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EFFECT OF PREGNANCY ON TISSUE DISTRIBUTION OF SALICYLATE IN RATS

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ABSTRACT:

The effect of pregnancy on tissue distribution of salicylate was studied by comparing both pharmacokinetic and protein-binding parameters between 20-day-pregnant rats and nonpregnant (control) rats. In the pregnant rats, the volume of distribution increased significantly ($p < 0.05$) from 164 ml/kg of the control to 225 ml/kg, and the total body clearance also increased significantly ($p < 0.05$) from 12.1 ml/hr/kg of the control to 19.8 ml/hr/kg. But these changes did not affect the plasma disappearance half-life of salicylate in the pregnant rats. The serum unbound fraction (f_s) of the pregnant rats at 8 hr after iv administration of salicylate increased remarkably from [illegible] of the control to 0.67. The f_s in the fetal serum (0.41) was lower than that in the maternal serum in spite of the lower albumin concentration in the fetal serum. A nonlinear serum protein binding was observed both in the control and in the fetal rats, but not observed in the pregnant rats. In the pregnant rats, the tissue-to-serum concentration ratios (*Kp*) of all tissues studied were larger than those in the control rats, and the values of *Kp* in the fetal were larger than those in the maternal. To elucidate these difference in *Kp* values between the pregnant and control rats, a mathematical model was proposed, where salicylate was distributed in the interstitial fluid, bound to the interstitial albumin, and translocated into the intracellular fluid according to the pH partition theory. The *Kp* values of most tissues in the control and pregnant rats were predicted successfully by using this model. It was concluded that a pronounced increase in f_s of the pregnant rats may primarily contribute to the increase in the *Kp* values of most tissues.

It has been reported that the pharmacokinetics of many drugs was affected by the physiological changes associated with pregnancy (1). In some drugs, significant changes in their plasma concentrations during pregnancy have been observed both in humans (2) and in rats (3).

The distribution volume and the total body clearance of many drugs, of which elimination is not rate-limited by the blood flow, are the function of their unbound fraction in plasma (4, 5). The plasma or serum protein-binding of some drugs is decreased during pregnancy (6, 7). In the rats, pronounced increase in the serum unbound fraction (f_s) of acidic drugs, especially salicylic acid and sulfisoxazole, were observed on the 20th day of pregnancy (8). Furthermore, with regard to salicylic acid, both the plasma level and protein binding in fetal plasma were greater than those in maternal plasma (6, 7). However, few quantitative [illegible] are available on the pharmacokinetics and tissue distribution [illegible] salicylic acid in pregnancy. The purpose of the present study, therefore, is to investigate the influence of pregnancy on various pharmacokinetic parameters and tissue distribution of salicylic acid, and to assess the factors which are responsible for the changes of its disposition in pregnancy.

Materials and Methods

Chemicals. SA,[1] salicyluric acid were purchased from Koso Chemical Co. (Tokyo, Japan) and Sigma Chemical Co. (St. Louis, MO), respectively. [*carbonyl*-^{14}C]SA (51.7 mCi/mmol) was purchased from New England Nuclear Corp. (Boston, MA) and was found to be at least 97% pure by thin layer chromatography. All other chemicals were commercially available and of analytical grade.

[1] Abbreviation used is: SA, sodium salicylate.

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Animals. Adult female rats (Wistar ST strain, Shizuoka Jikkendobuts Co., Hamamatsu, Japan) weighing between 230 and 260 g, were mated with males overnight. The day on which the spermatozoa was first found in vaginal smear was counted the 1st day of pregnancy. The animals were housed under conditions of controlled temperature and lighting with free access to food and water at all times. All nonpregnant rats, which were as old as pregnant rats, were used as the control.

***In Vivo* Experiments.** All rats were fasted for 16–18 hr before operation. Under light ether anesthesia, the femoral vein and artery were cannulated with PE-50 polyethylene tubing. Cannulated rats were kept in the spine position on a fixed board and recovered from anesthesia prior to the injection of SA. Each rat received a single dose of 10 mg/kg of SA containing 5 μCi/kg of [^{14}C]SA in physiological saline through the femoral vein cannula. Blood samples were obtained at 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hr in polyethylene centrifuge tubes (Beckman Instruments, Fullerton, CA). Serum was separated by centrifugation for 20 sec in a table-top Microfuge (Beckman Instruments). We preliminarily found by TLC analysis (the conditions for TLC analysis are described later) that the concentrations of metabolites in plasma at every sampling time were negligible in the control, pregnant, and fetal rats. Therefore, the concentration of SA in serum was determined by scintillation counting of 50 μl of serum in 10 ml of scintillation fluid [1 liter of toluene, 1 liter of Triton X-100, 4 g of diphenyloxazole, and 0.1 g of 1,4-bis[2-(5-phenyloxazolyl)] benzene] with an external standard.

The rats were decapitated at 8 hr after iv injection of SA and various tissues were excised for the determination of SA concentrations. After decapitation of the pregnant rats, approximately 10 fetuses were obtained from the mother. Serum and tissues from these fetuses were combined and used for measuring the SA concentrations. The radioactivity in the tissues of the control, pregnant, and fetal rats, except that in kidney and liver, was measured in a liquid scintillation spectrophotometer (Packard model 3255, Packard Instruments Corp., Downers Grove, IL) after oxidizing in an automatic sample oxidizer (Packard model 306). The concentrations of SA in the kidney and liver were determined after separation from its metabolites according to the modified methods of Gonzalez *et al.* (9) and Chrastil and Wilson (10) as described below. Each tissue was homogenized in 2 ml of distilled water for every gram

of tissue. One ml of homogenate was transferred into a 10-ml centrifuge tube containing approximately 1 g of sodium chloride and 0.3 ml of 6 N HCl. After addition of 1 ml of ether, the samples were shaken vigorously for 30 min. After centrifugation, 300 μl of the organic phase was applied to a TLC plate of silica gel 60 (without fluorescent indicator, Merck, Tokyo). Authentic nonlabeled SA and SU dissolved in ether were spotted as the reference. The plate was developed immediately with ether:n-butyric acid (10:1, v/v) mixture. The spots on the dried plate were visualized under UV light and then were scraped into vials containing 10 ml of scintillation fluid described above. The recovery of SA from this system was 97.8 ± 3.7%.

The tissue-to-serum partition coefficient (*Kp*) was calculated using the serum and tissue concentrations of SA at 8 hr post-iv injection and was corrected according to the method of Chen and Gross (11).

Serum Protein Binding. Serum protein binding was determined by an ultrafiltration or an equilibrium dialysis method. Bindings of SA to serum were measured using serum obtained at 8 hr post-iv injection of SA in the same control, pregnant, and fetal rats used in *in vivo* experiments, by an ultrafiltration method using a membrane cone (Amicon Centriflo ultrafiltration membrane filter cone, type CF-25, Lexington, MA), which provided approximately 10% of the initial serum volume after centrifugation at 1000*g* for approximately 3 min. Two hundred μl of serum sample was applied to the membrane cone after incubation at 37°C for 5 min. The concentrations of [^{14}C]SA in the filtrate were determined as described above. The adsorption of [^{14}C]SA to the membrane was negligible.

The bindings of SA were also measured over wide ranges of SA concentrations (10–250 μg/ml) by equilibrium dialysis. Serum obtained from two each of control, pregnant, and fetal (from the same pregnant rats) rats was used in the equilibrium dialysis at 37°C for 6 hr using a microequilibrium dialysis cell (12). Twenty μl of serum and an equal volume of 0.13 M Sorensen's phosphate buffer (pH 7.4) containing 250 nCi/ml of [^{14}C]SA, and various concentrations of nonlabeled SA (25–500 μg/ml) were added to each side. [^{14}C]SA concentrations were determined in the buffer side (unbound concentration) and serum side (total concentration) as described above. The determination of albumin in serum was carried out using a commercial kit (Albumin B Test, Wako Pure Chemical Co., Ltd., Osaka, Japan) based on the method using bromcresol green (13).

Blood-to-Plasma Concentration Ratio of SA. The blood from five rats was combined, yielding two pools of each serum from the pregnant and control rats. The pooled blood of the pregnant and control rats was incubated with 100 nCi/ml of [^{14}C]SA and various amounts of nonlabeled SA (20–160 μg/ml) at 37°C for 20 min. After centrifugation, an aliquot of the plasma was removed and the concentration of [^{14}C]SA was assayed as described above.

Data Analysis. The data were analyzed by an iterative least squares method using a HITAC M 200/280H digital computer using SALS ·ogram (14).

Statistical Analysis. All means are presented ±SE. Student's *t* test was utilized to estimate a significant difference between the control and pregnant or fetal rats.

Results

SA Elimination from Serum. The serum disappearance curves of SA after iv administration of 10 mg/kg in the control and the 20-day-pregnant rats are shown in fig. 1. The disappearance of SA followed a monoexponential curve in both the control and pregnant rats. Various pharmacokinetic parameters computed are listed in table 1. The serum concentrations of SA in the pregnant rats were significantly lower than those in the control rats, although the serum half-lives in both the control and pregnant rats were almost the same. These findings can be attributed to an approximately 40% increase in the distribution volume (V_d) of the pregnant rats compared to that of the control rats as shown in table 1. In the pregnant rats, an increase in the total body serum clearance (CL) was also calculated. The serum concentration of SA in the fetal rats was not significantly different from that in the maternal rats at 8 hr after iv administration.

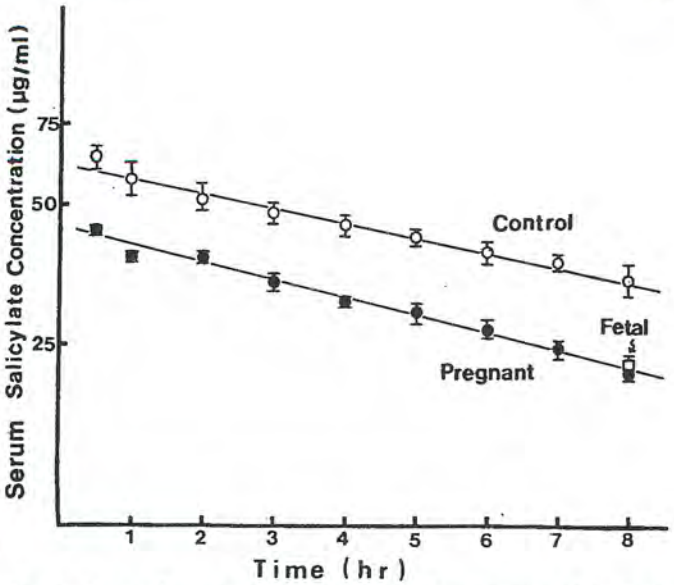

FIG. 1. *Serum disappearance of SA after iv administration of 10 mg/kg in control (○) and pregnant rats (●) and fetal rats (□).*

Each *point* and *vertical bar* represents the mean ±SE of three rats. *Lines* were calculated by fitting to monoexponential equations using a least squares method.

TABLE 1

Pharmacokinetic parameters of salicylate in pregnant and nonpregnant female (control) rats

Values are mean ± SE of three rats.

	Control	Pregnant
Body weight (g)	238 ± 1	316 ± 4[a]
V_d (ml·kg^{-1})	164 ± 11	225 ± 5[a]
β (hr^{-1})	0.0742 ± 0.0020	0.0878 ± 0.0080
CL_{tot} (ml·hr^{-1}·kg^{-1})	12.1 ± 0.6	19.8 ± 1.7[a]

[a] Significantly different from the control rats ($p < 0.05$).

Serum Protein Binding. The serum protein binding of SA was measured by an ultrafiltration method using the serum obtained at 8 hr post-iv injection of SA. The average serum concentrations of SA in the control, pregnant, and fetal rats were 34.3 ± 2.7, 21.7 ± 1.5, and 22.5 ± 0.8 μg/ml, respectively, and the corresponding serum unbound fractions were 0.137 ± 0.021, 0.667 ± 0.018, and 0.413 ± 0.030, respectively. To compare the binding isotherms among sera of control and pregnant rats and fetuses, SA bindings were measured by an equilibrium dialysis by changing the initial concentration of SA from 25 to 500 μg/ml and the results are shown in fig. 2. The serum unbound fraction of SA in the control rats and fetuses increased with the increased concentration of SA, while that in the pregnant rats was constant irrespective of the concentration of SA. The binding parameters calculated are listed in table 2. In the fetal rats the dissociation constant, K_d, was increased, while the binding capacity, $n(p)$, was decreased compared to the control, but after correction by the albumin concentration in each serum, numbers of binding sites (n) on albumin were almost the same. In the pregnant rats, the serum protein binding of SA was decreased to a great extent and this confirmed the results obtained by an ultrafiltration method in the different groups of rats. The binding of SA to

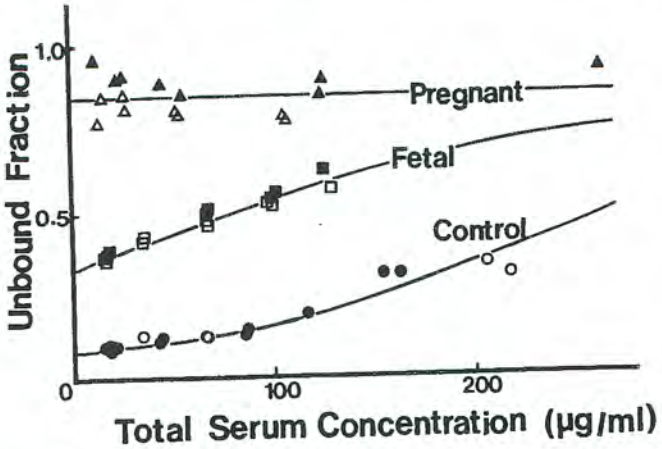

FIG. 2. *Serum protein binding of SA in control (○, ●), pregnant (△, ▲), and fetuses of the same pregnant rats (□, ■).*

The *points* represent the experimental values as the unbound fraction of the total serum concentration. Sera obtained from two control rats (rat A, ●; rat B, ○), two pregnant rats (rat C, ▲; rat D, △), and 10 fetuses of the same pregnant rats C (■) and D (□) were separately used for the binding study. The *lines* were calculated by fitting to a Langmuir-type equation (see *Materials and Methods*).

TABLE 2

Parameters for serum protein binding of SA and serum albumin concentration in rats

Values are the mean value of two independent experiments.

	Control	Pregnant	
		Maternal	Fetal
Albumin concentration (mM)	0.513	0.457	0.294
Serum binding			
K_d (mM)	0.083	0.188[a]	0.244
$n(P)$ (mM)	0.964		0.486
n	1.9		1.7

[a] $n(P)/K_d$ (dimensionless).

serum of the pregnant rats was linear over a wide range of SA concentrations, suggesting the decrease in the binding affinity compared to the control rats.

Blood-to-Plasma Concentration Ratio of SA. The ratios of blood-to-plasma concentration of SA over the range of blood concentrations (20–160 μg/ml) were determined in the control and pregnant rats. The ratios were almost constant over the concentration ranges of SA studied and the average ratios calculated were 0.60 in the control and 0.74 in the pregnant rats.

Serum pH and Hematocrit Value. The pH of freshly prepared serum from the control and pregnant rats were 7.50 ± 0.05 (n = 4) and 7.66 ± 0.06 (n = 4), respectively. Hematocrit values of the blood from the control and pregnant rats were 0.46 ± 0.02 (n = 4) and 0.38 ± 0.02 (n = 4), respectively.

Tissue Distribution of SA. Tissue-to-serum partition coefficients (Kp) at 8 hr post-iv injection of 10 mg/kg SA in the control and pregnant rats are listed in table 3. The Kp values of all tissues studied, except liver, were significantly higher in the pregnant rats than those in the control rats. In the fetus, the Kp values of all tissues, except lung and kidney, were significantly increased compared to those of the mother. Especially, the Kp values in the fetal brain was much higher than those in the control and maternal rats.

TABLE 3

Tissue-to-serum partition coefficients (kp) of SA in rats

Values are mean ± SE of three rats.

Tissue	Control	Pregnant	
		Maternal[a]	Fetal[b]
Brain	0.060 ± 0.005	0.093 ± 0.012[c]	0.336 ± 0.023[d]
Heart	0.187 ± 0.030	0.325 ± 0.016[c]	0.547 ± 0.043[d]
Lung	0.192 ± 0.033	0.394 ± 0.025[e]	0.410 ± 0.002
Liver	0.228 ± 0.028	0.339 ± 0.042	0.498 ± 0.045[f]
Kidney	0.438 ± 0.033	0.540 ± 0.004[c]	0.590 ± 0.055
Gastrointestinal	0.205 ± 0.028	0.322 ± 0.016[c]	0.506 ± 0.033[d]
Muscle	0.116 ± 0.019	0.222 ± 0.026[c]	0.505 ± 0.038[d]
Skin	0.244 ± 0.019	0.369 ± 0.021[c]	0.585 ± 0.009[d]
Bone	0.142 ± 0.013	0.203 ± 0.016[c]	ND[g]
Placenta		0.597 ± 0.057	

[a] Kp value of the maternal rats.
[b] Kp value of the fetal rats.
[c] Significantly different ($p < 0.05$) from the control.
[d] Significantly different ($p < 0.01$) from the maternal tissue.
[e] Significantly different ($p < 0.01$) from the control.
[f] Significantly different ($p < 0.05$) from the maternal tissue.
[g] Not determined.

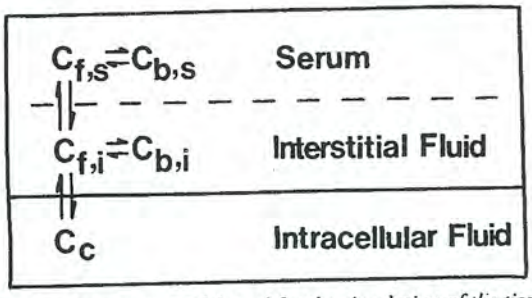

FIG. 3. *One-organ model used for the simulation of the tissue distribution of SA in the noneliminating organ.*

Discussion

In the present study, we examined the effect of pregnancy on the tissue distribution of SA by comparing the change in pharmacokinetic parameters during pregnancy.

The most remarkable change observed in the pregnancy was the decrease in the protein binding of SA in serum, which caused a remarkable increase in f_s of the pregnant rats (from 0.14 in the control to 0.67) (see *Results*). This finding coincides well with that reported by Stock *et al.* (8). Since the serum albumin concentration was not significantly decreased during pregnancy (table 2), a considerable decrease in the binding affinity of SA to albumin was suggested. This may be explained by endogenous inhibitor(s) which increased during pregnancy (8). Previously Tsuji *et al.* (15) proposed a mathematical model to estimate the tissue distribution of β-lactam antibiotics. In this model, the tissue was divided anatomically into three fluid compartments: (a) the capillary blood, (b) the interstitial fluid, and (c) the intracellular fluid. By use of this model, they demonstrated that the interstitial albumin plays a major role to determine the antibiotic levels in various tissues (15). In the present study, we utilized this model (fig. 3) to elucidate the determinant of the tissue distribution of SA during pregnancy. Since SA binds primarily to albumin in serum (9) and rats examined were decapitated before preparation of tissue samples, the tissue distribution of SA appeared to be in the interstitial and intracellular fluids. Therefore, the binding of SA to albumin in the interstitial

fluid could not be neglected. In order to simulate the concentrations of SA in various tissues, the following assumptions were made: 1) SA which distributes into the interstitial fluid can bind only to albumin which exists in this compartment as well as in serum; 2) the pH difference exists between the interstitial and intracellular fluids, and SA distributes into the intracellular fluid according to the pH partition theory; 3) the binding of SA to the components of the cell is negligible (16). The ratio of the amount of SA in the interstitial space (A_I) to that in the intracellular space (A_C) is given by a following equation (see Appendix 2)

$$\frac{A_I}{A_C} = \frac{ISF}{ICF \cdot R} \cdot \left[(1 - AR) + \frac{AR}{f_s}\right] \quad (1)$$

where *ISF*, *ICF*, *AR*, and *R* are the tissue interstitial fluid fraction, the tissue intracellular fluid fraction, and the interstitial fluid-to-serum concentration ratio of albumin, and the intracellular fluid-to-serum unbound concentration ratio of SA, respectively (see Appendix 1 for details). The ratio (A_I/A_C) was calculated by changing the f_s of SA as shown in fig. 4 using the values of *ISF*, *ICF*, and *R* obtained from the control rats. According to eq. 1, when the f_s is small (*i.e.* in the control rats), SA distributes primarily into the interstitial space, whereas if the f_s increased, the ratio A_I/A_C decreased and when f_s approached unity, the value of A_I became close to that of A_C. In the case of pregnant rats, the f_s of SA increased to 0.67 and the ratio A_I/A_C calculated from eq. 1 is approximately 0.86.

To elucidate the mechanism of the increase in the K_p values of the pregnant rats, the K_p values of the control and pregnant rats were also simulated according to the model described above. During pregnancy, the binding of SA to serum decreased (table 2), while the serum pH increased (see *Results*). It was reported that the interstitial space increased during pregnancy (17). Considering these findings, the calculation was performed using the parameters listed in table 4. The serum pH used was actually measured to be 7.50 in the control and 7.66 in the pregnant rats. The intracellular pH was assumed to be 7.0 (18) and not to be changed during pregnancy. The value of 3.0 was used as the pK_a for SA (19). The ratios of concentrations of the unbound drug between the interstitial and intracellular fluids (R) were calculated by eq. 4 in Appendix 2, and those of the control and pregnant rats were 0.316 and 0.219, respectively.

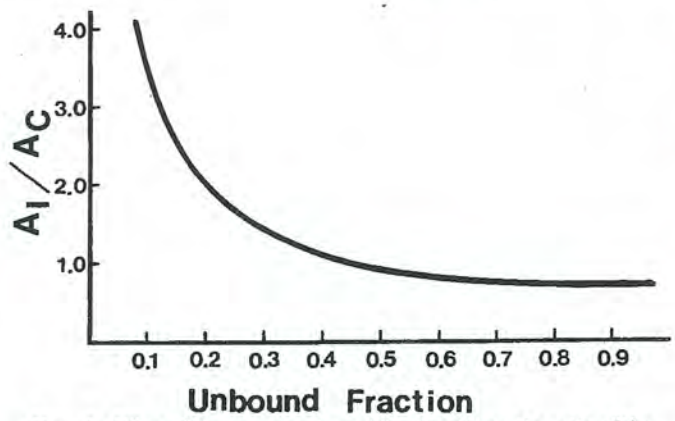

FIG. 4. *Effect of the serum unbound fraction (f_s) on the ratio of the amount of SA distributed between the ISF and ICF.*

A_I and A_C denote the amount of SA in the interstitial and intracellular fluids, respectively. The *line* was simulated by eq. 1 in the text, using parameters of *ISF* = 0.15, *ICF* = 0.55, *AR* = 0.5, serum pH 7.40, and intracellular pH 7.00.

TABLE 4

Tissue total fluid (TW), intracellular fluid (ICF), interstitital fluid (ISF), and albumin concentration ratio (AR) between ISF and serum in the rat

Parameters are represented as the percentage of tissue weight.

Tissue	Control			Pregnant			AR^d
	TW	*ICF*[a]	*ISF*	TW_p[b]	ICF_p	ISF_p[c]	
Heart	77[e]	63[e]	14[e]	81	63	18	0.5
Lung	78[e]	53[e]	25[e]	86	53	33	0.5
Liver	70[f]	57[f]	13[f]	74	57	17	0.5
Gastrointestinal	75[f]	54[f]	21[f]	81	54	27	0.9
Muscle	71[f]	59[f]	12[f]	75	59	16	0.6
Skin	60[f]	30[g]	30[g]	69	30	39	0.6
Kidney	78[f]	58[f]	20[f]	84	58	26	0.5

[a] $ICF = TW - ISF$.
[b] $TW_p = TW + (ISF_p - ISF)$.
[c] $ISF_p = ISF \times 1.3$ (17).
[d] AR = (interstitial albumin concentration)/(serum albumin concentration). Obtained from the literature (15, 32).
[e] Obtained from the literature (33).
[f] Obtained from the literature (34).
[g] Obtained from the literature (15).

TABLE 5

Comparison between observed and calculated tissue-to-serum partition coefficients (Kp value) in control and pregnant rats

Tissue	Control		Pregnant			
	Kp_{obs}[a]	Kp_{cal}[b]	$Kp_{cal,1}$[c]	$Kp_{cal,2}$[d]	$Kp_{cal,3}$[e]	Kp_{obs}[a]
Heart	0.187	0.105	0.250	0.284	0.243	0.325
Lung	0.192	0.166	0.321	0.388	0.354	0.394
Liver	0.228	0.099	0.229	0.263	0.226	0.339
Kidney	0.438	0.140	0.290	0.340	0.303	0.540
Gastrointestinal	0.205	0.216	0.317	0.375	0.341	0.322
Muscle	0.116	0.105	0.229	0.264	0.226	0.222
Skin	0.244	0.210	0.324	0.402	0.383	0.369

[a] Observed values calculated by using the mean concentrations (N = 3) of serum and each tissue at 8 hr after iv bolus injection of SA (10 mg/kg).
[b] Calculated by eq. 2 using parameters listed in table 4.
[c] Calculated by eq. 2 using parameters of the control except f_s. The values of f_s in the pregnant rat was used (see text).
[d] Calculated by eq. 2 using parameters of the control except f_s and *ISF*. The values of f_s and *ISF* in the pregnant were used (see text).
[e] Calculated by eq. 2 using parameters of the control except f_s, *ISF*, and serum pH, for which those of the pregnant rat were used (see text).

The Kp_{cal} value in various tissues is given by a following equation (see Appendix 2 for details)

$$Kp_{cal} = [ISF \cdot (1 - AR) + ICF \cdot R] f_s + ISF \cdot AR \quad (2)$$

To determine the contribution of parameters which affects the *Kp* value in the pregnant rats, three parameters, *i.e.* f_s, *ISF*, and serum pH, in eq. 2 were changed from the values of the control rats to those of the pregnant rats as follows. First, only the value of f_s was changed to that of the pregnant rats and $Kp_{cal,1}$ thus was calculated. Second, the values of f_s and *ISF* were changed to those of the pregnant rats and $Kp_{cal,2}$ was calculated. Finally, all three parameters were changed to those of the pregnant rats and $Kp_{cal,3}$ was calculated. The results of the calculation are summarized in table 5. In both the control and pregnant rats, the values of Kp_{cal} showed good agreements with the observed values of *Kp* except for kidney, liver, and heart. Among three values of Kp_{cal}, $Kp_{cal,1}$ showed a comparable value to the observed value of *Kp*,

suggesting that the increase in f_s during pregnancy is the most dominant determinant which affects the change in the tissue distribution of SA during pregnancy. The discrepancy between the calculated and observed *Kp* values in the kidney and liver might be in part explained by the binding to the cytosol proteins such as ligandin (20) and Dv protein (21). However, in the heart it is difficult to explain the reason at this time.

In all tissues studied, the *Kp* values of the fetal rats were higher than those corresponding *Kp* values of the maternal rats (table 3). Especially in the brain, the *Kp* value of the fetal rats was remarkably higher than that of the maternal rats, and this may be explained by the immaturity of an efflux mechanism of SA in the fetal brain as previously suggested (9). Though the protein binding of SA in the fetal serum was higher than that of the maternal serum (fig. 2), the *Kp* values of all fetal tissues studied were larger than those of the maternal tissues (table 3). As an explanation for these results, a large volume of interstitial space in the fetal rat may be conceivable. According to Friis-Hansen (22), the extracellular space of the fetus was 43% of the total body weight of the fetus.

The placental transfer of several drugs has been studied in the pregnant sheep (23–26), while little data including the f_s value of the drug both in the maternal and fetal serum was available in the pregnant rats (27, 28). In the present study, we observed a fetal/maternal serum concentration ratio of 1.04 (fig. 1). Two mechanisms have been proposed for the maintenance of a concentration gradient of SA in serum between mother and fetal serum at steady state, *i.e.* the difference in the protein binding between the fetal and maternal serum (29) and the difference in the degree of drug ionization in the fetal and maternal serum (30). SA is a weak acid ($pK_a = 3.0$) (19) and is almost ionized at the physiological pH. The mean value of pH of the maternal serum was 7.66 (see *Results*), while that of the fetal serum was 7.37 as reported by Kennedy *et al.* (31). The distribution ratio of the unbound concentration (ionized and un-ionized) of SA between the fetal serum (C_f) and maternal serum (C_m) was calculated to be 0.51 by the following equation based on the assumption that only the un-ionized form can penetrate across the placental membrane (30)

$$\frac{C_f}{C_m} = \frac{1 + 10^{pH_f - pK_a}}{1 + 10^{pH_m - pK_a}} \tag{3}$$

If we further consider the difference in the serum unbound fractions between the maternal serum ($f_s = 0.67$) and fetal serum ($f_s = 0.41$), the serum concentration ratio of SA (fetal/maternal) could be calculated by $(0.51/0.41)/(1/0.67) = 1.24/1.49 = 0.83$. This ratio was comparable to that observed (1.04) (fig. 1), indicating that both the difference in the serum protein binding and pH between the maternal and fetal serum could explain the fetal/maternal concentration ratio.

In conclusion, the remarkable increase in f_s during pregnancy may primarily contribute to the increase in the *Kp* values of most tissues in the pregnant rats.

Appendix 1: Nomenclature

Kp, tissue-to-serum partition coefficient of SA
f_s, serum unbound fraction of SA
n, number of binding sites on albumin
Kd, dissociation constant of serum protein binding (mM)
$C_{b,s}$, concentration of bound drug in serum (mM)
$C_{b,i}$, concentration of bound drug in the tissue interstitial fluid (mM)
$C_{f,s}$, concentration of unbound drug in serum (mM)
$C_{f,i}$, concentration of unbound drug in the tissue interstitial fluid (mM)
C_c, concentration of drug in the tissue intracellular fluid (mM)
R, intracellular fluid-to-serum unbound concentration ratio of SA
AR, interstitial fluid-to-serum concentration ratio of albumin
P, concentration of albumin in serum (mM)
P_i, concentration of albumin in the tissue interstitial fluid (mM)
V, volume of the tissue (ml)
V_i, volume of the tissue interstitial fluid (ml)
V_c, volume of the tissue intracellular fluid (ml)
TW, tissue total fluid fraction $(V_i + V_c)/V$
ISF, tissue interstitial fluid fraction (V_i/V)
ICF, tissue intracellular fluid fraction $(V_c/V = TW - ISF)$
pH_s, serum pH
pH_c, pH of the intracellular fluid

Appendix 2

If only an un-ionized drug can cross the cell membrane by a passive diffusion and there is a pH difference between the intracellular and interstitial fluids, the intracellular fluid-to-serum concentration ratio of SA (*R*) is given by (19)

$$R = \frac{C_c}{C_{f,i}} = \frac{1 + 10^{pH_c - pK_a}}{1 + 10^{pH_s - pK_a}} \tag{4}$$

The ratio of the amount of SA in the interstitial space to that in the intracellular space of the tissue is given by eq. 5 based on the assumption that the binding of SA to the components of cell is negligible.

$$\frac{A_I}{A_c} = \frac{V_i \cdot (C_{f,i} + C_{b,i})}{V_c \cdot C_c} \tag{5}$$

and $C_{b,i}$ is described by a Langmuir type equation as follows:

$$C_{b,i} = \frac{nP_i \cdot C_{f,i}}{K_d + C_{f,i}} \tag{6}$$

where the values of *n* and K_d were equal to those obtained from the serum protein-binding experiments.

Since $C_{f,i}$ and P_i are equal to $C_{f,s}$ and $AR \cdot P$, respectively, eq. 7 can be derived from eq. 6 as follows:

$$C_{b,i} = AR \cdot C_{b,s} \tag{7}$$

and $C_{b,s}$ is given by

$$C_{b,s} = \left(\frac{1 - f_s}{f_s}\right) \cdot C_{f,s} \tag{8}$$

Substituting eqs. 4, 7, and 8 into eq. 5

$$\frac{A_I}{A_C} = \frac{V_i}{V_c \cdot R} \cdot \left[(1 - AR) + \frac{AR}{f_s}\right] \tag{9}$$

Since *ISF* and *ICF* are equal to V_i/V and V_c/V, respectively, eq. 1 in the text is obtained.

The calculated tissue-to-serum partition coefficients, Kp_{cal}, is given by

$$Kp_{cal} = \frac{V_i \cdot (C_{f,i} + C_{b,i}) + V_c \cdot C_c}{V \cdot (C_{f,s} + C_{b,s})} \tag{10}$$

Substituting eqs. 4, 7, and 8 into eq. 10, eq. 2 in the text thus is obtained.

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