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Distribution of Thiourea following Intravenous and Oral Administration to Rats[1]

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^{14}C-Thiourea was given by intravenous or oral administration at low dose (100 μCi/0.16 mg/kg) or high dose (100 μCi/160 mg/kg) to rats, then plasma levels were determined and whole-body autoradiography was carried out.

The gel filtration on Sephadex G-10 of plasma obtained following intravenous administration of ^{14}C-thiourea showed that the radioactive label was covalently bound to macromolecular fractions such as protein or peptide.

Plasma levels of radioactivity due to unchanged thiourea and the tissue distribution patterns of radioactivity following intravenous administration of ^{14}C-thiourea were considerably different after a low dose and a high dose. The elimination of thiourea from plasma in the case of high dose ($t_{1/2\beta}$; 7.0 h) was greatly delayed as compared with that after the low dose ($t_{1/2\beta}$; 0.69 h). The whole-body autoradiograms indicated that, at low dose, very high radioactivity was presented in the lung, thymus and Harder's gland at 60 min following intravenous administration, whereas when the high dose was administered the radioactivity was distributed almost homogeneously throughout the body. The remarkable dose dependency in thiourea distribution might be brought about by the saturation of irreversible covalent binding sites of plasma and tissue macromolecules in the case of high dose administration.

Keywords——thiourea; distribution; elimination; dose dependency; plasma level; whole-body autoradiography; pharmacokinetics; gel filtration

Introduction

In previous reports,[2,3] the distribution kinetics of creatinine and urea, which are considered to pass through the water-filled pores of biological membranes easily, were discussed on the basis of experiments on plasma levels and whole-body autoradiography. It was clarified in these studies that the differences of pharmacokinetic parameters between a low dose and a high dose (5700 times and 15000 times the low dose for creatinine and urea, respectively) were not great.

Thiourea is a weakly basic (pK_a 0.96) and water-soluble compound with a relatively low molecular weight (M.W. 76.1), like creatinine and urea. It was reported,[4–7] however, that when ^{35}S- or ^{14}C-labelled thiourea was administered to rats or mice intravenously or intraperitoneally, the radioactivity was gradually concentrated in the thyroid gland, lung, liver and Harder's gland, in contrast to creatinine[2] and urea[3] which were distributed homogeneously throughout the body in the β-phase. The mechanism of accumulation of radioactivity may involve the formation of covalent (disulfide) bonds between thiourea and tissue protein, as was asserted in the case of thyroid gland.[8] This interesting behavior led us to study the effect of dose levels on the distribution kinetics of thiourea, since no such study has yet been reported.

In this report, studies on plasma levels and whole-body autoradiography following intravenous or oral administration of thiourea at low dose (0.16 mg/kg) and high dose (160 mg/kg) to rats were performed. Additionally, the effect of pre-administration of thiouracil, which has a similar distribution pattern to that of thiourea in the body,[6] and the change of distribution pattern in 3-week-old rats were investigated by whole-body autoradiography.

Materials and Methods

Chemicals——^{14}C-Thiourea (specific activity, 9.6 mCi/mmol) was purchased from New England Nuclear, Boston, Mass., U.S.A. The radiochemical purity was greater than 97%. All other chemicals were of analytical grade and were used without further purification.

Animals——Male Wistar rats, 8 to 9 weeks old, were purchased from Shizuoka Agricultural Cooperative of Experimental Animals, Hamamatsu, Japan, for all experiments. In the whole-body autoradiography experiments, 3-week-old rats were also used. All rats were chronically cannulated into the left external jugular vein with silicone polymer tubing (Dow Corning, Tokyo, Japan) by the method of Upton.[9] In the case of oral administration, rats were fasted for at least 15 h before experiments.

Urinary, Fecal and Expiratory Excretion following Intravenous and Oral Administration——Rats were given 10 μCi/kg of ^{14}C-thiourea (0.16 mg/kg as thiourea) intravenously into the external jugular vein or orally by gastric intubation. The rats were housed individually in metabolic cages (KN-450, Natsume, Tokyo, Japan), which were equipped to capture $^{14}CO_2$ in a mixture of ethanolamine and methanol (1: 2, v/v), or in conventional metabolic cages (KN-646(B), Natsume).

Plasma Levels of ^{14}C-Thiourea following Intravenous Administration——Rats were given 100 μCi/kg of ^{14}C-thiourea at low dose (0.16 mg/kg as thiourea) or high dose (160 mg/kg as thiourea) into the external jugular vein. Blood samples (250 μl) were withdrawn periodically into small heparinized and ice-cooled tubes, and plasma samples (100 μl) were obtained by centrifuging the tubes at 3000 rpm for 15 min. Plasma samples were divided into two fractions. One fraction (50 μl) was used for the determination of total radioactivity, and the other fraction (50 μl) was used to determine unchanged thiourea by gel filtration chromatography, as will be mentioned later.

Whole-Body Autoradiography following Intravenous and Oral Administration——Rats (8 to 9 weeks old) were given intravenously 100 μCi/kg of ^{14}C-thiourea at low dose (0.16 mg/kg as thiourea), with or without intravenous pre-administration of thiouracil (100 mg/kg) 5 min before the thiourea administration, or at high dose (160 mg/kg as thiourea). Immature rats (3-week-old) were given only the low dose. In other experiments, rats were orally given 100 μCi/kg at low dose or high dose by gastric intubation. These rats were sacrificed at 5 or 60 min by soaking them in dry ice-acetone (−78°C) under light anesthesia with ether. Sections (40 μm) were obtained with a microtome (Yamato 1111, Tokyo, Japan) at about −25°C, and attached to SALOTAPE (Hisamitsu Pharmaceutical Co., Ltd., Tosu, Japan). After being dried in a freeze-dryer for a few days, the sections were placed in contact with X-ray films (No. 150, Fuji Photo Film Co., Ltd., Tokyo, Japan) for 20 d at 4°C. A densitometer (PDA-11, Konishiroku, Tokyo, Japan) was used for determining the optical density of autoradiograms.

Gel Filtration——Plasma (50 μl) or urine (200 μl) samples were applied to a 1×15 cm column packed with Sephadex G-10 (Pharmacia Fine Chemicals, Uppsala, Sweden) at room temperature, and then eluted with distilled water. The eluate was fractionated by means of an automatic fraction collector.

Radio-Thin-Layer Chromatography of the Eluates from Sephadex G-10 Chromatography——The fractions were concentrated in an evaporator and directly spotted on the TLC-plates (Avicel SF, Funacoshi Pharmaceutical Co., Ltd., Tokyo, Japan) with non-radiolabelled thiourea. The plates were developed with the solvent system of acetone: methanol: water=5: 2: 1, and the distribution profiles of radioactivity were determined with a radio-thin-layer chromatogram scanner (JTC-203, Aloka, Tokyo, Japan).

Radioactivity Measurement——The radioactivity was determined in a Mark II liquid scintillation spectrometer (Nuclear-Chicago Corporation, Des Plaines, Ill., U.S.A.). All samples were determined with 10 ml of toluene-Triton X-100 liquid scintillator (PPO 5 g, POPOP 300 mg, toluene 700 ml, Triton X-100 300 ml). The counting efficiencies were automatically determined by the external standard ratio method and cpm was converted to dpm.

Results

Measurement of Unchanged Thiourea in Plasma and Urine

Typical gel filtration chromatography profiles on Sephadex G-10 of plasma and urine in 3 h following intravenous administration of ^{14}C-thiourea (low dose, 0.16 mg/kg) are shown in Fig. 1. As the radioactivity was separated into two fractions in both plasma and urine, the radio-thin-layer chromatography of each fraction of urine was carried out. The TLC chromatograms for low and high molecular fractions in urine are shown in Fig. 2. It is apparent from Fig. 2 that the bulk of the radioactivity contained in the low molecular fraction was that of unchanged thiourea, and that most of the radioactive materials contained in the high molecular fraction were strongly adsorbed by cellulose. As ^{14}C-thiourea was almost completely recovered from the column packed with Sephadex G-10, it was concluded that the total radioactivity contained in the low molecular fraction which was separated on Sephadex

G-10 was equal to the radioactivity of unchanged thiourea in urine, and that the difference between the total radioactivity and the radioactivity in the low molecular fraction was equal to the radioactivity of the metabolites. Similar considerations should apply to plasma.

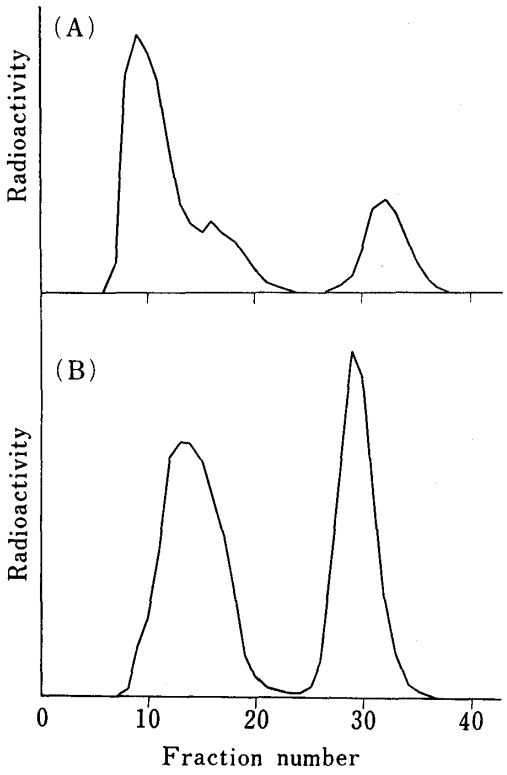

Fig. 1. Typical Gel Filtration Chromatograms on Sephadex G-10 of Plasma (A) and Urine (B) at 3 h following Intravenous Administration of ^{14}C-Thiourea

Dose, 10 μCi/kg (0.16 mg/kg as thiourea); column size, 1×15 cm; eluent, distilled water; volume of fractions, 250 μl; sample applied, plasma 50 μl, urine 200 μl.

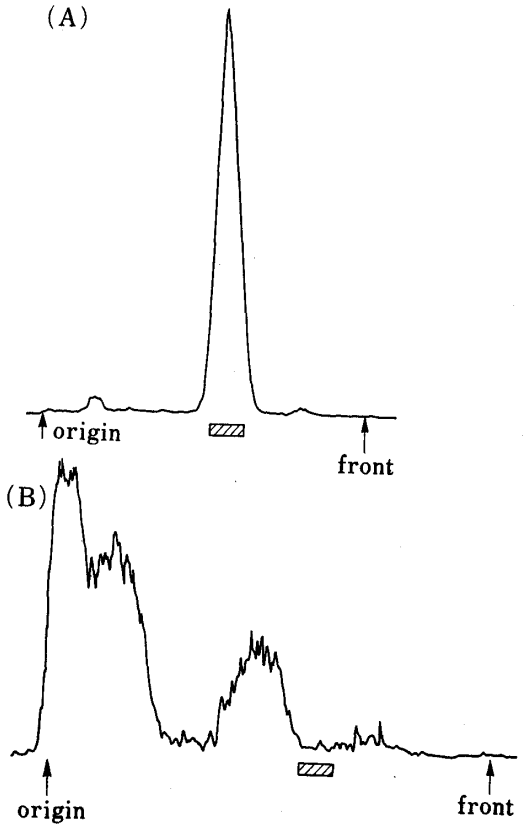

Fig. 2. Typical Radio-Thin-Layer Chromatograms for Low (A) and High (B) Molecular Fractions of Urine in Fig. 1.

Solvent system, acetone: methanol: water=5: 2: 1. The hatched area indicates the dark spot detected as thiourea under a UV-lamp.

Urinary, Fecal and Expiratory Excretion following Intravenous and Oral Administration

The percentages of radioactivity recovered from urine, feces and expired air following intravenous and oral administration of ^{14}C-thiourea (low dose, 0.16 mg/kg) to rats are summarized in Table I. It is apparent that the total recovery in 24 h was about 80% in both routes of administration, and that urine was the major excretion route of the radioactivity.

The gel filtration on Sephadex G-10 of the urine showed that about 30% of the dose was excreted into the urine as unchanged form in 24 h following intravenous administration at

Table I. Urinary, Fecal and Expiratory Excretion following Intravenous or Oral Administration[a] of ^{14}C-Thiourea to Rats

Biological sample	Recovery (% of dose±S.D., 0—24 h)	
	Oral (n=3)	Intravenous (n=3)
Urine	74.5±1.1	77.1±1.9
Feces	1.5±0.8	2.0±0.5
Expired air	2.3±0.3	2.6±0.4
Total	79.2±1.8	81.7±1.1

a) Dose: 10 μCi/kg (0.16 mg/kg as thiourea).

low dose. The experiment at high dose, however, could not be carried out because rats died within a few hours following intravenous administration.

Plasma Levels of ^{14}C-Thiourea following Intravenous Administration

Plasma levels of radioactivity from unchanged thiourea following intravenous administra-

Table II. Pharmacokinetic Parameters for ^{14}C-Thiourea following Intravenous Administration at Low Dose and High Dose (Value for Parameter±Standard Error[a])

Parameter	Low dose[b] (n=7)[c]	High dose[d] (n=7)[c]
A, dpm/ml	1.70×10^{5} ±3.04×10^{4}	1.26×10^{5}[e] ±1.27×10^{4}
B, dpm/ml	2.44×10^{5} ±2.13×10^{3}	1.76×10^{5}[e] ±1.13×10^{4}
α, min^{-1}	5.17×10^{-1}±8.40×10^{-2}	6.61×10^{-2}[e]±1.13×10^{-2}
β, min^{-1}	1.67×10^{-2}±8.56×10^{-5}	1.66×10^{-3}[e]±4.64×10^{-4}
k_{10}, min^{-1}	2.78×10^{-2}±2.02×10^{-3}	2.79×10^{-3}[e]±6.54×10^{-4}
k_{12}, min^{-1}	1.95×10^{-1}±5.28×10^{-2}	2.57×10^{-2}[e]±6.00×10^{-3}
k_{21}, min^{-1}	3.11×10^{-1}±3.11×10^{-2}	3.93×10^{-2}[e]±1.10×10^{-2}
V_1, ml/kg	5.36×10^{2} ±4.00×10^{1}	7.35×10^{2}[e] ±2.68×10^{1}
V_2, ml/kg	3.35×10^{2} ±1.00×10^{2}	4.81×10^{2} ±1.76×10^{2}
AUC, dpm·min/ml	1.49×10^{7} ±1.68×10^{5}	1.08×10^{8}[e] ±3.04×10^{7}
$(V_d')_{extrap}$,ml/kg[f]	9.10×10^{2} ±7.49×10^{0}	1.26×10^{3}[e] ±8.09×10^{1}
$(V_d')_{\beta}$,ml/kg[g]	8.92×10^{2} ±9.29×10^{1}	1.24×10^{3}[e] ±4.53×10^{2}
$t_{1/2\beta}$, h	6.92×10^{-1}±3.55×10^{-3}	6.96×10^{0}[e] ±1.95×10^{0}

a) W. E. Deming, "Statistical Adjustment of Data," John Wiley and Sons, Inc., New York, 1946.
b) 0.16 mg/kg.
c) The number of input data.
d) 160 mg/kg.
e) Significantly different from the value for the low dose at $p<0.01$.
f) $(V_d')_{extrap}=$(dose)/B.
g) $(V_d')_{\beta}=V_1\cdot k_{10}/\beta$.

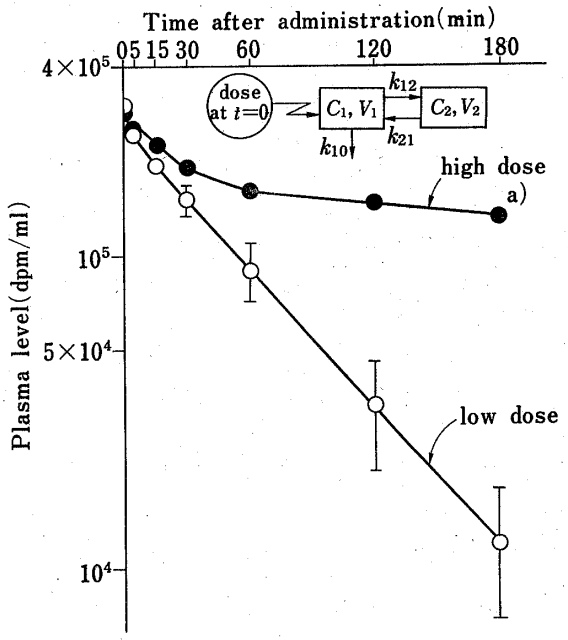

Fig. 3. Plasma Levels of ^{14}C-Thiourea following Intravenous Administration at Low Dose and High Dose

Each point represents the mean±S.D. for three to four animals (a): for one animal). The points without S.D. have smaller S.D. than the circles. The plots are computer-fitted curves (weight $(i)=1/C_i^2$).
○, low dose; ●, high dose.

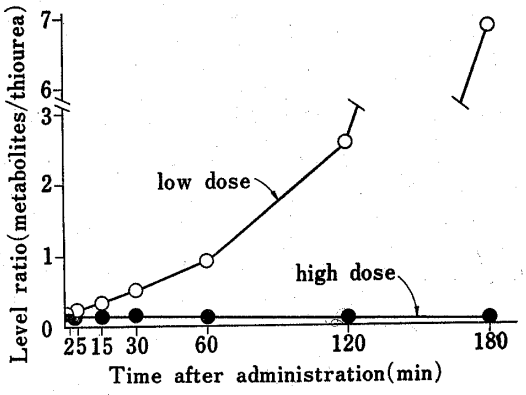

Fig. 4. The Concentration Ratios of Metabolites to Unchanged Thiourea in Plasma following Intravenous Administration at Low Dose and High Dose

The mean concentrations were used to calculate the ratios.
○, low dose; ●, high dose.

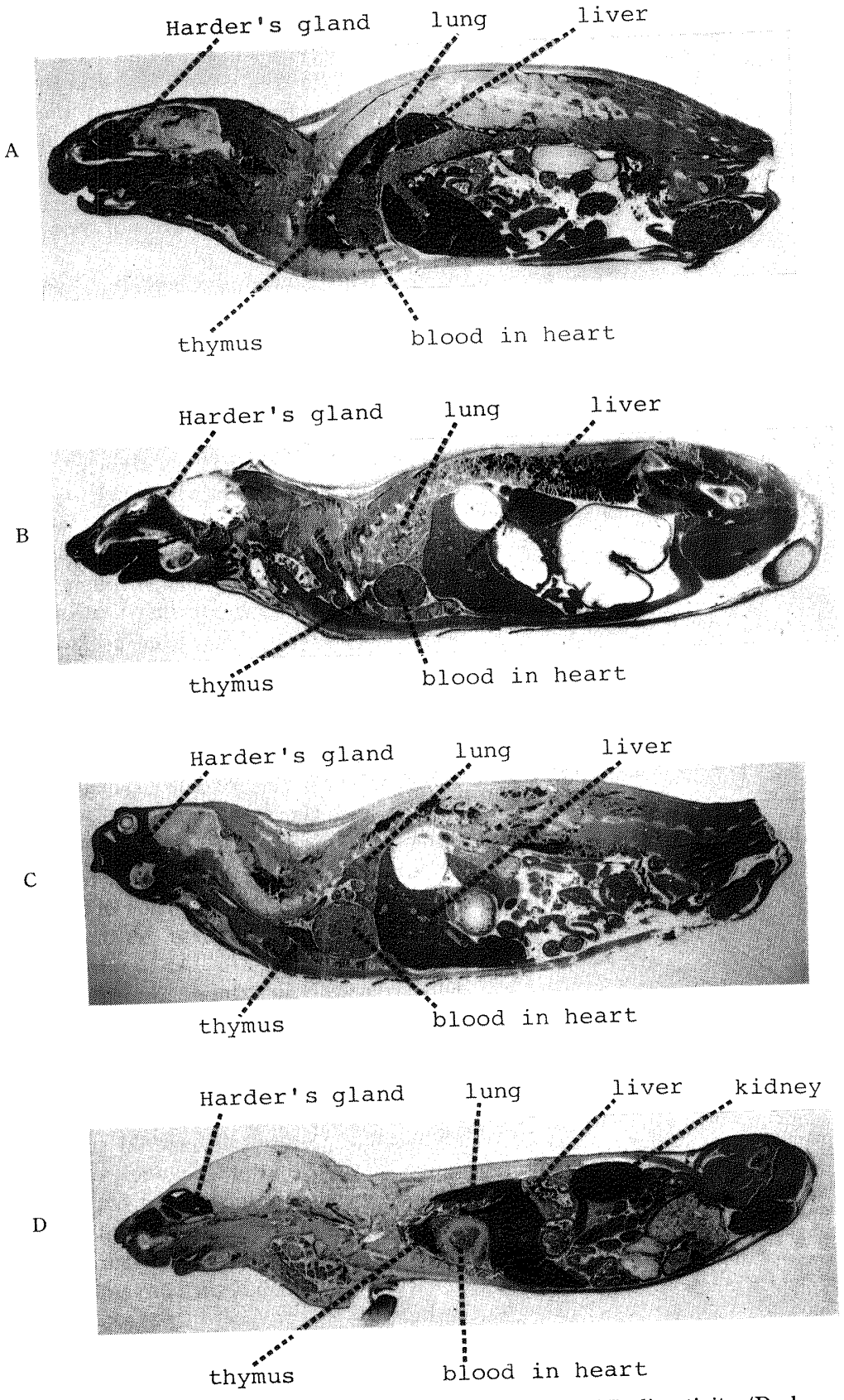

Fig. 5. Autoradiograms showing the Distribution of Radioactivity (Dark Area) at 60 min following Intravenous Administration of ^{14}C-Thiourea

A, low dose (0.16 mg/kg); B, high dose (160 mg/kg); C, low dose with the pre-administration of thiouracil (100 mg/kg) D, low dose to a immature rat (3-week-old).

tion at low dose and high dose, which were analyzed by a two-compartment open model, are shown in Fig. 3 and the estimated pharmacokinetic parameters are summarized in Table II. Significant differences ($p<0.01$) were observed in all parameters except V_2, and the differences were especially marked in the parameters α, β, k_{10}, k_{12}, k_{21}. At high dose, the parameters relating to elimination, β and k_{10}, were one-tenth of the values at low dose, and the parameters relating to transfer between compartments 1 and 2, k_{12} and k_{21}, were about one-eighth of those at low dose. In contrast to the large differences found in these rate constants, the volume of distribution parameters, such as V_1, V_2, $(V_d')_{extrap}$ and $(V_d')_\beta$, were not very different at the two dose levels.

The ratios of metabolites to unchanged thiourea in plasma are plotted *versus* time in Fig. 4. The ratio at low dose rose with time, but that at high dose never rose above 0.25.

Whole-Body Autoradiography following Intravenous Administration

In Fig. 5, whole-body autoradiograms at 60 min following intravenous administration to rats at low dose (Fig. 5-A), at high dose (Fig. 5-B), and at low dose with pre-administration of thiouracil (Fig. 5-C), and to a immature rat at low dose (Fig. 5-D) are shown. In Fig. 5-A, it can be seen that the radioactivity was distributed at much higher concentrations in the lung, thymus and Harder's gland than in blood and the other tissues or organs. With the high dose (Fig. 5-B), however, the radioactivity was distributed almost homogeneously throughout the body, and a similar distribution pattern is apparent in Fig. 5-C (after pre-administration of thiouracil, which has distribution characteristics similar to those of thiourea[6]).

When a immature rat (3-week-old) was used (Fig. 5-D), the radioactivity was highly concentrated in the liver as well as in the lung, thymus and Harder's gland. The most notable difference between Fig. 5-A and Fig. 5-D is the extent of transfer of radioactivity to the liver.

The optical density ratios of liver or lung to heart blood in these autoradiograms are illustrated in Fig. 6. At 5 min, the optical density ratios in these autoradiograms were virtually the same in both liver and lung. At 60 min, however, the ratios at low dose (○) were considerably different from those obtained at high dose (●), after pre-administration of thiouracil (△) or in a 3-week-old rat (▲) in both organs, except that no change in the liver was caused by pre-administration of thiouracil. It is also apparent that the optical density ratio for the liver was larger in immature rat (▲) than in mature rat (○), but the converse was true for the lung.

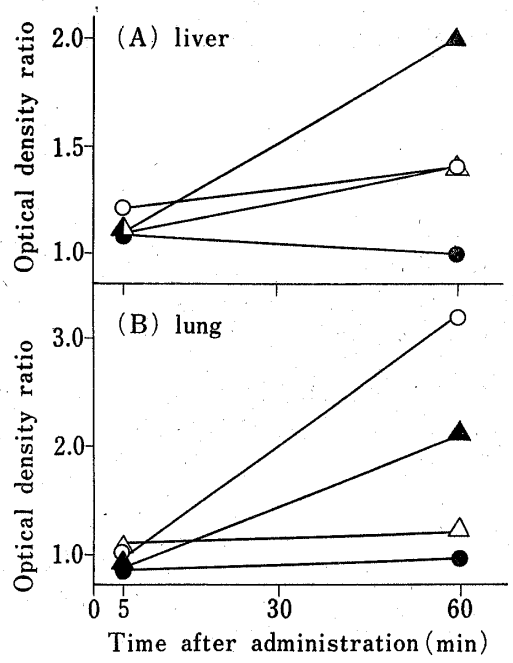

Fig. 6. Optical Density Ratios of Liver (A) or Lung (B) to Heart Blood in Autoradiograms following Intravenous Administration of ^{14}C-Thiourea

○, low dose; ●, high dose; △, low dose with the pre-administration of thiouracil (100 mg/kg); ▲, low dose to immature rats.

A whole-body autoradiogram at 24 h following intravenous administration to a rat at low dose is shown in Fig. 7. Relatively high radioactivity was still present in the lung, liver, kidney, thymus and Harder's gland. The radioactivity in these organs and tissues might represent the amount (about 20%) of the dose which was not recovered in the 24 h following intravenous administration (Table I).

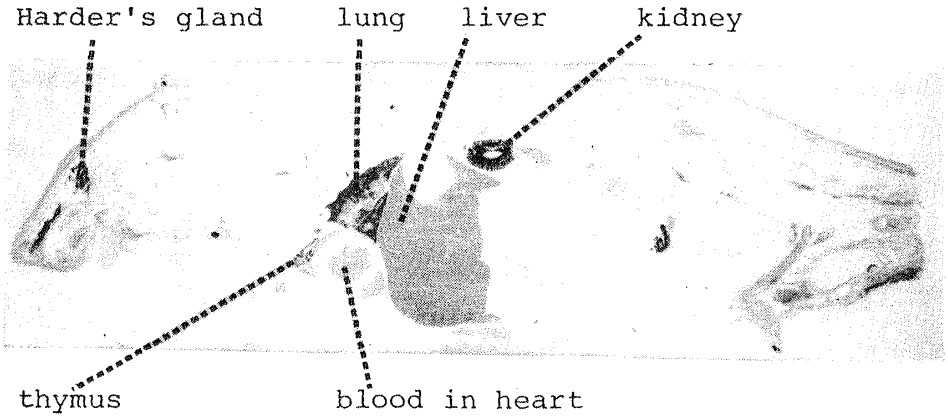

Fig. 7. Autoradiogram showing the Distribution of Radioactivity (Dark Area) at 24 h following Intravenous Administration of ^{14}C-Thiourea

Whole-Body Autoradiography following Oral Administration

Whole-body autoradiograms at 60 min following oral administration to rats at low dose and high dose are shown in Fig. 8, and the optical densities and optical density ratios to heart

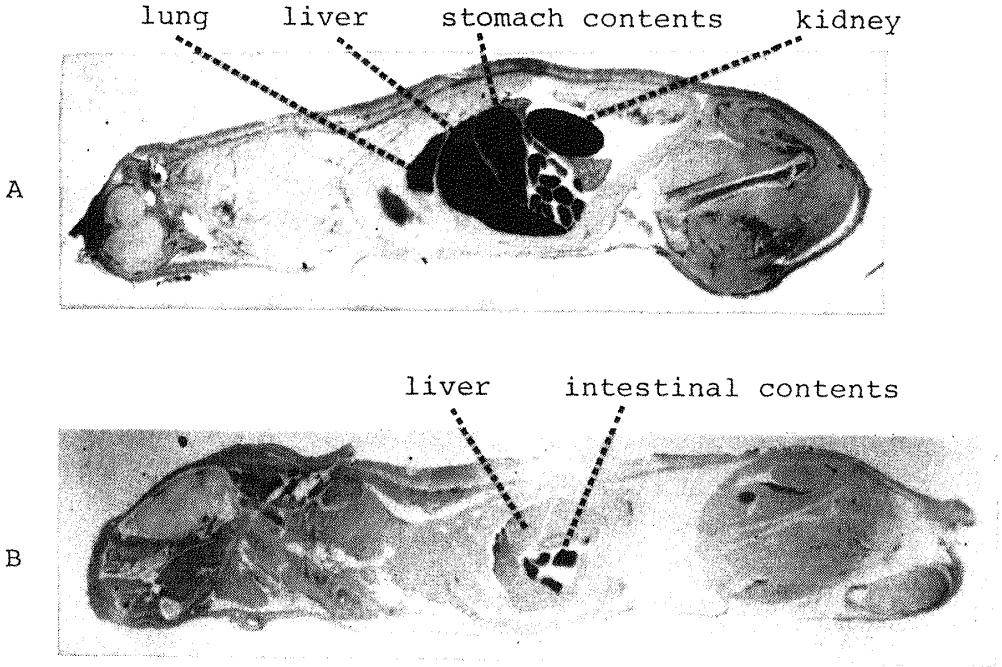

Fig. 8. Autoradiograms showing the Distribution of Radioactivity (Dark Area) at 1 h following Oral Administration of ^{14}C-Thiourea at Low Dose and High Dose

A, low dose; B, high dose.

TABLE III. The Optical Density of Liver or Lung in Autoradiograms at 60 min following Intravenous or Oral Administration at Low Dose and High Dose

Organ	Low dose		High dose	
	Intravenous	Oral	Intravenous	Oral
Liver	0.26 (1.4)[a]	0.30 (2.3)	0.16 (1.0)	0.09 (1.8)
Lung	0.58 (3.2)	0.24 (1.8)	0.15 (0.94)	0.08 (1.6)

a) The numbers in parentheses indicate the optical density ratio to heart blood.

blood are listed in Table III for the liver and lung. Although ^{14}C-thiourea remained significantly in the gastrointestinal tract to be absorbed following the administration of the low dose, the optical density in the liver (0.30) was much the same as that in the case of intravenous administration (Fig. 5-A, 0.26). When the optical density ratios of liver to heart blood are compared, an apparent difference between intravenous (1.4) and oral (2.3) administration was seen. In lung, however, the reverse result was obtained.

Discussion

By means of studies on the tissue levels of radioactivity,[4,5] or whole-body autoradiography[6,7] in mice and rats following the administration of ^{35}S- or ^{14}C-labelled thiourea (1 mg/rat,[4] 0.6 mg/kg,[5] 0.05 mg/mouse[6] as thiourea), it was clarified that the radioactivity was concentrated in the thyroid gland, lung, liver, Harder's gland, *etc.* From these experimental data, we considered that these tissues might show saturation characteristics for the transfer of thiourea and/or its metabolites, and we therefore investigated the dose-dependent distribution, which was not remarkable in the cases of creatinine[2] and urea[3] reported previously.

The peak of unchanged thiourea was not observed in the TLC chromatogram (Fig. 2-B) for the high molecular fraction (Fig. 1-B) of the radioactivity in urine, and therefore it was assumed that the interaction between thiourea and macromolecules in the body was not a usual drug-macromolecule interaction, but covalent binding. This might be based on the cleavage of disulfide bonds in the macromolecules followed by the formation of irreversible covalent (disulfide) binding between the macromolecules and thiourea, as suggested by Maloof and Soodak for thyroid tissue,[8] and/or on the formation of irreversible covalent (disulfide) binding between SH-groups of macromolecules and thiourea. In this paper, the authors regarded the covalently bound product of thiourea and macromolecule as a metabolite.

The pharmacokinetic parameters relating to elimination, β and k_{10}, were surprisingly different at low dose (0.16 mg/kg) and high dose (160 mg/kg) (Fig. 3, Table II). The decreased total body clearance ($k_{10} \cdot V_1$) in the case of high dose was considered to be partly caused by the saturation of such irreversible covalent binding between macromolecules in plasma and thiourea as mentioned above, thus bringing to a halt of the metabolism of thiourea. In fact, the plasma total radioactivity following intravenous administration of ^{14}C-thiourea at high dose consisted largely of the radioactivity of unchanged thiourea (Fig. 4).

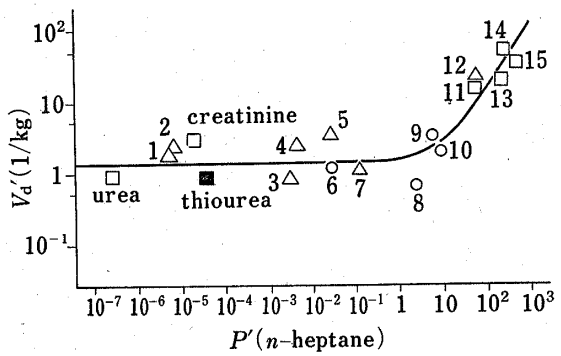

Fig. 9. Relationship between V_d' and P' for Basic Drugs

○, rabbits; □, ■, rats; △, dogs. 1, ephedrine; 2, norephedrine; 3, *N*-acetyl-4-aminoantipyrine; 4, tolazoline; 5, quinidine; 6, antipyrine; 7, chlordiazepoxide; 8, *p*-toluidine; 9, 3,4-xylidine; 10, *o*-chloroaniline; 11, promazine; 12, fluphenazine; 13, trimeprazine; 14, chlorpromazine; 15, triflupromazine. Values of $(V_d')_{extrap}$ were provisionally used as V_d' in this paper for the chemicals, for which blood levels were analyzed by means of a two-compartment open model. The value of P' for thiourea was calculated from the equation,[13] $\log P'_{n\text{-heptane}} = 1.848 \times \log P'_{octanol} - 2.223$.

Watanabe and Kozaki[10,11] have described the relationship between the apparent volume of distribution (V_d') of basic drugs and the apparent partition coefficients (P') for *n*-heptane, that is, the V_d' value is almost constant in the region of low P', but increases in the region of high P'. The value of apparent partition coefficient for thiourea (7.24×10^{-2}) was reported for octanol as oil phase.[12] The authors calculated the apparent partition coefficient of thiourea when *n*-heptane was used as the oil phase by the method of Leo and Hansch.[13] When the point obtained from $(V_d')_{extrap}$ at low dose (9.10×10^2 ml/kg) and the calculated

value of apparent partition coefficient (4.68×10^{-5}) was incorporated into the figure described by Watanabe and Kozaki, it was clear that thiourea was fairly close to the solid line calculated according to the drug distribution model[10,11] (Fig. 9).

The whole-body autoradiogram at 60 min following intravenous administration of the low dose (Fig. 5-A) showed remarkable concentration of radioactivity in the lung, thymus and Harder's gland. This result is in good agreement with the reported autoradiographic studies in mice.[6,7] However, when the high dose was administered (Fig. 5-B), or when the pre-administration of thiouracil was performed (Fig. 5-C), the radioactivity was distributed almost homogeneously. The above distribution properties suggested that the irreversible covalent binding mentioned above might occur not only with the plasma macromolecules but also with the tissue macromolecules, and that the saturation of the binding formation might occur in Fig. 5-B and Fig. 5-C where thiouracil was expected to pre-occupy the binding sites of the macromolecules in the body. The effect of the high dose on tissue distribution of radioactivity was remarkable in both the lung and liver (Fig. 6). However, no effect of pre-administration of thiouracil was observed in the liver, in contrast to the lung. The difference of covalent binding characteristics (extent or rate) to the liver between thiouracil and thiourea might be responsible for this. Hollinger *et al.*[5] reported that the radioactivity binding to lung protein was greater in mature rats than in immature rats following intraperitoneal administration of ^{14}C-thiourea (0.6 mg/kg). They directly determined the level of radioactivity in the tissues and organs removed from rats. When we applied the autoradiographic technique to clarify the difference of radioactivity distribution between mature and immature (3-week-old) rats, the result for the lung was similar to the data of Hollinger *et al.* However, it was found that the level of radioactivity in the liver was considerably different between mature (Fig. 5-A) and immature (Fig. 5-D) rats, and that the radioactivity bound to liver was more in immature rats than in mature rats, in contrast to the case of the lung.[5] Growth-related quantitative and/or qualitative changes in macromolecules of lung and liver may exist.

When thiourea is absorbed from the gastrointestinal tract, it should arrive in the liver first and foremost. Therefore, we expected to observe the greatest value of optical density ratio in liver to heart blood after oral administration of a low dose. As expected, Fig. 8 and Table III show that the transfer of radioactivity to liver was greater following oral administration at low dose than in the case of high dose or in the case of Fig. 5-A (intravenous administration at low dose).

It is interesting that the radioactive carbon atoms of ^{14}C-thiourea were selectively and irreversibly transferred to macromolecules in plasma and tissues, and studies designed to investigate the effects of disease states, which are accompanied by changes of plasma or tissue macromolecules, on thiourea distribution may be worth pursuing.

References and Notes

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