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Thyroid Function Studied in Paired Maternal-Cord Sera and Sequential Observations of Thyrotropic Hormone Release during the First 72 Hours of Life

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Extract

Pituitary thyrotropic hormone (TSH) was measured in seven normal newborns followed during the first 72 hr of life. These infants demonstrated an acute unsustained release of TSH in the early minutes of postnatal life. Peak levels occurred between 15 and 30 min after birth, increasing from cord levels ranging from 3.2–10.7 μunits/ml to a maximal elevation in sera of 25–163 μunits/ml. This rise was followed by a steep decline in serum levels at an initial rate comparable to that reported for the half-life of the hormone. A slower rate of decay was noted after 120 min.

The TSH levels in 20 normal paired maternal and cord samples indicated that cord levels were consistently higher ($t = 5.90$, $P < 0.001$). Maternal levels were comparable to those found in nonpregnant adult females; however, cord levels of TSH were higher than that expected for normal children. Specificity of the TSH assay would indicate that immunoreactive TSH in maternal and cord sera was of pituitary origin.

Maternal thyroxine levels were significantly greater than those of cord sera ($t = 3.31$, $P < 0.01$), reflecting similarly higher levels of thyroxine-binding globulin (TBG) in maternal specimens ($t = 7.31$, $P < 0.001$). No significant differences ($t = 1.11$, $P > 0.2$) existed when levels of free thyroxine (FT_4) in maternal and cord sera were compared.

Speculation

The relatively high levels of TSH in cord serum in the presence of normal or high normal free thyroxine concentrations would suggest that the hypothalamic-pituitary responsiveness to thyroxine in the fetus is altered. Free thyroxine concentrations that maintain TSH in the euthyroid range in normal children are apparently unable to do so in the fetus. Any other hypothesis for this observation must necessarily postulate a persistent stimulus to TSH secretion *in utero*. One such postulated stimulus could be a relatively low serum concentration of free triiodothyronine (T_3) in the fetus.

Introduction

Laboratory findings suggestive of hyperthyroidism in the neonate were first described by Danowski *et al.* [2] in 1951. Subsequently, many studies have examined different aspects of thyroid physiology in the first hours of life. Thyroid metabolism in the newborn, however, is still not totally understood. In this study we have assessed some of the more important variables

of pituitary-thyroid function in an attempt to clarify the nature of this apparent hyperthyroid state.

Materials and Methods

Twenty paired maternal and cord sera samples were studied. Informed consents were obtained from all subjects in accord with the Helsinki Declaration. All the mothers were normal, healthy, pregnant women and delivered normal infants by the vaginal route without complication. Epidural anesthesia was routinely used.

Seven of the 20 newborns were followed during the first 2 or 3 days of life. Blood was drawn at 15, 40, 60, and 120 min, and at 6, 24, 48, and 72 hr of life. Venipunctures were done to obtain maternal specimens, and the cord, 6-hr and 24-hr specimens from the infants. The other blood samples from infants were obtained by capillary bleeding. Cord and maternal sera were drawn simultaneously at the time of delivery.

The TSH assay was performed by a double antibody technique [21]. Highly purified human thyrotropic hormone (HTSH) (2.5 μg) was iodinated with 400 μCi ^{125}I by the method of Greenwood, Hunter, and Glover [12]. Serum samples were prepared in an incubation volume of 1.0 ml composed of 100 μl HTSH antisera [30], 1:20,000; 100 ml human chorionic gonadotropin (HCG) (20 IU); 100 μl 0.1 M; 100 ml ^{125}I-HTSH (0.1 ng); 400 μl serum; and 200 μl phosphate-buffered saline with 0.1% bovine serum albumin. Results are expressed as microunits per milliliter of Human Thyrotropin Research Standard A [31]. All standards contained 400 μl bovine serum. Appropriate amounts of bovine serum were added to samples so that each tube contained at least 400 μl serum. Two microliters normal rabbit serum were added to each tube. After incubation for 6 days at 4°, sheep antirabbit gamma globulin antiserum, which was titrated for maximal precipitation, was added. Specimens were centrifuged and counted after incubation for 24 hr at 4°. Samples were assayed in triplicate, and all sera were measured in the same assay. Several specimens and one serum sample from a hypothyroid subject were assayed at different dilutions to demonstrate proportionality. Intraassay variability at 95% confidence limits for a single serum, which was measured 10 times in duplicate on the sensitive portion of the standard curve, was 4.7%. All normal sera measured less than 7.0 μunits/ml. Forty percent of sera from normal adults and children was below the limits of sensitivity (1.25 μunits/ml). Paired TSH values from venous and capillary serum obtained simultaneously were comparable (Table 1).

Table 1. Comparison of TSH levels[1]

Sample	TSH, μunits/ml	
	Venous	Capillary
1	2.6	2.9
2	2.3	2.6
3	1.6	2.1
4	10.0	8.6
5	5.3	5.5

[1] Paired sera samples obtained from venous and capillary blood.

Serum thyroxine concentrations were determined by the Murphy-Pattee method [32]. Results are expressed in micrograms thyroxine per 100 ml. All determinations were corrected for a recovery of 77%. Coefficient of variation for intraassay variability at 95% confidence limits was 8.0%.

Thyroxine-binding globulin (TBG) was assessed by reverse flow paper electrophoresis in glycine acetate buffer at pH 8.6 [15]. Each sample was enriched with <6 μg/100 ml tracer ^{131}I-T_4. Cord sera were assayed for maximal binding capacity with 200 and 300 μg/100 ml and maternal sera with 300 and 400 μg/100 ml of added cold thyroxine, respectively. A current of 12.0 ma was applied for 18 hr, and the electrophoresis strips were dried and scanned for radioactivity in an automatic gas flow counter. The distribution of radioactivity was assessed by manual planimetry. The coefficient of variation for intra- and interassay variability at 95% confidence limits was 11.2 and 16.7%, respectively.

Free thyroxine was assayed by the method of Sterling and Brenner [28]. Purity of the ^{131}I-T_4 [33] was determined by thin layer chromatography on cellulose plates with the use of a two-dimensional system [5]. Thyroxine accounted for 94.6%, iodide for 3.0%, triiodothyronine for 1.3%, and diiodotyrosine for 1.1% of the radioactivity, respectively. The ^{131}I-T_4 was dialyzed against 8 liters of 0.15 M potassium phosphate buffer, pH 7.4, for 48 hr prior to use in the assay [27]. All samples were assayed in duplicate in a single assay at a dilution of 1:25. The free thyroxine fraction (FT_4F) was corrected for the dilution, and the absolute free thyroxine concentration (FT_4) was calculated as the product of the FT_4F and the serum thyroxine concentration. The coefficient of variation for intraassay variability at 95% confidence limits was 5.7%.

Results

An acute increase in TSH levels occurred within the first minutes of life in the seven newborns studied. Peak values occurred between 15 and 30 min and

Table II. TSH, thyroxine, free thyroxine, and thyroxine-binding globulin concentration in 20 paired samples of maternal and cord sera

Samples	TSH, μunits/ml		T_4, μg/100 ml		TBG, μg/100 ml		FT_4F, $\times 10^{-2}$/100 ml		FT_4, ng/100 ml	
	Maternal	Cord	Maternal	Cord	Maternal	Cord	Maternal	Cord	Maternal	Cord
1	<1.25	3.2	15.9	15.9	44.6	28.3	1.90	2.10	3.02	3.34
2	<1.25	2.1	18.2	13.0	44.6	22.3	2.10	2.68	3.82	3.48
3	2.3	6.8	17.1	10.7	32.1	21.7	1.85	2.99	3.16	3.20
4	<1.25	10.7	16.3	18.2	43.4	28.0	2.15	2.45	3.50	4.46
5	1.35	8.7	15.0	19.5	41.8	24.5	2.43	2.09	3.65	4.08
6	<1.25	2.6	11.4	11.4	39.4	27.3	2.17	1.97	2.47	2.25
7	<1.25	4.4	17.7	18.7	39.8	32.7	—	—	—	—
8	4.0	4.0	15.6	16.3	52.5	26.7	2.14	2.26	3.34	3.68
9	1.3	2.1	17.7	19.7	38.2	16.1	2.38	2.70	4.21	5.32
10	1.7	7.8	14.8	9.9	81.9	28.0	2.47	2.96	3.66	2.93
11	2.0	3.1	12.4	7.8	61.5	—	2.23	5.16	2.77	4.02
12	5.1	7.2	13.5	11.8	67.1	33.7	3.17	3.37	4.28	3.98
13	1.8	8.2	10.4	8.2	64.5	31.4	2.76	2.92	2.87	2.39
14	<1.25	2.5	10.9	9.1	44.8	27.5	2.09	3.27	2.78	2.98
15	<1.25	7.8	9.8	10.7	36.7	22.1	—	—	—	—
16	1.8	5.0	10.6	9.0	45.0	18.5	2.04	3.37	2.16	3.03
17	<1.25	6.9	13.0	11.4	59.8	20.8	3.28	4.11	4.26	4.69
18	<1.25	4.0	13.8	11.2	28.1	20.6	2.70	2.98	3.73	3.34
19	<5.7	6.2	13.5	8.8	28.1	16.0	2.70	—	3.65	—
20	1.7	6.5	15.6	9.9	33.7	26.2	—	—	—	—
Number	20	20	20	20	20	19	17	16	17	16
Mean ± SD	2.0[1]±1.3	5.5±2.5	14.2±2.6	12.6±4.0	46.3±14.1	24.7±5.1	2.39±0.41	2.96±0.82	3.37±0.64	3.57±0.84
Paired t test	$t = 5.90$		$t = 3.31$		$t = 7.31$		$t = 4.66$		$t = 1.11$	
	$P < 0.01$		$P < 0.01$		$P < 0.001$		$P < 0.001$		$P > 0.2$	

[1] Limits of sensitivity of the method were 1.25 μunits/ml.

maximal values ranged between 25 and 163 μunits/ml. No correlations were found between the height of the peak and level of TSH in the cord. The TSH disappeared very rapidly, falling to 50% of the peak values 2 hr after birth and to 20% at the end of day 1. By 48–72 hr the values had returned to the levels found in cord serum. The TSH levels in the newborns, between 30 and 120 min after birth, fell in as an exponential decay. The calculated half-life ranged from 44 to 80 min. After 120 min the decay was much slower.

Data obtained from the paired maternal and cord sera samples are recorded in Table II. Maternal TSH levels ranged from <1.25 to 5.7 μunits/ml and were within the limits for normal nonpregnant women (<1.25–7.0 μunits/ml). The TSH levels in cord sera (2.1–10.7 μunits/ml) were higher than those found in normal children 5–12 years of age (<1.25–7.0 μunits/ml). All cord values were within the range of sensitivity of the assay, whereas 40% of the sera samples found in normal adults and children are below the range of sensitivity. In each of the 20 cord samples, the TSH values were higher than those found in corresponding maternal sera. The difference was significant by the paired t test ($t = 5.90$, $P < 0.01$).

Thyroxine levels in maternal sera were significantly higher than those in the corresponding cord samples ($t = 5.90$, $P < 0.01$). This was not uniform for all individual pairs, however, as some showed no difference or higher values in the cord sample.

The TBG capacity measurements also indicated higher levels in maternal than in cord sample ($t = 7.31$, $P < 0.001$). This was true for all individual pairs, although some cord values overlapped the maternal range when each group was considered separately.

The FT_4F level was significantly lower in the maternal specimens than in the cord specimens ($t = 4.66$, $P < 0.001$), probably reflecting the higher TBG capacity. The mean cord level of FT_4 was slightly higher than the mean maternal level, but it was not statistically different when examined by the paired t test.

Discussion

Amounts of thyrotropic hormone in maternal and cord blood have been previously measured using a bioassay by Yamazaki and co-workers [29]. These authors found no differences between maternal and cord sera. Our data, along with those of Fisher *et al.* [8] and

Robin *et al.* [26], demonstrate significantly higher cord levels for immunoreactive TSH. However, in light of the recent identification of a human chorionic placental thyrotropin (HCT) [13, 14] and the demonstration that maternal and cord sera contain high levels of HCG [9], establishing that the immunoreactive TSH measured in maternal and cord sera is TSH of pituitary origin becomes exceedingly important in interpreting these data.

The antihuman pituitary TSH antiserum [30] reacts very poorly with HCT. Hershman and Starnes [14] noted that 100 μg purified placental thyrotropin with a potency of 1.5 munits/mg on bioassay is equivalent to only 3 μunits HTSH in the immunoassay. Fisher *et al.* [9] reported that 2000 times more HCT than HTSH, by weight, was required to produce equivalent displacement of radioiodinated HTSH from this particular antibody. In addition, HCT as measured by bioassay was found to be near nonpregnant levels in maternal sera at term [13]. The possibility that this hormone accounts for any of the measurable TSH in maternal or cord sera would thus seem remote.

Cross-reactivity of the antiserum [30] with HCG and luteinizing hormone (LH) has been previously demonstrated [20]. After adsorbing the antiserum with large amounts of HCG (20 IU), however, a very significant and specific dose-response curve for HTSH remained and cross-reactivity was abolished [20]. Fisher *et al.* [9] also noted that increasing the HCG from 30 to 60 units decreases the percentage of bound counts only 2%. This observation was confirmed in our laboratory. The LH Ag cross-reacts in the adsorbed system only to the extent that it is contaminated with TSH [20]; therefore, LH and HCG are not measured in this assay system.

Additional evidence of homogeneity of the assayable TSH was demonstrated by the observation that serum samples assayed in different dilutions showed a parallel displacement comparable to that of the human pituitary standard curve. It would be reasonable to conclude that immunoreactive TSH in the maternal and newborn serum is in fact TSH of pituitary origin.

Whether the high levels of TSH in cord blood represent actual fetal levels of the hormone, and whether the stimulus responsible for the postnatal release of TSH is operative at term remain moot. Fisher and Odell [8] were able to demonstrate similarly high levels of TSH in infants' scalp vein blood drawn prior to delivery. In a recent study [11] we have investigated the thyroid function of 21 human fetuses (10–24 weeks gestational age) and have shown that TSH was detected by radioimmunoassay in significant amounts in nearly all instances. After 16 weeks gestation almost all TSH levels were comparable to those seen at term (>4 μunits/ml).

It also is unlikely that maternal TSH contributes to the high concentrations seen in cord sera. We were able to demonstrate that TSH concentrations in maternal sera are lower than those in cord samples (Table I), and that these differences are present during most of fetal life [11]. These data are in accord with the observation in animals that TSH is unable to cross the placenta in significant amounts [24]. It seems likely, then, that the TSH measured at birth in cord samples are true reflections of the fetal environment and are only of fetal origin.

The explanations for the high fetal and cord TSH levels remain speculative. The fetal hypothalamic-pituitary mechanism apparently is not suppressible by free thyroxine levels which are at the normal or upper normal range for older infants (Table II). Other explanations require that some abnormal stimulus to TSH secretion *in utero* is present during most of fetal life.

The acute release of TSH observed in normal newborns during the first minutes of life agrees with the observations of Fisher and Odell [8]. The phenomenon occurred only in the newborns and not in the mothers. Serum TSH levels, determined in two mothers before delivery, and 1, 2, and 24 hr after delivery, were constant. These findings are also in accord with those of Fisher and Odell [8].

The release of TSH during the early minutes of life occurs acutely and probably without sustained hypersecretion during the subsequent 120 min. The half-life during this period (44–80 min) is comparable to that reported by Odell *et al.* for the radioiodinated TSH (39–67 min) [19], but it is slightly longer than has been estimated for a pulse injection of HTSH (40 min) [1]. The slower decay rate after 120 min and the persistance of cord levels at 72 hr of life could represent either a hypersecretion of TSH at this time or a slowing of the metabolic clearance rate during the period of fetal adjustment to extrauterine life.

The explanation for the striking increase in TSH levels is obscure. The stress of delivery is not the stimulus for TSH secretion, as babies born by cesarean section present the same TSH release phenomenon [8]. There is considerable evidence in animals that cold is a stimulus for TSH secretion [4, 16]. In human adults, however, Odell *et al.* [22] were unable to show TSH release during cold exposure. Fisher and Oddie [7] have shown that prevention of neonatal cooling minimized the usual marked increase in protein-bound

plasma iodine (PBI) at the end of day 1. Similarly, Fisher and Odell [8] demonstrated that cooling babies at 3 hr of life produces a release of TSH, proving that exposure to a relatively cold environment is a TSH stimulus in the newborn. These authors, however, were unable to prevent the TSH peak from occurring by heating the babies just after birth. Possibly this was because a very short exposure to cold could not be avoided. The question of the role of cold in the acute release of TSH, then, remains unanswered, and a totally satisfactory explanation of the acute TSH release immediately after birth awaits further study.

Other thyroid variables at birth have been assessed many times, and the literature offers many contradictory observations. Some investigators have found higher mean PBI and butanol-extracted iodine values in maternal blood at delivery than in cord blood [3, 6, 10, 18], while others have found no difference [2, 9, 17, 22, 23, 25]. We have shown that maternal blood thyroxine levels are slightly, but significantly, higher than those in cord samples. This difference probably reflects the higher maternal TBG.

Some investigators have reported higher cord values for FT_4 [9, 24, 25]; others have found values comparable to those of maternal serum [3]. Because FT_4 is calculated as the product of total thyroxine and FT_4F in serum, the discrepancies in FT_4 reported from different laboratories can partly be explained by different methods used to estimate thyroxine in serum. This fact does not allow any confidence in statements concerning maternal-fetal FT_4 difference at term. In addition, we have recently shown that maternal FT_4 levels are consistently higher than FT_4 levels found in fetuses of from 11 to 20 weeks gestation [11]. This observation would suggest that maternal-fetal FT_4 difference at term has little meaning in terms of early fetal environment.

Summary

Seven normal newborns monitored during the first 72 hr of life had an acute, but unsustained, release of pituitary TSH in the early minutes of postnatal life followed by a steep decline in serum levels at an initial rate comparable to that reported for the half-life of the hormone. The slower decay rate noted after 120 min and the persistance of hormone in blood at 72 hr of life would indicate that either a hypersecretion of TSH occurs at this time or there is a slowing of the metabolic clearance rate of TSH during the period of fetal adjustment to extrauterine life.

The TSH levels in 20 normal cord sera samples were consistently higher than paired maternal specimens. Maternal levels were comparable to those of nonpregnant adult females; however, levels of TSH found in cord specimens were higher than those expected for normal children. The specificity of the TSH assay would indicate that the immunoreactive TSH present in maternal and cord sera was of pituitary origin.

Maternal thyroxine levels were significantly greater than those found in cord sera, reflecting correspondingly higher levels of TBG in maternal specimens; FT_4, however, was found to be not significantly different.

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