Table I—Effect of Pregnancy on Sulfate Concentrations in Plasma of Caucasian Women [a]

	Pregnant (n = 7)	Nonpregnant (n = 9)	Significance
Plasma sulfate, mM	0.410 ± 0.035	0.333 ± 0.038	$p < 0.005$
Age, yr	24.4 ± 4.2	27.2 ± 5.5	NS
Gestation age, wk	35.8 ± 3.8		
Total protein, g/dl	6.08 ± 0.29	6.43 ± 0.35	NS
Albumin, g/dl	3.28 ± 0.19	4.14 ± 0.20	$p < 0.001$

[a] Results expressed as mean ± SD.

pooled citrated plasma from nonpregnant women with known concentrations of added sodium sulfate for preparation of a standard curve. All samples were assayed on the same day, and the results were corrected for sample dilution with citrate anticoagulant solution. Total plasma protein concentrations (14) and albumin fraction[2] were also determined. Results were examined by one-way ANOVA, and possible associations between sulfate concentration and other variables were determined by correlation analysis[3].

The results of this study are summarized in Table I. Plasma sulfate concentrations were significantly higher in the pregnant women, but the quantitative difference between the pregnant and nonpregnant women was relatively small. Plasma albumin concentrations were significantly decreased in late pregnancy, consistent with previous observations (15). The difference in sulfate concentration between pregnant and nonpregnant subjects remained when smokers were eliminated (0.427 ± 0.039 mmole/liter in four pregnant women and 0.328 ± 0.036 in eight nonpregnant women, $p < 0.005$). Eight Black women (including two smokers) in their third trimester of pregnancy were also studied; their plasma sulfate concentration (0.360 ± 0.072 mmole/liter) is not significantly different from those of the Caucasian pregnant and nonpregnant women, respectively. There is a significant negative correlation between the plasma sulfate concentration of the 15 pregnant women (Black and Caucasian combined) and their plasma albumin concentration ($r = -0.548$, $p < 0.05$). No other significant correlations were found.

The results of this investigation are in qualitative agreement with those of Tallgren (11) with respect to Caucasian women, but the quantitative difference between pregnant and nonpregnant women is considerably smaller in our study. Our results for nonpregnant females are similar to those described (11), but the inorganic sulfate concentrations in pregnant women are appreciably lower in our study. The intake of proteins rich in sulfur-containing amino acids is an important determinant of endogenous inorganic sulfate levels (11, 16), but it appears unlikely that the difference between the two groups of pregnant women is due to diet, since the two nonpregnant control groups had similar sulfate concentrations. Tallgren's analytical procedure (11) requires incubation of the serum sample for 4 hr at 37° and pH <1, which may favor hydrolysis of endogenous steroid sulfates, while the procedure used by us requires no such incubation. However, the plasma concentrations of these conjugates in pregnancy (17, 18) are much too low to account for the observed differences.

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Changes in Plasma Protein Binding of Drugs after Blood Collection from Pregnant Women

Keyphrases □ Protein binding—plasma, changes after blood collection from pregnant women □ Unesterified fatty acids—plasma, changes after blood collection from pregnant women

To the Editor:

Drug-protein binding in plasma obtained from 20-day pregnant rats can decrease rapidly *in vitro* after blood collection (1) due to lipolysis and the consequent increase in the concentrations of unesterified fatty acids[1]. These

[2] By Gilman Sepratek Electrophoresis System.
[3] BMPD-79 statistical package.

[1] Results to be published.

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changes are much less pronounced in plasma of nonpregnant rats (1). Since there are pronounced differences in the drug-protein binding characteristics of rat and human plasma and serum (2, 3), this investigation was designed to determine if the rapid and pronounced decrease in drug-protein binding and increase in fatty acid concentrations observed in plasma of pregnant rats occur also in plasma of pregnant women.

Blood samples were obtained from 15 healthy women, 18 to 29 years old, who were in the third trimester of pregnancy and who were not taking any medications. The blood was drawn from an antecubital vein into a heparinized plastic syringe, and plasma was separated immediately by 1-min centrifugation. A 0.1-ml aliquot of the plasma was immediately extracted into hexane for determination of fatty acid concentrations by gas chromatography (4). Fifty microliters of an aqueous solution of either sodium phenytoin or sodium salicylate was added to another 5- to 6-ml aliquot of plasma to yield final concentrations of ~20 and 200 μg/ml (in terms of the acid), respectively. Two milliliters of this plasma was immediately ultrafiltered (35 min) through a cellophane membrane (Visking, molecular weight cut-off 12,000–14,000), another 2 ml was maintained at 37° for 6 hr and then ultrafiltered after removal of 0.1 ml for determination of fatty acid concentrations, and 0.4 ml was placed in a dialysis cell for equilibrium dialysis (6 hr) against an equal volume of 0.13 *M* sodium–potassium phosphate buffer, pH 7.4, at 37°. A 0.1-ml portion of the plasma phase was collected after dialysis for fatty acid assays. Phenytoin and salicylic acid concentrations in ultrafiltrate, dialysate, and plasma phases were determined by high-performance liquid chromatography, as previously described (5), except that *o*-methoxybenzoic acid was used as the internal standard for the salicylate assay. The equilibrium dialysis procedures were repeated ~1 week later, but at 25°. In view of the concentration dependence of salicylate protein binding, sodium salicylate in a concentration approximately equal to that found previously in the ultrafiltrates was added to the buffer solution. No such precaution was required with respect to phenytoin because of the relative concentration independence of its plasma-free fraction value in the therapeutic concentration range. The plasma for the repeat dialysis procedure had been stored frozen at −20°.

The results of the protein binding determinations are summarized in Table I. Neither incubation of the plasma at 37° nor the 6-hr incubation at 25° associated with equilibrium dialysis had any significant effect on the plasma protein binding of phenytoin. These same procedures caused a pronounced decrease of phenytoin protein binding in plasma of pregnant rats (1). Technical difficulties related to the need for immediate ultrafiltration in the clinic prevented measurements at 37°, except for equilibrium dialysis. The phenytoin free-fraction value at 37° (0.175 ± 0.016) is considerably higher than the corresponding value in nonpregnant individuals (≃0.1) and is in good agreement with previous determinations of phenytoin free fraction in pregnant women (6, 7).

Table I—Changes in Plasma Protein Binding of Phenytoin and Salicylate after Blood Collection from Pregnant Women in the Third Trimester of Pregnancy

Procedure	Free Fraction in Plasma × 100, mean ± *SD*	
	Phenytoin[a]	Salicylate[b]
Immediate ultrafiltration at 25°	9.29 ± 2.32	28.2 ± 6.9
Ultrafiltration at 25° after 6 hr at 37°	9.89 ± 2.41	34.9 ± 7.8[c]
Dialysis for 6 hr at 25°	10.9 ± 1.5	28.7 ± 3.7
Dialysis for 6 h at 37°	17.5 ± 1.6	

[a] Concentration, 22.8 ± 1.8 μg/ml; $n = 8$. [b] Concentration, 203 ± 15 μg/ml; $n = 7$. [c] Significantly different from value after immediate ultrafiltration ($p < 0.05$). The ratio of free-fraction values, 6 hr/immediate, is 1.27 ± 0.26.

Table II—Changes in Concentrations of Unesterified Fatty Acids in Plasma after Blood Collection from Pregnant Women in the Third Trimester of Pregnancy

Fatty Acid	Fatty Acid Concentration, μM[a]			Concentration Ratio, 6 hr/immediate
	Immediately after Plasma Separation	6 hr at 37° after Plasma Separation	After 6-hr Dialysis at 37°	
Linoleic	46.4 ± 30.2	63.2 ± 30.5	60.0 ± 25.2	1.67 ± 0.60
Oleic	123 ± 109	143 ± 102	136 ± 102	1.32 ± 0.23
Palmitic	118 ± 63	157 ± 65	146 ± 64	1.46 ± 0.34
Stearic	61.3 ± 25.6	67.3 ± 21.6	58.6 ± 19.0	1.17 ± 0.26
Total	348 ± 218	430 ± 213	401 ± 205	1.36 ± 0.27

[a] Mean ± *SD*, $n = 15$.

The process of equilibrium dialysis at 25° had no apparent effect on the plasma protein binding of salicylate, but incubation of the plasma for 6 hr at 37° decreased protein binding slightly (Table I). Results of equilibrium dialysis at 37° are not listed in the table, because the buffer phase was not spiked with salicylate to prevent a decrease in drug concentration in the plasma phase. However, the free-fraction values (0.242 ±0.035) are in good agreement with those of an earlier study under similar experimental conditions (8).

The results of fatty acid concentration determinations are summarized in Table II. Some lipolysis occurred in plasma during 6 hr of incubation at 37°, but the fatty acid concentrations increased only by 17–67% on average during incubation and somewhat less during equilibrium dialysis. Under similar conditions, total fatty acid concentrations in plasma from pregnant rats increase by ~500% when heparin is used as the *in vitro* anticoagulant and by 200% when EDTA is used as the anticoagulant[1]. Heparin added *in vitro* to plasma from nonpregnant humans has no apparent effect on the protein binding of phenytoin and salicylic acid (3).

In summary, it has been found that the drug-protein binding characteristics of plasma from healthy women in the third trimester of pregnancy are relatively stable, at least with respect to the drugs tested. These observations are consistent with the relatively small increase of fatty acid concentrations due to *in vitro* lipolysis in plasma during incubation or dialysis at 37° for 6 hr after blood collection. While all plasma samples should be processed to minimize *in vitro* lipolysis, this precaution applies much more to pregnant rats (and perhaps other species) than to pregnant women.

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Drug Kinetics in Low-Flux (Small) Anatomic Compartments

Keyphrases □ Pharmacokinetics—low-flux peripheral compartment □ Cochlear perilymph—aminoglycoside kinetics

To the Editor:

Small anatomic compartments may be the site of action or toxicity of drugs. Because the kinetics in the anatomic compartment may differ significantly from those in the sampled compartment, pharmacokinetic modeling of such drugs requires the development of explicit expressions for the time course of drug concentrations in the anatomic compartment. Classical compartmental analysis is based on large (kinetic) compartments, which exchange enough drug that measurable alterations in the concentration of drug in the sampled compartment result. A small (anatomic) compartment may not give rise to a detectable alteration in drug concentration in the sampled compartment, because its drug flux is small. In such a case, the anatomic compartment can not be represented by a peripheral kinetic compartment. This communication describes the derivation of an equation for the time course of drug concentrations in such small compartments based on a modified compartmental analysis.

Consider an n-compartment linear mammillary system with first-order rate constants and elimination from the central compartment (denoted by subscript 1) only. Let there be an $(n + 1)$th compartment that exchanges drug with the central compartment but for which the flux of drug into and out of the compartment is so minute that it does not measurably alter levels of the drug in the central compartment. This compartment will be called the low-flux peripheral compartment and will be labeled LF.

The differential equations governing this model are:

$$\frac{dC_1}{dt} = \sum_{j=2}^{n}\left(k_{j1}\frac{V_j}{V_1}C_j - k_{1j}C_1\right) - k_{10}C_1 \quad \text{(Eq. 1)}$$

$$\frac{dC_j}{dt} = k_{1j}\frac{V_1}{V_j}C_1 - k_{j1}C_j,\ n \geq j > 1 \quad \text{(Eq. 2)}$$

$$\frac{dC_{LF}}{dt} = k_{1LF}\frac{V_1}{V_{LF}}C_1 - k_{LF1}C_{LF} \quad \text{(Eq. 3)}$$

where

C_j = concentration of drug in the jth compartment;
V_j = pharmacokinetic volume of distribution of drug in the jth compartment;
k_{ab} = first-order transfer rate constant from the ith to the jth compartment;
k_{10} = first-order elimination rate constant from the central compartment;
C_{LF} = concentration of drug in the low-flux compartment;
k_{1LF}, k_{LF1} = first-order transfer rate constants into and out of the low-flux compartment.

If the drug enters the central compartment as a result of bolus injection, the drug concentration in that compartment, as a function of time, will be a sum of exponentials (1):

$$C_1(t) = \sum_{i=1}^{n} a_i e^{-\lambda_i t} \quad \text{(Eq. 4)}$$

where a_i = coefficient of ith exponential term and λ_i = exponent of the ith exponential term. The general equation for the drug concentration in the jth peripheral compartment is found by the use of Laplace transforms (2):

$$C_j(t) = \frac{V_1}{V_j}\sum_{i=1}^{n}\frac{k_{1j}}{(k_{j1} - \lambda_i)}a_i(e^{-\lambda_i t} - e^{-k_{j1}t}) \quad \text{(Eq. 5)}$$

The equation for the drug concentration in the low flux compartment is:

$$C_{LF}(t) = \frac{V_1}{V_{LF}}\sum_{i=1}^{n}\frac{k_{1LF}}{(k_{LF1} - \lambda_i)}a_i(e^{-\lambda_i t} - e^{-k_{LF1}t}) \quad \text{(Eq. 6)}$$

By similar means, expressions can be found for the drug concentration in the low-flux compartment following entry of the drug into the central compartment by first-order absorption or continuous infusion.

As an example of the application of this model, Eq. 6 is used to describe the kinetics of amikacin, an aminoglycoside antibiotic, in cochlear perilymph, presumably a low-flux compartment. The data are taken from a study performed in guinea pigs, as reported by Brummett *et al.* (3). The concentrations of amikacin were determined in serum and in cochlear perilymph over time following a single subcutaneous injection of the drug (as detailed in Ref. 3). The subcutaneous absorption of amikacin was very rapid, resulting in a monoexponential serum drug concentration *versus* time curve. Therefore, Eq. 4, and consequently Eq. 6, can be used in this case even though the injection was not intravenous.

The equation for the serum drug concentration *versus* time curve is:

$$C_{serum}(t) = 489e^{-0.6992t\,(\text{in hr})} \text{ in micrograms per milliliter}$$

The experimental data and corresponding predicted values are presented in Table I. The perilymph drug concentration *versus* time curve is fitted to a biexponential

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