compound nor can it be used as a substitute for chromatography to isolate specific steroids.

In summary, this is rapid (20 min) and practical method for the economical repurification of radiolabeled steroids. It can be applied to both triitated and iodinated steroids. Results compare favorably with those after various chromatographic methods that require more manipulations, more personnel time, and expensive equipment.

References

Salivary Estriol Concentrations during Normal Pregnancies, and a Comparison with Plasma Estriol

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Saliva would have advantages over plasma or urine for monitoring estriol during pregnancy. Specimen collection, after stimulation of flow by citric acid, is non-invasive and simple. We measured concentrations of unconjugated estriol in saliva and compared them with those in plasma in normal pregnancies, and found a good correlation ($r = 0.79$). In addition, trends of concentrations in saliva and plasma were statistically compared and found to be highly correlated. The variation among individuals in the saliva/plasma concentration ratio suggested that some inter-individual factor(s) may affect this relationship. The normal reference interval for unconjugated estriol concentration in saliva from 20 weeks of gestation to term was established.

Additional Keyphrases: reference interval, concentration ratio, saliva/plasma, fetal status

The measurement of plasma estriol during pregnancy is widely used for monitoring fetal well being. However, urine collections for this purpose are inconvenient for the woman and are not always reliable and accurate, and venepunctures for blood assays require clinic visits and can be stressful. On the other hand, collection of saliva is non-invasive and can even be done at home. We have previously shown in a small number of women that the concentration of unconjugated estriol increases in saliva as pregnancy progresses (1). Here, we extend those findings and compare estriol concentrations in saliva and plasma.

Subjects and Methods

We studied 50 women who attended the antenatal clinic at Christchurch Women's Hospital before 25 weeks of gestation (as estimated from the last menstrual period and usually confirmed with at least one measurement of fetal biparietal diameter). The women had clinically normal pregnancies. We collected into plain glass tubes about 2 mL of saliva, stimulated by placing on the tongue a small square of dry filter paper that had been soaked in citric acid. This technique has been previously demonstrated not to affect the concentration of estriol measured, as compared with that in unstimulated specimens (1). After centrifuging the saliva to remove sedimentable material, the supernate was decanted and stored at $-20$ °C. Ordinarily, we collected plasma and saliva together between 0700h and 1000h; occasionally this was not possible, but the correlation between concentrations of estriol in plasma and saliva appeared not to be altered by the increased time intervals.

Estriol in saliva was measured by RIA as previously described (1), which briefly is as follows. An ether extract of saliva was evaporated, the residue was redissolved in borate buffer, and two aliquots were assayed by mixing with a solution of antiserum [raised against estriol-6-(O-carboxymethyl)oxime-bovine serum albumin; Steraloids Inc., Wilton, NH 03086] and triitated estriol in borate buffer containing bovine serum albumin. Antibody-bound and free fractions were separated by precipitation with saturated ammonium sulfate. Plasma samples were assayed similarly (2), except that the volume of ether phase evaporated after extraction was less. Standard curves of appropriate ranges were constructed.

Results

We established the range of salivary estriol concentrations for each week of gestation throughout pregnancy (for weeks 20 through 40) and calculated the mean concentrations (Figure 1).

Comparing the corresponding concentrations of estriol in saliva and plasma, we found good correlation ($r = 0.79$) between 320 pairs of saliva and plasma specimens from 50 women. For 18 women with $r >0.90$ who had five or more paired samples collected during their pregnancies, the plasma to saliva concentration ratio ranged from 6.1 to 14.0. Therefore, the ratio of estriol concentrations was often relatively constant for a given woman, but was not constant from woman to woman. The range of correlation coefficients for 39 women with five or more paired samples was 0.99–0.38 (mean 0.81); there were only three women for whom we observed poor ($r <0.50$) correlations.

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Examining the changes in estriol concentration during pregnancy, we noted a general similarity of trends for estriol in saliva and plasma. Results from four women are illustrated in Figure 2. To compare statistically the trends from 31–32 weeks to 35–36 weeks, we collected paired specimens from 32 women at both dates, and each result was placed relative to the median at the appropriate interval of gestation. That is, trends in saliva and plasma concentration were determined as being above the median at 31–32 weeks to above the median at 35–36 weeks, or above to below, or below to below. The similarity of trends of concentrations in the plasma and saliva samples was highly statistically significant \( p < 0.001 \), chi-square test.

**Discussion**

In this study we have established the normal reference interval for concentrations of unconjugated estriol in saliva during pregnancy and have shown the distribution of values to be generally similar to that for plasma. An asymmetrical distribution about the mean was observed. The ranges at each gestation interval appeared similar to those in other recently published studies (3–5). Other investigators have reported concentrations of less well-defined estrogen fractions, in which estrogens other than estriol reacted in the RIA method, or in which estriol conjugates contributed an undefined fraction of the total (6–8).

The high correlation between the estriol concentration in saliva and that in a paired plasma sample is consistent with the concept that salivary steroid concentrations largely reflect those in plasma. Plasma and saliva samples in separate individuals often were very highly correlated; however, because of the wide range of plasma/saliva ratios, one cannot accurately predict estriol concentrations in saliva from those in plasma. The relationship between estriol concentrations in saliva and plasma in different individuals may depend upon unknown variables. This finding, derived from studying individuals for extended periods during their pregnancies, has important implications if salivary estriol assay is applied uncritically to clinical assessments. Additionally, on a few occasions we saw a very poor correlation between concentrations in saliva and plasma.

Although absolute concentrations differed by about 10-fold, the trends of concentrations of estriol in saliva and plasma were similar. Therefore clinical information similar to that obtained from plasma trends would be expected from saliva. In this study, we used a novel, convenient method for objectively comparing trends (as well as absolute concentrations) of estriol in saliva and plasma.

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**References**