[Chem. Pharm. Bull. 22(10)2427—2433(1974)]

UDC 547.91.09 : 615.33.015.4.033

Studies on Absorption, Distribution, Metabolism, and Excretion of a New Macrolide Antibiotic, SF-837. II.[1] Pharmacokinetic Studies on SF-837, M1, and M2 Substances

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(Received February 25, 1974)

The absorption studies by means of rat ligated loop technique indicated that this antibiotic was absorbed from the intestine most efficiently in the form of unaltered SF-837, in contrast to the low absorption of the metabolites. Most of the absorption occurred in the middle of small intestine of the rat where SF-837 was remarkably changed into M1. The metabolic pathway of this antibiotic was established as SF-837→M1→M2 from the urinary and biliary excretion study. The biliary to urinary excretion ratio of this antibiotic increased with decreasing dosage, as evidence by the infusion study. From comparative studies on portal and femoral vein infusion of M2, the biliary to urinary excretion ratio was found to be larger after portal vein infusion than the femoral vein infusion. This difference was in particular remarkable at a low speed of infusion (25—200 μg/rat/hr).

Absorption, metabolism, and excretion of the antibiotic, SF-837 (Medemycin), using rats, dogs, and men as test animals, were described in our previous paper.[1] It has been indicated in the previous study that SF-837 was metabolized to substances designated as M1 (4″-depropionyl-SF-837) and M2 (14-hydroxy-4″-depropionyl-SF-837) in these animals after its intravenous and oral dosing, and that M1 was mostly excreted in the urine and M2 mainly in the bile. Excretion of intact SF-837, on the other hand, was very small in the urine and negligible in the bile.

In order to disclose the fate of SF-837 *in vivo*, in particular, in connection with the metabolism of this antibiotic, it seemed to be of importance to compare pharmacokineticaly on their intestinal absorption and excretion of SF-837 and its metabolites.

The intestinal absorption was studied by means of the rat ligated loop technique, and urinary and biliary excretion by means of intravenous administration of these substances at a variety of dose levels, or infusional administration.

Experimental

Materials——SF-837, M1, and M2 used in this investigation were in the form of free base purified in this laboratory by silica gel column chromatography. M1 was prepared by incubating SF-837 with rat liver homogenate,[3] and M2 was isolated from the bile collected from the rat after subcutaneous administration of SF-837.[3] The chemical and biochemical purity of these materials were confirmed by means of the method described following. The thin-layer chromatograms (Eastman chromatogram sheet 6061, solvent system: benzene: acetone=2: 1, $CHCl_3$: MeOH=10: 1) of these materials were sprayed with a solution of 10% H_2SO_4 and heated at about 80° for 3 minutes. SF-837, M1, and M2 appeared as violet, reddish violet, and bluish violet spot in that order. The other TLC plates developed simultaneously were put on the agar plate of *Sarcina lutea* and after 2 hours the TLC plates were taken away and the agar plates were incubated to yield a single inhibition zone.

Intravenous solutions (5—50 mg/ml of the saline solution for the rapid intravenous injection and 50—400 μg/ml of the saline solution for the infusion study) were prepared by dissolving the test compound in an aqueous solution containing a sufficient amount of tartaric acid to dissolve the material.

1) Part I: T. Shomura and K. Umemura, *Chem. Pharm. Bull.* (Tokyo), **21**, 1824 (1973).
2) Location: *Morooka-cho, Kohoku-ku, Yokohama, 222, Japan.*
3) S. Inouye, T. Shomura, T. Tsuruoka, S. Omoto, T. Niida, and K.Umemura, *Chem. Pharm. Bull.* (Tokyo), **20**, 2366 (1972).

Samples injected into the ligated loop were prepared as fine suspensions (8 mg/0.4 ml of the suspension for each loop) in 0.8% sodium taurocholate (Difco Lab., Detroit 1, Michigan USA).

Absorption from Rat Ligated Loop——Male albino Donryu rats (Nippon Rat Co., Tokyo), wieghing 230—250 g, were used after fasting for 16—24 hr. Rats were anesthetized with pentobarbitone-sodium (35 mg/kg, intra-muscular injection), the central mid-line incision was made, the intestine or stomach was pulled out and each of two loops of about 10—11 cm long were prepared from the parts of the gastrointestinal tracts, *i.e.* duodenum, upper, middle, and lower parts of the small intestine, rectum, and whole stomach by ligating the two terminals. Then 0.4 ml of the sample suspension was injected into the lumen of each loop with a syringe, the loop was returned into the abdomen, and the incision was sutured. After a given interval, the loop was removed and the liquid content was washed three times with 2 ml each of cold saline. After cutting the loop open, the mucosal surface was washed three times with 3 ml each of saline. The combined washings were extracted with 30 ml of AcOEt in alkaline condition with 1N NaOH. The cutted loop was homogenized with a Teflon homogenizer in 10 ml of cold saline and centrifuged. The precipitate was washed with 10 ml of cold saline. The combined saline solutions were extracted with 30 ml of AcOEt under alkaline condition. The AcOEt extracts were then evaporated to dryness under a slight vacuum, adjusted to 5 ml with AcOEt, and 2—5 μl was directly applied on a TLC plate for analysis. The recovery in the lumen and tissue estimated by the above procedure was over 96% for 4 experiments. The amount of the antibiotic absorbed from the loop was calculated by subtracting the residual amount in the lumen and in the tissue from the dose administered.

Urinary and Biliary Excretion——Male albino Donryu rats weighing 230—280 g were used for the study of rapid intravenous injection, and the same strain rats weighing 250±2 g for the infusion study. Urine and bile fistula were operated by the method described in a previous paper[1] to examine excretion of substances in urine and bile. In the study of rapid intravenous administration, 1 ml/kg solution of the substance was administered rapidly through the femoral vein. For the infusion study, 3 ml of the saline solution was infused with an automatic micro-infusion pump (Manostat Cassette Pump, New York, N.Y. USA) into the femoral or portal vein at a constant rate (0.5 ml/hr). The rate of 0.5 ml/hr corresponded to that of 25—200 μg/hr of the substance. Urine and bile samples were taken at given intervals after the administration, and the content was examined by the TLC analysis described below.

Analytical Methods——Fractional estimation of the intact antibiotic and its metabolites in the urinary and biliary samples was made by the method described in the previous report, with a slight modification; the concentrated AcOEt extract was adjusted to 100—200 μl in volume and 2—20 μl of the adjusted extract was applied on the TLC plate. Densitometry of the TLC spots visualized with H_2SO_4 was performed by the use of Shimadzu Dual Wavelength TLC Scanner Model CS-200. The AcOEt extracts from the lumens were also treated in the same manner.

Results and Discussion

Absorption of SF-837 from Rat Gastrointestinal Tract

Absorption of SF-837 during 1 hr after its injection into the lumen of the ligated loop prepared from various sections of rat gastrointestinal tract are shown in Table I. It was found that SF-837 was absorbed from the whole of the small intestine, in particular from the middle part where approximately 57% of the dose (8 mg/10 cm of the loop) was disappeared from the lumen during 1 hr. Absorption of the substance from the stomach was found to be relatively low. About 6—38% of SF-837 was changed to M1 in 1 hr in the whole of the small intestine and rectum. The rate of metabolism to M1 was largest in the middle of small intestine where maximum absorption of SF-837 occurred. Accordingly SF-837 was transformed in part to M1 during passage through the rat gastrointestinal tract.

In the stomach, a small amount of SF-837 was decomposed into another substance which showed *Rf* 0.1 on TLC (solvent system: benzene: acetone=2: 1). This substance was considered to be demycarosyl-SF-837. The *Rf* value of this substance corresponded to the standard demycarosyl-SF-837 which was obtained by chemical degradation of SF-837. We presumed that it was formed by hydrolysis of SF-837 by the low pH of the gastric juice.

Although it has been presumed that the absorption of SF-837 from the stomach might be influenced by sodium taurocholate in the sample suspension, the absorption of the suspension without sodium taurocholate showed no significant difference from that containing the salt.

Table I. Absorption[a] of SF-837 Substance from Different Section of Rat Gastrointestinal Tract

Section	Residual amount in gastrointestinal lumen and tissue (mg, $n=4$)[b]			Amount absorbed into blood	
	As SF-837	As M1	As other substance[c]	(mg)	(%)[d]
Stomach	5.83±0.40	—	0.53±0.06	1.65±0.20	20.9±2.4
Stomach	5.69±0.55	—	0.94±0.31	1.37±0.03	17.2±0.4[e]
Duodenum[f]	4.65±0.31	0.62±0.08	—	2.72±0.39	34.0±4.8
Small intestine					
upper	3.39±0.36	1.14±0.12	—	3.70±0.41	46.2±5.2
middle	2.37±0.28	1.43±0.07	—	4.52±0.32	56.7±4.0
lower	3.63±0.45	0.90±0.16	—	3.64±0.19	45.5±4.0
Rectum	4.64±0.33	0.30±0.11	—	2.61±0.41	32.7±5.1

a) The amount of the antibiotic absorbed from the loop was calculated by subtracting the residual amount in the lumen and in the tissue from the dose given. dose per loop: 8 mg/0.4 ml suspension in 0.8% taurocholate solution
b) Mean value ± standard error, 4 animals used for each experiment
c) *Rf* value of this substance was about 0.1 (solvent system was benzene: acetone=2: 1).
d) Percentage transferred into blood to dose in 1 hr (mean value ± standard error).
e) Not containing sodium taurocholate.
f) The common bile duct was ligated.
animal used: male Donryu rat, body wt. 230—250 g

Absorption of the Metabolites (M1 and M2)

Table II shows the intestinal absorption of M1 and M2 from the ligated loop of the middle of small intestine of a rat. The absorption of M1 and M2 was not so rapid as that of the parent substance. Particularly noteworthy was the extremely slow absorption of M2. SF-837 excreted into bile in the form of M2, indicating that the enterohepatic circulation of this substance was not so large.

Table II. Absorption[a] of SF-837 and Its Metabolites from Ligated Loop of Rat Small Intestine

Substance	Amount absorbed druring 1 hr[b] (%)
SF-837	56.7±4.0
M1	17.9±1.1
M2	5.6±0.1

a) the same method as Table I
b) Percentage transferred into blood to dose in 1 hr. (mean value ± standard error)

Urinary and Biliary Excretion of Metabolites after Intravenouse Administration

Fig. 1 and 2 illustrate the urinary and biliary excretion of M1 and M2 after rapid intravenous administration (50 mg/kg) of these metabolites to rats. As shown in these graphs, M1 and M2 were excreted into urine and bile after administration of the M1, while only M2 was excreted after administration of the M2. As reported in a previous paper, after administration of the parent substance, SF-837, M1, and M2 appeared in urine and bile, and no other major metabolite was observed. These results indicated that SF-837 was transformed to M1 and M2, and M1 to M2, but M2 remained intact without further transformation to either M1 or the parent antibiotic. Therefore, metabolic pathway operating for this antibiotic in the rat is that SF-837 is metabolized first to M1 and then to M2.

The dose-responce curves in the urinary and biliary excretion during the first 1.5 hr after intravenous administration of SF-837, M1, and M2 are shown in Fig. 3, 4, and 5. As clearly

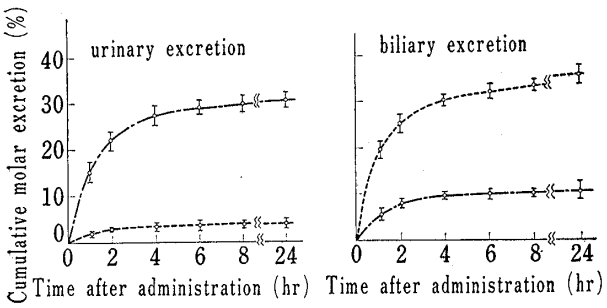

Fig. 1. Urinary and Biliary Excretion of M1 and Its Metabolite in Rat after Intravenous Administration (50 mg/kg)

animal used: male Donryu rat, body wt. 250—280 g
determination by densitometry of TLC by extraction method
plotting: mean vale ± standard error (n=4)
⊥—⊥: M1 ⊥---⊥: M2

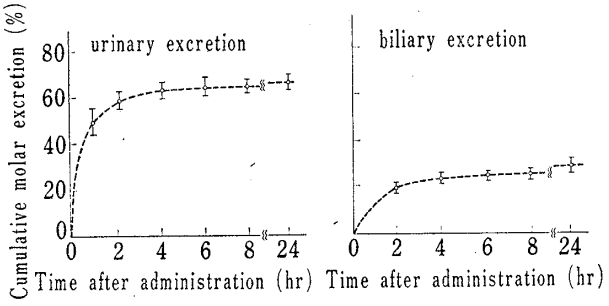

Fig. 2. Urinary and Biliary Excretion of M2 in Rat after Intravenous Administration (50 mg/kg)

animal used: male Donryu rat, body wt. 250—280 g
determination by densitometry of TLC by extraction method
plotting: mean value ± standard error (n=4)
⊥---⊥: M2

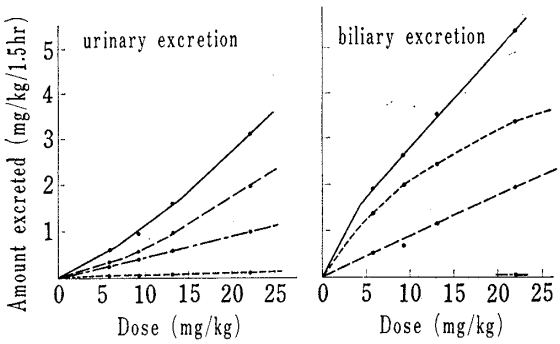

Fig. 3. Dose-response of Urinary and Biliary Excretion of SF-837 and Its Metabolites after Intravenous Administration of SF-837

The responce was observed at 1.5 hr after dosing.
Each point is the average of two determinations on different rats.
animal used: male Donryu rat, body wt. 230—250 g
determination by densitometry of TLC by extraction method
—●—: total, --●--: M1, --●--: M2, --●--: SF-837

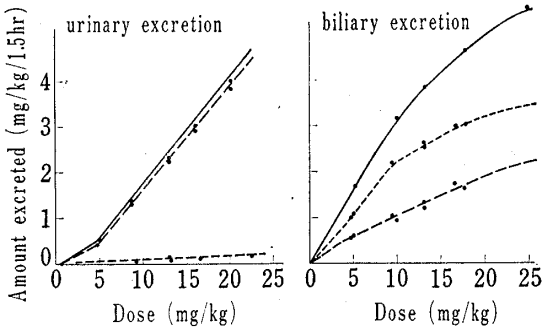

Fig. 4. Dose-responce of Urinary and Biliary Excretion of M1 and Its Metabolite after Intravenous Administration of M1

The responce was observed at 1.5 hr after dosing.
Each point is the average of two determinations on different rats.
animal used: male Donryu rat, body wt. 230—250 g
determination by densitometry of TLC by extraction method
——: total, -----: M1, ------: M2

seen from these graphs, biliary excretion of these substances showed a convergent pattern in many cases, but the urinary excretion showed a divergent curve as the dose increased. From these results, we considered that the urinary excretion of these substances were complementary to the biliary excretion at higher dosing. Accordingly, in the case of a relatively lower dose, the ratio of biliary excretion to the urinary was expected to increase as the dose decreases. This was verified in Fig. 6 which illustrates the dose-response of the biliary to urinary excretion ratio calculated from the above experiments (Fig. 3, 4, and 5). Comparison of the ratio of three substances showed that the biliary excretion ratio was the largest in case of SF-837, followed by M1 and M2 in that order.

Fig. 7 is the dose-response curve of the biliary to urinary excretion ratio of M2 by the rapid intravenous injection of SF-837, M1, and M2. As easily seen from this graph, the biliary excretion ratio of M2 was very large when SF-837 or M1 was administered but this ratio became considerably low when M2 was administered. This large difference in the excretion ratio may be explained by a mechanism whereby M2 formed in the liver from the parent antibiotic or M1 and then was immediately excreted in bile at the rate surpassing its diffusion

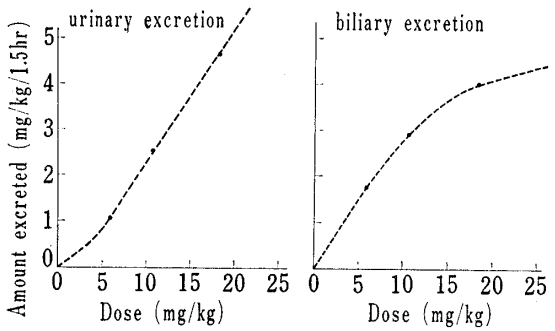

Fig. 5. Dose-responce of Urinary and Biliary Excretion of M2 after Intravenous Administration of M2

The response was observed at 1.5 hr after dosing.
Each point is the average of two determinations on different rats.
animal used: male Donryu rat, body wt. 230—250 g
determination by densitometry of TLC by extraction method.

------: M2

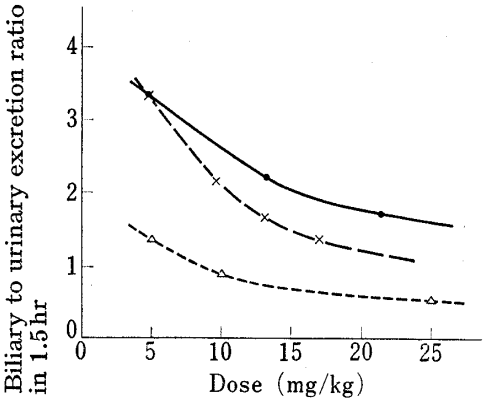

Fig. 6. Total Biliary to Urinary Excretion Ratio *vs.* Dose Plot after Intravenous Administration of SF-837, M1, and M2.

This plot was calculated from Figs 3, 4, and 5.
—●—: from SF-837
--×--: from M1
---△---: from M2

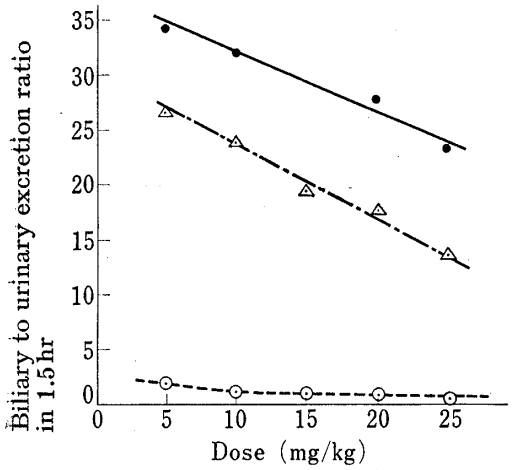

Fig. 7. Biliary to Urinary Excretion Ratio of M2 *vs.* Dose Plot after Intravenous Administration of SF-837, M1, and M2

This plot was calculated from Fig. 3, 4, and 5.
—●—: from SF-837
—△—: from M1
------⊙------: from M2

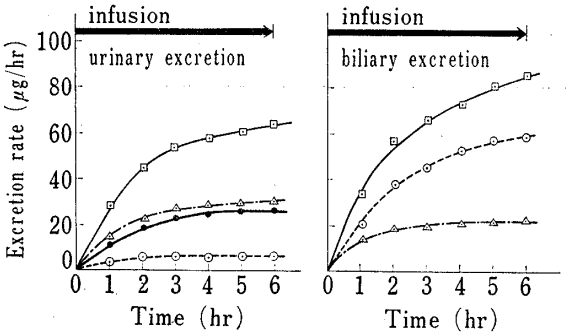

Fig. 8. Urinary and Biliary Excretion Rate of SF-837 Its Metabolites after Femoral Vein Infusion of SF-837

infusion speed: 200 μg/0.5ml/rat/hr
plotting: mean value (n=4)
animal used: male Donryu rat, body wt. 250±2 g
—⊡—: total —△—: M1 substance
------⊙------: M2 substance —●—: SF-837 substance

into blood. In the case of the administration of M2, a considerable amount of M2 was excreted into urine as well as in bile.

From these facts it was considered that there was a hepatic excretion system into bile for each of these three substances and they can be excreted into bile independently.

However, their transport rates from blood to the hepatic excretion system appeared to be not so different because there is a considereable evidence that both the sinusoidal epithelium and the liver cell are exceptionally porous and may not present a barrier to the inward diffusion of most foreign compounds.[4]

Fig. 8, 9, and 10 show the result of infusion of SF-837, M1 and M2 from femoral vein by their urinary and biliary excretion. As is clear from these graphs, the pattern of urinary and biliary excretion after their infusional administration was also similar to that shown after their rapid intravenous injection.

4) D.J. Back and T.N. Calvery, *Brit. J. Pharmacol.*, **44**, 534 (1972).

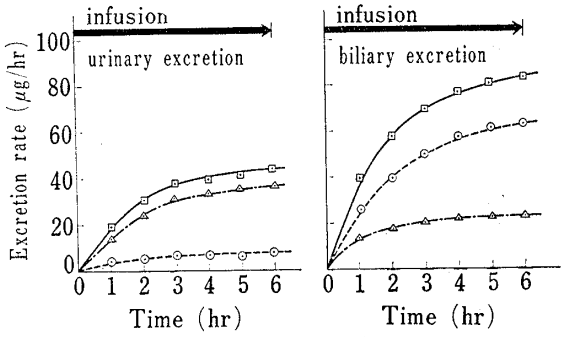

Fig. 9. Urinary and Biliary Excretion Rate of M1 and Its Metabolite after Femoral Vein Infusion of M1

infusion speed: 200 μg/0.5 ml/rat/hr
plotting: mean value (n=4)
animal used: male Donryu rat, body wt. 250±2 g
—⊡—: total
—△—: M1 substance
------⊙------: M2 substance

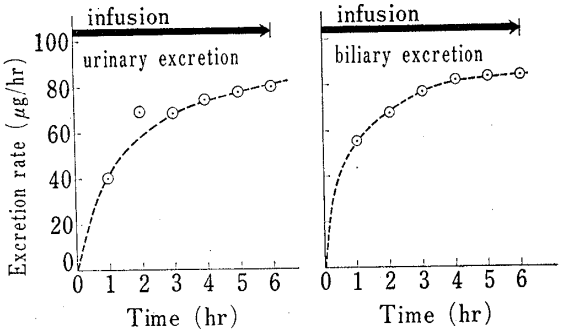

Fig. 10. Urinary and Biliary Excretion Rate of M2 after Femoral Vein Infusion of M2

infusion speed: 200 μg/0.5 ml/rat/hr
plotting: mean value (n=4)
animal used: male Donryu rat, body wt. 250±2 g
------⊙------: M2 substance

TABLE III. Comparison of Excretion Rate of M2 according to Infusion Speed from Femoral Vein

	Time (hr)	Infusion rate[a] (μg/hr) 200	100	50	25
		n=4	n=4	n=4	n=2
Amount excreted in urine (μg)[b]	1	40.0±2.9	22.3±0.5	7.3±1.5	0.22±0.01
	2	69.4±4.3	31.7±1.4	12.6±1.2	3.77±0.09
	3	68.8±2.3	35.2±2.0	13.3±1.7	4.46±0.23
	4	77.4±4.1	36.3±4.0	14.4±3.2	5.23±0.29
	5	81.1±3.8	40.7±5.7	13.0±1.2	4.93±0.18
	6	82.1±3.1	39.1±5.5	13.1±1.3	5.26±0.02
Amount excreted in bile (μg)[b]	1	55.8±5.1	24.9±2.1	12.5±0.6	5.61±0.41
	2	67.6±6.3	34.9±2.5	16.7±0.5	6.47±0.41
	3	76.9±4.0	36.4±2.3	16.9±0.6	6.95±0.05
	4	81.6±3.2	46.3±5.1	18.5±0.5	7.40±0.01
	5	85.0±7.9	49.9±6.0	18.1±0.3	7.40±0.23
	6	87.2±7.0	49.3±5.5	18.0±0.5	7.28±0.08

a) infusion medium: 25—200 μg/0.5 ml saline, infusion period, 0—6 hr
b) mean value of amount excreted ± standard error.
n=number of experiments.
animal used: male Donryu rat, body wt. 250±2 g

TABLE IV. Comparison of Biliary to Urinary Excretion Ratio after Portal and Femoral Vein Infusion of M2 Substance

Infusion speed μg/hr	Biliary to urinary excretion ratio[a] Mean value±standard error: Femoral vein	Portal vein
200	1.06±0.14(84.6),[b] n=4	3.63±0.67(54.9), n=2
100	1.25±0.36(88.6), n=4	4.04±0.85(39.3), n=2
50	1.37±0.20(62.2), n=4	—
25	1.57±0.25(50.2), n=2	5.91±1.21(29.6), n=2

a) Ratio of excreted amount in bile to urine at 5—6 hr during infusion period.
b) Percentage of amount excreted in bile and urine to amount infused at 5—6 hr during infusion period.
n=number of experiment

Table III shows the effect of the infusion speed from femoral vein to the excretion rate of M2. As the infusion speed became slower, the biliary and urinary excretion of M2 decreased markedly.

The comparative study was carried out by two different administration routes, one was the portal vein infusion and the other the femoral vein infusion by which the substance would not pass through the liver before distribution. Infusion through these two different routes was carried out at various speed (25—200 μg/0.5 ml/rat/hr) and its results are shown in table IV and Fig. 11, plotted for values during 5—6 hr of infusion period.

These results clearly showed a difference in the biliary to urinary excretion ratio between portal and femoral vein infusion. With lower speed of infusion, the biliary excretion ratio was larger by portal vein than by femoral vein infusion, but at a higher speed of infusion, this difference was narrowed.

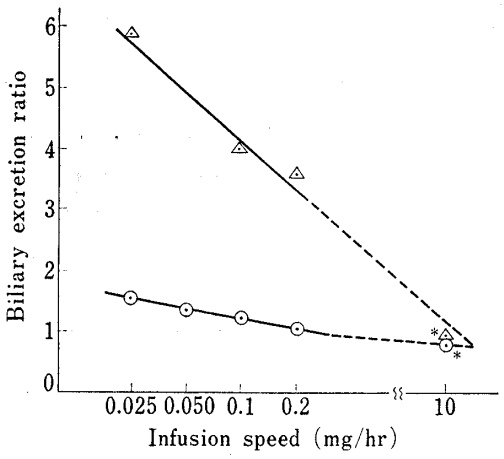

Fig. 11. Comparison of Biliary to Urinary Excretion Ratio after Portal or Femoral Vein Infusion of M2

plotting: mean value ($n=4$)
*$n=1$
*the result of experiment one
—△—: portal vein —⊙—: femoral vein

From these results, it was concluded that the biliary excretion of M2 was larger than its urinary excretion, and that this difference was larger after its portal vein infusion but, at a higher rate (10 mg/hr), the biliary excretion of M2 became saturated, and consequently, its urinary excretion increased. As a result, the difference in excretion after portal and femoral vein infusion became smaller.

The percentage of the amount excreted into bile and urine to that infused during the consecutive 5—6 hr was fairly small in spite of the apparent equilibrium (Table IV). This percentage, however, decreased markedly when the infusion speed became lower. It was further noted that the percentage after portal vein infusion was much smaller than that after femoral infusion. From these facts, it can be considered that a substance infused *via* the portal vein received decomposition and metabolism by the liver more than that *via* the femoral route.

Acknowledgement The authors thank Miss Kiyoko Kojima for her invaluable technical assistance and cooperation.