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SERUM TOTAL ESTRIOL IN ABNORMAL NEWBORN INFANTS

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SUMMARY

Serum total estriol was determined in blood samples obtained from 128 newborn infants admitted to a newborn intensive care unit. Forty of the infants were studied by serial sampling. The estriol was assayed following hot acid hydrolysis of the serum, using a highly specific antibody to estriol. The mean values of serum total estriol, on the first day of life, ranged from 959 ± 413 (S.D.) ng/ml in infants <30 wks gestation to 1127 ± 489 (S.D.) ng/ml in infants 33–35 wk gestation. Mean values fell rapidly postnatally, to 151 ± 131 (S.D.) ng/ml in 11–25 day infants, and 39 ± 20 (S.D.) ng/ml in infants 35–73 days of age. There were no significant differences between total estriol values, on the first day of life, of infants with and without hyaline membrane disease. The $t_{1/2}$ of the endogenous total estriol could be determined in 21 infants. The $t_{1/2}$s tended to be less with increasing gestational age, the regression equation being $y = 5.98 - 0.128 \times (r = -0.51)$. Intrauterine growth retarded and dysmature infants >35 wk gestation had a mean serum total estriol level of 950 ± 477 (S.D.) ng/ml on the first day of life. This value is similar to the mean of the appropriate for gestational age infants >35 wk, 1028 ± 440 (S.D.) ng/ml. Very slow declines in serum total estriol were found in 4 infants with intestinal hypomotility who developed necrotizing enterocolitis. The cause may be enterohepatic shunting of estriol as a result of the delay in clearing meconium from the intestinal tract.

INTRODUCTION

A predominance of estriol over the other principal estrogens, estrone and estradiol-17β, in circulating blood and in urine is a characteristic of human pregnancy. Estriol is produced in the placenta principally from fetal-derived 16α-hydroxylated neutral steroid precursors, the most important of which is 16α-OH-dehydroepiandrosterone sulfate [1]. Most of the estriol is transferred across the placenta to the mother, but a substantial amount circulates back to the fetus. In addition, the fetus is able to produce estriol by hepatic 16α-hydroxylation of circulating estrone and estradiol-17β [2].

Ninety percent, or more, of the circulating estriol in the fetus and newborn infant is conjugated [3, 4, 5]. The sulfate conjugate is predominate [3, 4] with lesser amounts of estriol present in the sulfo-glucuronide double conjugate form, as demonstrated by isotopic studies in midgestation fetuses [5] and in an anencephalic infant at term [6]. A rapid decline of serum estriol postnatally would be expected because production ceases with separation of the fetus from the placenta. The postnatal changes in serum concentration of total estriol (unconjugated plus all conjugates) have been studied only by Schwers and Wolter[7] who investigated full-term infants in the first 3 days of life. They found the total estriol level to fall exponentially with an approximate half-life ($t_{1/2}$) of 20 h. In this present study we have measured serum total estriol in premature and term newborn infants by a sensitive radioimmunoassay procedure following acid hydrolysis of serum samples. In the first day of life, the mean serum total estriol concentrations were similar in infants with and without hyaline membrane disease. Premature infants of varying gestational ages were studied by serial sampling of blood during the first several weeks of life. We found the duration of the $t_{1/2}$ of endogenous serum total estriol to be inversely correlated with length of gestation.

EXPERIMENTAL

Patients

The results to be discussed below are based on analyses of blood samples obtained from 128 newborn infants admitted to St. Paul Children's Hospital Newborn Intensive Care Unit, 40 of whom were studied by serial sampling. The infants were transferred from the hospital where they were born for various indications, the major diagnoses being immaturity or immaturity plus signs of respiratory distress. Excluded from this study were infants with multiple anomalies, infants of diabetic mothers (including gestational diabetes) and infants with chronic fetal viral infections. Infants with intrauterine growth retardation (IUGR) and term or post-term infants with signs of dysmaturity were analyzed separately from infants

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who showed no fetal growth retardation. For purposes of the analyses presented in this paper, patients who were appropriate in size for gestational age were divided into three groups: those with hyaline membrane disease (HMD) who survived, those with HMD who died in the first two weeks of life, and those without hyaline membrane disease. Patients in this last group had diagnoses of prematurity and/or benign respiratory distress (transient tachypnea of the newborn) [8]. HMD was diagnosed using the classical criteria of dyspnea, tachypnea and cyanosis in room air in association with a reticulogranular pattern in the peripheral lung fields on chest x-ray. Gestational age was determined from the date of the last normal menstrual period of the mother and was confirmed in all cases by physical examination. IUGR was diagnosed when the infant had a birth weight below the tenth percentile of normal weight for gestational age, using the intrauterine growth standards of Lubchenco *et al.* [9]. The clinical investigation was approved by the University of Minnesota Committee on the Use of Human Subjects in Research.

Procedures for total serum estriol assay

Materials. Borate buffer, pH 7.8, was used throughout. Unflavored gelatin, 1 gm/l, and sodium azide, 1 gm/l, were added to the stock solution. Nonradioactive estriol was purchased from Schwarz-Mann Laboratories (Orangeburg, NY) and was recrystallized four times before use. Estriol-3-sodium sulfate was purchased from Steraloids, Inc. (Wilton, NH). [6,7-^{3}H]-estriol (42.2 Ci/mmol) and [2,4,6,7-^{3}H]-estriol (95.6–112 Ci/mmol) were purchased from New England Nuclear Corporation (Boston, MA). Each was diluted to 50 ml in methanol for storage at −20°. Ether was "USP for Anesthesia" grade in 1/4 lb cans from Mallinckrodt Chemical Works (St. Louis, MO).

Acid hydrolysis. Fifteen ml glass-stoppered conical centrifuge tubes were used in the hydrolysis and extraction steps. They were acid washed before each use. 50 μl serum was added to a tube, followed by about 2000 c.p.m. [6,7-^{3}H]-estriol (about 13 pg) [10] in 10 μl methanol. The acid hydrolysis procedure was that of Nachtigall, *et al.*[11] with the modification that, following the addition of water, the tube was incubated for 30 min at 37°. Hydrolysis was carried out in a glycerine bath at 103–105° for 45 min.

Extraction. Seven ml of ether from a freshly opened can were added to each tube. Following vigorous shaking for 30 s, the tube was immersed in acetone-dry ice. When the aqueous phase froze, the ether was decanted. The extraction was repeated and the combined ether extracts were washed once with 0.1 vol. water. The ether phase was decanted following a further freezing of the water phase. The washed ether extract was dried under nitrogen in a 41° water bath and the residue was taken up in 1.0 ml methanol. A 0.5 ml aliquot was taken for determination of [^{3}H]-estriol recovery.

Radioimmunoassay. Triplicate aliquots of 15 μl and triplicate aliquots of 30 μl were taken of the extract residue which had been dissolved in 1.0 ml methanol. Each aliquot was transferred to a methanol washed disposable 16 × 100 mm glass culture tube and dried under N_2 in a 41° water bath. 0.5 ml buffer was added to each tube and the tube was shaken on a Turbo Mixer® (TechniLab Instruments, Inc., Pequannock, NJ) and heated for 10 min at 37° to dissolve the dried residue. Standard tubes were prepared by addition of 0.5 ml aliquots of solutions containing 10–700 pg estriol per 0.5 ml buffer. The antiserum (R8-14) used was generously supplied by Dr. Uwe Goebelsmann and has been described previously [12]. 0.1 ml of diluted (1/8000) antiserum was added to each assay tube, the tube was shaken for 1 s, then 0.1 ml of buffer containing about 5000 c.p.m. [2,4,6,7-^{3}H]-estriol (about 11.5 pg) was added. After shaking for 1 s, the tubes were left at room temperature for 30 min to equilibrate.

Separation of bound from free steroid was carried out by adding 0.7 ml saturated $(NH_4)_2SO_4$ solution [13] to each tube, followed by shaking for one s. After 10 min at room temperature, the tubes were centrifuged at 4° for 15 min at 3400 rev/min. The tubes were then placed in ice and 0.5 ml was rapidly transferred from each to a glass liquid scintillation vial. 10 ml of a toluene fluor (5 gm PPO, 0.3 gm dimethyl POPOP, 20 ml absolute ethanol, and toluene to 1 l.) were added to each scintillation vial, the vial was shaken vigorously on a Turbo-Mixer® for 20 s, and then allowed to equilibrate for 30 min in the dark. The two phase system in each vial was then counted in a liquid scintillation counter.

The standard curve was plotted on a log-logit plot. No sample value was acceptable unless it fell between 20% and 80% on the logit scale [14]. The approximate amounts of estriol corresponding to 20% and 80% on the standard curves were 200 pg and 15 pg, respectively. A 100 ng standard sample was included in each standard curve for determination of the "*N*" value.

Specificity and accuracy. The specificity of the antiserum used (R8-14) [12] is high. All non-estrogenic steroids cross-reacted at less than 0.1%. Estrogens cross-reacted to the following extent (estriol = 100%): estradiol-17β—2.3%; estradiol-17α—0.4%; estrone—0.1%; 16-epiestriol—1.0%; 16-keto-estradiol-17β—0.3%; 16α-OH-estrone—0.8%; estetrol (15α-OH-estriol)—0.9%. In our laboratory, the antiserum used cross-reacted 22% with estriol-3-sulfate; therefore, we elected to hydrolyze the plasma before assaying. Because of the high specificity of the antiserum, we did not separate estriol from other estrogens by solvent partition or chromatographic methods.

Procedural losses were estimated from each serum sample analyzed through the addition of [6,7-^{3}H]-estriol before hydrolysis [10]. The mean recovery of [^{3}H]-estriol was 79.2 ± 3.4 (S.D.)% (n = 213). By using [6,7-^{3}H]-estriol, we avoided the large loss of

counts seen when estriol labeled at the C-2 and C-4 positions is used as an internal standard for hot acid hydrolysis [15]. Interassay variability was estimated from 32 assays of charcoal stripped adult male serum to which estriol-3-sulfate, 1500 ng/ml, had been added. The serum contained 1108 ng/ml estriol. In each assay, 15 μl and 30 μl of the reconstituted extract residue were processed. The results in ng/ml were: 15 μl aliquots (mean ± S.D.) 914 ± 155, *CV* (coefficient of variation) = 17%, (recovery 82.5 ± 14.0%); 30 μl aliquots—955 ± 131, *CV*—13.7%, (recovery 86.2 ± 11.8%). Intraassay variability was estimated from two series of assays of the standard serum, each series containing 10 specimens. The coefficients of variation were 6.5% and 9.8%.

RESULTS

Serum total estriol concentrations in normally grown newborn infants

In Table 1 are listed the means, standard deviations and ranges of serum total estriol values in non-growth retarded newborn infants admitted to an intensive care unit for various indications. This group of infants includes those with immaturity alone, and those with respiratory distress due to hyaline membrane disease or benign respiratory distress. The mean values in the 1–24 h postnatal period for infants in all gestational age groups are similar, ranging from 959–1127 ng/ml. Mean values of total estriol (ng/ml) fell during the first weeks of life to 151 ± 131 (S.D.) in infants 11–25 days of age and to 39 ± 20 (S.D.) in infants over 25 days of age (ages 35–73 days).

A goal of this study was the investigation of the usefulness of the serum total estriol level, obtained on admission to the newborn intensive care unit, as an indicator of the prognosis of the infant presenting with signs of respiratory distress. Thus, the serum total estriol levels at 1–24 h, of all gestational age groups, as listed in Table 1, were analyzed according to the presence or absence of HMD and the severity of the HMD. No more than one serum sample from any infant is included in this analysis. The mean ±S.E. of the serum total estriol values during the first day of life for non-growth retarded infants with no HMD, infants with HMD who survived, and infants with HMD who died in the first two weeks of life are illustrated in Fig. 1 for four gestational age groups: less than 30 wk, 30–32 wk, 33–35 wk, and 36–40 wk. There are no significant differences between the total estriol values of the various diagnostic categories within any of the gestational age groups.

In order to investigate the relation of 1–24 h total estriol levels with gestational age, the regression lines of total estriol levels as related to gestational age,

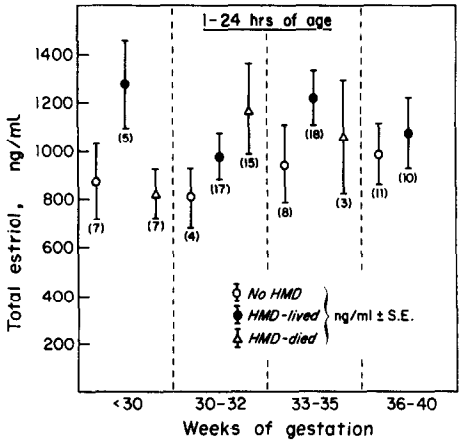

Fig. 1. The mean ± S.E. of the total serum estriol levels at 1–24 h of age in non-growth retarded newborn infants with no hyaline membrane disease (no-HMD), infants with hyaline membrane disease who survived (HMD-lived), and infants who died of hyaline membrane disease (HMD-died). The numbers in parentheses are the number of samples in the category.

Table 1. Serum total estriol concentrations (ng/ml) in non-growth retarded newborn infants

Gestation length (wks)	Postnatal age				
	1–24 h	2–5 days	6–10 days	11–25 days	>25 days
<30 Mean	959	705	341		
S.D.	413	390	167		
Range	447–1931	210–1609	39–539		
No.	19	20	11		
30–32 Mean	1039	611	156	151	39
S.D.	560	243	74	131	20
Range	363–2691	194–1202	62–137	33–400	21–73
No.	36	17	15	11	6
33–35 Mean	1127	588			
S.D.	489	323			
Range	421–2651	61–1317			
No.	29	16			
>35 Mean	1028				
S.D.	440				
Range	397–2032				
No.	21				

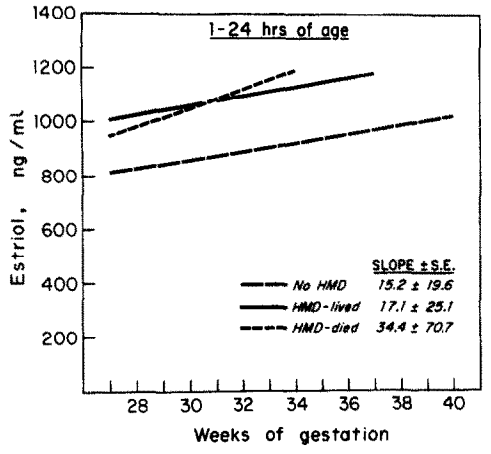

Fig. 2. Regression lines of serum total estriol levels vs gestational age in non-growth retarded infants 1–24 h of age. The abbreviations are defined in the legend for Fig. 1.

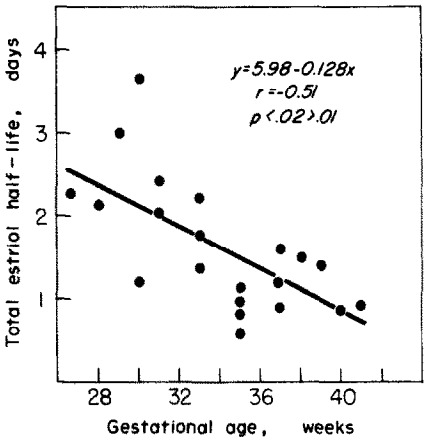

Fig. 3. The relationship of the $t_{1/2}$ of endogenous serum total estriol to the gestational age at birth in 21 infants studied in the postnatal period.

for each of the three diagnostic categories, were calculated. Fig. 2 illustrates these regression lines, together with the values for the slope ±S.E. Each slope is positive, showing a trend of rising first day total estriol levels with increasing geatational age. However, none of the slopes is significantly different from a slope = 0.

Total estriol half-lives in the neonatal period

Twenty-one infants were studied sufficiently, by serial blood sampling, so that there were at least three samples during the period of falling total estriol levels, thus allowing the calculation of a half-life value. Of the 21 infants, 15 had an exponential decline beginning on the first day of life, four had relatively stable levels until day two, and two had relatively stable levels until day three, when the exponential declines began. The half-life values of the total estriol levels determined for each infant were calculated by the method of least squares. The correlation coefficients varied from $r = -0.96$ to $r = -0.99$ for the individual calculated exponential decay curves.

The half-life values are plotted in relation to gestational age at birth in Fig. 3. The half-lives tend to be less with increasing gestational age. The regression equation, $y = 5.98 - 0.128x$, calculated by the method of least squares, has a correlation coefficient of $r = -0.51$, $P < 0.02 > 0.01$.

Relation of serum total estriol and serum albumin in newborns 1–6 hours old

Of the 105 samples obtained on the first day of life, 41 samples were collected before seven h of age and had serum albumin concentrations as well as total estriol determined. There was no correlation of the serum albumin and total estriol levels in these infants, the correlation coefficient being $r = -0.05$. This correlation was looked for because of evidence that the estriol conjugates circulating in the newborn infant are bound to albumin [16].

Serial total estriol concentrations in prematures with necrotizing enterocolitis

Four infants of those studied by serial blood sampling developed necrotizing enterocolitis, an intestinal disease seen principally in stressed premature infants. They ranged from 27–30 wk gestation. All four had prolonged high serum total estriol levels, with a marked rise in serum concentrations occurring in one of the infants at 7–11 days of age. Their serum total estriol values are shown plotted against time in Fig. 4. These infants had intestinal hypomotility from birth, with poor tolerance of oral feedings and episodes of intestinal distension prior to the onset of necrotizing enterocolitis.

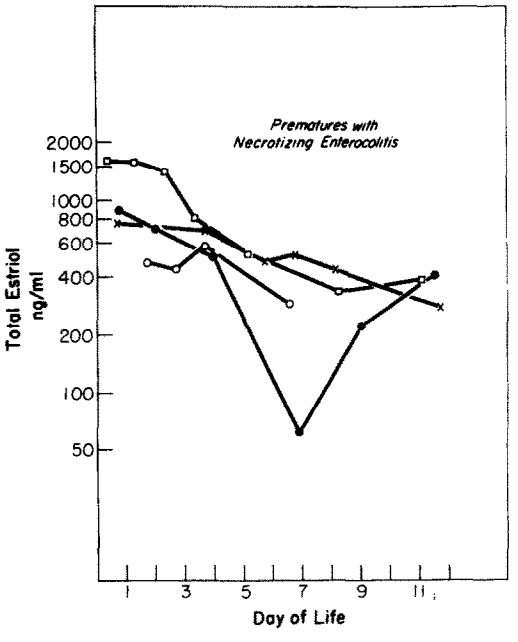

Fig. 4. Serial serum total estriol values in four premature infants with intestinal hypomotility who developed necrotizing enterocolitis.

Serum total estriol concentrations in intrauterine growth retarded and dysmature infants

Total estriol concentrations in 12 infants with either intrauterine growth retardation or physical signs of dysmaturity were studied in the first 24 h of life. Two infants, 33–35 wk gestation, had levels of 1206 and 1213 ng/ml which were similar to the mean value of normally grown infants of that gestational age (Table 1). The 10 infants over 35 wk gestation had a mean total estriol value in the first 24 h of 950 ± 477 (S.D.) ng/ml. The seven infants with IUGR in this group had a mean total estriol of 964 ± 396 (S.D.) ng/ml. These values were similar to the mean value for normally grown infants of that length of gestation and postnatal age, 1028 ± 440 (S.D.) ng/ml.

DISCUSSION

The tendency for serum total estriol concentrations during the first day of life to be higher with increasing gestational age at birth (Fig. 2) is consistent with the finding of Conly, *et al.*[17] that umbilical venous total estriol levels were higher in full-term than in premature infants. The mean serum total estriol levels in newborn infants 1–24 h of age, found in this present study (Table 1), ranged from 959–1127 ng/ml in all gestational age groups. These values are similar to the mean value of 1030 ng/ml found by Conly *et al.*[17] for umbilical venous total estriol concentration in normal premature infants. However, they report [17] significantly lower cord total estriol levels in premature infants with HMD and other forms of respiratory distress. This is in contrast to the findings reported in this paper of no significant differences between serum total estriol levels on the first day of life in infants with no HMD, infants with fatal HMD, and infants with HMD who survive. In fact, except in the less than 30 wk gestational age group, the mean serum total estriol values of newborns with no HMD are less than mean values of both groups of infants with HMD, though the differences are not significant (Fig. 1). The differences between our findings and those of Conly *et al.*[17] may be due to the differences in time of sampling, i.e. umbilical venous as compared to postnatal sampling. Another difference between the studies is that our group with no HMD includes infants with transient tachypnea of the newborn, thus not fitting the criteria for normal prematures used by Conly *et al.*[17].

Our findings are consistent with those of Fenner *et al.*[18] who found no significant difference in urinary estriol excretion, in the first hours of life, between newborns with and without HMD. However, other studies have demonstrated significantly lower than normal urinary estriol excretions on the first day of life in infants who developed HMD [19, 20]. The urinary excretion of estriol or the serum level of total estriol could be expected to be lower than normal in infants with HMD if the disturbances in fetal health and placental function, predisposing the infant to HMD, were present over a long period prenatally. However, if the fetal and placental abnormalities were intrapartum, these might not be reflected in the infant's serum or urinary total estriol levels in view of the long $t_{1/2}$ of total estriol in the newborn.

The only previous study of the rate of decline of endogenous serum total estriol in the postnatal period was by Schwers and Wolter[7] who found a $t_{1/2}$ of approximately 20 h in term infants. In addition, the decline of urinary total estriol over the first four days of life in full-term infants has a $t_{1/2}$ of approximately 24 h [21]. These values are similar to the $t_{1/2}$ value of 20.5 h that can be calculated for infants of 40 wk gestation from the regression equation derived from our data (Fig. 3). The disappearance rate of serum total estriol is much more rapid in postpartum women, the $t_{1/2}$ ranging from 1/2 to 6 h [22].

In this present study, we found the $t_{1/2}$ of total estriol to be negatively correlated with length of gestation (Fig. 3). The cause of the high $t_{1/2}$ at term, as compared to the postpartum woman, and the even longer $t_{1/2}$ in the small premature infant probably lies in the combined effects of the types of conjugates circulating in the newborn, the extent of protein binding of these conjugates, and the relatively low glomerular filtration rates in premature infants. The circulating estriol conjugates in the neonates, estriol-3-sulfate and estriol-3-sulfate-16-glucosiduronate [5, 6], are cleared more slowly by the kidney than are the other important conjugates, estriol-3-glucosiduronate and estriol-16-glucosiduronate [23]. In contrast to the newborn infant, all four conjugates of estriol circulate in the pregnant woman. The differences in renal clearance of the conjugates are attributed to differences in their extent of protein binding [16]. It is not known if the protein binding of estriol conjugates is different in the neonatal period than in the adult. Newborn infants, and particularly premature infants, are well recognized to have low glomerular filtration rates as compared to adult values [24].

Four infants, 27–30 wk gestation, had prolonged high serum total estriol levels and all developed necrotizing enterocolitis (Fig. 4). Each infant had intestinal hypomotility and intolerance of oral feedings from birth. The persistent high levels of total estriol could not be explained solely by their immaturity as other prematures of similar gestational ages had well-defined total estriol disappearance rates (Fig. 3). The infants were not in shock and had adequate urine output during the period of blood sampling, so the observation cannot be explained by a severe limitation of renal function. A more likely explanation is that there was enterohepatic circulation of estriol as a result of the delay in clearing meconium from the intestinal tract. Meconium contains very large amounts of estriol sulfate and estriol glucosiduronate, 10 mg/100 gm [25]. The estriol glucosiduronates may be hydrolyzed by β-glucoronidase in the intestinal mucosa, the free estriol absorbed, and then sulfuryl-

ated in the fetal liver [26]. Enterohepatic circulation of estriol may also explain the delay in onset of an exponential disappearance curve for 2–3 days after birth in six of the 21 infants for whom $t_{1/2}$ values could be calculated.

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