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Perinatal Changes in a Digoxin-like Immunoreactive Substance

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ABSTRACT. An endogenous digoxin-like immunoreactive substance(s) (DLIS) exists in the serum of premature and full term infants not receiving digoxin. We followed serum changes in DLIS concentration sequentially over the first 14 postnatal days in 24 premature neonates who did not receive digoxin in the intensive care nursery. All infants had measurable levels (>0.6 ng/ml) of DLIS in their serum. There was a distinct peak in DLIS concentration in 19 of 24 infants occurring at 4 ± 1.6 (SD) days after birth (range, 1–8 days). No peak was found in five infants. The peak serum level of DLIS obtained in the first 8 days of life was negatively correlated with gestational age and birth weight.

DLIS levels in amniotic fluid remained constant from 16 to 33 weeks of gestation but rose from 33 wk to term. DLIS concentrations in umbilical artery, umbilical vein, and maternal serum at normal full term delivery suggested that DLIS was of fetal origin. DLIS and digoxin concentrations are additive when present in the same serum sample if measured by standard radioimmunoassay methods. (*Pediatr Res* 18:1097–1099, 1984)

Abbreviations

DLIS, digoxin-like immunoreactive substance
EGA, estimated gestational age

We (1, 2) and others (3) have recently reported that DLIS occurs in the serum of premature and full term infants not receiving digoxin therapy. The presence of this endogenous substance(s) in this age group may result in falsely elevated apparent serum levels of digoxin. The degree of analytical interference varies considerably with the immunoassay kit used for analysis of digoxin (2). This variability is a function of the relative cross-reactivity of the antibody to DLIS. However, all kits we have examined to date show some degree of cross-reactivity (2).

The aims of this study were to investigate longitudinal changes in DLIS in the premature infant and to measure DLIS levels in the antenatal period, in amniotic fluid and at normal full term delivery in umbilical arterial, umbilical venous, and maternal blood.

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MATERIALS AND METHODS

Patients. All patients were cared for in Grace Hospital Maternity Unit or in the Intensive Care Nursery at Children's Hospital, Vancouver. Amniotic fluid samples were obtained under ultrasound control from pregnant women not receiving digoxin in whom amniocentesis was indicated for clinical reasons. The gestational age at the time of amniocentesis was determined by maternal dates and confirmed by ultrasound examination. Umbilical arterial and venous samples were obtained at delivery by double cord clamping in the usual way. Residual serum was obtained from blood samples submitted for clinically referred analyses and pooled within each sampling day for each infant. None of the mothers or infants included in this study had received digoxin.

Methods. DLIS was measured using the NML Digoxin RIA method with antibody lot No. DB 157 (NML Laboratories, Inc., Dallas TX) following the manufacturer's instructions. The same antibody lot number was employed for all studies to ensure that all results were comparable. Previous studies (2) had demonstrated antibody lot DB 157 to have the highest degree of cross-reactivity to DLIS of eight different antibody lots tested from seven different manufacturers. Amniotic fluid levels were determined after prior dilution (1:1) with the zero digoxin standard in order to correct for a previously observed matrix effect.

This study was given prior approval by the University of British Columbia Screening Committee for Research and other Studies involving Human Subjects. Basic statistical analyses were computed using a family regression program and a Hewlett Packard 9815A calculator.

RESULTS

The DLIS concentration in the serum of 24 premature infants not receiving digoxin was followed sequentially during the first 14 days of life. The apparent digoxin level due to DLIS ranged from 0.6–5.3 ng/ml and showed a distinct peak at a mean of 4 ± 1.6 (SD) days after birth (range, 1–8 days) in 19 of 24 cases. No DLIS peak was found in five of the 24 cases, but the peak may have been missed in two of these infants because the first sample was not drawn until the third postnatal day. Figure 1 shows the different types of longitudinal profiles for serum DLIS concentration in six representative infants. DLIS concentration was followed in 13 neonates for periods longer than 14 days after birth. A second distinct rise in DLIS concentration which occurred anywhere from 12–33 days after birth was found in four cases.

The highest DLIS levels and the most marked changes in DLIS concentration occurred in the most premature infants. Full term infants tended to have lower and relatively constant serum concentrations of DLIS (data not shown). Peak serum DLIS levels obtained in premature infants during the first 8 days of life showed a strong negative correlation with gestational age ($r^2 = 0.75$; $p < 0.001$) and birth weight ($r^2 = 0.45$; $p < 0.001$) (Fig. 2) [EGA 28.3 ± 2.3 wk (SD); birth weight, 1.14 ± 0.36 kg (SD)].

We determined DLIS concentrations in amniotic fluid from 36 different mothers taken between 16 and 43 wk of gestation. DLIS concentration was relatively constant in amniotic fluid from 16–33 wk gestation but increased after 33 wk (Fig. 3).

We compared DLIS concentrations in umbilical artery, umbilical vein, and amniotic fluid taken at the time of delivery in 11 healthy full term neonates. In nine of these, maternal serum DLIS levels were compared to umbilical vein DLIS levels. DLIS concentration in serum from the umbilical vein was consistently higher than DLIS found in the mother's serum (Fig. 4). There was no difference between the concentration of DLIS in the umbilical vein and the umbilical artery (Fig. 4). DLIS concentration was consistently higher in the amniotic fluid than the umbilical vein at the time of delivery (Fig. 4).

In order to document the additive effect of DLIS on digoxin in an infant, DLIS was followed for 3 wk after birth using two different digoxin radioimmunoassay kits: NML and Clinical Assays (Cambridge, MA). The Clinical Assays kit had approximately one-quarter the cross-reactivity with DLIS as compared to NML (Lot No. DB 157) (2). DLIS accounted for the methodological differences in concentration of digoxin after digoxin was administered to this neonate on day 22 (Fig. 5).

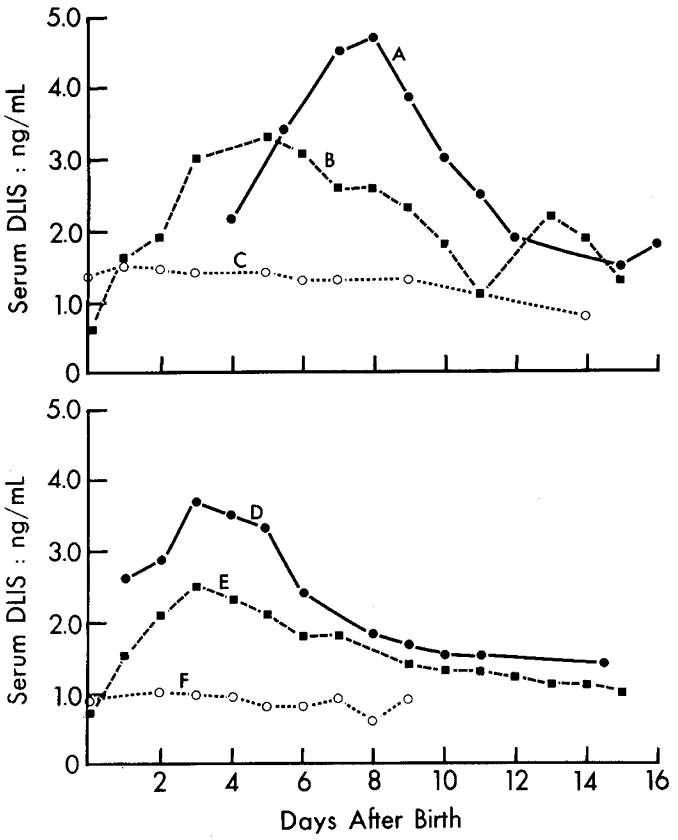

Fig. 1. Change in serum DLIS concentration in six premature neonates with time after birth. *Baby A*, birth weight 1.01 kg, EGA 27 wk; *baby B*, 1.30 kg, EGA 27 wk; *baby C*, 0.94 kg, EGA 33 wk; *baby D*, 1.10 kg, EGA 27 wk; *baby E*, 1.12 kg, EGA 28 wk; *baby F*, 2.28 kg, EGA 32 wk.

DISCUSSION

We have previously shown that within day variations of DLIS concentration in neonatal serum are insignificant while day to

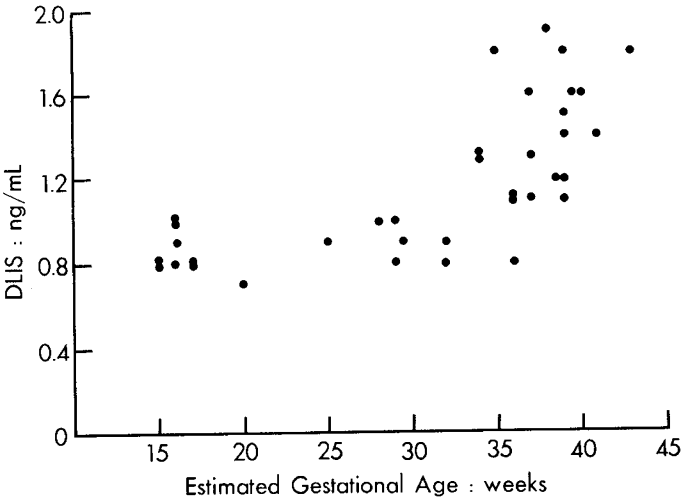

Fig. 3. Relationship between DLIS concentration in amniotic fluid and the estimated gestational age at the time of sampling ($n = 36$).

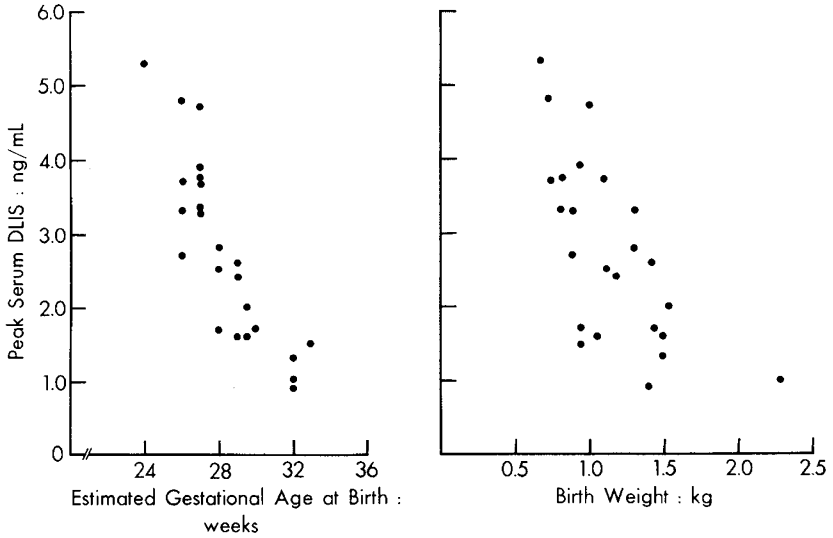

Fig. 2. Relationship between peak serum DLIS concentration [occurring between 1 and 8 days after birth; mean 4 ± 1.6 (SD) days] and the estimated gestational age and weight at birth in 28 premature neonates. The statistical parameters are discussed in the text.

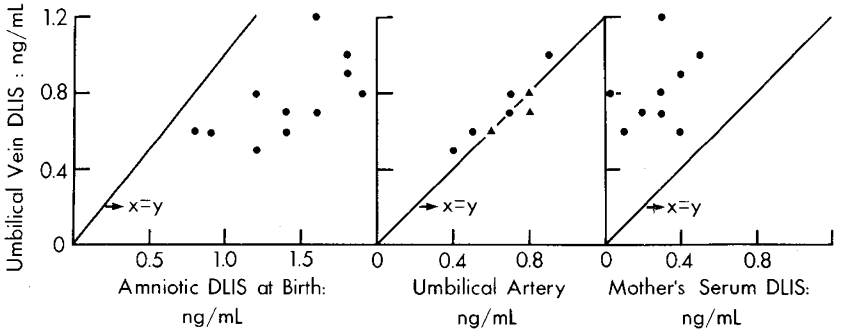

Fig. 4. Relationship between DLIS concentration in umbilical vein and concentration of DLIS in amniotic fluid, umbilical artery, and mother's serum at the time of birth in healthy full term neonates. ▲, two data points.

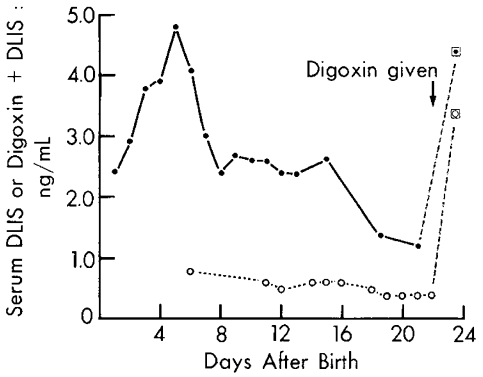

Fig. 5. Change in serum DLIS ± digoxin concentration in a premature infant with time after birth. ●——●, serum DLIS concentration as measured by the NML digoxin kit (Lot No. DB 157); O---O, serum DLIS concentration as measured by the Clinical Assays digoxin kit. Digoxin was administered to this baby on day 22 after birth.

day variations may be quite considerable (2). Within day pooling of left-over blood samples for each infant was therefore considered appropriate to provide one sample per infant for each sampling day. Most of the infants had a distinct peak in DLIS in the first 8 days of life, with the peak level being highest in the most premature infants. In some infants, a secondary peak appeared later, while in others DLIS remained relatively constant for long periods of time. Although peak levels of DLIS correlated with prematurity, high DLIS levels could not be clearly attributed to significant events in the clinical course of these infants. We have not yet found any apparent explanation for the secondary rise in DLIS seen in some infants.

During pregnancy, the low levels of DLIS in the maternal blood and higher levels in amniotic fluid suggest that DLIS is a fetal product as confirmed by DLIS behavior after delivery. The lack of arteriovenous difference in DLIS at delivery suggests that there is no major flux of DLIS between fetus and mother across the placenta. DLIS levels in amniotic fluid increased from 33 wk to term suggesting increased synthesis and/or excretion of DLIS into amniotic fluid.

The difficulties in interpreting clinically indicated digoxin levels are clearly demonstrated by the infant whose DLIS levels were followed by two different digoxin kits and was subsequently digitalized (Fig. 5). The contribution of DLIS is additive to exogenously administered digoxin in producing the measured "digoxin" level. The problem is compounded further by the unpredictable day to day fluctuations of DLIS and the variable sensitivity to DLIS of different antibody batches.

It is known that very low birth weight infants are unable to maintain sodium balance on a standard salt intake (4, 5). This phenomenon is due to a greater basal secretion of sodium that improves significantly with increasing gestational or postnatal age. Although the mechanism for this impaired sodium conservation in preterm infants has not been elucidated, it is tempting to speculate that DLIS may be a natriuretic factor and as such be partly involved in this mechanism.

The biochemical identity and physiological significance of DLIS remain unknown. The premature infant appears to provide an excellent opportunity for further investigating the nature of this substance.

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