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Reactiveness of Fetal Pituitary to Stressful Stimuli. Does the Maternal ACTH Cross the Placenta? (26528)

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Normally the concentration of ascorbic acid in the adrenal of healthy adult animals is reduced by adrenocorticotrophin (ACTH) released from the anterior pituitary gland. The same pituitary-adrenal relationship probably exists in the fetus, since ACTH release by the fetal pituitary and responsiveness of fetal adrenals has been shown(1-4). However, the question has not been fully resolved as to whether the source of the ACTH to which the fetal adrenals respond is the fetal or the maternal hypophysis. The question of placental permeability to ACTH arises. It is generally believed that the pituitary hormones, being proteins, do not cross the placental barrier under normal conditions. Yet some authors claim that under certain experimental conditions ACTH may cross the placenta(5,6). The authors have shown that subcutaneous injection of epinephrine into embryos after the 19th day caused a significant depletion of ascorbic acid from the adrenals of the fetus(7). In this paper the effect of epinephrine injection into the fetus was determined in normal and in decapitated fetuses.

Materials and methods. All experiments were carried out on rats on the 22nd or last day of pregnancy under ether anesthesia. The sequence of operation on the embryos was from ovarian to the cervical end of one horn of the utreus, then from the ovarian to the cervical end of the other horn. First, 2 control untreated embryos were removed through a small incision in the uterine wall, blotted on filter paper and weighed on a torsion balance. After decapitation, adrenals were removed and cleaned under 10-fold magnification, pooled and weighed in a closed vessel on an automatic analytical balance. Later the ascorbic acid content of the adrenals was determined by a modification(8) of the Roe and Kuether method. The next pair of embryos was similarly treated to also serve as control. The values from these 2 pairs of embryos established the 100% value for adrenal ascorbic acid concentration for the experimental embryos of the same female rat. In all experiments 4 adrenals were pooled for ascorbic acid determination. The next 4 fetuses were decapitated as follows: A circle of stitches of a thick surgical thread was placed on the uterine wall just above the head of the fetus. An incision in the middle of this circle allowed the head to peep through. The stitches were tightened around the throat of the fetus, then the head was cut off just above the ligature. This decapitation does not disturb the normal intrauterine position of the fetus. Removal of the 4 control fetuses takes only 1 to 2 minutes. It takes about 4 minutes to decapitate the first pair of fetuses (Decapitation I) and 4 additional minutes to decapitate the second pair of fetuses (Decapitation II). When the fetuses were to be further treated, epinephrine (20 μg/fetus), or ACTH* (50 milliunits/fetus) was injected subcutaneously in aqueous solution into the fetus through the uterine wall immediately after decapitation. The remaining fetuses were left untreated to determine to what extent the adrenals were stimulated by the laparotomy of the mother as well as by the operations on and injections into the other embryos. Exactly one hour after

* ACTH "Prolek" 1 mg contains 25 I. U.

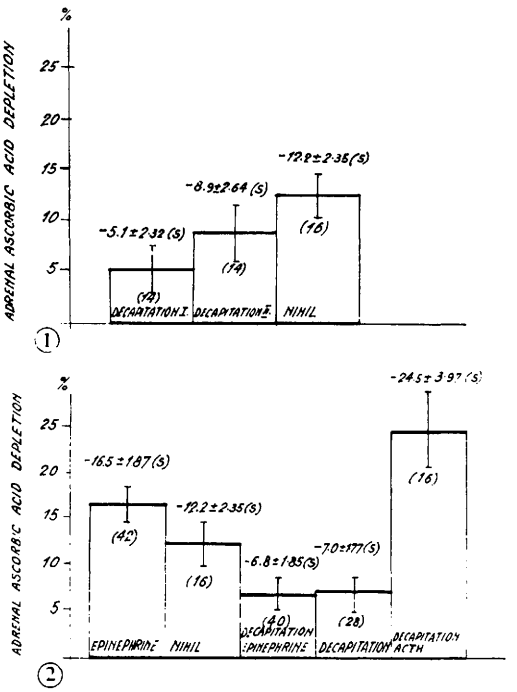

FIG. 1 and 2. Adrenal ascorbic acid depletion of 22-day-old rat fetuses. Vertical bars represent mean values, vertical lines, stand. error of means. Numbers within bars represent number of observations. S = significant depletion from control.

injection of epinephrine or ACTH under ether anesthesia, the decapitated fetuses and the remaining untreated fetuses were removed from the uterus and adrenal ascorbic acid concentration measured as above. Adrenal ascorbic acid depletions are expressed as the percentage change from ascorbic acid concentration in the adrenals of the 2 pairs of control embryos.

Results. The depletion of ascorbic acid from adrenals of the fetuses increased with time and amount of manipulation of the pregnant uterus (Fig. 1). There was only 5.1% depletion in the fetuses first decapitated (6 min), 8.9% depletion in those decapitated 4 minutes later (10 min), and 12.2% depletion one hour later in those that were untreated and killed at end of experiment (1 hour and 10 min). When 20 μg of epinephrine were injected subcutaneously into decapitated fetuses there was no further depletion of ascorbic acid (6.8% *vs.* 7.0%), (Fig. 2). When 50 mU of ACTH were injected subcutaneously into decapitated fetuses depletion was increased from 7.0% to 24.5%. In other rats, 20 μg of epinephrine were injected in fetuses without decapitation and an insignificantly greater depletion was found than in uninjected fetuses (16.5% *vs.* 12.2%).

Discussion. The failure of epinephrine, in decapitated fetuses, to cause greater depletion of ascorbic acid from the adrenals than that found in decapitated controls (6.8% *vs.* 7.0%), was expected because of the absence of the fetal pituitary. Epinephrine was injected into intact fetuses as a stressful stimulus but the data indicate that the stress of the operative conditions was sufficient to cause nearly as much depletion (16.5% *vs.* 12.2%). The much greater depletion in the intact fetuses than in decapitated fetuses (16.5% and 12.2% *vs.* 6.8% and 7.0%) proves that the fetal pituitary plays the dominant role in causing adrenal ascorbic depletion. The diminished reactiveness of adrenals of decapitated fetuses is excluded by highly significant adrenal response to exogenous ACTH (24.5%). The small, but significant depletion of adrenal ascorbic acid in decapitated uninjected fetuses (7.0%) may be caused either by maternal ACTH which crossed placenta or by fetal ACTH discharged from the fetal pituitary before its removal. The latter possibility is somewhat supported by the evident tendency of greater depletion of adrenal ascorbic acid by the pair of fetuses decapitated later in relation to those operated first (8.9% *vs.* 5.1%), and especially by the greater depletion obtained in intact fetuses (12.2%). If maternal ACTH were the cause of the depletion, then the same depletion should be found in all 3 groups. It may be concluded that the ascorbic acid depletion from the adrenals of the fetuses is due to fetal ACTH and not to ACTH from blood of the mother.

Summary. Stressful stimulus in intact fetuses, but not in decapitated fetuses, caused adrenal ascorbic acid depletion. ACTH injection into decapitated fetuses was followed by considerable adrenal ascorbic acid depletion. These data support the concept that the placenta is not permeable to ACTH.

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Effects of Conjugated Bile Acids on *in vivo* Cholesterol Metabolism in the Mouse.* (26529)

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Considerable data have accumulated on the effects of various bile acids on rates of cholesterol synthesis in different tissues(1,2). If one couples these data with the finding that certain bile acids are principal end products of cholesterol metabolism(3,4), it appears most probable that these substances are active in the control of certain phases of cholesterol metabolism. However, free bile acids—not the glycine and taurine conjugates—were employed in this work. It is, therefore, possible that some of the observed effects were due to exhaustion of the supply of amino acids used in conjugate formation; *viz.*, glycine, serine, and cysteine. A deficiency of any of these could have a serious effect on cholesterol metabolism and on metabolism in general. To check this possibility, the glycine and taurine conjugates of a number of bile acids were synthesized and their effects on cholesterol metabolism compared with those of the free bile acids.

Methods. The conjugates of cholic, deoxycholic, and hyodeoxycholic acid were synthesized according to the method of Norman(5), with some modification. The purity of the conjugates was checked by paper chromatography(6) and elementary analysis.

Elementary Analysis of Bile Acid Conjugates

		Calc.	Found
Glycocholic acid	N	3.01	3.25
Sodium Taurocholate	S	5.96	5.70
Glycodeoxycholic acid	N	3.12	3.32
Taurodeoxycholic "	S	6.15	6.04
Glycohyodeoxycholic acid	C	69.45	70.0
	H	9.64	10.37
	N	3.12	3.07

One hundred female albino mice of the Webster strain, weighing 21-26 g, were placed on a cholesterol free diet(7) for a 2-week period. They were then divided into 10 equal groups and diets supplemented as shown in Table I. All diets were fed *ad lib.*

After 3 weeks each animal received an intraperitoneal injection of 5 μc sodium acetate-1-C^{14}. One hour later the animals were sacrificed and livers removed for analysis of cholesterol and cholesterol-x-C^{14}(4). Cholesterol isolated from tissues was purified through the dibromide(8).

Results and discussion. Effects of free bile acids and of bile acid conjugates, are, in most cases, qualitatively similar (Table I). The conjugates of cholic and deoxycholic acids raise liver cholesterol levels to a greater extent than the free bile acids, but they do not have this increased effect on acetate-1-C^{14} incorporation rates. However, these rates were lowered one-sixth to one-tenth by free cholic and deoxycholic acid, so that any further lowering would be difficult to detect with any certainty because of high deviations.

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