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Serum Growth-Promoting Activity in Normal and Hypotrophic Fetuses at Midpregnancy

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ABSTRACT. Blood from 24 human fetuses aged 19–24 wk was collected by ultrasound-guided puncture of the umbilical cord *in utero*, performed for prenatal diagnosis of mother to fetus transmissible infections. Fetal serum growth-promoting activity (thymidine activity) was measured by its effect on ^{3}H-thymidine incorporation into human lectin-activated lymphocytes. Ten blood samples were obtained at 19–22 wk of pregnancy and 14 at 23–24 wk. The pregnancies were maintained and the fetuses delivered, free of infection, at 38–40 wk, nine of them being small for date and 15 having a normal weight for gestation age. The bioassayable thymidine activity was significantly lower in the hypotrophic (0.84 ± 0.04 U/ml) than in the normal fetuses (1.28 ± 0.09 U/ml) whatever the time of sampling. Thymidine activity was significantly negatively correlated with gestational age in the normal for date fetuses, not in the small for date. It is suggested that early measurement of thymidine activity in fetal blood might be of value in the assessment of fetal growth despite the fact that the tissue growth factors may be more important in fetus than are the serum factors. (*Pediatr Res* 22: 39–40, 1987)

Abbreviations

Sm-C, serum somatomedin-C
TA, thymidine activity
IGF, insulin-like growth factor
NFD, normal for date
SFD, small for date
SGA, small for gestation age

Our understanding of the factors regulating fetal growth in humans and animals is quite limited. Fetuses that are SGA usually are discovered by ultrasonography after the 24th wk of pregnancy. The etiologic factors present before this time may include humoral growth-regulating factors acting during the 2nd trimester of pregnancy. To evaluate the growth-promoting activity of serum in normal living human fetuses aged less than 24 wk, we utilized two previously developed techniques: collection of fetal blood *in utero* for purpose of prenatal diagnosis (1, 2) and measurement of the stimulating effect of serum on the incorporation of tritiated thymidine into lectin-activated human lymphocytes (3, 4). Comparing the results in normal and hypotrophic fetuses was the aim of this study.

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MATERIALS AND METHODS

Fetal serum samples were obtained by direct puncture of the umbilical cord *in utero*. This procedure was performed to determine whether rubella or toxoplasmosis infection occurring during pregnancy had contaminated the fetus. The ultrasound-guided technique and its results in the prenatal diagnosis of mother to fetus transmissible infection have been published previously (1, 2, 5). One and one-half to 3 ml of fetal blood were collected. Contamination of fetal blood with maternal blood was assessed by screening red blood cell size by Coulter counter: any sample with significant maternal contamination was excluded. After clotting at room temperature, serum was decanted and stored at −80° C for later hormone evaluation.

Satisfactory samples were collected from 24 fetuses at 19 to 24 wk of pregnancy which were proven not to have been contaminated. The fetal age was calculated from a) the 1st day of the last menstrual period and b) the ultrasound measurements. All fetuses were delivered at term (38 to 40 wk of pregnancy). Newborns were divided into two groups on the basis of birth weight: 15 newborns whose weight was in the normal range (NFD newborns) and nine whose weight was below the 3rd percentile for age according to French standards (SFD newborns) (6). The group of NFD newborns included six females and nine males whose mean ± SEM weight at birth was 3181 ± 92 g. The group of SFD newborns included six females and three males weighing 2496 ± 23 g. These mean birth weights were significantly different ($p < 0.001$). All infants were delivered vaginally. Apgar scores were 8 to 10 at birth. None had malformation or gross abnormality.

Serum growth-promoting activity was measured as stimulating activity upon 3H-thymidine incorporation into human lectin-activated lymphocytes, as detailed in previous reports from this laboratory (3, 4, 8). Lymphocytes from normal adult males were purified with Ficoll Telebrix. Cells were suspended at concentrations of 1×10^5/250 μl of RPMI medium in microtiter plates with phytohemagglutinin, 4 μg/ml, and gentallin, 0.2 mg/ml, then incubated 67 h at 37° C in 5% CO_2 humidified atmosphere. Then ^{3}H-thymidine, 0.5 μCi, was added in each well and the incubation time prolonged for 5 h. The cells were harvested, washed three times on the glass-fiber membranes of a Mash II apparatus, dried, and put in 3 ml of Packard Filter Count scintillation liquid. The tritium radioactivity was counted in a spectrometer and expressed as the ratio of the radioactivity of the sample studied to the mean radioactivity of cells incubated without serum. Each individual serum was studied in triplicate at six levels of dilution, 0.03 to 1.25%. The TA concentration was determined by comparing thymidine uptake of unknown samples to that of a pool of sera from eight normal adult males whose TA is arbitrarily assigned a value of 1 ml. The slope ratio assay of Burn *et al.* (7) was used for this calculation. Finney's

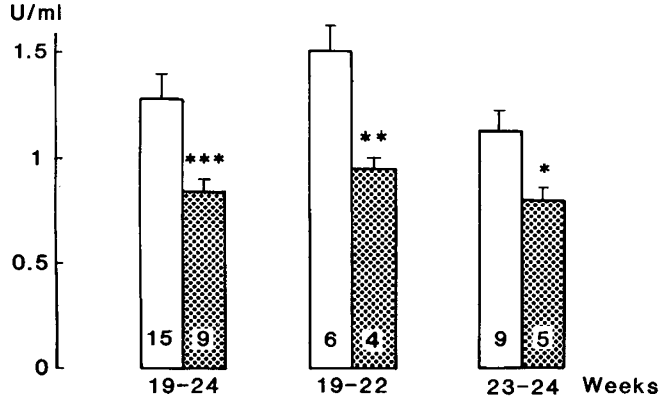

Fig. 1. Mean and SEM values of bioassayable thymidine activity in normal (*white columns*) and SFD fetuses (*gray columns*). *Left columns* show values in 24 fetuses aged 19–24 wk, the *middle columns* show values in 10 fetuses aged 19–22 wk, the *right columns* show values of 14 fetuses aged 23–24 wk. * $p < 0.05$, **, $p < 0.01$, ***, $p < 0.005$.

"g" test (7) was used to compare the results. The coefficient of variation within each assay was 5 to 10%, and the interassay variation was 10%. The statistical study of the results was done using the Student's *t* test.

RESULTS

The mean ± SEM TA was 1.28 ± 0.09 U/ml in the 15 NFD and 0.84 ± 0.04 U/ml in the nine SFD fetuses whose samples were obtained at 19 to 24 wk of pregnancy. These results differ significantly, $p < 0.005$. Considering samples obtained at 19–22 wk of gestation, the mean TA value was 1.49 ± 0.11 U/ml in the NFD ($n = 6$) and 0.93 ± 0.06 in the SFD ($n = 4$); these values differ significantly ($p < 0.01$). In the 14 of 24 samples obtained at 23–24 wk of gestation, the mean TA was 1.12 ± 0.10 U/ml in the NFD ($n = 9$) and 0.78 ± 0.05 in the SFD ($n = 5$) ($p < 0.05$). These results are summarized in Figure 1. The mean TA values at 19–22 and at 23–24 wk differend significantly in the NFD ($p < 0.05$), but not in the SFD infants. The NFD fetuses of this study had a TA negatively correlated with their gestation age ($r = -0.58$, $p < 0.05$). No such correlation was found in the SFD group or in the whole of the 24 fetuses studied.

DISCUSSION

In a previous work (8) using measurements of both bioassayable thymidine activity and radioimmunoassayable somatomedin-C in 48 healthy fetuses aged 21 to 28 wk, we demonstrated that TA is high before 24 wk, then seems to decrease. In contrast the mean Sm-C level is very low before 24 wk and increases thereafter, suggesting that the humoral control of fetal growth at midpregnancy is related to mechanisms other than direct regulation by Sm-C/IGF I. Similarly, radioimmunologically measured levels of IGF I in plasma obtained from 20 normal fetuses by fetoscopic sampling before abortion at age 15–23 wk (9) have been reported to be 36 ± 11 ng/ml, with IGF II values of 162 ± 55 ng/ml. These levels are much lower than those found in cord blood of term newborns (84 ± 58 and 264 ± 176 ng/ml, respectively). In our earlier study (8), the IGF I and the TA levels were, respectively, correlated positively and negatively with the gestational age.

We had insufficient serum to measure Sm-C/IGF I in the fetuses of the present series, since the risk of collecting more than 3 ml of blood via the cord blood sampling technique intrauterine is considered hazardous and at least 2 ml were needed for toxoplasmosis and rubella studies. In a previous study at birth (10) we reported that cord blood serum of SFD newborns contains less Sm-C/IGF I and has significantly less thymidine activity at term than serum of normal birth weight infants. The present results show that this difference in bioassayable TA is existing early in pregnancy, before the 23rd wk and as early as the 19th wk, a time where measurable Sm-C/IGF I is very low even in healthy fetuses.

These results suggest that growth-promoting factors other than Sm-C/IGF I regulate fetal growth early in gestation. These factors may include the embryonic somatomedin described by Sara *et al.* (11) and perhaps IGF II (9). Early measurement of growth-promoting activity in fetal blood could contribute to a better understanding of fetal development and offer a complementary means of investigation of abnormalities of fetal growth. However, it is likely that cellular autocrine growth regulation of fetal growth is as important as regulation by blood factors and may involve local production of somatomedin-C/IGF I (12, 13).

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