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METABOLISM OF PREDNISOLONE BY THE ISOLATED PERFUSED HUMAN PLACENTAL LOBULE

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Summary—Previous studies of the metabolism of 11β-hydroxy corticosteroids by placental tissue have indicated that the only product is the C_{11}-oxidized metabolite. In the present study we have re-examined the metabolism of prednisolone in the isolated, perfused, dual recirculating human placental lobule, using a perfusate based on tissue culture medium 199. Four metabolites were identified in both the maternal and fetal compartments in 6 h perfusions by comparison of relative retention times measured by HPLC and capillary gas chromatography (GC) and of mass spectra recorded by capillary gas chromatography-mass spectrometry (GC–MS) with those of authentic reference standards. The steroids were derivatized as the MO–TMS ethers for mass spectral measurements. Analysis of samples from five perfusion experiments resulted in the following percentage conversions after 6 h perfusion (mean ± SD, maternal and fetal perfusate, respectively): prednisone (49.1 ± 7.8, 49.1 ± 6.6), 20α-dihydroprednisone (0.84 ± 0.29, 0.81 ± 0.35), 20β-dihydroprednisone (39.1 ± 6.7, 39.2 ± 5.9), 20β-dihydroprednisolone (6.8 ± 2.7, 6.3 ± 1.6) and unmetabolized prednisolone (4.1 ± 1.8, 4.6 ± 2.1). No evidence was found for metabolites formed by 6β-hydroxylation or cleavage of the C_{17}–C_{20} bond.

INTRODUCTION

The successful use of maternal antepartum corticosteroid administration for the prevention of respiratory distress syndrome (RDS) in premature infants has been well documented since the pioneering work of Liggins and Howie [1] in 1972. Cortisol and its synthetic analogues, betamethasone, dexamethasone and prednisolone, are effective as therapeutic agents by inducing maturation of fetal lung [2–5].

The placenta has a large capacity for active corticosteroid metabolism by the enzyme 11β-hydroxysteroid dehydrogenase (11β-OHSDH) [6]. In addition, other enzyme systems with steroid lyase, hydroxylase, dehydrogenase, isomerase, conjugative, deconjugative and/or aromatase activity have been demonstrated in this tissue [7]. Despite this metabolic capacity, few reports have dealt specifically with placental metabolism of one of the corticosteroids, prednisolone. Blanford and Murphy [8] reported 51% metabolism of prednisolone to its 11-oxo metabolically-inactive form, prednisone, by minces of mid-gestational or term human placentae. Corresponding metabolism of cortisol, dexamethasone and betamethasone was demonstrated with 67, 1.8 and 7.1% conversion to the respective 11-oxo compounds. Levitz *et al.* [9] provided evidence of more extensive conversion of prednisolone to the same product (68% in maternal artery samples) by the isolated perfused human placental lobule, with cortisol, dexamethasone and betamethasone also being metabolized (37, 25 and 18%, respectively, maternal artery samples) to the same products. Prednisolone was also shown to undergo transformation to prednisone by the human placenta *in vivo* [10].

These studies provided evidence for oxidation at C_{11} as the only metabolic modification of these corticosteroids in placental tissue. Placental microsomes and tissue slices contain 3α- and 20α- and/or 20β-hydroxysteroid dehydrogenase in addition to 11β-OHSDH [11, 12], and the human placenta *in vitro* metabolizes progesterone to 20α-dihydroprogesterone in increasing amounts as pregnancy advances [13]. The open column or paper chromatographic techniques employed in these early reports of placental metabolism of prednisolone may have lacked the sensitivity to detect further metabolism,

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particularly of a low grade nature. In rat kidney perfusion studies, cortisone, 20-dihydrocortisone and 20-dihydrocortisol of unknown stereochemistry have been identified as the main metabolites of cortisol [14].

This study was designed to re-investigate the placental metabolism of prednisolone by the use of an *in vitro* perfusion technique [15] combined with the more sensitive analytical techniques of HPLC, capillary gas chromatography (GC) and capillary gas chromatography–mass spectrometry (GC–MS) for product identification.

EXPERIMENTAL

Materials

Tissue culture medium (M199) was obtained from Difco (Detroit, MI, U.S.A.), heparin and gentamicin injections were from David Bull Labs (Melbourne, Australia). Sodium hydrogen carbonate and glucose were purchased from Ajax Chemicals (Auburn, Australia), while prednisolone, prednisone, 6α-methylprednisolone, dextran (approx. M_w 40,500), cholesteryl butyrate, methoxyamine hydrochloride (in pyridine), hydroxyalkoxypropyl dextran and hexamethyldisilazane were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). 20β-Dihydroprednisone and 20β-dihydroprednisolone were obtained from the Schering Corp. (Kenilworth, NJ, U.S.A.), and 20α-dihydroprednisone from the Steroid Reference Collection (London, U.K.). Trimethylsilylimidazole (TMSI) was from Pierce Chemical Co. (Rockford, IL, U.S.A.). [2,4,6,7-^{3}H]Prednisolone (47.7 Ci/mmol) and [*N-methyl*-^{14}C]antipyrine (50 mCi/mmol), were purchased from Amersham Int. (Bucks., U.K.). Scintillation fluid (Ready Safe) was from Beckman Instruments Inc., (Fullerton, CA, U.S.A.). All solvents were of analytical grade and were glass distilled prior to use.

Placental perfusion

The perfusion method used was adapted from that of Miller *et al.* [15]. The perfusate was M199, supplemented with heparin (25 IU/ml) and gentamicin (100 mg/l), glucose (2 g/l), sodium bicarbonate (2.9 g/l) and dextran (fetal 29 g/l; maternal 7.5 g/l). Term human placentae were obtained at caesarean section within 5 min of delivery from healthy women with no significant drug history and transported immediately in cold, oxygenated perfusate to the laboratory (housed in an adjacent building). An artery–vein pair to an intact peripheral lobule was cannulated for fetal circuit perfusion with maternal perfusion being established by insertion of two cannulae approx. 0.8 cm through the decidual plate.

The volume of perfusate in the maternal and fetal circuits was 150 and 100 ml, respectively, with the pH maintained at 7.35 to 7.45 by the addition of sodium bicarbonate. Perfusion was maintained for 7 h in a dual recirculating mode with maternal and fetal flow rates of 25 and 3 ml/min, respectively. Tissue temperature was maintained at 37°C and physical viability of the preparation was determined by measuring the utilization of glucose and oxygen and production of human placental lactogen and lactate [16].

Initial pH, oxygen and carbon dioxide tensions were measured in each circuit (ABL300 blood gas analyser, Radiometer, Copenhagen, Denmark) to confirm a minimum oxygen transfer (fetal vein–artery $\geq$90 mmHg) [16]. The maternal perfusion was briefly interrupted and [^{3}H]prednisolone (7–10 μCi, HPLC purified), non-radiolabelled prednisolone (150 μg) and [^{14}C]antipyrine (5–10 μCi, used as an internal marker of transfer) were added directly to the maternal reservoir. This was mixed and a sample taken as a blank. Maternal circulation was resumed (time zero) and perfusion continued for a further 6 h. Samples (100 μl) were drawn with glass syringes (SGE, Melbourne, Australia) at regular intervals from ports placed in each of the maternal and fetal artery and vein circuits to allow monitoring of transfer of the radiolabelled chemicals from maternal to fetal compartments. At the end of the 6-h period remaining maternal and fetal perfusates were centrifuged and the supernatants stored at −20°C for analysis.

Extraction of perfusate samples

Samples of maternal and fetal perfusates were extracted using Sep-pak cartridges (Waters Associates, Milford, MA, U.S.A.) in the same manner as Cannell *et al.* for serum [17]. An internal standard (6α-methylprednisolone, 500 ng/ml final concentration) was added to each sample (10 ml) prior to extraction. The bound steroids were eluted with 5 ml of methanol (HPLC grade) and the methanol removed under a stream of nitrogen at 40°C prior to reconstitution for analysis by HPLC or derivatization and analysis by GC.

HPLC analysis

Evaporated extracts were reconstituted in mobile phase (100 μl) and an aliquot (50 μl) analysed according to the method of Cannell *et al.* [18]. Eluant fractions (10 s) from the HPLC were collected in glass scintillation vials to which scintillation fluid was added and the radioactivity measured (Tricarb 2000CA scintillation counter, Packard, U.S.A.). The radioactivity measurements (dpm) were corrected for background and represented graphically vs HPLC retention time. Total radioactivity in each peak was calculated and expressed as a percentage of sum of the radioactivity contained in all peaks of the sample (i.e. % total product).

GC analysis

Perfusate samples (30 ml), to which internal standard (cholesteryl butyrate, 10 μg) had been added, were extracted and the eluants evaporated in 1 ml glass vials. Methoxyamine hydrochloride (100 μl, 1 mg/ml in pyridine) was added to each, the samples were heated for 30 min at 60°C, TMSI added and the vials heated for a further 2 h at 100°C. The vials were cooled and excess derivatizing reagent was removed using a modification of the method of Axelson and Sjovall [19]. Briefly, a small column of hydroxyalkoxypropyl dextran, prepared in hexane, was washed with a solvent mixture of hexane, pyridine, hexamethyldisilazane and 2,2-dimethoxypropane in the proportions 87:1:2:10 (v/v). The derivatized steroids were taken up in this solvent mix and eluted from the column with approx. 5 ml of the same solvent. The solvent was evaporated with a gentle nitrogen stream at 40°C. The purified samples were reconstituted in 200 μl of the same solvent (except heptane was substituted for hexane) prior to analysis.

Steroid analyses were performed on a model 3500 capillary gas chromatograph fitted with a model 8035 autosampler (Varian Instruments, CA, U.S.A.), a direct on-column injector and a flame ionization detector. A Heliflex WCOT column (30 m × 300 μm i.d.; Alltech Associates Inc., Deerfield, IL, U.S.A.) was used for separation, using hydrogen (average linear velocity 35 cm/s) as the carrier gas. The temperatures of the injector and column oven were programmed as follows: injector 90–230°C at 200°C/min, hold at 230°C; column 90–230°C at 50°C/min, 230–270°C at 1°C/min. Detector temperature was maintained at 250°C, and injection volume was 0.8–1.0 μl.

GC–MS analysis

The samples prepared for GC analysis were also used for analysis by GC–MS. The instrument used was a Hewlett–Packard model 5840 gas chromatograph coupled with a model 5985B mass spectrometer. A Hewlett–Packard HP1 methylsilicone column, 12.5 m × 200 μm i.d., was used for separation with helium as the carrier gas (flow 1 ml/min). A 1:5 split injection was used for sample introduction onto the column with the injector port temperature maintained at 250°C. The column temperature program used was: injection temperature 180°C, maintained for 1 min; 180–220°C at 20°C/min; 220–290°C at 2°C/min. The transfer line between the GC and the MS was maintained at 290°C. Mass spectrometer conditions employed were as follows: ion source temperature 200°C; ion source pressure 3×10^{-6} torr; mass scan 50 to 700 a.m.u. (calibrated with *s*-triazine); ionization mode electron impact 70 eV).

RESULTS

HPLC analysis

Analysis of maternal and fetal perfusate samples from five 6-h perfusion experiments by HPLC, GC and GC–MS revealed prednisolone to be metabolized to four compounds. A representative HPLC analysis of perfusate from the maternal and fetal compartments is shown in Fig. 1. The retention times of these metabolites and closely corresponding reference standards are listed in Table 1. There was no evidence for highly polar metabolites such as 6β-hydroxylation products or for lyase cleavage of the C_{17}—C_{20} bond from experiments using either radiolabelled or unlabelled substrates. No evidence for 20α-dihydroprednisolone production

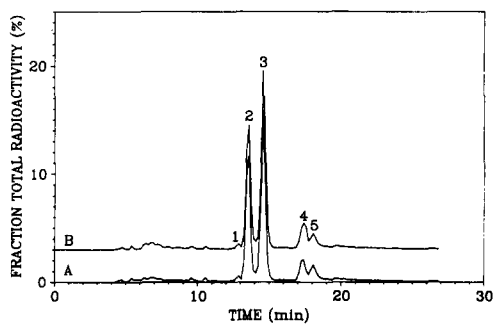


Fig. 1. Radiochemical HPLC analysis of maternal (A) and fetal (B, offset) perfusate from a 6-h recirculating perfusion. [^{3}H]Prednisolone and metabolites were extracted from perfusate and analysed by HPLC. Aliquots (10 s) were collected and the radioactivity content determined. Peak identities are listed in Table 1.

Table 1. Relative retention of prednisolone and metabolites from HPLC analysis of perfusate

Component[a]	Relative retention[b]	Peak identity[c]
1	0.71	20α-Dihydroprednisone
2	0.75	20β-Dihydroprednisone
3	0.81	Prednisone
4	0.96	20β-Dihydroprednisolone
5	1.00	Prednisolone

[a]Unknown components are numbered according to Fig. 1.
[b]Retention times were normalized relative to prednisolone in each analysis.
[c]Peak identity assigned by comparison with authentic standards.

Table 2. Composition (%) of prednisolone metabolites in maternal and fetal perfusate from 6-h recirculating perfusions

Product	Maternal perfusate[a]	Fetal perfusate[a]
20α-Dihydroprednisone	0.84 ± 0.29	0.81 ± 0.35
20β-Dihydroprednisone	39.1 ± 6.7	39.2 ± 5.9
Prednisone	49.1 ± 7.8	49.1 ± 6.6
20β-Dihydroprednisolone	6.8 ± 2.7	6.3 ± 1.6
Prednisolone	4.1 ± 1.8	4.6 ± 2.1

[a]Each value is the result of 5 perfusions expressed as mean ± SD.

was found (relative retention compared with prednisolone, 0.84).

The relative distributions of the metabolites expressed as a percentage of total product are recorded in Table 2. The product distributions in maternal and fetal perfusate were esentially identical after 6 h. This precludes any active transport mechanisms for these compounds which would be indicated if significant concentration gradients existed. The release of metabolites into the two compartments, however, could be asymmetric and accompanied by unrestricted transfer and equilibration during this time.

GC analysis

Analysis of perfusion samples by GC confirmed the HPLC findings of multiple metabolites of prednisolone. A chromatogram from a typical analysis is shown in Fig. 2. Peaks 1–5, by comparison of their retention times with those of relative standards, correspond with the following: peak 1, prednisone; peak 2, 20α-dihydroprednisone; peak 3, prednisolone; peak 4, 20β-dihydroprednisone; peak 5, 20β-dihydroprednisolone (Fig. 3). The relative retention

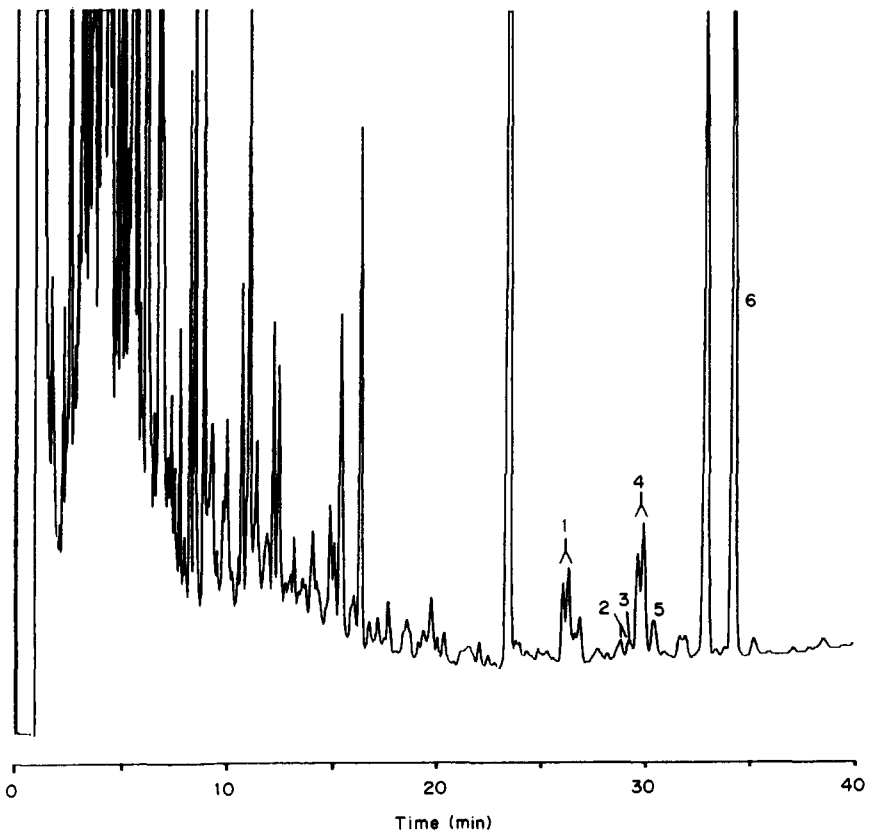
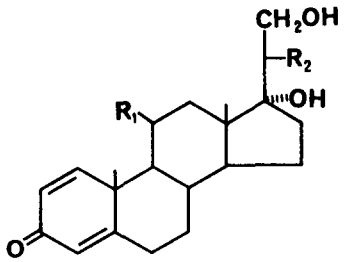


Fig. 2. GC analysis of prednisolone and its metabolites from a 6-h recirculating perfusion. Peak identities are: 1, prednisone; 2, 20α-dihydroprednisone; 3, prednisolone; 4, 20β-dihydroprednisone; 5, 20β-dihydroprednisolone and 6, cholysteryl butyrate (internal standard). The large peak eluting before cholesteryl butyrate is 3β-hydroxy-5-cholesten-3-one (relative retention 0.96), which can be used as an alternative internal standard.



	R1	R2
Prednisolone	-OH	=O
Prednisone	=O	=O
20α-Dihydroprednisone	=O	····OH
20β-Dihydroprednisone	=O	—OH
20β-Dihydroprednisolone	-OH	—OH

Fig. 3. Structure of prednisolone and metabolites identified in placental perfusates.

times of the metabolites and standards are listed in Table 3. No peak was observed for 20α-dihydroprednisolone which has a relative retention of 0.935 normalized to the internal standard, cholesteryl butyrate.

GC–MS analysis

The mass spectra of authentic standards of 20α-dihydroprednisone and 20β-dihydroprednisone were essentially identical as were the mass spectra of the corresponding metabolites of prednisolone. The identity of each peak in the GC analysis (Fig. 2) of a typical 6-h sample from a perfusion with prednisolone, could be assigned by comparison of relative retention times (Table 3) and mass spectra with those of known standards. The mass spectra of 20α-dihydroprednisone (peak 2 in Fig. 2) and 20β-dihydroprednisone (peak 4) were identical with a standard of 20α-dihydroprednisone [Fig. 4(A)] and the fragmentation pattern of 20β-dihydroprednisolone (peak 5) was identical with that of an authentic standard [Fig. 4(B)]. The absence

Table 3. Relative retention of prednisolone and metabolites from GC analysis of perfusate from 6-h recirculating perfusions

Unknown component[a]	Relative retention[b]	Reference standard
1	0.762	Prednisone
	0.769	
2	0.841	20α-Dihydroprednisone
	0.853	
3	0.853	Prednisolone
4	0.865	20β-Dihydroprednisone
	0.873	
5	0.887	20β-Dihydroprednisolone
6	1.000	Cholesteryl butyrate

[a]Unknown components are numbered according to Fig. 2.
[b]Retention times were normalized relative to the internal standard (cholesteryl butyrate).

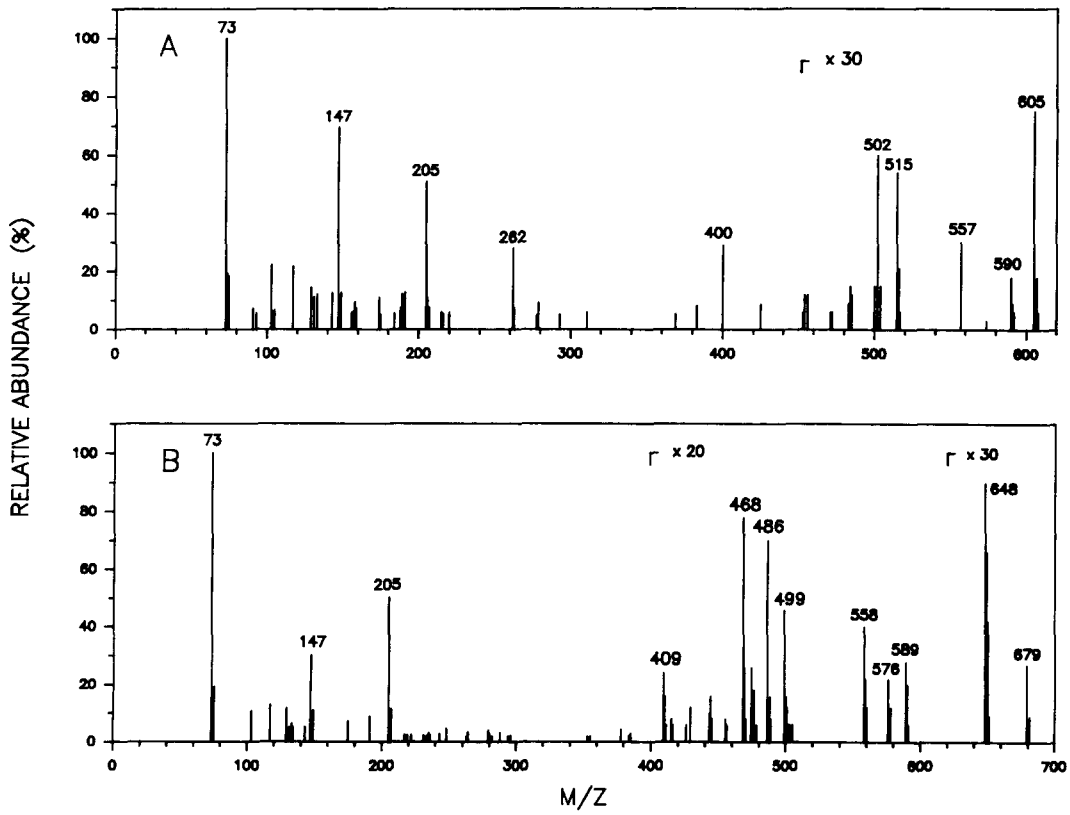


Fig. 4. (A) Mass spectrum of the MO–TMS derivative of 20α-dihydroprednisone. (B) Mass spectrum of the MO–TMS derivative of 20β-dihydroprednisolone.

of mass spectral evidence for the formation of 20α-dihydroprednisolone confirmed the result found by GC.

From the molecular ions of the MO–TMS ether derivatives of the steroids listed in Fig. 3, all functional hydroxyl and carbonyl groups are derivatized as TMS ethers or methoximes, respectively except for the C_{21} carbonyl in the prednisone series. This is based on the observation that the C_3 methoxime is formed in the corresponding prednisolone derivatives. The fragmentation patterns of the assigned 20-dihydro compounds were consistent with the presence of a hydroxyl at C_{20}. 20α-Dihydroprednisone and 20β-dihydroprednisone each lose a derivatized fragment ion of m/z 103, corresponding to a $(M\text{-}CH_2OTMS)^+$ loss of C_{21}, to produce an ion at m/z 576. Cleavage of the C_{17}—C_{20} bond results in a fragment of m/z 205 with a relative abundance >40% in each case. 20β-Dihydroprednisolone loses ions of m/z 103 and 205, corresponding to losses of the side chain. Other characteristic ions in the mass spectra of the MO–TMS derivatives of all three compounds are exhibited at m/z 605 and 679 (M^+, 20α-/20β-dihydroprednisone and 20β-dihydroprednisolone, respectively), 590 and 664 $(M\text{–}CH_3)^+$, 574 and 648 $(M\text{–}OCH_3)^+$, 515 and 589 $(M\text{–}TMSOH)^+$, 502 and 576 $(M\text{–}CH_2OTMS)^+$, 484 and 558 $(M\text{–}31\text{–}90)^+$ and 425 and 499 $(M\text{–}TMSOH\text{–}TMSOH)^+$.

DISCUSSION

The placenta performs a number of essential and highly differentiated functions, from mediating maternal–fetal exchange to endocrine functions such as the synthesis of progesterone and estrogen. The high concentrations of 11β-OHSDH in placental tissue may form a barrier for the transfer for the large amount of maternal cortisol to the fetal compartment [6, 20]. The placenta has been shown to be capable of performing most of the biotransformation reactions found in the liver, and several examples of each of the four major drug metabolizing reactions (oxidation, reduction, hydrolysis and conjugation) have been shown to occur in placental homogenates [21]. Reduction of progesterone to 20α-dihydroprogesterone has provided evidence for 20α-OHSDH activity in the human placenta [13].

Analysis of perfusates from 6-h perfusion experiments by HPLC, GC and GC–MS has shown the isolated, perfused human placental lobule to be capable of metabolizing prednisolone to at least four compounds. These derivatives have been qualitatively identified by these analytical techniques as prednisone, 20α-dihydroprednisone, 20β-dihydroprednisone and 20β-dihydroprednisolone, with quantitation by HPLC resulting in the percent conversion to each product as listed in Table 2. No evidence was found for metabolites formed by 6β-hydroxylation, cleavage of the C_{17}—C_{20} bond or for the formation of 20α-dihydroprednisolone in these perfusions.

These results are in contrast with previous reports of placental corticosteroid metabolism. Levitz *et al.* [9] reported conversion of prednisolone, betamethasone, dexamethasone and cortisol by perfused human placental lobules or placental minces to be restricted to the respective 11-keto derivatives only. These workers found conversion of prednisolone to prednisone in maternal and fetal vein perfusate samples to be 68 and 86%, respectively in perfusions performed in a non-recirculating mode (i.e. both maternal and fetal circuits open), with the results presented being from one analysis only. Blanford and Murphy [8] reported conversion of the same corticosteroids to their respective 11-keto steroids in minces of human placental tissue, with the conversion of prednisolone to prednisone (51.4 ± 6.1%) being similar to that found in this perfusion study (49.1 ± 7.8% in maternal perfusate). The Sephadex-LH20 or paper chromatographic separation techniques used by Levitz *et al.* [9] and Blanford and Murphy [8] may have lacked the sensitivity or specificity required to detect any 20-reduced metabolites formed in those studies. It is also possible that the rates of reduction of the 20α- or 20β-dehydrogenase enzymes in the placenta are slower than that of the 11β-dehydrogenase, resulting in the formation of 20-reduced derivatives in 6-h perfusion experiments, but not in single-pass style experiments as conducted by Levitz.

The results presented here are consistent with the isolation of 20-reduced corticosteroids from placental tissue reported previously. Berliner *et al.* [22] identified 20β-dihydrocortisone in human placental extracts, while this compound and the 20α-epimer were isolated from this tissue by Neher and Stark [23] in 1961. Injection of [^{3}H]cortisol and [4-^{14}C]cortisone into the intact feto–placental circulation, prior to interruption of gestation, provided evidence for extensive placental interchange of cortisol and

cortisone, however no C_{20}-reduced metabolites were isolated from the placental tissue [24]. In another study, dexamethasone was administered before birth and was cleared more rapidly than expected from the fetus. This discrepancy could not be accounted for by the small amounts of 11-dehydrodexamethasone formed, however the formation of other metabolites could explain this difference [25]. Although the reduction of cortisol and cortisone to the 20-reduced derivatives has been reported for a variety of tissues [26], the corresponding metabolites have not previously been found for the metabolism of prednisolone by placenta and this may be due to a lack of sensitivity in analytical techniques or in combination with low grade metabolism.

The 20-reduced metabolites of prednisolone have been identified in human urine: Gray *et al.* [27] in 1956 found prednisone, prednisolone, their respective 20β-dihydro metabolites and a fifth substance suggestive of 20α-dihydroprednisone in the urine of patients receiving oral prednisone or prednisolone. Vermeulen [28] also identified prednisone, prednisolone and 20β-dihydroprednisone in the urine of prednisone-treated subjects. These results were verified by administration of [^{14}C]prednisolone to subjects and isolation of the 20β-dihydrometabolites of both prednisone and prednisolone [29]. Traces of 1,4-androstadiene-3,11,17-trione, formed by the oxidative loss of the side chain from C_{17} in prednisone, were also isolated. The first evidence for reduction to the 20α-epimer was provided by Vermeulen and Caspi [30], where 20α-dihydroprednisolone was isolated from metabolism of prednisolone. Hydroxylation at the 6-position has resulted in approx. 6% of a prednisolone dose being excreted as 6β-dihydroprednisolone [31] with this compound proposed as an indicator of enhanced prednisolone catabolism [32].

The structural requirements for glucocorticoid activity include an 11β-hydroxyl moiety and a —CH_2COCH_2OH side chain at C_{17} with an oxo group at C_{20} [33]. This activity is attenuated or lost by either reduction of the C_{20} carbonyl group or oxidation of the C_{11} hydroxyl group. It is therefore unlikely that any of the metabolites identified in this study would have retained glucocorticoid activity, however any other physiological activity or pharmacological significance, if any, associated with these metabolites is unknown.

The product distribution found in this study using a dual recirculating system highlights the more complex metabolic profile which may not be apparent in the open-circuit, dual recirculating type perfusions. On this basis a more complex metabolic profile would be expected for the placental metabolism of other corticosteroids than has previously been reported [9].

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REFERENCES

1. Liggins G. C. and Howie R. N.: A controlled trial of antepartum glucocorticoid treatment for prevention of respiratory distress syndrome in premature infants. *Pediatrics* **50** (1972) 515–525.
2. Dluholucky S., Babic J. and Taufer I.: Reduction of incidence and mortality of RDS by administration of hydrocortisone to mother. *Archs Dis. Child.* **51** (1976) 420.
3. Papageorgiou A. N., Desgranges M. F., Masson M., Colle E., Shatz R. and Gelfand M. M.: The antenatal use of betamethasone in the prevention of respiratory distress syndrome: a controlled, double-blind study. *Pediatrics* **63** (1979) 73–79.
4. Caspi E., Schreyer P., Weinraub Z., Reif R., Levi I. and Mundel G.: Prevention of the respiratory distress syndrome in premature infants by antepartum glucocorticoid therapy. *Br. J. Obstet. Gynec.* **83** (1976) 187–193.
5. Horvath I., Mehes K., Fias I., and Symodits S.: Prevention of respiratory distress syndrome by antenatal maternal steroid treatment. *Acta Pediat. Acad. Sci. Hung.* **17** (1976) 303–306.
6. Osinski P. A.: Steroid 11β-ol dehydrogenase in human placenta. *Nature* **187** (1960) 777.
7. Gowler D. N.: The role of cytochrome *P*450 in steroidogenesis and properties of some of the steroid transforming enzymes. In *Biochemistry of Steroid Hormones* (Edited by H. Makin). Blackwell Scientific, London, 2nd edn (1984) pp. 230–292.
8. Blanford A. T. and Murphy B. E. P.: *In vitro* metabolism of prednisolone, dexamethasone, betamethasone and cortisol by the human placenta. *Am. J. Obstet. Gynec.* **127** (1977) 264–267.
9. Levitz M., Jansen V. and Dancis J.: The transfer and metabolism of corticosteroids in the perfused human placenta. *Am. J. Obstet. Gynec.* **132** (1978) 363–366.
10. Beitins I. Z., Bayard F., Ances I. G., Kowarski A. and Migeon C. J.: The transplacental passage of prednisone and prednisolone in pregnancy near term. *J. Pediat.* **81** (1972) 936–945.
11. Meigs R. A. and Engel L. L.: Metabolism of neutral steroids by human tissues. *Fedn Proc.* **17** (1958) 274.
12. Meigs R. A. and Engel L. L.: The metabolism of adrenocortical steroids by human tissues. *Endocrinology* **69** (1961) 152–162.
13. Milewich L., Gant N. E., Schwarz B. E., Chen G. T. and MacDonald P. C.: Initiation of human parturition. IX. Progesterone metabolism by placentas of early and late human gestation. *Obstet. Gynec.* **51** (1978) 278.
14. Reach G., Nakane H., Nakane Y., Auzan C. and Corvol P.: Cortisol metabolism in the isolated perfused rat kidney. *Steroids* **30** (1977) 621–635.

15. Miller R. K., Weir P. J., Maulik D., and di Sant'agnese P. A.: Human placenta *in vitro*. Characterization during 12 hours of dual perfusion. *Contr. Gynec. Obstet.* **13** (1985) 77–84.
16. Cannell G. R., Kluck R. M., Hamilton S. E., Mortimer R. H., Hooper W. D. and Dickinson R. G.: Markers of physical integrity and physical viability of the perfused human placental lobule. *Clin. Exp. Pharmac. Physiol.* **15** (1988) 837–844.
17. Cannell G. R., Galligan J. P., Mortimer R. H. and Thomas M. J.: Extraction of steroids from plasma with reversed phase C_{18} cartridges. *Clin. Chim. Acta* **122** (1982) 419–423.
18. Cannell G. R., Mortimer R. H., Maquire D. J. and Addison R. S.: Liquid chromatographic analysis of prednisolone, prednisone and their 20-reduced metabolites in perfusion media. *J. Chromat.* **563** (1991) 341–347.
19. Axelson M. and Sjovall J.: Separation and computerised GC–MS of unconjugated neutral steroids in plasma. *J. Steroid Biochem.* **5** (1974) 733–738.
20. Lopez Bernal A., Flint A. P. F., Anderson A. B. M. and Turnbull A. C.: 11β-Hydroxysteroid dehydrogenase activity (EC. 1.1.1.146) in human placenta and decidua. *J. Steroid Biochem.* **13** (1980) 1081–1087.
21. Juchau M. R.: Mechanisma of drug biotransformation reactions in the placenta. *Fedn Proc.* **31** (1972) 48–52.
22. Berliner D. L., Jones J. E. and Salhanick H. A.: The isolation of adrenal-like steroids from the human placenta. *J. Biol. Chem.* **223** (1956) 1043.
23. Neher R. and Stark G.: Nachweis von corticosteroisen in menschlicher placenta und isolierung von 16α-hydroxy-estrosteron. *Experientia* **15** (1961) 510–512.
24. Pasqualini J. R., Nguyen B. L., Uhrich F., Wiqvist N. and Diczfalusy E.: Cortisol and cortisone metabolism in the human foeto-placental unit at midgestation. *J. Steroid Biochem* **1** (1970) 209–219.
25. Kream J., Mulay S., Fukushima D. K. and Solomon S.: Determination of plasma dexamethasone in the mother and the newborn after administration of the hormone in a clinical trial. *J. Clin. Endocr. Metab.* **56** (1983) 127–133.
26. Jenkins J. S.: The metabolism of cortisol by human extrahepatic tissues. *J. Endocr.* **34** (1966) 51–56.
27. Gray C. H., Green M. A. S., Holness N. J. and Lunnon J. B.: Urinary metabolic products of prednisone and prednisolone. *J. Endocr.* **14** (1956) 146–154.
28. Vermeulen A.: Isolation of 1,4-pregnadiene-3,20-dione-11β,17α,21-triol from the urine of patients treated with metacortandracin. *J. Clin. Endocr. Metab.* **16** (1956) 163–164.
29. Vermeulen A.: The metabolism of 4-[^{14}C]-prednisolone. *J. Endocr.* **18** (1959) 278–291.
30. Vermeulen A. and Caspi E.: Metabolism of 1-dehydrosteroids in man. II. Isolation of 20α and 20β-hydroxy metabolites. *J. Biol. Chem.* **234** (1959) 2295–2297.
31. Nakagawa T.: Metabolism of [^{14}C]-prednisolone and [^{14}C]-prednisone in healthy adult men. *Chem. Abstr.* **77** (1972) 29310b.
32. Frey F. J. and Frey B. M.: Urinary 6β-hydroxyprednisolone excretion indicates enhanced prednisolone catabolism. *J. Lab. Clin. Med.* **101** (1983) 593–604.
33. Gower D. B.: Biosynthesis of the corticosteroids. In *Biochemistry of Steroid Hormones* (Edited by H. L. J. Makin). Blackwell Scientific, London, 2nd edn (1984) pp. 117–121.