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Studies on Absorption, Distribution, Metabolism and Excretion of a New Macrolide Antibiotic SF-837.[1] I. Absorption, Metabolism and Excretion of SF-837

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The comparative absorption, metabolism, and excretion of SF-837 substance, a new macrolide antibiotic, was studied in rat, dog and man. Blood, urine and bile analysis were performed on these animals collected at several times after either oral ingestion or intravenous administration of a dose. SF-837 was found to be rapidly absorbed in rats and once in the body, was metabolized to M1 substance and M2 substance in rats, dogs and men.

By densitometric analysis of thin-layer chromatograms, the urinary antimicrobial activities were analyzed falling in with 2.0% of unchanged SF-837, 18.0% of M1 and 1.7% of M2, and the biliary were analyzed falling in with 0.02% of unchanged SF-837, 5.8% of M1 and 47.3% of M2, within 24 hours after intravenous administration of SF-837 (50 mg/kg).

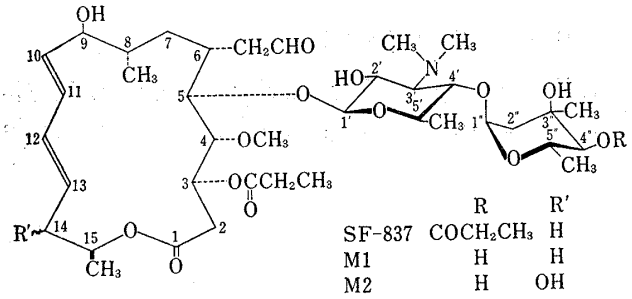
Namely the main metabolite in the urine was M1 substance and that in the bile was M2 substance.

The antimicrobial activity in the blood was derived mostly from M1 substance, not only by intravenous administration but also oral in rats.

Similar results were obtained also from dogs and men.

These results indicated that SF-837 was metabolised mainly to M1 substance and then M2 substance in rats, dogs and men.

Antibiotic SF-837 is a new macrolide which was isolated in this laboratories[3] from *Streptomyces mycarofaciens nov. sp.*. The structure of this antibiotic (Chart 1)[4] was reported as in the leucomycin-carbomycin family, and the biological activity of this antibiotic was also published.[5]



	R	R'
SF-837	$COCH_2CH_3$	H
M1	H	H
M2	H	OH

Chart 1. Structure of SF-837 and Its Metabolites, M1 and M2 Substance

The isolation and structure of the two metabolites (Chart 1) of this antibiotic were previously described in this Journal;[6] M1 substance which was mainly appeared in the urine is 4″-depropionyl-SF-837 and M2 substance which was eliminated in the bile is 14-hydroxy, 4″-depropionyl-SF-837.

This report summarized the results of the study of the absorption, metabolism and excretion of SF-837 in rats, dogs and men. The densitometric analysis of the

1) T. Shomura, I. Komiya, and K. Umemura, Presented at the 92nd Annual Meeting of Pharmaceutical Society of Japan, Abstracts of Papers, IV-75p (1972).
2) Location: *Morooka-cho, Kohoku-ku Yokohama, 222, Japan.*
3) T. Tsuruoka, T. Shomura, N. Ezaki, H. Watanabe, E. Akita, S. Inouye, and T. Niida, *J. Antibiotics* (Tokyo), **24**, 452 (1971).
4) S. Inouye, T. Tsuruoka, T. Shomura, S. Omoto, and T. Niida, *J. Antibiotics* (Tokyo), **24**, 460 (1972).
5) K. Kawaharajo, T. Yoshida, T. Watanabe, K. Miyauchi, B. Nomiya, S. Tada, and S. Kuwahara, *Chemotherapy*, **20**, 633 (1972).
6) S. Inouye, T. Shomura, T. Tsuruoka, S. Omoto, T. Niida, and K. Umemura, *Chem. Pharm. Bull.* (Tokyo), **20**, 2366 (1972).

thin-layer chromatography (TLC) were used to study the quantitative estimation of the unchanged SF-837 and its metabolites (M1, M2 substance) in the blood, urine and bile of rats, dogs and men, after intravenous and oral administration.

Material and Method

SF-837 used in this investigation was in the form of the base which assayed 1000 mcg per mg. Both M1 and M2 substance used for the quantitative estimation in the blood, urine and bile were the standard materials which were prepared from the urine or bile following dosing SF-837 in rats[6] and purified by silica gel column chromatography. Intravenous solutions (50 mg/g of the solution for the rats, 100 mg/g for the dogs) were prepared by dissolving the drugs in the aqueous solutions of containing the sufficient amount of tartaric acid. Oral dose were provided for the rats as suspensions (200 mg/3 g of the suspension) in 0.8% of the solutions of sodium taurochorate and for the men in capsules (1000 mg/8 pieces for a man) filled with the loose powder.

Male albino rats (Donryu, 250—280 g) and male Beagles (Satsuma, 10—13 kg) were used. Male albino rats were performed the urinary fistulous operation from the bladder and the biliary from the common bile duct, under anaesthetizing with pentobarbitonesodium (35 mg/kg *i.p.*), for urinary and biliary excretion experiments. Adult male Beagles were anaesthetized with the same anaesthetic (35 mg/kg *i.v.*); the common bile duct was cannulated with polyethylene tube and the gallbladder was not ligated. Each ureter was cannulated with polyethylene tube about 2—3 cm from the hilus of the bladder. The operated dogs were used to study the blood levels and also urinary and billiary excretion experiments.

During the urine and the bile collected, the rats were housed in the restraint cages and the dogs were fixed on the operation tables and anaesthesia was maintained with pentobarbitone, if necessary. In the oral administrations, animals were used after fasting for 18 hours.

In the rat experiments, bile and urine were collected at given times, blood samples were taken from the heart of a rat each 0.1 ml at given times for the determination of the antimicrobial activity. Total bleedings were done from the individual rat hearts at each time for the quantitative estimation of the unchanged drug and its metabolites.

In the dog experiments, boold samples were taken from the forelimb vein of the dog 5 ml for 10 and 30 minutes after intravenous administration, 10 ml for 60 minutes, 20 ml for the 2 and 4 hours; urine and bile samples were taken at the given times.

The man studies were done on the subjects receiving SF-837 orally; and one group of the subjects were analyzed in urinary excretion, and another (a man) was experimented in biliary excretion from the hepatic bile after extomy operation of the gallbladder.

Thin-Layer Chromatography——TLC was carried out with Eastman Chromagram Sheet 6061 (silica gel) and solvent system, benzene–acetone (2: 1), or chloroform–methanol (10: 1).

Quantitative Estimation of the Unchanged Drug and Its Metabolites in Blood, Urine or Bile——Blood samples (2 ml) were heparinized and hemolyzed with ten times volumes of cold water (20 ml). The hemolyzed solutions were adjusted to pH 9—10 with 4 ml of 1N NaOH

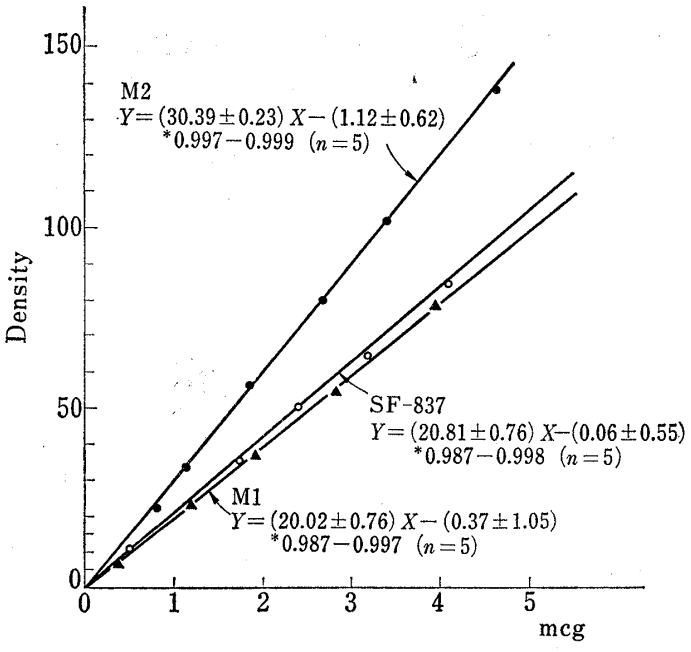


Fig. 1. Working Curves of SF-837, M1 and M2 by Densitometry of TLC

used plate: Eastman chnomagram sheet 6061 silica gel
solvent system: benzene–acetone(2:1)
densitometer: ATAGO DENSITOMASTER CHEMIC
*: relative coefficient

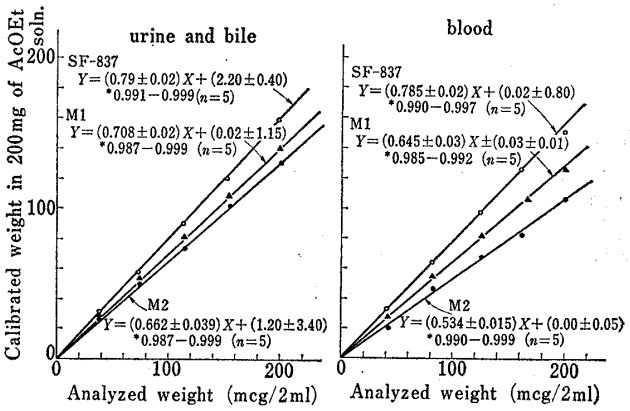


Fig. 2. Calibration Curves of SF-837, M1 and M2 in Urine, Bile and Blood

and extracted with 40 ml of ethylacetate, and 1 ml of the solution of $(NH_4)_2SO_4$ (40%), under vigorously shaken condition (during 10 minutes), and centrifuged immediately. The ethylacetate extracts (30 ml) were evaporated under slight vacuum, until the desired small volume was reached. These concentrated ethylacetate extracts were adjusted to the weight of 200 mg with ethylacetate and directly applied (weighing 3—20 mg of the solution) on to the TLC plate. For the quantitative estimation of unchanged drug and its metabolites, the chromatograms were sprayed with a solution of H_2SO_4 (10%) and heated at about 80°C during about 3 minutes. The TLC plates were put between two glass plate in order to prevent the fading. The plates were applied on the densitometer (ATAGO Chemic), at 500 mμ. This method was particularly useful for the concentrations of the unchanged drugs and its metabolites in blood of the order 10—200 mcg/ml. For the concentration of SF-837 and its metabolites smaller than 10 mcg/ml, larger amount of the samples could be used.

The urine or bile samples (2 ml) were added 2 ml of water and extracted with 10 ml of ethylacetate after adjusting to pH about 8 with 1 ml of $NaHCO_3$ (7.5%). The ethylacetate extracts (8 ml) were treated similarly to the blood estimation.

These quantitative estimations were done from the working curves (Fig. 1, 2), for which the standard substances (SF-837, M1, M2 substance) in blood, urine and bile were used.

Bioassay——The levels of antimicrobial activity in blood, urine or bile were assayed microbiologically using Sarcina lutea (paper disk or cup-plate diffusion method).

Result and Discussion

Explorations of the Metabolites of SF-837 in Blood, Urine and Bile.

Fig. 3, 4 and 5 illustrated the densitograms of the metabolites in the blood, urine and bile following intravenous or oral administration of SF-837 in rats (Fig. 3), dogs (Fig. 4), and men (Fig. 5). These results were derived from the ethylacetate extracts under alkaline conditions from the body fluids; and no principal metabolite which was not extractable with ethylacetate was observed. As clearly from these densitograms, the metabolism of SF-837 in rat, dog and human was similar in the extent of these animals.

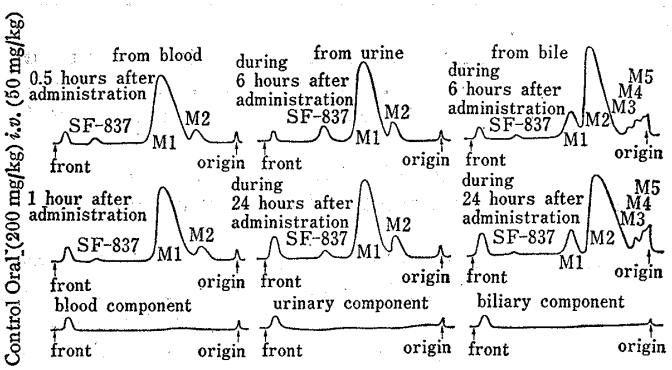


Fig. 3. Densitogram of Thin-Layer chromatogram of Ethylacetate Extract obtained from Rat Blood, Urine and Bile, after Administration of SF-837

TLC: Eastman chromagram sheet 6061
solvent system: benzene-acetone=2:1
color: 10% H_2SO_4 heat(80°)

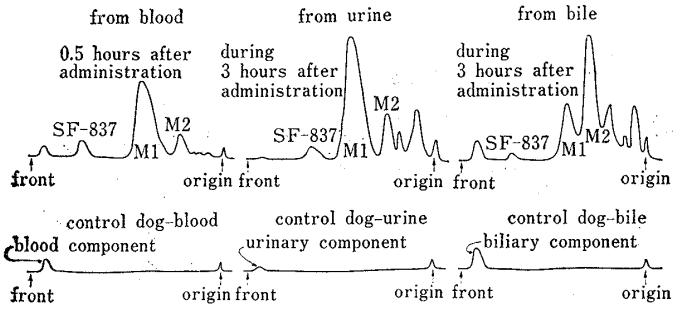


Fig. 4. Densitogram of Thin-Layer Chromatogram of Ethylacetate Extract obtained from Dog Blood, Urine and Bile, after Intravenous Administration of SF-837 (50 mg/kg)

TLC: Eastman chromagram sheet 6061
solvent system: benzene: acetone=2:1
color: 10% H_2SO_4 heat (80°)

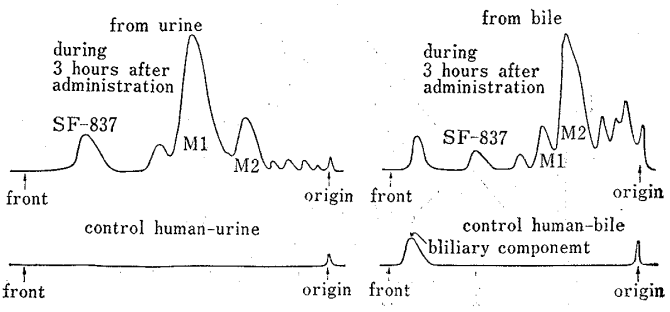


Fig. 5. Densitogram of Thin-Layer Chromatogram of Ethylacetate Extract obtained from Human Urine and Bile, after Oral Administration of SF-837 (1000 mg/man)

TLC: Eastman Chromagram sheet 6061
solvent systm: benzene: acetone=2:1
color: 10% H_2SO_4 heat (80°)

Blood Concentrations of SF-837 in Rats and Dogs

Fig. 6, 7 and 8 illustrated the blood antimicrobial activity profiles following intravenous (50 mg/kg) or oral (200 mg/kg) administration of SF-837 in rats and dogs. The blood concentration values of the antimicrobial activities in rats or dogs following intravenous dosing

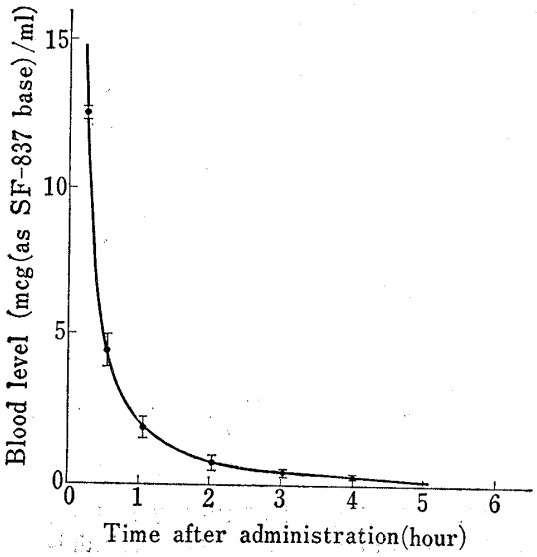


Fig. 6. Apparent Blood Level as Antimicrobial Activity of SF-837 in Rats after Intravenous Administration (50 mg/kg)

used animal:rat (Donryu, male) body wt. 250—280 g
determination: bioassay, paper-disk method(S. lutea)
plotting: mean value±S.E. (n=4)

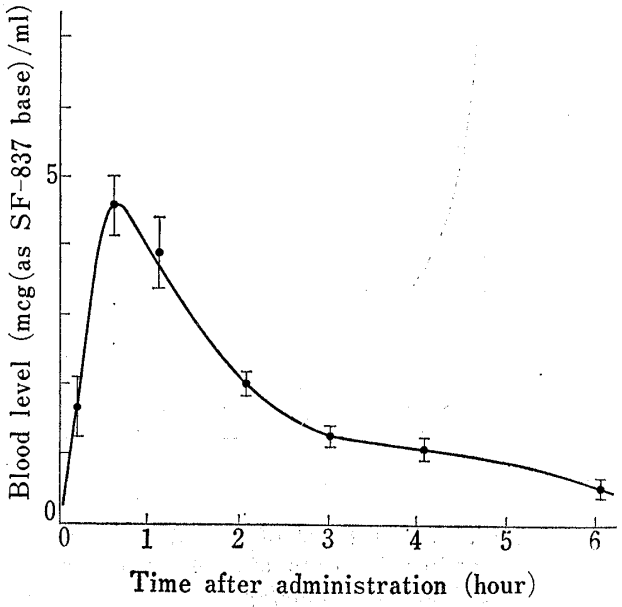


Fig. 7. Apparent Blood Level as Antimicrobial Activity of SF-837 in Rats after Oral Administration (200 mg/kg)

used animal:rat (Donryu, male) body wt. 250—280 g
Determination: bioassay,paper-disk method (S. lutea)
plotting: mean value±S.E. (n=4)

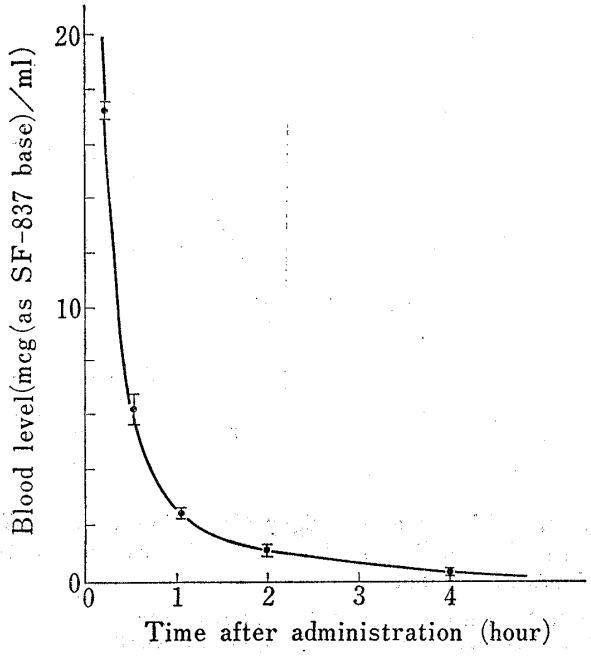


Fig. 8. Apparent Blood Level as Antimicrobial Activity of SF-837 in Dogs after Intravenous Administration (50 mg/kg)

used animal: dog (Satsuma bengle body wt. 10—13 kg
determination: bioassay, paper-disk method (S. lutea)
plotting: mean value±S.E.(n=3)

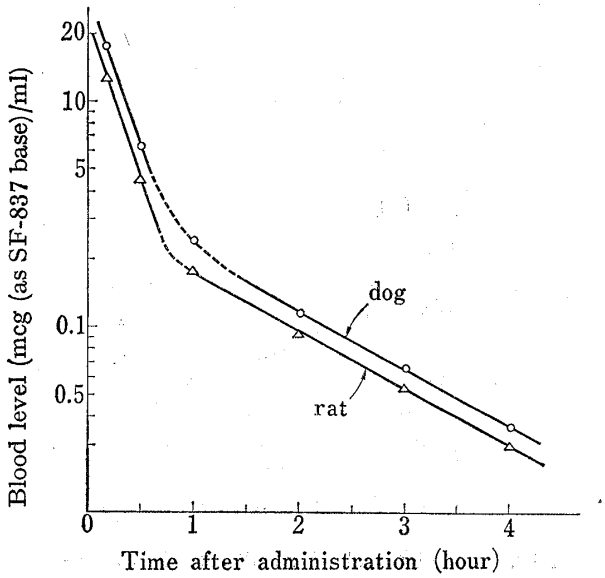


Fig. 9. Semilogarithmic Plots of Blood Antimicrobial Activities in Rats and Dogs after Intravenous Administration of SF-837 at the Dose of 50 mg/kg

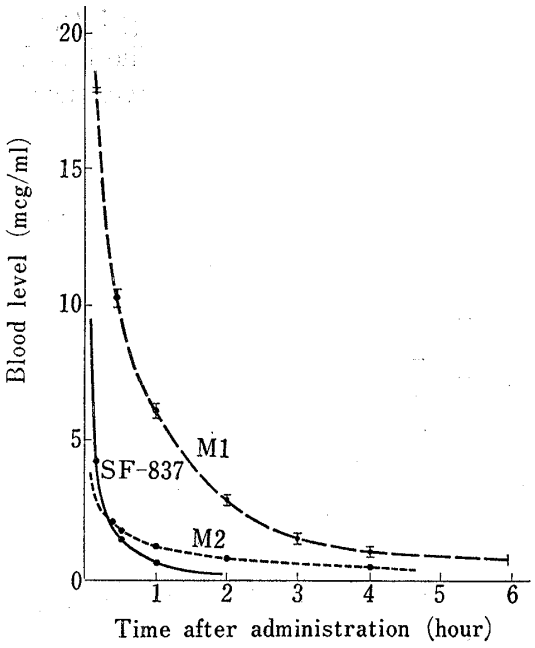


Fig. 10. Blood Level of SF-837 and Its Metabolites in Rats after Intravenous Administration (50 mg/kg)

determination: densitometry of TLC by extraction method
plotting: mean value±S.E. (n=4)

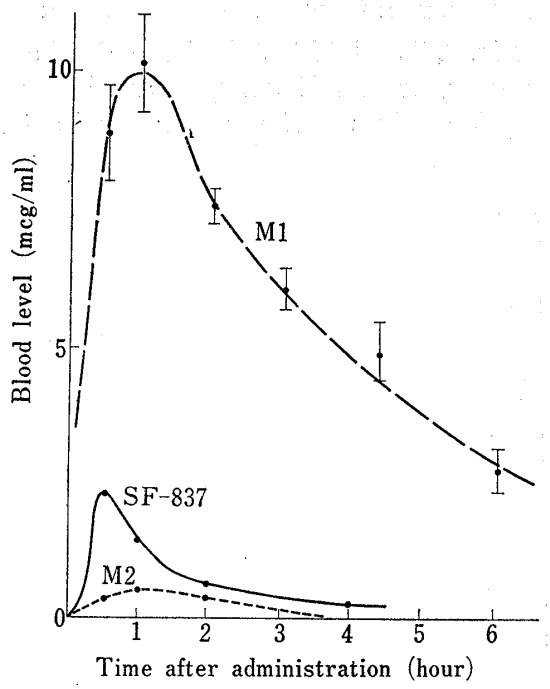


Fig. 11. Blood Level of SF-837 and Its Metabolites in Rats, after Oral Administration (200 mg/kg)

determination: densitometry of TLC by extraction method
plotting: mean value±S.E. (n=4)

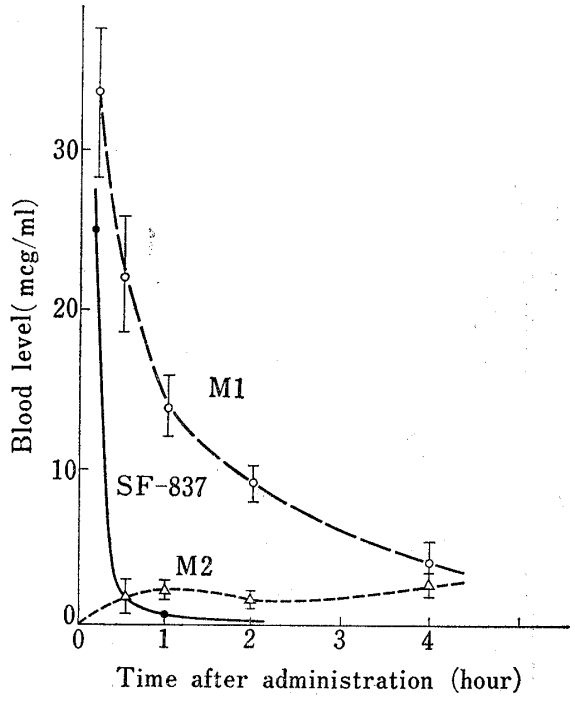


Fig. 12. Blood Level of SF-837 and Its Metabolites in Dogs after Intravenous Administration (50 mg/kg)

determination: densitometry of TLC by extraction method
plotting: mean value±S.E. (n=3)

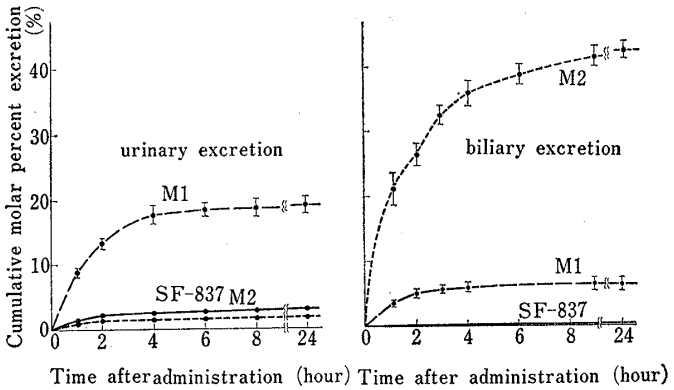


Fig. 13. Urinary and Biliary Excretion of SF-837 and Its Metabolites in Rats after Intravenous Administration (50 mg/kg)

used animal: rat (Donryu, male) body wt. 250—280 g
determination: densitometry of TLC by extraction method
plotting: mean value±S.E. (n=4)

were decreased very rapidly; and in all experiments when blood samples were drawn and determined early enough, the practical shape and the blood concentrations were remarkably similar. The semilogarithmic plots of the previous experiments were illustrated in Fig. 9. The blood curves following intravenous administration (50 mg/kg) had two linear phases. The slope of the initial linear segment of the curve bore the evidence of the distribution and the metabolism on which the drug was changed to the lower active or inactive metabolite. Fig. 10, 11 and 12 illustrate the blood levels of the unchanged drug and its metabolite profiles following intravenous (50 mg/kg) or oral (200 mg/kg) administration of SF-837 in rats and dogs. As clearly from these results, SF-837 was very rapidly metabolized to M1 substance in the animal body, and the main substance composed the blood antimicrobial concentration was M1 substance. It is necessary to reconsider that the antimicrobial assay method which has been used to the measurement of blood or body fluid concentration of an antibiotic does not always indicate the substance concentration. Because the antimicrobial activity of M1 substance is about one-forths of SF-837; this fact may be one of the reasons of appearing of the apparent low blood concentration.

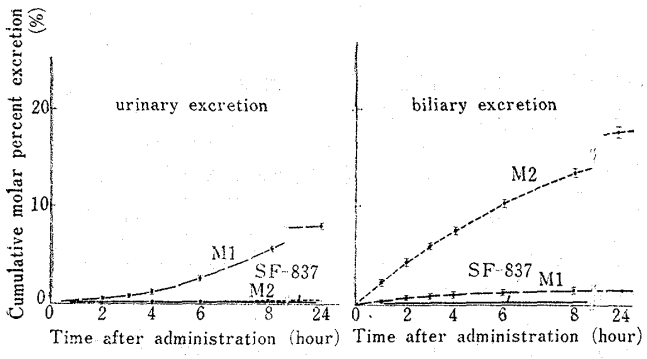


Fig. 14. Urinary and Biliary Excretion of SF-837 and Its Metabolites in Rats after orally Administration (200 mg/kg)

used animal: rat(Donryu, male) body wt. 250—280 g
Determination: densitometry of TLC by Extraction method
plotting: mean value±S.E. ($n=4$)

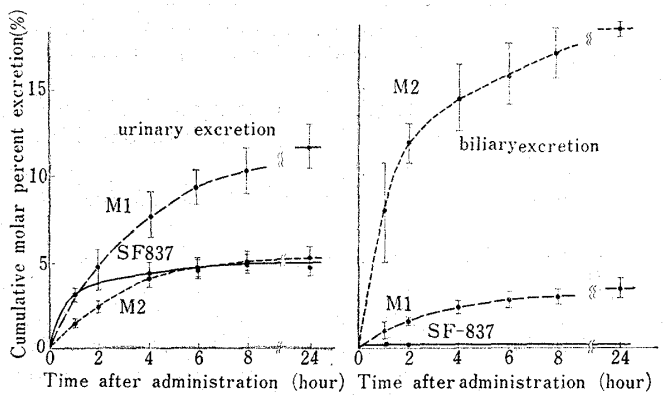


Fig. 15. Urinary and Biliary Excretion of SF-837 and rts Metabolites in Dogs after Intravenous Administration (50 mg/kg)

used animal: dog (Satsuma beagle) body wt. 10—13 kg
determination: densitometry of TLC by extraction method plotting: mean value±S.E. ($n=4$)

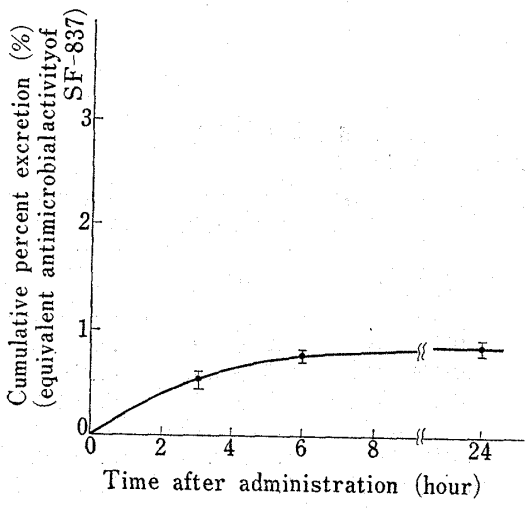


Fig. 16. Urinary Excretion of Antimicrobial Activity in Humans after Oral Administration of SF-837 (1000 mg/man)

determination: bioassay, paper-disk method and cup method (S. lutea)
plotting: mean value±S.E. ($n=3$)

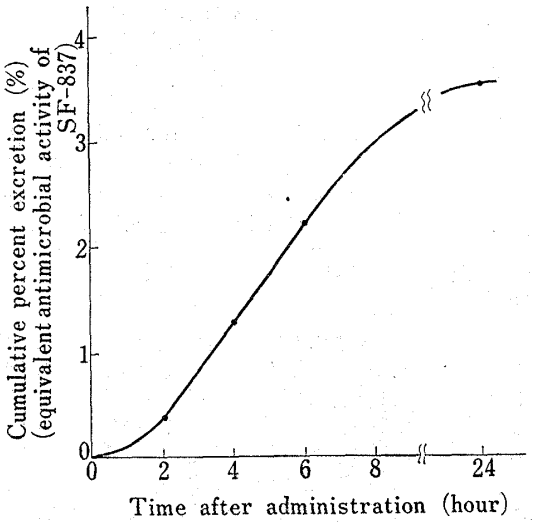


Fig. 17. Biliary Excretion of Antimicrobial Activity in Human after Oral Administration of SF-837 (1000 mg/man)

determination: bioassay, paper-disk method and cup method (S. lutea)

Table I. Urinary and Biliary Excretion of Antimicrobial Activity following Intravenous (50 mg/kg) or Oral (200 mg/kg) Administration of SF-837 in Rat

Excretion rout	Intravenous administration		Oral administration	
	in urine (%) ±S.E. (n=4)	in bile (%) ±S.E. (n=4)	in urine (%) ±S.E. (n=4)	in bile (%) ±S.E. (n=4)
Time after administration				
10 min	1.04±0.16			
30 min	2.57±0.27			
1 hr	3.80±0.45	2.89±0.33	0.12±0.07	0.35±0.20
2	5.94±0.45	5.15±0.53	0.27±0.15	0.67±0.10
3			0.38±0.18	0.99±0.08
4	6.55±0.51	5.98±0.52	0.58±0.20	1.32±0.10
6	6.74±0.51	6.29±0.52	0.87±0.40	1.54±0.10
9	6.83±0.51	6.53±0.52	1.68±0.43	2.12±0.16
24	7.12±0.54	6.91±0.47	2.35±0.41	2.64±0.19

used animal: rat (Donryu, male) body wt. 250—280 g
determination: bioassay, paper-disk method (S. lutea)

Table II. Urinary and Biliary Excretion of SF-837 and Its Metabolites after Intravenous Administration (50 mg/kg) in Rat

Route	in urine (%)±S.E. (n=4)			in bile (%)±S.E. (n=4)		
metabolite / time	SF-837	M1	M2	SF-837	M1	M2
10 min	0.49±0.09	2.15±0.29	0.316±0.063			
30 min	1.06±0.16	5.64±0.43	0.778±0.172			
1 hr	1.39±0.30	9.24±0.56	0.895±0.145	0.016±0.004	4.45±0.49	22.1 ±2.10
2	1.95±0.31	14.31±0.91	1.40 ±0.17	0.019±0.006	5.77±0.79	29.7 ±2.60
4	2.08±0.19	17.05±1.20	1.58 ±0.20	0.022±0.009	5.83±0.82	36.2 ±2.40
6		17.77±1.22	1.68 ±0.24			38.7 ±2.40
8		18.13±1.26	1.68 ±0.24			
9						40.6 ±2.40
24	2.08±0.91	19.31±1.28	1.70 ±0.28	0.022±0.009	5.84±0.82	43.70±2.00

total amount of excretion during 24 hr after administration (%)
SF-837: 2.10±0.19
M1: 25.14±2.10
M2: 45.46±2.28
Total: 72.70±4.57

Table III. Urinary and Biliary Excretion of SF-837 and Its Metabolites after Oral Administration (200 mg/kg) in Rat

Route	in urine (%)±S.E. (n=4)			in bile (%)±S.E. (n=4)		
metabolite / time (hr)	SF-837	M1	M2	SF-837	M1	M2
1	0.070±0.070	0.20±0.10	0.006±0.004	0.003±3.000	0.26±0.56	2.24±0.51
2	0.148±0.147	0.51±0.11	0.029±0.012	0.006±0.000	0.50±0.07	4.45±0.53
3	0.178±0.177	0.79±0.17	0.051±0.024	0.007±0.000	0.72±0.09	6.39±0.51
4	0.198±0.197	1.30±0.32	0.077±0.37	0.008±0.000	0.94±0.10	8.59±0.30
6	0.208±0.207	2.64±0.95	0·214±0.079	0.008±0.000	1.15±0.15	10.76±0.48
9	0.213±0.212	5.88±0.87	0.502±0.113	0.008±0.000	1.46±0.20	13.89±0.20
24	0.213±0.212	8.54±0.80	0.734±0.165	0.008±0.000	1.81±0.22	18.20±1.12

total amount of excretion during 24 hr after administration (%)
SF-837: 0.22±0.21
M1: 10.35±1.02
M2: 18.93±1.28
Total: 29.50±2.51

The blood concentrations of M2 substance following the dosing of SF-837 were very low levels during the experiment after both intravenous and oral administration.

Following oral administration of SF-837 in the rats, maximum blood level was achieved in 0.5—1 hour; and the concentration of M1 substance at the time was 10 mcg/ml; it was presumed that this antibiotic was absorbed very rapidly and efficiently, and then metabolized to the M1 substance immediately. These experiments in the rat were indicated that no important differences in blood levels of unchanged SF-837 and its metabolites could be attained following oral doses and intravenous injections.

Urinary and Biliary Excretion of SF-837 in Rats, Dogs and Men

In rat, dog and man experiments SF-837 and its metabolic transformation products were excreted in the urine and bile, after oral (200 mg/kg per rats and dogs, 1000 mg per man) or intravenous (50 mg/kg) administration (Fig. 13—17, Table I, II, III). Excretion of antimicrobial activity was about 7.1% of the dose in the urine, 7.9% in the bile, within 24 hours after intravenous administration of SF-837 (50 mg/kg) in rats (Table I). Excretion of antimicrobial activity was about 2.4% of the dose in the urine, 2.7% in the bile, within 24 hours, after orally dosing (200 mg/kg) in rats (Table I). By the quantitative estimations in these experiments, the urinary antimicrobial activities were analyzed falling in with about 2.0% of unchanged SF-837, 18.0% of M1 and 1.7% of M2 substance, and the recovery rate in the urine and bile was 72.7%, within 24 hours after intravenous administration of SF-837 at the dose of 50 mg/kg in rats (Table II). Namely the main metabolite in the urine was M1 substance and that in the bile was M2 substance.

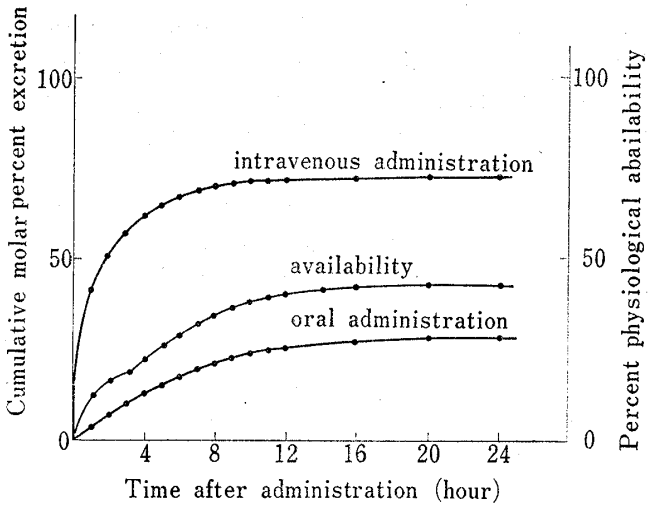


Fig. 18. Availability of Absorption of SF-837 from Rat (by Convolution Analysis from Total Excretion of SF-837 and Its Metabolites in Urine and Bile after Intravenous Administration (50 mg/kg) and Oral Administration (200 mg/kg))

Folowing oral administration in the rat, the urinary excretion curves of SF-837 and its metabolites were indicated the delayed shapes (Fig. 14).

Similar results were obtained also from dogs (Fig. 15) and men (Fig. 16, 17 and Fig. 5).

The absorption-availability (Fig. 18) of this antibiotic in rats was analyzed 40% by the calculation of the convolution analysis of the total excretion in the urine and bile following oral (200 mg/kg) to the intravenous (50 mg/kg) administration.

About the metabolic pathway operated for this compound in those animals the authors presumed that the SF-837 was metabolized at first to the M1 substance in the liver or the body fluid and then transformed to the M2 substance in the liver: and M2 substance excreted immediatly in the bile because of the structure of these metabolites and M2 substance was little appeared in the blood.

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