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Serum Growth-Promoting Activity Measured as [^{3}H]Thymidine Incorporation into Human Activated Lymphocytes and Serum Transferrin Levels in Newborns and Mothers

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ABSTRACT. Serum growth-promoting activity measured as [^{3}H]thymidine incorporation into human activated lymphocytes and serum transferrin levels were measured during the perinatal period in newborns and mothers. Both thymidine activity (TA) and transferrin levels were significantly increased at the time of delivery in mothers compared to control women, and there was a progressive return to control levels in the first 5 postpartum days. A significant correlation was found between TA and placental weight. In the newborns, TA was low in cord blood after vaginal delivery but not in the cord blood from babies born by cesarean sectio. In premature newborns, TA was lower than in full term newborns. In all newborns during the first 24 postnatal hours, there was an increase in TA with levels rising above adult cotrol values: levels in cord blood were positively correlated with birth weight but not with thymidine activity. These data afford complementary insights into the humoral controls of growth in newborn infants. (*Pediatr Res* 19: 220–223, 1985)

Abbreviations

SM, somatomedin
TA, thymidine activity
SGA, small for gestational age
IGF, insulin-like growth factor
RIA, radioimmunoassay

Clinical and experimental data support the hypothesis that the GH-dependent SM mediate the growth-promoting action of growth hormone in mammals. However, low fetal SM levels have been reported, contrasting with high fetal plasma growth hormone concentrations and the rapid linear growth usually observed during the fetal and perinatal periods (1, 15, 17, 19, 25). This paradoxical finding raises many questions about the mechanism(s) regulating fetal and neonatal growth. We developed a bioassay for serum growth-stimulating activity which measures [^{3}H]thymidine uptake into lectin-activated human lymphocytes (35, 36). Using this bioassay we previously reported age-related variations of TA from birth to adulthood (4, 37). The aim of ths work was to evaluate TA in the serum of human newborns and their mothers. In addition, we conducted measurements of serum transferrin levels because in a previous work we suggested a possible relationship between transferrin and growth rate in children (9) and wanted to assess transferrin levels at birth, a time of rapid growth.

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MATERIALS AND METHODS

Serum samples were collected from the following subjects. 1) Blood was collected immediately after vaginal delivery from 108 normal full term newborns (group A), 17 premature newborns, 24–37 wk of gestational age (grpup B), 10 normal full term newborns immediately after cesarean section delivery (group C), and 48 SGA newborns (group D). All babies were healthy and received no medication or iron supplementation. The birth length and weight were in the normal range for gestational age in groups A, B, and C. The SGA infants had no known cause of gestational abnormality.

2) Eighty-four mothers of full term newborns from group A, 21 mothers of premature newborns from group B, and 18 mothers after cesarean section delivery (group C) were studied. All mothers were healthy and free of pregnancy complications.

Seventeen young nonpregnant women using no medication or hormonal contraception were studied.

4) Nineteen young healthy adult males using no medication were included in the study.

After informed consent, peripheral vein blood samples were obtained from adult controls and mothers. None had received any medication or infusion at the time of sampling. In the newborn infants, umbilical cord blood samples (arterial and venous) were collected at birth and venous blood samples were obtained at days 1, 3, 5, and 15. The sera were stored at −20° C. Just before the assay, sera were heated 40 min at 60° C. Serum samples from each mother and child pair were studied in the same assay.

Serum growth-promoting activity was measured as [^{3}H]thymidine incorporation into human lectin-activated lymphocytes (3, 35, 36). Eight doses were tested in triplicate for each serum, from 0.015 to 2.5% final concentration in 200-μl total incubation volume. Plotting the results as the square root of serum concentration *versus* the uptake ratio (number of counts in each serum-stimulated well, divided by the mean of counts in serum-free wells), one obtains a straight line dose-response relationship, the slope of which depends on the stimulating activity of the serum studied (5, 36). Unknown sample results were compared to the reference serum (pool of sera from healthy male adults) results using the Burn's slope ratio assay (5); the activity of this reference serum was arbitrarily fixed as 1 unit/ml. Finney's g test (5) was used to compare results. The coefficient of variation within each

assay was 5 to 10% and the variation between assays was 10 to 15%.

Transferrin levels were measured in the same samples, using the single radial immunodiffusion technique of Mancini *et al.* (23) on M Partigen plates (Behring Co.). Antiserum to human transerrin was obtained by immunizing rabbits. Sera and standards were introduced undiluted into the well in the agarose gel layer. The volume required per well was 5 μl. After a diffusion period of 2 days, the precipitate diameters were measured with an accuracy of 0.1 mm, using a scaled magnifying glass against a black background with lateral illumination.

Statistical analysis was performed using Student's *t* test.

RESULTS

Thymidine activity. In newborns, the mean ± SEM of TA values found in each group are given in Figure 1.

In cord blood of full term newborns after spontaneous delivery, the TA was 0.77 + 0.04 unit/ml, significantly lower than in normal adults (1.04 ± 0.06 units/ml, $p < 0.01$) but significantly higher than the values found in premature newborns (0.46 ± 0.08 unit/ml, $p < 0.05$). In contrast, in the cord blood from term newborns delivered by cesarean section, the TA was relatively elevated (1.28 ± 0.21 units/ml). The increase of TA in the 12 or 24 h following spontaneous delivery was highly significant in both full term (*A*) and premature (*B*) newborns ($p < 0.001$ in both cases); the increase was not significant in the babies delivered after cesarean section (*C*). The correlation of individual levels of TA at birth and at 5 days of age in 22 full terms and 10 premature infants (Fig. 2) also was significant ($r = 0.787$, $p < 0.001$). No correlation was found between thymidine activity in cord blood and birth weight or placental weight.

The possibility that low TA values in cord blood could result from the presence of an inhibitor was considered. This seemed unlikely because of the linearity of the dose-response relationship in samples drawn from cord blood. Moreover, we used the technique introduced by Salmon (28) for demonstrating inhibitors of somatomedin in the serum of starved rats. Mixing vol/vol aliquots of cord sera having low TA with aliquots of reference serum having an activity of 1 unit/ml did not decrease the measured TA below the level observed by 1:1 dilution of reference serum.

In the mothers, the mean (±SEM) TA found in each group is shown in Figure 3. Compared with the control value for nonpregnant women (0.90 ± 0.04 unit/ml), the TA was increased at delivery in all mothers: mothers of full term newborns (2.09 ± 0.14, $p < 0.001$), mothers of premature infants (1.33 ± 0.18, $p < 0.001$), and mothers delivered by cesarean section (1.84 ± 0.18, $p < 0.01$). The serum TA in mothers at the time of delivery was significantly higher ($p < 0.001$) in groups A and B than the levels in respective cord blood; TA activities were similar in maternal and cord blood of group C. In each group of mothers,

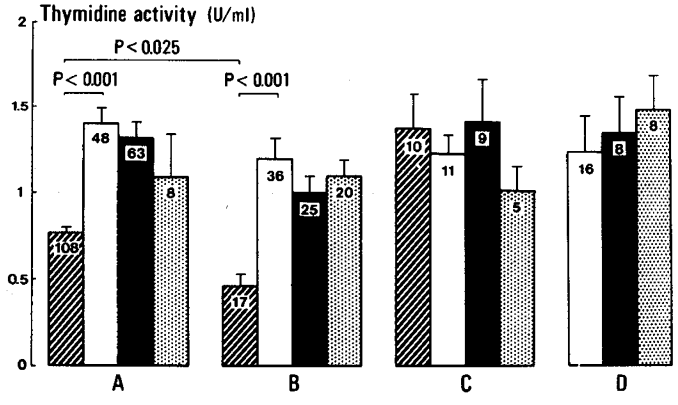

Fig. 1. Thymidine activity (±SEM, units/ml) in newborns: normal full term (*A*), premature (*B*), after cesarean delivery (*C*), and small for gestational age (*D*). Cord blood, ▨; 1 day, □; 5 days, ■; and 15 days ▦ of age. Numbers of subjects are indicated on *bars*.

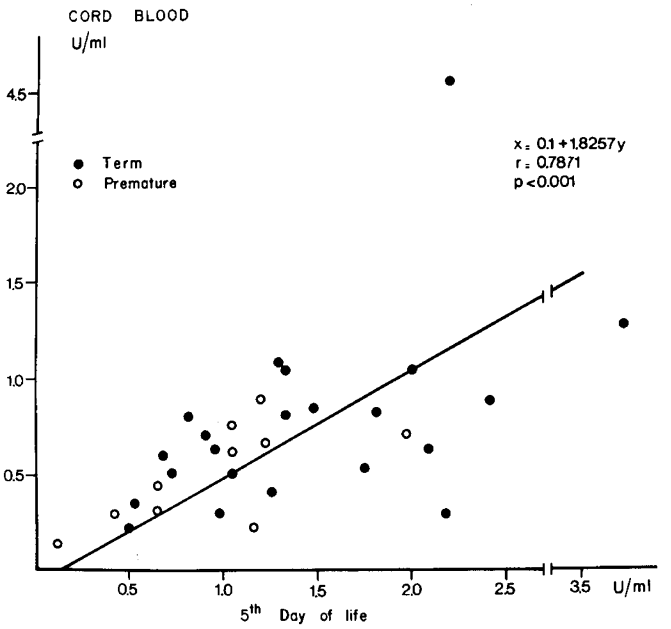

Fig. 2. Correlation between individual levels of thymidine activity at birth and 5 days of age, in 22 full term (●) and 10 preterm newborns (○).

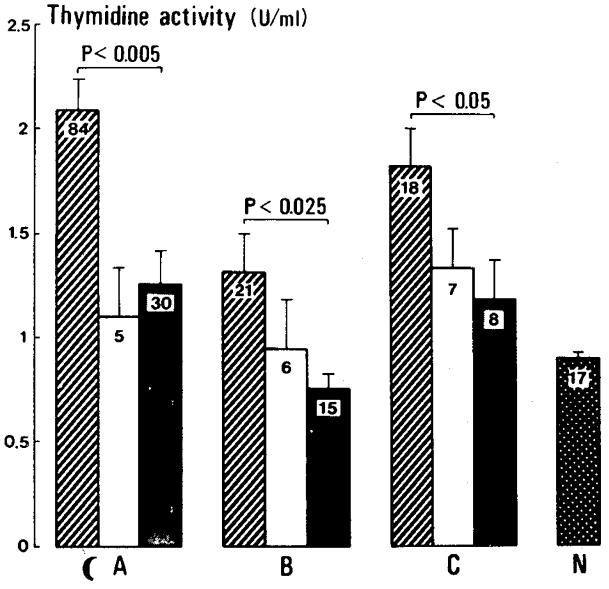

Fig. 3. Thymidine activity (±SEM, units/ml) in mothers of full term (*A*) and premature (*B*) infants, in mothers before cesarean delivery (*C*), and in nonpregnant women (*N*). At time of delivery, ▨; at day 3, □; at day 5 ■ following delivery.

we observed a decrease in TA during the 5 days following delivery ($p < 0.005$, group A; $p < 0.025$, group B; $p < 0.05$, group C). A positive correlation was observed between the TA in mothers and the placental weight ($r = 0.409$, $p < 0.05$).

Transferrin levels. Only samples obtained from newborns and mothers in groups A and B were assayed. No significant difference between the two groups was observed. In mothers ($n = 11$) at delivery, the mean (±SEM) transferrin level was 4.62 ± 0.3 g/liter, significantly higher than in control female adults ($n = 12$, 2.73 ± 0.11 g/liter, $p < 0.001$) and higher than in respective paired cord blood samples (2.27 ± 0.5 g/liter, $p < 0.001$); there was no correlation between individual mother and newborn values. In newborns studied on day 1 ($n = 22$) and day 5 ($n = 18$), no significant variation of transferrin levels was observed when compared to the cord blood levels.

Serum transferrin levels in newborns were positively correlated

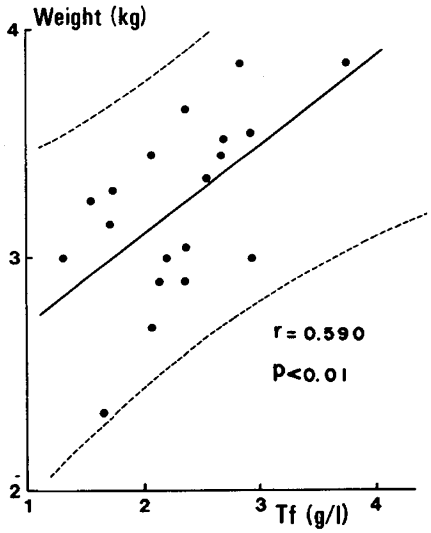

Fig. 4. Correlation between transferrin (*Tf*) levels in cord blood and birth weight.

with birth weight (Fig. 4). There was no significant correlation with thymidine activity.

DISCUSSION

In previous papers (30, 36), we reported that the TA of serum, measured as [^{3}H]thymidine incorporation into lectin-activated human lymphocytes, was GH-dependent *in vivo* and that TA is increased by somatomedins added *in vitro*. Thus, we related TA to somatomedin activity as defined by Daughaday *et al.* (7), although other authors using modified experimental conditions have not been able to duplicate our results (20, 27). We will relate our present data to results of earlier somatomedin bioassays and radioligand assay results in the perinatal period.

The thymidine activity of serum is elevated in women at the time of parturition, with a striking decline following delivery and return to the average adult level at day 5. This increase seems to be related to gestational age, since the level of TA is lower in the mothers of premature than in the mothers of full term newborns. The TA in mothers correlates with the weight of placenta ($r = 0.409$, $p < 0.05$). These data differ from those reported by Svan *et al.* (32) using a radioreceptor assay for somatomedin A in humans, from data of Kastrup *et al.* (22) measuring the sulfation activity determined by chick embryo assays in humans, and from data of Daughaday *et al.* (8) using a radioimmunoassay for IGF 1 in rats: all report low levels in late pregnancy. In contrast, our maternal results agree with those reported by Furlanetto *et al.* (16) measuring somatomedin C by a specific radioimmunoassay in humans, those of Daughaday *et al.* (8) using a RIA for IGF 2 in rat, and those of Wilson *et al.* (39) using a specific RIA for IGF 1 and IGF 2 and a measure of SM peptide content by radioreceptor assay. It is likely that methodological conditions, such as the precise time of sampling, and variations of assay types, possibly measuring different growth factors, are responsible for the discrepancies in the results.

The relatively low TA in newborns in the present study is in agreement with the low cord blood somatomedin levels reported using bioassays (14, 17, 22, 33), radioreceptor assays (12, 19), and radioligand assays (1, 2, 13, 15). The elevated TA in cord blood from full term newborns delivered by cesarean section was unexpected. We know of no other reports of measurements of growth-promoting activity or somatomedins comparing cesarean-born and vaginal-born infants. The effect of uterine contraction, circulatory modifications, and relative anoxia during delivery could be involved in the differences found. No correlation was found between TA in mothers and their newborns in the different groups A, B, and C. This suggests a fetal regulation distinct from that of the mothers.

The lower levels of TA in preterm than in full term newborns agree with the previous reports based upon measurement of sulfation activity (14, 26, 22), radioreceptor assay (10), and RIA levels of IGF 1 and IGF 2 in human cord blood (2). This difference between preterm and full term newborns is clearly related to gestational age. In infants with intrauterine growth retardation, Foley *et al.* (14) and Tato *et al.* (33) using bioassay, and d'Ercole *et al.* using a radioreceptor assay (11) reported very low somatomedin values, independently of the duration of gestation. This suggests that the nutritional factors involved in dysmaturity play a role in the low level of serum TA.

The mechanism of the decrease in TA in mothers during the postpartum period is not clear. A role for GH in mothers was excluded since there is evidence that matenal growth hormone secretion is suppressed during pregnancy (40). A major role of prolactin seems unlikely since prolactin remains elevated for several days in the postpartum period (16). It remains to be detemined whether the elevated concentration of cortisol observed during pregnancy (6), or the real stress of parturition play any role in the increase of TA. Another explanation might be that the human placenta is a site of production of TA, in which case the postpartum decline could be the result of expulsion of the placenta. Moreover, the possible influence of specific lymphocyte growth factors, still not measured in newborns and mothers, could have to be considered.

The neonatal increase of TA in the first days of life agrees with previous reports using somatomedin bioassay (22, 34) or direct SM C measurement (21). Our findings differ in that postnatal somatomedin levels have been reported to remain below those in normal adult serum, whereas TA rapidly exceeds normal adult values in full term as well as in small-for-gestational age newborns. Daughaday *et al.* (8) showed that IFG 1 is present in higher concentrations than previously reported in term fetal rat serum, and that IGF 2 is selectively elevated in rats during fetal and neonatal life. Gluckman *et al.* (18) have shown that IGF 1 concentrations in human newborns are lower than adult values, correlating with gestational age and with birth weight, and that IFG 2 concentrations are similar to adult values and do not correlate with gestational age or with birth size or IGF 1 values. Moses *et al.* (24) reported higher levels of MSA in fetal than in maternal rat sera, and Sara *et al.* (29) reported increased levels of fetal brain RRA-SM in the fetal circulation. D'Ercole *et al.* (11) suggested that the relatively low levels of SM in fetal rat serum may reflect low levels of the SM-binding protein rather than an absolute deficiency of biologically active SM. The identification of specific somatomedin receptors in fetal tissues opens the possibility that SM stimulates the growth of the fetus. Moreover, there is some evidence of fetal production of SM (10).

Presently available data on growth regulation at birth remain conflicting (2, 8, 18, 41). Measurements of IGF 1 and IGF 2 by specific RIA confirm the low levels of these growth factors in human newborns (2, 41). This is surprising, since linear growth is proceeding at a rapid rate at this time. However, in agreement, we found no correlation between TA in cord blood and birth weight. In contrast there is a correlation between TA and growth rate in children (4, 37). Thus, the physiological significance of our data remains an open question. Perhaps cord blood which is a mixture of placental and fetal blood is not the best sample in which to measure growth factors in newborns. The significance of data using human lymphocytes for measurement of TA also could be questioned since somatomedin have been reported to stimulate (30) or not to stimulate (20, 27) the transformation of T cells.

Finally, whenever allowed by the volume of samplings, we measured the serum transferrin level because of a possible relationship between transferrin and SM activity in children (9). Moreover, Vada *et al.* (38) presented evidence for a specific transferrin receptor in the brush border membrane of the human

syncytial trophoblast. We observed high transferrin levels at delivery in the mothers and low levels in the cord blood, but there was no correlation between individual mother and newborn values. However, the correlation between transferrin in cord blood and the birth weight would support a possible role of transferrin during fetal life.

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