

Editor: Mitsuru Hashida

Full title

**Absorption Characteristics of Dextrans with Different Molecular Weights from the Liver Surface Membrane in Rats: Its Implication for Targeting to the Liver**

Abbreviated title for running headlines

DEXTRAN ABSORPTION FROM LIVER SURFACE

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manuscript: 19 pages

figure captions: 2 page

figures: 5 pages

tables: 3 pages

Journal of Drug Targeting, 4 (3), 141-150, 1996

## Abstract

We examined the importance of molecular weight on the absorption from the liver surface in rats using fluorescein isothiocyanate-dextran (FDs) with molecular weights of 4,400 (FD-4), 9,300 (FD-10), 40,500 (FD-40) or 69,000 (FD-70). After application of FDs (5 mg) to the rat liver surface employing a cylindrical glass cell (i.d. 9 mm), each FD appeared gradually in the plasma, and the *in vivo* behavior was explained by two-compartment model with first-order absorption. The absorption ratios of FDs from the rat liver surface at 6 h, calculated from the amount recovered from the glass cell, decreased with an increase in the molecular weight (44.5 % for FD-4, 29.3 % for FD-10, 5.1 % for FD-40 and 2.2 % for FD-70). A linear relationship was observed between the absorption rate constant and the reciprocal value with square root of molecular weight of the model compounds. The limit of absorption from the rat liver surface was extrapolated to be at a molecular weight of 70,000. Furthermore, absorbed FDs were accumulated in the liver, as high liver / plasma concentration ratio as compared with that of i.v. administration.

We clarified the molecular weight dependence of drug absorption from the liver surface in rats. Moreover, the liver surface application appeared to be a promising route with enhancing the efficacy of drug targeting to the liver.

**KEYWORDS:** Absorption mechanism, administration route, dextran, molecular weight, rat liver surface, pharmacokinetics

## INTRODUCTION

Previously (Nishida *et al.*, 1994), we reported the possibility of the drug absorption from the liver surface in rats, by using organic anions as model drugs, aiming to develop a new administration route for improving the treatment of liver disease. Furthermore, we examined the effect of protein binding on the drug absorption from the liver surface, and demonstrated that protein binding tended to suppress the absorption of model drugs and that its inhibitory effect was closely correlated with the tightness of their binding to bovine serum albumin (Nishida *et al.*, 1995b).

Since molecular weight seemed to play an important role in drug absorption from the liver surface membrane, the absorption mechanism of macromolecules itself needs to be carefully examined for clinical application of macromolecules such as biologically active peptides. There are some reports concerning the molecular weight dependence of peritoneal transport (Flessner *et al.*, 1985a; Leypoldt *et al.*, 1987a; Nagy *et al.*, 1989; Seymour *et al.*, 1987; Yamaoka *et al.*, 1995b) and the accumulation of macromolecules in several organs after intraperitoneal administration (Flessner *et al.*, 1992; Seymour *et al.*, 1991). However, the absorption characteristics of macromolecules across the surface membrane of a particular organ involving the liver is still unknown.

In the present study, we selected four types of fluorescein isothiocyanate-dextran with various molecular weights as model macromolecules and studied their *in vivo* pharmacokinetics after application to the rat liver surface. Dextran is a glucose polymer which is available in different molecular weights, and has been suggested to be possible macromolecular carriers for delivery of drugs (Larsen, 1989; Sezaki *et al.*, 1989). Additionally, dextran is fairly resistant to metabolic degradation and their

*in vivo* fate has been characterized fully in rats (Mehvar & Shepard, 1992; Mehvar *et al.*, 1994; Nishida *et al.*, 1991).

## **MATERIALS AND METHODS**

### **Chemicals**

Fluorescein isothiocyanate-dextrans (FDs) with average molecular weights of 4,400 (FD-4), 9,300 (FD-10), 40,500 (FD-40) or 69,000 (FD-70), and bovine serum albumin (BSA; fraction V) were obtained from Sigma Chemical Co. (St. Louis, U.S.A.).

Evan's blue (EB) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals were reagent grade products.

### **In vivo experiment**

All animal procedures in the present study conformed to the Guidelines for Animal Experimentation in Nagasaki University.

Male Wistar rats (230 - 250 g) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the left femoral artery was cannulated with a polyethylene tube (i.d. 0.5 mm, o.d. 0.8 mm; Dural Plastics, Dural, Australia). After the middle abdomen was cut open about 3 cm, the common bile duct was cannulated with a polyethylene tube (i.d. 0.28 mm, o.d. 0.61 mm; Becton Dickinson & Co., Parsippany, U.S.A.). The body temperature of the rats was kept at 37°C with a heat lamp during the experiment. The test solution of FDs was prepared in an isotonic phosphate buffer (pH 7.4) to yield a concentration of 5 mg/0.1 ml, and administered as follows.

I.v. administration: The test solution (50 mg/ml X 0.1 ml) was injected into the jugular vein in rats.

Application to the liver surface: A cylindrical glass cell (i.d. 9 mm, effective area 0.64 cm<sup>2</sup>) was attached to the rat liver surface at the area of the left lobe with Aron Alpha (Sankyo Co. Ltd, Tokyo, Japan), and the test solution (50 mg/ml X 0.1 ml) was

added to the glass cell directly. In other experiments, EB (2 mg/ml) - BSA (5 % W/V) solution was applied to the rat liver surface. The top of the glass cell was sealed with a piece of aluminum foil to prevent the evaporation of the applied solution.

The blood samples were collected at selected times from the heparinized cannula inserted into the femoral artery over a 3- or 6-h period, and were centrifuged at 15,000 rpm for 5 min. The bile samples were collected at appropriate time intervals. At 3 or 6 h after the dosing, the urine remaining in the bladder was collected directly with a syringe, followed by excision of the liver. For application to the liver surface, the solution remaining in the glass cell at 6 h after dosing was withdrawn by more than five washings with saline before the liver was excised. Also, the time course studies on the distribution of FD-4 and FD-10 in the plasma, bile, urine and glass cell were performed.

### **Analytical method**

The concentrations of FDs as fluorescence in the plasma, bile, urine and solution remaining in the glass cell were measured by a spectrofluorometer at excitation and emission wavelengths of 489 and 515 nm, respectively. The FDs concentration in the liver was determined as follows. The excised liver was homogenized in an equal volume of its weight of distilled water. After 10 ml of acetone was added to 5 ml of the liver homogenate, the mixture was shaken for 15 min, followed by centrifugation for 15 min at 3,000 rpm. The FDs concentration as fluorescence in the resulting supernatant was determined in the same manner as described above.

The concentration of EB (EB-BSA) was determined spectrophotometrically at 615 nm after dilution with an isotonic phosphate buffer (pH 7.4).

## Compartment model analysis

First, the plasma concentration ( $C_p$ ) profile of FDs after i.v. administration was fitted to the biexponential equation described as follows, by the nonlinear least-squares method (Yamaoka *et al.*, 1981).

$$C_p = \frac{\text{Dose}(\alpha - K_{21})}{V_c(\alpha - \beta)} e^{-\alpha \cdot t} + \frac{\text{Dose}(K_{21} - \beta)}{V_c(\alpha - \beta)} e^{-\beta \cdot t} \quad (1)$$

Hybrid parameters  $\alpha$  and  $\beta$  are defined as  $\alpha + \beta = K_{12} + K_{21} + K_{el}$  and  $\alpha \cdot \beta = K_{21} \cdot K_{el}$ .  $V_c$  is the volume of the central compartment.  $K_{el}$  is the first-order elimination rate constant from the central compartment.  $K_{12}$  and  $K_{21}$  are the first-order transfer rate constants between the central and peripheral compartment. The total body clearance ( $CL_{total}$ ) and area under the plasma concentration profile ( $AUC_p$ ) was obtained by  $K_{el} \cdot V_c$  and  $\text{Dose}/CL_{total}$ , respectively.

Next, in the same way, the plasma concentration profile of FDs after application to the rat liver surface was fitted in a two-compartment model incorporating first-order absorption, by the nonlinear least-squares method (Yamaoka *et al.*, 1981). In this model, the equation for plasma concentration is given as follows.

$$C_p = \frac{F \cdot \text{Dose} \cdot K_a}{V_c} \left\{ \frac{K_{21} - K_a}{(\beta - K_a)(\alpha - K_a)} e^{-K_a \cdot t} + \frac{K_{21} - \alpha}{(\beta - \alpha)(K_a - \alpha)} e^{-\alpha \cdot t} + \frac{K_{21} - \beta}{(\alpha - \beta)(K_a - \beta)} e^{-\beta \cdot t} \right\} \quad (2)$$

$K_a$  is the first-order absorption rate constant for absorption into the blood stream from the rat liver surface.  $F$  is the availability of FDs after application to the rat liver surface. In the case of curve-fitting of plasma concentration profile of FDs after application to the rat liver surface, the pharmacokinetic parameters ( $\alpha$ ,  $\beta$ ,  $K_{21}$ ,  $V_c$ ) obtained from the plasma concentration profile after i.v. administration were substituted

into Eq. (2).

## **RESULTS**

### **In vivo elimination of FDs after i.v. administration**

First, the plasma concentrations of FDs with different average molecular weights were determined for 3 h after i.v. administration to rats at a dose of 5 mg, and were illustrated in Figure 1. All FDs showed biphasic elimination from the plasma, as shown in Figure 1. Table 1 summarizes the pharmacokinetic parameters for FDs calculated on the basis of the two-compartment model. FD-4 and FD-10 showed a large clearance from the plasma, while FD-40 and FD-70 had a small total body clearance. Thus, a marked difference was observed in systemic clearance between FD-10 and FD-40. This phenomenon was well consistent with the previous finding that dextrans with low molecular weight were cleared from the body by tubular filtration, while the vascular permeability at the glomeruli was negligible at a molecular weight of 30,000, owing to restriction by pore size of vascular beds in the kidney (Brenner *et al.*, 1978; Leypoldt *et al.*, 1987b).

### **Plasma concentration profiles of FDs after application to the rat liver surface**

Figure 2 illustrates the plasma concentration profiles of FDs with different molecular weights after application to the rat liver surface at a dose of 5 mg. All FDs were absorbed from the liver surface, followed by progressive appearance into the plasma. For FD-40 and FD-70, the plasma levels either plateaued or continued to rise until 6 h after dosing. Also, the appearance of FDs in the plasma was delayed with an increase in the molecular weight of FDs, suggesting that the absorption rate of FDs with high molecular weight is low.

### **Recovery of FDs in the glass cell, bile, urine and liver**

Table 2 shows the recovery in 6 h of FDs with different molecular weights in the glass cell, bile, urine and liver after application to the rat liver surface at a dose of 5 mg, along with that in 3 h after i.v. administration for comparison. The absorption ratios of FDs from the rat liver surface at 6 h were calculated from the amount recovered from the glass cell, as 44.5 % for FD-4, 29.3 % for FD-10, 5.1 % for FD-40 and 2.2 % for FD-70. An increase in the molecular weight of FDs resulted in the decrease of absorption rate from the rat liver surface.

We added the data of BSA labeled with EB (EB-BSA) after application to the rat liver surface. The absorption ratio of EB-BSA in 6 h was calculated to be  $3.6 \pm 0.8$  % of dose (mean  $\pm$  S.E., N=5) from the amount recovered from the glass cell.

Similar to i.v. administration, each FD was excreted mainly into the urine in proportion to the absorbed amount after application to the rat liver surface, while the biliary recovery was less than 1 % of the dose. A considerable amount of FDs was found in the liver at 6 h after application to the rat liver surface (Table 2). The recovery of FDs in the liver was slightly larger than that after i.v. administration (3 h after dosing), although FDs applied to the rat liver surface were not absorbed completely. Also, the liver / plasma concentration ratio of FDs at 6 h after application to the rat liver surface was high (9.3 for FD-4, 11.5 for FD-10, 3.8 for FD-40 and 3.0 for FD-70), as compared with that of i.v. administration at 3 h (2.9 for FD-4, 5.6 for FD-10, 0.3 for FD-40 and 0.03 for FD-70).

#### **Distribution of FD-4 and FD-10 in the glass cell, plasma, bile and urine after application to the rat liver surface**

To assess the absorption characteristics from the rat liver surface membrane, we studied the distribution pattern of FDs after application to the rat liver surface. We selected

FD-4 and FD-10 as a model, because their absorbability was relatively good (Table 2).

Figure 3 shows the time courses of distribution of FD-4 and FD-10 in the glass cell, plasma, bile and urine after application to the rat liver surface at a dose of 5 mg. The amount of FD-4 and FD-10 remaining in the plasma determined as  $C_p \times V_{\text{plasma}}$  was small ( $< 1\%$  of dose), where  $V_{\text{plasma}}$  (plasma volume in rat) was estimated from reported values (45 ml/kg) by Bischoff *et al.* (1971). The amount of FD-4 and FD-10 remaining in the glass cell declined at different rates, as shown in Figures 3A and 3B. A cumulative amount of FD-4 and FD-10 excreted into the urine increased with time, with the decrease in the amount of FD-4 and FD-10 in the glass cell

A semi-log plot of time course of the amount of FD-4 and FD-10 remaining in the glass cell gave a straight line (correlation coefficient: 0.99 for FD-4 and 0.95 for FD-10), as shown in Figure 4. This suggests that the absorption of FDs from the rat liver surface proceeds via first-order kinetics, similar to the case of small molecule phenol red (Nishida *et al.*, 1995a). The first-order absorption rate constant  $K_a$  for FD-4 and FD-10 was calculated to be  $157.2 \times 10^{-5}$  and  $92.4 \times 10^{-5} \text{ min}^{-1}$ , respectively (Table 3).

### **Compartment model analysis of plasma concentration profile after application to the rat liver surface**

We constructed the two-compartment model with first-order absorption based on the biexponential elimination after i.v. administration, to explain the *in vivo* behavior of FDs after application to the rat liver surface.  $K_a$  values of FDs were estimated by the elimination profile from the glass cell (Figure 4) or curve-fitting of plasma concentration profile according to Eq. (2), and are summarized in Table 3. On the other hand, F values of FDs were also obtained by curve-fitting of plasma concentration profile, and calculated to be approximately 100 % for every FD. In the case of FD-40

and FD-70, the  $K_a$  value through the elimination profile from the glass cell was estimated by using the absorption ratio at 6 h after dosing, because we could not obtain a correct first-order elimination pattern owing to their low absorption rate.

To clarify the validity of the pharmacokinetic model, we performed the simulation of plasma concentration according to Eq. (2) by use of the parameters given in Table 3, and compared it with the experimentally observed data (Figure 2). Each symbol is the experimental value, and the solid and broken lines show the simulation curves reconstructed employing the estimated  $K_a$  and F values obtained through the elimination profile or curve-fitting (Table 3), respectively. In the case of simulation employing the parameters through the elimination profile, the simulation curves was reconstructed by assuming that F is 100 %. In general, good agreement was observed between the simulation curves and experimentally observed values of FDs in both methods as shown in Figure 2, suggesting the validity of this pharmacokinetic model. As listed in Table 3,  $K_a$  values of FDs calculated by the curve-fitting correlated closely with those calculated by the elimination profile, supporting the present fitting procedure.

## DISCUSSION

We examined the absorbability of macromolecules from the liver surface and its dependence of molecular weight, and demonstrated clearly that FDs with different molecular weights as model macromolecules were absorbed from the liver surface at different rates and that the absorption rate obeyed the first-order kinetics. Also, we indicated that the *in vivo* behavior of FDs after application to the rat liver surface was well described by a two-compartment model with first-order absorption. The calculated  $K_a$  for FDs decreased with the increase in the molecular weight (Table 3), suggesting that the drug absorption from the liver surface was affected greatly by the molecular weight. When considering the clinical application of macromolecular drug, it is important to examine the molecular weight dependence of absorption from the liver surface. Therefore, we compared the absorbability of several model compounds having different molecular weights.

The following equation has been proposed with respect to the drug absorption from the stomach and small intestine via passive diffusion (Koizumi *et al.*, 1964a,b), on the basis of the principle that the diffusibility of a substance in uniform solutions is approximately inversely proportional to the square root of the molecular weight of drug ( $\sqrt{Mw}$ ).

$$\frac{1}{\sqrt{Mw} \cdot K_a} = A + \frac{B}{P_a} \quad (3)$$

Where  $P_a$  represents the partition coefficient, and constants A and B are correction factor to  $P_a$  and constant for diffusion, respectively.

Because each model compound has high hydrophilicity, the  $P_a$  is supposed to be identical approximately and the right side of Eq. (3) can be transformed as the fixed

number. We examined the relationship between the  $K_a$  and the reciprocal value with square root of molecular weight ( $1/\sqrt{Mw}$ ) of the model compounds with different molecular weights, as illustrated in Figure 5. The values for phenol red (PR, Mw: 354), bromphenol blue (BPB, Mw: 670) and bromsulphonphthalein (BSP, Mw: 838) were reported previously (Nishida *et al.*, 1994). As shown in Figure 5, the increase in the molecular weight (i.e., the decrease in the  $1/\sqrt{Mw}$ ) of the model compounds resulted in the decrease of the  $K_a$ . A linear relationship was observed between the  $K_a$  and  $1/\sqrt{Mw}$  of the model compounds (correlation coefficient: 0.96), suggesting that the model compounds are absorbed from the rat liver surface membrane via simple passive diffusion.

The liver is covered by serous membrane containing monolayer squamous epithelial cells. And a space between the serous membrane and hepatic parenchymal cell is supported by connective tissue, in which the capillary of portal vein and hepatic artery circulates. Therefore, the drug penetrated across the capillary wall in the connective tissue is flowed into the sinusoid. Also, the possibility of direct diffusion of drug into the parenchymal cell must be taken into consideration. The absorption of the model compounds from the liver surface might occur through the intercellular gap and pore in the serous membrane and connective tissue rather than the transcellular lipid route, since the hydrophilicity of the model compounds is high. However, the rate-limiting step in the absorption from the liver surface was unknown from our present investigation.

The molecular weight when the  $K_a$  value is 0 (x-intercept) was extrapolated to be 71,195 from the plot in Figure 5. We consider that the limit (so-called threshold value) of the molecular weight of the drug absorbed from the rat liver surface is

approximately 70,000, which is larger than that restricting the movement of macromolecules from the peritoneum (Mw: 50,000) reported by Flessner *et al.* (1985b) and Hirszel *et al.* (1984).

FDs applied to the rat liver surface were accumulated in the liver to some extent (Table 2). For the mechanism of accumulation of drug in the liver, the following route is considered, judging from the anatomical feature of the liver surface membrane. The drug flowed into the blood (sinusoid) circulation was taken up by the liver, and/or the drug diffused directly into the parenchymal cell. The percent of dose recovered from the liver decreased gradually from 5.4 % for FD-4 to 2.0 % for FD-70 (Table 2). The recoveries of FD-4 and FD-10 in the liver were small, despite their good absorbability from the rat liver surface. The reason for this phenomenon was considered as follows, absorbed FD-4 and FD-10 were rapidly cleared by urinary excretion, and/or FD-4 and FD-10 taken up by the liver flowed out into the systemic circulation.

The amount of FD absorbed into the systemic circulation for 6 h was calculated by dividing  $AUC_p$  for 6 h after application to the rat liver surface by  $AUC_p$  of i.v. administration (Table 1), as 44.9 % for FD-4, 29.9 % for FD-10, 2.7 % for FD-40 and 0.5 % for FD-70. Therefore, the ratio of amount remaining in the liver to the amount absorbed into the systemic circulation at 6 h was calculated to be 0.12 for FD-4, 0.16 for FD-10, 1.03 for FD-40 and 4.06 for FD-70. This indicates that targeting efficacy was enhanced with an increase in the molecular weight and was marked for FD-40 and FD-70.

Moreover, the liver / plasma concentration ratio of FDs after application to the rat liver surface was high ranging from 3.0 to 11.5, as compared with that of i.v.

administration. The specific moieties for the liver need to be introduced to macromolecules, for enhancement of hepatic uptake and reduction of toxicity in the normal organ outside the liver. The specific bioactive recognition system in the liver was appropriately added to macromolecules for more effective targeting, such as a receptor for terminal galactose (Ashwell and Harford, 1982) and/or non-specific association by electric cationic charge (Nishida *et al.*, 1991; Takakura *et al.*, 1990; Yamaoka *et al.*, 1995a).

Consequently, we clarified the absorption characteristics of FDs as model macromolecules from the liver surface in rats, and implied the possibility of this new administration route to improve the strategy for specific delivery of therapeutic agents to the diseased region in the liver. According to the change in hepatic function, sinusoidal blood flow and nature of liver surface membrane and so on, the absorbability from the liver surface appears to be altered in the diseased liver. Thus, a further investigation on this point is required to be performed in the future, for clinical application.

#### **ACKNOWLEDGEMENTS**

We wish to thank Kaori Uemura and Yukiko Suetsugu for skilled technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

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TABLE 1  
*Pharmacokinetic parameters for FDs with different molecular weights after i.v. administration in rats at a dose of 5 mg*

Compound	$K_{12}$ ( $\text{min}^{-1}$ )	$K_{21}$ ( $\text{min}^{-1}$ )	$K_{el}$ ( $\text{min}^{-1}$ )	$V_c$ (ml)	$CL_{total}$ (ml/min)	$AUC_p$ ( $\mu\text{g/ml}\cdot\text{min}$ )
FD-4	0.292 $\pm 0.045$	0.108 $\pm 0.025$	0.137 $\pm 0.038$	13.7 $\pm 0.7$	1.875 $\pm 0.488$	2664 $\pm 693$
FD-10	0.167 $\pm 0.053$	0.131 $\pm 0.031$	0.158 $\pm 0.018$	13.2 $\pm 0.9$	2.078 $\pm 0.231$	2397 $\pm 267$
FD-40	0.020 $\pm 0.002$	0.016 $\pm 0.004$	0.011 $\pm 0.002$	11.4 $\pm 0.5$	0.112 $\pm 0.032$	39872 $\pm 11392$
FD-70	0.011 $\pm 0.001$	0.017 $\pm 0.003$	0.003 $\pm 0.001$	9.5 $\pm 0.4$	0.025 $\pm 0.004$	175439 $\pm 28070$

Each value is the mean  $\pm$  S.E. of four experiments.

TABLE 2  
*Recovery (%) of FDs with different molecular weights at 6 or 3 h after application to the liver surface (LS) or i.v. administration in rats, respectively, at a dose of 5 mg*

Compound	Glass cell	Bile		Urine		Liver	
	LS	i.v.	LS	i.v.	LS	i.v.	LS
FD-4	55.5 ±2.5	0.4 ±0.1	0.4 ±0.1	66.6 ±10.8	24.5 ±5.1	3.3 ±0.2	5.4 ±0.1
FD-10	70.7 ±2.7	0.5 ±0.1	0.4 ±0.1	73.0 ±2.6	18.9 ±3.2	3.9 ±1.6	4.9 ±0.1
FD-40	94.9 ±1.8	2.0 ±0.5	0.9 ±0.2	32.4 ±1.4	2.3 ±0.8	2.2 ±0.3	2.8 ±0.2
FD-70	97.8 ±1.0	2.6 ±0.2	0.5 ±0.1	5.8 ±1.8	0.3 ±0.1	1.1 ±0.3	2.0 ±0.3

Each value is the mean ± S.E. of at least four experiments.

TABLE 3  
*First-order absorption rate constant  $K_a$  ( $\text{min}^{-1} \times 10^{-5}$ ) for FDs with different molecular weights after application to the rat liver surface*

Source	FD-4	FD-10	FD-40	FD-70
Elimination profile <sup>a</sup>	157.2	92.4	14.6	6.1
Curve-fitting <sup>b</sup>	156.4	117.3	12.3	3.7

<sup>a</sup>  $K_a$  was estimated from the elimination profile of FDs amount remaining in the glass cell after application to the rat liver surface at a dose of 5 mg.

<sup>b</sup>  $K_a$  was obtained from the curve-fitting of the plasma concentration profile of FDs after application to the rat liver surface at a dose of 5 mg.

## Figure captions

FIGURE 1. Plasma concentration profiles of FD-4 ( $\square$ ), FD-10 ( $\circ$ ), FD-40 ( $\triangle$ ) and FD-70 ( $\nabla$ ) after i.v. administration in rats at a dose of 5 mg. Curves show simulated functions based on the parameters shown in Table 1. Each point represents the mean  $\pm$  S.E. of at least four experiments.

FIGURE 2. Plasma concentration profiles of FD-4 (A), FD-10 (B), FD-40 (C) and FD-70 (D) after application to the rat liver surface at a dose of 5 mg. Each point represents the mean  $\pm$  S.E. of at least four experiments. Solid and broken curves show simulated functions based on the pharmacokinetic parameters of FDs (Tables 1 and 3) calculated by elimination profile from the glass cell or by curve-fitting of the plasma concentration profile, respectively.

FIGURE 3. Time courses of amount of FD-4 (A) and FD-10 (B) in the glass cell ( $\circ$ ) and plasma ( $\bullet$ ), and their cumulative amount excreted into the bile ( $\square$ ) and urine ( $\triangle$ ) after application to the rat liver surface at a dose of 5 mg. Each point represents the mean  $\pm$  S.E. of at least four experiments.

FIGURE 4. Semi-log plots of time courses of the amount of FD-4 ( $\square$ ) and FD-10 ( $\circ$ ) remaining in the glass cell after application to the rat liver surface at a dose of 5 mg. Each point represents the mean  $\pm$  S.E. of at least four experiments.

FIGURE 5. Relationship between the molecular weight (Mw) and first-order absorption rate constant ( $K_a$ ) of the FDs ( $\circ$ ) and the other model compounds ( $\bullet$ ) with different molecular weights. The  $K_a$  value was calculated from the amount remaining in the glass cell at 6 h after application to the rat liver surface. The values for phenol red (PR), bromphenol blue (BPB) and bromosulphonphthalein (BSP) were

reported previously (Nishida *et al.*, 1994). Each point represents the mean of at least four experiments.

approximate position: p. 9, line 3  
Nishida, K., Sato, N., Sasaki, H., Nakamura, J.

**Fig. 1**

approximate position: p. 9, line 18  
Nishida, K., Sato, N., Sasaki, H., Nakamura, J.

**Fig. 2**

approximate position: p. 11, line 4  
Nishida, K., Sato, N., Sasaki, H., Nakamura, J.

**Fig. 3**

approximate position: p. 11, line 12  
Nishida, K., Sato, N., Sasaki, H., Nakamura, J.

**Fig. 4**

approximate position: p. 14, line 1  
Nishida, K., Sato, N., Sasaki, H., Nakamura, J.

**Fig. 5**