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Urinary Excretion of Epinephrine and Norepinephrine During Fasting in Late Pregnancy in the Rat[1]

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ABSTRACT. Twenty-four hr urinary excretions of catecholamines did not differ in pregnant, post partum and age-matched virgin rats when they were given unrestricted access to food. Significant differences became manifest when food was withheld: Fasting from day 19–21 of gestation elicited significant increases in urinary epinephrine on each of the 2 days and increased urinary norepinephrine on the second. Contrariwise, urinary catecholamines were not increased when the same animals were fasted again on day 10–12 post partum or when age-matched virgin rats were subjected to comparable dietary deprivation. It has been suggested that the differences during fasting between gravid and nongravid rats may be ascribed to the greater homeostatic challenge that is posed by starvation in late pregnancy, and by the hypoglycemia that may occur under these circumstances. The potential contributions of enhanced sympathoadrenal activity to the metabolic response to starvation during gestation have been discussed. (*Endocrinology* **84: 447**, 1969)

COMPLEX interactions between intra-uterine and extra-uterine factors have been implicated (1) in the accelerated mobilization of fat, lipemia and ketonemia that occurs during starvation in late pregnancy (2–7). However, the possibility that heightened autonomic activity may act as a contributory mediator in these changes has not been evaluated previously.

The possibility merits consideration. After overnight fast in the third trimester of human pregnancy, plasma glucose remains in the normoglycemic range but is significantly lower than in post partum or nongravid females (8–10). When more stringent fasting is instituted by withholding food from pregnant rats (6) or guinea pigs (4) for one or more days near term, frank hypoglycemia may supervene. Thus, pregnancy constitutes one of the few physiological situations in which mild dietary deprivation can elicit meaningful diminutions of blood sugar. Since it has been known for more than 4 decades that hypoglycemia may elevate circulating catecholamines (11), we have compared the urinary excretion of norepinephrine and epinephrine during fasting in virgin, pregnant and post partum rats.

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Materials and Methods

Twenty-four hr urine collections were analyzed for catecholamines to evaluate the effects of starvation. Primiparous pregnant and age-matched virgin rats were secured from Charles River Laboratories, Wilmington, Mass. Animals had been mated at 41–47 days of age. On the 15th day of timed gestation, the pregnant animals and their paired virgin controls were placed into individual wire-bottomed metabolism cages (Acme Metal Products, Inc., Chicago, Ill.), in air-conditioned animal quarters (22–24 C), and provided with unrestricted access to Purina pellets and water. Only water was provided during the periods of fasting.

Two groups of pregnant rats (Series I and II) were deprived of food for 2 consecutive 24-hr periods from day 19–21 and 20–21 of their 22-day gestation period. Animals were then refed, permitted to deliver spontaneously, and litters were removed to prevent nursing. Repeat fasts were instituted on days 10–11 and 11–12 post partum. In Series II, comparable fasts were also performed at identical time intervals with the age-matched virgins. In addition, in Series II, urines were secured during the 24 hr immediately preceding each fast to assess the excretion of catecholamines *in the fed state.*

Urines were collected for 24-hr periods. Collections were terminated at 11 AM by gently lifting the animals and stroking their abdomen to encourage final emptying of bladders. To minimize excitement, experimental animals were not subjected to

TABLE 1. Effect of pregnancy and fasting on the urinary excretion of epinephrine and norepinephrine in the female rat†

	Ante partum			*Post partum*		
Days after mating:	18–19	19–20	20–21	9–10	10–11	11–12
Dietary status:	Fed	Fasted	Fasted	Fed	Fasted	Fasted
Series I						
A. Pregnant animals (n = 5)						
Epinephrine	—	1.09 ± 0.17	1.68 ± 0.29	—	0.81 ± 0.08	0.77 ± 0.07
p					< .05	< .05
Norepinephrine	—	1.33 ± 0.18	2.80 ± 0.29	—	1.21 ± 0.07	1.01 ± 0.12
p					NS	< .01
Series II						
A. Pregnant animals (n = 5)						
Epinephrine	0.95 ± 0.09	1.57 ± 0.13**	2.91 ± 0.21***	0.94 ± 0.03	0.93 ± 0.08	1.24 ± 0.14
p				NS	< .01	< .001
Norepinephrine	1.83 ± 0.23	1.46 ± 0.14	2.70 ± 0.22*	1.56 ± 0.05	1.07 ± 0.10**	1.28 ± 0.10
p				NS	NS	< .01
B. Age-matched virgin controls (n = 5)						
Epinephrine	1.13 ± 0.10	0.97 ± 0.04	1.07 ± 0.09	0.72 ± 0.10	1.09 ± 0.10	0.80 ± 0.10
p	NS	< .01	< .001	.05	NS	NS
Norepinephrine	2.04 ± 0.30	0.89 ± 0.23*	1.17 ± 0.24	1.10 ± 0.27	0.99 ± 0.14	1.06 ± 0.04
p	NS	< .02	< .01	NS	NS	NS

† Urines were collected directly (Series I) or under mineral oil (Series II) for 24-hr periods as described in the text. Mean ± SEM values for the excretions of epinephrine and norepinephrine (μg/24 hr) are summarized above. In the pregnant animals of Series I and II, p denotes the significance of the differences between ante vs. post partum excretions under similar dietary conditions. For Series II, age-matched virgin female rats were subjected to 2 periods of fasting at the same time intervals as the pregnant animals, and p denotes the significance of the differences between the virgin animals and the corresponding ante and post partum groups. Asterisks denote the significance of the changes (Fed vs. Fasted) during each day of the fast within each group (* $p < .05$; ** $p < .01$; *** $p < .001$; the absence of an asterisk indicates that statistically significant differences could not be demonstrated).

further handling and blood was not sampled.[1] At the completion of each collection, animal cages were rinsed with deionized-distilled water and washings were added to the urine collections. Total volumes were adjusted to 100 ml, filtered through Whatman #1 paper, and stored in polyethylene bottles at −18 C prior to assay.

In Series I, urines were permitted to drain directly down the "fast runoff" metal funnels, into narrow-mouth graduated cylinders which contained 0.5 ml of 6N HCl. Recovery experiments corroborated the stability of catecholamines in the acidified solutions within the cylinders. However, the substantial dead space along the sides of the funnels and the exposure to air in transit introduced the theoretical possibilities of drying and oxidative damage during collection. Therefore, the collection procedure was modified for Series II: The metal funnels were filled with light mineral oil to within 2 cm of the wire bottoms of the cages. An additional finer wire grating was placed 1 cm below the surface of the mineral oil to isolate the feces completely. Pieces of polyethylene tubing, closed at the bottom by a clamp, and containing 0.5 ml 6N HCl, were attached to the snouts of the funnels. At the completion of each 24-hr period, the clamps were opened and the accumulated acidified urine was drained into graduated cylinders and processed as above *but under oil throughout*. Possible losses of catecholamines into the mineral oil were evaluted by adding D,L-noradrenaline-7-^{14}C (New England Nuclear Corporation, Boston, Mass.) to acidified solutions. When such mock collections were kept under mineral oil at room temperature for 24 hr and then stored under mineral oil at −18 C for 1 week, recoveries of radioactivity in the aqueous phase ranged from 99.5 to 99.9%.

Assays for epinephrine and norepinephrine were performed by the trihydroxyindole method of Crout (12). As judged by fluorescence, recovery of catecholamines added prior to adsorption onto alumina ranged from 70.3 to 81.2% (mean ±SEM: 76.6 +0.59%). In 6 additional studies with rat urine, 81.6–87.7% (mean ±SEM: 84.0 ±0.38%) of added noradrenaline-7-^{14}C was recovered. Absolute values for catecholamine excretion in the present experiments were based on internal standards of epinephrine and norepinephrine which were run with each urine specimen. Replicate assays of in-

[1] In separate experiments (Knopp, R. H., H. Ruder, E. Herrera, and N. Freinkel, manuscript in preparation), we found that the concentration of sugar in blood derived from the tail of unanesthetized animals under these conditions averaged (mg/100 ml; mean ±SEM): 98.7 ±1.9 [20], 92.4 ±1.5 [18] and 76.9 ±1.7 [18] in *fed*, and 64.5 ±1.4 [23], 68.7 ±1.9 [17] and 43.4 ±1.3 [17] in *48-hr fasted* virgin, post partum and 19-day pregnant rats, respectively. The brackets denote the number of animals in each category. These findings of hypoglycemia during fasting in late pregnancy in the rat are essentially confirmatory of similar studies previously published by Scow, Chernick and Brinley (6).

dividual specimens by this technique did not differ by more than 18%.

Results

Findings are summarized in Table 1. During 2 days of fasting in Series I, greater quantities of catecholamines appeared in the urine ante partum than post partum. Epinephrine was more abundant on each of the 2 days; for norepinephrine, the increased excretion was confined to the second day of the fast. More detailed characterizations were secured in Series II. Pregnant, post partum and virgin female rats did not differ in their 24-hr excretions of catecholamines when access to food was unrestricted (Table 1, Series II, "fed" animals). Significant differences became manifest when food was withheld for 48 hr: In pregnant rats, epinephrine excretion was increased on each day of the fast and urinary norepinephrine became more abundant on the second day. Contrariwise, urinary catechols were not increased by starvation post partum or during either of the 2-day fasts in age-matched virgin animals. Indeed, the nongravid animals exhibited some tendency to diminished excretion of norepinephrine on the first day of dietary deprivation (Table 1, Series II, "fasted" animals).

Although results were qualitatively similar in Series I and Series II, the absolute values for catecholamines in the latter studies were somewhat higher. Since all other aspects of experimental technique remained constant, it would appear that more quantitative collections were possible with the use of mineral oil.

Discussion

The present studies have demonstrated that the urinary excretion of catecholamines is increased by fasting during late gestation in the rat. Comparable starvation did not elicit heightened catecholamine excretion during the post partum period or in age-matched virgin animals. Since differences between pregnant and nongravid animals could not be demonstrated when access to food was unrestricted, the augmented excretion of epinephrine and norepinephrine during fasting in late pregnancy presumably cannot be ascribed to gestational changes in the distribution, metabolism or renal handling of catecholamines. Instead, it most likely reflects a true augmentation of sympathoadrenal function under these circumstances.

Precise mediation remains to be elucidated. Progressive lowering of blood sugar during starvation in late pregnancy has been recognized for some time and frank hypoglycemia has been demonstrated in the fasted pregnant rat by Scow *et al.* (6), and confirmed by us (13). The continuing removal of maternal glucose and gluconeogenic precursors in the absence of exogenous fuels has been implicated in the hypoglycemia (1,5,10,14). Although this explanation has not been proven, and others are tenable, its attractiveness is reinforced by the known correlations between the size of the litter and the magnitude of fall in blood sugar when the mother is deprived of food late in gestation (4–6). Our observation that the excretion of epinephrine is increased before that of norepinephrine, and to a relatively greater degree, could be consonant with a hypoglycemic basis for the enhanced elaboration of catecholamines, since the adrenal medulla is selectively activated by reduction of blood sugar (15–17). However, fasting stresses multiple other aspects of homeostasis, and the failure of catechol excretion to rise in nongravid rats might merely indicate that the homeostatic challenges of starvation are less severe in the absence of the conceptus.[6] In any event, certain metabolic benefits of the increased sympatho-adrenal activity should be recognized. In the least, it could abet the mobilization of fat, formation of ketones, and resistance to insulin by which maternal glucose could be spared. Additionally, the enhancing effect of catecholamines upon gluconeogenesis (21) could contribute to the augmented efficiency for forming glucose from smaller precursors which we have recently demonstrated in the fasted pregnant rat (13).

Previous reports concerning sympatho-adrenal function prior to labor in late gestation are sparse, and inconclusive (18, 22–26). However, none of the authors have specified the dietary status of their subjects. Moreover, serial observations in individual subjects have been limited (18, 23), a fact which may restrict definitive interpretations in view of the relatively wide range of "normal" catechol values in grouped populations. The present experiments suggest that the problem may merit renewed evaluation and with refinements that would detect changes of the magnitude that we have described. The desirability for such efforts is compounded by the recent demonstration that altered elaboration of catecholamines may be triggered in human subjects by even minor decreases in blood

[6] The relatively small elaboration of catecholamines by the fetus (18) and the uncertainties concerning their placental transfer (19) render it doubtful whether maternal urine receives meaningful contributions from fetal catecholamines. Nonetheless, it should be noted that the neonatal rat can respond to induced hypoglycemia with heightened release of epinephrine from the adrenal medulla (20).

sugar, which remain within the range of normoglycemia (27).

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