Developmental Aspects of the Pituitary-Adrenal Axis Response to Hemorrhagic Stress in Lamb Fetuses In Utero

JAMES C. ROSE, ALASTAIR A. MACDONALD, MICHAEL A. HEYMANN, and ABRAHAM M. RUDOLPH, *The Cardiovascular Research Institute, and the Departments of Physiology, Pediatrics, and Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, California 94143*

ABSTRACT Plasma ACTH and corticosteroid concentrations were measured by radioimmunoassay in chronically catheterized fetuses of 32 pregnant sheep. Fetal plasma ACTH levels 38±5 pg/ml (means±SEM) were slightly ($P < 0.05$) lower than maternal 54±4 pg/ml levels. No general rise in fetal plasma ACTH concentration was noted before 140 days gestation; however, fetal plasma corticoid levels began to increase after about 125 days. This suggested that an increase in fetal adrenal responsiveness to endogenous ACTH occurred during gestation.

Hemorrhage of 15% of estimated blood volume decreased mean arterial pressure from 54±3 to 36±3 torr and increased plasma ACTH from 30±5 to 130 ±30 pg/ml in fetuses older than 0.80 gestation. In fetuses younger than 0.67 gestation, 15% hemorrhage caused no change in plasma ACTH levels despite a significant fall in mean arterial pressure. This suggests that system(s) subserving the ACTH response to mild hemorrhage are either absent or nonfunctional in the younger fetuses. The hemorrhage-induced increase in plasma ACTH levels was associated with a small rise in plasma corticoids in fetuses younger than 0.94 gestation. In older fetuses, a similar increase in plasma ACTH was associated with a pronounced increase in plasma corticoid levels. This also suggests that an increase in adrenal responsiveness to endogenous ACTH occurs during gestation. No detectable changes in maternal plasma ACTH or corticoids were found in response to fetal hemorrhage, thus the fetal pituitary-adrenal axis can autonomously respond to stress.

INTRODUCTION

Numerous investigators have demonstrated that hemorrhage increases pituitary-adrenal activity in adult animals (1–4). However, limited information is available concerning the response of the fetal pituitary-adrenal system to hemorrhage and, to our knowledge, all previous work on this topic has been done on acutely exteriorized fetuses (5, 6). Various stresses associated with exteriorization and the presence of anesthesia may have significant effects on fetal pituitary adrenal activity in such acute studies. Therefore, a specific aim of the present investigation was to examine the pituitary-adrenal response to hemorrhage in the fetus in utero in the absence of the complicating effects of exteriorization and anesthesia. Consequently, all experiments were performed on fetal lambs bearing chronically implanted catheters. We thought that results obtained with this preparation would be more representative of the fetal pituitary-adrenal response to a stimulus in utero. Also, we evaluated the response of the pituitary-adrenal axis to hemorrhage at various gestational ages to assess whether or not there were developmental differences.

In addition, we simultaneously monitored plasma ACTH and corticoid levels in relatively undisturbed fetuses in utero (0.46–0.99 gestation) to ascertain whether or not the well-documented increase in fetal plasma corticoid levels before parturition is accompanied by an increase in plasma ACTH concentration.

Portions of this work were previously reported at the 59th Annual Meeting of the Endocrine Society, 1977 (Abstract 172).

J. C. Rose was the recipient of Postdoctoral Fellowship NS-00712 from the National Institutes of Health. His present address is the Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem, N. C. 27103.

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METHODS

We studied the fetuses of 32 pregnant sheep of Dorset and Hampshire mixed breeding. The gestational ages of the lambs were known and ranged from 68 to 146 days (0.46 to 0.99 gestation). The animal management and anesthetic and surgical procedures used were as described earlier (7). In fetuses older than 95 days, polyvinyl catheters (inside diameter [ID] 0.038 inch, outside diameter [OD] 0.066 inch)[1] were inserted into the saphenous artery and vein of one or both fetal hind limbs and advanced to the descending aorta and proximal inferior vena cava, respectively.

In fetuses younger than 90 days gestation, we exposed quaternary or tertiary tributaries of the umbilical artery and vein through a small uterine incision. Polyvinyl catheters (ID 0.030 inch, OD 0.048 inch) were inserted and advanced so as to lie in a major placental artery and vein close to the umbilical cord. In one fetus, aged 106 days, and twins, aged 87 days, polyvinyl catheters (ID 0.038 inch, OD 0.066 inch) were inserted into a carotid artery and jugular vein and advanced to the common carotid artery and superior vena cava, respectively.

The skin and uterine incisions were sutured and all catheters were led to the left flank of the ewe where they were protected by a Teflon cloth pocket sewn onto the flank. 1,000,000 U of penicillin G and 500 mg of Kanamycin were injected into the amniotic cavity and the saphenous vein of the sheep on the day of surgery and each day thereafter.

All catheters were filled with heparin solution and sealed with metal plugs. The animals were allowed at least 48 h to recover from surgery before the study. All blood samples, pressures, and heart rates were obtained while the unsedated ewe stood quietly in her stall. We drew arterial blood samples (1 ml from the fetus and 2 ml from the ewe) into lightly heparinized syringes, immediately transferred them to chilled centrifuge tubes, and separated the plasma by centrifugation at 1,000 *g* for 10 min at 4°C. The plasma was then aspirated, aliquoted, and stored frozen for subsequent hormone analysis. Generally, we collected baseline blood samples and did experiments between 9 a.m. and 12 noon. We have not presented any observations made within 72 h of fetal death. Fetal weight was estimated from the data of Winters and Feuffel (8) and fetal-placental blood volume from the data of Creasy et al. (9). The animal was hemorrhaged by withdrawing blood from an arterial catheter at the rate of 1.5% of estimated blood volume per minute. We removed either 5 or 15% of estimated fetal-placental blood volume. Strict aseptic procedures were used.

At the onset of all and end of 19 observation periods, a fetal arterial blood sample was obtained for the measurement of hematocrit; pH, Pa_{O_2}, and Pa_{CO_2}, were measured with a Radiometer blood-gas analyzer (Radiometer Co., Copenhagen, Denmark) and appropriate electrodes. In fetal preparations, heart rate, arterial and venous blood pressures, and amniotic and maternal arterial pressures were monitored continuously throughout the studies. All pressures were measured with P_{23}Db (Statham Instruments Div., Gould Inc., Oxnard, Calif.) pressure transducers. Fetal arterial and venous pressures are reported with respect to intrauterine pressures. Fetal heart rate was monitored continuously with a cardiotachometer triggered from the fetal arterial pressure signal. All pressure and heart rate recordings were made on a Beckman direct-writing recorder (Beckman Instruments, Inc., Fullerton, Calif.).

Blood samples for hormone analysis were obtained simultaneously from the fetus and mother before and at 3, 11, 15, 25, and 40 min after the start of the hemorrhage. Approximately 30 min after hemorrhage, the shed blood was returned to the hemorrhaged animal via a venous catheter at a rate identical to that used for withdrawal. An appropriate volume of warm isotonic saline also was administered to the fetus to compensate for the volume of blood set aside for hormone analysis. We studied a few animals on more than one occasion, in which case at least 48 h elapsed between experiments.

Because the concentrations of ACTH and corticoids in blood samples drawn from the three fetuses with carotid artery catheters did not differ significantly from the values in samples collected from femoral or umbilical arteries, these values have been included in the calculation of means.

ACTH measurement. The antisera used in the assay was produced in rabbits, as described previously (10). On a molar basis, it shows 100% cross-reactivity with α_0^{1-39} ACTH, greater than 90% cross-reactivity with the N-terminal α^{1-24} fragment of the molecule, and less than 30% cross-reactivity with N-terminal α^{1-17} fragment; also, it does not cross-react with the α^{1-10}, α^{1-10} amide, α^{11-19}, α^{11-24}, or α_0^{25-39} fragments of the molecule. Luteinizing hormone, growth hormone, thyroid-stimulating hormone, and prolactin do not cross-react in the assay. We used synthetic human ACTH (140 U/mg), generously provided by Doctors C. H. Li and J. Ramachandran, for both standards and iodination. The hormone was iodinated as described by Berson and Yalow (11).

ACTH was extracted from unknown plasma samples and standards (in hypophysectomized dog plasma) as described by Rees et al. (12) with minor modifications.

The assay buffer was that of Berson and Yalow (11). On the day of assay, appropriate dilutions of standards, samples, and antisera were pipetted into 10 × 75-mm plastic test tubes and incubated at 4°C for 2 days. After this, iodinated ACTH was added to each tube, and the incubation was continued for an additional 4 days. At the end of the incubation period, antibody bound and free hormone were separated with dextran-coated charcoal (13). Damage control tubes containing no antisera were included in every assay. Both bound and free fractions were counted in a Packard autogamma spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) and B to F ratios were calculated. The limit of sensitivity of the assay was 1.5 pg. Parallelism was observed between purified ovine ACTH, ACTH extracted from plasmas of rat, dog, fetal and adult sheep, and monkeys, and the synthetic human ACTH used for standards. Three different plasma pools were analyzed in multiple assays to determine the interassay coefficient of variation. Values observed were 170±15 (mean±SD), 116±16, and 55±8 pg/ml for coefficient of variation of 9, 14, and 15%, respectively. The intraassay coefficient variation was 6% when one sample was analyzed 10 times in a single assay. We analyzed all samples from a single experiment in the same assay to minimize the effects of interassay variation.

Plasma ACTH levels in undisturbed, intact, and adrenalectomized rats measured with the assay were similar to levels reported elsewhere (12, 14, 15). Plasma samples obtained from hypophysectomized dogs and dexamethasone-treated humans contained no detectable ACTH, and surgical stress in intact dogs (16) produced a prompt increase in plasma ACTH to levels comparable to those reported by others (17).

Steroid measurements. We measured the plasma corticosteroid levels (corticoids) by radioimmunoassay (Clinical Assays Inc., Cambridge, Mass.). We tested the specificity of the antiserum supplied in the kit and found the following cross-reactivity: corticosterone 17%, 17α hydroxyprogester-

[1] *Abbreviations used in this paper*: ID, inside diameter; OD, outside diameter.

one 30%, progesterone 5%, 11-desoxy-cortisol 16%, and desoxycorticosterone <2%. This agrees well with data furnished by the manufacturer. They also report that cortisone, testosterone, and tetrahydrocortisol cross-react <3% with this antiserum. Intra- and interassay coefficients of variation were 5 and 7%, respectively.

Statistical analyses. Observations were summarized and presented as means±SEM. Statistical tests were done by analysis of covariance and unpaired *t* test, except for sets of paired observations which were evaluated by paired *t* test (18).

RESULTS

Basal concentrations of plasma ACTH and corticoids during pregnancy. No differences were detected in maternal plasma ACTH (54±4 pg/ml) and corticoid (3.0±2 ug/dl) concentrations at various stages of pregnancy.

Fetal plasma ACTH (38±5 pg/ml) concentrations were slightly lower than maternal levels ($P < 0.05$).

Higher fetal ACTH concentrations were found after 140 days gestation and were associated with the delivery of a liveborn lamb within 72 h of the peak value (Fig. 1). We did not find any general rise in fetal plasma ACTH concentration before 140 days. However, there was a significant increase in fetal plasma corticoid concentrations (Fig. 1); the corticoid levels began to rise after about 125 days, and maximal levels were found in fetuses older than 140 days. Fig. 2 illustrates this plasma corticoid increase with plasma ACTH remaining relatively unchanged in a single fetus. The association between ACTH and corticoid concentrations in fetal plasma was tested by analysis of covariance, and it was found that fetuses older than 125 days had higher concentrations of plasma corticoids than younger fetuses with the same plasma ACTH concentration (Fig. 3).

Fetal hemorrhage: changes induced in plasma ACTH and corticoids. Maternal plasma ACTH and corticosteroid concentrations did not change significantly as a consequence of fetal hemorrhage. The effects on the fetus of 15 or 5% hemorrhage were examined in 23 experiments on 18 fetuses aged 100–144 days; five animals were studied more than once. The results are summarized in Table I*a*. The larger hemodynamic stimulus induced a fall in blood pressure and a rise in fetal plasma ACTH and corticoid concentrations, whereas the smaller hemorrhage produced no significant changes in the parameters measured. These effects are exemplified in Fig. 4 which shows the effects of simultaneous hemorrhage in twin fetal lambs; one had a 15% and the other a 5% blood loss. 3 days later, the volumes removed in the lambs were reversed. In each case, the smaller hemorrhage did not alter arterial pres-

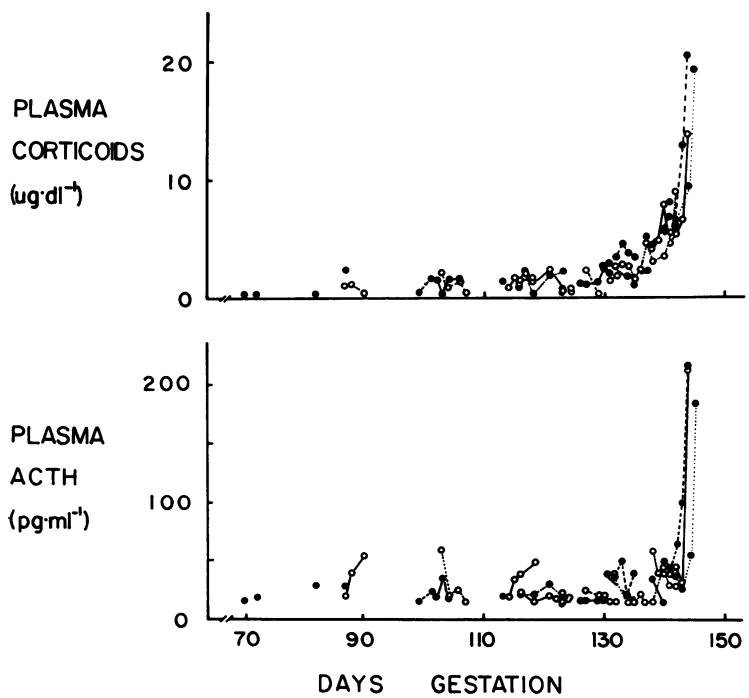


FIGURE 1 Plasma ACTH (lower) and corticoid (upper) levels in 25 fetal lambs from 70 to 146 days gestation. Individual open and closed circles denote measurements in different animals. Circles connected by lines represent serial measurements in the same animal.

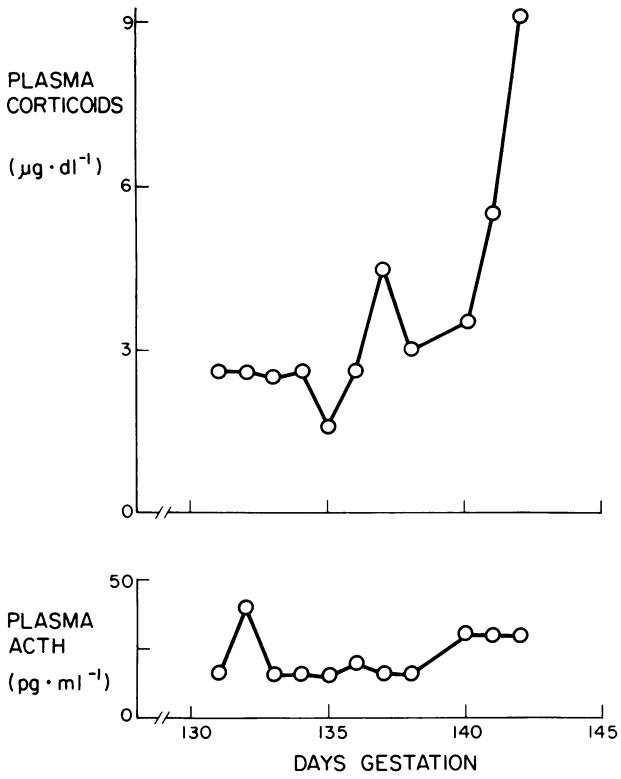


FIGURE 2 Daily measurements of plasma ACTH and corticoid concentrations in a fetal lamb in utero from day 131 to day 142 of gestation.

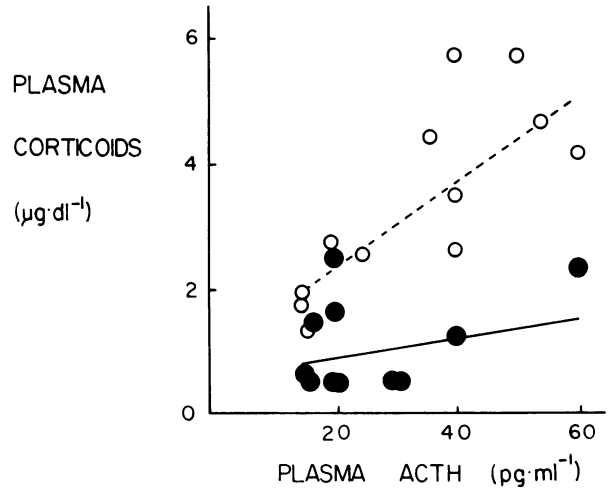


FIGURE 3 Plot of basal plasma ACTH vs. basal plasma corticoid concentrations in fetuses (●) <125 and (○) >125 days gestation. Slopes of the regression lines for animals <125 (——) and >125 days (– – –) are different ($P < 0.001$). The mean plasma corticoid concentration is higher ($P < 0.001$) in the older animals whereas plasma ACTH concentrations are not different in the two groups.

sure, plasma ACTH, or corticoids, whereas the 15% blood loss decreased arterial mean pressure and increased concentrations of ACTH and corticoids.

We also found age-related differences in the response of the fetal hypophysis to a 15% hemorrhage (Table I*b*). In seven fetuses aged 68–99 days, hemorrhage produced no change in plasma ACTH (20±6 pg/ml) in spite of a significant ($P < 0.005$) decrease in mean arterial pressure from a control of 38±2–28±2 torr. In 17 studies on 14 older fetuses of 100–144 days gestation, there was a significant increase ($P < 0.001$) in plasma ACTH concentrations from a control of 30±5–130±30 pg/ml after hemorrhage. Blood pressure in these animals fell from 49±3 to 33±3 torr.

There were also age-related differences in the responsiveness of the fetal adrenal to endogenous ACTH. A ratio made of the hemorrhage-induced change in plasma ACTH concentration and the resulting change in plasma corticoid concentration (Fig. 5) indicated that in all but one of the fetuses aged between 100 and 135 days gestation, adrenal responsiveness to plasma ACTH was low, but that by 139 days the adrenal responsiveness to similar changes in plasma ACTH concentration was increased.

DISCUSSION

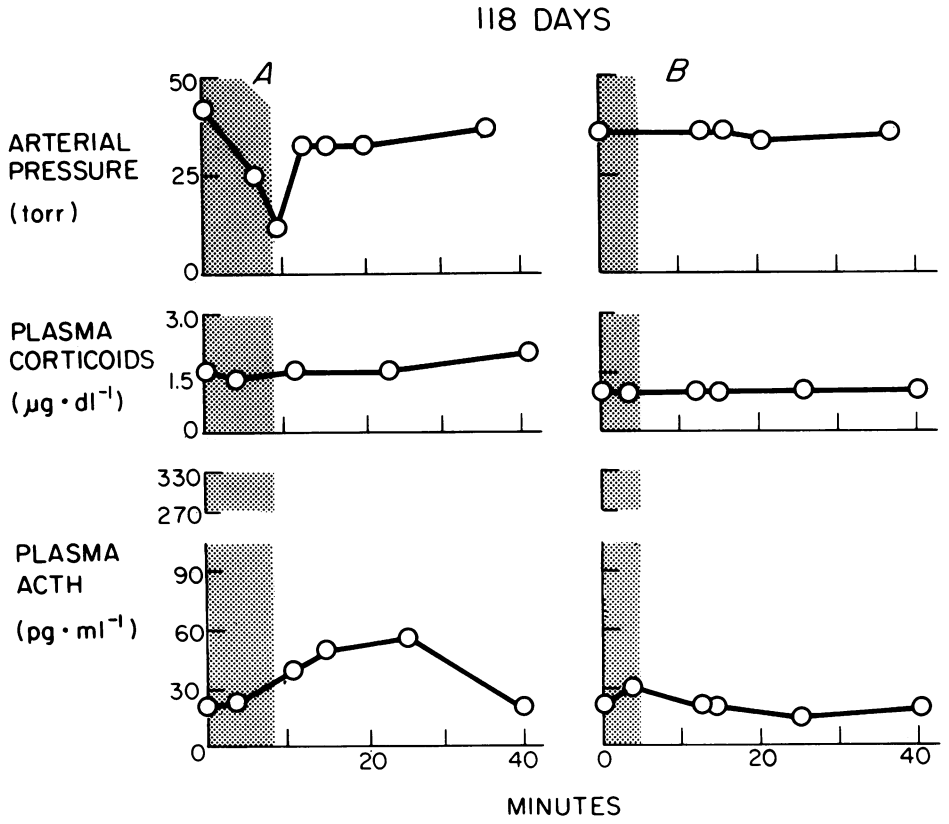
In the present studies, we monitored plasma ACTH and corticoids in relatively undisturbed fetal sheep in utero from mid-gestation to term. Fetal plasma cor-

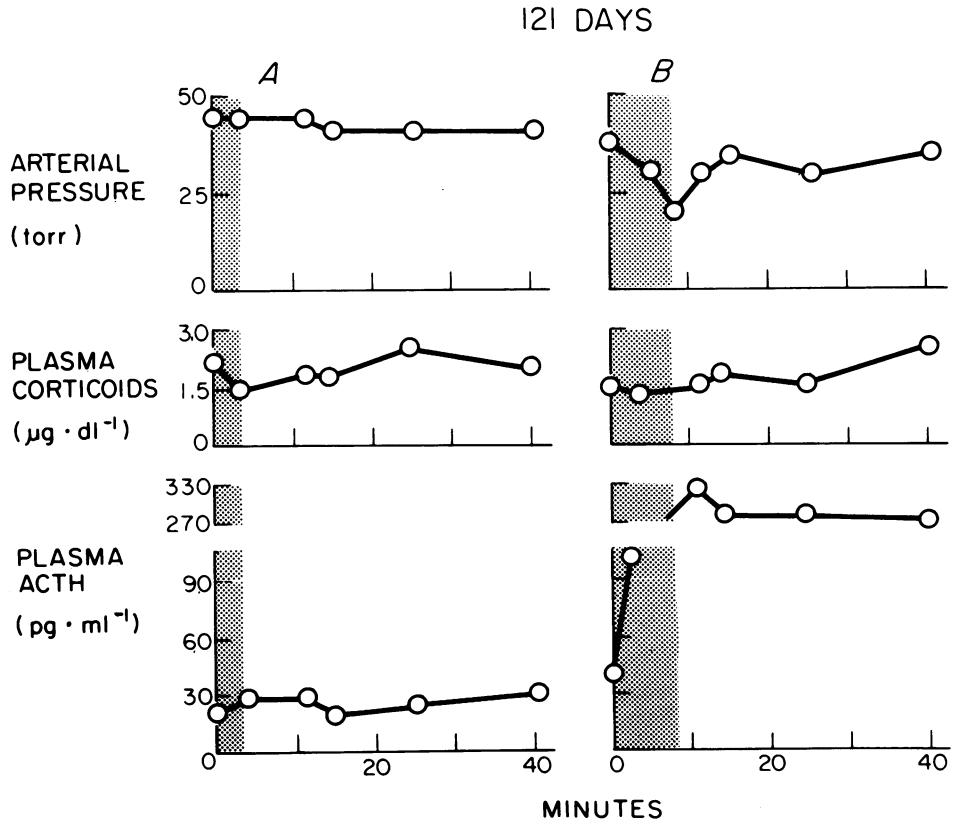
TABLE I

Changes in Fetal Arterial Mean Pressure, ACTH concentration, and Corticoid Concentration after Hemorrhage of 5 or 15% of Estimated Blood Volume of Older Fetal Lambs (0.80–0.98 Gestation), and of 15% Hemorrhage of Younger Fetal Lambs (0.48–0.68 Gestation)

	Arterial pressure	Plasma ACTH	Plasma corticoids
	torr	*pg/ml*	*μg/dl*
a. Older fetal lambs			
Control	49±4	20±5	2.6±1.0
5%	47±4	30±4	2.4±1.1
n	6	6	6
P	NS	NS	NS
Control	49±3	30±5	3.5±0.7
15%	33±3	130±30	5.5±1.1
n	17	17	17
P	<0.01	<0.01	<0.01
b. Younger fetal lambs			
Control	38±2	20±3	1.2±0.4
15%	28±2	20±5	1.5±0.4
n	7	7	7
P	<0.01	NS	NS

Results are ± SEM.


118 DAYS
A
B
ARTERIAL PRESSURE (torr)
50
25
0
PLASMA CORTICOIDS (μg · dl⁻¹)
3.0
1.5
0
PLASMA ACTH (pg · ml⁻¹)
330
270
90
60
30
0
20
40
MINUTES


121 DAYS
A
B
ARTERIAL PRESSURE (torr)
50
25
0
PLASMA CORTICOIDS (μg · dl⁻¹)
3.0
1.5
0
PLASMA ACTH (pg · ml⁻¹)
330
270
90
60
30
0
20
40
MINUTES

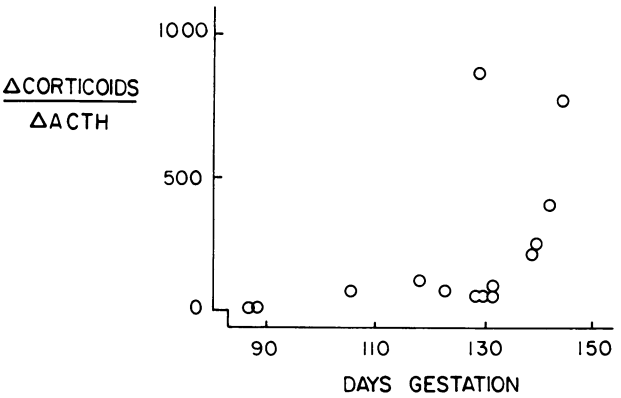


FIGURE 5 Ratio of the hemorrhage-induced change in plasma ACTH and resulting change in corticoids in animals studied once during the latter half of gestation.

ticoid concentrations were low but detectable as early as 0.5 gestation, and the increase observed after 125 days gestation was similar to that described by others (19–25). The increase in fetal plasma corticoids that occurs before birth has been suggested as instrumental in initiating labor in this species (26, 27).

Transfer of glucocorticoids from the maternal to the fetal compartment does not appear to explain the increased steroid levels in the fetus because transplacental passage of corticoids is low in this species (28). Results of several studies (29–31) suggest that the elevated fetal plasma corticoid concentrations near term reflect an increased corticoid secretion rate by the fetal adrenal. Our results showed that maternal plasma corticoid levels were unchanged when fetal plasma corticoids were increasing prepartum; this, too, suggests that the elevated fetal corticoid levels do not result from placental transfer of steroid from mother to fetus. A similar disasssociation of maternal and fetal plasma corticoid levels has been noted by others (19, 22, 23).

The basal fetal plasma ACTH levels reported here are not only considerably lower than the values reported in studies using acutely exteriorized lambs (5, 6, 32, 33), but also lower than the values in chronically catheterized fetal sheep (24, 25, 34–38). Indeed, in our chronically catheterized animals, the mean resting ACTH concentration was slightly lower in fetal than in maternal plasma and was similar to basal ACTH values observed by others in adult animals (12, 14–17, 35–39). The ACTH levels reported here imply that the fetal animals used in the present studies were relatively undisturbed, and the higher levels reported (5, 6, 24, 25, 32, 38) are what might be expected in stressed animals.

Our finding that the prepartum increase in fetal plasma corticoids occurs before a significant increase in fetal plasma ACTH concentration (Figs. 1 and 2) is of particular interest because it suggests that the elevated fetal plasma corticoid levels observed before parturition are not caused by fetal ACTH. We recognize that single observations in the fetus may not reveal episodic elevations in plasma ACTH concentration if fetal hormone secretion is pulsatile; however, the lack of evidence (24, 25, see above) for increased ACTH secretion occurring before the preparturient plasma corticoid increase, implies that the fetal adrenal increases in responsiveness to endogenous ACTH during gestation. Results of in vitro studies on fetal adrenal responsiveness to ACTH are consistent with such a possibility (40–42), and increased adrenal responsiveness to exogenous ACTH has been reported (31).

Little is known about the control of ACTH secretion in the fetus. A few observations imply that the fetal pituitary can secrete ACTH in response to stress. In the acutely exteriorized fetus, massive hemorrhage causes an increase in plasma ACTH concentration (5, 6). Maternal hypoxia also increases plasma ACTH concentration in mother and fetus in this acutely exteriorized preparation (5, 33). However, exteriorization is a severe stress to mother and fetus; thus, results obtained with this model may not accurately represent responses in utero.

Maternal hypoxia is also an effective stimulus in the chronic preparation. However, in any study where manipulation activates maternal as well as fetal ACTH secretion, it is extremely difficult to rule out the possibility that some humoral factor involved in the maternal stress response (for example, corticotropin releasing factor) may cross the placenta and have a profound effect on fetal ACTH secretion. In the present investigations, we subjected chronically catheterized fetuses to hemorrhage to study the effects of this hemodynamic stimulus on the pituitary-adrenal axis in utero. It is evident that hemorrhage induced an increase in plasma ACTH concentration in fetuses greater than 100 days gestation. The changes in plasma ACTH levels after hemorrhage probably were not mediated via changes in fetal blood gases, hematocrit, or pH (Table II). Although decreased hormone clearance might have resulted from the reduction in umbilical blood flow produced by hemorrhage (43) and could partially account for the elevation in ACTH

FIGURE 4 Effects of simultaneous hemorrhages in twin fetal lambs on mean arterial pressure, plasma corticoid, and ACTH concentrations. Shaded area indicates period of blood withdrawal. At 118 days twin A received a 15% and twin B a 5% hemorrhage. 3 days later the volumes removed in the lambs were reversed.

TABLE II

Fetal Blood pH, Pa_{O_2}, Pa_{CO_2}, and Hematocrit before and after 15% Hemorrhage

	pH	Pa_{O_2}	Pa_{CO_2}	Hematocrit
Prehemorrhage	7.34±0.01	22±0.7	43±1.2	31±1.4
Number of observations	24	22	23	24
Posthemorrhage	7.35±0.02	21±1.7	44±1.0	29±1.8
Number of observations	18	16	17	18
P	NS	NS	NS	<0.005

Results are ± SEM.

that occurred, the rapidity of the increase in plasma ACTH concentration after initiation of hemorrhage strongly suggests an increase in secretion. It is clear that the increase in fetal plasma ACTH concentration occurred in the absence of any detectable change in activity of the maternal pituitary-adrenal axis. Our studies clearly demonstrate that the pituitary-adrenal axis of the fetal sheep does possess autonomy and can respond to fetus-specific stimuli.

Signals affecting activation of the pituitary-adrenal system by hemorrhage include hypotension, hypovolemia, and the rate of change of volume with respect to time (3, 44). In the present experiments, 5% hemorrhage did not cause a significant decrease in fetal blood pressure nor did it increase fetal plasma ACTH levels. Hemorrhage of 15% of estimated fetal blood volume consistently decreased fetal mean arterial pressure and, in 16 of 17 experiments with fetuses older than 100 days gestation, increased fetal ACTH secretion. This suggested that the stimulus producing fetal ACTH secretion was either hypotension or hypovolemia in excess of 5% blood volume. Further work is needed to assess individual effects of hypotension and hypovolemia on activation of the fetal pituitary-adrenal system.

There was an age-related difference in the fetal ACTH response to 15% hemorrhage. Hemorrhage of this magnitude increased plasma ACTH levels only in animals greater than 100 days gestation. No significant increase in plasma ACTH occurred in animals less than 100 days gestation in spite of a decrease in mean arterial pressure after hemorrhage. The observation that pituitary concentration of ACTH does not appear to change during the latter half of gestation (5, 6) would suggest that the absence of the ACTH response to hemorrhage in animals less than 100 days gestation could not be attributed to a lack of hormone stored in the fetal pituitary gland.

If the degree of hypotension were responsible for activating receptors mediating the ACTH secretory response to hemorrhage, then hemorrhage in the younger animals should have stimulated ACTH secretion because the nadir in mean arterial pressure after blood withdrawal was significantly lower in these animals. Thus, one of the components contributing to the release of ACTH in response to 15% hemorrhage may be absent or nonfunctional in animals less than 100 days gestation.

Baroreceptors have been implicated in regulation of ACTH secretion in the adult (44–47), and in the fetus, the sensitivity of the baroreflex to changes in arterial pressure has been shown to increase with gestational age (48). The change in pressure after hemorrhage was greater among older animals (>100 days) and might have produced a stronger stimulus to ACTH secretion than did a similar blood loss in the younger animals (<100 days). Whatever the explanation, we have demonstrated a difference in the fetal pituitary-adrenal stress response apparently related to maturation of the system(s) subserving the response.

The absence of an increase in plasma ACTH concentration after 15% hemorrhage in fetuses younger than 100 days gestation is in contrast to reports by Alexander et al. (5, 6) who noted markedly elevated levels of plasma ACTH after hemorrhage. Their observations were made on acutely exteriorized fetuses that had high resting plasma ACTH concentrations. Furthermore, the magnitude of hemorrhage they used was quite large (50–60% of fetal blood volume). The discrepancy between their results and ours may be due to either one or a combination of the above factors.

That fetuses younger than 139 days showed only slightly increased plasma corticosteroid concentrations to the elevated plasma ACTH concentration produced by hemorrhage, whereas older fetuses responded with larger increases in corticosteroid concentrations, is consistent with in vitro studies that demonstrated increased responsiveness of the fetal adrenal after 139 days gestation (41–42). The change in adrenal responsiveness may be explained, in part, by the increase in adrenal cortical tissue occurring after day 135 of gestation (30, 49). However, in vitro studies have shown that the adrenal cortex from fetuses older than 139 days secretes more corticosteroids per milligram of tissue than the adrenal cortex from younger fetuses (41–42). This suggests that, at about 139 days, cellular changes take place either in receptor sensitivity or intracellular modulation of corticosteroid production and secretion.

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