Ontogenesis of Gastric Acid Secretion in Fetal Rat

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Summary

The pH of gastric fluid was measured in rat fetuses during the last 3 days of gestation. On day 19, the gastric pH was close to neutral. During the night of day 20, the pH was clearly lowered (6.11 ± 0.15 units), this decrease becoming more marked on the following day. At birth (day 22), just before the first feeding, the pH of gastric fluid reached the mean value of 2.98 ± 0.14 units. This drop in gastric pH was concomitant with an increase in chloride concentration whereas the gastric PCO_2 remained constant. These results imply that in term rat fetuses, the gastric mucosa secretes fixed acid, very likely hydrochloric acid.

The administration of acetazolamide (inhibitor of carbonic anhydrase) to 20-day-old fetuses did not suppress the spontaneous acidification of gastric fluid, although the enzyme activity was reduced by approximately 80%. Moreover, the gastric pH in acetazolamide-injected fetuses was markedly lower than in the noninjected littermates. The administration of NaCl solution (acetazolamide vehicle) had no effect on the carbonic anhydrase activity but clearly decreased the pH of gastric fluid. Thus, the drop of gastric pH produced by injection of acetazolamide or saline solution alone probably results from a stress effect of puncture.

In fetuses from adrenalectomized, metopirone-treated mothers, the injection of NaCl solution no longer had effect on the pH of gastric fluid whereas triamcinolone injection produced a clear decrease in the gastric pH 3 hr later.

Speculation

Corticosteroids could play an important role in the development of H^+ generation processes in parietal cells during the fetal period of life.

Electron microscopic studies have shown that in some mammals species, including man, parietal cells of the gastric mucosa begin to develop during intrauterine life (14, 15, 25). It has been observed that gastric acid secretion takes place before birth in guinea pig (28), lamb (16), and rabbit (30). Newborn human infants have an intragastric pH ranging from 5.5 to 7; this pH then strongly decreases by 4 to 5 hr of age (1, 13). As far as we know, there is no available information concerning the factor(s) responsible for the development of H^+ generation processes in parietal cells.

In rat fetuses, we have observed a sharp drop in the pH of the gastric content on the last day of gestation (11). Because it has been previously reported that the activity of carbonic anhydrase markedly increases near term (15, 29), it was tempting, as a first approach to the problem, to hypothesize a cause-effect relationship between these two events. Thus, we have studied the effect of carbonic anhydrase inhibition on the development of gastric acid secretion. We have, in addition, studied the effects of adrenalectomy and corticosteroid administration. Some of our results suggest that the adrenal gland could be implicated in the functional differentiation of parietal cells.

MATERIALS AND METHODS

ANIMALS

Sherman rats, fed a commercial diet (U.A.R., France), were used for this study. To obtain fetuses of known age, females were mated overnight. Gestation was detected by palpation 2 wk later; the day after the night of mating was considered as day zero of gestation. In this strain, delivery occurs in the night of day 21.

CHEMICAL

Acetazolamide was purchased from Theraplix (Paris, France), and triamcinolone was purchased from Sigma Chemical Co. (Saint Louis, MO). Metopirone was a gift of the Ciba laboratory, (Basel, Switzerland).

EXPERIMENTAL PROCEDURES

Pregnant rats were adrenalectomized under ether anaesthesia on day 16 of gestation and then received 10 mg metopirone by intraperitoneal injections twice a day until the time of sacrifice. Such treatment suppresses, or at least strongly reduces, the circulating corticosteroids in fetal blood (10). The effectiveness of this treatment in fetuses is indicated by the enlargement (compensatory hypertrophy) of fetal adrenal glands as shown in Tables 3 and 4.

Acetazolamide and triamcinolone were administered at doses of 100 and 20 μg , respectively, in 20 μl 0.15 M NaCl per fetus. Some fetuses (NaCl-injected fetuses) were given injections of 20 μl of the NaCl solution alone. Subcutaneous injections into fetuses were done through the uterine wall after laparotomy of the mother under light ether anaesthesia.

pH AND PCO_2 DETERMINATIONS

At the time of sacrifice, pregnant rats were laparotomized under pentobarbital anaesthesia. Samples of amniotic fluid, fetal blood, and fetal gastric fluid were collected into type D 551 capillary tubes (Radiometer, Copenhagen, Denmark), avoiding any air bubbles. Determinations of pH and PCO_2 were immediately done at 37°C using a Blood Micro System 3 Radiometer apparatus.

CHLORIDE DETERMINATION

The concentration of chloride in fluid samples was measured by potentiometric titration with silver nitrate (27) using a silver electrode PK 499 (Radiometer) connected to a TTT 2 Radiometer titrator and an autoburette ABU 12 (Radiometer) filled with 0.1 M AgNO_3 solution.

CARBONIC ANHYDRASE ASSAY

Stomachs were excised from fetuses, cut open along the visceral axis, washed in ice-cold distilled water, and weighed. Whole stomachs were homogenized (Potter Teflon-glass homogenizer) in distilled water. The proportion of homogenizing solution to tissue being 30/1 (v/w), it was necessary to pool stomachs of three to six

Fig. 1. Evolution of the pH of fetal rat gastric fluid on the last 3 days of gestation. Mean $\pm$ standard error (vertical bars) with number of experiments. pH determinations were done at 37°C in anaerobic conditions.

fetuses according to their age. The homogenate was squeezed through a double layer of gauze; part of this suspension was used for protein determination (22). Protein concentration in the homogenate was approximately 600 $\mu\text{g}\cdot\text{ml}^{-1}$; preliminary experiments have shown that the hydration reaction was linear for concentrations ranging from 150 to 1000 $\mu\text{g}\cdot\text{ml}^{-1}$.

The activity of carbonic anhydrase (EC 4.2.1.1) was assessed by measuring the rate of hydration of carbon dioxide following the pH-stat method described by Hansen and Magid (12) with slight modifications. The reaction chamber (a polyethylene cupel) was filled with 2.5 ml of Sørensen phosphate buffer, (pH 7.2) and 0.5 ml of either gastric homogenate or buffer solution for determination of the rate of the uncatalyzed reaction. The reaction was started by introducing a gas mixture (10% CO_2 , 90% N_2) at a flow rate of 300 $\text{ml}\cdot\text{min}^{-1}$ into the medium, temperature being maintained at 4°C by a circulating refrigerant. The pH was measured by a combined glass-calomel electrode (GK 230, Radiometer) connected to the TTT 2 titrator and held at the constant value of 7.2 units by addition of 0.05 M NaOH solution automatically delivered by the ABU 12 autoburette. Measurements were carried out for a period of 2 min during which the rate of hydration reaction was linear. Results were expressed as $\mu\text{moles of H}^+$ produced per mg^{-1} protein per minute ($\mu\text{moles H}^+\cdot\text{mg}^{-1}\text{Prot}\cdot\text{min}^{-1}$); the activity of the carbonic anhydrase is the difference between the rates of H^+ production in the presence and in the absence (uncatalyzed reaction) of homogenate.

Although fetuses were carefully bled, traces of blood were present in the gastric homogenate. A correction for the carbonic anhydrase activity due to the presence of erythrocytes was made by measuring the concentration of haemoglobin (5) in the homogenate and the carbonic anhydrase activity per mg haemoglobin in fetal erythrocytes.

All data are presented as means $\pm$ S.E. and evaluated for significance by the unpaired Student *t* test.

RESULTS

SPONTANEOUS ACIDIFICATION OF THE GASTRIC CONTENT IN FETAL RAT

As shown in Figure 1, between the morning of day 19 and the afternoon of day 20, there was a slight, but not significant, decrease

in the gastric pH (6.85 ± 0.09 and 6.52 ± 0.11 ; $P > 0.05$). During the night of day 20, the pH was clearly lowered (6.11 ± 0.15), this decrease becoming more marked on the following day (day 21). At birth, just before the first feeding, the pH of gastric fluid reached the mean value of 2.98 ± 0.14 units.

Between days 19 and 21, the acidification of the gastric fluid was associated with a clear increase in the chloride ion concentration (Table 1). During this period, the pH and chloride concentration in blood plasma and amniotic fluid remained constant. In addition, there was no change in the PCO_2 of gastric fluid which was close to that of blood.

DEVELOPMENT OF THE GASTRIC CARBONIC ANHYDRASE ACTIVITY IN FETAL STOMACHS

As shown in Figure 2, no carbonic anhydrase activity was observed in the gastric tissue homogenate on days 17 and 18. A very low but significant activity appeared for the first time on day 19: $8.0 \pm 1.6 \mu\text{moles H}^+\cdot\text{mg Prot.}^{-1}\cdot\text{min}^{-1}$. From this day, the carbonic anhydrase activity rose, reaching a level five times higher on the last day of gestation: $37.2 \pm 5.0 \mu\text{moles H}^+\cdot\text{mg Prot.}^{-1}\cdot\text{min}^{-1}$.

EFFECT OF ACETAZOLAMIDE ON THE ACIDIFICATION OF GASTRIC FLUID

A single dose of acetazolamide was injected in the evening of day 20. Sampling was done 16 hr later in the morning (10:00 AM) of day 21. This treatment strongly inhibited the gastric carbonic anhydrase activity (-75%) in both acetazolamide-injected fetuses and noninjected littermates, meaning that the enzyme inhibitor easily diffuses from the injected fetuses to the others (Table 2). The level of enzyme activity in acetazolamide-treated litters was close to that observed in 19-day-old fetuses.

Surprisingly, the gastric pH in the acetazolamide-injected fetuses was markedly lower than in the noninjected littermates. In

Table 1. pH, PCO_2 and Cl^- concentration in fetal blood plasma and gastric and amniotic fluids on the last 3 days of gestation¹

Day of gestation	Gastric content	Blood plasma	Amniotic fluid
19			
pH	6.85 ± 0.09^2 (6) ³	7.27 ± 0.02 (5) ⁴	7.25 ± 0.02 (6) ⁴
Cl^-	104.9 ± 1.2 (7)	101.5 ± 0.5 (5) ⁴	104.0 ± 0.4 (6)
20			
pH	6.66 ± 0.04 (19)	7.26 ± 0.03 (9) ⁴	7.26 ± 0.01 (7) ⁴
Cl^-	114.4 ± 2.0 (20) ⁵	99.2 ± 1.6 (10) ⁴	102.4 ± 3.3 (9) ⁴
PCO_2	61.9 ± 3.0 (15)	64.7 ± 1.7 (17)	
21			
pH	4.61 ± 0.15 (21) ⁵	7.29 ± 0.01 (6) ⁴	7.26 ± 0.01 (7) ⁴
Cl^-	138.5 ± 1.9 (19) ⁵	99.5 ± 1.6 (10) ⁴	101.1 ± 3.3 (9) ⁴
PCO_2	60.7 ± 2.2 (10)	62.0 ± 1.7 (16)	

¹ PCO_2 is expressed as mm Hg and Cl^- concentration as mmole l^{-1} . All animals were sacrificed at 10: AM; pH and PCO_2 determinations were done in anaerobic conditions at 37°C.

² Mean $\pm$ S.E.

³ Numbers in parenthesis, number of experiments.

⁴ Differences from gastric fluid values statistically significant ($P < 0.05$).

⁵ Differences from the preceding day of gestation statistically significant ($P < 0.05$).

Fig. 2. Development of carbonic anhydrase activity in fetal stomach. Mean $\pm$ standard error with number of experiments. Enzyme activity corrected for blood contamination (see "Material and Methods") is expressed as μ moles H^+ per mg of protein per minute.

Table 2. Effect of carbonic anhydrase inhibition on the pH of gastric fluid in 21-day-old fetuses

Treatment of fetuses ¹	Carbonic anhydrase activity	Gastric pH
Acetazolamide injected	9.2 $\pm$ 2.7 ² (5) ^{3,4}	3.46 $\pm$ 0.26 (5) ⁵
Noninjected	9.2 $\pm$ 4.3 (5) ⁴	5.18 $\pm$ 0.47 (5)
NaCl injected	39.8 $\pm$ 2.6 (7)	3.97 $\pm$ 0.15 (8) ⁵
Noninjected	40.0 $\pm$ 1.9 (7)	4.85 $\pm$ 0.20 (8)
Controls	37.2 $\pm$ 0.8 (5)	4.80 $\pm$ 0.20 (10)

¹ During the evening of day 20, mothers were laparotomized under light ether anaesthesia, and fetuses were given injections of either 100 μ g of acetazolamide in 20 μ l of 0.15 M NaCl or the saline solution alone (vehicle); animals were sacrificed 16 hr later (day 21; 10:00 AM). Noninjected animals are fetuses from the same litters as those given injections; controls are fetuses from nonlaparotomized mothers.

² Mean $\pm$ S.E. Activity of carbonic anhydrase (μ mol H^+ · mg⁻¹ Prot · min⁻¹) is corrected for blood contamination.

³ Numbers in parentheses, number of experiments.

⁴ Differences from controls or NaCl-injected litters are statistically significant ($P < 0.001$).

⁵ Differences from controls or noninjected fetuses are statistically significant ($P < 0.001$).

the latter, the mean gastric pH value did not significantly differ from that found in fetuses from nonlaparotomized mothers sacrificed at the same time (controls). The administration of NaCl solution (acetazolamide vehicle) had no effect on the carbonic anhydrase activity but clearly decreased the pH of gastric fluid.

There was no significant difference between the pH values in acetazolamide and NaCl-injected fetuses.

ROLE OF CORTICOSTEROIDS

In the 20-day-old fetuses from adrenalectomized, metopirone-treated mothers, NaCl injection had no effect on the pH of gastric fluid when compared with that of noninjected fetuses of the same litter, whereas triamcinolone clearly decreased the gastric pH. In both fetuses from sham-adrenalectomized mothers and controls, the injection of NaCl alone decreased the gastric pH (Table 3). On day 21, the lack of circulating corticosteroids produced a retardation of the spontaneous acidification of gastric fluid without any significant change in the carbonic anhydrase activity (Table 4).

DISCUSSION

The stomach of near-term rat fetuses is filled with a liquid (approximately 30 μ l) which mainly originates from swallowed amniotic fluid (11, 21). Because there is no change in the composition of amniotic fluid during the last days of gestation, the drop in pH concomitant with an increase in chloride concentration occurring in fetal stomach must reflect the onset of physiologic

Table 3. Effects of NaCl and triamcinolone injections on the gastric pH in 20-day-old fetuses from adrenalectomized, metopirone-treated mothers

Treatment ¹	Gastric pH	Adrenal gland (mg · 100 g ⁻¹ body wt)
Fetuses from adrenalectomized metopirone-treated mothers		
Triamcinolone injected	5.92 $\pm$ 0.12 ² (10) ^{3,4}	
NaCl injected	6.76 $\pm$ 0.06 (10) ⁵	109.1 $\pm$ 3.6 (20) ⁶
Noninjected	6.85 $\pm$ 0.06 (12)	
Fetuses from sham adrenalectomized mothers		
NaCl injected	6.16 $\pm$ 0.06 (9) ⁷	67.5 $\pm$ 5.4 (10)
Noninjected	6.84 $\pm$ 0.04 (9)	
Controls		
NaCl injected	6.12 $\pm$ 0.09 (11) ⁷	64.7 $\pm$ 4.3 (9)
Noninjected	6.83 $\pm$ 0.08 (12)	

¹ Pregnant rats were adrenalectomized on day 16 of gestation and received 10 mg metopirone twice a day until sacrifice (day 20). Three hr before sampling, some fetuses received by subcutaneous injection either 20 μ g triamcinolone in 20 μ l of 0.15 M NaCl solution or 20 μ l of NaCl solution alone (NaCl injected). Noninjected animals are fetuses from the same litters as NaCl-injected ones. Controls are fetuses from nonadrenalectomized mothers sacrificed at the same time as those from adrenalectomized or sham-operated mothers.

² Mean $\pm$ S.E.

³ Numbers in parentheses, number of experiments.

⁴ Differences from NaCl-injected or noninjected fetuses statistically significant ($P < 0.01$).

⁵ Differences from NaCl injected of sham-operated or control groups statistically significant ($P < 0.01$).

⁶ Differences from sham-operated or control groups statistically significant ($P < 0.001$).

⁷ Differences from noninjected statistically significant ($P < 0.01$).

Table 4. Effect of maternal adrenalectomy and metopirone administration on the gastric pH and carbonic anhydrase activity in 21-day-old fetuses

Treatment ¹	Gastric pH	Carbonic anhydrase activity	Adrenal gland wt (mg · 100 g ⁻¹ body wt)
Fetuses from adrenalectomized, metopirone-treated mothers	5.81 ± 0.18 ² (12) ^{3,4}	35.5 ± 1.3 (9) ⁵	106.2 ± 3.9 (13) ⁵
Fetuses from sham adrenalectomized mothers	4.21 ± 0.16 (10)	38.9 ± 2.1 (7)	69.1 ± 0.3 (10)
Controls	4.31 ± 0.10 (9)	40.7 ± 1.7 (9)	63.7 ± 0.3 (9)

¹ Pregnant rats were adrenalectomized on day 16 of gestation and received 10 mg metopirone twice a day until sacrifice (day 21 at 10:00 AM). Controls are fetuses from nonadrenalectomized mothers sacrificed at the same time as those from adrenalectomized or sham operated mothers. Animals were sacrificed in the morning of day 21 (10:00 AM).

² Mean ± S.E.

³ Numbers in parenthesis, number of experiments.

⁴ Differences from sham operated or controls statistically significant ($P < 0.001$).

⁵ No significant difference from sham operated or controls.

activity of the gastric mucosa. The fact that gastric PCO_2 remains constant implies that fixed acid, very likely hydrochloric acid, is secreted. We have recently presented in a short abstract (8) direct *in vitro* evidence for the presence of an active H^+ transport by the stomach of 20- to 21-day-old rat fetuses. Our results are in opposition to data in the literature (15, 16, 23, 32) which report that the pH of gastric content in near-term fetuses and newborns is close to neutral. Such a discrepancy is probably due to the use by previous investigators of inadequate techniques for measuring pH with sufficient accuracy in such small amounts of fluid. Differentiated parietal cells appear for the first time in gastric mucosa of rat fetuses on day 19 of gestation (15) and acquire the capacity to secrete acid some hours later (present data). Thus, the close relationship between ultrastructural and functional differentiation of parietal cells previously observed in the rabbit (14, 30) is also found in the rat. In man, typical gastric parietal cells have been observed from the fifth month of gestation (25), but the relatively high pH values observed at birth (1) does not allow to establish such a relationship.

In adult stomach, the role of carbonic anhydrase which catalyzes the generation of H^+ from CO_2 is not clearly defined (7). It was noted that carbonic anhydrase activity increases after stimulation of gastric secretion, in rat (24, 26) and guinea pig stomach (19). The effects of enzyme inhibitors on the rate of acid secretion are controversial. Administration of acetazolamide to histamine-stimulated dogs markedly decreases acid secretion (18). However, the carbonic anhydrase inhibitor does not modify the *in vitro* production of H^+ by isolated gastric mucosa of rabbits (9). During fetal life, gastric carbonic anhydrase activity has been observed in man (2) and rat (15). In the latter (present data), we did not find any cause-effect relationship between the increase of carbonic anhydrase activity and the appearance of acid secretion. The spontaneous acidification of gastric content occurred normally in acetazolamide-treated fetuses, although carbonic anhydrase activity was reduced by approximately 80%; moreover, the secretion of acid could be prematurely induced by injection of saline solution in spite of the very low activity of enzyme. The small residual activity of carbonic anhydrase might be sufficient to sustain acid production, and it would be interesting to obtain a complete inhibition of enzyme, but higher doses of acetazolamide appeared to be lethal for fetuses. In any case, our results clearly indicate that the rapid development of the activity of carbonic anhydrase is not a limiting factor for the installation of acidifying processes in gastric mucosa of fetal rats.

The drop in gastric pH produced by the injection of acetazolamide or saline solution probably results from "a stress effect of puncture." Inasmuch as this effect was no longer observed in fetuses from adrenalectomized, metopirone-treated mothers and triamcinolone was able to stimulate the gastric acid secretion in these fetuses, one may think that an increase in the level of circulating endogenous corticosteroids may account for this so-

called stress effect of puncture. Corticosterone has been detected in the plasma of fetal rats, the highest hormone level being found on day 19 of gestation (4, 17). More recently, the presence of specific glucocorticoid receptors has been demonstrated in the cytosol of fetal stomach (20). Yeomans *et al.* (31) have shown in cultured fetal rat stomach that cortisol promotes the cytologic differentiation and maturation of gastric mucosa. Therefore, the high gastric pH that we have observed on day 21 in fetuses from adrenalectomized, metopirone-treated mothers could have been caused by a delayed organogenesis of gastric mucosa. However, this trophic effect of steroids could not explain the rapidity of the response of fetal stomach (3 hr) injection of triamcinolone. Such a short-term effect of corticosteroids on the acidification of gastric content seems more compatible with a stimulation of H^+ generation processes in already differentiated parietal cells than with a corticosteroid-induced architectural maturation of the mucosa. In the adult, it is generally recognized that corticosteroids are necessary for the maintenance of normal gastric acid secretion; adrenalectomy leads to a marked fall in acid concentration of the gastric juice (3). However, results concerning the effect of corticosteroid treatment are conflicting; some authors have observed an increased acid secretion whereas others fail to obtain any evidence of a rise in the gastric acid secretion (6). Our own results strongly suggest that corticosteroids play a role in the development of H^+ generation processes in parietal cells during the fetal period of life, and it would be interesting to know whether corticoids act directly on parietal cells or indirectly by the way of stimulating factors as histamine, gastrin, or others (?).

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