Ontogeny of Human Gastric Lipase and Pepsin Activities

DANIEL MÉNARD, SOPHIE MONFILS, and ERIC TREMBLAY

Groupe de Recherche en Biologie du Développement, Département d'Anatomie et de Biologie Cellulaire, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, Québec, Canada

Background/Aims: **The developmental profile of human gastric lipase activity as well as the secretory capacity of the immature gastric mucosa are still unknown. The aims of this study were to establish tissue activity levels for lipase and pepsin in the various anatomical regions of the developing stomach and to assess whether lipase is secreted by the fetal gastric mucosa.** ***Methods:*** **Lipase and pepsin activities were assayed in 49 specimens of different gestational ages. Gastric explants were cultured in chemically defined medium for up to 5 days, and enzymic activities were measured in tissues and in the culture media.** ***Results:*** **Lipolytic activity was present in gastric tissues at 10–13 weeks and steadily increased for up to 20 weeks, whereas pepsin activity did not vary significantly over the periods of study. There was a clear decreasing gradient of lipase activity; the highest activity was in the fundic area, and the lowest activity was in the antrum. Quantitative pepsin activity did not vary over the gastric regions. During culture, total lipolytic and pepsin activity increased 3.8-fold, and both enzymes were secreted into the culture medium.** ***Conclusions:*** **Gastric lipase appears as early as 10–13 weeks. Adult distribution of the enzyme became established by 16 weeks' gestation. The secretion of lipase into the organ culture suggests that the gastric mucosa is the main source of lipolytic activity in gastric aspirates of premature infants.**

Knowledge of the function of the human stomach has expanded over the last decade to include a significant role in fat digestion.[1–3] Human gastric lipase consists of a 379–amino acid polypeptide with an approximate molecular mass of 49,000 as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.[4] The characteristics of gastric lipase such as optimum acid pH and its ability to function without bile salts or cofactors are advantageous in gastric lipolysis.[3,5,6] In the presence of normal pancreatic function, free fatty acids and monoglycerides produced by gastric lipolysis facilitate the subsequent hydrolysis of triglycerides by pancreatic lipase.[2,7] However, in physiological (preterm or full-term infants) and pathological pancreatic insufficiency (cystic fibrosis), the importance of gastric lipolysis increases and it assumes a greater role in the digestion of dietary triglycerides.[3,8,9]

The localization and distribution of lipase activity in the human adult stomach has been investigated[10,11] and recently correlated with that of pepsin activity.[12] Further studies showed that gastric lipase is not only able to hydrolyze short- and medium-chain triglycerides at significant rates but also long-chain triglycerides,[8,13,14] which are the natural components of dietary fat. Normal development of gastric lipolytic activity is therefore essential for normal digestion of fat. Lipase activity in biopsied or surgical specimens of human gastric mucosa was found to be relatively constant between the ages of 20 and 60 years with a significant decrease above 60 years of age.[10,11] Recently, lipase and pepsin activities were quantitated in human gastric biopsy specimens between the ages of 3 months and 26 years with no significant differences between the different age groups.[12] There are no data on the presence and distribution of lipase and pepsin activities during ontogeny of the human gastric mucosa. Although information is available on lipase activity in gastric aspirates of premature (23 weeks' gestation) and full-term infants,[8,15] the exact origin of the activity remains uncertain. Therefore, the aim of this investigation was to establish tissue activity levels for lipase and pepsin between 10 and 20 weeks' gestation and to study enzymic levels in various anatomical regions of the developing stomach. In addition, we used our organ culture technique, which enables morphological and physiological maintenance of human fetal gastric mucosa in vitro[16] and excludes the possibility of contamination by other sources, to assess whether these enzymes are secreted from the developing gastric mucosa.

Materials and Methods

Specimens

Tissues from 49 fetuses varying in age from 10 to 20 weeks' gestation (postfertilization) were obtained after legal

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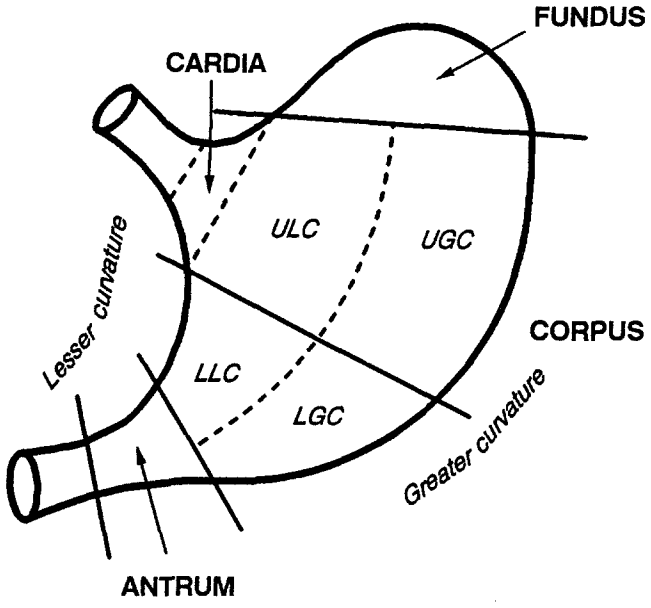

Figure 1. Areas of the human fetal stomach from which tissue specimens were taken for determination of lipase and pepsin activities. ULC, upper lesser curvature; UGC, upper greater curvature; LLC, lower lesser curvature; LGC, lower greater curvature.

abortion. The project was in accordance with the requirements of the institutional review committee for the use of human tissue. The stomach was immersed in Leibovitz L-15 medium (room temperature) containing garamycin (40 μg/mL) and mycostatin (40 μg/mL) and brought to the culture room within 30 minutes. For developmental studies of gastric enzymic activities, the cardiac and pyloric regions were removed and the gastric corpuses were slit longitudinally along the greater and the lesser curvatures, washed in culture medium, frozen in liquid nitrogen, and stored at −70°C. Analyses was performed within 1–4 weeks. For determination of enzymic activities in the different regions of the stomach, specimens were obtained from various areas (Figure 1), and the assays were performed immediately.

Organ Culture

The cardiac and pyloric regions were removed, and the gastric corpuses were slit longitudinally along the greater and the lesser curvatures, washed in culture medium, and cut into explants (5 × 5 mm). These explants were then randomly transferred onto lens paper (Canadian Laboratories Supplies Ltd., Montréal, Québec, Canada) with the mucosal side up. The lens paper covered a stainless steel grid overlying the central well of a Falcon organ culture dish (Falcon Plastics, Los Angeles, CA). Leibovitz L-15 medium supplemented with garamycin (40 μg/mL) and mycostatin (40 μg/mL) was added in sufficient quantity to wet the lens paper. Tissues were cultured at 37°C in an environment of 5% CO_2/95% air and saturated water vapor. Culture medium was renewed after day 1 and every 2 days thereafter.[16] After appropriate culture periods, the explants were removed, rinsed with saline, weighed, and frozen in liquid nitrogen. When indicated, corresponding culture media were frozen and also kept at −70°C for further analysis.

Biochemical Determinations

Preparation of homogenates. Whole stomach or specific gastric regions (Figure 1) were homogenized at 4°C in a polytron using citrate-phosphate buffer, pH 6.0. Homogenates were immediately used for biochemical determinations.

Lipase assay. Lipolytic activity was measured using a long-chain triglyceride substrate (tri-[1-^{14}C]oleic), and the emulsion was prepared as described previously.[17] The assay system contained, in a final volume of 200 μL, 1.2 μmol labeled triglyceride; 10 μmol citrate-phosphate buffer, pH 6.0; 0.1 μmol bovine serum albumin (free fatty acid; Sigma Chemical Co., St. Louis, MO); 2 μmol/L Triton X-100[10,15]; and 50–100 μL of tissue homogenate. Incubation was performed in duplicate at 37°C in a Dubnoff shaking bath, and the reaction was stopped after 30 minutes by the addition of 3.25 mL of a mixture of methanol/chloroform/heptane (1.41:1.25:1.00, vol/vol/vol). Released free [^{14}C]oleic acid was separated by liquid-liquid partition[18] and quantitated by liquid scintillation spectrometry. Activity was expressed as nanomoles of free fatty acid produced per minute per milligram of protein or tissue. The effect of pH on gastric lipolytic activity was evaluated over a range from 2.5 to 9.0 as previously described.[8] As shown in Figure 2, lipolytic activity from gastric tissues collected from two fetuses (18 and 20 weeks' gestation) and assayed separately was highest at pH 6.0. Therefore, this pH, which is compatible with the gastric milieu during development,[19] was used for all measurements.

Pepsin assay. Pepsinogen was assayed by the method of Anson and Mirsky using acid-denaturated hemoglobin as substrate.[20] Briefly, pepsinogen was measured in the reaction mixture containing 0.9 mL 2.0% bovine hemoglobin (H-2625;

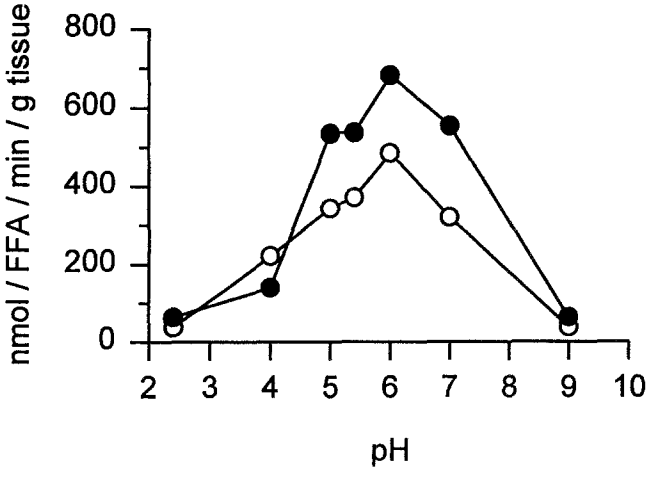

Figure 2. Effect of pH on lipolytic activity in two gastric specimens (fundus and body) from fetuses at 18 (○) and 20 (●) weeks' gestation.

Sigma Chemical Co.) in 0.06 mol/L HCl and 0.1 mL of tissue homogenate. The reaction was performed at 37°C for 30 minutes and stopped by the addition of 2 mL of 10% trichloroacetic acid. The mixture was then centrifuged at 13,000 rpm for 20 minutes, and the absorbance of the supernatant was read at 280 nm against a zero time blank.[21] Activity was expressed as the change in absorbance at 280 nm · min^{-1} · mg^{-1} of protein or tissue. In this report, the term pepsin will be used rather than pepsinogen because, at a pH of <2.5, the latter is activated to yield pepsin. Protein was quantitated by the method of Lowry et al.[22] with bovine serum albumin as the standard.

Results are expressed as mean ± SEM. Statistical significance was established at 95% and determined by analysis of variance followed by Student's *t* test when significance was indicated.

Results

Developmental Studies

Lipase activity in the whole stomach (fundus and corpus) was determined in 42 specimens between 10 and 20 weeks' gestation (Figure 3). Lipolytic activity was already present at low levels between 10 and 13 weeks' gestation. Lipase activity increased steadily up to 20 weeks. The same pattern was recorded when activity was expressed as nanomoles of free fatty acids per minute per milligram of tissue (data not shown). Pepsin activity was assessed between 16 and 20 weeks in 15 specimens (Figure 4). Although present at 16 weeks, pepsin activity did not vary significantly over the studied period. Again the same pattern was recorded when activity was expressed as units per milligram of tissue (data not shown).

The distribution of lipase and pepsin activities over the different gastric regions (Figure 1) was investigated

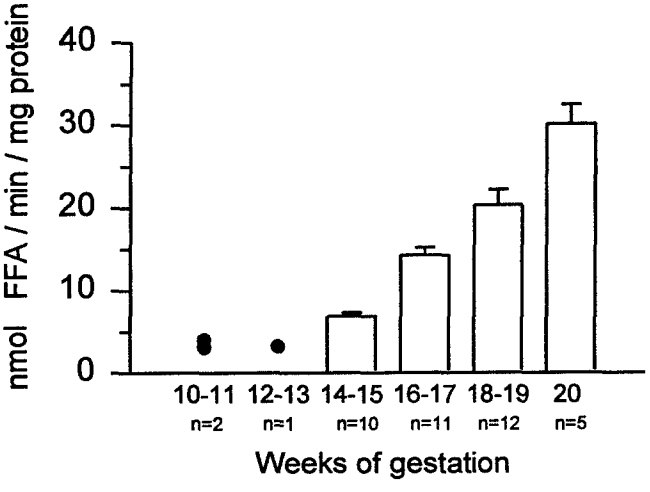

Figure 3. Lipase activity in gastric specimens (fundus and body) between 10 and 20 weeks' gestation. *Small numbers* represent the number of specimens used. Data are expressed as mean ± SEM.

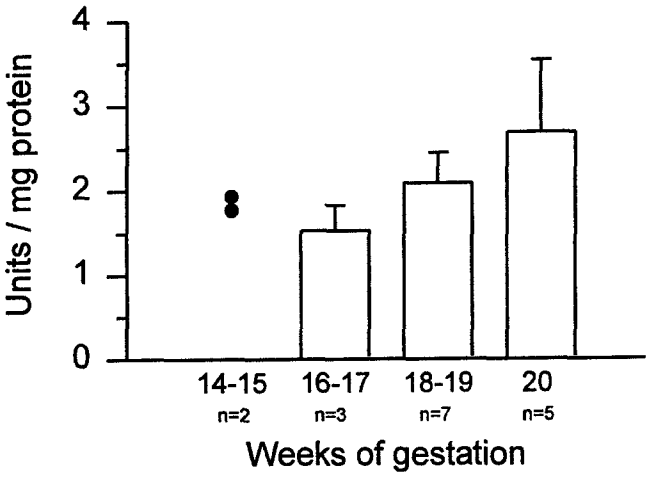

Figure 4. Pepsin activity in gastric specimens between 16 and 20 weeks' gestation. *Small numbers* represent the number of specimens used. Date are expressed as mean ± SEM.

in three specimens (Figure 5*A* and *B*). Lipase activity was not evenly distributed throughout the stomach. Activity was highest in the fundic area and increased with gestational age. A clear decreasing gradient of lipase activity was evident from the upper greater and lesser curvatures to the lower greater and lesser curvatures, the activity being the lowest in the antrum. As opposed to lipase, quantitation of pepsin activity did not show any particular distribution over the gastric regions, although a steady increase was observed in the antrum between 16 and 20 weeks' gestation.

Organ Culture

Human fetal gastric explants were cultured up to 5 days in chemically defined serum-free medium, and lipase and pepsin activities were determined in explants as well as in the culture media (Figure 6*A* and *B* and Tables 1 and 2). Total activity (tissue plus medium) for both enzymes significantly increased (3.8-fold) during the culture compared with that of tissue alone at the beginning of the culture. Although both enzymic activities were secreted into the culture medium, different patterns were recorded. A rapid and important secretion of lipase activity was noted in the medium as early as 24 hours of culture, the enzymic activity being higher than that recorded in the gastric explants. On the other hand, secretion of pepsin enzymic activity in the medium remained lower than that quantitated in the corresponding gastric explants.

Discussion

The present study establishes that gastric lipase activity, using long-chain triglyceride as substrate, is

already detectable at 10–13 weeks' gestation. This activity steadily increases between 14 and 20 weeks' gestation in good correlation with the development of gastric glands.[23–25] It also confirms and extends preliminary data obtained in one specimen of human fetal stomach reporting lipolytic activity (using tributyrin as substrate) at 18 weeks' gestation.[26] The presence of low but stable tissue pepsin activity between 16 and 20 weeks is in accordance with an immunoenzymologic study[27] showing the presence of low concentrations of various molecular forms of pepsinogen up to the third semester.[28] To our knowledge, this is the first report on the comparative development of these enzymic activities during the formation of human gastric glands.

The distribution of lipase activity in the human fetal stomach at 16, 18, and 20 weeks was also investigated (Figure 5). Quantitation of lipase showed that even at 16 weeks, activity was highest in the fundic area with a decreasing gradient of activity down to the antrum. The increase of lipase activity as established between 14 and 20 weeks (Figure 3) occurred in all gastric regions except in the antrum where it remained very low. Of note is that lipase activity recorded in the fetal fundus at 20 weeks represents 30% of the mean lipase activity (403 nmol free fatty acids · min^{-1} · mg $protein^{-1}$) measured in the upper greater curvature (fundus) in biopsy specimens of adult subjects.[11] No significant differences in pepsin activity levels were noted in the various gastric regions between 16 and 20 weeks in accordance with the overall developmental profile (Figure 4). In contrast to lipase, the antrum had the highest activity of all gastric regions at 20 weeks. Recently, pepsin activity has been reported in biopsy specimens of gastric body and antrum of infant and adult subjects.[12] Considerable pepsin activity was present in the gastric antrum at all ages, although the

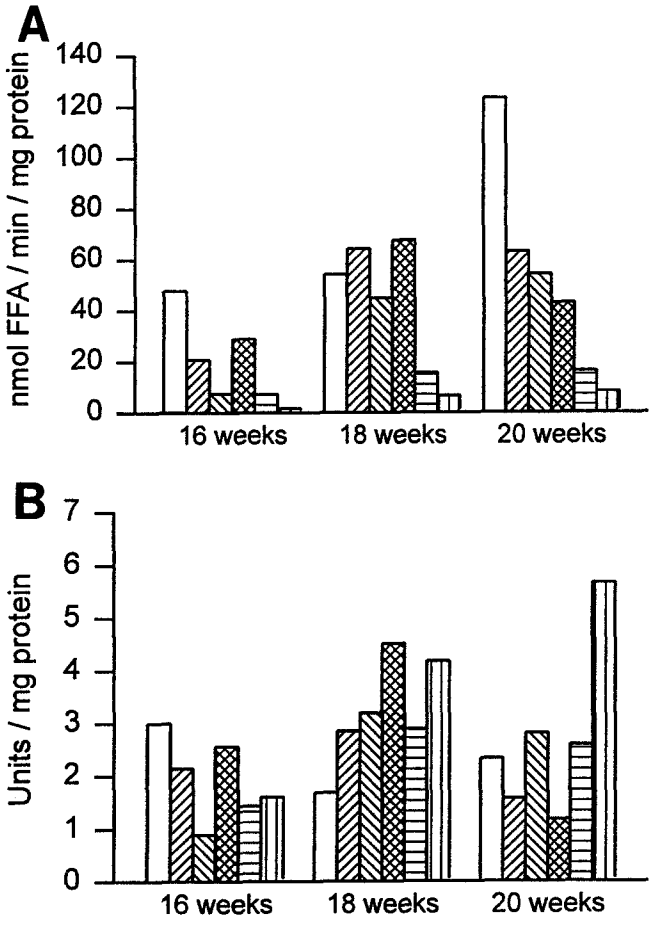

Figure 5. Distribution of (*A*) lipase and (*B*) pepsin activities in the stomach of 16-, 18-, and 20-week-old fetuses. □, Fundus; ▨, upper greater curvature; ▧, upper lesser curvature; ▩, lower greater curvature; ▤, lower lesser curvature; ▥, antrum.

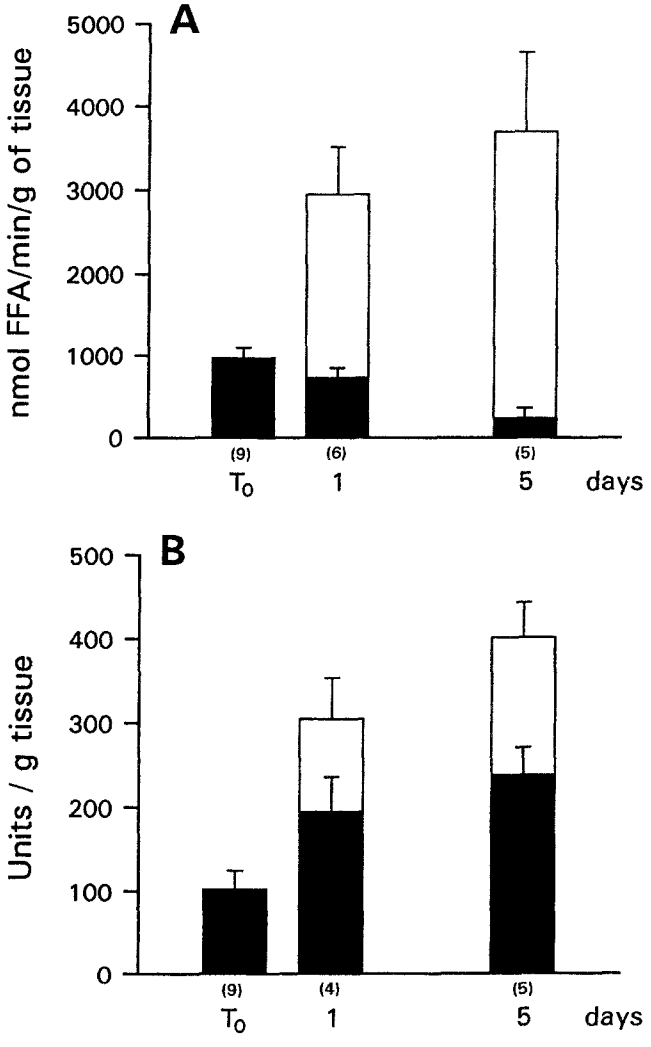

Figure 6. (*A*) Lipase and (*B*) pepsin activities in gastric explants (■) and culture media (□) at the beginning of the culture (T_0) and after 1 and 5 days of culture. Numbers in parentheses represent the number of experiments. Data are expressed as mean ± SEM.

Table 1. Lipase Activities of Cultured Explants and Corresponding Media

Culture	Control (n = 9)	1 day (n = 6)	5 days (n = 5)
Tissue	974 ± 124	741 ± 115	250 ± 110[a]
Medium	—	2580 ± 614	3443 ± 980
Total	974 ± 124	2950 ± 567[a]	3693 ± 968[a]

NOTE. Enzymic activity was expressed as nanomoles of free fatty acids per minute per gram of tissue. Results are expressed as mean ± SEM.
[a]$P < 0.05$ compared with control values.

gastric body biopsy specimens did have the highest activity. Our data therefore illustrate that the adult regional distribution of lipase activity is already in place at 16 weeks' gestation, whereas that of pepsin is not yet established even at 20 weeks. It is not clear why high lipase and pepsin activities do not always coincide. Obviously, this differential distribution recorded in infant[12] and adult[12] human stomach is already in place as soon as the gastric glands develop (10–20 weeks' gestation) in the different gastric areas. A similar nonparallel distribution was reported in many species.[3,29] The cellular localization of gastric lipase and pepsin in human adult fundic mucosa showed that the two enzymes were always found together in the chief cells.[30] Assuming such a cellular localization in all gastric regions, it is therefore possible that, although located in the same cell type, certain gastric regions are richer in lipase and others contain higher pepsin levels. The cellular and subcellular localization of these enzymes requires further studies especially in human fetal and infant stomach to elucidate this aspect.

We used our recently described organ culture technique for human fetal stomach[16] to assess whether gastric enzymes are secreted from cultured gastric explants. As shown in Figure 6, both lipase and pepsin activities were found in the medium and a 3.8-fold increase of the total activity (tissue plus medium) was recorded. These data are compatible with a continued synthesis and secretion of both enzymes by the gastric explants and are in accordance with the sustained or increased protein ([^{3}H]-leucine incorporation) and glycoprotein ([^{3}H]glucosamine incorporation) syntheses reported in fetal gastric tissues in organ culture.[16] A similar phenomenon was observed in rabbit fundic mucosal biopsy specimens in which a continuous protein synthesis occurred during the 24-hour culture period accompanied by an increase of the total pepsin activity (tissue plus medium).[31] Although lipase and pepsin activities showed the same increase level (3.8-fold) during the culture and were secreted by the explants, the secretion patterns differed (Figure 6). This observation is puzzling in relationship to the in vivo coupled secretion of both enzymes after pentagastrin stimulation in adult subjects.[32] However, in patients with duodenal ulcers, the secretion pattern deviated from the normal parallelism, indicating that under certain physiological or pathological circumstances, the secretion of both enzymes could not be parallel.[32] Although studies with isolated gastric glands showed a similar pattern of response to secretagogues in the secretion of lipase[3,32] and pepsinogen,[28,34] very few studies dealt with the simultaneous secretion of both enzymes.[3,32] The possibility that the organ culture itself alters the secretory property of the cells cannot be ruled out. But even then, it would affect it differently, therefore supporting a different secretion pattern for both enzymes during the fetal period. No studies have been conducted to date on the maturation of the lipase secretory mechanism. Our data show for the first time that both enzymes are indeed secreted from the developing human gastric glands but the secretion patterns deviate from the normal parallelism observed in adult human mucosa.[32] Whether this phenomenon is specific to the fetal period or present in the premature or newborn infant remains to be addressed.

The presence of lipase activity in human fetal gastric mucosa and the demonstration of the ability of this mucosa to secrete lipase are of importance to clarify the origin of the lipolytic activity that Hamosh et al.[8] and Lee et al.[15] found in gastric aspirates of premature infants as early as 23 weeks' gestation. These investigators postulated two possible sources for the lipolytic activity, namely, the tongue lingual serous glands and/or the gastric glands. The demonstration that human adult preduodenal lipase is entirely of gastric origin[10] and a recent report of the absence of lipolytic activity in the tongue-esophagus tissues in two fetuses aged 27 weeks[26] combined with the present data strongly suggest that the gastric mucosa is the main source of lipolytic activity in gastric aspirates of premature infants. The present study also provides direct evidence that pepsinogen is also secreted early during development (16–20 weeks) in good

Table 2. Pepsin Activities of Cultured Explants and Corresponding Media

Culture	Control (n = 9)	1 day (n = 4)	5 days (n = 5)
Tissue	103 ± 23	196 ± 40[a]	239 ± 32[a]
Medium	—	110 ± 25	163 ± 25
Total	103 ± 23	305 ± 49[a]	401 ± 42[a]

NOTE. Enzymic activities were expressed as units per gram of tissue. Results are expressed as mean ± SEM.
[a]$P < 0.05$ compared with control values.

accordance with the electrophoretic studies of amniotic fluids (20 weeks' gestation) in which different molecular forms of pepsinogen were found.[28,35]

In conclusion, this investigation shows that gastric lipase appears very early in the developing human gastric glands and can be abundantly secreted. Increasing evidence shows that initial digestion of dietary fat in the stomach of infants and adults is a prerequisite for efficient intestinal lipolysis.[3] Recently, the human fetal small intestine (14–20 weeks) was shown to absorb and incorporate long-chain fatty acid into esterified lipids, to elaborate and secrete four lipoprotein classes, and to transport newly synthesized lipids.[36,37] Therefore, the early appearance and secretion of gastric lipase supports the hypothesis that this enzyme has a significant nutritional role during human development in hydrolyzing amniotic fluid triglycerides[38] and providing fatty acids for intestinal metabolism. Further investigations are needed to establish the specific regulatory mechanism(s) involved in the development of human gastric lipase synthesis and secretion.

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Address requests for reprints to: Daniel Ménard, Ph.D., Département d'Anatomie et de Biologie Cellulaire, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4. Fax: (819) 564-5445.

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