Estrogen-Induced Transcortin Increase and Progesterone and Cortisol Interactions: Implications from Pregnancy Studies

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ABSTRACT

The concentrations of progesterone, cortisol, estradiol, and transcortin binding capacity (TBC) were measured in plasma samples of women during normal pregnancy. Between 10 weeks and 20 weeks gestation, the mean of TBC increased linearly, and the mean increase in TBC for a given estradiol increment was constant until the estradiol concentrations reached approximately 30 nmol per liter. The results were consistent with the increase in TBC having been induced by estradiol; however, there was an inherent upper limit of response. Progesterone and cortisol were each linearly related to TBC, but the ratios of progesterone:TBC and cortisol:TBC showed no systematic trend throughout the period studied, and there was no systematic relationship between TBC and the progesterone:cortisol ratio. There was, however, a linear relationship between TBC and the progesterone + cortisol sum, such that a unit increase in TBC was accompanied by an approximate unit increase in the total concentration of the two main transcortin binding steroids. Some cases of spontaneous abortion or habitual abortion might be due to aberrant metabolic influence on progesterone of binding protein; in the instances studied, no evidence was found for this.

Introduction

For some years the behavior of steroids during pregnancy has been investigated. Until a short time ago, the use of steroids was advocated to monitor the well being of pregnancy. A realization of the imperfections of this technique accompanied by the introduction of physical ultrasound methods has led to a decline in its popularity in a number of laboratories. Nevertheless, there are many who believe that biochemical monitoring will continue to be an impor-
tant part of obstetric care for some time. Our investigations have led us to conclude that, in their present form, the measurements of plasma metabolites are of limited use in an unscreened population.\textsuperscript{1,4}

The possibility is intriguing that more clearly defining the events occurring in individual women will enable the data to be interpreted more usefully and increase the efficiency of biochemical screening of fetal well being. This report documents a stage of these continuing studies.

Progesterone, along with cortisol, is bound with high affinity by transcortin. The concentration of transcortin can be affected by pharmacological estrogens,\textsuperscript{8} but few attempts have been made to define the effect of physiological estrogen variations. This study determined some of the relationships between progesterone, cortisol, estradiol, and transcortin binding capacity (TBC) during normal pregnancy and during some pregnancies complicated by habitual or threatened abortion in which progesterone deficiency has been postulated. By examining the concentrations in individual women rather than the group-means, a number of details were able to be revealed. In the process, increases were observed during pregnancy of the mean concentrations of progesterone, cortisol, estradiol, and TBC. Since these have been recorded previously by us and others, they are reported only briefly here.

Methods

There were three groups of women in this study: (I) those women \((n = 32)\) whose obstetric history and current pregnancy were normal. They each provided three to nine blood samples between five and 40 weeks of pregnancy. (II) a group of women \((n = 10)\) who had two or more previous spontaneous abortions. (III) Women \((n = 8)\) who had threatened abortions and provided one to four samples each before 20 weeks gestation prior to carrying their pregnancies to term. This investigation was approved by the local ethical committee, and each patient gave informed consent before providing the samples.

Blood was taken between 0900 h and 1030 h by venepuncture into heparinised tubes, and the plasma was stored at \(-20^\circ\text{C}\). Progesterone, cortisol, estradiol, and TBC were measured by radioassays. Progesterone was measured as previously described.\textsuperscript{1} Plasma \((50\ to\ 100\ \mu\text{l})\) was extracted with redistilled ether; after evaporation, the residue was incubated with 0.3 ml of a solution of antiprogestrone serum and \((1,2,6,7\text{-}^3\text{H(N)})\text{progesterone}\* in 0.1 M borate buffer containing bovine serum albumin \((0.1\ g\ per\ 100\ ml)\). The antibody-bound and -free fractions were separated using dextran-coated charcoal (DCC). The interassay CV was 6.2 percent. Estradiol was measured similarly,\textsuperscript{3} except that 50 percent saturated ammonium sulphate was used to precipitate the antibody-bound fraction. The interassay CV was 9.3 percent. Cortisol was measured using Coat-a-Count solid phase RIA kits.\textsuperscript{\dagger} The interassay CV was 5.7 percent. Transcortin binding capacity was measured using a modification of the method of Moore et al.\textsuperscript{6} Plasma samples were divided into 100 \(\mu\text{l}\) aliquots and stripped of steroid with DCC. Half the aliquots of samples were heated to \(60^\circ\) for 30 min to denature the transcortin; a solution of \((1,2,6,7\text{-}^3\text{H(N)})\text{cortisol}\ (\text{NEN})\) together with cold carrier cortisol was added to all tubes at \(37^\circ\). Unbound steroid was adsorbed to DCC, and, after centrifugation, the supernatants were counted. The difference in the amount of cortisol bound in the tubes containing unheated
and heated plasma sample provided an index of transcortin binding capacity. The interassay CV was 7.4 percent.

**Results**

The mean concentration of all four measured entities increased in plasma during pregnancy. Between 10 and 40 weeks gestation, the mean progesterone concentration increased by more than four fold, cortisol by approximately two fold, estradiol by more than 10 fold, and TBC by approximately two fold.

The mean TBC increased approximately linearly from 10 weeks to 20 weeks, and then the curve flattened. The increase in TBC was related to that of estradiol such that up to an estradiol concentration of 30 nmol per liter there was a linear relationship with a very good correlation ($r = 0.72$) (figure 1). After that concentration of estradiol had been attained, there were no significant increases in TBC for increments in estradiol. Overall, higher estradiol concentrations generally corresponded to higher TBC values; of the samples which contained TBC at a level $>700$ nmol per liter, all except two had estradiol $>5$ nmol per liter. Conversely, if the sample contained estradiol at a concentration $>20$ nmol per liter, then TBC was $>700$ nmol per liter. In individual women for estradiol of similar concentrations (50 to 70 nmol per liter), TBC could vary two fold (700 to 1560 nmol per liter). Although the average trend was for a lower TBC:estradiol ratio at estradiol $>30$ nmol per liter, there was no upper concentration limit of estradiol universal for all women beyond which estradiol did not induce further increases in TBC.

The relationship between TBC and binding steroids was investigated. There was a linear relationship ($F = 62.1$) between progesterone and TBC in the samples. The mean ratio of progesterone:TBC in individual samples was $0.25 \pm 0.11$ (mean $\pm$ SD); between cortisol and TBC ($F = 230.2$), the mean cortisol:TBC ratio being $0.91 \pm 0.21$. There was also a linear relationship between progesterone and cortisol ($F = 39.2$), the mean progesterone:cortisol ratio was $0.28 \pm 0.14$. In contrast, the ratios of progesterone:TBC and cortisol:TBC showed no systematic trend during the period, and the relationship between TBC and the progesterone:cortisol ratio in samples was random ($F = 1.5$). Thus, as TBC increased, there was not a trend in the women for progesterone and cortisol to increase in a constant ratio.

On the other hand, TBC and the total ligand concentration (progesterone + cortisol) had a linear relationship ($F = 27.1$). The (progesterone + cortisol):TBC slope was 1.10, implying that a unit increase in TBC was associated with an approximate unit increase in the sum of progesterone and cortisol; the results were consistent with a metabolic relationship between the binding protein and the ligands. It has been sug-

![Figure 1](image-url) **Figure 1.** The relationship between estradiol concentration and transcortin binding capacity in women with normal pregnancies. The closed circles are the interval means ($\pm$ SE) and the solid line is the regression curve calculated using all samples with estradiol concentration up to 30 nmol per liter.
gested that high progesterone concentrations found in pregnancy would dynamically displace some of the expected amount of cortisol from transcortin. Such a relationship between transcortin and the sum (progesterone + cortisol) should appear in the form of a negative correlation in the residuals of TBC versus progesterone against residuals of TBC versus cortisol. No such negative correlation was observed, the result having implications for the explanation of certain Cushingoid symptoms.

A group of women with a history of recurrent abortions, but whose currently studied pregnancy continued to term, was investigated. Their levels of progesterone, cortisol, estradiol, and TBC were within the normal range. The progesterone:TBC and cortisol:TBC ratios were 0.22 ± 0.14 and 0.77 ± 0.21. There were no detected significant differences in the interactions between steroids and binding protein in the pregnancies studied (t-test). A group of women with threatened abortions and whose pregnancies subsequently continued to term were also investigated. Samples were taken before 20 weeks gestation. The ratios in these women were progesterone:TBC, 0.17 ± 0.04, and cortisol:TBC, 0.96 ± 0.47. There were again no apparent differences from the women with normal pregnancies (t-test). Two women did not carry their pregnancies to term after threatened abortion; they had lower progesterone and estradiol than normal, consistent with previous observations.3

Discussion

This study compared concomitant variations in four components of an endocrinological system during pregnancy. The variations observed were due to physiological adjustments rather than pharmacological manipulations.

The results were consistent with suggestions that increases in TBC above non-pregnancy levels are caused by estrogens.11 This investigation revealed that different women either had different sensitivity to estradiol or had different TBC-producing capacities. There were also different lag phases and threshold concentrations of estradiol necessary to produce a rise in TBC. Nevertheless, all women showed a similar relationship between estradiol and TBC. The TBC rose linearly as estradiol increased to 30 nmol per liter. Above a certain concentration of estradiol, the TBC secretory system appeared unable to respond proportionately. The study thus provided information of the response to physiological estrogenic supply in contrast to previous studies where pharmacological estrogen doses of uncertain physiological relevance have been employed.

The results suggested there are limitations to the proposal that TBC response invariably reflects the estrogenic potency of an administered agent.5,7,11 Although the slope of the TBC:estradiol graph was linear after the required threshold of estradiol had been reached and there was a good linear correlation between TBC and estradiol, there was a range between women. Also agents with high estrogenic potency might not, without dilution or prior evidence, be distinguished from those of medium potency because of the refractoriness of the response at high estrogen activity.

It has been suggested that the maximum concentration of TBC is reached after 14 days of estrogen.8 The reduction in the relative responsiveness at higher estradiol suggested that the actual maximum of estradiol which will induce further increases in TBC is in fact that level of estradiol attained several days earlier than the apparent limit.

It was observed by the present authors that the (progesterone + cortisol):TBC slope was approximately one during the
period investigated, indirectly revealing (by the law of mass action) that there is an increase in the amount of steroid not bound to protein later in pregnancy. This implies altered feedback controls if free hormone is the biologically active entity.

The study produced data on interacting processes, involving maternal liver (TBC), the maternal adrenal (cortisol), and two pathways in the placenta (estradiol and progesterone). The endocrinological responsiveness to the changing milieu of pregnancy was complemented by the inherent physiologic buffering which the results revealed in the multiproductive system, e.g., a bounded response of TBC to estradiol, and transcortin-cortisol and -progesterone equilibria not being mutually perturbed. Such buffering must be considered during evaluation of tests when clinically monitoring pregnancies.

Women with threatened abortion or a history of recurrent abortions were examined to investigate the possibility that some pregnancy loss might be due to inadequate control of progesterone by binding protein. However, no evidence could be found for such a syndrome in the patients studied, and offspring who have been born to mothers with less than normal transcortin levels\(^2\,5\,10\) were normal.

Further definition of the relationships that reflect the endocrinology of pregnancy will assist in understanding the basic processes involved and in enhancing methods of clinical assessments.

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References