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Quantitative Relations of Fetal and Maternal Pituitary-Adrenal Systems

I. EFFECTS OF MATERNAL HYPOPHYSECTOMY

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ABSTRACT Even though certain aspects of the fetal pituitary-adrenal system have been extensively studied, much remains to be learned of its basic development and function. In the present work, the effect of maternal hypophysectomy upon quantitative pituitary-adrenal relations in mother and fetus was investigated in pregnant beagle dogs. At 57 days gestation in each of seven normal animals and seven animals 3 wk posthypophysectomy, a cannula for collection of adrenal effluent was placed in a single fetus *in utero* under halothane anesthesia. A timed fetal adrenal sample was obtained; ACTH (10 mU) was injected into the fetus; 3 min thereafter a second fetal adrenal sample was collected and fetal and maternal peripheral arterial samples were drawn. All fetuses and their adrenal glands were weighed. Concentrations of cortisol and corticosterone were determined by a modification of the double-isotope dilution derivative method of Kliman and Peterson.

Mean peripheral cortisol concentrations in mother and fetus were 92 and 94 ng/ml, respectively (ratio 1.0), in normal pregnancies and 11 and 54 ng/ml, respectively (ratio 0.2), in maternal hypophysectomy pregnancies. Weights of fetal adrenal gland pairs of 32 and 44 mg, respectively, in normal and hypophysectomy pregnancies indicate increased fetal ACTH secretion in response to lowered circulating cortisol in the fetus secondary to maternal hypophysectomy. These data demonstrate the presence of an active pituitary-adrenal feedback mechanism in the dog fetus which is partly influenced by maternal pituitary-adrenal function. The shift in the maternal-fetal ratio of peripheral cortisol concentrations from 1.0 to 0.2 occasioned by maternal hypophysectomy neither supports nor rules out the presence of specific placental mechanisms affecting relative concentrations of cortisol in mother and fetus. It does suggest, however, that the relative steroid input into maternal and fetal compartments is one of the factors which influences such concentration ratios. Concentrations of cortisol were significantly higher in fetal adrenal effluent (pre-ACTH) than in fetal peripheral plasma in normal pregnancies, which demonstrates secretion of cortisol by the fetus and shows that corticosteroid of maternal origin does not lead to complete suppression of fetal pituitary-adrenal function. Cortisol secretion rates in response to exogenous ACTH were essentially the same in fetuses in normal and hypophysectomy pregnancies (132 and 128 ng/min, respectively). Thus, fetal adrenal responsiveness to ACTH, i.e., maximum secretory capacity, is not enhanced by increased ACTH stimulation sufficient to induce adrenal hypertrophy in the same fetuses.

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INTRODUCTION

Corticosteroid metabolism involves multiple factors in individuals after birth but is of even greater complexity in the fetus. Specifically involved in the fetus are a developing biochemical capacity for steroid synthesis within the fetal adrenal gland, a maturing fetal central nervous system-hypophyseal-adrenal control mechanism, and functional endocrine relations with the placenta and the mother. The human fetal adrenal cortex, from midpregnancy and even earlier, has been demonstrated to possess enzyme systems capable of forming diverse steroid molecules from acetate but to have, at the same time, a significant deficiency of the enzymes 3β-hydroxysteroid dehydrogenase and 3-ketosteroid-Δ^4-Δ^5-isomerase

necessary for the conversion of pregnenolone (3β-hydroxypregn-5-en-20-one) to progesterone (pregn-4-ene-3,20-dione). The human fetal gland is, however, believed to synthesize corticosteroids, such as cortisol (11β,17,21-trihydroxypregn-4-ene-3,20-dione), utilizing as a precursor progesterone largely derived from the placenta, an illustration of one form of interdependency between the fetus and the placenta in steroid metabolism (1–5). The fetal adrenal glands of several lower mammalian species, although less extensively studied than in man, have been shown in vitro to form corticosteroids from endogenous precursors (1). Fetal secretion of cortisol and corticosterone (11β,21-dihydroxypregn-4-ene-3,20-dione) has been demonstrated directly in sheep, dogs, and monkeys by analysis of adrenal venous effluent (6–9). In man and experimental animals, primarily on the basis of morphologic observations, a functioning fetal pituitary-adrenal feedback mechanism has been demonstrated and shown to be influenced by maternal corticosteroid secretion (1, 10, 11). There is also strong evidence for pituitary-adrenal responsiveness to stress in fetal rats near term (11). Although, as noted, much is known about the fetal pituitary-adrenal system, a great deal more information is required to complete our basic understanding of its development and function. This is particularly true with regard to the regulation of fetal corticosteroid secretion and of maternal influences on that regulation; it was in this regard that the present work was undertaken.

METHODS

Experimental preparation and design. This study was carried out in pregnant purebred beagle dogs, with known breeding dates, obtained from a closed colony (Hazelton Research Animals, Inc., Cincinnati, Ohio). They were worm-free by stool examination at the time of air shipment to our laboratory approximately 1 mo after the beginning of pregnancy. Experimental animals were assigned to either a normal control group or a maternal hypophysectomy group. In the hypophysectomy group the maternal pituitary was removed under halothane anesthesia on the 33rd to 49th day of gestation by the transbuccal approach generally as described by Aschner (12) and McLean (13). Hypophysectomy animals received daily intramuscular injections of progesterone (Parke, Davis & Co., Detroit, Mich.), 25 mg (to within 24–48 h of the fetal experiment), penicillin, 300,000 U, and streptomycin, 0.25 g.

Fetal adrenal cannulations were performed in the normal group at a mean gestational age of 57 days (range 56–58 days) and in the maternal hypophysectomy group at 56.4 days (range 55–60 days). Maternal laparotomy was performed under halothane anesthesia. In a single fetus, ECG electrodes were implanted as described by Jackson, Clarke, and Egdahl (14), and a fetal electrocardiogram was recorded. A fetal marsupializing incision was performed by the method of Jackson and Egdahl (15) which is characterized by retention of the fetus within the uterus and negligible amniotic fluid loss. Fetal adrenal cannulation was carried out as described by Jackson and Piasecki (8). Briefly, a cannula was inserted into the distal inferior vena cava of the fetus, and a choker was placed around the proximal vena cava. With the choker tightened, the catheter drained an isolated segment of the vena cava receiving the effluent of the two adrenal glands and the two kidneys.

Immediately after completion of the fetal adrenal cannula, a fetal electrocardiogram was recorded; the fetal heart rate was noted in order to assess the general condition of the fetus at the time of the actual experiment. The vena caval choker was tightened, and a heparinized fetal adrenal blood sample of approximately 1.0–1.5 ml was collected for 1 min. The choker was released, and 10 mU of ACTH (Parke, Davis & Co.) was injected. 3 min later, a second 1-min fetal adrenal collection was made. A 5- to 10-ml volume of blood was immediately withdrawn from the fetal aorta. Simultaneously with the collection of the fetal aortic blood, a systemic blood sample was withdrawn from the mother. Samples were centrifuged at 2,500 rpm for 10 min; blood and plasma volumes were recorded; and the plasma was removed and stored in a freezer. After collection of blood specimens in the mother and operated fetus, all fetuses were removed from the uterus and weighed. The adrenal glands of all fetuses were excised and weighed in pairs on a Mettler balance type B6.

In hypophysectomized mothers, upon completion of fetal and maternal blood sampling as above, maternal adrenal cannulation was performed by the technique of Hume and Nelson (16) in order to quantitate maternal adrenal function and thus assess the completeness of pituitary ablation.

Analytical procedures. Fetal adrenal and peripheral plasma and maternal peripheral plasma samples were analyzed for cortisol and corticosterone by a modification of the double-isotope dilution derivative method of Kliman and Peterson (17). 4-^{14}C ring-labeled steroid was used as the recovery marker. A fourth chromatographic step was added which utilized the solvent systems, cyclohexane-dioxane-methanol-water (4:4:2:1) for cortisol and cyclohexane-benzene-methanol-water (4:2:4:1) for corticosterone. Samples were counted for ^{3}H and ^{14}C in a Packard Tri-Carb Liquid Scintillation Spectrometer, model 3214.

5-ml adrenalectomized dog plasma blanks were run with each assay. Mean blank ± SE for cortisol in all assays was 1.4±0.4 ng and for corticosterone was 3.0±0.8 ng. The ratios of cortisol actually measured in each sample to the equivalent blank were 275±55 in the normal group and 113±17 in the hypophysectomy group. The smallest individual sample to blank ratio for cortisol was 15. Assayed corticosterone to blank ratios were greater than 10 except for certain maternal and fetal peripheral samples in the hypophysectomy group.

Maternal adrenal samples (obtained only for confirmation of completeness of hypophysectomy) were analyzed by a modification (18, 19) of the Silber-Porter method (20).

RESULTS

Experiments were completed in seven normal pregnant animals. Maternal hypophysectomy was performed at 49 days gestation in four pregnant dogs, of which three died and one aborted. 13 pregnant dogs were hypophysectomized at 33–39 days gestation, of which five died, one aborted, and seven survived with their pregnancies intact and served as the hypophysectomy group. In that group maternal adrenal corticosteroid secretory rates

TABLE I

Corticosteroid Data (Mean ±SE)

Group	Concentration plasma				Fetal secretory rates*					
	Peripheral arterial		Fetal adrenal				Relative to fetal weight		Relative to adrenal weight	
	Maternal	Fetal	Pre-ACTH	Post-ACTH	Pre-ACTH	Post-ACTH	Pre-ACTH	Post-ACTH	Pre-ACTH	Post-ACTH
	ng/ml				*ng/min*		*ng/min/kg*		*ng/min/mg*	
Cortisol										
Normal	92 ±5	94 ±8	194 ±37	308 ±60	72 ±27	132 ±34	288 ±106	532 ±133	1.89 ±0.57	3.75 ±0.61
Hypophysectomy	11 ±1	54 ±11	233 ±33	284 ±39	101 ±14	128 ±29	465 ±71	587 ±126	2.52 ±0.42	3.19 ±0.73
Corticosterone										
Normal	30 ±4	20 ±2	63 ±13	70 ±10	30 ±10	33 ±6	117 ±36	134 ±25	0.83 ±0.26	1.01 ±0.18
Hypophysectomy	2 ±1	4 ±1	47 ±8	64 ±12	25 ±4	34 ±9	113 ±18	157 ±38	0.61 ±0.10	0.85 ±0.21

* Calculated from concentration in adrenal plasma less systemic plasma concentration.

(mean±SE, 0.34±0.1 μg/min during operative stress and 0.41±0.08 μg/min post-ACTH) were in keeping with ablation of the maternal pituitary. Maternal adrenal cortices were also hypotrophic with diffusely pale cells, extensive pyknosis in the fasiculata-reticularis layers, and scattered areas of necrosis.

Fetal heart rates (recorded as an indicator of general fetal condition) in normal pregnancies ranged from 180 to 230 beats/min at the start of the fetal adrenal cannulation procedure and from 180 to 210 beats/min at the time of sample collection. In the hypophysectomy group, fetal heart rates ranged from 175 to 220 initially and from 135 to 190 at the start of sampling.

Cortisol data. Maternal and fetal cortisol data in normal and maternal hypophysectomy groups are included in Table I and Figs. 1–3. Comparisons of various results, including probabilities of statistical significance determined by Student's *t* test (21), are presented in the figure legends.

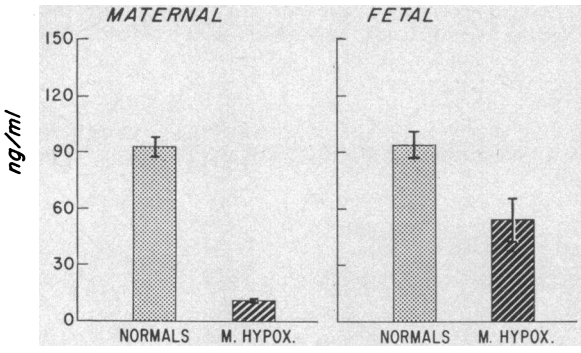

FIGURE 1 Maternal and fetal mean peripheral plasma cortisol concentrations in normal and maternal hypophysectomy groups. *P* values for various comparisons are as follows: normal vs. hypophysectomy groups, maternal ($P < 0.001$), fetal ($P < 0.025$), maternal vs. fetal, normal group ($P < 0.9$), hypophysectomy group ($P < 0.01$).

Systemic cortisol concentrations were markedly higher in normal mothers than in those having undergone hypophysectomy, as seen in Fig. 1. Fetal values were also higher in normal than in hypophysectomy pregnancies. Maternal and fetal systemic concentrations in normal pregnancies were virtually the same, whereas fetal values were significantly greater than those in the mother in the presence of maternal hypophysectomy.

As depicted in Fig. 2, fetal pre- and post-ACTH adrenal concentrations were significantly greater than the fetal peripheral concentrations in both normal and maternal hypophysectomy groups, indicating active secretion of cortisol by the fetus.

Fetal secretory rates for cortisol were calculated from the sample collection time, adrenal plasma volume, and adrenal plasma concentration. Since the total quantity of cortisol measured in fetal adrenal samples represented not only the steroid secreted by the adrenal glands but also that contained in arterial blood delivered to the adrenal glands and that contained in renal venous blood collected with the adrenal effluent, systemic cortisol concentrations were subtracted from adrenal concentrations in order to calculate corrected, true secretory rates. Rapid deterioration of the fetus due to blood volume depletion after collection of the large fetal peripheral arterial sample necessitated the use of this single value for correcting both pre- and post-ACTH fetal cortisol secretory rates.

Fetal cortisol secretory rates in normal and hypophysectomy pregnancies are compared directly and relative to fetal body and adrenal weights in Fig. 3. Fetal pre-

ACTH secretory rates for cortisol were somewhat greater in hypophysectomy than in normal pregnancies, but the differences were not statistically significant in any of the three modes of comparison. The fetal post-ACTH cortisol secretory rates were virtually the same in the fetuses of normal and hypophysectomized mothers.

Corticosterone data. Maternal and fetal corticosterone data are included in Table I.

The systemic concentration of corticosterone was significantly higher in normal than in hypophysectomized mothers ($P < 0.001$), just as it was in fetuses in normal compared with hypophysectomy groups ($P < 0.001$). The maternal systemic concentration of corticosterone was significantly higher than that in the fetus in normal pregnancies ($P < 0.05$). In the presence of maternal hypophysectomy, however, both maternal and fetal values were quite low with little difference between them.

As was the case for cortisol, fetal pre- and post-ACTH adrenal corticosterone concentrations were significantly greater than fetal peripheral arterial concentrations both in normal pregnancies and in the presence of maternal hypophysectomy ($P < 0.005$). The pre- and post-ACTH fetal secretory rates for corticosterone were not significantly different in normal from those in hypophysectomy pregnancies either upon direct comparison or relative to fetal body or adrenal gland weights.

Fetal autopsy data. Mean body weight±SE of cannulated fetuses in the normal group was 242±9 g with a range of 196–269 g. In the hypophysectomy group the body weight of cannulated fetuses was 221±12 g with a range of 179–263 g. In three of the hypophysectomized dogs, from one to two fetuses were found to have died and been partially resorbed. Adrenal weights (combined right and left) in fetuses actually studied in normal animals were 33.10±3.36 mg and in hypophysectomized mothers were 41.88±3.58 mg.

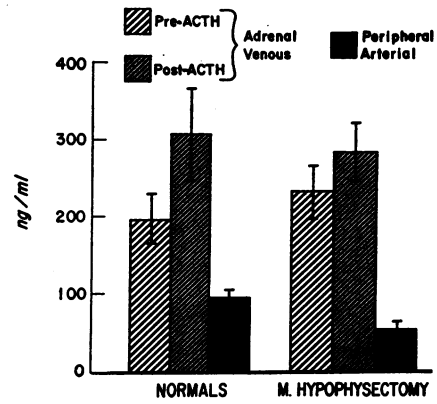

FIGURE 2 Mean plasma concentrations of cortisol in fetal adrenal effluent, pre- and post- ACTH, compared with those in fetal peripheral arterial blood in normal and hypophysectomy pregnancies. P values for comparisons between values are as follows: normal pregnancies, pre-ACTH vs. peripheral ($P < 0.05$), post-ACTH vs. peripheral ($P < 0.02$); hypophysectomy pregnancies, pre-ACTH and post-ACTH vs. peripheral ($P < 0.005$ in both cases).

A comparison of fetal adrenal gland weights in normal and hypophysectomy pregnancies based on measurements in all nonoperated fetuses is shown in Fig. 4. Fetal adrenal gland weights were significantly greater in hypophysectomy than in normal pregnancies both upon direct comparison and relative to fetal body weight.

Histologic sections of fetal adrenal glands (stained with hematoxylin and eosin) showed a clear-cut zona glomerulosa in normal fetuses. Fasiculata and reticularis zones were also present with an indistinct division

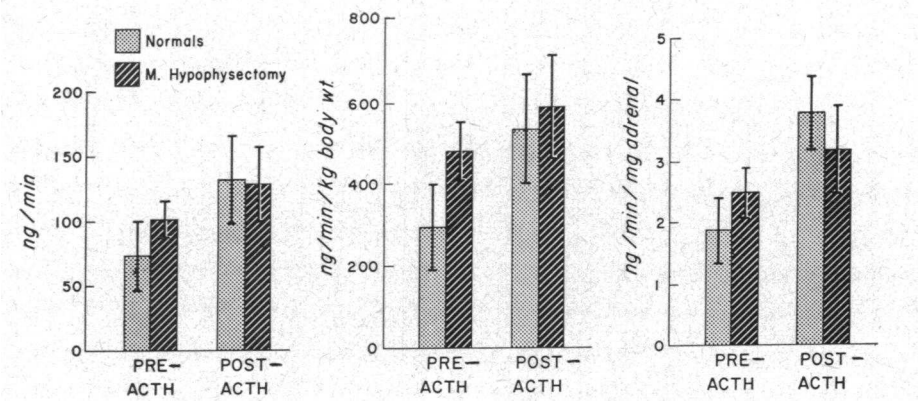

FIGURE 3 Fetal pre- and post-ACTH mean cortisol secretory rates in normal and hypophysectomy pregnancies, compared directly and relative to fetal body and adrenal gland weights. P values for comparisons between normal and hypophysectomy groups are as follows: direct, pre-ACTH ($P < 0.4$) post-ACTH ($P < 0.9$); relative to body weights, pre-ACTH ($P < 0.3$) post-ACTH ($P < 0.8$); relative to adrenal gland weights, pre-ACTH ($P < 0.5$) post-ACTH ($P < 0.6$).

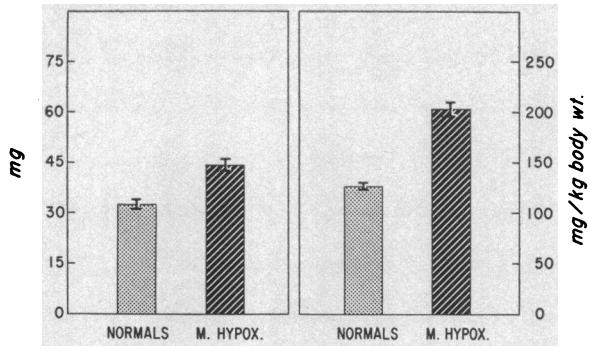

FIGURE 4 Mean weights of adrenal gland pairs in all nonoperated fetuses in normal and hypophysectomy pregnancies compared directly and relative to fetal body weights. P values are as follows: normal vs. hypophysectomy groups, direct ($P < 0.001$), relative to body weights ($P < 0.001$).

between the two. There was considerable granularity and some degree of vacuolization within the cytoplasm of the zona fasiculata-reticularis. No striking qualitative difference in fetal adrenal glands derived from hypophysectomy pregnancies could be found as compared with those derived from normal pregnancies.

DISCUSSION

In this work we were concerned with relations between the pituitary-adrenal systems of mother and fetus. More particularly, the quantitative aspects of this relationship and the influence of the mother in control of fetal pituitary-adrenal function were of interest. The investigation was pursued through comparison in normal and hypophysectomized dogs of maternal and fetal systemic corticosteroid levels, fetal corticosteroid secretory rates, and fetal adrenal morphology. While all samples were analyzed for cortisol and corticosterone, conclusions have been based directly on cortisol data. Corticosterone results, however, are largely supportive of these conclusions.

Maternal hypophysectomy was shown to be effective in each experiment by demonstration in the mother of decreased systemic levels of corticosteroids, loss of adrenal secretory responsiveness to operative stress and exogenous ACTH,[1] and histologically atrophic adrenal cortices. Progesterone was administered to the hypophysectomized animals in order to maintain pregnancy in the absence of normal ovarian function secondary to pituitary ablation (22–25). Hypophysectomized dogs were maintained for approximately 3 wk before performance of definitive experiments to allow maximal fetal response to alterations in maternal endocrine function.

[1] Loss of ACTH responsiveness by the dog adrenal has been shown to occur approximately 48 h after hypophysectomy (B. T. Jackson, unpublished data).

Although experiments were performed acutely, under halothane anesthesia, at the time of operation for fetal adrenal cannulation, fetuses were maintained *in utero* throughout, loss of amniotic fluid was insignificant, and the uterus was only partially exposed and was protected by a plastic drape. Fetal heart rates at the time of sampling were somewhat lower in the maternal hypophysectomy group than in the normal group but were quite similar to those found in a previous study of fetal glucocorticoid secretion in normal mongrel dogs (8). There was also a wide overlap in fetal heart rates in the hypophysectomy group with those in a prior study (26) conducted also under halothane anesthesia in which fetal pH's and blood gasses were generally at physiologic levels. Fetal heart rates in both normal and hypophysectomy groups were in the range of those seen in canine fetuses undergoing other types of operations in our laboratory that have characteristically been associated with long-term fetal survival.[2]

The sensitivity of the steroid assay used appears to have been adequate for determinations of cortisol as indicated by sample cortisol content to blank ratios ranging from 15 to over 200. Sample to blank ratios were lower for corticosterone than for cortisol but were generally greater than 10.

The cortisol data in normal purebred beagles reported herein show no significant differences from results obtained in a prior study of normal mongrel dogs in this laboratory (8) which offers supplemental validation of the normal control data in the present investigation. Present data also confirm the active secretion of corticosteroids by the normal dog fetus as previously reported (8).

Peripheral concentrations of cortisol, essentially equal in normal mothers and fetuses, were significantly lower in both after maternal hypophysectomy. The decrease was much more marked in maternal than in fetal levels, yielding a maternal-fetal cortisol ratio of about 0.2. Milkovic and Milkovic (27), on the other hand, found in pregnant rats 12 h after maternal adrenalectomy that systemic concentrations of corticosterone were reduced by 50% from control values in the mother, but were unchanged in the fetus. The marked dissimilarity in elapsed time between maternal manipulation and collection of samples (ca. 3 wk vs. 12 h) probably accounts for the disparate fetal response to ablation of maternal adrenal function in the two studies, although the significance of species differences cannot be discounted.

The finding of higher concentrations of corticosteroids in maternal than in fetal plasma in intact dogs, man, rats, and sheep (27–30) has suggested the presence of a specific mechanism controlling the relative amounts of these substances in mother and fetus, but only in sheep

[2] Jackson, B. T., and G. J. Piasecki, unpublished data.

has such a factor been demonstrated directly (30). The aforementioned shift in the maternal-fetal ratio of peripheral cortisol concentrations from 1.0 to 0.2 occasioned by maternal hypophysectomy does not support the presence of a predominating mechanism of this type in the dog. It does indicate, however, that the relative steroid input into maternal and fetal compartments significantly influences such concentration ratios. The findings in a number of previously reported studies also support the importance of the input factor in this regard (*27–29*, 31).

It is now well accepted that one of the chronic effects of ACTH upon the adrenal is the maintenance of the mass of the gland (*32*). Increased fetal adrenal gland weights in fetuses in hypophysectomy pregnancies, therefore, indicate increased fetal ACTH secretion. The increased ACTH production in the fetus is assumed to be secondary to the decreased systemic concentrations of corticosteroids which were demonstrated to be present and implies, therefore, the presence of an active pituitary-adrenal feedback mechanism in the dog fetus late in gestation just as has been established previously in the fetal rat (1) and suggested in the neonatal dog (33).

The fetal pituitary-adrenal system is obviously influenced functionally by that of the mother as is shown by the decreased circulating levels of cortisol and the apparent increase in ACTH secretion which occur in the fetus in response to decreased adrenal secretion in hypophysectomized mothers. These changes could be accounted for either by a loss of cortisol normally transferred transplacentally from the mother to the fetus or by increased fetal metabolic clearance of cortisol through placental transfer to the depleted mother or by both. Direct suppression of fetal pituitary-adrenal function by cortisol of maternal origin does seem likely but is clearly limited in extent as the fetal system does remain functional. That fetal ACTH secretion is not more strikingly suppressed in such a species as the dog in which corticosteroids are readily transferred across the placenta (28) is an interesting point, but its full discussion is beyond the scope of this paper.

Evidence has already been presented for clearly increased chronic trophic (weight maintaining) ACTH effects in fetuses of hypophysectomized mothers. An additional chronic effect of ACTH on the adrenal gland also exists: the maintenance of adrenal cortical responsiveness, that is, the capacity of the adrenal to respond acutely to ACTH with the secretion of corticosteroids (34, 35). Elevated ACTH stimulation of the adrenal gland over a period of time would be expected to result in increased responsiveness of the gland (*36*, *37*). In the present investigation adrenal responsiveness was compared in fetuses in normal and hypophysectomized mothers by determination of the maximum capacity of the fetal glands to secrete cortisol in response to exogenous ACTH. No significant difference was found between the two groups in this respect, in spite of the evident differences in long-term ACTH secretion. This result could be explained in a number of ways. Increased ACTH secretion in fetuses of hypophysectomized mothers, although quantitatively adequate to induce adrenal hypertrophy, may have been inadequate to bring about increased responsiveness. Some aspect of immaturity of the fetal adrenal gland may limit its capacity for increased enzyme formation, e.g., cholesterol side-chain desmolase (38), in response to greater ACTH stimulation. On the other hand, some fetal adrenal enzyme system, little affected by ACTH and not ordinarily rate limiting in the adult, may be deficient in the fetal gland and thus rate limiting there. It is also conceivable that some precursor of maternal origin necessary for cortisol formation by the fetus may be a limiting factor. Although little information pertinent to this finding is available in the literature, there have been two reports suggesting in fetal rats a similar dichotomy between the chronic effects of ACTH in maintaining adrenal weight and the capacity for steroid synthesis (39, 40).

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