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CATABOLIC REGULATION OF BLOOD CORTISOL IN PREMATURE AND TERM BABOON NEONATES

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SUMMARY

The metabolism of I.V. administered 4-[^{14}C]-cortisol (F) was examined in 3 premature (cesarean section at 160, 167, and 174 days gestation, term = 184 days) and 8 spontaneously delivered term (184 ± 2 days) baboon neonates (*Papio papio*). Three premature (Group 1) and 4 spontaneously delivered animals (Group 2) were studied 2–18 h after delivery and the remainder on the 5th ($N = 2$) or 12th ($N = 2$) day of life (Group 3). [^{14}C]-Metabolites were isolated by chromatography and crystallization. More than 10% of ^{14}C was in liver, 3–9% in intestine and brain, ≤ 2% in lung, kidney, heart, spleen, and urine, and < 0.2%/g in blood and muscle 30 min. after injection. Most ^{14}C (77.5 ± 1.6%) in all tissues was unconjugated. Extrahepatic tissues were primarily responsible for oxidation of F to cortisone. Formation of tetrahydrocortisol (THF), tetrahydrocortisone (THE), two hydroxylated metabolites and glucuronoside conjugates was restricted to liver. Alterations in F metabolism (decreased hydroxylated metabolites, increased THF and THE glucuronides) were not evident until 5–12 days after delivery.

It is concluded that extrahepatic 11β-hydroxysteroid dehydrogenase is important in achieving a low F:E ratio in fetal blood. The similarity in F catabolic patterns of premature and day 1 term newborns makes it unlikely that changes in metabolism can account for the increased fetal blood F concentrations prior to parturition.

INTRODUCTION

Recently, interest in the production and metabolism of glucocorticoids during the perinatal period has increased because of the potential role of cortisol (F) in fetal maturation, parturition, and maintenance of homeostasis in the newborn. The mechanisms maintaining a lower ratio of F to E (cortisone) concentrations in fetal than maternal blood [1–3] and which account for the prepartal rise in fetal F levels [4–6] are obscure. Studies in humans [7] and nonhuman primates [3] have explored maternal and placental factors in determining F and E levels in fetal plasma. We have examined whether fetal catabolism contributes to the maintenance of low blood F:E ratios. In addition, changes in F metabolism by fetal tissues could be responsible for the increased F levels in late gestation. Therefore, we have compared F catabolism in newborn baboons delivered by cesarean section, shortly before term, with that in spontaneously delivered term neonates.

MATERIALS AND METHODS

Animals. A total of 11 newborn baboons, divided into 3 groups, were examined. Group 1 (Premature) consisted of 3, otherwise normal, animals (2♂, 1♀) delivered at 160, 167, and 174 days gestation (term: 184 days) by cesarean section. The remaining 8 normal baboons delivered spontaneously per vagina, at 184 ± 2 days. Four (3♂, 1♀) were studied 2–18 h after delivery (Group 2; Day 1), and 2 (1♂, 1♀) at 5 days and 2 (1♂, 1♀) at 12 days of age (Group 3; Day 5–12). The latter baboons were reared as previously described [8].

Experimental. Using a 23 gauge scalp vein needle, newborns were injected *via* a peripheral arm or leg vein with 15–25 μCi 4-[^{14}C]-F (S.A. 55 Ci/mol; New England Nuclear Corp.) dissolved in 2.5 ml (9:1 v/v) pyrogen-free saline–ethanol. Thirty minutes later, 10 ml blood were removed by heart puncture and the animals killed by I.V. administration of 5 ml sodium pentobarbital (65 mg/ml). Tissues were quickly removed, placed on ice, freed of adhering fat and mesentery, weighed, and stored at −20°C until processed. Urine was collected in a cloth diaper and/or was obtained from the bladder at autopsy. The following tissues were removed: brain, skeletal muscle (gastrocnemius), liver, lung, heart, stomach and intestines including contents (henceforth called intestine), spleen, kidneys, and adrenals. Metabolites in skeletal muscle and adrenals were not isolated.

Processing of tissue. Tissues, other than urine and blood, were diced, suspended in 50–500 ml chilled 0.9% saline and homogenized in a Waring Blender (brain) or a Sorvall-Omni-Mixer for 1 min., at 4°C, and filtered through cheese-cloth. ^{14}C content was determined by liquid scintillation spectrometry using automatic external standardization to correct for quenching [9]. Homogenates were extracted 3 times with 2 vol. of ethyl acetate to remove unconjugated materials. Extracts of blood, spleen, kidneys, and heart were partitioned between hexane and meth-

anol–water (9:1 v/v) to remove low polar nonradioactive materials. Extracts of liver, brain, intestine, and lung were defatted in 70% methanol overnight at −20°C. Unconjugated [^{14}C]-metabolites were purified by sequential paper chromatography as previously described [9]. In addition, F and E were crystallized to constant specific activity. The glucuronoside fraction was obtained following incubation of homogenate residues for 96 h at 37°C (0.1 M sodium acetate buffer, pH 5.5) with bacterial β-glucuronidase (5000 units/g tissue; Sigma). Extraction and purification of radioactive metabolites was as described above. Sulfo-conjugates were solvolyzed using the methods of Burstein and Lieberman [10]. Unconjugated metabolites were isolated from blood, liver, intestine, and lung of all animals and from kidneys, spleen, heart, urine, and brain of 1 animal from Group 1 and 2 animals from each of Groups 2 and 3.

Statistical analyses were by independent and dependent Student's 't' tests.

RESULTS

Mean intestinal weight increased ($P < 0.001$) between 0 and 5–12 days of age, while that of lung decreased ($P < 0.05$) from 18.7 g prior to parturition to 11.5 g at delivery (Table 1). These changes were also evident when corrected for body weight. There were no significant differences in weights of any other tissues or in body weight. More than 10% of injected [^{14}C]-F was found in liver; the intestine and brain accumulated 3–9%. Tissue accumulation was similar in all animals except in urine and intestine, presumably due to increased weight, where ^{14}C content was greatest ($P < 0.02$) in 5–12 day old animals (Table 2).

The majority of radioactive material in all tissues was unconjugated. The percentage unconjugated [overall mean ± S.E. (N) = 77.5 ± 1.6 (68)] did not vary between groups (Tables 3–5). The nature of this fraction in blood, liver, and intestine is presented in Table 3. Most ^{14}C in blood was associated with F, E, and compounds more polar than 6β-hydroxy-F when chromatographed in chloroform–ethyl acetate–methanol–water (25:75:50:50, by vol.). In the present report, these latter metabolites are referred to collectively as the high polar fraction. In liver, the major F metabolites were THF, THE, and high polar materials and their concentrations (d.p.m./g) were greater ($P < 0.001$) than those in blood (d.p.m./ml). Decreased amounts of ^{14}C high polar materials were present in liver of 5–12 day old baboons. In both liver and blood the F:E ratio was greater than 2. By contrast, in intestine this ratio was ≦ 1. In blood of premature and day 1 animals, this ratio was greater

Table 1. Body and organ weights of baboon neonates

	Wet weight (g, mean ± S.E.)		
	Premature (N = 3)	Day 1 (N = 4)	Day 5–12 (N = 4)
Body wt.	803 ± 61	848 ± 45	859 ± 57
Liver	21.1 ± 1.1	21.5 ± 1.5	25.2 ± 1.9
Intestine	24.2 ± 3.1	23.4 ± 1.4	46.2 ± 4.3
Brain	77.3 ± 3.3	73.4 ± 5.2	85.1 ± 3.5
Lung	18.7 ± 2.8	11.5 ± 0.5	10.6 ± 0.4
Kidneys	3.4 ± 0.4	3.8 ± 0.3	4.5 ± 0.2
Heart	4.3 ± 0.7	4.4 ± 0.5	4.8 ± 0.4
Spleen	1.5 ± 0.2	1.5 ± 0.1	1.9 ± 0.2
Adrenals (combined)	0.29*	0.32 ± 0.02	0.41 ± 0.07

* N = 2, adrenals from one animal lost.

Table 2. Distribution of [^{14}C]-F and its metabolites in organs of baboon neonates

Organ	% Dose [^{14}C]-F ± S.E.		
	Premature (N = 3)	Day 1 (N = 4)	Day 5–12 (N = 4)
Blood*	0.18 ± 0.01	0.20 ± 0.05	0.15 ± 0.02
Liver	11.2 ± 0.8	10.3 ± 0.8	10.8 ± 0.8
Intestine	3.5 ± 0.6	4.3 ± 0.5	9.1 ± 1.0
Brain	4.5 ± 0.2	4.4 ± 0.5	3.5 ± 0.1
Lung	2.1 ± 0.2	2.1 ± 0.5	1.3 ± 0.3
Kidney	0.8 ± 0.2	1.0 ± 0.1	1.4 ± 0.5
Heart	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Urine	0.5 ± 0.2	0.3 ± 0.1	1.6 ± 0.4
Spleen	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1
Skeletal Muscle†	0.13 ± 0.03	0.12 ± 0.04	0.09 ± 0.03

* %/ml whole blood.

† %/g wet weight. Assuming that blood vol. is 10% of body weight and skeletal muscle = 45% of body weight, > 80% of injected [^{14}C]-F is recovered.

Table 3. Distribution of unconjugated [^{14}C]-F and its metabolites in blood, liver, and intestine of baboon neonates*

Tissue	Group (N)	d.p.m. × 10^{-2}/g unconjugated	% unconjugated†	d.p.m. × 10^{-2}/g F	E	THF	THE	H.P.‡	Ratio F/E
Blood	1 Premature (3)	725 ± 66	78 ± 6	442 ± 51	126 ± 31	19 ± 7	16 ± 1	99 ± 30	4.0 ± 1.1
	2 Day 1 (4)	817 ± 147	87 ± 3	492 ± 85	120 ± 37	41 ± 16	18 ± 8	142 ± 28	4.6 ± 0.9
	3 Day 5–12 (4)	527 ± 73	75 ± 6	265 ± 49	97 ± 5	14 ± 3	13 ± 3	101 ± 11	2.6 ± 0.4
Liver	1 Premature (3)	1760 ± 478	65 ± 13	272 ± 91	85 ± 36	248 ± 181	168 ± 36	772 ± 270	3.5 ± 0.4
	2 Day 1 (4)	1756 ± 153	76 ± 6	528 ± 106	124 ± 40	321 ± 84	198 ± 61	586 ± 51	6.6 ± 3.4
	3 Day 5–12 (4)	1281 ± 256	59 ± 8	276 ± 61	104 ± 49	161 ± 64	241 ± 79	295 ± 58	3.8 ± 1.1
Intestine	1 Premature (3)	648 ± 168	88 ± 6	159 ± 61	350 ± 138	21 ± 6	26 ± 2	56 ± 12	0.6 ± 0.2
	2 Day 1 (4)	754 ± 48	86 ± 4	277 ± 47	258 ± 42	48 ± 9	62 ± 11	108 ± 16	1.1 ± 0.1
	3 Day 5–12 (4)	713 ± 110	78 ± 8	178 ± 30	235 ± 76	41 ± 3	29 ± 7	148 ± 32	0.9 ± 0.2

* Mean ± S.E. All values normalized to dose of 21.92 μCi.
† d.p.m. unconjugated divided by blood or organ total d.p.m. ^{14}C.
‡ High polar fraction.

Table 4. Difference in [^{14}C]-F and [^{14}C]-E concentrations between blood and organs of neonatal baboons*

Tissue	N†	F (d.p.m. × 10^{-2}/g)		E (d.p.m. × 10^{-2}/g)	
Blood	5	336 ± 61		106 ± 21	
Kidney	5	66 ± 17	< 0.01	937 ± 81	< 0.01
Heart	5	265 ± 72	> 0.05	130 ± 24	< 0.02
Spleen	5	176 ± 67	< 0.02	240 ± 36	< 0.01
Brain	5	111 ± 33	< 0.01	80 ± 14	< 0.05
Lung	10	313 ± 73	> 0.05	158 ± 14	< 0.02

* Mean ± S.E. No differences in F or E concentrations were observed between groups, therefore values were pooled and analyzed by paired '*t*' test. In all tissues, except blood, F + E = 78 ± 2% of unconjugated fraction which = 79 ± 2% of organ total ^{14}C.

† For kidney, heart, spleen, and brain, 1 animal from Group 1 and 2 from each of Groups 2 and 3 were studied. For lung, all animals, except 1 from Group 3, were examined. Respective values for F and E in these 10 animals were: (d.p.m. × 10^{-2}/g) F: 421 ± 44; E: 117 ± 16. The ratio ($\bar{x}$ ± S.E.) of F:E in urine of 6 animals (1 from Group 1, 2 from Group 2, and 3 from Group 3) was 2.3 ± 0.7.

($P < 0.05$) than that in 5–12 day old baboons (Table 3). This was due to a decrease ($P < 0.05$) in the concentration of [^{14}C]-F in blood of 5–12 day old animals. In the remaining tissues investigated, F and E accounted for about 80% of the unconjugated metabolites (Table 4). In heart, urine and brain, the ratio of F:E was > 1 but in lung, spleen and kidney, it was < 1. The concentration of E in the latter 2 tissues greatly exceeded ($P < 0.01$) that in blood. In brain, the concentrations of F and E were lower ($P < 0.05$) than in blood.

Only in liver was the concentration of [^{14}C]-metabolites conjugated with glucuronic acid greater than that in blood. The major metabolites isolated from this fraction were THF, THE, and high polar materials, which together accounted for about 80% of the ^{14}C (Table 5). There was a decrease ($P < 0.01$) in the proportion of high polar metabolites and an increase ($P < 0.05$) in the proportion of THF and THE formed in 5–12 day old animals compared with values in younger animals. The formation of sulfo-conjugates was limited, accounting for < 5% of ^{14}C in all tissues examined. This fraction was not examined further.

DISCUSSION

In baboon neonates, a variety of tissues interact in the metabolism of F. Intestine, kidney, spleen and lung have extensive 11β-hydroxy steroid dehydrogenase activity since E is the single metabolite formed in concentrations (d.p.m./g) significantly greater than those found in blood (Tables 3 and 4). In kidney, the concentration of E is 9 times that in blood while that of F is 6 times lower. Most of this renal E is resorbed rather than excreted since the ratio of F:E in urine was > 1. This was confirmed when urine was collected for 3 days following [^{14}C]-F administration [8]. The extensive 11β-hydroxy steroid dehydrogenase activity of these extrahepatic tissues is important in achieving a low F:E ratio in fetal blood [1–3]. *In utero*, the baboon placenta would contribute to the maintenance of this ratio since, as determined in additional experiments, this organ has extensive 11β-hydroxy steroid dehydrogenase activity (Table 6).

In liver the concentrations of THF, THE, and high polar metabolites* were greater than those in blood (Table 3), identifying this organ as the site of hydroxylation and ring A reduction. There was no evidence of 4-ene-reductase or hydroxylase activities in any other tissue. It appears that ring A reduction [11] and subsequent hydroxylation are favored by an 11-oxo rather than an 11β-hydroxyl function since the ratio of unconjugated THF:THE was 1.2 but that of F:E exceeded 3 (Table 3). In addition, the ratio of unknown I* (a derivative of THF) to unknown II (a derivative of THE) in the high polar fraction was < 1. This is consistent with our earlier finding [8] that, when incubated with newborn baboon liver homogenates, > 45% E or THE was converted to unknown II but < 10% F and < 25% THF was metabolized to unknown I. Thus, we suggest that hepatic metabolism is facilitated by prior extrahepatic conversion of F to E.

The limited capacity to form glucuronoside conjugates resides almost exclusively in liver since only in this tissue was the concentration of conjugates greater than that in blood. The majority of conjugated materials were high polar (Table 5). Sulfo-conjugation of F and its metabolites occurred to a very minor extent in baboon neonates.

* The high polar fraction consisted primarily of two hydroxylated metabolites, designated unknown I and II. In incubations with neonatal liver, unknown I could be derived from tetrahydrocortisol (THF), while unknown II could be obtained from tetrahydrocortisone (THE) [8].

Table 5. Distribution of [^{14}C]-F metabolites in the glucuronoside fraction of baboon liver*

Group (N)	d.p.m. × 10^{-2}/g	% Glucuronoside†	d.p.m. × 10^{-2}/g THF	d.p.m. × 10^{-2}/g THE	d.p.m. × 10^{-2}/g H.P.	% of fraction as: THF	% of fraction as: THE	% of fraction as: H.P.
1 Premature (3)	301 ± 100	13 ± 6	18 ± 6	13 ± 4	216 ± 85	9 ± 4	7 ± 4	67 ± 7
2 Day 1 (4)	175 ± 36	8 ± 2	11 ± 5	23 ± 9	105 ± 30	7 ± 4	15 ± 5	57 ± 5
3 Day 5–12 (3)	320 ± 57	13 ± 4	53 ± 32	99 ± 36	111 ± 25	18 ± 12	32 ± 13	36 ± 4

* Values are mean ± S.E. and normalized to dose of 21.92 μCi.

† d.p.m. released by glucuronidase hydrolysis divided by organ total ^{14}C.

Table 6. Oxidation of F to E by baboon placental homogenates*

Time (Min)	μg F → E/min/g wet weight Placenta 1	Placenta 2
10	0.97	1.21
30	2.37	1.96
60	4.18	2.40
Rate (μg/min/g)	0.063	0.024
Capacity (mg F → E/day/placenta)	9.04	3.98

* Placentas were obtained from 2 baboons following cesarean delivery of viable newborns at 162 and 166 days gestation. After removal of membranes and large blood vessels, placental cotyledons were cleared of blood, weighed (99, 120 g), and homogenized in a Waring Blender in Robinson's Buffer, pH 7.4 [13]. Assays were performed on 8,700 *g* supernatant. Duplicate incubations were performed at 37°C, pH 7.4 for 10, 30, and 60 min. Reaction mixtures, in 10 ml Robinson's Buffer, pH 7.4, contained 0.01 mM TPN^+ and 5–10 μCi 1,2-[^{3}H]-F (18.75 μg). Reactions were begun by addition of placental homogenate (1 ml, 3–4 g tissue). E was isolated as described in Materials and Methods.

Significant alterations in F metabolism in baboon neonates were not evident until 5–12 days after delivery. These include (a) decreased hepatic formation of unconjugated and conjugated high polar metabolites, (b) a proportional increase in THF and THE formation in the glucuronoside fraction of liver, (c) decreased [^{14}C]-F concentrations in blood, (d) decreased [^{14}C]-F:[^{14}C]-E ratios in blood, and (e) an increase in the amount of ^{14}C appearing in urine. These changes resulted in a pattern of metabolism resembling more closely the situation in nonpregnant animals [9] and probably reflect cessation of estrogen stimulation.

The similarity in F catabolic patterns of premature and term newborns (Tables 3 and 5) makes it unlikely that changes in metabolism can account for the increased fetal blood F concentrations prior to parturition [4–6]. Presumably, such increases are attributable to enhanced F production by the fetal adrenal [12] or increased transfer from the maternal circulation.

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