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Metabolism of Drugs. LXXXVI.[1] The Metabolic Fate of Nitrofuran Derivatives. (4).[2] The Portal Absorption of Nitrofuran Derivatives and the Absorption Rate as a Function of Age in Rats

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The absorption route of ^{14}C-AF-2(2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide) and ^{14}C-nitrofurazone(5-nitro-2-furfural semicarbazone) was examined with thoracic duct or portal vein cannulated rats. As a result, it was found that the radioactivity was almost exclusively absorbed *via* portal system from intestinal tract after administration of the above labeled compounds to rats. In addition, the present study revealed the direct evidences concerning the metabolism of the above nitrofuran derivatives in the intestinal mucosa of rats and the absorption of their metabolites from the intestinal wall. Furthermore, it was demonstrated that the absorption rate of radioactivity was lower in 7 weeks old rats than in over 8 weeks old rats after oral administration of ^{14}C-AF-2 and ^{14}C-NF-161 (2-amino-5-[2-(5-nitro-2-furyl)-1-(2-furyl)vinyl]-1,3,4-oxadiazole).

Previously, we reported the excretion and absorption of nitrofuran derivatives after oral administration of the ^{14}C-labeled compounds to rats.[4] In the subsequent studies, it was found that a part of nitrofuran derivatives given orally to rats was metabolized by the small intestinal mucosa and that the absorption rate of the radioactivity had close relationship with the metabolic rate.[5] Furthermore, it was shown that the initial reaction in the metabolism of 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) and 5-nitro-2-furfural semicarbazone (nitrofurazone) described above are mainly catalyzed by xanthine oxidase, while that of 2-amino-5-[2-(5-nitro-2-furyl)-1-(2-furyl)vinyl]-1,3,4-oxadiazole (NF-161) is catalyzed by alternative enzyme beside xanthine oxidase.[2]

It was examined in this study, whether the absorption of ^{14}C-AF-2 and ^{14}C-nitrofurazone from the rat intestinal tract to the circulation was made through the lymphatic or the portal system. In addition, the absorption rate of the radioactivity as a function of age after oral administration of ^{14}C-AF-2 and ^{14}C-NF-161 was also described.

Experimental

Materials——^{14}C-AF-2 [2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (acrylamide-3-^{14}C)], ^{14}C-NF-161 [2-amino-5-(2-(5-nitro-2-furyl)-1-(2-furyl)vinyl)-1,3,4-oxadiazole (vinyl-2-^{14}C)] and ^{14}C-nitrofurazone [5-nitro-2-furfural semicarbazone (formyl-^{14}C)] were kindly supplied by Ueno Pharmaceutical Co., Ltd. The radiochemical purity of these labeled compounds was examined by the thin-layer chromatography with solvent system of AcOEt–hexane–AcOH (12 : 8 : 1). The specific radioactivity of above compounds was as follows: ^{14}C-AF-2 0.20 μCi/mg, ^{14}C-NF-161 0.22 μCi/mg and ^{14}C-nitrofurazone 0.12 μCi/mg.

Animal and Treatment——Male Donryu rats were used in all experiments and maintained on MF diet (Oriental Yeast Co., Ltd.). Fig. 1 shows the growth curve of these animals during 14 weeks after birth.

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3) Location: *a) Maidashi, Fukuoka*; *b)* Present address: *Research Laboratory, Shionogi Pharmaceutical Co., Ltd., Sagisukami, Fukushimaku, Osaka.*
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^{14}C-AF-2, ^{14}C-NF-161 and ^{14}C-nitrofurazone were dissolved in 10% N,N-dimethylformamide and administered to rats at a dose of 2.5 mg/kg, 0.7 mg/kg and 2.5 mg/kg, respectively.

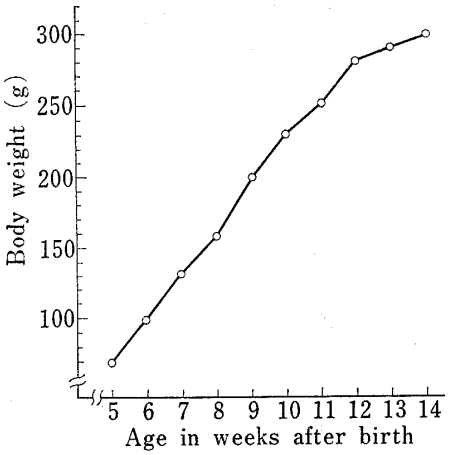

Fig. 1. Curve showing the Relation between Body Weight and Age of Male Donryu Rat

Cannulation of Common Bile Duct——The cannulation of common bile duct of rats was performed as described in the previous paper.[4] After this operation, 10 ml of 5% glucose–0.9% NaCl (1:1) mixture was slowly injected subcutaneously to each of these rats everyday. The rats were kept warm with a 60 W lamp. The drugs were administered orally to the rats 24 hr after the operation. The bile and urine were separately collected during 24 hr after the medication for measurement of the radioactivity.

Cannulation of Thoracic Duct——Rats weighing 200 to 300 g were fasted overnight and then administered orally with 1 ml of cream of cow's milk 1 hr prior to the operation. The cannulation of thoracic duct was conducted under ether anesthesia by slight modified Bollman's technique.[6] A polyethylene tubing (PE 50 Clay-Adams, external diameter 0.8 mm) with J curved end and beveled tip was inserted into the thoracic duct and secured. The free end of the tubing was passed to an exit at the lower end of the incision line of the abdominal wall. After operation, the cannulated rat was restrained in a cage designed by Bollman. From the following day, the lymph flow in most case was satisfactory for a sufficient length of time with fairly good physical condition of the animals, and the volume excreted during 24 hr varied from 60 to 150 ml. The drugs were administered orally to the rats 24 hr after this operation. The lymph and urine were separately collected during 24 hr after the medication, and the radioactivity in these samples was measured.

Cannulation of Portal Vein——Rats weighing 200 to 300 g were fasted overnight and then anesthetized with ether. The cannulation of femoral vein was first conducted with polyethylene tubing (Igarashi No. 5, external diameter 0.8 mm), which was connected to the blood infusion syringe, provided with the infusion liquid which was prepared according to the modified method of Kvetina, and Guaitani as follows. Arterial-venous blood was collected from rats of the same age, defibrinated and heparinized (0.12 ml of 5% heparine for 30 ml of blood). Ringer's solution consisted of NaCl 8.6 g, KCl 0.3 g, $CaCl_2$ 0.33 g and distilled water to make 1000 ml, with glucose 1 g and polyvinylpyrrolidone 35 g. Such a solution is mixed with defibrinated blood in the ratio of 2:1 and filtered through gauze. Next, the portal vein was cannulated with polyethylene tubing (Hibiki No. 4, external diameter 1 1/3 mm) and then the tubing was fixed in the portal vein by the ligation. Infusion syringe is immediately operated at such a rate as compensate the blood loss, followed by intraduodenal administration of the drugs.

Determination of Nitrofuran Derivatives in Blood——The blood was collected from portal vein of rats after administration of ^{14}C-AF-2 or ^{14}C-nitrofurazone. After centrifugation, an aliquot of the plasma was counted for total radioactivity (unchanged compound plus metabolites). Other aliquot of the plasma was extracted three times with two volumes of AcOEt by shaking for 10 min. After centrifugation, the organic phase was removed and its aliquot was counted for radioactivity (unchanged compound). In this case, thin-layer chromatography showed that the radioactivity in the organic phase is only due to unchanged nitrofuran derivative. The preliminary studies showed that ^{14}C-AF-2 and ^{14}C-nitrofurazone, which were added to the blood, incubated at 37° for 1 hr and centrifuged, were extracted into AcOEt in the recovery of 85.1 and 82.3% as unchanged compound, respectively. However, it was not known what the unextractable materials are.

Radioisotope Methods of Analysis——The radioactivity of all samples was measured using Packard Scintillation Spectrometer (Model 3375) and was corrected for quenching by an external standard method. The samples of urine, bile, lymph and plasma were counted in a toluene phosphor (0.4% PPO and 0.01% POPOP in toluene) with BBS-3 (Beckman bio-solve No-3) and 30% ascorbic acid.

Results and Discussion

Absorption Route from Gastrointestinal Tract

The lymphatic and portal systems are known to play the important role in the absorption of lipids, vitamins and foreign compounds from the gastrointestinal tract. Namely, the

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intestinal lymphatics have been shown to constitute the major route of absorption for cholesterol,[8] long-chain fatty acids,[9] other dietary lipids[10] and vitamins (A,[11] D,[12] E,[13] K[14] and coenzyme Q[15]). In addition, scattered reports in the literature have indicated that to a lesser extent other compounds including some fat-soluble dyes[16] and a few chemicals such as *p*-aminosalicylic acid (PAS),[17] tetracycline,[17] octadecanoic acid,[18] octadecanol,[18] p,p'-DDT[18] and 1,2,4-trimethoxypropenyl benzene.[15] On the other hand, the portal route appears to be more important for the intestinal absorption of griseofulvin,[19] benzoic acid,[18] aniline,[18] *p*-aminobenzoic acid,[18] antipyrine,[18] estradiol[18] and so on. Until now, however, no report concerning the absorption route of nitrofuran derivatives has been made.

Table I shows the percentage of radioactivity appearing in thoracic duct lymph and urine for 24 hr after oral administration of ^{14}C-AF-2 and ^{14}C-nitrofurazone to rats.

TABLE I. Percent Recovery of ^{14}C in Lymph and Urine during 24 hr after Oral Administration of ^{14}C-AF-2 and ^{14}C-Nitrofurazone to the Rat

	Lymph (% of dose)	Urine (% of dose)
AF-2	0.7	18.1
Nitrofurazone	2.2	56.8

Values in Table represent the mean values of two animals.

As can be seen in Table I, small amount of radioactivity appeared in thoracic duct lymph in both cases of above compounds, whereas the radioactivity recovered from urine accounted for about 18% of the dose for AF-2 and 57% for nitrofurazone, respectively. These percent recovery of radioactivity in 24 hr urine was coincided with the data of previous paper, describing that about 17% and 46% of the administered radioactivity recovered in 24 hr urine after oral administration of ^{14}C-AF-2 and ^{14}C-nitrofurazone to intact rats as the solution in 10% N,N-dimethylformamide.[5] This fact suggested that after oral administration of above ^{14}C-compounds to rats, the radioactivity was almost exclusively absorbed through portal vein

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from gastrointestinal tract. Accordingly, the following experiment was carried out in order to prove this point.

After intraduodenal administration of ^{14}C-AF-2 and ^{14}C-nitrofurazone to rats with cannulated portal veins, the venous blood was collected at intervals indicated in Table II.

Table II. Percent Recovery of ^{14}C in Portal Vein Plasma and Percentage of Unchanged Compound in Total Radioactivity after Intraduodenal Administration of ^{14}C-AF-2 and ^{14}C-Nitrofurazone to the Rat

	Time (min)	Radioactivity (% of dose)	Unchanged compound (%)
AF-2	0—30	5.7	48.9
	30—50	3.1	21.0
Nitrofurazone	0—30	4.5	48.2
	30—40	2.0	31.9

Values in Table represent the mean values of two animals.

As can be seen in Table II, the amounts of radioactivity appearing in the blood were 8.8% of the dose for AF-2 during 50 min and 6.5% for nitrofurazone during 40 min, respectively, supporting the above assumption.

So far, the absorption of some drugs *via* portal system has been demonstrated indirectly by the facts that little radioactivity appears in the lymph after intraduodenal administration of the radioactive compounds to the thoracic duct cannulated rats, whereas large amounts of radioactivity appeared in the urine,[15,19] or that lymph/plasma radioactivity ratio is low after treatment with radioactive drugs to the cannulated rats.[18] However, the present study showed directly that the nitrofuran derivatives such as AF-2 and nitrofurazone can be absorbed into portal vein by the method of cannulation of portal vein in rats.

As described above, the radioactivity appearing in the thoracic duct lymph represented only a small percentage of the dose after treatment with ^{14}C-AF-2 or ^{14}C-nitrofurazone. These low levels of radioactivity in the lymph are probably not due to their direct absorption into the lymphatics, but rather to their ability to the body water compartment, of which lymph is a part.

Previously, we reported that the absorption rates of radioactivity from gastrointestinal tract for 48 hr after oral administration of ^{14}C-NF-161 (oxadiazole-2-^{14}C) and ^{14}C-NF-161 (vinyl-2-^{14}C) to rats were 28% and 64% of the dose, respectively. This evidence indicated that less than 28% of NF-161 given orally to the rat was absorbed as unchanged compound and at least 36% of the dose as its metabolites which might be formed in intestinal mucosa. Also, it was demonstrated that AF-2 and nitrofurazone given orally to rats were metabolized by small intestinal mucosa.[5] Accordingly, a part of such nitrofuran derivatives appears to be absorbed from intestinal tract as metabolites similar to NF-161. In order to elucidate this problem, the composition of the radioactivity in the portal venous blood was examined. As the last column of Table II shows, about 50% of the radioactivity appearing for the first 30 min was due to the unchanged AF-2 and nitrofurazone, and the remaining radioactivity due to their polar metabolites. In AF-2, only 21% of the radioactivity appearing for next 20 min was present as the unchanged compound. In nitrofurazone, about 32% of the radioactivity appearing for next 10 min was due to the unchanged compound. These results suggested that after oral administration of nitrofuran derivatives to rats, the percentage of their metabolites which transfer from the intestinal mucosa increases as time passes. Thus, the present study provided the direct evidences concerning the metabolism of nitrofuran derivatives in the intestinal mucosa of rats and the absorption of their metabolites from the intestinal wall.

Effect of Age on Absorption Rate from Gastrointestinal Tract

It seems to be important to elucidate the relationship between animal age and absorption rate from gastrointestinal tract. However, such report is not available so far. In the previous paper,[4,5] it was shown that after oral administration of ^{14}C-nitrofuran derivatives to intact rats, the total radioactivity excreted in urine and feces is approximately equal to that administered. Furthermore, it was indicated that the total radioactivity in urine, bile and gastrointestinal tract was coincided with that administered to common bile duct cannulated rats, and the radioactivity in urine and the summation of radioactivity in bile and gastrointestinal tract were equal to that in urine and feces of intact rats described above, respectively. In these cases, most of radioactivity was recovered in urine and bile during the first 24 hr. From these results, the summation of radioactivity appearing in urine and bile was regarded as the absorbed radioactivity. In the present study, the same procedure was employed in order to obtain the absorption rate, which was expressed as percent of the dose.

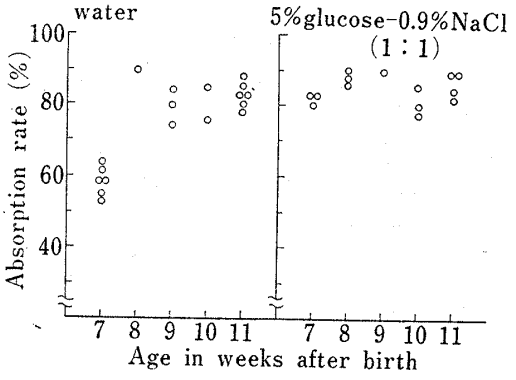

Fig. 2. Absorption Rate of Radioactivity from Gastrointestinal Tract as a Function of Age of the Rat, after Oral Administration of ^{14}C-AF-2, and Effect of 5% Glucose–0.9% NaCl (1 : 1) Mixture on the Absorption Rate

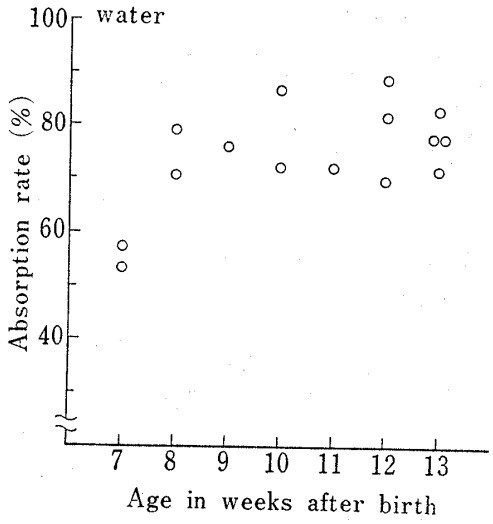

Fig. 3. Absorption Rate of Radioactivity from Gastrointestinal Tract as a Function of Age of the Rat, after Oral Administration of ^{14}C-NF-161

Fig. 2 shows that when ^{14}C-AF-2 was administered orally to the common bile duct cannulated rats, which were allowed for free access to water, the absorption rate of radioactivity was lower in 7 weeks old group (average 58%) than in over 8 weeks old groups (average 82%). In addition, similar result was obtained under the same conditions after oral administration of ^{14}C-NF-161 as shown in Fig. 3. In this case, the average of the absorption rate of radioactivity from gastrointestinal tract was 56 and 78% for 7 weeks old and over 8 weeks old groups, respectively. In the present study, the absorption rate in under 6 weeks old rats was not examined, because the cannulation of their common bile duct is difficult. Furthermore, when ^{14}C-AF-2 was administered orally to the cannulated rats, which were allowed for free access to 5% glucose–0.9% NaCl (1:1) mixture instead of water, the absorption rate of radioactivity in 7 weeks old group increased up to those in the older groups, which were unaffected by the above mixture as shown in Fig. 2.

Fig. 4 shows the percent recovery of radioactivity appearing in 24 hr urine and bile of the common bile duct cannulated rats, which was allowed for free access to water or 5% glucose–0.9% NaCl (1: 1) mixture, after oral administration of ^{14}C-AF-2. These data provided the basis of the absorption rate described above. The excretion rate of radioactivity in urine was approximately same level in all cases, whereas that of radioactivity in bile was considerably

lower in 7 weeks old group which was allowed for free access to water than in other groups. In this case, there was no difference in bile flow rate among 7 weeks old rats which were allowed for free access to water and 5% glucose–0.9% NaCl (1 : 1) mixture. The reason why the lower absorption rate of 7 weeks old group was reflected only on the biliary excretion is not clear at present.

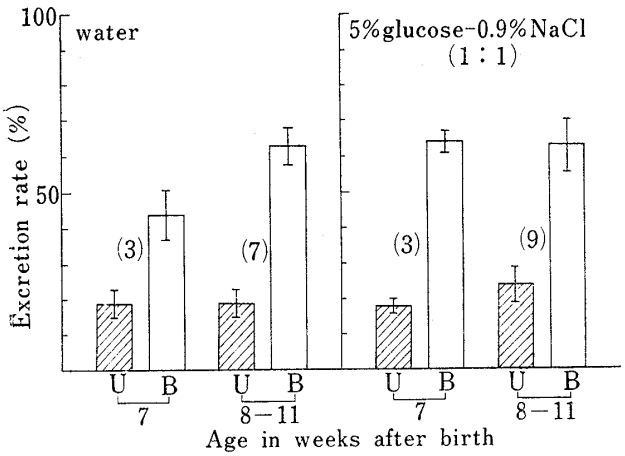

Fig. 4. Excretion Rate of Radioactivity in Urine and Bile as a Function of Age of the Rat, after Oral Administration of ^{14}C-AF-2, and Effect of 5% Glucose–0.9% NaCl (1: 1) Mixture on the Excretion Rate

U: urine, B: bile Each value is expressed as the mean ±SD with the number of experiments in parentheses.

It is easily understood that drug absorption from the gastrointestinal tract is affected by physico-chemical properties of drugs and by physiological conditions of animals. However, little is known about the relationship between the absorption of drugs from gastrointestinal tract and the age of animals. The present study demonstrated that the absorption rate of radioactivity was different between 7 weeks old rats and over 8 weeks old rats following oral administration of ^{14}C-AF-2 or ^{14}C-NF-161. In this case, the body weight was 100—140 g in 7 weeks old rats and 120—160 g in 8—9 weeks old rats. Accordingly, the absorption rate seemed to be correlated with age rather than body weight. It has been considered that rats become sexually mature at about 50—60 days of age.[20] Also, earlier investigations of the change in chemical composition of rat body with age revealed that rats become chemically mature at about 50 days of age.[21] From these facts, it appears probable that the low absorption rate in 7 weeks old rats described above results from the immaturity of their physiological states. However, the detailed relationship among age, physiological conditions and absorption of drugs from gastrointestinal tract in rats is left to be studied.

After oral administration of ^{14}C-AF-2, the absorption of radioactivity in 7 weeks old rats increased when water in tap was replaced by 5% glucose–0.9% NaCl (1: 1) mixture as shown in Fig. 2. In this case, subcutaneous injection of the above mixture (10 ml) every day to rats, which were allowed for free access to water, had no effect on the absorption of radioactivity. From this fact, it was considered that the absorption of radioactivity was influenced by the glucose and/or NaCl present in the intestinal tract. Previously, Fisher[22] and also Smyth and Taylor[23] reported the effect of glucose on the water absorption from small intestinal tract. Recently, Kitazawa and Ito.[24] presented the effect of glucose on drug absorption. However, it is difficult to explain reasonably the phenomenon observed in the present study from the available data.

Acknowledgement This work was supported partially by a Grant-in Aid for Scientific Research provided by the Ministry of Education to which the authors are indebted.

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