Studies on the Metabolic Fate of ^3H -TJN-101 (I) : Absorption, Distribution and Excretion following Single Oral Administration to Rats.

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Summary

We investigated the absorption, distribution and excretion of ^3H -TJN-101 following single oral administration of the drug in rats.

1. The concentration of radioactivity in blood increased rapidly after oral administration of 4 mg/kg of ^3H -TJN-101 in male rats and reached $C_{\max}$ of 816 ng/ml (TJN-101 base) at 0.75 hr. Thereafter, the radioactivity decreased, with a half-life of 1.1 hr up to 4 hr and with a half-life of 7.6 hr from 6 to 24 hr after dosing. The concentration dropped below the detection limit at 48 hr. The area under the concentration-time curve ($\text{AUC}_{0-24\text{hr}}$) was $3.12 \mu\text{g}\cdot\text{hr}/\text{ml}$. Following oral administration of 0.8 mg/kg of ^3H -TJN-101 in male rats, both $C_{\max}$ and $\text{AUC}_{0-24\text{hr}}$ were 12% of the values of the 4 mg/kg group. $C_{\max}$ and $\text{AUC}_{0-24\text{hr}}$ following oral administration of 20 mg/kg of ^3H -TJN-101 were 5.4 and 9.2 times of the values of the 4 mg/kg group, respectively.

2. In female rats, at 0.75 hr after oral administration of 4 mg/kg of ^3H -TJN-101, $C_{\max}$ was 682 ng/ml, and it decreased, with a half-life of 2.9 hr up to 8 hr after dosing. $C_{\max}$ and $\text{AUC}_{0-24\text{hr}}$ were 84% and 1.3 times of those in the male rats, respectively.

3. Tissue radioactivity after oral administration of 4 mg/kg of ^3H -TJN-101 reached the maximum level within 0.25 to 0.75 hr, being higher in the liver, and lower in the central nervous system, testis, and eyeball.

4. Within 168 hr after oral administration of 4 mg/kg of ^3H -TJN-101, radioactivity in the urine and feces in male rats was 13.6% and 83.1% of administered dose, respectively, and that in female rats was 20.9% and 73.9% of the dose, respectively.

5. Within 48 hr after dosing, 91.1% of the total radioactivity was detected in the bile and 4.5% in the urine, as determined simultaneously.

6. Within 48 hr after intraduodenal administration of radioactive bile obtained from other rats which had been administered ^3H -TJN-101 orally, 52.0% of injected radioactivity was excreted in the bile and 4.0% in the urine.

Introduction

TJN-101 (Gomisin A ; (+)-(6S, 7S, R-biar)-5, 6, 7, 8-tetrahydro-1, 2, 3, 12-tetramethoxy-6, 7-dimethyl-10, 11-methylenedioxy-6-dibenzo [*a, c*] cyclooctenol), is one of the lignan components isolated from *Schisandra* fruits¹⁾.

This compound has shown an inhibitory effect on the liver injuries induced by some hepatotoxic chemicals^{2~4)}, and it has been shown to prevent immunologically induced acute hepatic failure⁵⁾. Moreover, as it demonstrated an inhibitory effect on hepatic fibrosis⁶⁾ and a promotive effect on liver regeneration^{6,7)}. Therefore, it could be expected to have clinical effect in the treatment of hepatitis. In the present study, we investigated the absorption, distribution and excretion of radioactivity following single oral administration of tritium-labeled TJN-101 (³H-TJN-101) to rats. The authors also report the plasma protein binding of the radioactivity, both *in vitro* and *in vivo*.

Materials and Methods

1. Labeled compound

³H-TJN-101 (Lot No. TP-182) was synthesized in accordance with the reaction conditions shown in Fig. 1. The specific activity of ³H-TJN-101 was 699MBq/mg (18.9mCi/mg) and the radiochemical purity, based on thin-layer chromatography (TLC), was more than 98%. A silica gel plate (Silica gel 60 F₂₅₄, df=0.25mm, E. Merck) was used for the TLC, and the solvent systems were ethyl acetate/toluene (1/1, v/v), chloroform/acetonitrile (20/1, v/v), or ethyl acetate alone. The labeled compound was stable during the experiment.

Fig. 1 Chemical structure of TJN-101 and synthetic route for ³H-TJN-101

2. Animals

Male and female Sprague Dawley rats (specific pathogen-free, Charles River Japan Inc.) weighing about 250 and 170 g, respectively, were used. They were housed at a temperature of $23 \pm 2^\circ\text{C}$ and a humidity of $55 \pm 10\%$, and were given a commercial pellet diet (Oriental Yeast Co. Ltd., MF) and water. All animals in this experiment were used after more than one week of pre-breeding. The animals were fasted overnight before and for 4hr after dosing, but were given water *ad libitum*.

3. Drug administration

The ³H-TJN-101 was diluted with non-labeled TJN-101 and prepared as a suspension in 5% gum arabic solution. Rats were given oral doses of 0.8, 4.0, and 20mg/kg. Trace

amounts of tritium were about 148MBq/kg (4mCi/kg) for whole-body autoradiography and 4.44MBq/kg (120 μ Ci/kg) for the other studies, and the volume of administration was 2 ml/kg in all experiments.

4. Determination of radioactivity

The radioactivity of each sample was determined by a direct or a combusted method. In the direct method, the radioactivity of the sample was determined with a liquid scintillation counter (model LSC-903, Aloka) after the samples were dissolved in 13ml of liquid scintillator (Atomlight, Du Pont NEN Research product, U.S.A.). In the combusted method, the sample was put into a paper cup filled with filter paper and either dried at 40°C for more than 24hr in an electric drying oven (dry method) or not dried (wet method), following which was combusted with an automatic combustor (model ASC-112, Aloka). Thereafter, the radioactivity of the sample was determined using the same procedures as for the direct method, after the sample was dissolved directly in 12ml of liquid scintillator (Biofluor, Du Pont NEN Research Products). Quenching was corrected using an automatic external standard.

5. Blood levels

After oral administration of ^3H -TJN-101 to male rats ($n=4$) at doses of 0.8, 4.0, and 20mg/kg, or at 4.0mg/kg to female rats ($n=4$), blood samples (50 μ l) were collected at fixed times from the caudal vein. The radioactivity in blood was determined by the dry method, with only the 4 mg/kg male group also being measured by the wet method. Sampling periods were 5, 15, 30, 45min, and 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168hr after dosing.

6. Distribution

1) whole-body autoradiography

Each rat was sacrificed under ether anesthesia at 0.25, 0.75, 2, 8, 24 and 72hr after oral administration of ^3H -TJN-101 (4mg/kg). The hair was immediately cut with electric clippers, and the nasal cavity and anus of the rats were then filled with 5% carboxymethyl cellulose (CMC) solution. The rats were then frozen by dry ice-acetone (-70°C) and kept in a freezer (-20°C). The limbs and tail were removed and the carcass was embedded with 5% CMC on the stage of a microtome and frozen by dry ice-acetone. Adhesive tape (No.810, Sumitomo 3M Co. Ltd.) was attached and sections (35 μ m) were prepared with a Cryo-Microtome (450MP, Palmstilernas mekanska verkstad) at about -25°C . The sections were freeze-dried at -25°C for 3 days and placed in contact with tritium-sensitive films at 4°C for 35 days. The films were developed and whole-body autoradiograms were obtained.

2) Tissue levels

Rats were killed by exsanguination from the abdominal aorta under ether anesthesia at 0.25, 0.75, 2, 8, 24, and 72hr after oral administration of ^3H -TJN-101 (4mg/kg, $n=4$); the tissues or organs were then isolated immediately. Whole tissues or organs and aliquots were weighed exactly and radioactivity was determined by the dry method as

described above. The radioactivity of plasma samples was also measured by the direct method, as described above, and the ratio of unstable tritium was calculated from the determinations of both methods.

7. Excretion in urine and feces

After oral administration of ^3H -TJN-101 (4 mg/kg) to male and female rats ($n=4$), the animals were kept separately in metabolic cages (KN-646B, Natsume Seisakusho Co. Ltd.). Urine (kept under ice-cold conditions) and feces were periodically collected separately for up to 168hr. Urine samples were made up to 100ml with water, and the radioactivity of 1 ml of this solution was determined after dissolving it directly in 13ml of liquid scintillator (Atomlight, Du Pont NEN Research Products) (1). One ml of the same urine solution was then dried at 50°C, and the radioactivity was measured after adding 1 ml of water and 13ml of Atomlight (2). The ratio of unstable tritium was calculated from the balance of (1) and (2).

Feces were homogenized with 50% methanol and made up to 300ml. The radioactivity was determined by the dry method, using 0.5ml of homogenate. For the measurement of residual ^3H -TJN-101 radioactivity in the body, rats were sacrificed under ether anesthesia at 168hr after ^3H -TJN-101 administration and frozen. The carcasses were then suitably sectioned and the sections were dissolved by heating reflux for 72hr in 400ml of 0.5N sodium hydroxide and 80ml of toluene. The aqueous and toluene layers were diluted to 900ml and 100ml, respectively. For the measurement of radioactivity, 0.5ml of the aqueous layer and 1 ml of the toluene layer, respectively, were added to 13ml of Atomlight and the residual radioactivity in the carcass was calculated.

8. Excretion in bile

A polyethylene tube was inserted into the bile duct of ether-anesthetized male rats (bile-duct-cannulated rat, $n=4$). After complete recovery from anesthesia, ^3H -TJN-101 (4 mg/kg) was given orally. The animals were then fixed in Ballman cages and the bile and urine were collected periodically, for up to 48hr under ice-cold conditions. Feces were also collected for up to 48hr after dosing. The bile samples obtained were made up to 100ml with water, and the radioactivity of 1 ml of this solution was measured by the direct method. The unstable tritium ratio and the excretion ratio of the radioactivity of ^3H -TJN-101 in urine and feces were determined as outlined above. For the measurement of the residual radioactivity ratio in the gastrointestinal lumen, rats were sacrificed under ether anesthesia at 48hr after dosing and the gastrointestinal contents were removed. These were homogenized with 50% methanol and made up to 300ml, and 0.5ml of the homogenate was used for radioactivity measurement by the dry method. After the gastrointestinal contents were removed, the residual ratio of radioactivity in the carcass was calculated as described above.

9. Enterohepatic circulation

^3H -TJN-101 (4 mg/kg) was administered orally to certain bile-duct-cannulated rats ($n=4$) and the bile was collected up to 4hr later. This radioactive bile ($667\mu\text{g}/24.7\text{MBq}$

(19.4 μ Ci)/4ml/kg) was then injected into the duodenum of other bile-duct-cannulated rats (n=4). The animals were then fixed in Ballman cages and bile and urine were collected, under ice-cold conditions, periodically for up to 48hr. Feces were also collected for up to 48hr after dosing. The radioactivity in the bile, urine, and feces was measured as described above. The animals were sacrificed at 48hr after dosing, and the residual ratio of ^3H -TJN-101 radioactivity in the gastrointestinal lumen and carcass was also measured, as described above.

10. Protein binding

1) *In vivo*

Plasma samples, obtained from tissue level study at 0.25, 0.75, 2, and 8hr after dosing were used to determine protein binding by equilibrium dialysis. One ml of each plasma sample was put into a dialysis bag (#24/32, Visking Co.) with 1 ml of 1/15M phosphate buffer (pH7.4). The bag was suspended in a test tube containing 10ml of 1/15M phosphate buffer (pH7.4), and the test tube was shaken for 24hr at 4°C. After dialysis, the radioactivity of the outer solution and the plasma were determined by the dry method, and the percentage of radioactivity bound to protein was calculated from the difference between their radioactivity levels. Further, one ml of plasma sample taken at 0.75hr after dosing and 1ml of 1/15M phosphate buffer (pH7.4) were put into a dialysis bag in order to study the reversibility of the binding. The bag was suspended in a test tube containing about 45ml of 1/15M phosphate buffer (pH7.4) and the tube was shaken at 4°C; the outer solution was exchanged until its radioactivity dropped below the detection limit. After dialysis, the inner solution was diluted to 5 ml with 1/15M phosphate buffer (pH7.4). The radioactivity of one ml of this solution was measured by the dry method, and the ratio of residual radioactivity in the dialysis bag was calculated. The adsorption of ^3H -TJN-101 to the dialysis bag was then investigated according to the following procedure. Namely, one ml of ^3H -TJN-101 solution (0.5 μ g/ml) dissolved in 1/15M phosphate buffer (pH7.4) and the same volume of 1/15M phosphate buffer (pH7.4) were put into a dialysis bag. The bag was then suspended in 10ml of 1/15M phosphate buffer (pH 7.4), and shaken for 24hr at 4°C. After dialysis, the radioactivity of one ml of outer solution was determined, and the adsorption ratio was calculated.

2) *In vitro*

^3H -TJN-101 was dissolved in 1/15M phosphate buffer (pH7.4), and one volume of the resultant solution was added to nine volumes of rat, dog, or human plasma, following which plasma solution containing 0.02, 0.1, 0.5, or 2.5 μ g/ml of ^3H -TJN-101 were prepared. Each sample was incubated in a water bath at 37°C for 10min and used for dialysis as described above, in the *in vivo* procedure. The radioactivity in plasma before dialysis and the radioactivity in the outer solution after dialysis were determined by the dry method and protein bindings were calculated. The reversibility of binding was also investigated, in 0.5 μ g/ml plasma, as described above.

11. Calculation of pharmacokinetic parameters

The values of pharmacokinetic parameters were, in principle, calculated from the mean

blood concentration-time curve of radioactivity in four animals. The values of area under the concentration-time curve (AUC) were calculated by the trapezoidal method. Apparent half-life values were estimated using the least square method of actual data.

Results

1. Blood concentration

Fig. 2 shows the blood concentrations of radioactivity, measured by the dry or the wet method, after the oral administration of ^3H -TJN-101 (4 mg/kg) to male rats. Maximum blood concentration appeared at 0.75hr after oral administration; the values were 816 ± 101 and $834 \pm 101 \text{ ng/ml}$ (TJN-101 base) determined by the dry and wet method, respectively. Thereafter, blood concentration decreased, with a half-life of 1.1hr up to 4hr and with a half-life of 7.6hr from 6 to 24hr as determined by the dry method. The area under the concentration-time curve ($\text{AUC}_{0-24\text{hr}}$) was $3.12 \mu\text{g} \cdot \text{hr/ml}$. As detected by the wet method, blood concentration decreased with a half-life of 1.5hr from the maximum to 4hr and with a half-life of 64hr from 6 to 168hr. $\text{AUC}_{0-168\text{hr}}$ was $18.0 \mu\text{g} \cdot \text{hr/ml}$. The ratios of unstable tritium in blood were 2%, 14%, 40%, 65%, and 86% at 0.75, 2, 4, 12, and 24hr after dosing, respectively; on and after 48hr, only unstable tritium was detected.

Fig. 3 shows the blood concentrations of radioactivity, measured by the dry method, after the oral administration of ^3H -TJN-101 at doses of 0.8, 4.0 and 20mg/kg to male rats. Blood concentrations increased relatively fast and reached their maxima at 0.25, 0.75, and 2hr for each of these doses, respectively. The increase of maximum concentration (C_{max}) was related to increased doses at values of 94 ± 14 , 816 ± 101 , and $4430 \pm 116 \text{ ng/ml}$, respectively. A similar tendency was observed for $\text{AUC}_{0-24\text{hr}}$ values of 0.362, 3.12, and $28.7 \mu\text{g} \cdot \text{hr/ml}$.

Fig. 2 Blood concentration of radioactivity after single oral administration of ^3H -TJN-101 (4 mg/kg) to male rats

Data are expressed as the mean values $\pm$ S.E. of four animals.

○ — ○ Dry

● — ● Wet

Fig. 3 Blood concentration of radioactivity after single oral administration of ^3H -TJN-101 to male rats

Data are expressed as the mean values $\pm$ S.E. of four animals.

- — 0.8 mg/kg
- — 4 mg/kg
- ▲ — 20 mg/kg

Fig. 4 Blood concentration of radioactivity after single oral administration of ^3H -TJN-101 (4 mg/kg) to female rats

Data are expressed as the mean values $\pm$ S.E. of four animals.

- — Male
- — Female

Blood concentrations of radioactivity, measured by the dry method, after oral administration of ^3H -TJN-101 (4 mg/kg) to female rats are shown in Fig. 4. Blood concentration reached maximum at 0.75 hr after dosing, with a value of $682 \pm 14 \text{ ng/ml}$, and then decreased, with a half-life of 2.9 hr up to 8 hr and with a half-life of 17 hr from 12 to 48

Table I Pharmacokinetic parameters of radioactivity in blood after single oral administration of ^3H -TJN-101 to rats

Sex	Dose (mg/kg)	$t_{\max}$ (hr)	$C_{\max}$ ng eq./ml	$t_{1/2}$ (hr)	AUC_{0-24} $\mu\text{g eq.}\cdot\text{hr}/\text{ml}$
male	4	0.75	816 ± 101	1.1 (0.75-4hr) 7.6 (6-24hr)	3.12
male	0.8	0.25	94 ± 14	1.4 (0.25-4hr) 10 (6-24hr)	0.362
male	20	2	4430 ± 116	2.7 (2-12hr)	28.7
female	4	0.75	682 ± 14	2.9 (0.75-8hr) 17 (12-48hr)	4.03

Each values are calculated from the mean blood concentration-time curve of radioactivity determined by the dry method.

hr after administration. $\text{AUC}_{0-24\text{hr}}$ was $4.03\mu\text{g}\cdot\text{hr}/\text{ml}$.

The Pharmacokinetic parameters of radioactivity in blood are summarizes in Table I.

2. Distribution study

Fig.5 shows whole-body autoradiograms at 0.25, 0.75, 2, 8, 24, and 72hr after oral administration of ^3H -TJN-101. At 0.25 and 0.75hr, the radioactivity in the gastrointestinal contents was the highest, followed by that in the liver, gastrointestinal wall, and urine in the urinary bladder. The radioactivity in the hypophysis, thyroid, harderian gland, submaxillary gland, heart, lung, kidney, adrenal, and brown fat was higher than that in the blood. Most other tissues and organs had the same levels of radioactivity as those found in the blood, and the radioactivity in the central nervous system (CNS), eyeball, and testis were lowest. At 2hr, higher radioactivity was observed in the gastrointestinal contents, liver, part of the eyeball, gastrointestinal wall, and urine in the urinary bladder. Most other tissues and organs had the same levels of radioactivity as those found in the blood. At 8hr, higher radioactivity was observed in the intestinal contents, liver, part of the eyeball, gastrointestinal wall and urine in the urinary bladder. At this time, the lower radioactivity in the thyroid, kidney, skin, and most other tissues and organs was trace level. At 24hr, although radioactivity was generally decreased, higher radioactivity was observed in the liver, gastrointestinal wall, and intestinal contents. The thyroid, skin, urine in the urinary bladder, and other tissues and organs had trace levels of radioactivity. At 72hr, although the radioactivity was greatly decreased, radioactivity in the liver and intestinal contents was still detected at a significant level.

Tissue levels of radioactivity and the percent distribution of radioactivity in the tissues at 0.25, 0.75, 2, 8, 24, and 72hr after single oral dosing are summarized in Table II and III, respectively. The maximum level of radioactivity was observed at 0.25 or 0.75hr after dosing in all tissues except the colon. At 0.25hr, the stomach and liver had much higher levels of radioactivity than other tissues; the levels being 49 and 13 times higher, respectively, than the plasma level ($1.14\mu\text{g}/\text{ml}$). About 12% of the dose was found in the liver at this time, and the concentrations of radioactivity in the adrenal, kidney, harderian gland, pancreas, and brown fat were relatively high. The radioactivity in each tissue

Table II Tissue concentrations of radioactivity after single oral administration of ^3H -TJN-101 (4 mg/kg) to male rats

Tissue	Concentration of radioactivity (μg equiv. of TJN-101/g or ml)					
	0.25 hr	0.75 hr	2 hr	8 hr	24 hr	72 hr
Plasma	1.14 $\pm$ 0.20 (1.00)	0.74 $\pm$ 0.19 (1.00)	0.29 $\pm$ 0.03 (1.00)	0.11 $\pm$ 0.01 (1.00)	0.02 $\pm$ 0.00 (1.00)	0.01 $\pm$ 0.00 (1.00)
Blood	1.02 $\pm$ 0.16 (0.89)	0.64 $\pm$ 0.15 (0.86)	0.26 $\pm$ 0.02 (0.90)	0.10 $\pm$ 0.01 (0.91)	0.02 $\pm$ 0.00 (1.00)	0.01 $\pm$ 0.00 (1.00)
Cerebrum	0.58 $\pm$ 0.11 (0.51)	0.32 $\pm$ 0.11 (0.43)	0.07 $\pm$ 0.01 (0.24)	0.01 $\pm$ 0.00 (0.09)	N.D. ^{a)}	N.D.
Cerebellum	0.56 $\pm$ 0.12 (0.49)	0.32 $\pm$ 0.10 (0.43)	0.08 $\pm$ 0.01 (0.28)	0.01 $\pm$ 0.00 (0.09)	N.D.	N.D.
Hypophysis	1.09 $\pm$ 0.22 (0.96)	0.90 $\pm$ 0.24 (1.22)	0.27 $\pm$ 0.03 (0.93)	0.07 $\pm$ 0.00 (0.64)	N.D.	N.D.
Medulla spinalis	0.53 $\pm$ 0.12 (0.46)	0.42 $\pm$ 0.16 (0.57)	0.10 $\pm$ 0.00 (0.34)	0.02 $\pm$ 0.00 (0.18)	N.D.	N.D.
Thyroid	1.57 $\pm$ 0.28 (1.38)	1.13 $\pm$ 0.30 (1.53)	0.50 $\pm$ 0.10 (1.72)	0.19 $\pm$ 0.04 (1.73)	0.10 $\pm$ 0.02 (5.00)	0.09 $\pm$ 0.04 (9.00)
Eyeball	0.34 $\pm$ 0.06 (0.30)	0.28 $\pm$ 0.08 (0.38)	0.14 $\pm$ 0.01 (0.48)	0.04 $\pm$ 0.00 (0.36)	0.01 $\pm$ 0.00 (0.50)	N.D.
Submaxillary gland	1.81 $\pm$ 0.30 (1.59)	1.19 $\pm$ 0.33 (1.61)	0.37 $\pm$ 0.03 (1.28)	0.08 $\pm$ 0.00 (0.73)	0.02 $\pm$ 0.00 (1.00)	0.01 $\pm$ 0.00 (1.00)
Harderian gland	2.59 $\pm$ 0.51 (2.27)	2.24 $\pm$ 0.70 (3.03)	0.64 $\pm$ 0.03 (2.21)	0.13 $\pm$ 0.01 (1.18)	0.05 $\pm$ 0.00 (2.50)	0.02 $\pm$ 0.00 (2.00)
Trachea	1.12 $\pm$ 0.12 (0.98)	0.97 $\pm$ 0.23 (1.31)	0.33 $\pm$ 0.04 (1.14)	0.08 $\pm$ 0.01 (0.73)	0.03 $\pm$ 0.00 (1.50)	N.D.
Thymus	0.91 $\pm$ 0.18 (0.80)	0.69 $\pm$ 0.19 (0.93)	0.23 $\pm$ 0.02 (0.79)	0.04 $\pm$ 0.00 (0.36)	0.01 $\pm$ 0.00 (0.50)	N.D.
Heart	1.89 $\pm$ 0.27 (1.66)	1.21 $\pm$ 0.33 (1.64)	0.37 $\pm$ 0.03 (1.28)	0.09 $\pm$ 0.01 (0.82)	0.02 $\pm$ 0.00 (1.00)	0.01 $\pm$ 0.00 (1.00)
Lung	1.61 $\pm$ 0.27 (1.41)	1.06 $\pm$ 0.28 (1.43)	0.35 $\pm$ 0.03 (1.21)	0.09 $\pm$ 0.01 (0.82)	0.02 $\pm$ 0.00 (1.00)	0.01 $\pm$ 0.00 (1.00)
Liver	14.77 $\pm$ 0.13 (12.96)	8.24 $\pm$ 1.40 (11.14)	4.56 $\pm$ 0.36 (15.72)	3.25 $\pm$ 0.34 (29.55)	1.27 $\pm$ 0.11 (63.50)	0.70 $\pm$ 0.04 (70.00)
Kidney	2.62 $\pm$ 0.37 (2.30)	1.90 $\pm$ 0.46 (2.57)	0.71 $\pm$ 0.07 (2.45)	0.24 $\pm$ 0.02 (2.18)	0.05 $\pm$ 0.01 (2.50)	0.02 $\pm$ 0.00 (2.00)
Spleen	1.24 $\pm$ 0.20 (1.09)	0.90 $\pm$ 0.25 (1.22)	0.31 $\pm$ 0.03 (1.07)	0.07 $\pm$ 0.00 (0.64)	0.02 $\pm$ 0.00 (1.00)	0.01 $\pm$ 0.00 (1.00)
Pancreas	2.39 $\pm$ 0.44 (2.10)	1.70 $\pm$ 0.39 (2.30)	0.54 $\pm$ 0.08 (1.86)	0.14 $\pm$ 0.02 (1.27)	0.04 $\pm$ 0.01 (2.00)	0.01 $\pm$ 0.00 (1.00)
Adrenal	4.45 $\pm$ 0.56 (3.90)	2.86 $\pm$ 0.82 (3.86)	0.93 $\pm$ 0.05 (3.21)	0.16 $\pm$ 0.00 (1.45)	0.04 $\pm$ 0.01 (2.00)	N.D.
Fat	1.44 $\pm$ 0.34 (1.26)	5.12 $\pm$ 2.54 (6.92)	3.82 $\pm$ 0.42 (13.17)	0.19 $\pm$ 0.04 (1.73)	0.02 $\pm$ 0.00 (1.00)	N.D.
Brown fat	2.23 $\pm$ 0.54 (1.96)	3.81 $\pm$ 1.63 (5.15)	1.25 $\pm$ 0.06 (4.31)	0.10 $\pm$ 0.01 (0.91)	0.03 $\pm$ 0.00 (1.50)	0.02 $\pm$ 0.00 (2.00)
Testis	0.37 $\pm$ 0.08 (0.32)	0.51 $\pm$ 0.17 (0.69)	0.22 $\pm$ 0.01 (0.76)	0.04 $\pm$ 0.01 (0.36)	0.01 $\pm$ 0.00 (0.50)	0.00 $\pm$ 0.00 (0.00)
Epididymis	0.60 $\pm$ 0.11 (0.53)	0.88 $\pm$ 0.29 (1.19)	0.50 $\pm$ 0.04 (1.72)	0.08 $\pm$ 0.01 (0.73)	0.01 $\pm$ 0.00 (0.50)	0.00 $\pm$ 0.00 (0.00)
Muscle	0.90 $\pm$ 0.14 (0.79)	0.80 $\pm$ 0.22 (1.08)	0.28 $\pm$ 0.02 (0.97)	0.06 $\pm$ 0.00 (0.55)	0.01 $\pm$ 0.00 (0.50)	N.D.
Bone marrow	1.12 $\pm$ 0.21 (0.98)	0.67 $\pm$ 0.18 (0.91)	0.34 $\pm$ 0.02 (1.17)	0.07 $\pm$ 0.01 (0.64)	0.02 $\pm$ 0.00 (1.00)	N.D.
Skin	1.26 $\pm$ 0.35 (1.11)	1.54 $\pm$ 0.54 (2.08)	0.48 $\pm$ 0.03 (1.66)	0.12 $\pm$ 0.00 (1.09)	0.06 $\pm$ 0.01 (3.00)	0.03 $\pm$ 0.00 (3.00)
Aorta	1.79 $\pm$ 0.35 (1.57)	1.70 $\pm$ 0.36 (2.30)	0.56 $\pm$ 0.03 (1.93)	0.13 $\pm$ 0.01 (1.18)	0.02 $\pm$ 0.00 (1.00)	N.D.
Sciatic nerve	0.83 $\pm$ 0.18 (0.73)	0.93 $\pm$ 0.31 (1.26)	0.32 $\pm$ 0.01 (1.10)	0.05 $\pm$ 0.01 (0.45)	N.D.	N.D.
Stomach	56.10 $\pm$ 16.92 (49.21)	11.51 $\pm$ 5.89 (15.55)	0.81 $\pm$ 0.14 (2.79)	0.13 $\pm$ 0.03 (1.18)	0.06 $\pm$ 0.01 (3.00)	0.01 $\pm$ 0.00 (1.00)
Duodenum	9.89 $\pm$ 2.40 (8.68)	5.07 $\pm$ 1.98 (6.85)	1.21 $\pm$ 0.34 (4.17)	1.02 $\pm$ 0.17 (9.27)	0.10 $\pm$ 0.03 (5.00)	0.01 $\pm$ 0.00 (1.00)
Jejunum	8.67 $\pm$ 3.71 (7.61)	4.75 $\pm$ 1.21 (6.42)	0.89 $\pm$ 0.06 (3.07)	1.46 $\pm$ 0.81 (13.27)	0.06 $\pm$ 0.03 (3.00)	0.01 $\pm$ 0.00 (1.00)
Colon	1.23 $\pm$ 0.10 (1.08)	1.32 $\pm$ 0.40 (1.78)	0.68 $\pm$ 0.14 (2.34)	7.84 $\pm$ 2.02 (71.27)	0.82 $\pm$ 0.16 (41.00)	0.08 $\pm$ 0.01 (8.00)
Urinary bladder	1.02 $\pm$ 0.17 (0.89)	1.14 $\pm$ 0.20 (1.54)	0.54 $\pm$ 0.04 (1.86)	0.64 $\pm$ 0.27 (5.82)	0.03 $\pm$ 0.01 (1.50)	N.D.
Plasma (wet) ^{b)}	1.17 $\pm$ 0.19 (1.03)	0.80 $\pm$ 0.19 (1.08)	0.38 $\pm$ 0.03 (1.31)	0.25 $\pm$ 0.01 (2.27)	0.17 $\pm$ 0.01 (8.50)	0.10 $\pm$ 0.01 (10.00)

Data are expressed as the mean values $\pm$ S.E. of four animals.

Figures in parentheses indicate the ratio of concentration in tissue to that in plasma.

a) : Not detected b) : Data were obtained from wet plasma samples.

Fig. 5 Autoradiograms showing the distribution of radioactivity after single oral administration of ^3H -TJN-101 (4 mg/kg) to a male rat

Table III Percentage distribution of radioactivity after single oral administration of ^3H -TJN-101 (4 mg/kg) to male rats

Tissue	Tissue content (% of dose)					
	0.25 hr	0.75 hr	2 hr	8 hr	24 hr	72 hr
Blood ^{a)}	1.63±0.26	1.02±0.24	0.42±0.03	0.15±0.01	0.04±0.00	0.02±0.00
Cerebrum	0.08±0.02	0.04±0.02	0.01±0.00	0.00±0.00	— ^{b)}	—
Cerebellum	0.02±0.00	0.01±0.00	0.00±0.00	0.00±0.00	—	—
Hypophysis	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	—	—
Thyroid	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Eyeball	0.01±0.00	0.01±0.00	0.00±0.00	0.00±0.00	0.00±0.00	—
Submaxillary gland	0.08±0.01	0.05±0.01	0.02±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Thymus	0.06±0.01	0.05±0.02	0.01±0.00	0.00±0.00	0.00±0.00	—
Heart	0.16±0.02	0.11±0.03	0.03±0.00	0.01±0.00	0.00±0.00	0.00±0.00
Lung	0.16±0.03	0.11±0.03	0.04±0.00	0.01±0.00	0.00±0.00	0.00±0.00
Liver	11.98±2.47	6.71±1.11	3.55±0.25	2.99±0.24	1.63±0.23	0.97±0.05
Kidney	0.58±0.08	0.41±0.10	0.15±0.01	0.06±0.00	0.01±0.00	0.01±0.00
Spleen	0.07±0.01	0.06±0.02	0.02±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Pancreas	0.13±0.03	0.08±0.02	0.02±0.00	0.01±0.00	0.00±0.00	0.00±0.00
Adrenal	0.02±0.00	0.01±0.00	0.00±0.00	0.00±0.00	0.00±0.00	—
Fat ^{a)}	1.81±0.43	6.38±3.17	4.76±0.51	0.24±0.05	0.02±0.00	—
Testis	0.09±0.02	0.11±0.03	0.05±0.00	0.01±0.00	0.00±0.00	0.00±0.00
Epididymis	0.02±0.00	0.03±0.01	0.02±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Muscle ^{a)}	9.04±1.40	7.99±2.18	2.76±0.19	0.63±0.04	0.14±0.02	—
Skin ^{a)}	6.97±1.94	8.44±2.95	2.63±0.16	0.67±0.03	0.38±0.04	0.22±0.03
Stomach	7.55±2.24	1.53±0.82	0.11±0.02	0.02±0.00	0.01±0.00	0.00±0.00
Urinary bladder	0.01±0.00	0.01±0.00	0.00±0.00	0.00±0.00	0.00±0.00	—

Data are expressed as the mean values ± S.E. of four animals.

a) : Blood, muscle, fat, and skin volume were assumed to be 6.4%, 40%, 5%, and 22% of body weight, respectively.

b) : Not calculated, because tissue concentration was not detected.

and organ gradually decreased, except in the liver, it was lower than 0.5% of the dose at 24hr after ^3H -TJN-101 administration. At 72hr, radioactivity in the thyroid, liver, and skin was 6%, 5%, and 2%, respectively, of the maximum level, and, in other tissues and organs, radioactivity was less than 1% of the maximum level. The ratio of unstable tritium in plasma was 3%, 8%, 24%, 56%, 88%, and 90% of the concentrations found at 0.25, 0.75, 2, 8, 24, and 72hr after dosing, respectively.

3. Excretion in urine and feces

The cumulative excretion of radioactivity in the urine and feces of male and female rats after oral administration of ^3H -TJN-101 is shown in Table IV and V. The radioactivity excreted in urine and feces was 13.6% and 83.1%, respectively, of the dose at 168 hr after dosing in male rats, and 20.9% and 73.9%, respectively, in female rats. The ratio of unstable tritium in total urine was 1.7% of the dose in male rats and 1.0% of the dose in female rats. The residual radioactivity in the carcass at 168hr after dosing was 2.0% and 0.9% of the dose in male and female rats, respectively.

Fig. 6 Cumulative excretion of radioactivity in bile, urine, and feces after single oral administration ^3H -TJN-101 (4 mg/kg) to bile-duct cannulated male rats

Data are expressed as the mean values $\pm$ S.E. of four animals.

Amount in gastrointestinal contents at 48hr ; $0.2 \pm 0.1\%$ of dose.

Residual in carcass at 48hr ; $2.0 \pm 0.1\%$ of dose.

■ — ■ Bile
● — ● Urine
▲ — ▲ Feces

Table IV Cumulative excretion of radioactivity in urine and feces after single oral administration of ^3H -TJN-101(4mg/kg) to male rats

Sampling period (hr)	Excretion of radioactivity (% of dose)		
	Urine	Feces	Total
0- 4	3.7 ± 0.4 (3.6 ± 0.4)	— a)	—
8	5.9 ± 0.7 (5.7 ± 0.6)	0.0 ± 0.0	5.9 ± 0.7
12	8.2 ± 0.4 (7.9 ± 0.3)	—	—
24	11.0 ± 0.6 (10.4 ± 0.5)	47.2 ± 14.3	58.2 ± 13.8
48	12.5 ± 1.0 (11.7 ± 0.9)	75.0 ± 5.1	87.5 ± 4.1
72	12.9 ± 1.0 (11.8 ± 1.0)	80.9 ± 3.0	93.8 ± 2.1
96	13.2 ± 1.1 (11.9 ± 1.0)	82.1 ± 3.1	95.2 ± 2.2
120	13.3 ± 1.1 (11.9 ± 1.0)	82.6 ± 3.0	95.9 ± 2.0
144	13.4 ± 1.1 (11.9 ± 1.0)	82.9 ± 2.9	96.3 ± 2.0
168	13.6 ± 1.1 (11.9 ± 1.0)	83.1 ± 2.9	96.6 ± 2.0

Figures in parentheses were obtained from dried urine samples.

Residual in carcass at 168hr was $2.0 \pm 0.3\%$ of the dose.

Data are expressed as the mean values $\pm$ S.E. of four animals.

a) : Not determined

Table V Cumulative excretion of radioactivity in urine and feces after single oral administration of ^3H -TJN-101 (4mg/kg) to female rats

Sampling period (hr)	Excretion of radioactivity (% of dose)		
	Urine	Feces	Total
0- 4	4.7 $\pm$ 0.8 (4.7 $\pm$ 0.8)	— a)	—
8	9.2 $\pm$ 1.4 (9.0 $\pm$ 1.3)	0.0 $\pm$ 0.0	9.2 $\pm$ 1.4
12	13.0 $\pm$ 0.8 (12.7 $\pm$ 0.7)	—	—
24	18.2 $\pm$ 1.4 (17.8 $\pm$ 1.3)	46.2 $\pm$ 8.7	64.4 $\pm$ 8.0
48	20.0 $\pm$ 1.7 (19.4 $\pm$ 1.6)	67.6 $\pm$ 3.7	87.6 $\pm$ 2.7
72	20.4 $\pm$ 1.8 (19.7 $\pm$ 1.7)	72.1 $\pm$ 2.3	92.6 $\pm$ 1.3
96	20.6 $\pm$ 1.8 (19.8 $\pm$ 1.7)	73.2 $\pm$ 2.2	93.7 $\pm$ 1.2
120	20.7 $\pm$ 1.8 (19.8 $\pm$ 1.7)	73.5 $\pm$ 2.2	94.2 $\pm$ 1.2
144	20.8 $\pm$ 1.8 (19.8 $\pm$ 1.7)	73.7 $\pm$ 2.2	94.5 $\pm$ 1.2
168	20.9 $\pm$ 1.8 (19.9 $\pm$ 1.7)	73.9 $\pm$ 2.2	94.8 $\pm$ 1.2

Figures in parentheses were obtained from dried urine samples.

Residual in carcass at 168 hours was 0.9 $\pm$ 0.1% of dose.

Data are expressed as the mean values $\pm$ S.E. of four animals.

a) : Not determined

Table VI Cumulative excretion of radioactivity in bile, urine, and feces from bile duct-cannulated male rats after intraduodenal injection of bile sample (0-4 hr) obtained following single oral administration of ^3H -TJN-101 (4mg/kg) to male rats

Sampling period (hr)	Excretion of radioactivity (% of injected radioactivity)		
	Bile	Urine	Feces
0- 1	0.6 $\pm$ 0.1 (0.6 $\pm$ 0.1)	— a)	—
2	2.4 $\pm$ 0.5 (2.3 $\pm$ 0.4)	—	—
4	14.5 $\pm$ 3.9 (13.9 $\pm$ 3.7)	—	—
6	34.2 $\pm$ 3.4 (33.1 $\pm$ 3.2)	1.9 $\pm$ 0.3	—
8	41.6 $\pm$ 2.5 (40.2 $\pm$ 2.5)	—	—
12	47.0 $\pm$ 1.7 (45.3 $\pm$ 1.6)	2.5 $\pm$ 0.5	0.0 $\pm$ 0.0
24	50.9 $\pm$ 1.2 (49.0 $\pm$ 1.2)	3.8 $\pm$ 0.1	35.0 $\pm$ 3.4
48	52.0 $\pm$ 1.2 (49.9 $\pm$ 1.2)	4.0 $\pm$ 0.2	39.2 $\pm$ 2.4

Figures in parentheses were obtained from dried bile samples.

Amount in gastro-intestinal contents at 48hr was 1.7 $\pm$ 0.9% of the injected radioactivity.

Residual in carcass at 48hr was 1.6 $\pm$ 0.2% of the injected radioactivity.

Data are expressed as the mean values $\pm$ S.E. of four animals.

a) : Not determined

4. Excretion in bile

The cumulative excretion of radioactivity in bile, urine, and feces after oral administration of ^3H -TJN-101 is shown in Fig. 6. The percent excretion of radioactivity in bile was 80.3%, 88.0%, and 91.1% of the dose at 4, 12, and 48hr, respectively, after administration. The excretion of radioactivity in urine and feces, determined simultaneously, was

Table VI Binding of radioactive substances to plasma protein after single oral administration of ^3H -TJN-101 (4 mg/kg) to male rats

Time (hr)	Binding Ratio (%)
0.25	34.8 $\pm$ 2.5
0.75	32.5 $\pm$ 1.3
2	29.6 $\pm$ 0.7
8	43.8 $\pm$ 5.8

Data are expressed as the mean values $\pm$ S.E. of four animals.

Table VII Binding of ^3H -TJN-101 in male rat, dog, and human plasma protein *in vitro* at ^3H -TJN-101 concentrations of 0.02, 0.1, 0.5, and 2.5 $\mu\text{g/ml}$

Concentration ($\mu\text{g/ml}$)	Binding Ratio (%)		
	Rat plasma	Dog plasma	Human plasma
0.02	66.4 $\pm$ 1.1	60.0 $\pm$ 1.3	95.4 $\pm$ 0.2
0.1	63.1 $\pm$ 1.6	55.6 $\pm$ 2.0	95.4 $\pm$ 0.5
0.5	56.5 $\pm$ 1.3	56.3 $\pm$ 2.3	95.2 $\pm$ 0.5
2.5	46.3 $\pm$ 0.7	46.1 $\pm$ 1.1	93.2 $\pm$ 0.3

Data are expressed as the mean values $\pm$ S.E. of four experiments.

4.5% and 2.5%, respectively, of the dose at 48hr after administration. Residual radioactivity in the gastrointestinal contents and the carcass at 48hr after dosing was 0.2% and 2.0% of the dose, respectively.

5. Enterohepatic circulation

^3H -TJN-101 was administered to the bile-duct-cannulated rats, thereafter bile was collected for up to 4hr. Radioactivity excreted into the bile was about 80% of the dose. The radioactive bile was injected into the duodenum of other bile-duct-cannulated rats. The percent absorption of radioactive materials in the bile was 14.5%, 50.9%, and 52.0% of the administered radioactivity in the bile at 4, 24, and 48hr after dosing, respectively, as shown in Table VI. The excretion of radioactivity in urine and feces, determined simultaneously, was 4.0% and 39.2% of the injected radioactivity at 48hr after injection. The residual radioactivity in the gastrointestinal contents and the carcass at 48hr after injection was 1.7% and 1.6%, respectively, of the injected radioactivity.

6. Protein binding

The ratio of plasma protein binding of radioactive substances *in vivo* following oral administration of ^3H -TJN-101 (4mg/kg) to male rats is shown in Table VII. The ratio of binding was 34.8%, 32.5%, 29.6%, and 43.8% of the radioactivity in plasma at 0.25, 0.75, 2, and 8hr after dosing, respectively. To investigate the reversibility of binding, the

radioactivity of the inner solution after infinite dialysis was measured, so that 4.8% of the total plasma radioactivity (0.75hr) remained in the dialysis bag. Adsorption of radioactivity to the dialysis bag was not observed.

In the *in vitro* study, the percent binding of radioactivity to rat plasma protein was 66.4%, 63.1%, 56.5%, and 46.3% at ^3H -TJN-101 concentrations of 0.02, 0.1, 0.5, and $2.5\mu\text{g/ml}$, respectively (Table VII). On the other hand, the percent binding of radioactivity was 60.0%, 55.6%, 56.3%, and 46.1% to dog plasma protein, and 95.4%, 95.2%, and 93.2% to human plasma protein at the same ^3H -TJN-101 concentrations as shown above for the rat. When studying the reversibility of binding, we found that the radioactivity of the inner solution after infinite dialysis was 1.6% and 0.8% of the total plasma radioactivity in rat and dog, respectively, at a ^3H -TJN-101 concentration of $0.5\mu\text{g/ml}$. In human plasma, significant radioactivity of the outer solution was still observed after 96hr dialysis, and 65.0% of added radioactivity remained in the inner solution.

Since denaturation of the plasma protein was observed, at this time, the dialysis was then finished.

Discussion

After oral administration of ^3H -TJN-101 (4 mg/kg) to male rats, volatile (unstable) tritium was excreted in urine (1.7% of the dose at 168hr after administration), but not excreted in expired air (data not shown). Accordingly, biotransformation of ^3H -TJN-101 to unstable tritium seemed to be relatively low. We speculated that our present study offer sufficient information regarding the metabolic fate of TJN-101 and its metabolites, which have the same basic structure. The quantity of unstable tritium is very small in terms of percentage of the dose, but it has a large ratio in blood and plasma for a long period after dosing. It is necessary to take the concentration of unstable tritium into account when performing a distribution study.

Following oral administration of ^3H -TJN-101 (4mg/kg), blood radioactivity concentration increased rapidly and reached a maximum at 0.75hr after dosing. After oral administration, the excretion of radioactivity in feces was 83.1% of the dose and that in urine was only 13.6% of the dose within 168hr after dosing. On the other hand, in bile-duct-cannulated rats after dosing, the main excretory route was bile, with 91.1% of the radioactivity being thus excreted 48hr after dosing. Urinary excretion of radioactivity, determined simultaneously, was only 4.5% of the dose. It was confirmed that the gastrointestinal absorption of TJN-101 was very high, more than 95% of the dose being thus absorbed. We have already reported a similar result which was obtained from an absorption study using an *in situ* loop method⁸⁾ with non-labeled TJN-101. Moreover, it was suggested that the absorption rate was extremely fast, because excretion of radioactivity in bile had almost ended 4hr after dosing. This rapid absorption was also supported by our result showing that the t_{max} of blood concentration was 0.75hr after oral dose.

Since a large quantity of radioactivity was excreted in bile, we carried out a study of enterohepatic circulation, in which radioactive bile was injected into the duodenum of bile-duct-cannulated rats. About 60% of the injected radioactivity was reabsorbed and excreted mainly in bile. Therefore, it was clear that TJN-101 was excreted mainly in bile

and that it repeated the enterohepatic circulation after being absorbed from the gastrointestinal lumen; finally being excreted mainly in feces. While excretion of the radioactivity in bile had almost ended 4hr after dosing, excretion in urine and feces continued for more than 24hr after dosing. Blood concentration from 4hr to 8hr after dosing changed at almost the same levels. It was considered that these levels were affected by the enterohepatic circulation.

TJN-101 was administered orally to male rats at doses of 0.8, 4.0, and 20mg/kg to investigate dose-relation. $C_{\max}$ and AUC of the 0.8mg/kg group were 12% of the values in the 4mg/kg group, and the $C_{\max}$ and AUC of the 20mg/kg group were 5.4 and 9.2 times larger, respectively, than those in the 4mg/kg group. The $t_{\max}$ had a tendency to delay when doses were increased. It is suggested that the dose-dependency was not authentic. However, these results in affected by not only unchanged TJN-101 but also its metabolite. We have already confirmed the dose-relationship of unchanged TJN-101⁸⁾.

For investigating sex differences in the metabolic fate of TJN-101, ^3H -TJN-101 was given orally to female rats at a dose of 4mg/kg. No fundamental differences were observed in $t_{\max}$, $C_{\max}$ and AUC except that the elimination half-life from $t_{\max}$ to 8hr after dosing was longer in the females. Furthermore, urinary excretion in the female rat was about 7% more than that in the male rat, and the ratio of unstable tritium to total radioactivity excreted in urine in the female rat was less than that in the male rat. Thus, there is no sex difference in the absorption phase, however, the discrepancy in the excretion phase and in the ratio of unstable tritium to total radioactivity excreted in urine is due to metabolic differences between the male and female rats.

After oral administration of ^3H -TJN-101 to male rats, the radioactivity in most tissues reached its maximum level within 0.25 to 0.75hr, being much higher in the liver, which is the target organ, and lower in the central nervous system, testis, and eyeball. Tissue levels in other organs were equal to or higher than those in plasma. It is suggested that TJN-101 and its metabolites are transported rapidly to various tissues after oral administration and it is considered that low plasma protein binding *in vivo* is related to this favorable distribution in tissues and organs. Elimination of radioactivity was slightly slower in the liver and thyroid than in other tissues and organs, but radioactivity was rapidly eliminated in the other tissues in the same manner as it was from plasma, and radioactivity did not tend to remain in the tissues.

We carried out two studies on plasma protein binding. In the *in vitro* study, rat, dog, and human plasma were used to investigate species differences. The percent binding of radioactivity to rat and dog plasma protein was about 60% at concentrations of 0.02 to 0.5 $\mu\text{g}/\text{ml}$ of ^3H -TJN-101, which decreased by 46% at a concentration of 2.5 $\mu\text{g}/\text{ml}$. On the other hand, the percentage of radioactivity bound to human plasma protein was about 95% at each concentration, so that a species difference was observed between humans and experimental animals. In the *in vivo* study, the percentage of plasma protein binding was lower than that *in vitro* at each sampling point. This showed that binding of the metabolites to plasma protein was lower than that of unchanged TJN-101; a result also supported by our previous report⁸⁾. Infinite dialysis was carried out to investigate the reversibility of binding, and high radioactivity in the dialysis bag was observed only in

the case of human plasma. However, as 98% of the radioactivity in plasma was extracted with methanol (unpublished data), it may be concluded that the binding to plasma protein was reversible.

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