	9.45 ± 1.70	16.14 ± 1.92
24 hr	0.33 ±0.05	0.07 ±0.01	1.68 ± 0.13	0.07 ±0.00	1.11 ± 0.08	0.55 ± 0.03
Half-life (min)	37		53		42	45

mean±standard error, $n=3$

a) CEZ-Na equivalents μg/g of wet weight

pearance of the antibiotic from the maternal blood stream after a single intravenous injection.

In summary, it can be stated that, after an intravenous administration of CEZ-Na-7-^{14}C or CEZ-Na-3-^{14}C to mice, the radioactivity was very broadly and rapidly distributed throughout most organs and tissues except the central nervous system, followed by a rapid elimination into the urine and bile. The radioactivity was nearly completely eliminated within 24 hours after administration, and no tendency of accumulation was noted in any organ or tissue. Results from both CEZ-Na-7-^{14}C and CEZ-Na-3-^{14}C were in good agreement in many aspects. These comments are summarized in Table II. The degree of blackening was determined semiquantitatively by visual evaluation of the autoradiographic films.

Tissue Distribution in Rats

The tissue distribution in rats was determined to investigate; (1) time-course of radioactivity in main organs, (2) more detailed tissue distribution in male and female rats, and (3) any possible accumulation of CEZ in tissue after repeated doses.

1) Time-course of Radioactivity Levels in Brain, Liver, Lungs, Heart, Kidneys, Spleen, Serum and Blood

Table III shows the radioactivity levels in various organs and tissue of rats administered 20 mg/kg of CEZ-Na-7-^{14}C intramuscularly and killed afterwards. The radioactivity re-

Table IV. Tissue Levels and % of the Administered Dose of CEZ-Na-7-^{14}C at 30 min after a Single Dose in Rats (*i. m.*, 20 mg/kg)

Organ	♂		♀	
	μg/g[a]	%	μg/g	%
Brain	1.40± 0.42	0.05±0.01	0.74± 0.19	0.02±0.01
Stomach[b]	3.59± 2.09	0.44±0.28	45.02±26.49	1.62±0.99
Small intestine[b]	19.09± 3.55	4.27±0.61	62.28± 9.39	9.97±0.79
Large intestine[b]	3.80± 0.88	0.37±0.07	4.83± 0.09	0.46±0.01
Liver	28.22± 4.73	5.65±0.75	31.53± 1.96	6.04±0.46
Heart	12.89± 2.36	0.23±0.06	22.19±12.44	0.43±0.25
Lung	15.97± 2.21	0.33±0.04	18.79± 4.29	0.41±0.12
Kidney	129.15± 30.80	5.15±1.37	122.02±14.39	4.01±0.34
Urinary bladder	180.09±114.81	0.35±0.11	95.49±32.33	0.15±0.05
Pancreas	9.62± 1.09	0.13±0.03	10.95± 3.18	0.13±0.03
Spleen	5.50± 1.25	0.14±0.04	7.40± 2.32	0.08±0.02
Hypophysis	20.50± 9.39	trace	18.94± 0.47	trace
Thymus	5.28± 0.85	0.08±0.02	8.24± 3.60	0.08±0.02
Submaxillary gland	16.47± 2.61	0.13±0.04	11.38± 2.07	0.09±0.02
Adrenal	12.28± 1.44	0.01±0.00	10.63± 1.11	0.01±0.00
Thyroid	24.58± 4.48	0.01±0.00	28.07± 7.72	trace
Testis	4.16± 0.42	0.17±0.02	—	—
Ovary	—	—	16.72± 1.89	0.03±0.00
Seminal vesicle	18.38± 4.76	0.10±0.04	—	—
Uterus	—	—	24.45± 3.05	0.17±0.02
Prostate	11.05± 3.26	0.04±0.01	—	—
Abdominal mammary gland	—	—	12.41± 0.82	—
Injection site	182.89± 29.18	8.99±2.65	138.71±27.93	10.72±2.05
Urine[c]	106.77± 90.71	8.03±6.98	69.36± 3.92	5.24±1.07
Feces	0.69[d]± 0.34	trace	—	—
Blood[c]	59.49± 6.44	—	41.10± 3.11	—
Muscle (femoral)	7.44± 1.44	—	4.85± 0.30	—
Brown fat	18.07± 1.49	—	9.32± 1.53	—
Carcass	12.93± 1.45	43.04±4.24	10.29± 0.93	37.60±3.17
Total		77.71±1.95		77.24±3.90

mean±standard error, $n=3$

a) CEZ-Na equivalents μg/g of wet weight; *b*) including its contents; *c*) μg/ml; *d*) μg/g of dry weight

covered in organs and tissue was estimated as has been derived from unchanged CEZ, and was expressed in terms of tissue concentration as well as in its percentage to the administered dose. A cursory glance at the data suggests that very high levels of radioactivity were immediately attained in various organs and tissue except for the brain, peaked 15 minutes after administration, and then decreased. The high levels of radioactivity in the liver and kidneys throughout observation indicate that this antibiotic is excreted *via* urine and bile. The levels of radioactivity in the brain were extremely low throughout the 2-hour assay. The lower levels of radioactivity in the whole blood indicate that more CEZ was present in serum than in erythrocytes.

Plotting this data on a semi-logarithmic scale paper yielded curves that fit the kinetics of fitst-order decay in all organs examined. The slopes of the best straight lines were obtained by computer regression analysis and used to estimate the elimination half-lives of radioactivity in organs or tissue by Wagner method.[9] The half-lives shown in Table III indicated that rapid elimination of CEZ from the rat body.

Table V. Tissue Levels and % of the Administered Dose of CEZ-Na-7-^{14}C at 24 hr after a Single Dose in Rats (*i. m.*, 20 mg/kg)

Organ	♂		♀	
	μg/g[a]	%	μg/g	%
Brain	0.14± 0.02	0.01± 0.00	0.28± 0.05	0.01±0.00
Stomach[b]	0.73± 0.47	0.70± 0.05	0.93± 0.64	0.04±0.03
Small intestine[b]	0.47± 0.02	0.09± 0.01	0.45± 0.13	0.08±0.02
Large intestine[b]	2.90± 0.59	0.36± 0.06	9.01± 2.75	1.85±0.77
Liver	0.33± 0.05	0.07± 0.01	0.48± 0.08	0.10±0.02
Heart	0.24± 0.01	trace	0.31± 0.05	0.01±0.00
Lung	0.30± 0.02	0.01± 0.00	0.41± 0.05	0.01±0.00
Kidney	1.68± 0.13	0.07± 0.00	2.22± 0.08	0.08±0.00
Urinary bladder	0.35± 0.04	trace	0.50± 0.05	trace
Pancreas	0.26± 0.01	trace	0.44± 0.07	trace
Spleen	0.28± 0.03	trace	0.41± 0.07	trace
Hypophysis	2.26± 1.77	trace	0.86± 0.29	trace
Thymus	0.16± 0.02	trace	0.19± 0.01	trace
Submaxillary gland	0.49± 0.21	trace	0.23± 0.03	trace
Adrenal	0.48± 0.10	trace	0.53± 0.10	trace
Thyroid	0.44± 0.16	trace	4.09	trace
Testis	0.18± 0.02	0.01± 0.00	—	—
Ovary	—	—	0.44± 0.04	trace
Seminal vesicle	0.32± 0.09	trace	—	—
Uterus	—	—	0.48± 0.06	trace
Prostate	0.18± 0.02	trace	—	—
Abdominal mammary gland	—	—	0.40± 0.06	—
Injection site	1.38± 0.17	0.07± 0.01	1.43± 0.56	0.09±0.03
Urine[c]	158.65±11.78	81.99±10.23	124.53±19.17	74.54±2.01
Feces	110.14[d]±32.61	11.72± 4.22	188.43[d]±15.79	14.29±2.95
Blood[c]	1.11± 0.08	—	0.98± 0.14	—
Muscle (femoral)	0.24± 0.01	—	0.83± 0.53	—
Brown fat	0.32± 0.06	—	0.28± 0.03	trace
Carcass	0.33± 0.01	1.12± 0.06	0.48± 0.09	1.56±0.29
Total		95.60± 6.74		92.66±0.43

mean ± standard error, $n=3$

a) CEZ-Na equivalents μg/g of wet weight; *b*) including its contents; *c*) μg/ml; *d*) μg/g of dry weight

9) J.G. Wagner, *Drug Intelligence Clin. Pharm.*, **2**, 126 (1968).

2) Organ Distribution in Male and Female Rats after a Single Dose

A more detailed organ distribution in male and female rats, killed 30 minutes and 24 hours after receiving 20 mg/kg of CEZ-Na-7-^{14}C intramuscularly, is shown in Table IV and V. In male rats killed 30 minutes after administration, the highest levels of radioactivity were shown in the urinary bladder, kidneys, blood, liver, thyroid and hypophysis, followed by the submaxillary glands, lungs, heart, adrenals, prostate gland, small intestine (including its contents), seminal vescile and brown fat. There was little radioactivity in the brain. The injected site of the femoral muscle retained about 9 percent of the administered radioactivity. Similar patterns of radioactivity distribution were observed in female rats, wherein the reproductive organs showed radioactivity of similar degree to that in the male reproductive organs. In rats of both sexes, most radioactivity disappeared from the body in 24 hours, with only limited levels of radioactivity remaining in various organs other than the alimentary tracts (including the contents) (Table V).

3) Organ Distribution after Repeated Doses

Table VI shows distribution of radioactivity in various organs of 2 rats. A daily dose of 4 g/kg of unlabeld CEZ-Na was given for 21 days and, on the 22nd day, the same dose of CEZ-Na-7-^{14}C (4.87 μCi/rat) was administered. These animlas were killed 24 hours thereafter. Necrotic change caused by irriration, was observed at the site of injection, due to the repeated very high doses. Accordingly, counting was made in two divided fractions: The liquefactive necrotic area (I) and its surrounding areas (II). The residual radioactivity in the subcutaneous connective tissue at the injection site and the large intestine (including the contents) was fairly high, but differed little in the other organs or tissue as compared with the case of single dose (Table V).

Table VI. Tissue Levels and % of the Administerd Dose of CEZ-Na-7-^{14}C at 24 hr after Repeated[a] Dose in Rats (*s. c.*, 4 g/kg)

Organs	μg/g[b]	%	Organs		μg/g[b]	%
Brain	0.01	trace	Adrenal		0.03	trace
Stomach[c]	0.21	0.12	Thyroid		0.07	trace
Small intestine[c]	0.41	0.60	Testis		0.01	trace
Large intestine[c]	1.45	4.45	Seminal vesicle		0.02	trace
Liver	0.03	0.04	Prostate		0.02	trace
Heart	0.02	0.01	Injection site	I[d]	0.07	0.01
Lung	0.02	trace		II[d]	0.04	1.14
Kidney	0.08	0.02	Urine[e]		19.47	82.12
Urinary bladder	0.47	0.01	Feces[f]		9.52	2.98
Pancreas	0.04	trace	Blood[e]		0.05	—
Spleen	0.03	trace	Muscle (femoral)		0.04	—
Hypophysis	0.02	trace	Brown fat		0.12	—
Thymus	0.02	trace	Carcass		0.03	0.60
Submaxillary gland	0.02	trace	Total			92.10

mean of two male rats
a) A daily dose of 4 g/kg of unlabeled CEZ-Na was given for 21 days prior to the administration of labeled CEZ-Na.
b) CEZ-Na equivalents μg/g of wet weight.
c) including its contents.
d) see text
e) μg/ml
f) μg/g of dry weight

Acknowledgement The authors wish to thank Dr. H. Nakano, the director of this research laboratories, and Dr. K. Kariyone for guidance and encouragement. They also wish to thank Mr. M. Doi and his groups for their assistance in the experimental work, and Dr. Y. Morimoto in kinetic analysis of the data.