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PLASMA STEROID ASSAY IN THE ASSESSMENT OF FOETOPLACENTAL FUNCTION

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SUMMARY

The significance of blood concentration as opposed to urinary output of steroid hormones in pregnancy is examined. The nature of the normal pregnancy curve for such steroids as oestriol, oestradiol and progesterone is considered. The spread of values from one patient to another is reported and the findings on the variability from time to time in the same subject. It is concluded that total plasma oestriol (conjugated plus unconjugated steroid) is the entity most likely to give insight into the fetal state in obstetric disease. The changes in plasma oestriol encountered in retarded fetal growth and in pre-eclamptic toxaemia are described.

Much of scientific progress comes from advances in methodology. Few advances in methodology have wrought more change in endocrinology than the introduction of radioimmunoassay. Many institutions are now changing from measurements in urine to measurements in blood. It is the purpose of this investigation to examine some of the changes in concept which need to accompany the change from urine to blood.

For plasma assays must not be thought of as a technologically sophisticated version of a urinary assay. The information conveyed by a plasma assay may be of the same nature as that carried by the 24 h urinary output but the elements which go to compose it are quite different. In both instances we are trying to reason back to the amount of steroid being produced by the fetoplacental unit. In the case of plasma assays all we have is the value of the steroid *concentration*; a figure which owes as much to the size of the compartment in which the steroid is contained as to the rate at which it is being produced. Women at the same stage of gestation and carrying fetuses and placentae of similar size have very different plasma concentrations of steroids such as oestriol or progesterone, a finding which could in part be accounted for by differences in plasma volume. Nor is plasma the only maternal compartment in which the steroids are contained. They move into the interstitial fluid and a proportion is held within the cells [1]. The bile is yet another steroid containing compartment [2]. If there were free movement of steroid molecules between these compartments it would be legitimate to regard changes in plasma concentration from time to time in the same subject as representing changes in the rate of steroid production by the fetoplacental unit and the wide differences in plasma concentration between similar subjects as being in part due to differences in the total size of the containing compartments. However, different forms of the same steroid move between the plasma and the interstitial fluid at different rates and once within the enterohepatic circulation steroids move back to the plasma in an irregular and impeded fashion by reabsorption from the gut. Clearly the rate of production by the fetoplacental unit is not the only factor determining the plasma concentrations of a steroid.

There is a further element of complexity in the nature of a plasma steroid concentration. Urine is at the end of the line, there is nowhere that a steroid can go once it is in the urine. But in plasma steroids are subject to ebb and flow and the rate of outflow from the plasma is just as important as the rate of inflow in determining the plasma concentration. Besides movement into other compartments there are several outflow routes from the plasma for steroids. They can be removed by further metabolism as in the case of progesterone, or by renal excretion as with oestriol glucosiduronate or by excretion in faeces as with pregnanediol [3]. Urinary assays give a summary of the events of the previous 24 h, plasma assays indicate only a momentary point of balance between a number of forces.

Given the very different nature of urinary and of plasma assays it is surprising that there should be any correspondence between the two. For conjugated forms of oestriol the correlation between the plasma concentration and the 24 h urinary output is 0·68 [4] while for unconjugated oestriol it is 0·59 [5]. This is not a close correlation and it cannot be expected that in an individual high or low plasma values would very frequently be found when the urinary output is high or low. But the fact that there is a correlation augurs well for the suggestion that these measurements reflect a common factor, the rate of steroid production by the fetoplacental unit.

Plasma steroid assays are of most immediate clinical interest in late pregnancy and it is on this stage of gestation that we propose to concentrate attention. In our view three general considerations determine the clinical significance of plasma steroid assays in late pregnancy. The first is the trend of values with

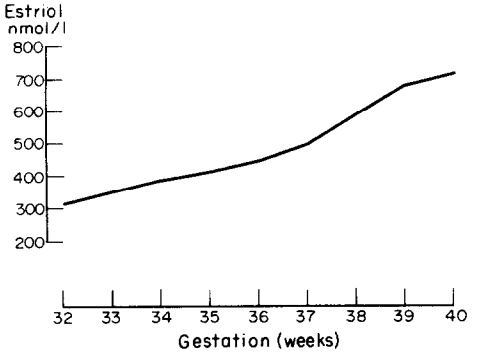

Fig. 1. The mean curve for total plasma oestriol during pregnancy.

advancing gestation, ie the shape of the mean curve. The second is the variability from one subject to another and the third is the variability of the value from time to time in the same subject. We have applied these criteria to the steroid measurements of normal volunteers at 38 weeks gestation. Total plasma oestriol (conjugated plus unconjugated) was measured by the method of Wilson[6] as was the unconjugated plasma oestriol. Plasma oestradiol-17β (unconjugated) was measured by the method of Hotchkiss[7] and plasma progesterone by the method of Johansson[8].

The mean total oestriol during pregnancy is shown in Fig. 1. The shape of the curve for unconjugated oestriol is essentially the same although the actual values are perhaps one tenth of the total oestriol. The important feature of this curve is the increase in the slope during late pregnancy. The mean urinary oestriol curve shows the same sharply upward inclination, and it has been surmised that this reflects a new, specifically fetal, element in oestriol biogenesis [9]. Whatever the reason, this upward trend in plasma oestriol over the last 6 weeks of pregnancy bears strongly on the clinical significance of the measurement. Although of course not every individual shows a consistent increase in plasma oestriol concentration in late pregnancy, a flattening of the curve in late pregnancy may be the only indication that oestriol biogenesis is not proceeding normally. Plasma oestradiol has much more of a tendency to flatten off over the last few weeks of pregnancy as can be seen in Fig. 2. Many normal subjects show no rise in the last few weeks and by virtue of this less significance can be attached to a flattening of the oestradiol curve in late pregnancy.

The shape of the mean progesterone curve in late pregnancy is intermediate between that of oestriol which rises sharply and oestradiol which tends to flatten out. The findings are shown in Fig. 3. The upward slope of plasma oestriol in late pregnancy adds a diagnostic dimension to the measurement of this steroid which is present to a lesser extent in progesterone measurements and not at all in oestradiol measurements. Because they are set against a more level normal curve it is more difficult to distinguish casual non-significant falls in progesterone or oestradiol from a pathological decline in the production of the steroid. In terms, therefore, of the shape of the normal curve, plasma oestriol measurements are preferable for clinical purposes to those of progesterone and of oestradiol.

The spread of values from one patient to another is much the same for all the steroids we have been considering. The results for plasma assays show a slightly wider scatter than for the subject to subject variation of 24 h urinary oestriol output which, over a large series of patients, was found to have a coefficient of variation of 28% [5]. As compared with this the subject-to-subject variation of a number of plasma steroids ranged from 31 to 37% (Table 1). Tests at other stages of gestation show an equally wide scatter from one normal subject to another. There is likely to be a considerable overlap between the normal and the pathological universe in any of the steroids examined. In view of this the diagnostic value of any single assay is limited. A detailed analysis of the characteristics of the test subjects in respect of features such as parity, maternal size, birthweight of the fetus etc. failed to show any association with steroid concentration. In terms of subject-to-subject variation there is nothing to choose between the steroids examined.

Note has already been made of the snapshot quality of a plasma steroid assay. The value applies only to that moment in time when the blood was drawn. It could be that the plasma concentration of a steroid

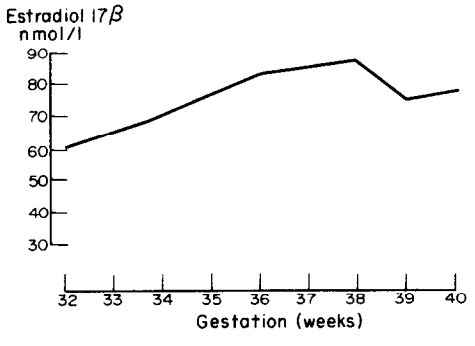

Fig. 2. The mean curve for unconjugated plasma oestradiol during pregnancy.

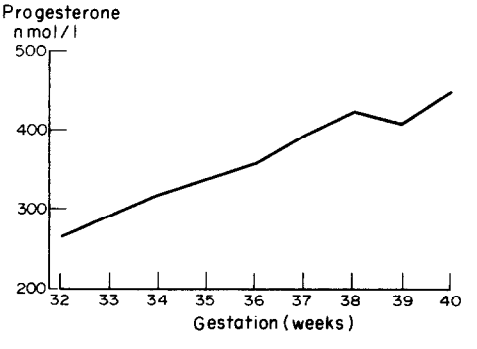

Fig. 3. The mean curve for plasma progesterone during pregnancy.

Table 1. Subject to subject variation in plasma and urinary steroids at 38 weeks gestation

Assay	Coefficient of variation (%)
24 h urinary oestriol excretion	28
Total plasma oestriol concentration	34
Unconjugated plasma oestriol concentration	32
Plasma oestradiol concentration	37
Plasma progesterone concentration	31

might change greatly from minute to minute. Of course the most important variation from the clinical point of view is the variation from day to day in the same subject under standard conditions. The application to the assessment of fetoplacental function must stand or fall by this. The subject-to-subject variability is so large that only the trend from day to day or week to week can indicate what is happening to fetoplacental function in an individual. Masson and Wilson[10] measured the total plasma oestriol in ten women at 38 weeks pregnancy daily for 6 days. They then calculated the coefficient of variation for each patient about the mean level over the 6 days. The overall mean coefficient of variation of all ten patients was 13·2%. In a later study [4] the coefficient of variation of unconjugated plasma oestriol was found to be 30·9% from day to day. The much higher variability of unconjugated oestriol is not surprising. Total oestriol is 90% conjugated steroid and the outflow for this is by renal excretion. The outflow mechanism for unconjugated oestriol is by further metabolism (conjugation) in the liver. It is likely that irregularities in the rate of metabolism of unconjugated oestriol contribute to the high variability of its plasma concentration.

The variability of plasma oestradiol was studied in similar fashion by Chan and Klopper[11]. They did assays on five patients for five successive days and found the average coefficient of variation of conjugated oestradiol to be 7·9% and of the unconjugated steroid 7·2%. The ratio of unconjugated to conjugated oestradiol is much smaller in plasma than in urine and further metabolism of unconjugated oestradiol must, like unconjugated oestriol, be an important outflow mechanism for this steroid. The fact that unconjugated oestradiol is a great deal more stable than unconjugated oestriol may be due to the fact that quite different enzyme systems are involved in the further metabolism of oestradiol. From the point of view of variability there is nothing to choose between unconjugated or conjugated oestradiol and as measurements of the unconjugated moiety are simpler this would appear to be the assay of choice.

The day to day variability of plasma progesterone was studied in ten patients over 6 days. The mean coefficient of variation was found to be 18·9%, about twice the variability of oestradiol. The day to day variability is summarised in Table 2.

It can be seen that plasma oestradiol and total plasma oestriol (largely conjugated) are stable entities while unconjugated oestriol and progesterone vary greatly from day to day without apparent cause. By the criterion of variability from time to time plasma oestradiol or total plasma oestriol assays are most likely to be useful in the assessment of fetoplacental function.

A further element which may contribute to the variability of these plasma steroids is the possibility that there may be a diurnal variation in the production of steroids by the fetoplacental unit, just as there is a diurnal variation in the secretion of cortisol by the adrenal. In the experiments we have described, the blood was always drawn at the same time in the morning and diurnal variation would not have an effect. In any event the possibility of diurnal variation in steroid production by the fetoplacental unit is remote. It was indeed claimed by Selinger and Levitz[12] that there was a diurnal variation in total plasma oestriol. This was contradicted by Masson and Wilson[10] and the claim was subsequently withdrawn [13]. There is no need for strict standardization of time of intravenous sampling in using plasma steroid assays for the assessment of placental function.

The use of plasma steroid assays as a means of assessing fetal wellbeing depends on the assumption that some types of obstetric disease will affect ster-

Table 2. Day-to-day variation in the plasma concentration of various steroids at 37–39 weeks gestation

Steroid	Coefficient of variation (%)
Total plasma oestriol	13·2
Unconjugated plasma oestriol	30·9
Unconjugated plasma oestradiol	7·2
Conjugated plasma oestradiol	7·9
Progesterone	18·9

oidogenesis in the fetoplacental unit. As far as the urinary excretion of steroids such as oestriol is concerned, this contention is well supported by experimental findings. Whether a similar decline in plasma steroid concentration takes place in obstetric disease has not been so clearly demonstrated. It is unlikely that all kinds of obstetric pathology would affect steroidogenesis equally. It is a matter of some importance that the change in plasma steroid levels should not be examined in the context of some vague concept like "placental insufficiency" but that the findings should be examined separately in defined disease entities.

We have examined total plasma oestriol concentration at 38 weeks gestation in a group of patients suffering from pre-eclamptic toxaemia. Ten of these were patients presenting with hypertension (blood pressure above 140/90) in late pregnancy; a further 20 patients had albuminuria as well as hypertension. The findings are shown in Fig. 4.

The plasma oestriol concentration is lowered in pre-eclamptic toxaemia, being roughly half that of normal pregnancy. There is no significant difference between the group with hypertension only and those who had albuminuria as well as hypertension. Somewhat surprisingly the group with albuminuria, although presumably the more severe form of the disease, had an average total plasma concentration very slightly higher than those with hypertension only. It is possible that the significant factor is not whether the patient had albuminuria but whether she was carrying a growth retarded fetus, unable to fulfil its role in steroidogenesis. The pre-eclamptic patients were therefore categorized in terms of whether or not they gave birth to a growth retarded fetus (in the lowest 10th percentile of weight for gestation).

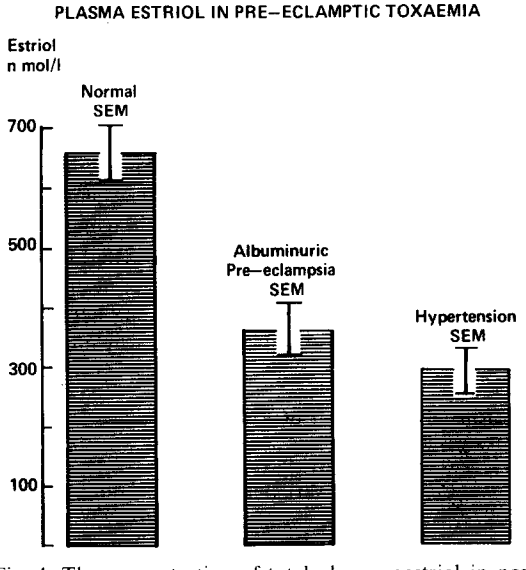

Fig. 4. The concentration of total plasma oestriol in normal pregnancy at 38 weeks gestation (mean and standard error of the mean) compared with the concentration of total plasma oestriol in pre-eclamptic pregnancy with albuminuria and pre-eclamptic pregnancy without albuminuria.

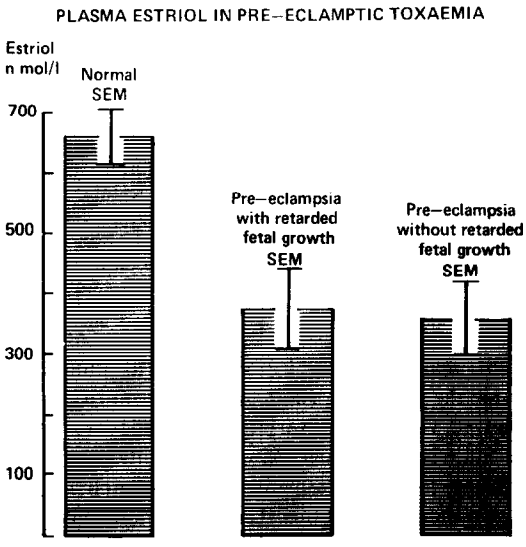

Fig. 5. The concentration of total plasma oestriol in normal pregnancy at 38 weeks gestation (mean and standard error of the mean) compared with the concentration of total plasma oestriol in pre-eclamptic pregnancy with fetal growth retardation and without fetal growth retardation.

The results are shown in Fig. 5. Although in pre-eclampsia the plasma oestriol is lowered there is no significant difference between the levels in those pre-eclamptic patients giving birth to a growth retarded baby and those who had a baby of normal weight.

Many clinicians believe that uterine blood flow is diminished in pre-eclamptic toxaemia. It is possible that in pre-eclampsia uterine blood flow may vary from time to time more than in normal pregnancy. If uterine blood flow affects placental steroidogenesis, as well it might, the hormone output from the placenta may be more variable in pre-eclampsia than in normal pregnancy. To test this we took blood samples at 2 h intervals over 24 h from a patient with pre-eclampsia and a similar set of samples from a normal subject at the same stage of gestation. The variability of a number of hormones is shown in Fig. 6.

Without exception the day to day variability of hormones was higher in pre-eclampsia. The highest variability occurred in the conjugated oestriol; the most promising fraction from the point of view of clinical control and one of the most stable ones in normal pregnancy. This high variability of oestriol in pre-eclampsia may not be entirely a disadvantage if the swings reflect clinical changes and indeed there is some evidence that the plasma oestriol concentration goes up and down in concert with the blood pressure [14].

Intra-uterine growth retardation is a condition which challenges clinical acumen and is a disease in which the obstetrician can use every bit of help the laboratory can give. Because the fetus itself is involved in the biogenesis of oestriol it is possible that growth retardation has particularly marked effects on the biogenesis of this hormone. We have

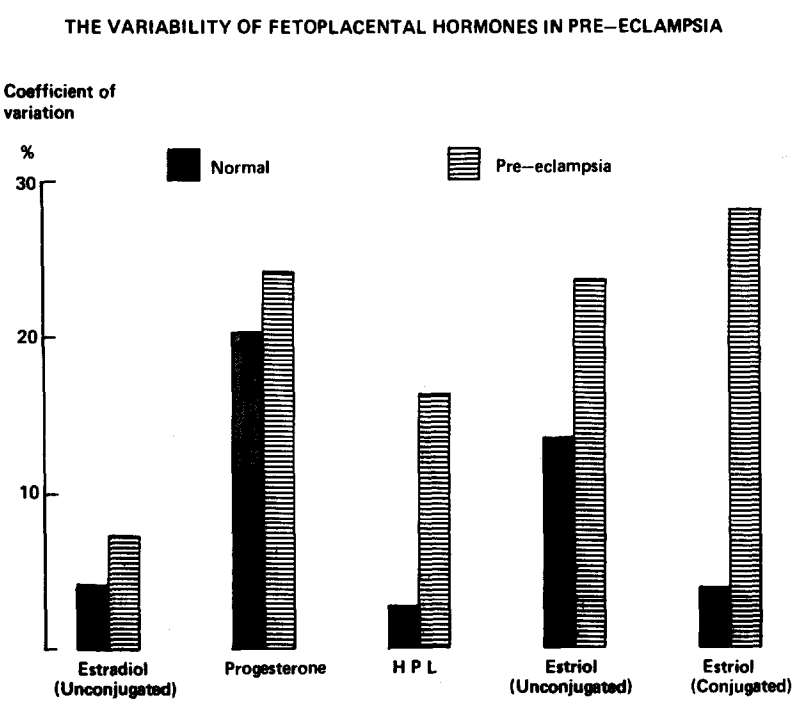

Fig. 6. Variability at 2 h intervals over 24 h in the plasma concentration of various hormones in a normal subject and in a patient with pre-eclampsia.

measured total plasma oestriol in 32 patients who subsequently gave birth to a growth retarded baby. The results are shown in Fig. 7. It can be seen that plasma oestriol concentration is significantly lower in women who are carrying a growth retarded fetus. Fetal growth retardation is not a homogenous condition with a single etiology. It is not possible to categorize to any extent the different varieties of fetal growth retardation, but it is possible to distinguish two broad categories, patients with hypertension in whom the growth retardation could be the consequence of reduced uterine blood flow, and patients without hypertension in whom a genetic factor might be operative. We therefore divided our growth retarded group into those who had hypertension and those who did not. The results are shown in Fig. 8. Clearly the operative factor is the presence of growth retardation, irrespective of the blood pressure.

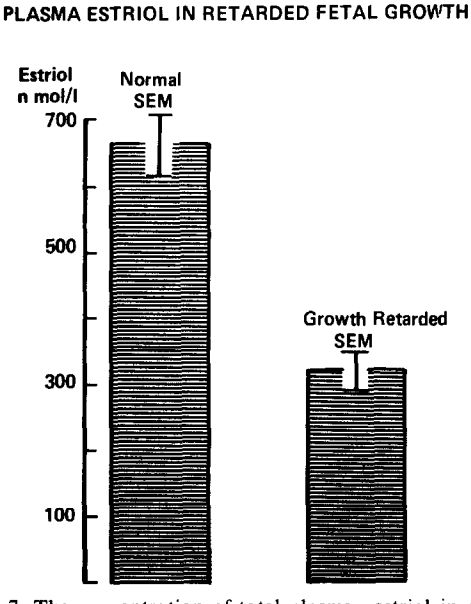

Fig. 7. The concentration of total plasma oestriol in normal pregnancy at 38 weeks (mean and standard error of the mean) compared with the concentration of total plasma oestriol in patients carrying a growth retarded fetus.

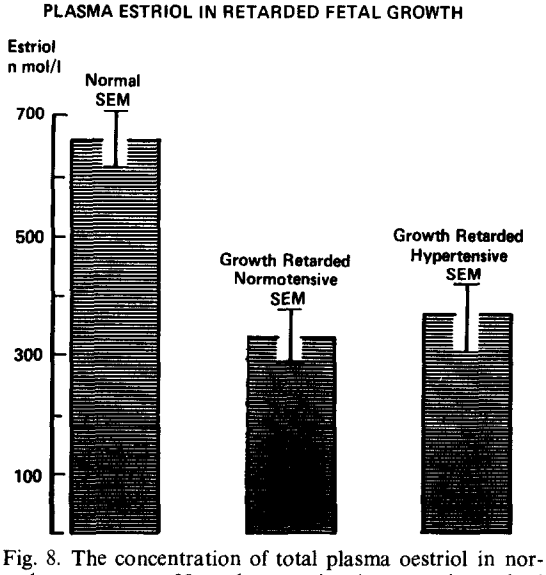

Fig. 8. The concentration of total plasma oestriol in normal pregnancy at 38 weeks gestation (mean and standard error of the mean) compared with the concentration of total plasma oestriol in normotensive patients with a growth retarded fetus and hypertensive patients with a growth retarded fetus.

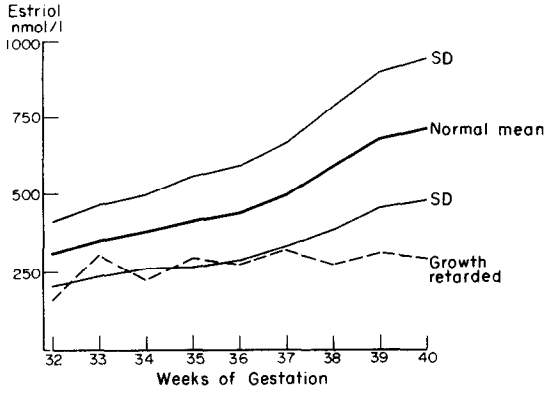

Fig. 9. The mean and standard deviation of total plasma oestriol concentration in late pregnancy compared with the weekly mean values in patients carrying a growth retarded fetus.

These studies have established nothing more than the fact that in diseases such as pre-eclampsia or retarded fetal growth the plasma oestriol tends to be lower than normal. We have dealt with group means. Inevitably they conceal a good deal of overlap in individuals. In particular we have not considered how far the trend over time in a particular obstetric disease may differ from the normal. Figure 9 shows the running mean of our growth retarded group contrasted with the normal universe. The characteristic of the growth retarded group is that the plasma oestriol does not rise in late pregnancy as it does in the normal.

None of this data bears upon a question of particular importance to the obstetrician. This is the problem of day to day management. Of course a sustained drop of more than 30% in any of these steroids indicates a real change in the plasma concentration. But how far does that necessarily mean a decline in steroid biogenesis by the fetoplacental unit? And how closely connected is a decline in steroid biogenesis with deterioration in the vital functions of the fetus? Selected anecdotal cases prove very little except the beliefs of the selector. When we know the function in pregnancy of any of these steroid hormones we shall have a maternal or fetal parameter to set against the steroid assay.

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