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Metabolism of 4-Ethoxy-2-methyl-5-morpholino-3(2H)-pyridazinone (M73101), a New Anti-inflammatory Agent. II.[1] Species Differences of Metabolism and Excretion[2]

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The species differences of metabolism of 4-ethoxy-2-methyl-5-morpholino-3(2H)-pyridazinone (M73101) were studied. The urinary and fecal metabolites in six animals (dog, mouse, rabbit, rat, monkey, and guinea pig) were analyzed qualitatively and quantitatively by thin-layer chromatography and gas-liquid chromatography in addition to gas chromatography–mass spectrum determination. From the results of qualitative analysis, seven to ten metabolites in urine were identified in each animal. From the results of quantitative analysis, total excretion percentage of unchanged M73101 and its metabolites in urine were 50.0, 48.4, 73.8, 56.1, 52.7, and 21.1% of the dose administered in dogs, mice, rabbits, rats, monkeys, and guinea pigs, respectively. The main metabolite excreted in urine was 5-[2-(carboxymethyloxy)ethylamino]-4-ethoxy-2-methyl-3(2H)-pyridazinone (M-9) in dogs, mice, rabbits, and rats and 5-(N-carboxymethyl-N-2-hydroxyethylamino)-4-ethoxy-2-methyl-3(2H)-pyridazinone (M-8) in monkeys and guinea pigs. For the fecal excretion, 59.4, 19.5, 17.7, and 15.3% of the dose administered were excreted in guinea pigs, mice, dogs, and rats, respectively, and rabbits and monkeys hardly excreted any metabolites. From the results of urinary and fecal excretion, the main excretion route was through liver and bile duct into feces in guinea pigs and was into urine in other five animals. For the effects of the dose administered on metabolism, dose-dependent variations of metabolism characteristic of guinea pigs were observed in biliary excretion, that is, main metabolite was M-8 in low dose (20 mg/kg) while M-9 in high dose (500 mg/kg) but such phenomenon was not found in rats.

Keywords——drug metabolism; species differences; identification and determination; effect of the dose on metabolism; metabolism of morpholino group; anti-inflammatory drug

Introduction

We previously reported on the metabolic fate of 4-ethoxy-2-methyl-5-morpholino-3(2H)-pyridazinone (M73101) in rabbits[1] and human,[4] in which there were observed some pronounced differences in metabolic pattern between two species. Thus, for example, in the biotransformation of morpholino group which involved the most important metabolic process in the metabolism of M73101, the oxidative cleavage of O–C bond was main in human, while split of N–C bond was main in rabbits. These observations prompted us to investigate the species differences in the metabolism of M73101 between several experimental animals used in pharmacological and toxicological studies of M73101 and to compare the results with those of human, because it has been now accepted to be very important that the species differences between experimental animals and human, if any, should be kept in mind in the extrapolation of observations from animals to man.

1) Part I: T. Hayashi, M. Sato, M. Ohki, and T. Kishikawa, *Chem. Pharm. Bull.*, (Tokyo), **26**, 3124 (1978).
2) A part of this work was presented at the 98th Annual Meeting of Pharmaceutical Society of Japan, April 1978, p. 161.
3) Location: *1658 Ohshinohara, Yasu-cho, Yasu-gun, Shiga, 520-23, Japan.*
4) T. Seki, T. Hayashi, M. Ohki, and T. Kishikawa, *Rinsho Yakuri*, **9**(2), 149 (1978).

The present paper deals with qualitative and quantitative analysis of metabolites of M73101 excreted in urine, feces and/or bile of dogs, mice, rabbits, rats, monkeys, and guinea pigs after oral administration. In addition, the effects of the dose administered on metabolism were investigated in rats and guinea pigs.

Experimental

Materials——M73101 was white needle crystal (mp 89—92°) synthesized according to the method described by Takahashi.[5] Authentic samples were synthesized in our laboratories.[6]

Human Study——The details about the administration of the drug and the determination of metabolites in urine were reported previously.[4]

Animal Studies——The animals used in these experiments were as follows: male albino rabbits (weighing about 3.0 kg), male Wistar rats (weighing about 200 g), male JCL-ICR mice (weighing about 25 g), male Hartley guinea pigs (weighing about 400 g), male beagle dogs (weighing about 9.0 kg) and female rhesus monkeys (weighing about 8.0 kg). Rabbits, rats, mice and guinea pigs received the drug orally after 16 hr-fasting in a dose of 100 mg/kg, and dogs and monkeys in a dose of 50 and 25 mg/kg after 16 hr-fasting, respectively. The urine and feces were collected for 0—24 and 24—48 hr after administration in all cases. Only in case of guinea pigs, the qualitative and quantitative determination of biliary excreta was carried out. In this experiment, the animals were anesthesized with urethane (1.2 g/kg) and then the common bile duct was cannulated with polyethylene tube through an abdominal incision. Bile was collected at 0—4, 4—8, and 8—24 hr intervals after administration.

Thin-Layer Chromatography (TLC)——TLC analysis was carried out in the same conditions as described previously.[1] The resulting chromatograms were visualized under ultraviolet (UV) light (2536 Å) or by spraying Folin Ciocalteu reagent.[7] The thin-layer radiochromatograms were obtained with an Aloka Thin-layer Chromatograms Scanner Model TRM-1B.

Gas-Liquid Chromatography (GLC)——Quantitative GLC analysis was carried out in the same conditions as described previously.[1]

Extraction and Determination of M73101 and Its Metabolites——Urine: Unchanged M73101 and its metabolites were fractionated as usual to the neutral and acidic fractions and each fraction was applied to GLC for qualitative or quantitative analysis. The details of these procedures were described in previous report.[4]

Feces: Feces were extracted with water after being finely ground. An aliquot of extracts was treated by the same procedures as in case of urinary analysis.

Bile: Biliary analysis was carried out by the same procedures as in case of urinary one.

Measurement of Radioactivity——Rats were given orally M73101 labelled with ^{3}H at the 6-position of pyridazinone ring (specific activity 50 μCi/mg) in a dose of 100 mg/kg. A definite volume of urinary sample was mixed with a given volume scintillator (15 ml) prepared with dioxane (750 ml), toluene (150 ml), methanol (100 ml), PPO (4 g) and naphthalene (100 g). Radioactivity was determined with a Beckman LS-100 liquid scintillation system.

GC-MS Determination——GC–MS spectra were recorded by a double focussing mass spectrometer (JMS-01SG) equipped with gas chromatography (JGC-20K) using a total ion monitor as a detector. Mass spectra (MS) were measured in following conditions: ion accelerating voltage; 6000 V, ionizing current; 200 μA, ionization energy; 30 eV, temperature of ionization chamber; 200—300°, and temperature of molecular separator; 280—300°. Gas-liquid chromatography was used in the following conditions: column; glass column packed with 2% OV-17 on chromosorb W(AW-DMCS), carrier gas; He(2 kg/cm^2), oven temperature; 175—200° (programming rate 2°/min).

Results

I Identification of Urinary Metabolites

Rabbit——As reported previously,[1] unchanged M73101 and ten metabolites have been identified in urine of rabbits. The structures of these metabolites were as follows: 4-Hydroxy-2-methyl-5-morpholino-3(2H)-pyridazinone(M-1), 5-[Bis(2-hydroxyethyl)amino]-4-ethoxy-2-methyl-3(2H)-pyridazinone (M-2), 4-Ethoxy-5-[2-(β-hydroxyethyloxy)ethylamino]-2-methyl-3-

5) T. Takahashi, M. Takaya, Y. Maki, and T. Satoda, Japan. Patent 47-24030 (1972) [C.P.I. 44179T (1972)].
6) M. Takaya, T. Yamada, and H. Shimamura, *Yakugaku Zasshi*, **98**, 1421 (1978).
7) E. Stahl, "Thin Layer Chromatography," Springer-Verlag, Berlin, 1969, p. 878.

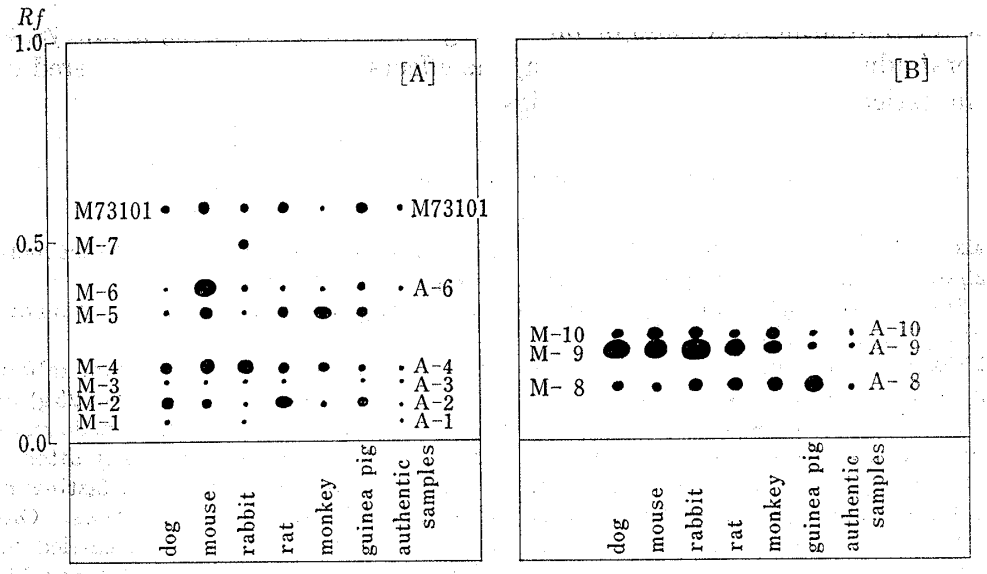

Fig. 1. Thin-Layer Chromatograms of Urinary Metabolites in Various Animals

[A]: neutral fraction of urinary extracts; solvent system A [ether-$CHCl_3$-EtOH (15: 10: 2, v/v)].
[B]: acidic fraction of urinary extracts; solvent system C [$CHCl_3$-EtOH-AcOH (25: 1: 1, v/v)].
A-1,2,3,4,6,8,9,10: synthesized authentic samples.

(2H)-pyridazinone(M-3), 4-Ethoxy-5-(2-hydroxyethylamino)-2-methyl-3(2H)-pyridazinone(M-4), 4-Ethoxy-5-(2-hydroxy-tetrahydro-1,4-oxazin-4-yl)-2-methyl-3(2H)-pyridazinone(M-5), 4-Ethoxy-5-morpholino-3(2H)-pyridazinone(M-6), 4-Ethoxy-2-methyl-5-(3-oxo-tetrahydro-1,4-oxazin-4-yl)-3(2H)-pyridazinone(M-7), 5-(N-Carboxymethyl-N-2-hydroxyethylamino)-4-ethoxy-2-methyl-3(2H)-pyridazinone(M-8), 5-[2-(Carboxymethyloxy)ethylamino]-4-ethoxy-2-methyl-3(2H)-pyridazinone(M-9), 5-Carboxymethylamino-4-ethoxy-2-methyl-3(2H)-pyridazinone(M-10). The eight metabolites other than M-1 and M-6 were the biodegradated products resulted from the oxidation of morpholino group.

Rat——The identification of urinary metabolites in rats was carried out by TLC, GLC, and GC–MS. Behaviours on TLC and GLC (*Rf* values and retention times) of each fraction obtained from rat urine were compared with those of authentic samples synthesized or isolated from rabbit urine. Thin-layer chromatogram and radio-chromatogram were depicted in Fig. 1 and 2, respectively. Apart from unchanged M73101, eight metabolites were found in rat urine; M-2, M-3, M-4, M-5, M-6, M-8, M-9, and M-10.

Mouse and Monkey——The thin-layer and gas-liquid chromatograms of neutral and

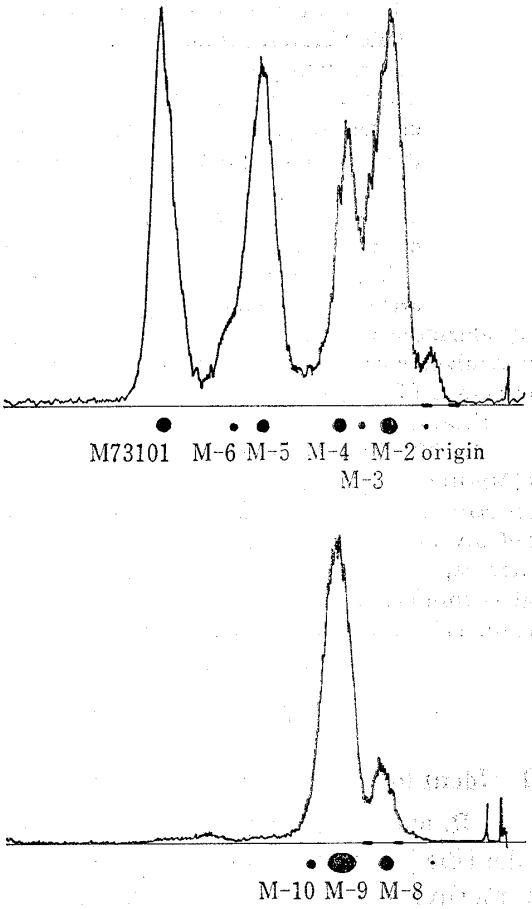

Fig. 2. Radiochromatogram of Urinary Metabolites in Rats

Upper chromatogram: neutral fraction, solvent system A.
Lower chromatogram: acidic fraction, solvent system C.

acidic fractions obtained from urine of mice and monkeys were compared with those of the authentic samples synthesized or isolated from rabbit urine. The unchanged M73101 and eight metabolites were found in urine of mice; M-2, M-3, M-4, M-5, M-6, M-8, M-9, and M-10, while the unchanged M73101 and seven metabolites were found in urine of monkeys; M-2, M-4, M-5, M-6, M-8, M-9, and M-10. Moreover, GC–MS analysis was successfully applied to detection of urinary metabolites of mice and monkeys as described later.

Guinea Pig and Dog——The identification of urinary metabolites in guinea pigs and dogs was carried out in the same manner as described in cases of mice and monkeys. Eight metabolites were found together with unchanged M73101 in urine of guinea pigs; M-2, M-3, M-4, M-5, M-6, M-8, M-9, and M-10, while in urine of dogs, nine metabolites were found as well as unchanged M73101; M-1, M-2, M-3, M-4, M-5, M-6, M-8, M-9, and M-10.

II GC–MS Analysis

As described in the preceding section, urinary metabolites in rats, mice, and monkeys were also determined by GC–MS, *i.e.* mass spectrum obtained from GC–MS analysis was compared with that of each authentic sample isolated from rabbit urine. The entire profile of

Table I. Urinary and Fecal Excretion of Unchanged M73101 and Its Metabolites in Various Animals and Human

	Dog		Mouse		Rabbit	
	Urine	Feces	Urine	Feces	Urine	Feces
M73101	0.3±0.1	Trace or n.d.	2.3±0.1	Trace or n.d.	1.0±0.1	Trace or n.d.
M-1	Trace		n.d.		Trace	
M-2+M-3	2.2±0.2		0.6±0.0		Trace	
M-4	1.8±0.1		1.7±0.1		2.3±0.3	
M-5	0.3±0.0		0.8±0.1		Trace	
M-6	Trace		3.1±0.1		0.5±0.2	
M-7	n.d.		n.d.		1.0±0.3	
M-8	2.7±0.5		8.7±0.8		16.4±2.1	
M-9	38.8±3.8	16.1±1.5	29.1±0.4	19.5±1.9	44.8±1.3	
M-10	3.9±0.3	1.6±0.3	2.2±0.3	Trace	7.8±0.5	
Total[a]	50.0±3.2	17.7±1.5	48.4±0.9	19.5±1.9	73.8±2.8	Trace
Total[b]	67.7±4.4		67.9±1.6		73.8±2.8	

	Rat		Monkey		Guinea pig		Human
	Urine	Feces	Urine	Feces	Urine	Feces	Urine
M73101	1.9±0.4	Trace or n.d.	Trace or n.d.	Trace or n.d.	1.1±0.2	Trace or n.d.	0.6
M-1	n.d.				n.d.		Trace or n.d.
M-2+M-3	2.5±0.2				1.0±0.0		
M-4	Trace				0.4±0.0		
M-5	0.8±0.0				1.0±0.1		
M-6	Trace				0.8±0.2		
M-7	n.d.				n.d.		
M-8	18.4±0.9		25.7±1.8		11.5±2.3	39.1±2.0	59.3
M-9	27.6±0.6	13.2±1.8	17.5±1.6		4.2±0.5	17.5±2.4	5.9
M-10	4.9±0.4	2.1±0.3	9.5±0.7		1.1±0.5	2.8±0.4	9.2
Total[a]	56.1±1.4	15.3±1.9	52.7±3.4	Trace	21.1±1.9	59.4±1.7	75.0
Total[b]	71.4±1.4		52.7±3.4		80.5±3.1		

Each value represents percentage of the dose administered (mean ±S.E.) for dog (n=5, 50 mg/kg), mouse (n=24, 100 mg/kg), rabbit (n=5, 100 mg/kg), rat (n=5, 100 mg/kg), monkey (n=5, 25 mg/kg), and human (n=2, 15 mg/kg) within 24 hr, and guinea pig (n=5, 100 mg/kg) within 48 hr.
a) Urine or feces.
b) Urine plus feces.

mass spectrum of each peak detected by total ion monitor showed a complete identity with that of each corresponding standard sample in all cases.

III Identification of Fecal Metabolites

The almost metabolites found in urine were detected also in feces of all animals though the amounts in feces were much less than those in urine in five animals except for guinea pigs as described later.

IV Quantitative Determination of Metabolites

The urine and feces were collected during every 24 hr for 2 days and unchanged M73101 and its metabolites were determined quantitatively by GLC. The excreted amount during 24—48 hr was much smaller or negligible except for the fecal significant excretion in guinea pigs. The amounts of unchanged M73101 and its metabolites excreted in urine and feces after oral administration to dogs, mice, rabbits, rats, monkeys, guinea pigs, and human were summarized in Table I. It was observed that M-8 and M-9 were excreted in larger quantities into the urine of all animals and human.

Dog, Mouse, Rabbit, and Rat——The amounts of unchanged M73101 in urine of dogs, mice, rabbits and rats were only 0.3%, 2.3%, 1.0% and 1.9% of the dose administered, respectively. The main metabolite in these four animals, dog, mouse, rabbit and rat, was M-9, and the amount of this metabolite recovered in urine during 0—24 hr after oral administration was 38.8%, 29.1%, 44.8%, and 27.6% of the dose administered and total amount of the excreta recovered in the urine during 0—24 hr was 50.0%, 48.4%, 73.8%, and 56.1% of the dose, respectively. Metabolites in feces were found in a considerable amount except in rabbits. M-9 was also the main metabolite in feces and the amount of this metabolite during 0—24 hr was 16.1%, 19.5% and 13.2% of the dose administered in dogs, mice, and rats, respectively. Total amount of excreta for 0—24 hr in urine and feces was 67.7%, 67.9%, 73.8%, and 71.4% in dogs, mice, rabbits, and rats, respectively.

Table II. Biliary Excretion of M73101 and Its Major Metabolites after Oral Administration of Various Doses in Guinea Pigs

	Dose	20 mg/kg	100 mg/kg	500 mg/kg
0— 4 hr	M73101	Trace	0.6	0.9
	M-8	24.8±4.9	13.5±0.9	3.0±1.1
	M-9	3.7±1.8	8.3±0.2	9.4±0.2
	M-10	Trace	0.4±0.1	0.2±0.0
	Ratio (M-8/M-9)	6.7	1.6	0.3
4— 8 hr	M73101	Trace	0.2	0.6
	M-8	15.4±2.5	11.0±1.1	2.9±0.9
	M-9	1.4	4.0±0.4	8.0±0.2
	M-10	Trace	0.7±0.1	0.3±0.0
	Ratio (M-8/M-9)	11.0	2.8	0.4
8—24 hr	M73101	n.d.	0.2	Trace
	M-8	4.4±1.4	13.9±2.2	3.0±1.1
	M-9	Trace	3.1±0.6	10.6±1.3
	M-10	Trace	1.1±0.2	0.8±0.1
	Ratio (M-8/M-9)	—	4.5	0.3
0—24 hr	M73101	Trace	1.0	1.5
	M-8	44.6±5.4	38.4±3.0	8.9±2.9
	M-9	5.1±1.8	15.4±0.5	28.0±1.6
	M-10	Trace	2.2±0.2	1.4±0.2
	Ratio (M-8/M-9)	8.8	2.5	0.3

Percentage of the dose administered (mean±S.E., $n=4$).

Monkey and Guinea Pig——As shown in Table I, unlike the four animals just mentioned above, the main metabolite in urine of monkeys and guinea pigs was M-8 and the excretion percentage of this metabolite in urine was 25.7% in monkeys during 0—24 hr and 11.5% in guinea pigs during 0—48 hr. Unchanged M73101 was also scarcely excreted in these two species as in four animals described above. Total amount of excreta recovered in urine during 0—24 hr was 52.7% in monkeys, while that in guinea pigs during 0—48 hr was only 21.1%. On the other hand, in feces, the poor excretion was observed in monkeys, but much larger amount of metabolites was found in guinea pigs, in which 59.4% of the dose administered was excreted in feces during 0—48 hr (46.2% during 0—24 hr, and 13.2% during 24—48 hr).

Human——For the purpose of reference, the results in human reported previously[4] were cited again in Table I. The main bioproduct excreted in urine was also M-8 as in monkeys and guinea pigs, and about 60% of the dose was recovered as M-8. Total amount of excreta in urine during 0—24 hr was 75.0% of the dose administered.

V Biliary Excretion

In guinea pigs, the amounts of unchanged M73101 and major metabolites (M-8, M-9 and M-10) excreted in bile were 22.8% of the administered dose during 0—4 hr, 15.9% during 4—8 hr, 18.3% during 8—24 hr, and therefore 57.0% during 0—24 hr in a dose of 100 mg/kg, as shown in Table II. Like in urine or feces, M-8 was the main metabolite also in bile, in which about 40% of the dose was excreted as M-8 during 0—24 hr, but the amount of unchanged M73101 was only 1% of the dose.

VI The Effects of the Dose Administered on Metabolism

Effects of various doses on the qualitative and quantitative aspects of metabolism of M73101 were investigated in rats and guinea pigs. The drug was administered at three levels of dose and the excreta in urine of rats and in bile of guinea pigs were determined. The results are shown in Tables II and III, in which the excretion percentages of unchanged M73101 and major metabolites (M-8, M-9, and M-10) after oral administration in each animal in doses of 20 mg/kg to 500 mg/kg are tabulated. As seen from Table III, in rats, the variation of metabolic pattern hardly arised with changes of the dose administered. On the other hand, in guinea pigs, a large difference of metabolic pattern was found between low- and high-dose levels as shown in Table II. In guinea pigs, the biliary excretion of M-8 extremely decreased with increase of the administered dose, *i.e.* 44.6%, 38.4% and 8.9% of the dose were recovered as M-8 for 24 hr at the dose of 20 mg/kg, 100 mg/kg and 500 mg/kg, respectively. On the contrary, the proportion of M-9 increased as the dose increased, *i,e.* 5.1%, 15.4% and 28.0% at the dose levels of 20 mg/kg, 100 mg/kg and 500 mg/kg, respectively. The excretion of unchanged M73101 was slight in all doses administered.

Table III. Urinary Excretion of M73101 and Its Major Metabolites after Oral Administration of various Doses in Rats

Dose	20 mg/kg	100 mg/kg	500 mg/kg
M73101	1.4±0.1	1.4±0.2	1.5±0.3
M-8	11.0±0.5	8.0±0.6	3.8±0.5
M-9	29.3±1.6	28.0±1.7	20.9±3.4
M-10	3.2±0.2	3.5±0.4	1.7±0.1
Ratio (M-8/M-9)	0.4	0.3	0.2

Percentage of the dose administered within 8 hr (mean±S.E., $n=4$).

Discussion

The metabolism of M73101 was studied using such experimental animals as dogs, mice, rabbits, rats, monkeys, and guinea pigs to know whether there were species differences in biotransformation of M73101. From the results of qualitative analysis of urinary metabolites in these animals, it has been revealed that eight to ten metabolites as well as unchanged M73101 were found in all species used, *i.e.* ten in rabbits, nine in dogs, eight in rats, mice and guinea pigs and seven in monkeys. In human, as reported previously,[4] seven metabolites

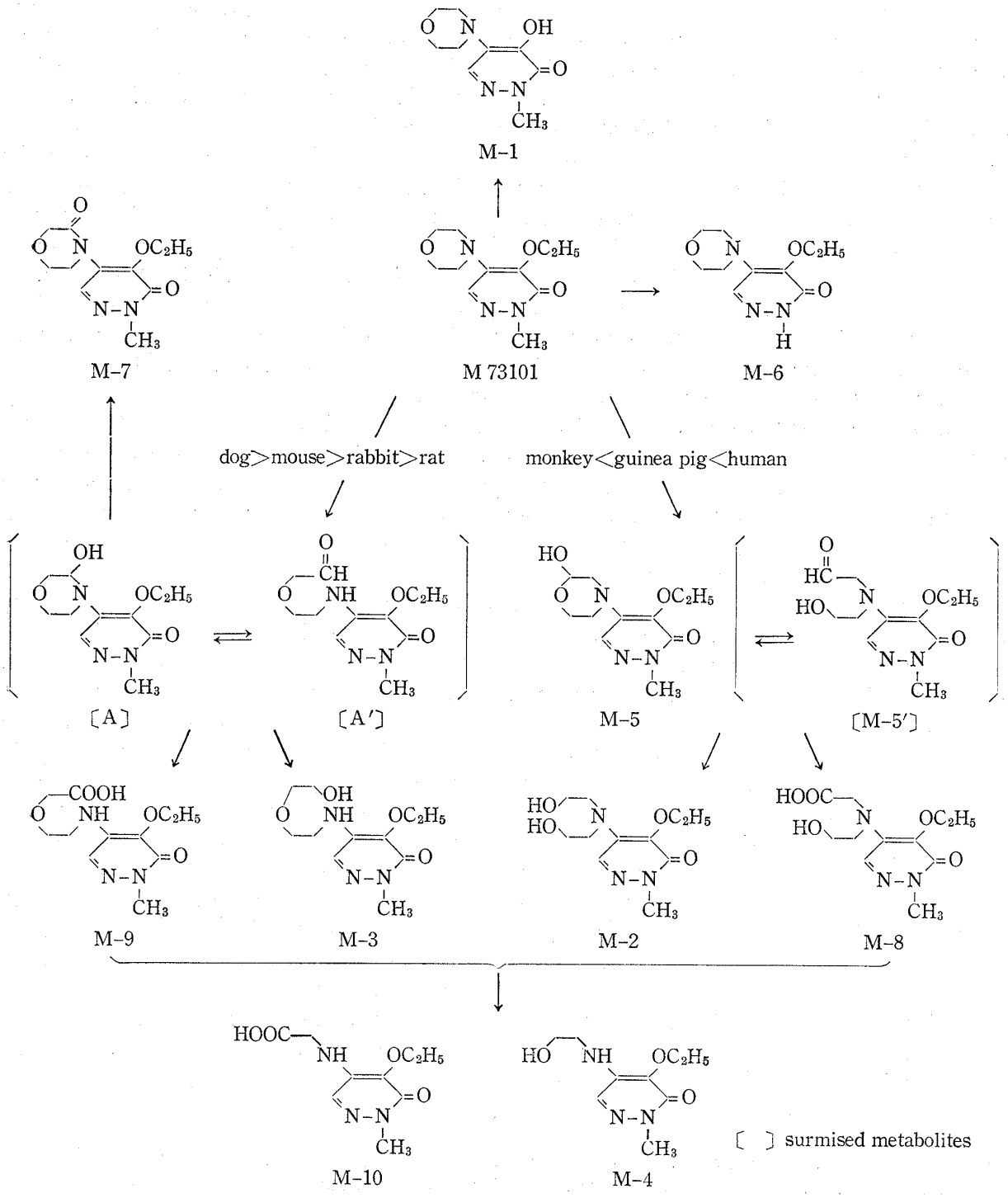

Fig. 3. The Proposed Metabolic Pathways of M73101

were identified. Among these ten metabolites, M-1 was found only in rabbits and dogs, M-7 only in rabbits, M-3 was found in all animals except for in monkeys and human, but other seven metabolites (M-2, M-4, M-5, M-6, M-8, M-9, and M-10) were common to all animals, which meant that the most parts of metabolic pathway might be the same in all species though a ratio of each metabolite fairly varied in each animal. The outline of the metabolic pathway is shown in Fig. 3, which was derived from the qualitative and quantitative determinations of urinary metabolites. The details about each metabolic process were explained in previous report.[1)]

We discuss the species differences from the view of quantitative points. In the results of quantitative analysis of urinary metabolites, the most striking difference between animals used was seen in the excretion percentage of M-8 and M-9. In dogs, mice, rabbits and rats, the main metabolite was M-9 but that in monkeys and guinea pigs was M-8, which was the case also in human. In order to simplify the results, the ratios of M-8 to M-9 (M-8/M-9) were given as follows; dog 0.1, mouse 0.3, rabbit 0.4, rat 0.7, monkey 1.5, guinea pig 2.7, and human 10.1, that is, in this order the proportion of M-8 increased at the expense of M-9. These facts strongly suggested that in morpholino biodegradation, the oxidation of carbon atom adjacent to nitrogen atom was dominant in dogs, mice, rabbits and rats, while the oxidative cleavage of O–C bond was dominant in monkeys, guinea pigs and notably in human, in which the ratio, M-8/M-9, was strikingly larger (10.1) and so the most pronounced difference between human and other animals was the position of oxidation in morpholino group.

With regard to fecal excretion, the most pronounced difference between six animals used was that in guinea pigs much larger quantities of fecal metabolites were found, and so we investigated the biliary excretion in this animal. The main site from which the metabolites were excreted was thought to be kidney (urine) in most animals other than guinea pigs, in which the major route of excretion might be into feces *via* bile duct, since the amounts of fecal excreta were 2.8 times of those in urine. These facts seemed to suggest the presence of the specific excretion mechanism in guinea pigs unlike other five animals used and human. The reabsorption into enterohepatic circulation and the secondary alteration of the metabolites by gut microflora did not seem to play an important role in guinea pigs because the amount of each metabolite in bile was almost equal to that in feces.

Some of works about the variation of metabolism by changes in the dose administered have been so far reported. One example is that on salicylamide[8)] in which the balance of sulfate and glucuronide formation was altered by change of dose and this phenomenon was interpreted by "saturation of metabolism". Another examples about testosterone[9)] and aminopyrine[10)] indicated that dose-dependent variations of metabolism could be produced by "substrate inhibition". In the present study, such dose-dependent alteration of metabolism was markedly recognized in biliary excretion of guinea pigs; the ratio of M-8 to M-9 at a dose of 20 mg/kg, 8.8 shifted to 0.3 at a dose of 500 mg/kg, namely the metabolic pattern in guinea pig bile was similar to that in human urine at a lower dose, while at higher dose it shifted to the pattern in mouse or rabbit urine. It has not been yet clarified what mechanism was involved at enzymatic levels in such dose-dependent variation of metabolism as observed in this experiment. It was probably suggested from the results of our preliminary experiments (unpublished data) that this change of metabolism were based on the saturation of enzyme system, that is, the biodegradation of morpholino group might be carried out by two microsomal oxidation systems with different affinities and capacities. On the contrary, in rats, the variation of metabolism by changes of dose was scarcely observed. This fact was thought to be a kind of the species differences and gives us some interesting problems to be solved.

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9) M. Jacobson, W. Levin, and R. Kuntzman, *Biochem. Pharmacol.*, **18**, 2253 (1969).
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The most metabolites among those described in this report had been already shown to have no pharmacological and toxicological activities.[1] It was certainly thought, therefore, that some types of species differences in metabolism discussed here might not cause species differences in pharmacological and toxicological responce in experiments using such animals as mentioned above.