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Response of the Hypothalamic-Hypophyseal Adrenal Axis to Stress: Observations in Fetal and Caesarean Newborn Rats.* (30621)

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A response of the hypothalamic-hypophyseal adrenal axis of the fetus to the stress of injected epinephrine has been reported(1,2,3). On the other hand, a lack of response of this axis to the stress of injected histamine has been reported(4).

The experiments to be reported were designed to resolve these conflicting reports. They also were designed to determine whether the hypothalamic-hypophyseal adrenal axis of a premature newborn obtained by Caesarean section is able to respond to the stress of injected epinephrine.

Materials and methods. In rats of the Sprague-Dawley strain (Simonsen Laboratories, Inc.), the adrenals of the experimental rats of a litter (E) were compared with those of an equal number of control rats of that litter (C).

The basic methods for production of stress by injected epinephrine and for determination of the ascorbic acid in the adrenals were as follows. An experimental rat was given 20 μg of epinephrine by subcutaneous injection. One hour later this rat and a control from the same litter were killed, and the adrenals of each rat were removed and cleaned of adherent fat. The adrenals of 2 or 3 rats of a kind were weighed on a Mettler balance with a sensitivity of 0.001 mg and put into a 4% solution of trichloracetic acid, thus making a pool of adrenals for study. The ascorbic acid in a pool of adrenals was determined by Abelson and Baron's modification(5) of the method of Roe and Kuether(6).

In the first experiment, a pregnant rat of the 22nd day was anesthetized with ether and subjected to laparotomy; *via* an incision in left uterine horn, 2 or 3 adjacent fetuses situated near the corpus uteri were removed and killed (Group 1-C, Table I). After the

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TABLE I. Concentration of Ascorbic Acid in Adrenals of Fetuses and Caesarean Newborns and Its Reduction in Response to Injected Epinephrine.

Rats					Adrenal ascorbic acid (μg/100 mg)		
Type	Group and subgroups	Condition	Given epinephrine	No.*	Concentration	Reduction from concentration in adrenals of littermate controls	Probability
Fetuses *in utero*	1 C	Intact	0	28 (11)	440 ± 21.6		
	E_1	Intact, given saline	0	28 (11)	420 ± 18.5	19.5 ± 15.6	.25
	E_2	Intact	+	28 (11)	389 ± 15.8	50.7 ± 13.2	.003
	E_3	Decapitated	+	28 (11)	432 ± 14.5	8.0 ± 11.2	.50
Caesarean newborns	2 C	Detached from placenta	0	34 (16)	424 ± 7.1		
	E		+	34 (16)	415 ± 7.6	9.1 ± 8.9	.33
	3 C	Not detached from placenta	0	33 (15)	306 ± 12.4		
	E		+	33 (15)	290 ± 13.0	14.8 ± 8.5	.11

* No. in parentheses indicates No. of observations. ± Standard error.

incised uterus had been sutured, the remaining 2 or 3 fetuses in that horn were given subcutaneous injections of 0.02 ml of saline (Group 1-E_1). Then, in the right uterine horn, 2 or 3 fetuses were decapitated *in utero* (2). These decapitated fetuses (Group 1-E_3) and the remaining nondecapitated fetuses in the right horn (Group 1-E_2) were given epinephrine. One hour after the several injections had been made—after the mother had recovered from the anesthesia—the mother was killed and the fetuses removed and killed.

In the second experiment begun on the 22nd day of pregnancy, all fetuses of a litter were removed from the uterus by severance of the umbilical cords, assembled into matched pairs (male-male and female-female) and allowed to breathe air (Group 2, Table I); any extra rats were discarded. The control member of a pair was killed about 15 minutes after the Caesarean delivery, to determine the initial level of ascorbic acid in the adrenals. The experimental member was given an injection of epinephrine about 15 minutes after the delivery; one hour later it was killed.

In the third experiment begun on the 22nd day of pregnancy, all fetuses of a litter were removed from the uterus in such manner that the Caesarean newborns remained attached to their placentae by their umbilical cords (Group 3, Table I). Each rat was placed on cotton moistened with saline, in a Petri dish; its umbilical cord and placenta were covered with the moistened cotton, in order to prevent drying; as the rat breathed air for 15 minutes, the color of the placenta changed from dark brown to bright red. Thereafter, the treatments were the same as those in Group 2.

The amount of ascorbic acid in the adrenals was expressed as μg per 100 mg of fresh adrenals. The reduction of the amount of ascorbic acid in the adrenals of rats given epinephrine was reckoned by subtraction (amount in experimental adrenals minus that in control adrenals). Statistical analysis of the data was made with the *t* test, the formula being

$$t = \frac{\text{mean}}{\text{S.E.}},$$

where mean is the average reduction of ascorbic acid and where S.E. (standard error) is calculated by the formula

$$\sqrt{\frac{d^2}{N(N-1)}}.$$

Results. In intact fetuses the injection of saline, a control procedure, caused no more than a 5%, though not significant, reduction in the amount of ascorbic acid in the adrenals (Group 1-E_1, Table I). In similar intact fetuses the injected epinephrine reduced the amount of ascorbic acid in the adrenals to the extent of about 12%, and this reduction was statistically significant (Group 1-E_2); probably an important factor in the demonstration of this significant change was the fact that the E and C fetuses were mem-

bers of the same litters. In fetuses decapitated *in utero,* in contrast, the injected epinephrine caused about a 2%, but not significant, reduction (Group 1-E_3).

In premature newborns obtained by severance of the umbilical cords the injected epinephrine caused about a 2%, yet not significant, reduction (Group 2-E). In those premature newborns which were obtained by Caesarean section but which remained attached to their placentae by their umbilical cords the injected epinephrine caused about a 5%, though not significant, reduction (Group 3-E).

Discussion. The observations largely resolve the conflicting reports mentioned in the introduction(1-4). Thus, the results in Group 1 (fetuses) support the view that in the rat the hypothalamic-hypophyseal adrenal axis begins to function before birth. They are in keeping with other reports that the decapitation of a fetus (hypophyseoprivus by decapitation) causes atrophic changes in the adrenals(7,8) and that the subjection of a fetus to unilateral adrenalectomy causes a compensatory hypertrophy of the remaining adrenal(9,10). Moreover, our results in Group 1-E_1 suggest that 3 of the procedures used—anesthesia, laparotomy and subcutaneous injection—do not constitute stimuli (stresses) which bring about a depletion of the ascorbic acid in the adrenals of the fetus. Also, the results in Group 1-E_3 suggest that injected epinephrine is a stressful stimulus only when the hypophysis is present (*cf.* results in Group 1-E_2).

Although our results in Group 1 are out of harmony with Schapiro and Geller's conclusion, they would seem not to be at variance with the results of their own experiment(4). A careful rereading of their paper makes it clear that they gave histamine to premature newborns obtained by Caesarean section (experiment *ex utero*), not to fetuses (not an experiment *in utero*).

Our results in Groups 2 and 3 suggest that in a Caesarean newborn the hypothalamic-hypophyseal adrenal axis does not respond significantly to injected epinephrine. This view is in line with the reports that in a rat born by spontaneous delivery there is, for a few days after birth, a temporary hiatus of time during which the hypothalamic-hypophyseal adrenal axis does not respond to experimental stress(11-14).

Our attempt to simulate experimentally one of the elements of prenatal life, in the rats of Group 3 (rats attached to their placentae by their umbilical cords), yielded results unlike those obtained in fetuses. Thus, in the rats of Group 3-E, the injected epinephrine did not significantly reduce the amount of ascorbic acid in the adrenals (*cf.* Group 1-E_2).

Any notion that the difference between the amounts of ascorbic acid in the adrenals of the controls of Groups 2-C and 3-C can be neither affirmed nor discredited by the present observations. Although this difference might merely reflect the fact that the Caesarean newborns of Group 2-C and those of Group 3-C were not members of the same litters, any firmer conclusion probably would not be warranted without additional experiment.

Summary. A fetal rat given epinephrine showed a depletion of the ascorbic acid in the adrenals, but a decapitated fetus did not, nor did an intact (nondecapitated) fetus given saline instead of epinephrine. In a premature newborn obtained by Caesarean section (detached from placenta or not detached), injection of epinephrine did not cause a depletion of the ascorbic acid. These observations support the views that in the rat the hypothalamic-hypophyseal adrenal axis begins to function before birth and that after delivery this axis temporarily becomes refractory to stimulation by injected epinephrine.

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Effect of Primidone (Mysoline) on Tissular Levels of Griseofulvin in the Rat. (30622)

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The antibiotic griseofulvin is a potent systemic agent in the treatment of superficial fungal infections in man(1). The effectiveness of griseofulvin appears to be related to its concentration in the blood, which, in turn, will depend mainly on the gastrointestinal absorption of griseofulvin(2) and on the rate of its metabolic degradation.

The finding of reduced blood-griseofulvin levels in rats(3) and humans(4) pretreated with phenobarbital may indicate enhanced rate of metabolism. In view of this possibility, we considered it interesting to investigate the structurally similar anticonvulsant agent 5-ethyl-5-phenylhexahydropyrimidine-4, 6-dione (primidone, Mysoline®) for its possible effect on griseofulvin content in rat blood and liver.

Methods. Ten female albino rats weighing about 150 g were used per group. The control group received saline. Over a period of 8 days the second group was given a daily oral dose of 20 mg/kg of phenobarbital sodium and the third group 20 mg/kg of primidone, respectively. On day 9 all animals received a single oral dose of 50 mg/kg of griseofulvin; so as to maximize gastrointestinal absorbtion(5), a fine-particle-state preparation (Grisactin®) was used. Since serum griseofulvin levels are highest 4 hours after oral dose(5), all animals were killed after 4 hours and the concentration of griseofulvin in the serum and liver was determined by a modification(6) of the fluorimetric method of Bedford, Child and Tomich(7). An Aminco-Bowman spectrophotofluorometer was used.

TABLE I. Effect of Primidone and of Phenobarbital on Levels of Griseofulvin in Rats.

Pretreatment (8 days)	Conc of griseofulvin* Serum, μg/100 ml	Liver, μg/g wet wt
Saline	4.77 ± .32†	17.9 ± .9
Primidone‡	2.89 ± .44 (−39%)	12.4 ± 1.4 (−31%)
Phenobarbital‡	2.96 ± .47 (−38%)	13.3 ± 1.3 (−26%)

* Four hours after 50 mg/kg griseofulvin *per os*. Average values of 2 experiments each with 10 rats per group.
† Standard error.
‡ Daily oral dose of 20 mg/kg.

Results. The average concentrations of griseofulvin in rat serum and livers as determined in 2 separate experiments are presented in Table I. Compared to untreated animals, repeated administration *per os* of Mysoline followed by a single oral dose of griseofulvin caused a statistically highly significant fall ($p<0.01$) of griseofulvin content in rat serum and livers. The degree of reduction was virtually identical with that detected in animals pretreated with phenobarbital which, in turn, are in agreement with those reported earlier(3).

Discussion. Recently, Conney and Burns (8) have reviewed the capacity of drugs to increase the activity of a wide variety of liver microsomal enzymes involved in drug metabolism. Among these is the enzyme system causing dealkylation of aromatic ethers. As such dealkylation appears to be an important reaction in the major pathway of griseo-

* Deceased.