Digestive Functions and Their Hormonal Regulation During Perinatal Development in Man and Experimental Animals

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Shortly after birth, infants start to consume milk, which is later replaced by a solid diet. Qualitatively and quantitatively, several major differences exist between the composition of the suckling's food, i.e., milk, and that of adults. Whereas suckling infants have to satisfy their energy and fluid requirements simultaneously, weaned infants have a choice. The energy intake per unit of body weight is substantially higher in newborns than in children and adults. Carbohydrates are represented in the milk by lactose (a β-disaccharide), whereas the majority of carbohydrates in the solid diet are complex polysaccharides containing α-linkages or sucrose (an α-disaccharide). In adults, lactose in milk and milk products represents only a small fraction of the carbohydrate intake. Similarly, there are qualitative differences in the proteins and lipids of milk and those contained in adult food.

In the last two decades, there has been considerable interest in the development of gastrointestinal function, both in man and in experimental animals (1–3). This chapter summarizes the most important facts concerning perinatal changes in digestion and absorption in man, as reviewed in detail elsewhere (3).

MAJOR FUNCTIONAL CHANGES IN THE DEVELOPING HUMAN GASTROINTESTINAL TRACT

Digestion and Absorption of Protein

1. Gastric peptidase activity is low during the first 3 postnatal months of life. After introduction of food, the pH of gastric contents remains high for longer in infants under 2 months of age than in children of 3 to 13 years of age.

2. Trypsin activity in newborns is low and reaches adult values at approximately 1 year of age.
3. Digestion of cow’s milk protein increases during the first 5 months of life. Absorption of intact proteins exists; it is accepted that it has an immunological significance.

Digestion and Absorption of Lipids

1. Some studies suggest that hydrolysis of lipids proceeds in newborns at a slower rate than it does later in life. Pancreatic lipase activity is low in newborns and shows a gradual increase during the first 6 months of life. It is helped in its action by other lipases (milk lipase and lingual/gastric lipase). The fact that glycerides are found in fecal lipids of newborns also appears to suggest that there is “defective” lipolysis in newborn infants.
2. The concentration of bile acids in newborns is low in the first year of life. Bile acids are initially mainly conjugated with taurine and later with glycine. During the first period of life the cholic acid/chenodeoxycholic acid ratio is high, and later on it decreases.
3. Absorption of cow’s milk lipids is less efficient and more dependent on bile acids than absorption of human milk lipids.

Digestion and Absorption of Carbohydrates

1. Pancreatic amylase activity is low during the early period of life (up to 2 years of age).
2. Activity of disaccharidases increases prenatally. Lactase activity appears later than sucrase activity. Digestive capacity for lactose decreases postnatally at varying rates in different human population groups.
3. Active transport of glucose already exists in the small intestine of the fetus. However, absorption of glucose appears to be less effective during the first 2 years of life than later.

EFFECTS OF HORMONES ON MATURING GASTROINTESTINAL TRACT

Numerous studies in experimental animals have shown that the development of many functions of the gastrointestinal tract—as of other systems—can be influenced by hormones. Because of their importance, I shall concentrate on the effects of (a) “classical hormones,” namely the glucocorticoids, and (b) an “emerging” hormone, namely epidermal growth factor.

Effect of Glucocorticoids

In Experimental Animals

The effect of glucocorticoids on the developing gastrointestinal tract in experimental animals has been studied extensively, and interesting results have been obtained
in man. The possible role of glucocorticoids in preventing necrotizing enterocolitis cannot be overlooked. In Table 1, I have summarized the effect of these agents on various functions in rats, mice, and pigs. In the list of references I have given preference to earlier studies. The effects on sucrase-isomaltase activity have been the most studied. Many laboratories have confirmed the original reports that glucocorticoids stimulate sucrase-isomaltase activity, while others have used changes in sucrase activity as a “marker” of steroid effects. Table 2 then gives a chronology of the sucrase studies. Interestingly enough, several studies have shown that the removal of the adrenal gland slows down but does not prevent the “programmed” developmental changes (2,4–6). Administration of exogenous glucocorticoids leads to a precocious increase in enzyme activity. This effect appears to be age-dependent; many effects are only detectable in a limited time “window” (1,2,4–6). In the confines of this chapter it is not possible to provide a complete list of references. I therefore apologize to those whose papers are not cited.

Human In Vitro Studies

Arsenault and Ménard (7) have shown that the addition of hydrocortisone to organ culture of human fetal (12- to 14-week-old) small intestine increased the activity of lactase, but not of sucrase. A later experiment performed in the same laboratory (8) showed that the addition of hydrocortisone (50 ng/ml) to the same cultures caused a doubling of lactase activity without change in lactase mRNA. The authors interpreted this as an indication of posttranscriptional modulation by hydrocortisone.

Human In Vivo Studies

Other studies have examined the effect of maternal treatment with steroids on several functions of the newborn. Watkins et al. (9) reported that premature infants born to mothers treated prenatally by dexamethasone had a threefold increase in the cholic acid pool and in cholic acid synthesis over corresponding controls. The functional significance of this effect remains uncertain, because in infants born to mothers treated with beta-methasone no effect on fat absorption has been shown (10). An interesting recent study showed that antenatal beta-methasone administration was associated with increased rate of duodenal contraction, number of contractions per burst, and intraluminal peak pressure in preterm infants (11).

Collaborative study in the USA (12) has shown that prenatal glucocorticoid treatment (with dexamethasone) led to a decreased incidence of necrotizing enterocolitis (NEC) in preterm infants (2% versus 7% in placebo controls). Another study performed in Argentina (13) confirmed these results; preterm infants of beta-methasone-treated mothers had a significantly lower incidence of NEC (3.4%) versus infants of placebo-treated mothers (14.4%). Postnatal treatment of infants with steroids decreased the incidence of NEC significantly as well, but to a lesser extent than prenatal treatment (6.9%).
### TABLE 1. Effect of adrenal steroids on gastrointestinal function in suckling animals

<table>
<thead>
<tr>
<th>Function</th>
<th>Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Differentiation of chief cells</td>
<td>↑</td>
<td>82, 83</td>
</tr>
<tr>
<td>Gastric proteases</td>
<td>↑</td>
<td>82, 84, 85</td>
</tr>
<tr>
<td>Gastric receptors</td>
<td>↑</td>
<td>86</td>
</tr>
<tr>
<td>Pancreas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>↑</td>
<td>87</td>
</tr>
<tr>
<td>RNA/DNA</td>
<td>↑</td>
<td>87</td>
</tr>
<tr>
<td>EFG content</td>
<td>↑</td>
<td>88</td>
</tr>
<tr>
<td>Lipase</td>
<td>↑</td>
<td>87, 89</td>
</tr>
<tr>
<td>Amylase</td>
<td>↑</td>
<td>90</td>
</tr>
<tr>
<td>Protease (trypsin)</td>
<td>↑</td>
<td>87</td>
</tr>
<tr>
<td>Small intestine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight of the jejunum</td>
<td>↑</td>
<td>Own unpublished results</td>
</tr>
<tr>
<td>Weight of the ileum</td>
<td>No change or decrease</td>
<td>Own unpublished results</td>
</tr>
<tr>
<td>Protein/DNA</td>
<td>No change</td>
<td>91</td>
</tr>
<tr>
<td>RNA/DNA</td>
<td>↑</td>
<td>92</td>
</tr>
<tr>
<td>Depth of crypts, mitotic index, rate of cell migration</td>
<td>↑</td>
<td>93</td>
</tr>
<tr>
<td>Appearance of enterocytes (i.e., presence of vacuoli, inclusions, and development of Golgi apparatus)</td>
<td>&quot;Matures&quot;</td>
<td>94</td>
</tr>
<tr>
<td>EGF content in jejunum</td>
<td>↑</td>
<td>88</td>
</tr>
<tr>
<td>EGF content in ileum</td>
<td>↓</td>
<td>88</td>
</tr>
<tr>
<td>Collagens (incl. mRNA)</td>
<td>↓</td>
<td>96</td>
</tr>
<tr>
<td>Lengthening of the microvilli</td>
<td>↑</td>
<td>95</td>
</tr>
<tr>
<td>Number of goblet cells</td>
<td>↑</td>
<td>94</td>
</tr>
<tr>
<td>Enzyme activities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>↑</td>
<td>97–99</td>
</tr>
<tr>
<td>Sucrase-isomaltase</td>
<td>↑</td>
<td>ADX: delays ↑</td>
</tr>
<tr>
<td>Lactase</td>
<td>↑</td>
<td>ADX: delays ↑</td>
</tr>
<tr>
<td>Acid β-galactosidase and several other lysosomal enzymes</td>
<td>↑</td>
<td>ADX: delays ↓</td>
</tr>
<tr>
<td>Non-specific esterase</td>
<td>ADX: delays ↑</td>
<td>102</td>
</tr>
<tr>
<td>Diamine oxidase</td>
<td>↑</td>
<td>104</td>
</tr>
<tr>
<td>γ-Glutamyltranspeptidase</td>
<td>↑</td>
<td>107</td>
</tr>
<tr>
<td>Other functions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absorption of antibodies</td>
<td>↓</td>
<td>94, 108, 109</td>
</tr>
<tr>
<td>Absorption of vitamin B₁₂</td>
<td>Abolished</td>
<td>110</td>
</tr>
<tr>
<td>Absorption of Cu</td>
<td>↓ absorption and ↑ secretion into the bile</td>
<td>110</td>
</tr>
<tr>
<td>Prevents experimental necrotizing enterocolitis</td>
<td>↑</td>
<td>111</td>
</tr>
</tbody>
</table>

ADX, removal of adrenal glands.
**TABLE 2. Adrenal cortex and sucrase-isomaltase (SI) in developing rodents**

| Glucocorticoid (GC) receptors are present (113,114) |
| GC induce SI activity (SIA) precociously; adrenalectomy delays the increase (101,115) |
| Inducibility of SIA increases with approaching time of normal increase (93,116) |
| Continuous administration of GC is necessary (101,117) |
| Increase of SIA is seen first in the crypt-villus junction area (93,116,118) |
| Increase of SIA is inhibited by actinomycin D (119) |
| Increase of SIA = appearance of a new protein (immuno; elpho) (118,120) |
| GC treatment leads to a parallel increase of SIA and mRNA (121,122) |

**Effect of Epidermal Growth Factor (EGF)**

This peptide has emerged in the last decade as an important regulator of gastrointestinal function. I believe that its cytoprotective role (see below), together with its presence in human milk (14-17), stresses its important role in the developing gastrointestinal tract. Noteworthy is the study of Wright et al. (18) who described active synthesis of EGF in the proximity of ulcers in patients with Crohn's disease. A recent study by Scott et al. (19) showing increased urinary secretion of EGF in infants with NEC points to a possible involvement of EGF in the response to this condition.

EGF was first recognized for its ability to stimulate precocious eye opening in newborn mice due to its mitogenic activity, and to inhibit gastric acid secretion in the dog (also called urogastrone activity) (20,21). EGF receptors were demonstrated in the human gastric mucosa (22-25), in the rodent small intestine (26-31), and in the human fetal small intestine and colon as early as 12 weeks of gestation (32,33).

Major sources of EGF are salivary glands and Brunner's glands in the duodenum (20). Large amounts of EGF are found in milks of various species (34). The amount of EGF in the gastrointestinal tract of the suckling rat is several times higher than in the adult rat. Whereas during fasting in adult rats the changes in gut EGF content are small, in suckling rats a profound decrease is observed (35). Refeeding suckling rats with rat milk or rat milk substitute with added EGF leads within 2 hours to a return to the values found in suckling rats that had not been fasted (36). EGF inhibits gastric acid secretion (37-41); it also inhibits pepsinogen secretion stimulated by a variety of secretagogues (42) and it stimulates intestinal ornithine decarboxylase (43,44), DNA synthesis in the gastric mucosa (45-48), gastric mucosal growth (38,48), and growth of the intestinal mucosa of adult rats (49) and suckling mice (50). EGF protects against experimental gastric ulcer formation at doses that have no effect on acid secretion (48,51). Interestingly, the addition of EGF to the jejunal perfusate in adult rats increased the absorption of $H_2O$, $Na^+$, $Cl^-$, and glucose within 20 minutes (52). Last, but not least, EGF affects gastrointestinal motility. In guinea pigs, EGF inhibited the response of the ileum to electrical stimulation *in vitro* and caused depression of spontaneous movement of the stomach *in situ* (53). In our laboratory, Shino- hara et al. (54) have shown that EGF in suckling rats acutely delays gastric emptying and slows down small intestinal propulsive motility.

EGF is trophic to the intestinal epithelium of parenterally fed rats (55) in doses
below that needed to inhibit gastric acid secretion. Goodlad et al. (55) found no effect after intraluminal administration, whereas Ulshen et al. (56) reported a trophic effect of intraileal EGF on the rat gastroduodenal mucosa.

EGF enhances the differentiation of the human fetal small intestine in organ culture (57), as well as in serially passaged normal human fetal colonic epithelial cells (58). Maturational effects were found in organ cultures of small intestine of developing rodents (59,60) and in in vivo experiments (61).

Interestingly, in fetal human intestinal cultures EGF increases lactase activity but decreases the activity of sucrase and the synthesis ("spontaneous" and hydrocortisone-stimulated) of DNA (57). In rat fetal small intestinal cultures no effect of EGF was seen on lactase activity, but incorporation of thymidine and proline into DNA and proteins, respectively, was increased (62). Increased intestinal proliferation was reported in a human infant with congenital microvillus atrophy who was fed intravenously and was treated with intravenous recombinant EGF (63). In weaned rats EGF given enterally and parenterally reduced mucosal permeability to HCl (40). Dembinski and Johnson (46) have shown that EGF stimulates oxyntic mucosal growth in unweaned rats. Administration of EGF increased sucrase and maltase activities in the small intestine of 3-day-old piglets (64), caused intestinal hypertrophy in fetal rhesus monkeys (65), and caused a large decline in lactase activity in the colon of newborn rats (66).

Interest in the possible luminal effects of EGF, i.e., milk-borne EGF in developing mammals, received its impetus from the work of Cohen and Taylor (67). They found that the oral administration of EGF caused precocious eyelid opening in newborn mice. "Survival" (i.e., resistance to proteolytic degradation in the stomach) is necessary if ingested EGF is to function within the gastrointestinal tract. As a first approach to this question in the human neonate, Britton et al. (68) have shown that degradation of $^{125}$I-human recombinant EGF by the gastric juices of preterm infants in vitro is negligible. Similar findings were obtained with gastric juices of suckling and weanling rats (69). Studies on the intestinal luminal content of EGF indicate that there are developmental changes, and whereas in suckling rats the degradation is low, it increases in weanling rats (69). Studies performed in vivo in suckling rats have shown that orogastrically administered $^{125}$I-EGF is degraded very little in the stomach and small intestinal lumen (70,71). Similar results are seen in suckling mice (72) and lambs (73). In mature rats the degradation is increased, but some $^{125}$I-EGF still remains intact and is absorbed (71). Newborn rats fed with artificial milk to which EGF was added showed an increase in DNA synthesis and content in the small intestine (74), an increase of DNA synthesis in the liver (75), and an increased stomach wet weight (76). The protein content of the colon in rats fed rat milk substitute with EGF was significantly lower and the DNA content significantly higher than in rats fed rat milk substitute only (77). The importance of EGF is further stressed by the observation that the intestine of suckling rats fed with pooled rat milk to which antibodies against EGF were added showed lower wet weight, DNA synthesis and content, and a lower RNA content (74). Orogastrically instilled EGF given to suckling rats between day 11 and 13 increased the cell labeling indices of fundic, antral, and ileal mucosae, and exocrine pancreas (78); in suckling rabbits it evoked an increase in wet weight of the
stomach and pancreas, increased DNA content in the ileum, and increase in sucrase activity concomitant with a decrease in lactase activity in the proximal segments of the small intestine (79), and precocious maturation of liver functions, i.e., increase in size of the bile salt pool, increased bile secretion, and increased activity of glucokinase (80). It is interesting that the urinary EGF output of 2-week-old breastfed infants was higher than in infants fed EGF-poor diets, i.e., bovine milk-based formulas or total parenteral nutrition (81).

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DISCUSSION

Dr. Guesry: New hormones are being discovered all the time—epidermal growth factor, neural growth factor, and so on—and there is a tendency to want to incorporate these hormones in infant formulas. This is a cause for anxiety because in nature the activity of such hormones is regulated by other hormones or factors, some of which may not even be known
about at present; if such controlling influences are absent or inadequate, there might be a risk of inducing tumor formation by the use of growth promoting hormones in foodstuffs.

Dr. Koldovsky: It is my belief that one day we shall have to add these factors to formulas, but not yet. It is clear that, for example, epidermal growth factor has inducing effects in tissue culture and probably in the whole animal when given in small doses, though in large doses it may have an inhibiting effect. It should be borne in mind that growth factors cooperate with each other, so more experience of their interactions needs to be gained before recommending their use. Human milk not only contains growth factors but also somatostatin, which opposes some of their actions.

Dr. De Curtis: A recent paper in the Journal of Pediatric Gastroenterology and Nutrition (1) dealt with the problem of supplementing infant formulas with the nucleotides that are present in human milk but not in formulas. What effects do such nucleotides have on intestinal development?

Dr. Koldovsky: Little is known about this, but contents of nucleotides and polyamines vary a greater or lesser extent in different formulas.

Dr. Guesry: We have considered adding them for 20 years now and each time we decide against it.

Dr. Orzalesi: I think it would be naive to assume that the gut will behave in the same way in any particular clinical situation. In fetal growth retardation, for example, there may or may not be stress accompanied by increased steroid output, there may or may not be nutrient deprivation, and so on. The interactions among various factors will determine how the gut responds. Thus you might have increased protection against necrotizing enterocolitis because of increased steroid production, or increased risk because of poor intestinal perfusion.

My question is, What do we know about the presence in amniotic fluid of the factors you have listed as being important for intestinal maturation? Babies swallow amniotic fluid, so are there important growth factors in the fluid that affect the development of the gut?

Dr. Koldovsky: All the hormones we could wish to find are present in the amniotic fluid! Steroids are present and increase through the duration of pregnancy. Epidermal growth factor, while not present early in gestation, starts to appear near term, when its concentrations are positively correlated with the size of the placenta (2). It is certainly possible that EGF and other hormones may have local effects in the gut but research is still needed in this area.

Dr. Delvin: We have shown in collaboration with Daniel Ménard that there are indeed other local factors that may be involved in gut development. For example, calcitriol, the active metabolite of vitamin D, has receptors in the gastrointestinal tract. By as early as 10 weeks of gestation vitamin D causes increased differentiation and increased proliferation of cells in organ culture, whereas by 20 weeks there is inhibition of proliferation but persistence of the differentiation effect. It has also been shown that vitamin D has effects on EGF. We therefore have to be careful before we start giving EGF, because we don’t know all the developmental implications.

Dr. El Mauhoub: What are the side effects of glucocorticoids in the amniotic fluid?

Dr. Koldovsky: Any side effects that we know of are the same as are seen in adults. For example, it is known that they may increase the risk of bleeding from the stomach. On the other hand they appear to reduce the risk of necrotizing enterocolitis. Steroids increase cholic acid synthesis in premature infants when given to the mother antenatally.

Dr. Fukagawa: Is anything known about the effects of maternal drug abuse on gastrointestinal development?

Dr. Koldovsky: I know of only one report where mothers received phenobarbitone for
therapeutic reasons. Premature infants born to such mothers had increased bile acid production.

*Dr. Singh:* Why does sucrase appear before lactase? It should be the other way around, since lactose is the only sugar in breast milk.

*Dr. Koldovský:* This is a puzzle and the answer is not known. It has been suggested that the “sucrase” is in fact not only sucrase but also an important brush-border structural protein.

*Dr. Schmitz:* Jack Welch in the United States and our group have shown that sucrase is present in the newborn macaque rhesus monkey. My proposition would be that this is an evolutionary trend originating from the fact that we had the same phylogenetic origin as the monkey and needed to be able to eat fruits very early in life.

*Dr. Donzelli:* You underlined the low activity of trypsin and lipase in low birthweight infants. It has been shown that trypsin activity is similar in preterm and small-for-dates babies, but this is not true for lipase. What is the reason for these differences?

*Dr. Koldovský:* All the pancreatic enzymes do not develop in the same way. Trypsin is among the fastest, amylase the slowest, and lipase somewhere in between. Some enzymes develop independently of duration of gestation, and are dependent on chronological time after birth. There is obviously a genetic plan and some regulation is determined by the events occurring after birth.

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