# Altered drug binding to serum proteins in pregnant women: therapeutic relevance[1]

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**Summary:** The binding of diazepam, phenytoin and valproic acid to serum proteins *in vitro* has been compared in pregnant women of different gestational ages and in controls. The unbound fraction of each of the three drugs was elevated during pregnancy (particularly during the last 8 weeks) probably due, at least in part, to a fall in serum albumin concentration. These findings may provide a partial explanation for the increase in the clearance of certain drugs during pregnancy and need to be taken into account when interpreting serum drug levels in clinical practice.

## Introduction

The physiological changes that occur during pregnancy may have a complex influence on drug disposition. Oestrogens and progestagens have been shown to act as modulators of mixed function oxidase activity (Feuer 1979), an observation that may provide an explanation for the described changes in the metabolic clearance of certain drugs during pregnancy and the puerperium (Krauer *et al.* 1980). Important distributional changes also occur due not only to the growth of the fetoplacental tissues, but also to a major expansion of the extracellular fluid space (Krauer *et al.* 1980). Since the relative contribution of these and other factors varies considerably from one patient to another, it is generally impossible to predict whether or not a clinically important alteration of pharmacological response during pregnancy will occur in a given individual.

In the case of drugs for which an optimal range of serum concentrations has been established (e.g. some antiepileptic drugs), it has been suggested that regular monitoring of serum levels during pregnancy will enable the clinician to detect promptly any important change in drug disposition and to take all corrective measures necessary to maintain an adequate therapeutic response. The use of serum levels as a guide to dosage adjustments, however, is based on the assumption that no significant changes in plasma protein binding occur during pregnancy. Although current methods for measuring drug levels do not discriminate between free (unbound) and protein-bound molecules, it is only the former that are available to diffuse across biological membranes and produce pharmacological effects at the receptor sites (Perucca & Richens 1980, Rowland 1980). If binding was impaired in pregnancy (e.g. a greater proportion of the total concentration was in the free form), total serum drug levels may no longer provide a reliable estimate of the amount of drug available to produce therapeutic effects.

Pregnancy is associated with appreciable changes in serum protein concentration, hormonal levels and free fatty acids (Krauer *et al.* 1980). Since all these factors are known to affect drug binding, we considered it of interest to examine the unbound fraction of a number of drugs in the serum of pregnant and control women. In this paper we present evidence that

[1] Paper read to Section of Medicine, Experimental Medicine & Therapeutics, 10 February 1981. Accepted 23 February 1981
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0141–0768/81/060422–05/$01.00/0

the binding of highly albumin-bound drugs is impaired as pregnancy progresses. These findings are discussed in the light of their therapeutic relevance.

## Methods

*In vitro study:* Experiments were performed *in vitro* by using serum samples collected from women at different stages of pregnancy and from non-pregnant control women of similar age. Most of the women in middle and late pregnancy were receiving iron and vitamin supplements. Women on drugs other than these were excluded from the study.

Three model drugs were selected for the investigation. These were valproic acid and phenytoin, two acidic drugs which bind predominantly to site I on the albumin molecule, and diazepam, a basic compound which is predominantly bound to albumin site II (Sjöqvist *et al.* 1980). Binding studies were performed by ultrafiltration at room temperature according to the method described by Lunde *et al.* (1970). Samples were spiked with therapeutic concentrations (350 μmol/l for valproic acid, 60 μmol/l for phenytoin and 1050 nmol/l for diazepam) of the drug in question prior to analysis. Serum albumin concentration was measured by spectrophotometry according to Doumas *et al.* (1971).

*Case report:* One epileptic woman receiving chronic treatment with phenytoin alone (400 mg daily) was followed prospectively before, during and after pregnancy. Total (free + protein-bound) serum phenytoin levels were determined at regular intervals by gas chromatography (MacGee 1970). Serum unbound phenytoin fraction and albumin concentration were measured according to the methods described above.

## Results

*In vitro study:* The serum unbound fraction of valproic acid, phenytoin and diazepam in the subjects included in the study is illustrated in Table 1. In women in early pregnancy the unbound fraction of diazepam was similar to that observed in controls, whereas the unbound fraction of phenytoin and valproic acid was slightly but significantly increased. In women 17–32 weeks pregnant the serum unbound fraction of all drugs was found to be moderately increased as compared to controls ($P<0.05$). A greater increase in unbound fraction was observed during the last weeks of pregnancy, the difference ranging from 25% on average in the case of phenytoin to approximately 50% in the case of valproic acid and diazepam ($P<0.01$). The serum albumin concentration was $45\pm3$ g/l in controls and $41\pm3$, $40\pm3$, and $36\pm3$ g/l in women in early, mid and late pregnancy respectively. For each of the three drugs, a negative linear correlation between serum unbound fraction and albumin concentration was found. The coefficients of correlation were $-0.80$ for phenytoin ($P<0.01$), $-0.57$ for diazepam ($P<0.01$) and $-0.73$ for valproic acid ($P<0.01$).

In order to examine the possibility that at least in the case of phenytoin the reduction in binding capacity was causally related to the fall in albumin concentration, pooled serum from

*Table 1. Serum unbound fraction of diazepam, phenytoin and valproic acid in pregnant women and in controls. Values represent mean ± s.d.*

| | | Serum unbound fraction (%) | | |
|---|---|---|---|---|
| | *n* | Diazepam | Phenytoin | Valproic acid |
| Controls | 10 | 1.8±0.4 | 9.7±0.8 | 9.4±1.6 |
| Early pregnancy (8–16 weeks) | 10 | 1.9±0.1 | 10.6±0.5■ | 11.5±1.5■ |
| Mid-pregnancy (17–32 weeks) | 10 | 2.1±0.2● | 10.9±0.8■ | 12.1±1.9■ |
| Late pregnancy (33–40 weeks) | 10 | 2.6±0.3■ | 12.6±1.1■ | 14.6±2.4■ |

●$P<0.05$ as compared to controls (Student's *t* test)
■$P<0.01$

control women was diluted with buffer (phosphate buffer 0.15 mol/l, pH 7.4) to yield a concentration of albumin equal to that observed in women in late pregnancy. Dilution resulted in an increase in the unbound fraction of phenytoin from 9.3% to 12.3% (means of three determinations), a value similar to that observed in the pregnant serum (12.9%).

*Case report:* The relationship between serum unbound phenytoin fraction and albumin concentration in a 33-year-old epileptic patient (DD) who received 400 mg phenytoin daily throughout pregnancy and the puerperium is illustrated in Figure 1. The unbound phenytoin fraction increased progressively during pregnancy, reached a maximum approximately at the time of delivery and returned to the pre-pregnancy level within the fourth month post-partum. Changes in serum albumin concentration were inversely correlated with the changes in unbound fraction. The magnitude of these changes was in agreement with that observed *in vitro* in non-epileptic women.

Serum total and unbound phenytoin concentration values in the same patient are shown in Figure 2. A considerable fall in total serum phenytoin levels was observed a few weeks prior to delivery. Due to the simultaneous increase in unbound fraction, the change in free concentration was less marked.

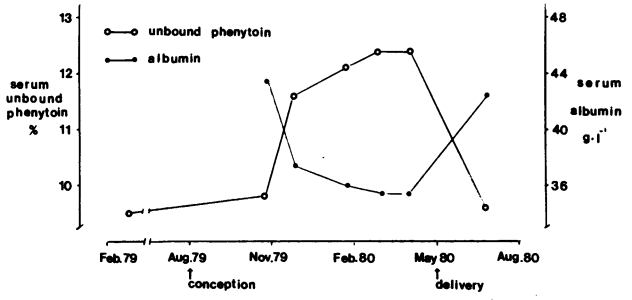


Figure 1. Relationship between serum unbound phenytoin fraction and albumin concentration in a 33-year-old epileptic patient (DD) who received phenytoin 400 mg daily throughout her pregnancy

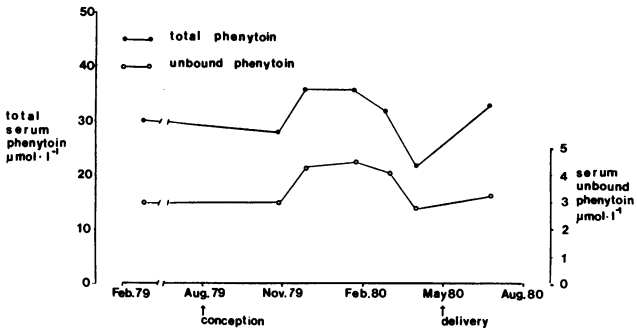


Figure 2. Serum total and unbound phenytoin concentration in patient DD

## Discussion

Changes in the serum protein binding of drugs during pregnancy have been little investigated to date. Most of the available information concerns drugs which are predominantly bound to albumin (Dean *et al.* 1979). Our results showing an increased unbound fraction of diazepam, phenytoin and valproic acid in pregnancy (especially during the last 8 weeks) are in close agreement with those recently reported by other authors for the same drugs as well as for phenobarbitone (Plasse *et al.* 1979) and salicylic acid (Dean *et al.* 1979, 1980).

On the other hand, an apparent discrepancy exists between our data and those reported by Hooper *et al.* (1974), who found no statistically significant difference in phenytoin binding between a small group of pregnant women and non-pregnant controls. The latter authors, however, gave insufficient details on the gestational age of their subjects and it is possible that their negative findings were accounted for by the inclusion in the study of women in early pregnancy.

The significant negative correlation between serum albumin concentration and unbound fraction of the three drugs suggests that the impairment in binding in middle and late pregnancy could be accounted for at least in part by a fall in serum albumin. For phenytoin, this interpretation is supported by the finding of an inverse relationship between serum albumin concentration and unbound fraction in patient DD. The fact that after dilution to equal concentrations of albumin control serum bound phenytoin to a similar extent as pregnant serum suggests that the affinity of albumin for phenytoin is not decreased in pregnant women, in contrast with the results of animal studies (Stock *et al.* 1980).

The altered drug binding in pregnancy has several therapeutic implications. Following acute administration, the impairment in binding capacity could result in higher free drug levels and, consequently, in potentiation of pharmacological response. For the drugs included in the present study, however, this effect should be only transient and no overall change of response would be expected during chronic administration. This is because all these drugs are subject to restrictive elimination (i.e. only the unbound drug can be cleared) and two of them, diazepam and phenytoin, also have a relatively large volume of distribution, i.e. the amount of drug in the serum is small compared with the total amount of drug in the body. Under these circumstances, the increase in free drug level should be rapidly compensated for and offset by enhanced drug elimination (clearance) and, especially for phenytoin and diazepam, greater distribution to storage sites in tissues. These effects will eventually lead to a new steady-state situation in which the unbound fraction remains increased, the total (i.e. free and bound) concentration is decreased and the free concentration, which determines the degree of pharmacological effect, is unchanged (Perucca & Richens 1980, Rowland 1980).

A decrease in binding capacity would provide an explanation for the observation that steady-state serum phenytoin levels frequently fall during the last weeks before delivery. Theoretically, the fall in total drug levels should be equal to the change in unbound fraction, i.e. approximately 25% in the case of phenytoin. The fact that in many patients the fall in serum phenytoin levels is much greater than this (Mygind *et al.* 1976) suggests that other mechanisms, such as an increase in drug metabolizing activity, may also be operating and that the concentration of free, pharmacologically active drug may also be reduced in some women in late pregnancy. The presence of additional mechanisms is further suggested by the observation that the serum levels of carbamazepine, the binding of which does not seem to be affected by pregnancy, also decline with increasing gestational age (Dam *et al.* 1979).

Another important implication of decreased protein binding is that in pregnant women serum levels of total drug may underestimate the concentration of free, therapeutically-active drug. This is illustrated by the case of patient DD, in whom the decrease in free concentration prior to delivery was less than the decrease in total drug level. The value of monitoring total serum phenytoin levels is reduced under these circumstances, unless the degree of change in binding capacity is known. An estimate of the latter can be obtained by measuring the serum albumin concentration. Our data indicate that a 25% fall in albumin concentration would produce an approximate 25% increase in unbound phenytoin fraction.

The increase in unbound fraction of diazepam and valproic acid in pregnancy is considerably greater than that observed with phenytoin. From the point of view of drug monitoring, the importance of these observations is reduced by the fact that serum levels of these drugs (diazepam in particular) are very seldom measured and used as a basis for dosage adjustments. Since both diazepam and valproic acid are subject to restrictive elimination, an increase in free drug concentration would not be expected to occur during chronic administration of these compounds to pregnant women. However, the possibility of a transient increase in free diazepam concentration (with a corresponding potentiation of

therapeutic and toxic effects in the mother and the fetus) cannot be excluded should this drug be given intravenously, e.g. for the treatment of status epilepticus during pregnancy. This possibility is particularly relevant in view of the evidence that relatively large doses of diazepam given to the mother during labour may adversely affect the vital parameters of the newborn (Mandelli *et al.* 1978).

Further work is required to examine the clinical implications of altered drug binding in greater detail. Particularly important in this respect is the study of the relationship between changes in degree of protein binding and fetal exposure to the drug. Whether the impairment in binding during pregnancy applies to all albumin-bound drugs is unclear. The fact that in the present study the change in unbound fraction differed markedly even between drugs (phenytoin and valproic acid) which bind to the same class of sites on the albumin molecule (Sjöqvist *et al.* 1980) suggests that it may not be possible to use information about the binding of one drug to predict changes in the binding capacity of another. The situation may be further complicated for drugs (mainly basic compounds) which bind extensively to serum proteins other than albumin (Piafsky 1980).

Drugs that would be particularly interesting to study in this context are those which show non-restrictive, flow-dependent elimination, e.g. propranolol or lignocaine. For these drugs a decrease in binding capacity may actually result in reduced metabolic clearance. This could lead not only to an altered relationship between serum levels and effect, but also to a long-lasting increase in free drug concentration and hence magnitude and duration of pharmacological response.

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