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Acidosis, Hypercalcemia, and Hyperphosphatemia in Rat Fetuses near Term and Effects of Maternal Acid/Base Loading (40682)[1]

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In most mammalian species examined, the levels of calcium (1–4) and of phosphorus (5–7) in the blood of the fetus at term exceed those of the dam. The difference in serum calcium between mother and fetus may, in part, be explained by the observation that the circulating level of this element in the dam falls in the final stages of gestation (5, 8, 9). In addition, in the many mammals investigated, the level of calcium in the blood of the fetus has been shown to increase as termination of pregnancy is approached (10–12). Finally, in the rat, fetal phosphatemia has been shown to parallel the rise in blood calcium in the last days before birth (13).

We have previously demonstrated that rat fetuses are acidotic near term, and that *in utero* hypercalcemia and early neonatal hypocalcemia are associated with low blood pH and normalization of blood pH, respectively (14). In view of this apparent association at term and immediately after birth, the present studies were undertaken: (i) to investigate whether in the final stages of gestation fetal blood pH shifts concurrently with reported alterations in the levels of calcium and phosphorus; and (ii) to test the hypothesis that changes in the circulating levels of calcium and phosphorus are causally related to blood pH in the developing mammal *in utero*, by either aggravating or alleviating the acidotic state of the conceptus by the maternal administration of ammonium chloride or sodium bicarbonate, respectively.

Materials and methods. Four- to eight-month-old pregnant albino rats and their offspring were used. The adult animals were maintained on Purina laboratory chow and tap water *ad libitum* and housed in a temperature-controlled room illuminated on a 14-hr-light/10-hr-dark cycle.

In order to obtain females at a known time of gestation, the animals were caged with males for a single overnight period. The day of mating was counted as Day 0, and experiments were terminated at preselected times during the last week of pregnancy.

Studies on fetuses in the final week of gestation. Beginning on Day 17 and then daily through the 22nd day of pregnancy, groups of animals were handled as follows:

The gravid female was lightly anesthetized with ether, a laparotomy was performed, and the pregnant uterus was exposed. Fetuses were sequentially bled and excised until the entire litter was sampled. Fetal blood was taken from the axilla while the placental and umbilical circulations were intact. Tail blood was drawn from the mother at the beginning and end of each experiment.

Studies on offspring of pregnant animals receiving either ammonium chloride or sodium bicarbonate orally. Pregnant animals were divided into three groups. Beginning on the 17th day of gestation, tap water was removed and the following substitutions were made. One group of animals was given a solution of 1.5% NH_4Cl, the second group received a 1.5% solution of $NaHCO_3$, and the remaining rats were given distilled water. Animals were allowed to drink freely and fluid intakes were monitored.

All experiments were terminated on the 22nd day of pregnancy and blood samples were obtained as described above.

Determination of blood pH, respiratory gases, total calcium, and inorganic phosphorus. Samples for determination of pH and respiratory gases were collected in heparinized Natelson blood pipets and kept in an ice bath until analyses were performed. Elapsed time between the taking of samples and their determination was between 1 and 4 hr. Measurements were made with a Corning model 160 pH/blood gas system.

Serum obtained from additional blood samples was analyzed for total calcium by

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atomic absorption spectrophotometry according to the method of Trudeau and Freier (15) and for inorganic phosphorus using the micromethod of Chen *et al.* (16).

Individual blood samples from older fetuses were used whenever adequate. Pooling of samples was necessary in the case of younger fetuses; however, even with pooling, amounts sufficient for pH and respiratory gases were not always available on the earliest days investigated.

Statistical analysis. Statistical evaluation was performed by the Student's *t* test for means.

Results. Blood pH, respiratory gases, calcium, and phosphorus in the last six days of gestation. Calcium and phosphorus. Maternal and fetal blood calcium levels are similar when first examined on Day 17 but beginning on Day 18 they shift in opposite directions with time (Fig. 1a). In the fetus, blood calcium declines sharply from Day 17 to 18 and subsequently increases until term. In the dam, calcium remains fairly constant from Day 17 to 19 (9.5–9.6 mg/100 ml) and then gradually declines to mildly hypocalcemic levels (8.75 mg/100 ml).

The difference between fetal and maternal blood phosphorus ranges between 3 and 5 mg/100 ml at each time point examined (Fig. 1b). Between Days 17 and 19 serum phosphorus initially declines and then rises in both the fetal and maternal organisms. Beyond Day 19, fetal phosphorus steadily increases, whereas maternal phosphorus falls significantly ($P < 0.001$) and remains depressed in the final days of pregnancy.

pH. The small size of blood samples obtainable from 17-day-old fetuses precluded pH determinations at this stage.

Relatively small but statistically significant decrements in maternal blood pH (Fig. 1c) were noted between Days 18 and 19 ($P < 0.001$) and between Days 20 and 21 ($P < 0.05$). By contrast, the fetus showed marked declines ($P < 0.001$) in pH between Days 19 and 20 (7.31 to 7.20) and between Days 21 and 22 (7.16 to 6.96) resulting in a state of pronounced acidosis.

Blood respiratory gases. Because analysis of blood gases requires an even larger sample than that needed for pH determinations, data on fetal pCO_2 and pO_2 could not be obtained for Days 17 and 18. The data presented in Fig. 1d, therefore, represent findings in animals from Day 19 through Day 22 plus Day 18 values of mother rats for comparison with maternal pH values at that time. Also, it should be pointed out that since blood from the axilla (fetus) and tail (dam) represents

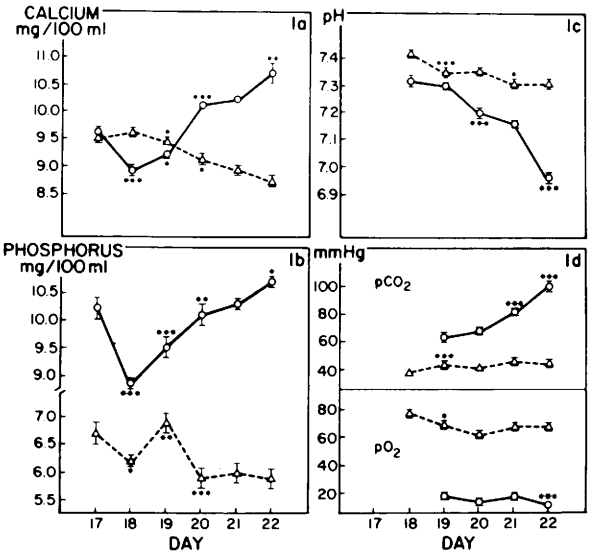

FIG. 1. Fetal and maternal blood pH, calcium, phosphorus, and respiratory gases in the last week of gestation. Each point represents the mean ± SE of either 21–52 maternal or 20–100 fetal samples. (○——○) Fetuses; (△- - -△) dams. For five of the mean values shown, the standard error is too small to be depicted. Asterisks indicate data which are significantly different from the preceding day: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

mixed arterial and venous samples, pCO_2 is higher and pO_2 lower than would be expected had determinations been done solely on arterial blood.

In the maternal organism, small but statistically significant ($P < 0.001$) increases in pCO_2 and a decline in pO_2 occur between Days 18 and 19. The elevated pCO_2 correlates well with the fall in blood pH previously noted at this time. From Day 19 through term, no significant alterations in maternal blood respiratory gases are seen.

In contrast, in the fetus, beginning on Day 19 and continuing through to term, pCO_2 increases in a linear fashion with highly significant ($P < 0.001$) increments occurring on the last 2 days of gestation. During the period examined fetal pO_2 remained low with a significant ($P < 0.001$) decrease noted between Days 21 and 22.

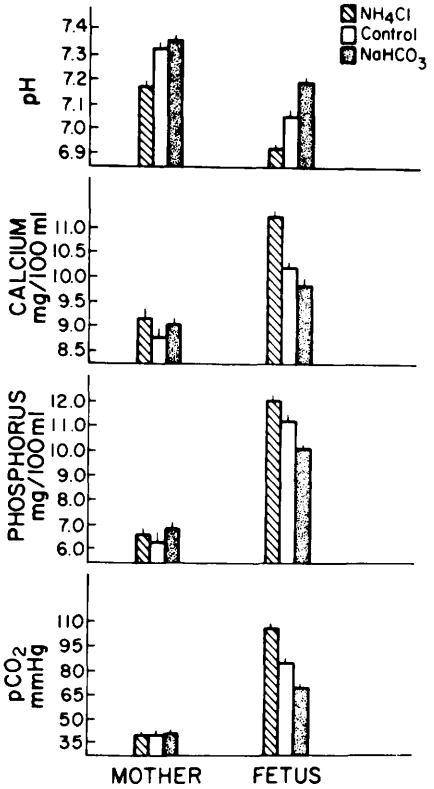

FIG. 2. Fetal and maternal blood pH, calcium, phosphorus, and pCO_2 on Day 22 of pregnancy. Gravid females were offered 1.5% NH_4Cl, 1.5% $NaHCO_3$, or distilled water in place of tap water on the preceding 6 days. Each bar represents the mean ±SE of 15–45 samples.

Administration of ammonium chloride or sodium bicarbonate to pregnant rats during the last six days of gestation (Fig. 2). Administration of NH_4Cl resulted, at term, in a highly significant ($P < 0.001$) depression of maternal blood pH whereas blood pH of $NaHCO_3$-treated dams was unaffected. Neither group of treated females, however, demonstrated any significant alterations in blood calcium, phosphorus, or respiratory gases.

In marked contrast to findings in rat mothers, the fetuses of alkali- or acid-treated dams were profoundly affected. When compared to control values, the blood of unborn offspring of NH_4Cl-treated mothers showed a pronounced decrease in pH (to 6.94) in concert with marked elevations of calcium, phosphorus, and pCO_2. Fetuses of bicarbonate-fed dams had blood with a substantially higher pH (to 7.20), lowered pCO_2, and decreased levels of calcium and phosphorus. All changes in fetal blood were highly significant ($P < 0.001$).

pO_2, not shown in Fig. 2, was unaltered in both mothers and their unborn offspring.

Discussion. At term the mammalian fetus has been reported to be hypercalcemic (1–4) and hyperphosphatemic (5–7), conditions which developed over a period of time in the final stages of gestation (10–13). The present report confirms the gradual buildup of calcium and phosphorus in fetal blood and demonstrates, in addition, that as circulating levels of these elements rise, blood pH falls.

Newly born animals have been reported to be acidotic, a condition which has been attributed to impaired placental circulation during the course of labor and delivery (17, 18), or, alternatively, to a buildup of lactic acid as a result of anaerobic metabolism (19). The acid/base status of the newborn may well be affected as a result of the delivery process; however, from the present study, it appears that like hypercalcemia and hyperphosphatemia, acidosis progressively develops *in utero*. Since blood levels of lactic acid are high at birth (19, 20), the accumulation of acid products of metabolism *in utero* probably contributes to the acidosis noted following delivery, and to the developing acidosis in the fetus in the last stages of gestation. However, a number of investigators have reported that fetal pCO_2 is elevated (14, 21–24)

and, in the present studies, was seen to increase steadily as term was approached. *In utero* acidosis then may well be both metabolic and respiratory in origin.

The present findings on fetal blood pH and calcium are in line with our earlier observations (14) in rat fetuses at term, and in neonates, where it was seen that high levels of blood calcium were associated with an elevated pCO_2 and acidosis *in utero*, and that with the onset of respiration following birth, serum calcium declined following a time course which paralleled increases in and ultimately normalization of blood pH. An association between pCO_2 and/or pH and circulating levels of blood calcium has also been inferred from reports in which blood calcium was increased in adult rats with metabolic and respiratory acidosis (24), in human infants born in a cyanotic state (25), and in fetal rats in response to maternal hypoxia (2). Similarly, blood phosphorus has been shown to be elevated in the presence of lactic acidosis (26), in response to exposure to CO_2 (27), and in the present studies, in association with developing *in utero* acidosis. If concentrations of calcium and phosphorus in fetal blood are related to acid/base status, then *in utero* hypercalcemia and hyperphosphatemia should be aggravated by a further fall in blood pH whereas increasing pH should be palliative. These indeed were the results when either NH_4Cl or $NaHCO_3$ was administered to pregnant females in the last week of gestation.

After 6 days of treatment, the pregnant females themselves responded to acid administration with a lowered blood pH whereas the hydrogen ion concentration in blood of dams receiving $NaHCO_3$ was unchanged. This is in line with observations in adult male rats subjected to acid or alkali loads for almost 1 year (28) but, in the case of $NaHCO_3$, the present results are at variance with short-term studies in pregnant patients wherein infused bicarbonate (29), or hyperventilation in humans (30) and sheep (31, 32), resulted in elevations of blood pH in the gravid females.

In this latter instance, the blood pH of the unborn offspring also rose, an observation which when taken together with the report of metabolic acidosis in laboring patients and their offspring in response to an infusion of fructose (33) lead to the conclusion that the acid/base status of the mother is readily reflected in the developing organism *in utero*.

Our findings in dams and offspring of the NH_4Cl group confirm this, but another explanation is required to account for the increased pH noted in fetuses of bicarbonate-treated mother rats who themselves appeared to be in acid/base balance. The cases mentioned earlier wherein both maternal and fetal pH were elevated involved bicarbonate infusion or hyperventilation in a short time frame (approximately 2 hr prior to delivery), possibly an inadequate period for compensation on the part of the female, and in these cases the prenatal animals do reflect the imbalance present in their mothers. In our hands, however, the extended period (almost 1 week) over which bicarbonate was administered was apparently of sufficient duration for the pregnant females to adjust to the base load and present with a normal blood pH at term. In spite of this, their offspring had blood pH levels higher than controls. Sodium bicarbonate is presumed to readily pass to the fetus by diffusion (34), and it would appear that, in contrast to the adult, the immature organism is incapable of compensating for the base load, and at birth is significantly less acidotic than its untreated counterparts.

It has been repeatedly demonstrated that the concentration of calcium in fetal blood is remarkably constant despite profound alterations in circulating levels of this element in the maternal organism. Thus fetal serum calcium is unaffected by maternal hypercalcemia resulting from administration of parathyroid hormone (35, 36), vitamin D (36), or calcium salts (37, 38) and is relatively unchanged when the mother is hypocalcemic as a result of an infusion of EDTA (38) or following removal of the maternal parathyroid glands (39). In contrast, in the present studies, the offspring of normocalcemic and normophosphatemic mothers had significantly increased or decreased levels of calcium and phosphorus in apparent response to shifts in the pH of fetal blood.

From the present and previous report (14) it would appear that before birth, circulating levels of calcium and phosphorus are related to the acid/base status of the organism *in*

utero. Acid loading of the pregnant female aggravates naturally occurring fetal acidosis and accentuates the attendant hypercalcemia and hyperphosphatemia; administration of bicarbonate is palliative and reduces the severity of the derangement in each parameter.

Finally, if, as we suggested earlier, neonatal hypocalcemia is associated with correction of fetal acidosis, then decreasing the severity of *in utero* acidosis should modify the degree of correction necessary, and the extent of the fall in blood calcium, following birth. No information is currently available on the blood mineral status of early neonates of bicarbonate-treated mothers. However, in the studies where $NaHCO_3$ was infused into female patients in labor (29), the infants were described as being in better acid/base and clinical condition than were their counterparts born to untreated mothers.

Summary. In the rat fetus, under normal conditions, blood calcium, phosphorus, and pCO_2 rise, and pH declines, during the last 6 days of gestation. Little or no correlation is seen between changes noted in the maternal organism and those occurring in her unborn offspring.

Oral administration of NH_4Cl to pregnant females results in a lowering of maternal blood pH, with no change in blood calcium, phosphorus, or respiratory gases. $NaHCO_3$ administration does not alter any of these parameters. In contrast, profound changes are observed in the fetuses of both acid- and alkali-treated dams. With NH_4Cl feeding, fetal pH falls while blood calcium, phosphorus, and pCO_2 rise; with $NaHCO_3$ administration, fetal blood pH increases and serum calcium, phosphorus, and pCO_2 decline.

These observations point strongly to an association between fetal blood pH and circulating levels of calcium and phosphorus, and suggest that the levels of these elements may be, in part, determined by the acid/base status of the organism *in utero.*

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