Journal of Steroid Biochemistry, Vol. 11, pp. 509 to 513
Pergamon Press Ltd 1979. Printed in Great Britain

CORTISOL AND CORTISONE IN HUMAN FETAL DEVELOPMENT

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SUMMARY

Although in adult life cortisone (E) circulates at only about 1/5 the concentration of cortisol (F), the situation is reversed in fetal life where the amount of E exceeds that of F. This is due to the high rate of metabolism of F to E by the placenta and other fetal tissues and the relative inability of the fetus to reduce E to F. Thus although the fetal adrenal produces F as early as 8 weeks of life, much of it is converted to E and secreted as such. This state appears to continue until late in pregnancy. An exception is the chorionic membrane which, like the maternal uterus, reduces E to F during the second half of gestation, thereby increasing amniotic fluid F. At about 35 weeks gestation, there is an alteration in fetal adrenal function as reflected by a 5-fold rise in amniotic fluid cortisol sulfate which parallels rising surfactant concentration and lung maturation. This change may be brought about at least in part by a dialyzable, heat-stable 11β-dehydrogenase inhibitor which is present in umbilical cord serum at delivery. It seems likely that conversion of F to E serves to maintain a low cortisol level throughout gestation until cortisol is required for maturational events to occur close to term.

Although it has been recognized for many years that cortisone (E) is a prominent steroid in the human placenta [1] and in fetal plasma [2] and amniotic fluid [3], this remains a puzzling observation. In the adult cortisol (F) and E are rapidly interconverted and as a therapeutic agent we more often prescribe E than F. However, so far as known, E itself is devoid of biological activity and depends for its therapeutic effectiveness on the enzyme, 11β-hydroxysteroid dehydrogenase which converts it to F. This reaction is therefore of great potential importance in the fetus where the concentration of E exceeds that of F.

In the adult the amount of F in serum is 5–10 times greater than that of E. As shown in Fig. 1, the ratio in the placenta is reversed so that F/E is only about 1/3 despite its large content of maternal blood. If the placenta is quickly put into liquid nitrogen before processing, much more cortisol is seen. This is because even at room temperature the placenta converts F to E so rapidly that the action proceeds while one is preparing the material for assay. The placenta is able to convert about 300 ng/g/min at term. The activity is largely destroyed by freezing or boiling

In cord blood, as Bro-Rasmussen *et al.*[2] observed as long ago as 1961, the ratio at delivery is about 1/2. We have found the concentration of F in the umbilical artery to be significantly higher ($P < 0.001$) than that in the vein after normal vaginal delivery with epidural anaesthesis indicating that the bulk of the cortisol is coming from the fetus rather than from the mother [4]. The highest F/E ratios at delivery were seen after vaginal delivery following labour of spontaneous onset while low values were seen after vaginal delivery following induction by oxytocin, at Caesarean section without labour and at spontaneous premature deliveries where the infants went on to develop the respiratory distress syndrome (RDS) [5, 6].

In the fetal adrenal [7], the F concentration is much higher than in cord serum but again there is a considerable amount of E, suggesting that both are produced there. If one incubates the fetal adrenal at mid-pregnancy with ACTH and progesterone, this production can be readily shown. In other fetal body tissues such as lung and kidney the F/E ratio is even lower than it is in serum [8].

In the amniotic fluid, however, the ratio rises at about 18 weeks and by term the F/E ratio exceeds 1.0. We found this intriguing and set out to find out why. We studied the *in vitro* conversion of a number of fetal tissues (Fig. 1) at 18–20 weeks but still found conversion of F to E but not the reverse. Finally, we looked at the amniotic membrane, which along with the placenta constitutes the sac containing the amniotic fluid. This membrane is made up of two layers which can be readily separated. The inner, very thin and transparent one is the amnion, and the outer, thicker one the chorion laeve. We found that the 11-oxidoreductive activity altered from oxidative (F to E) to reductive (E to F) in the chorion laeve at about 18 weeks and continued to be predominantly reductive until term [8] (Fig. 2). This was a most unexpected finding since the immediately adjacent placental tissue (chorion frondosum) is so strongly oxidative. Why should this one fetal tissue be different from all the others? Since the chorion laeve is in direct contact with the maternal uterus we looked there also. The decidua or inner lining was also strongly

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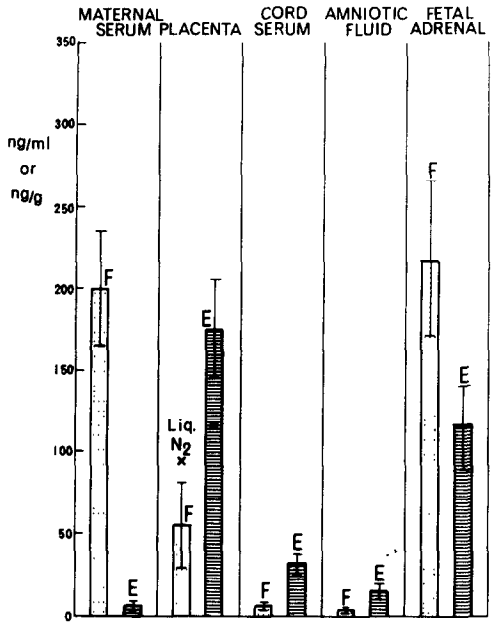

Fig. 1. Levels of cortisol (F) and cortisone (E) at 10–20 weeks. x indicates values when tissues were placed quickly into liquid nitrogen.

reductive while the underlying myometrium was weakly so. These observations led us to go back and look at the non-pregnant uterus which we found to be moderately strongly oxidative. When concentrations of F and E in the uterus were determined [9] it was found that the F/E ratio rose with pregnancy from 1 to 9, presumably due to the changing oxidoreductive activity which parallels that of the chorion laeve. The cortisol concentration of the uterine wall also rises 9-fold, a rise exceeding that of the serum where F rises 3-fold while the F/E ratio does not change appreciably [9].

Do these *in vitro* changes reflect the *in vivo* ones? The best evidence that they do is provided in a study by Pasqualini *et al.*[10] in 1970 who investigated the fate of labelled F injected into the umbilical vein just before hysterotomy at about 18 weeks. They showed that F was largely converted to E in all tissues except the liver. The only evidence to the contrary is that of Smith *et al.*[11] who, using tissue culture, found reductive activity but little or no oxidative activity in lung cells. This difference is likely due to the factors in the medium which included fetal calf serum.

In the "placenta" Pasqualini *et al.* found both reductive and oxidative activity but this was to be expected since the membranes were not removed.

Using our *in vitro* technique we have studied many tissues in order to gain some idea of the ontogeny of this reaction and of its overall significance (Fig. 3). It appears that while many tissues convert F to E in early fetal life this activity is not seen at the time of birth although it resumes to some extent in some tissues a few days later. In the case of the lung (Fig. 4) slight reductive activity was seen in infants [12]. The liver became strongly reductive over the first few months. The kidney and skin resumed their oxidative activity. These are summarized in Fig. 5.

What factors control oxidoreduction in these tissues? We know from the work of Osinski [13] in 1960 that in placenta the reaction can be pushed in either direction by cofactors and that the specificity is not

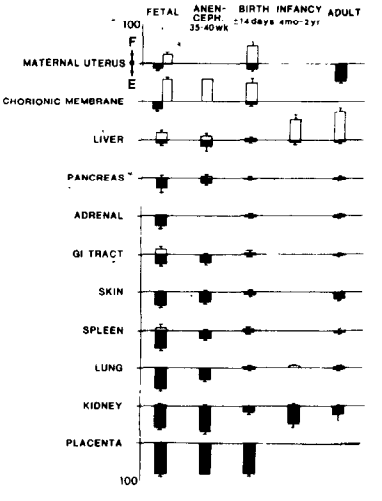

Fig. 3. Conversion of F $\rightleftharpoons$ E in fetal tissues and maternal uterus. Downward solid bars indicate conversion of F to E, upward open bars the conversion of E to F as %. (100% = width between bars).

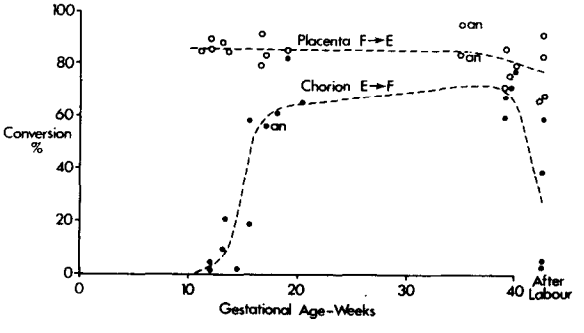

Fig. 2. F–E interconversion in placenta and chorion laeve. an = anencephalic. The dotted lines show mean trends. Reproduced from Ref. 8.

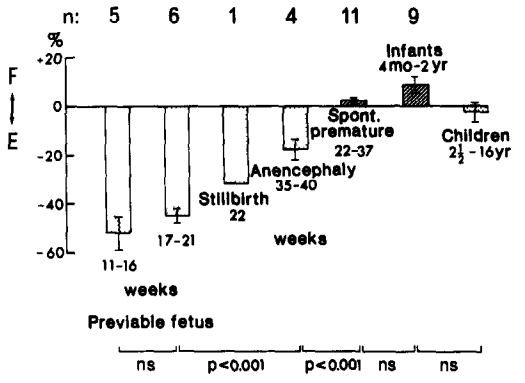

Fig. 4. Net F–E interconversion in human fetal lung. Net conversion to E is plotted downwards, that to F is plotted upwards. Reproduced from Ref. 12.

confined to F–E. Using the placenta as a test system, we looked at the effects of serum factors on the reaction (Fig. 6). Unlabelled F itself of course competes with labelled F to inhibit its conversion but this is relatively slight for endogenous concentrations, even those in maternal serum, and cannot account for the much greater inhibition seen with both maternal and cord serum. Large amounts of E had no effect so that product inhibition does not appear to be important.

Maternal serum greatly reduced the oxidative activity but did not increase the reductive activity. Its effectiveness was much decreased by heating to 60°C for 30 min, a procedure which denatures transcortin. Since the inhibitory factor was not dialyzable, it presumably is transcortin. This was further supported by the observation that even greater inhibition was seen using male serum from a man given high doses of oestrogen, known to raise transcortin levels, but was lower in serum taken before starting oestrogen therapy. Thus in these three instances the inhibition was proportional to the transcortin concentration. These data support the concept that transcortin prevents entry into the placental cells by binding the F in serum.

When cord serum, which is known to contain low concentrations of transcortin was used the results were quite different. Again there was inhibition but in this instance heating had no effect and the inhibitory factor was dialyzable and extractable into organic solvents. There thus appears to be a dialyzable, heat-stable, extractable inhibitor in cord serum at term, which is possibly a steroid acting competitively. Our current studies are aimed at identifying this substance.

If this substance controls F–E interconversion at term, it seems possible that the alteration of F metabolism brought about is responsible for all or part of the increase in F/E seen in cord blood at term, i.e. F rises because less is metabolized to E, rather than because F production is stimulated directly.

Another possibility is that F from amniotic fluid is absorbed by the fetus in sufficient quantities to influence serum concentrations. We have studied five fetuses where injection of tritiated F was made into the amniotic fluid about 30 min prior to hysterotomy. While the bulk of the tracer was still in the amniotic fluid, the concentrations of tracer in the cord blood averaged 10% of those remaining in the amniotic fluid.

How can we study the change in F metabolism occurring during those last few weeks of pregnancy? Since F is known to be produced by the chorion laeve as well as the fetal adrenal cortex, amniotic fluid F is an unsatisfactory indicator of intrafetal F. We therefore looked at conjugated F, particularly at F sulfate which is the predominant one and is thought to be produced probably entirely by the fetal adrenal cortex [14].

When we studied this entity (Fig. 7) a very different picture emerged from that of F alone. There was much less variability and the steady rise from about 33 weeks onward closely paralleled that of the palmitic/stearic ratio, a reliable indicator of fetal lung maturation [15]. We also confirmed the fact that corticosterone sulfate is also present in appreciable amounts at term, as previously shown by Giroud *et al.* [14].

Since the conjugated glucocorticoids appear to be so closely related to fetal lung maturation would it be possible that they might serve the same purpose in maternal urine? Our preliminary data shown in Fig. 8 suggests that they might, but it will require a great deal more work to determine if such an indicator may be clinically applicable.

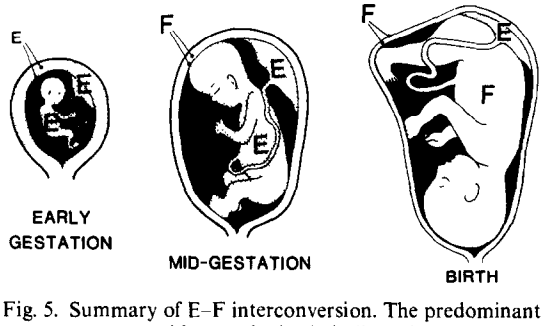

Fig. 5. Summary of E–F interconversion. The predominant steroid at each site is indicated.

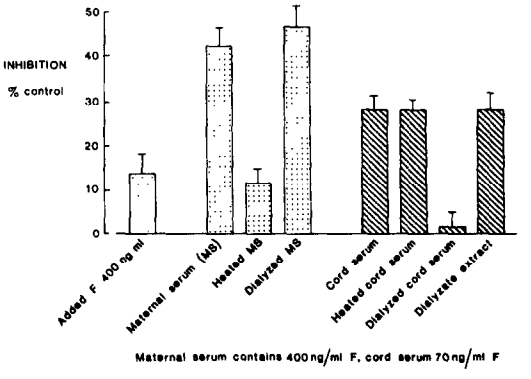

Fig. 6. Inhibition of placental conversion of F to E.

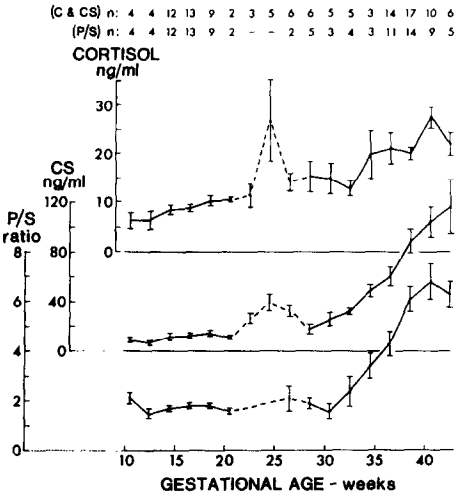

Fig. 7. Levels of cortisol and glucocorticoid conjugates (CS) and the palmitic/stearic ratio (P/S) in amniotic fluid throughout pregnancy. Means ± S.E. are shown. Reproduced from Ref. 15.

What is the biological significance of F–E interconversion? It is known that high F levels in early fetal life in many species can cause malformations and have many undesirable effects on development [16, 17]. It would thus appear that 11β-hydroxysteroid dehydrogenase, by converting F to its inactive analogue E, can maintain a low F level without compromising fetal adrenocortical production. This results first of all in preventing the passage of large amounts of F across the placenta and secondly by acting at the tissue level to divert F from the glucocorticoid receptors known to be present from the early weeks of pregnancy as in Fig. 9. In late pregnancy this metabolism decreases allowing maturational changes to occur, and is modified in postnatal life according to individual tissue requirements.

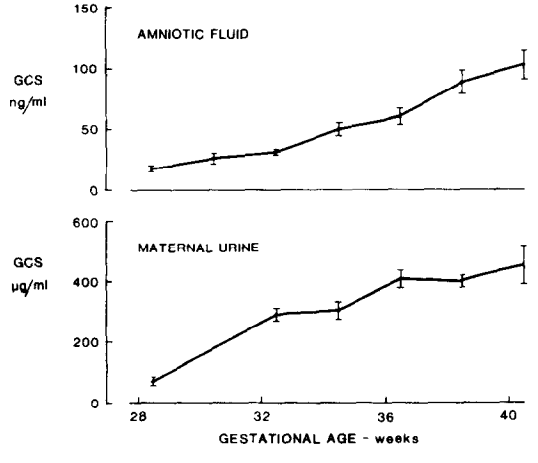

Fig. 8. Levels of glucocorticoid conjugates (GCS) in amniotic fluid and maternal urine compared in two different groups of patients. Means ± S.E. are shown.

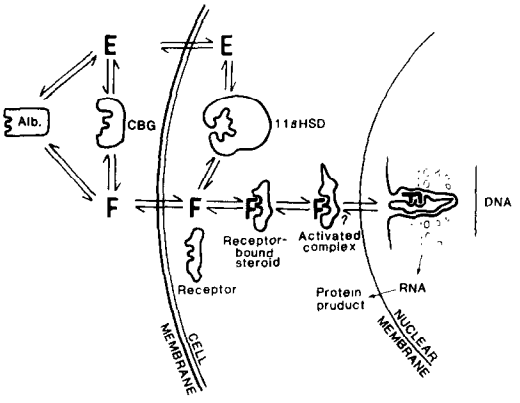

Fig. 9. Mechanism of cortisol action. The current concept is modified to include diversion of F from glucocorticoid receptors by enzymatic conversion to E.

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