correct value is used. Errors in the estimate of β are inherent due to biological and analytical variability, but their impact can be reduced drastically by expanding the sampling schedule to longer times. Since this is not always possible because of analytical and practical limitations, the use of statistical moments may not be appropriate if there is insufficient sampling to provide accurate and consistent estimates of β.

Routine analytical error in drug concentration data can affect estimates of MAT and MDT in two ways: first, by its effects on the calculation of $AUMC_0^t$ and AUC_0^t, which should be rather insignificant, and second, by its effects on the estimate of β, which in turn may affect the calculation of $AUMC_0^\infty$ and AUC_0^∞ significantly. The first case is illustrated in Table III by the values of MDT^b generated using the theoretical value of β (i.e. 0.1 hr^{-1}) for all extrapolations. The average ($n = 18$) estimated MDT values were good estimates of the theoretical values with coefficients of variation ranging from 23 to 32%. The effect of variations in β caused by random error and the subsequent impact on MDT^c is also shown in Table III. The average MDT^c values were again good estimates of the theoretical values but the coefficients of variation were quite large, ranging from 47 to 81%. With this sampling schedule ~8% of AUC_0^∞ and 31% of $AUMC_0^\infty$ were due to extrapolation, and this would not be uncommon for a typical bioavailability study. The large variability in these MDT values could affect the ability to statistically detect small differences in MDT values under such conditions.

Overall, the results of these studies suggest that the statistical moment approach to the analysis of bioavailability data may offer an attractive alternative to C_{max} and t_{max} or to the model-dependent methods for assessing the rate of drug absorption. However, accurate results and meaningful conclusions using this method are very much dependent on the experimental design of the studies from which the data are generated. To obtain optimal information, frequent sampling during the absorption phase is necessary. In addition, sufficient sampling during the terminal elimination phase is needed to minimize the impact of extrapolation error and to provide an accurate estimate of β. MAT and MDT values calculated from data generated *via* a less than rigorous experimental design may yield poor estimates of the actual values and can result in misleading conclusions. Therefore, an understanding of the influence of the various factors affecting the accuracy of the calculated MAT and MDT values, along with a well-defined pharmacokinetic profile of the drug, are essential to achieve an experimental design which will allow for the determination of meaningful estimates of mean absorption and dissolution times.

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Plasma Inorganic Sulfate Concentrations in Pregnant Women

Keyphrases □ Sulfate concentration—effect of pregnancy in humans □ Plasma sulfate concentrations—effect of pregnancy in humans

To the Editor:

The physiological process of sulfation has important biosynthetic and detoxification functions. It is necessary in the developing fetus for the formation of glycosaminoglycans, a structural component of cartilage and other tissues, and cerebroside sulfate, a constituent of the brain (1, 2). The sulfated glycosaminoglycans may also be involved in cell differentiation (2).

The biotransformation of certain phenolic drugs to sulfate conjugates is limited by the availability of inorganic sulfate (3, 4); the formation of such drug conjugates can cause depletion of endogenous inorganic (free) sulfate in animals and humans (4–6). Decreased sulfate availability in pregnant rats, due to administration of salicylamide, which produces sulfate depletion concomitant with salicylamide sulfate formation (3, 5), has been associated with decreased availability and decreased tissue incorporation of sulfate in the fetuses and may be the cause of teratogenic effects (7, 8). Depletion of endogenous sulfate can decrease the rate of sulfation of phenolic drugs, such as acetaminophen, and thereby decrease their clearance (4), while hypersulfatemia, such as occurs in renal dysfunction, can facilitate drug sulfate conjugate formation and thereby increase drug clearance (9). The maternal level of endogenous inorganic sulfate may, therefore, affect fetal development and fetal exposure to certain drugs and other xenobiotics.

The plasma or serum concentrations of most electrolytes tend to decrease slightly during pregnancy (10). We have found only one report concerning the effect of pregnancy on serum sulfate concentrations: Tallgren observed concentrations of 0.592 ± 0.275 mmole/liter (mean ± *SD*) in 118 Scandinavian women during their third trimester and 0.263 ± 0.091 mmole/liter in 42 age-matched nonpregnant controls (11). On the other hand, Lin and Levy recently determined serum sulfate concentrations in 20-day pregnant rats and nonpregnant controls and found no apparent difference between the two groups[1]. The effect of pregnancy on endogenous sulfate concentrations in women was, therefore, reexamined.

Seven Caucasian women in their third trimester of pregnancy and nine Caucasian nonpregnant women of similar age were the subjects in this study. They were medication-free for at least 1 week before the study and were in apparent good health. None of the controls were taking an oral contraceptive. Nine-milliliter blood samples were drawn from an antecubital vein into 10-ml capacity plastic disposable syringes containing 1 ml of trisodium citrate solution each. The citrated blood was centrifuged in plastic tubes at 12,000×*g* for 15 min at 25°, and the plasma was frozen pending assay. Inorganic sulfate concentration was determined by a modification (12) of the turbidimetric method of Berglund and Sörbo (13), using

[1] Results to be published.

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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

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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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