Prenatal Growth of Wistar Rats: Circadian Periodicity of Fetal Growth Late in Gestation [1]

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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

Received Dec. 22, '72. Accepted Mar. 5, '73.
[1] Supported by NIH grant 5-S01-RR05383 and American Cancer Society grant 1N40M.

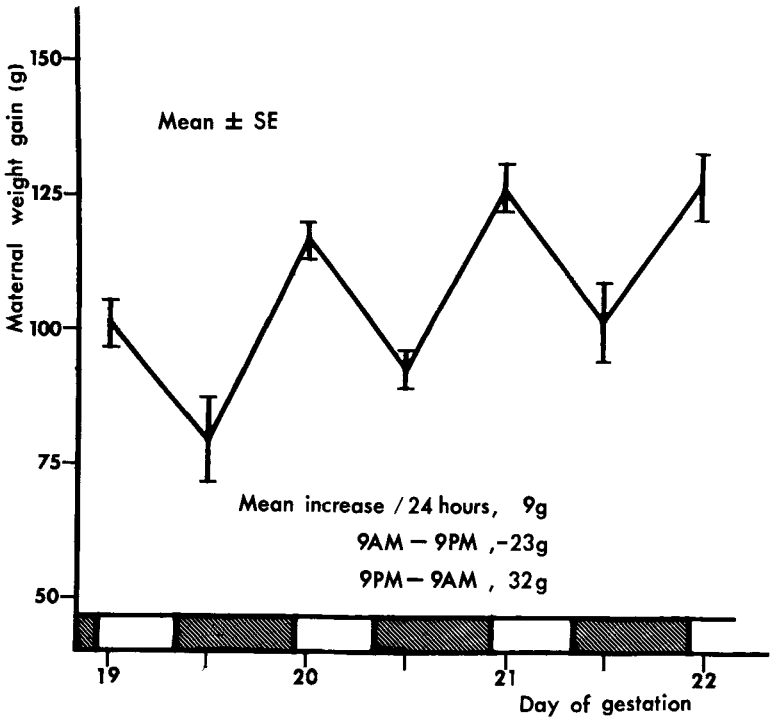

Fig. 1 Net weight gain from day 0.0 by pregnant CFN Wistar rats. Shaded and open bar along abscissa depicts the environmental lighting.

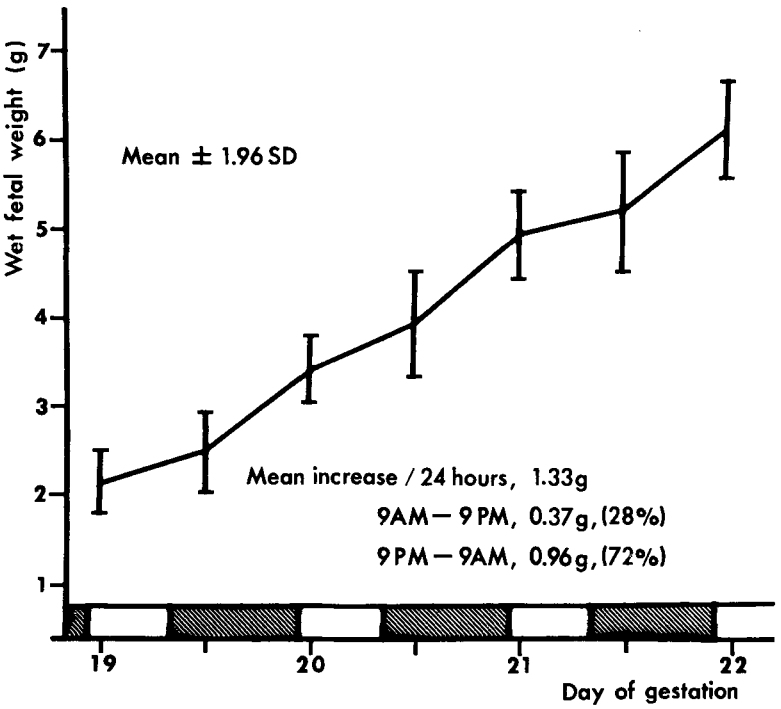

Fig. 2 Wet fetal weight (mean and middle 95% limits) of CFN Wistar rats in late gestation.

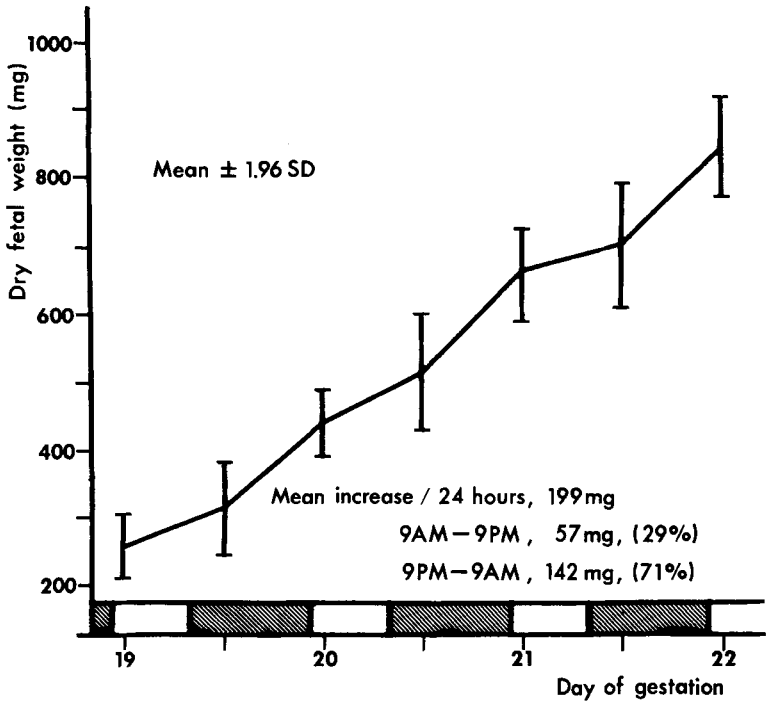

Fig. 3 Dry fetal weight (mean and middle 95% limits) of CFN Wistar rats in late gestation.

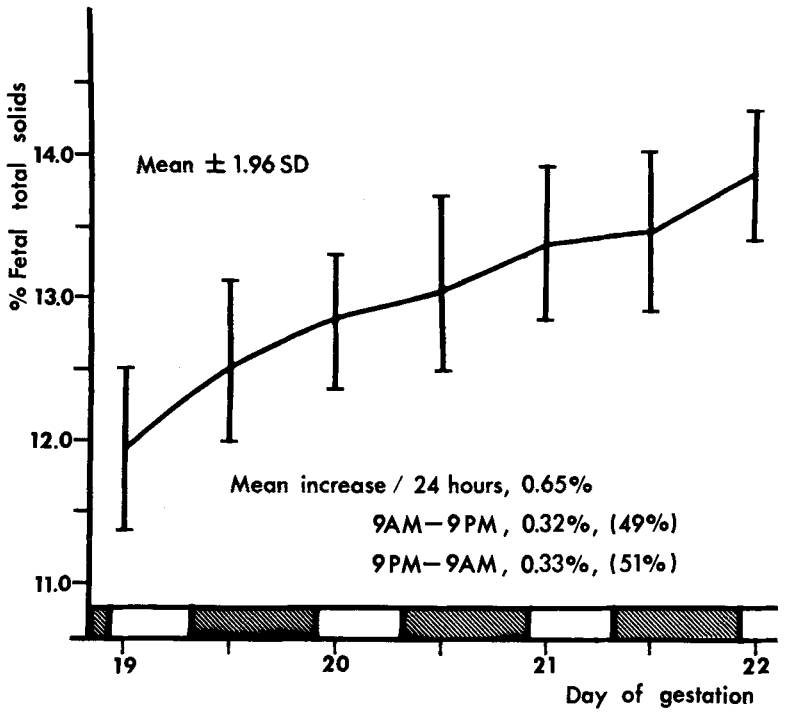

Fig. 4 Percentage of fetal total solids (mean and middle 95% limits) of CFN Wistar rats in late gestation.

TABLE 1

Growth of CFN Wistar fetal rats. Weight and solids content during the last 3 days of gestation

Age	Litters	Fetuses	Wet weight	Dry weight	Total solids
days	*n*	*n*	*g*	*mg*	%
				mean ± SE	
19.0	8	89	2.16 ± 0.02	258 ± 3	11.94 ± 0.03
19.5	6	54	2.49 ± 0.03	313 ± 5	12.58 ± 0.04
20.0	14	128	3.42 ± 0.02	440 ± 2	12.86 ± 0.02
20.5	20	199	3.93 ± 0.02	515 ± 3	13.09 ± 0.02
21.0	14	136	4.95 ± 0.02	664 ± 3	13.40 ± 0.02
21.5	16	161	5.22 ± 0.03	704 ± 4	13.49 ± 0.02
22.0	5	38	6.14 ± 0.05	854 ± 6	13.90 ± 0.04

of the cycle. Under the conditions of this experiment the rats sustained a mean loss of 6% of body weight during the daytime phase. Intake and output were measured at 12-h intervals in several pregnant rats from day 19.0 to 21.0. From the results it was estimated that about half of the daytime maternal weight loss was due to water deficit and the remainder to metabolism.

The number of litters and fetuses studied and their mean weights and percentage solids are given in table 1. In figure 2 mean wet fetal weight is plotted as a function of gestational age. Although fetal growth was approximately linear from day 19.0 to 22.0 (1.33 g/24 h) there was considerable acceleration-deceleration within each 24-h cycle. The mean fetal weight gain during the night was 72% of the total daily gain. Thus the period of rapid fetal weight gain paralleled the period of rapid maternal weight gain. The data for dry fetal weight (fig. 3) showed a pattern almost identical to that for wet fetal weight. The mean dry weight gain per 24 h was 199 mg and 71% of that was added during the night.

As the rat fetuses matured in utero there was a gradual increase in their relative content of total solids (fig. 4). The mean increases in their percentage solids content were equal during the day and night. As gestation advanced there was a deceleration of the "drying out" of the fetus, and this deceleration took place during the day. The change in rate of increase in solids during the day was −0.275%/12 h, while during the night it was +0.065%/12 h.

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.

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