

Concentrations of Progesterone in the Plasma of Mothers and Infants at Time of Birth

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Extract

A highly sensitive method utilizing competitive protein binding was used to study concentrations of progesterone in the plasma of mothers, in umbilical cord plasma, and in the plasma of full-term and premature infants in the immediate postpartum period.

Blood samples were obtained from 20 mothers during labor and from the cords of their infants at delivery. Most of these infants were also studied at 2 or 3 days of age. For purposes of comparison, a second series of samples was obtained from control subjects using needle aspiration to obtain samples from the umbilicus; 14 samples were studied.

In the mothers, concentrations of progesterone in plasma ranged from 46 to 387 ng/ml (mean: 129 ± 78 SD); in cord blood, progesterone levels ranged from 440 to 2,000 ng/ml (mean: $1,030 \pm 412$ SD); and in blood from the umbilical vein, concentrations of progesterone ranged from 310 to 720 ng/ml (mean: 562 ± 140 SD).

A total of 27 full-term newborns was studied at 24, 48, and 72 h of life. In four cases, blood was obtained from the same infant at 12, 24, and 72 h of life. The highest concentrations of progesterone were noted during the first 24 h of life, with levels ranging from 13 to 32 ng/ml (mean: 19 ng/ml) at 12 h, and from 2 to 32 ng/ml (mean: 16 ng/ml) at 24 h. By day 2, levels of progesterone had decreased to 3-20 ng/ml (mean: 10 ng/ml) and by day 3 were reduced to 0-16 ng/ml (mean: 8 ng/ml).

Thirty-two premature infants, with birth weights of from 1,317 to 2,465 g, and ranging in gestational age from 30 to 40 weeks, were also studied. In several cases serial blood samples were obtained by the same methods used with the full-term infants. Two sets of twins were included. In premature infants, as with full-term newborns, a range of 4-34 ng/ml (mean: 14 ng/ml) was found at 12 h of life, decreasing rapidly to 2-6 ng/ml (mean: 3 ng/ml) by 24 h of life. These low concentrations persisted throughout days 2 and 3.

There was no significant difference between concentrations of progesterone in plasma in full-term and premature infants at 2-3 days of age. It appeared that premature infants were as able to clear progesterone from the plasma as were the full-term infants.

Speculation

The data obtained in this study support the concept that large amounts of progesterone are delivered from the placenta to the fetus. This compound may be a precursor of C-21 steroids such as cortisol, synthesized by the fetal adrenal. Progesterone has also been shown to effect respiration in animals

and man [7, 8, 14, 15] and an effect on neonatal respiratory mechanisms and disease may occur, although this has not been demonstrated.

Maternal blood has a relatively large concentration of progesterone which diminishes rapidly following delivery of the placenta. Presumably the compound exerts some effect in the maintenance of pregnancy. Its exact role has not been established.

Introduction

Reports confirm that there is a marked increase in the concentration of progesterone and its metabolites in maternal blood and urine during pregnancy [12, 13, 19, 21]. There is a rapid decline following delivery of the infant and placenta [12, 13, 21]. Previous studies have included determination of progesterone concentration in maternal and cord blood, but only two studies are available [6, 10] regarding concentrations of progesterone in the blood of the neonate. Because methods used previously required large amounts of blood, study of young infants was not practical; now, however, the competitive protein-binding assay, described by MURPHY [16] and modified by NEILL *et al.* [17], is sufficiently sensitive so that measurements can be made with very small samples of blood. This technique [17] has been used in this investigation to measure concentrations of progesterone in blood from mothers, cords, and infants at delivery, and in mothers and babies during the immediate postpartum period. Data from studies of infants delivered prematurely were also compared with those obtained from babies delivered at term.

Materials and Methods

Group 1 consisted of 20 mothers at time of delivery of term pregnancy. Peripheral venous blood was obtained from these mothers on the delivery table during active labor. Immediately upon delivery of the placenta, blood was milked from the cord. Then, at 2–3 days postpartum, blood was again obtained from the mothers and infants. Blood was heparinized and the plasma was separated and frozen within 1 h. Blood obtained from the umbilical vein by needle aspiration was studied in a different series of 14 cases for comparison with cord blood.

Group 2 consisted of 27 full-term newborns, some of which were born to mothers in group 1. These infants were normal and the deliveries were uncomplicated. Blood was obtained between 12–24 h of life in 10 instances, 24–48 h in 12 instances, and up to 72 h of life in 13 instances. In four instances, blood was obtained from the same infant at 12, 24, 48, and 72 h of life.

Group 3 consisted of 32 premature infants ranging in birth weight from 1,317 to 2,465 g; it included two sets of twins. Length of gestation varied from 30 to 40 weeks. Blood was obtained from these infants at approximately 12, 24, 48, and 72 h of life. In three cases, serial blood samples were obtained.

The method used to determine concentrations of progesterone in plasma has been described in detail [17] and is based on the competitive binding of progesterone and corticosterone on the corticosteroid-binding globulin (CBG) of dog plasma. Extraction of 0.1–0.5 ml of plasma is performed and the extract is subjected to thin-layer chromatography (TLC). Markers appearing above and below the progesterone spot are used to locate the progesterone more accurately. Isatin, a yellow dye which has an R_f value identical to 17 α -hydroxyprogesterone and thus moves behind progesterone in our solvent system, is used as one marker. The other marker is an unknown substance in petroleum ether that fluoresces blue-white and that moves ahead of progesterone. During extraction, losses vary from 10 to 25% depending upon the purity of the petroleum ether used. The remaining loss occurs in the TLC sequence. Radioactive progesterone, added to pooled male plasma, has an average final recovery rate of 50%, and the results reported below have been corrected for this loss.

One milliliter of diluted male dog plasma, to which tritiated corticosterone was added, was pipetted into tubes containing dried eluates obtained from TLC. Tubes were then placed in a water bath, 45° for 5 min, mixed, and transferred to an ice bath for 10 min. Eighty milligrams of Florisil were added to each tube and the solution was mixed for 30 sec; an aliquot of the supernatant was removed and counted. A standard curve was prepared in duplicate using 1, 2, 5, and 10 ng of progesterone. The amount of progesterone in each sample was determined by comparing the values with a standard curve established with each assay. These values were corrected for procedural losses and dilution factors. The recovery of a given amount of progesterone added to pooled male plasma provided a control on substances that might interfere in the protein-binding assay; this control was processed with each assay.

The use of petroleum ether as an extraction medium confers some degree of specificity to this method be-

cause the cortisol and similar steroids abundant in plasma during pregnancy are removed even before the TLC step. Progesterone was separated from remaining compounds by TLC, using markers that moved on either side as well as in front of and behind the progesterone spot. Analysis of competitive protein binding shows a degree of specificity for individual steroids [16]. Results obtained with the competitive protein-binding method have compared favorably [9] with results obtained using a double isotope derivative method. The double isotope-derivative method for this comparison was a modification of that described by WOOLEVER and GOLDFEIN [20]. The correlation coefficient between the results of the two methods was 0.83.

Results

In group 1 (table I), the peripheral venous plasma obtained from the mothers at the time of delivery contained a mean concentration of 129 ng/ml of progesterone (range: 46–387 ng/ml; sd: ± 78 ng/ml). Umbilical vein blood obtained by needle aspiration contained a mean concentration of progesterone of 562 ng/ml (range: 310–720 ng/ml; sd: ± 140 ng/ml). Cord plasma had greater concentrations of progesterone (mean: 1,030 ng/ml; range: 440–2,000 ng/ml; sd: ± 412 ng/ml). By day 2 or 3 postpartum, levels of progesterone in maternal plasma had dropped to a mean of 8 ng/ml (range: 1–24 ng/ml; sd: ± 6 ng/ml). Similar concentrations of progesterone were found in the plasma of the infants (mean: 9 ng/ml; range: 0–17 ng/ml; sd: ± 4 ng/ml).

In group 2 (table II), which consisted of 27 full-term newborns, the highest concentrations of progesterone in plasma were noted during the first 24 h of life. After this time, most of the infants had cleared the plasma of significant amounts of progesterone. In five cases studied at 12 h of life, concentration levels ranged from 13 to 32 ng/ml (mean: 19 ng/ml). At 24 h, the range was 2–32 ng/ml (mean: 16 ng/ml). By day 2 of life, progesterone concentrations in plasma decreased to 3–20 ng/ml (mean: 10 ng/ml), and by day 3 postpartum, 0–16 ng/ml (mean: 8 ng/ml) were observed.

Group 3 consisted of 32 premature infants (table III). Here also the highest concentrations of progesterone were found during day 1 of life. A range of 4–34 ng/ml at 12 h of life (mean: 14 ng/ml) decreased rapidly to 2–6 ng/ml (mean: 3 ng/ml) by 24 h. These low levels persisted throughout days 2 and 3 postpartum.

We were unable to subject the data on newborns and prematures to statistical analysis since the exact time intervals were not known for each individual studied. Group comparison revealed no significant

difference between newborn and premature infants for the method used.

Discussion

It has been shown by LURIE *et al.* [13], using gas-liquid chromatography (GLC), that there is a steady increase in concentrations of progesterone in blood as pregnancy progresses. They reported a slight decrease before the onset of, and again during, active labor. In one case, LURIE *et al.* observed that a marked decrease in pro-

Table I. Full-term pregnancies¹

Mothers	Cords	Progesterone, ng/ml		
		Mothers, 2–3 days postpartum	Babies, 2–3 days postpartum	Umbilical vein ²
112	1,040	8	15	720
128	1,660	6	8	720
387	1,010	3	—	680
92	620	1	6	660
188	1,080	24	—	640
264	2,000	12	4	640
105	440	2	0	620
118	900	2	1	600
46	780	12	11	600
120	940	13	11	520
118	1,040	15	12	520
—	920	—	16	330
136	1,430	16	17	310
114	1,716	14	11	310
139	1,276	13	12	
66	835	6	10	
146	—	5	10	
55	515	4	5	
49	528	4	4	
69	845	6	5	
Range				
46–387	440–2,000	1–24	0–17	310–720
Mean \pm sd				
129 \pm 78	1,030 \pm 412	8 \pm 6	9 \pm 4	562 \pm 140

¹ Concentrations of progesterone in plasma in mothers, in cords at time of delivery, and in mothers and infants 2–3 days postpartum.

² The umbilical vein samples were obtained from another series of cases.

gesterone levels occurred within 10 min after delivery of the placenta. YANNONE *et al.* [21] have recently reported similar results although they did not note a decrease before the onset of labor. LLAURO *et al.* [12] have recently studied, in serial fashion, several women from 34 weeks gestation to onset of labor and after delivery; they report similar findings. Our data (table I) for progesterone in maternal plasma from peripheral veins agree reasonably well with those of LLAURO *et al.* [12] who found concentrations of $20.7 \pm 7.3 \mu\text{g}/100 \text{ ml}$ at the onset of labor, and with HARBERT *et al.* [10] who

found progesterone levels of $15.4 \mu\text{g}/100 \text{ ml}$ in the maternal vein. In this study, concentrations of progesterone in plasma from the umbilical vein were between the mean of $72.4 \mu\text{g}/100 \text{ ml}$ found by HARBERT *et al.* [10] and $37.2 \mu\text{g}/100 \text{ ml}$ found by ZANDER [22]. The discrepancy between the values in blood drained from the cord and in blood aspirated from the um-

Table II. Concentrations of progesterone in plasma in full-term newborn infants from 1-3 days of age

Infant no.	Progesterone conc in plasma, ng/ml			
	0-12 h	12-24 h	24-48 h	48-72 h
1			9	
2			20	
3		30		
4		32		
5			17	
6			3	
7				1
8			15	
9			8	
10		4		
11		6		
12		16		
13				2
14				0
15				1
16				11
17				11
18				12
19				16
20			17	
21			11	
22				12
23	32 ¹	14	5	10
24	13 ¹	2	11	10
25	13 ¹	8	3	5
26	15 ¹	9	3	4
27	20	16		
Range	13-32	2-32	3-20	0-16
Mean	19	16	10	8

¹ Blood was obtained from this infant at 12, 24, 48, and 72 h of life.

Table III. Concentrations of progesterone in plasma in premature infants from 1-3 days of age

Gesta- tion, wk	Birth wt, g	Progesterone conc in plasma, ng/ml			
		0-12 h	24 h	48 h	72 h
40	2,465		2		
36	2,438			1	
40	2,438				2
39	2,352			3	
38	2,297		2		
38	2,297		5		
36	2,270				2
36	2,252	8			
	2,247		5		
30	2,238			2	
40	2,225		2		
36	2,211	27			
40	2,175				4
32	2,175				3
40	2,156		2		
37 ¹	2,125	8	4		
30	2,120	6			
35	2,106			2	
36	2,097	10			
	2,070			4	
40	2,043	16			
35	1,975		6		
35 ¹	1,943	4	2	2	
40	1,930	34			
	1,930		2		
32-34 ¹	1,930	5	2	4	
30	1,898		4		
40	1,884				2
	1,702		2		
40	1,607	27			
	1,416			5	
	1,317			3	
Range		4-34	2-6	1-5	2-4
Mean		14	3	3	2

¹ Blood was obtained serially in these infants.

bilical vein is unexplained. LURIE *et al.* [13] found a progesterone concentration of 71.0 $\mu\text{g}/100$ ml in the intervillous pool compared with 61.5 $\mu\text{g}/100$ ml in the umbilical vein.

It has been known for some time that progesterone is produced by the human placenta in large amounts [19]. The reason for production of such concentrations of progesterone as pregnancy progresses to term has not been determined. Studies [4] performed during the late first and midtrimester (10–27 weeks of gestation) of pregnancy, in cases of threatened abortion, revealed no definite correlation with progesterone levels in plasma. Some patients aborted despite normal levels. Other studies [1, 21] have shown that progesterone concentration in plasma does not decrease prior to onset of labor. In cases of induced abortion [2], pregnanediol excretion in the urine decreased after the umbilical cord was severed, but while the placenta remained *in situ* a constant level of excretion was maintained.

There have been two studies of progesterone concentration in the plasma of newborn infants; one [6], using a bioassay method for progestins, revealed no detectable amounts in the plasma or serum of the newborn after 45 h postpartum; the second study [10] revealed that progesterone was detectable in 50% of the neonates studied at 12 or 24 h postpartum. To date, there have been no reports of progesterone in plasma in premature infants. A study of placental tissues of spontaneous abortions and premature births [11] showed that levels of progesterone in these tissues were less than in the placentas of normal pregnancies.

The major urinary metabolite of progesterone in the mother is pregnanediol. Urinary metabolites of progesterone have been studied during the newborn period utilizing GLC techniques [5, 19]. RAMOS *et al.* [18] revealed that pregnanediol glucuronide was present until day 5 of life, after which it was not detectable in most infants. These results are consistent with the changes in progesterone concentrations in plasma found in our study of full-term newborns during the first few days of life.

Conflicting reports of excretion of pregnanediol in the urine in premature births have been published. It is known that newborns do not convert progesterone into pregnanediol as effectively as adults [3], therefore, studies of metabolites in the urine often are not as satisfactory a method for investigating steroid metabolism as is measurement of plasma concentrations of the hormone. For this reason, we have attempted to establish the range of normal progesterone concentrations in the plasma of full-term and premature infants of varying birth weights and gestational ages. There appears to be no significant difference between premature and full-term newborns at 2–3 days of age.

From our results (tables II and III), it would appear that premature infants are able to clear the body of progesterone as well as the full-term infants studied here.

Summary

Twenty full-term births were studied to determine the range of normal levels of progesterone in plasma in peripheral venous blood of mothers at delivery, in cord blood, and in plasma of mothers and babies at 2–3 days postpartum.

Twenty-seven full-term newborns were studied at 1–3 days of age and the progesterone levels reported in the plasma were compared with the progesterone levels found in the plasma of 32 premature infants during the first 3 days of life. No significant difference was noted between these two groups. The premature infants were able to clear the plasma of progesterone as well as the term infants.

References and Notes

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