Prenatal Growth of Wistar Rats: Circadian Periodicity of Fetal Growth Late in Gestation

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ABSTRACT
Pregnant CFN Wistar rats demonstrated marked fluctuations of body weight within 24-h periods during late gestation. Maternal weight loss during the daytime phase of the daily cycle was accompanied by a slowdown in fetal growth. The period of rapid maternal weight gain that occurred during the night was paralleled by a period of rapid fetal growth. The periodicity of fetal growth was due to variation in accumulation of solids and not to diurnal fluctuations in relative water content.

Although complex equations are generally needed to describe animal growth over long periods of time, short-term growth often may be closely approximated by simple linear equations. It has been observed in this laboratory that weight gained by pregnant rats during the last 3 days of gestation is virtually linear; and the data of several reports (Stotsenburg, '15; Sikov and Thomas, '70; Wykoff, '71) indicate that weight gain of rat fetuses during the last 3 days of gestation also is essentially linear. However, maternal weight, when measured at intervals of less than 24 h, shows marked rhythmic fluctuation. This report deals with the association between maternal weight fluctuation and variation in the rate of fetal growth late in gestation of rats.

MATERIALS AND METHODS
Virgin female Wistar rats, purchased from Carworth, New City, N.Y., (200–250 g) were caged overnight with males and then examined for sperm by vaginal lavage. Pregnancy was assumed to have begun at 9 AM of the day sperm were found (day 0.0). The pregnant animals were allowed Rockland rat diet and tap water ad libitum. The lighting cycle was constant (light, 7:30 AM–4:30 PM). The pregnant rats were weighed and the gain from day 0.0 was determined at half-day intervals from day 19.0–22.0. Several rats cast their litters between days 21.5 and 22.0, and these were excluded from the study.

Eighty-three litters were delivered by cesarean section at half-day intervals from day 19.0 to 22.0. Fetal blood loss was prevented by electrocautery of the umbilical cords. The fetuses were cleaned, blotted free of surface moisture, and weighed. Each fetus was then frozen to −20°C and 12–24 h later was sliced into transverse sections (3–4 mm) while still frozen. The slices of each fetus were placed in individual weighing bottles and deep-frozen to −70°C until ready for drying. Drying was done by lyophilization at 0.1 μ Hg at −70°C for 72 h. These conditions have proven to give complete drying of tissues (unpublished data). The dried fetuses were weighed and the percentage of original (wet) weight due to total solids was calculated.

RESULTS
During the latter part of pregnancy the trend on the basis of once-a-day weighing was an approximately linear increase in maternal weight amounting to about 9 g per day (fig. 1). However, when maternal weight was measured at half-day intervals there was found to be a mean daytime (9 AM–9 PM) weight loss of 23 g and a mean nighttime (9 PM–9 AM) weight gain of 32 g. This pattern of weight gain agreed with the observation that 67% of the food and water used by these animals was consumed during the nighttime phase.
Mean ± SE

Mean increase / 24 hours, 9g
9AM – 9PM, -23g
9PM – 9AM, 32g

Fig. 1 Net weight gain from day 0.0 by pregnant CFN Wistar rats. Shaded and open bar along abscissa depicts the environmental lighting.

Mean ± 1.96 SD

Mean increase / 24 hours, 1.33g
9AM – 9 PM, 0.37g (28%)
9PM – 9AM, 0.96g (72%)

Fig. 2 Wet fetal weight (mean and middle 95% limits) of CFN Wistar rats in late gestation.
CIRCADIAN FETAL GROWTH

Fig. 3 Dry fetal weight (mean and middle 95% limits) of CFN Wistar rats in late gestation.

Fig. 4 Percentage of fetal total solids (mean and middle 95% limits) of CFN Wistar rats in late gestation.
The growth of CFN Wistar fetal rats. Weight and solids content during the last 3 days of gestation

<table>
<thead>
<tr>
<th>Age days</th>
<th>Litters n</th>
<th>Fetuses n</th>
<th>Wet weight g (mean ± SE)</th>
<th>Dry weight mg (mean ± SE)</th>
<th>Total solids % (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.0</td>
<td>8</td>
<td>89</td>
<td>2.16 ± 0.02</td>
<td>258 ± 3</td>
<td>11.94 ± 0.03</td>
</tr>
<tr>
<td>19.5</td>
<td>6</td>
<td>54</td>
<td>2.49 ± 0.03</td>
<td>313 ± 5</td>
<td>12.58 ± 0.04</td>
</tr>
<tr>
<td>20.0</td>
<td>14</td>
<td>128</td>
<td>3.42 ± 0.02</td>
<td>440 ± 2</td>
<td>12.86 ± 0.02</td>
</tr>
<tr>
<td>20.5</td>
<td>20</td>
<td>199</td>
<td>3.83 ± 0.02</td>
<td>515 ± 3</td>
<td>13.09 ± 0.02</td>
</tr>
<tr>
<td>21.0</td>
<td>14</td>
<td>136</td>
<td>4.95 ± 0.02</td>
<td>664 ± 3</td>
<td>13.40 ± 0.02</td>
</tr>
<tr>
<td>21.5</td>
<td>16</td>
<td>161</td>
<td>5.22 ± 0.03</td>
<td>704 ± 4</td>
<td>13.49 ± 0.02</td>
</tr>
<tr>
<td>22.0</td>
<td>5</td>
<td>38</td>
<td>6.14 ± 0.05</td>
<td>854 ± 6</td>
<td>13.90 ± 0.04</td>
</tr>
</tbody>
</table>

DISCUSSION

The observed periodicity of fetal growth was due to variation in the rate of accumulation of solids and not simply to diurnal swings in relative water content. The present data provide no clues as to whether or not this periodicity is evident in particular fetal tissues and, if it is, whether or not their periodicities are synchronized with that of the fetus as a whole. Fluctuations in weight occur in pregnant and nonpregnant rats, but it is not yet known how early in gestation diurnal fluctuations in fetal weight gain appear.

Presumably the fluctuations observed in maternal weight were due to diurnal variation in food and water intake and metabolism. It has been observed that rats are more active and their intake is greater during the night than the day (Slonaker, '12; Szymanski, '18). The rats in this study sustained a mean loss of 6% of body weight during the day, about equally distributed between water deficit and utilization of body stores. The periods of rapid fetal growth coincided with the periods of maternal weight gain. Although circadian variations of fetal growth rate may occur in response to intrinsic fetal factors, it seems likely that the rate variations are largely determined by maternal factors.

One can speculate about the role of circadian rhythms in influencing the action of teratogens. The wide variation in maternal weight during a 24-h period could have an important role in determining the effectiveness of a dose of some teratogens, particularly if a major component of that variation is change in the maternal body water compartments. Variations in maternal dilution and metabolism of a teratogen would in turn affect the amount of the agent reaching the fetus. Certainly the relation between the timing
of administration of growth-inhibiting agents and the phase or rate of fetal growth may be important in the expression of teratogenesis.

LITERATURE CITED


