Annu. Rev. Nutr. 14:147–67

GROWTH FACTORS IN MILK AS MEDIATORS OF INFANT DEVELOPMENT

S.M. Donovan and J. Odle
Division of Foods and Nutrition and Department of Animal Sciences, University of Illinois, Urbana, Illinois 61801

KEY WORDS: epidermal growth factor, insulin-like growth factors, insulin, intestine, neonate

CONTENTS

INTRODUCTION

Growth factors are composed of a heterogeneous group of proteins and peptides that initiate cellular growth and expression of differentiated function. Circulating growth factors may act in an analogous manner to classic endocrine hormones. In addition, most growth factors are synthesized in multiple cell types throughout the body and may act locally within a particular cell or upon adjacent cells via autocrine or paracrine mechanisms, respectively.

In the 1970s and 1980s, researchers demonstrated that milk is a potent stimulator of growth both in vivo and in vitro. Widdowson et al (122) documented the marked increase in gastrointestinal (GI) weight, length, and protein

0199-9885/94/0715-0147$05.00

and DNA content of colostrum-fed neonatal piglets in the first 24 h of life. More recent piglet studies have demonstrated stimulation of GI DNA (114) and protein synthesis (19) by both mature milk and, to a greater degree, colostrum. Colostrum-fed piglets also had greater fractional protein synthesis rates in liver, kidney, spleen, and skeletal muscle than piglets fed milk, which indicates that colostral factors may mediate growth of tissues outside of the GI tract (19). In 1978, Klagsbrun provided the first in vitro evidence that human milk contains mitogenic factors (58). At a concentration of 1% in the culture media, human milk was as effective as 5% human serum or 10% calf serum at stimulating DNA synthesis in fibroblasts. Human milk is potent mitogen for cultured hepatocytes (59) and enterocytes (55) as well.

In addition to the direct trophic effects of milk growth factors on the intestine, recent evidence suggests that we must consider the potential effect of these growth factors on the autocrine regulation of intestinal growth. For example, the addition of epidermal growth factor (EGF) to intestinal explants downregulated its own mRNA while upregulating the expression of TGF-α (80). Moreover, growth factors present in the serum of the neonate may also modulate GI growth via receptors on the serosal membranes of enterocytes. The ability of systemically administered insulin (78), EGF (63), and insulin-like growth factor-I (IGF-I) (5, 100) to stimulate GI growth and maturation supports this hypothesis.

This review discusses recent findings on growth factors in milk and their potential roles in the neonate. We focus on human milk, the human infant, and animal models used in neonatal research (e.g. rodents, piglets). The role of growth factors both in normal growth and development as well as their potential therapeutic role during recovery from intestinal injury is addressed. With the exception of insulin, this review does not discuss hormones in milk, which were the subject of several recent reviews (46, 62)

EPIDERMAL GROWTH FACTOR (EGF)

Background and General Physiological Function

The EGF family of growth factors currently includes EGF, transforming growth factor-α (TGF-α), amphiregulin, and a number of viral growth factors. Of this family, EGF and TGF-α are thought to be the major peptides involved in mammalian growth and development (22). Several reviews cover many facets of EGF biochemistry and physiology (21, 22, 61, 75, 93).

Human EGF (also known as urogastrone) is composed of 53 amino acids (~ 6 kDa), with ~ 50% sequence homology to rodent species. Structural similarities are sufficient to allow EGF from a given species to elicit effects in other species (75). EGF is translated into a 1200 amino acid prepro form,

which is rapidly processed to mature EGF in some tissues (e.g. mouse submaxillary gland), whereas others (e.g. kidney and mammary gland) accumulate the precursor (18, 97). The EGF family of mitogens possesses a common high-affinity receptor with an extracellular mitogen-binding site and a cytoplasmic domain possessing tyrosine-kinase activity. The details of the second-messenger cascade are not fully known but are the subject of active research. The cascade ultimately culminates in mitosis and/or differentiation of the target cells.

Milk Concentrations and Sources

EGF seems to act as a local regulator of prepartum mammary gland development (93). Studies in rodents have revealed that the EGF receptor is present in mammary tissue, that EGF stimulates mammary cell growth in vitro, and that EGF binding in mammary tissue increases 150% by days 10–15 of gestation (37). EGF concentration of human prepartum mammary secretions exceeds that of mature milk (12), which may imply a similar prepartum accumulation (Table 1).

Milk EGF may be derived from the maternal circulation or from mammary synthesis. The 30- to 1000-fold milk:plasma EGF concentration ratio coupled with low circulating EGF levels (i.e. < 0.1 nM; 50) indicates that mammary uptake of EGF from plasma would require an active transport mechanism. Indeed, the lactating goat mammary gland was shown to take up 83% of an infused ^{125}I-EGF dose. However, only 3% of the dose appeared in milk, which indicates that serum EGF is probably not the primary source of milk EGF (17). The presence of EGF mRNA in rodent mammary tissue and its modulation by lactogenic hormones (39) suggest that local EGF synthesis may contribute to milk EGF. Endogenous mammary EGF production may also explain the reported size heterogeneity of human (12, 28, 54, 89, 101) and rat (96) milk EGF. Whether these high molecular weight forms represent various degrees of proteolytic processing of prepro EGF, aggregation of mature 6 kDa EGF, or association of EGF with other proteins is not fully known. The predominant form of EGF in human milk was recently shown to be a 160–170 kDa heparin-binding glycoprotein that results from proteolytic cleavage of the cytoplasmic domain of prepro EGF (83). EGF species of high molecular weight have been reported to account for 0 (12, 28, 87, 101) to 50% (54) of total milk EGF activity. However, the active form of EGF may be produced within the stomach of the infant, since EGF forms of high molecular weight are labile to perturbations in pH (3).

Human milk EGF concentrations categorized by stage of lactation and method of analysis are presented in Table 1. Human milk values over a 40-fold range (3-133 nM) may be partially explained by normal changes over the course of lactation but could also result from methodological

Table 1 EGF in human mammary secretions

Days peripartum	EGF (nM)	Assay[a]	Reference
Prepartum	Average 58		
20 to −1 d	22–133	RIA	12
23 to −3 d	50.0	RIA	28
13 to −5 d	27.0	RRA	101
Colostrum	Average 25		
0 to 2 d	6–73	RIA	12
0 to 2 d	50.0	RIA	28
not specified	14.7	RIA	81
0 to 3 d	7.8 preterm; 6.5 term[b]	RIA	55
0 to 2 d	78 very preterm; 70 preterm; 54 term	RRA	99, 101
0 to 1 d	5.9	RRA	54
Mature milk	Average 10		
2 to 50 d	3–19	RIA	12
2 to 42 d	11.5	RIA	81
4 to 42 d	13.0	RIA	28
4 to 74 d	5.1 preterm; 5.3 term	RIA	55
banked milk	10.8	RIA	89
8 d	7.5	RRA	54
46 d	15 very preterm; 10 preterm; 4.9 term	RRA	99, 101

[a] RIA, radioimmunoassay; RRA, radioreceptor assay.
[b] Term 38–41 weeks gestation; preterm 31–36 wk; very preterm 26–30 weeks.

variation. The primary assays used are homologous radioimmunoassays (RIA) and heterologous radioreceptor assays (RRA). Direct methodological comparisons have shown that RIA and RRA are not always comparable (28, 55, 86). Therefore, data derived from differing assay conditions should be compared with caution.

With only one exception (81), EGF content in human prepartum secretions and colostrum was higher than in mature milk (Table 1). Milk EGF and protein decline in a parallel manner (101), resulting in a fairly constant protein:EGF ratio throughout lactation. EGF content of milk from mothers delivering prematurely was reported to be equivalent (55, 81) or higher (99) than that of term milk when assessed with RIA and RRA, respectively. Whether this discrepancy is related to presence of other growth factors (e.g. TGF-α) that may interact in the RRA remains to be determined.

For comparison, EGF values from other species are given in Table 2. The EGF content of mouse (13) and porcine milk (56) is higher than that of human milk, whereas levels in rat milk are lower (96). Concentrations ranging from 2–324 μg/liter have been reported in fresh cow milk; however, EGF is barely detectable in infant formulas (54, 55, 99).

Table 2 Concentrations of milk growth factors in various species

	Secretion				
	Prepartum[a]	Colostrum	Milk	Units	Reference
EGF					
Porcine	NR	1500 ± 525	160–240	μg/L	56
Bovine	NR	4–8	2–324	μg/L	54
Rat	NR	1.8	3.6–12.0	μg/L	96
Mouse	NR	55	130–427	μg/L	13
Insulin					
Human:					
Term[b]	24.5–44.2	21.5 ± 5	2.6 ± 0.3	μg/L	101
Preterm	NR	7.2 ± 1.4	1.4 ± 0.3	μg/L	99
V. preterm	NR	3.4 ± 1.1	1.5 ± 0.3	μg/L	99
Porcine	NR	300 ± 80	40 to 80	mU/L	56
Bovine	NR	37 ± 14	5.5 ± 0.6	μg/L	74
IGF-I					
Human:					
Term	NR	10.9 ± 5.3	7.1 ± 0.4	μg/L	11
Term	31	9.9	19.1	μg/L	29
Rat	NR	27.8 ± 2.0	16.2 ± 2.2	μg/L	33
Porcine	136.3 ± 22.6	39.0 ± 22	11.4 ± 1.4	μg/L	36
Bovine	2949 ± 1158	NR	5.0 ± 2.0	μg/L	121
IGF-II					
Human	NR	NR	2.7 ± 0.7	μg/L	34
Rat	NR	1.2 ± 0.2	<1.0	μg/L	33
Porcine	291 ± 64.5	82.3 ± 57.5	16.8 ± 5.8	μg/L	36
Bovine	1825 ± 608	NR	1.0 ± 0.1	μg/L	121
NGF					
Human	identified but not quantitated				124
Mouse	NR	NR	100 to 1100	μg/L	84
Relaxin					
Human	NR	327 ± 110	509 ± 53	ng/L	72
Bovine	NR	NR	1467 to 4770	ng/L	72
TGF-α					
Human	0–50	2.2–7.2	0 to 8.4	μg/L	27, 87
TGF-B1 & B2					
Bovine	detected but not qualified				57

[a] Colostrum 1 to 4 days postpartum; mature > 5 days postpartum.
[b] Term 38–41 weeks gestation; preterm 31–36 weeks; very preterm 26–30 weeks.

Effects on the Neonate

A plethora of studies in rodent species supports the hypothesis that milk-borne EGF conveys important regulatory signals to the developing offspring, including the timing of eyelid opening and tooth eruption as well as intestinal,

hepatic, pancreatic, and lung development (reviewed in 22, 60, 61, 76). These studies have vastly increased our knowledge of EGF physiology and function, and it is tempting to extrapolate these findings to humans. However, obvious differences in physiology and genetics and in the ontogeny (rate and sequence) of GI maturation (79) between species make direct comparisons difficult.

EGF is resistant to degradation within the gastric milieu of the suckling rat (15) and preterm infant (16), which suggests that milk-borne EGF may retain bioactivity in the neonatal GI tract. Recombinant human ^{125}I-EGF incubated for 1 h with gastric aspirates from preterm infants co-chromatographed with mature EGF and retained more than 75% of its ability to bind to EGF affinity columns or to EGF receptors (16). Developmental differences were observed in the small intestinal degradation of EGF. In suckling rats, EGF degradation was relatively low, but it increased 12-fold in weanling animals (15).

Data on the effect of EGF on the developing human GI tract are limited primarily to in vitro studies. Using intestinal explants from human fetuses (11–14 week gestation), Ménard and colleagues (77, 79) observed responses of various jejunal brushborder enzymes to EGF (77) and used quantitative autoradiography to demonstrate extensive EGF binding by crypt cells and in the basolateral infranuclear region of cells at the base of the villi (79). In contrast, other investigators have reported EGF-receptor immunoreactivity (80) and mRNA levels (6) extending to the villus tip. Absorption of orally administered ^{125}I-EGF has been clearly demonstrated in rats (120). A twofold increase in urinary EGF content of premature infants consuming breast milk vs cow milk–based infant formulas led Gale et al (42) to conclude that EGF is absorbed by the human infant. Whether this increase truly reflects absorption of EGF from breast milk or is due to increased renal EGF production (97) remains unknown.

In addition to its putative role in normal GI growth and development, EGF may also be beneficial during recovery from GI trauma. Weanling rats subjected to intestinal resection experienced a 19–25% increase in small intestinal regrowth when fed formula supplemented with 83 nM EGF for 7 days following surgery (98). Subcutaneous administration of EGF or TGF-α stimulated DNA synthesis and enhanced the healing of stress-induced gastric ulcerations in rats (63). Similarly, Petschow et al (90) treated adult rats with methotrexate to induce intestinal injury and demonstrated that after 6 days of feeding of EGF at 1 or 10 times that in human milk levels, intestinal leucine aminopeptidase and sucrase were increased by 80–250%. Most recently, we (125) developed a colostrum-deprived piglet model to study the role of growth factors in GI recovery from rotaviral damage. Addition of human recombinant EGF to formulas at 83 and 166 nM increased villus length and lactase specific

Table 3 Human insulin, IGF-I and -II receptors, and IGFBPs

Peptides	Molecular weight	Reported in milk
Insulin	5734 (α,β)	human (65, 99, 101)[a], porcine (56), bovine (75, 106)
IGF-I	7649	human (11, 29, 34, 117), bovine (121), goat (94), porcine (36, 115), monkey (123), rat (33)
IGF-II	7541	human (34), bovine (121), goat (95), porcine (36), rat (121)

Receptors	Molecular weight (kDa)	Affinity			Reported in intestine
		Insulin	IGF-I	IGF-II	
Insulin		100%	2%	2%	dog (44), rat (40)
α-subunit	130				
β-subunit	95				
Type I		1%	100%	10–30%	bovine (8), rat (67), porcine (107)
α-subunit	125				
β-subunit	95				
Type II	250	0%	0.1–0.2%	100%	bovine (8), porcine (107), rat (49, 67)

IGFBPs	Core protein mol wt (kDa)	Mol Wt by SDS-PAGE[b]	Reported in milk[c]
IGFBP-1	25	same	human (RIA; 117), 28 kDa IGFBP by LB: bovine (116), porcine (36)
IGFBP-2	31	same	human (LB; 34), rat (LB; 33), bovine (LB; 116), porcine (LB; 36)
IGFBP-3	29	~37, 43[b]	bovine (LB; 116), porcine (LB; 36), rat (LB; 33)
		150	human (C; 11, 29), porcine (C; 115), rat (C; 35)
IGFBP-4	26	25; 28[b]	bovine (LB; 116), porcine (LB; 36), rat (LB; 33)
IGFBP-5	28	same	N.R.[d]
IGFBP-6	23	same	N.R.

[a] Reference number
[b] Mol wt change due to post-translational modifications.
[c] Assay method: RIA radioimmunoassay; LB western ligand blotting; C Gel filtration chromatography
[d] Not reported

activity in a linear dose-response fashion when fed to virus-infected pigs for 8 days. At 83 nM, effects were noted only in the proximal portions of the small intestine, whereas effects from the higher EGF level extended further down the tract. Collectively, these studies suggest that supplementation with pharmacological levels of EGF may aid in the recovery of traumatized intestine, but further studies are required to determine safe and effective upper limits. In addition, the comparative safety and efficacy of intestinal as well as systemic administration of EGF on GI recovery needs to be assessed.

INSULIN, RELAXIN, AND INSULIN-LIKE GROWTH FACTOR-I AND -II

Background and General Physiological Function

All four members of the insulin-like family of peptides (insulin, relaxin, IGF-I, and IGF-II) have been reported in milk (Tables 2 and 3). IGF-I and IGF-II are 7.5 kDa polypeptides that retain 70% overall amino acid homology and a 50% homology with proinsulin (102). The biological actions of IGF-I and IGF-II are mediated primarily through the type I IGF receptor, which is structurally homologous to the insulin receptor and consists of two extracellular α-subunit ligand-binding domains joined by disulfide bonding to two β-subunits, each of which contains a transmembrane domain and a cytoplasmic tyrosine kinase domain (Table 3). In addition, IGF-II binds with high affinity to the type II IGF receptor/cation-independent mannose-6-phosphate receptor (type II/M6P receptor), a monomeric 250 kDa protein (49, 102). Insulin and type I and II IGF receptors have all been detected in the neonatal intestine (Table 3). Because of the homology between insulin and the IGFs, insulin at pharmacological doses stimulates mitogenesis via interaction with the type I IGF receptor. Likewise, high doses of IGF-I cause hypoglycemia as a result of stimulation of glucose uptake via the insulin receptor (102). Relaxin is primarily known for its role in cervical softening prior to parturition (53). However, in conjunction with prolactin, estrogen, and progesterone, relaxin promotes mammary growth and differentiation (48).

Unlike insulin and relaxin, the IGFs associate with a family of IGF-binding proteins (IGFBPs) (Table 3). Six genetically distinct but structurally related IGFBPs have been cloned and sequenced in the rat and human (110). IGFBP-3, the predominant serum IGFBP, is unique in that it binds to an additional 85 kDa protein known as the acid-labile subunit (ALS) to form a 150 kDa complex that does not cross the vascular epithelium (10), thereby increasing the half-life of serum IGFs. Additionally, IGFBPs function as more than just carrier proteins by modulating the interaction of IGFs with their cellular receptors (25).

Insulin

MILK CONCENTRATIONS AND SOURCES Insulin has been reported in human (65, 99, 101), porcine (56), and bovine (74) milk (Tables 2 and 3). Insulin accumulates in the bovine mammary gland in late gestation. This accumulation is reflected in 3- to 10-fold higher insulin levels in prepartum secretions and colostrum than in mature milk (74). If a similar mechanism occurs in humans, it may explain the lower milk insulin levels in women delivering prematurely (99). By measuring arteriovenous differences, Malven et al (74) demonstrated

that insulin is readily taken up from the maternal circulation by the lactating bovine mammary gland (3.94 U/day). In contrast to EGF, of which very little (3%) is transported into the milk (17), 62% of the insulin taken up by the mammary gland appeared in milk in an immunoreactive form (2.46 U/day) (74).

EFFECTS ON THE NEONATE Insulin was the first peptide shown to be absorbed from the neonatal GI tract in a biologically active form. Oral administration of pharmacological levels of insulin to the suckling rat [4 U/100 g body weight (BW)] (51), piglet (20 U/100 g BW), (2) or calf (3–50 U/100 g BW) (92) resulted in hypoglycemia, which indicates that insulin was absorbed intact and retained its ability to stimulate glucose uptake. Insulin receptors on jejunal and ileal brushborder membranes (40, 44) may allow for direct action upon the enterocyte and/or receptor-mediated uptake. Recent studies have both supported and contradicted these earlier observations on insulin absorption. In studies in which neonatal piglets (19) and calves (106) were fed colostrum or mature milk, serum insulin levels were two- and fourfold higher, respectively, in colostrum-fed neonates than in those fed mature milk. These results supported the concept of insulin absorption from the neonatal GI tract. Neither study determined whether this discrepancy was due to absorption of colostral insulin or to enhanced endogenous secretion, although endogenous insulin secretion is thought to be suppressed for at least 12–48 h postpartum (47). In contrast, two studies in which insulin was added to a formula (85 U/liter) (111) or administered orally (50 mg/100 g BW) immediately prior to feeding colostrum (47) did not result in a rise in serum insulin or a decline in blood glucose.

Enterally and systemically administered insulin accelerates GI growth and maturation. A single s.c. insulin injection (1.25 U/100 g per day) to eight-day-old mice induced changes not normally observed until weaning, including increased sucrase activity and a decrease in the crypt-to-villus cell transit time from six to four days (78). Intraperitoneal insulin injections (1.25 U/100 g per day) to suckling rats also induced sucrase activity; however, no such effect was observed when weanling rats were treated with the same dose of insulin. This finding suggests that the sensitivity of the enterocyte to insulin is developmentally regulated, or that insulin does not retain bioactivity in the GI tract of the weanling rat (20).

Piglet studies involving either subcutaneously (118) or orally (111) administered insulin further support a role for insulin in GI maturation. Gut closure in piglets occurs 18–36 h postnatally and is thought to be partially regulated by a humoral factor released postprandially, since fasted piglets have delayed gut closure (70). Svendsen et al (118) investigated whether insulin is the humoral factor involved in gut closure by subcutaneously injecting insulin (50 U/100 g) to colostrum-fed newborn piglets at 3 and 6 h postpartum. By 12 h

postpartum, insulin-treated piglets showed a 70% reduction in the macromolecular transport compared with control (colostrum-fed) piglets. The authors suggested that insulin-induced changes in enterocyte basement membrane proteoglycan synthesis may be a potential mechanism for the enhanced gut closure. Although this study used systemically administered insulin, colostral insulin may also play a role in GI maturation since insulin intake may be as high as 200 mU/day per day and may be transmitted into the blood (2).

Effects of oral insulin intake were studied in piglets consuming formula with or without supplemental porcine insulin (85 U/liter) between days two and nine postpartum (111). Formula intake, weight gain, and serum insulin levels did not differ significantly between treatment groups. However, piglets consuming formula + insulin (mean intake 16 U/day) exhibited increased ileal mucosal weight, protein, RNA and DNA content, and lactase and maltase activities. These results suggest that luminal insulin may act directly on the enterocyte. A subsequent study (112) showed that the elevation in ileal lactase activity in piglets consuming insulin-supplemented formula was not due to increased ileal lactase mRNA expression or to changes in the relative proportion of the precursor and active forms of the enzyme. The exact mechanism by which insulin exerts its action remains to be determined, but it may involve decreasing the rate of protein degradation, as has been shown in liver, cardiac and skeletal muscle, and kidney (82).

Relaxin

MILK CONCENTRATIONS AND SOURCES Relaxin mRNA is present in the corpus luteum in sows and rats (4) and in the mammary gland (88) and endometrium of guinea pigs (69). Endometrial mRNA rapidly declines postpartum, whereas mammary and ovarian expression are detectable during lactation. Relaxin has been reported in human and bovine milk (72) (Table 2). The mean concentration in human milk increased from 195 to 438 ng/liter between days 3 and 6 postpartum, after which time levels remained relatively constant through 34 weeks postpartum. In contrast, plasma relaxin levels declined from 83 to 32 ng/liter between days 3 and 6 postpartum and were undetectable after day 7 postpartum, which suggests that the mammary gland is the primary source of relaxin. Mean relaxin content of fresh bovine milk was 2916 ng/liter. In pasteurized samples, levels were approximately half that amount (1414 ng/liter) (72).

EFFECTS ON THE MAMMARY GLAND Whether milk relaxin plays a direct role in the neonate has not been investigated. However, studies using monoclonal antibodies during the second half of pregnancy to neutralize endogenous relaxin have demonstrated a specific role for relaxin in regulating morphological development of the nipple required for successful lactation (52, 64). Antibody-

treated rats had significantly shorter nipples and altered morphological characteristics, including smaller lactiferous ducts and blood vessels. In addition, tissue histological changes were apparent in antibody-treated rats. For example, a greater percentage of the nipple was composed of collagen, and these rats had shorter elastin fibers than control animals (64). The mechanism by which relaxin affects nipple structure and whether milk-borne relaxing mediates a similar response in the neonate remain to be determined.

IGF-I, IGF-II, and IGF-Binding Proteins

MILK CONCENTRATIONS AND SOURCES IGF is a potent stimulator of both mitogenesis and galactopoiesis in cultured mammary cells (108) and is thought to mediate most of the action of growth hormone on the mammary gland (31). IGF-I has been reported in the milk of all species investigated thus far (Tables 2 and 3). Although milk IGF-II has been less well characterized, it has been present in all species studied (Tables 2 and 3). IGF-I and IGF-II are higher in prepartum secretions and colostrum but decline sharply during the first 2–4 days of lactation. Colostral IGF levels in the human (11) and rat (33) are 2- to 3-fold higher than those in mature milk, whereas bovine (121) and porcine (36, 115) colostrum contains 10- to 500-fold higher levels than mature milk. Whether differences in prepartum mammary IGF accumulation are a reflection of the fact that production species have been selected for high milk production remains unknown. In addition, a truncated form of IGF-I (des-tripeptide IGF-I) has been reported that accounts for 50% of the IGF-I in bovine colostrum (41) but only 3% in bovine milk (109). This truncated variant of IGF-I, which lacks the first three amino terminal amino acids, has a reduced affinity for several IGFBPs and has a 5- to 10-fold higher biological activity in in vitro bioassays (105). Read et al (100) recently reported that growth of the rat GI tract was particularly sensitive to the action of des-IGF-I. Whether des-IGF-I is present in the milk of other species has not been determined. IGF is unaffected by pasteurization of bovine milk (79° C, 45 s) (26) or by heat treatment of banked human milk (56° C, 30 min) (34) but is either removed or destroyed during the processing of infant formulas (26, 55, 85).

Because of the low abundance of mammary IGF-I mRNA (119), maternal serum seems to be the primary source of milk IGF. Type I and II IGF receptors are present in mammary tissue (32) and may function to sequester IGF from the maternal circulation. In most species in which IGF-II is the predominant serum IGF (human, goat, and pig), IGF-II is also the major milk IGF. In the rat, however, serum IGF-I levels are 80 times higher than those of IGF-II, and IGF-I is predominant in milk (33) (Table 2). Recent studies by Prosser et al (94, 95) using close-arterial infusions of ^{125}I-IGF-I (94) or ^{125}I-IGF-II (95) into a mammary gland of lactating goats demonstrated that both peptides are

transported from serum into milk; however, the methods of transport appear to differ. Inclusion of unlabeled IGF-I in the infusate reduced the specific activity of ^{125}I-IGF-I in milk, which indicates that the IGF-I transport mechanism is competitive and saturable (94). Conversely, unlabeled IGF-II in the infusate did not lower the specific activity of milk ^{125}I-IGF-II (95), which suggests that IGF-II is transported nonspecifically.

Four of the six known IGFBPs (IGFBP-1 through -4) have been reported in milk, but their presence is species dependent (Table 3). IGFBPs can be separated into fractions of high molecular weight (150 kDa) or low molecular weight (< 50 kDa) by size-exclusion chromatography. The 150 kDa IGFBP-3/ALS peak has been demonstrated in human (11, 29), rat (35), and porcine (115) milk (Table 3). Milk is the only physiological fluid other than serum and lymph that contains the 150 kDa IGFBP-3 complex. Using western ligand blotting technique, milk has also been shown to contain IGFBP-2 (33, 34, 36, 116), IGFBP-1 (117), and IGFBP-4 (33, 36, 116) (Table 3). Whether milk IGFBPs protect milk-borne IGFs from digestion remains unknown. However, IGFBPs appear to affect IGF action in the intestine as evidenced by the greater effectiveness of IGF analogs, which bind poorly to IGFBPs, in stimulating GI growth (100).

EFFECTS ON THE NEONATE Several factors suggest that IGFs will prove resistant to digestion and thus may retain bioactivity within the GI tract of the human infant. First, IGFs and IGFBPs appear to be quite stable to acidic conditions (pH = 2) and extreme temperature (26, 34). Second, gastric acid secretion and small intestinal enzyme activities in the infant are only 10–60% of adult levels (68). Finally, the presence of IGFBPs and protease inhibitors in milk (71) may further protect the IGF peptide from digestion.

Type I and II IGF receptors have been detected on both mucosal (67, 107) and serosal surfaces (67) of the intestine. These findings support the view that both systemically (100) and orally administered (8) IGF stimulate GI proliferation. Higher binding of both IGF-I and IGF-II to crypt cells than to villus cells provides a mechanism by which IGFs may mediate the proliferation of the intestinal mucosa (67). In the piglet, binding of [^{125}I]-IGF-I to intestinal receptors was highest at birth (32%), declined at 3 (18%) and 5 (15%) days postpartum, but recovered by 21 days postpartum (27%) (107). Lastly, the reported Kd for the type I receptor in the piglet GI tract (~ 1 nM) (107) and the type II receptor (~ 0.1 nM) (102) falls within the range of normal milk IGF levels in all species.

Recent studies investigated the potential role of milk-borne IGF in the neonate. Oral administration of ^{125}I-IGF-I to suckling rats has shown that the majority (78%) of the IGF-I was retained, primarily in the stomach and intestinal lining (91), where it may exert a local effect. In calves, small amounts

of orally administered ^{125}I-IGF-I were transported into the circulation (9). The effect of feeding bovine milk replacer alone (devoid of IGF) or with supplemental IGF-I (750 μg/liter) for seven days was investigated in calves (8). No difference in serum IGF-I levels was observed between the two diets for the first three days; however, ingestion of milk replacer + IGF-I resulted in an acute, transient decrease in serum insulin (within 2 h) and an increase in serum prolactin levels (4–8 h postingestion). In addition, IGF ingestion increased thymidine incorporation into jejunal and ileal intestinal explants prepared at termination of the experiment. The enhanced DNA synthesis may be due to an upregulation in type I IGF receptors observed in jejunal and ileal microsomal membranes in the IGF-supplemented group (8). Taken together, these studies suggest that orally administered IGF at least partially survives digestion, binds to the GI tract, upregulates its own receptor, and may stimulate cellular proliferation. In addition, IGF can be absorbed into the blood, where it may affect the secretion of other hormones.

As was previously noted for EGF, IGF may also play a role in recovery of the GI tract. Recent studies investigated the effect of systemic IGF administration to rats after gut resection or to rats in a catabolic state as a result of streptozotocin-induced diabetes or dexamethasone treatment (5, 100). Administration of IGF-I for seven days resulted in increased weight gain and selective growth of the spleen, stomach, and intestine. These effects were augmented by the use of IGF analogs, which bind poorly to IGFBPs (e.g. des-IGF-I or LR^{3}IGF-I). Whether enterally administered IGF would be equally effective, particularly in the gut resection model, remains to be assessed.

OTHER PEPTIDE GROWTH FACTORS IN MILK

In addition to those discussed thus far, several other growth factors have been identified in milk (46). With the exception of nerve growth factor (NGF), the effect of oral administration of these factors on the neonate has not been studied. NGF is present in mouse milk at concentrations of 30–1000 μg/liter (45, 84) and has also been identified in human milk (124). Appearance of ^{125}I-NGF in the blood of neonatal rodents following oral (1) or ileal (113) administration suggests that NGF may survive gastric digestion and may be absorbed intact. NGF is crucial to the survival of specific neurons of the peripheral nervous system (14). Neuronal hypertrophy was observed in mice fed NGF for the first week of life (1).

Recent studies have greatly increased our knowledge of the potential role of TGF-α and TGF-β in intestinal development. TGF-α and -β are present in milk (27, 87) (Table 2) and appear to be important in mammary gland development (30, 93). In addition, local production of TGF-α and -β within the intestine (7, 80) suggests that they act as autocrine and/or paracrine regulators

of GI function. Intestinal TGF-α expression was increased threefold in jejunal explants exposed to 17 nM EGF (80). Whether EGF in milk regulates in vivo intestinal TGF-α production remains to be studied.

As noted previously, TGF-α is a member of the EGF family of peptides and can stimulate mitotic events through the EGF receptor. TGF-α levels in human milk (0–8.4 μ/g per liter) (27) are low compared with EGF and are relatively constant during the first week postpartum (87). TGF-α expression has been quantified in rat (6) and human fetal (80) GI tracts. TGF-α mRNA was found throughout the human fetal GI tract but was most abundant in the duodenum (80). Differential isolation of enterocytes along the villus-crypt axis of the jejunum revealed that both TGF-α mRNA and immunoreactive TGF-α were present in villus cells, but crypt cells did not stain, which suggests that TGF-α is associated with differentiated rather than proliferating cells. As with EGF (63) and IGF (5, 100), TGF-α administration augments GI recovery after an insult (63). Subcutaneous infusion of TGF-α into rats reduced the severity of ethanol- or stress-induced gastric lesions and accelerated the rate of gastric recovery when administered immediately following stress. Intragastric administration of TGF-α was without effect.

The TGF-β growth factor family was recently studied in rat (30, 104) and bovine (73) mammary tissue. Together with other locally produced growth factors, TGF-β growth factors are thought to modulate development and differentiated function (103) of the mammary gland. The β1 and β2 isoforms have been identified in bovine milk (57) and as such may play a role in GI development of the suckling neonate. Unlike traditional growth factors, TGF-β1 inhibits proliferation and induces terminal differentiation of intestinal epithelial cells in vitro (66). Consistent with this observation is the finding of mRNA in mouse jejunum and colon for the three isoforms (7). Expression in jejunal mucosa was greatest in cells located on the villus tip but was not detected in cells of the crypt. Using intestinal IEC-6 epithelial cells in an in vitro wounding model, Ciacci et al (24) showed that TGF-β1 inhibited proliferation but accelerated the rate of healing by stimulating the migration of cells into the wound area. TGF-β also stimulates IgA production by intestinal lymphodal cells (23) and may thus play a role in the immunological protection of the GI mucosal surface.

CONCLUDING REMARKS

The sources and levels of growth factors in milk have been fairly well described; however, much less research has been conducted on their potential roles in the neonate. In particular, little is known about the mechanisms underlying the variety of responses observed following oral administration of growth factors. In addition, the potentially complex interactions among growth

factors and hormones in milk have only begun to be considered. For example, human milk stimulates growth of cultured cells by 2- to 50-fold (29, 56), but physiologically relevant concentrations of pure EGF or IGF added alone could only account for 4–17% of this increase. This observation suggests that other factors acting independently or synergistically with IGF and EGF must be taken into account. Lastly, the effect of exogenously administered peptide growth factors on endogenous production of that peptide (80), its receptor (8), or other growth factors (80) needs to be addressed before we consider supplementing infant formulas with growth factors.

This review has focused primarily on the role of milk growth factors in the intestine. However, colostrum- and milk-derived growth factors (i.e. insulin, IGF, EGF, and NGF) may also mediate the growth of tissues not directly associated with the GI tract and therefore may have greater implications for overall growth and development of the neonate. Moreover, growth factors appear to enhance recovery of the compromised GI tract (prematurity, GI resection, rotaviral infection). The potentially therapeutic role of oral or systemic administration of growth factors during compromised states may lead to their future application in the human infant, particularly in the premature or postsurgical infant.

The apparently normal growth and development of healthy infants receiving formulas devoid of growth factors indicate that they are not essential for survival of the infant. However, as summarized by Ellis & Picciano (38), epidemiological assessment of the health consequences of formula feeding versus breast-feeding suggests that the relative risk of developing various illnesses through early adulthood is elevated in formula-fed infants. Several of these illnesses are related to the intestine, including diarrhea, necrotizing enterocolitis, colitis, and Crohn's disease. Importantly, the apparent protective effect of breast-feeding in decreasing the risk of colitis and Crohn's disease persists through young adulthood. Bioactive compounds (growth factors, hormones, enzymes, and immune components) present in breast milk but absent in formulas may be responsible for these effects.

Literature Cited

1. Aloe L, Calissano P, Levi-Montalcini R. 1982. Effects of oral administration of nerve growth factor and its antiserum on sympathetic ganglia of neonatal mice. *Dev. Brain Res.* 4:31–34
2. Asplund JM, Grummer RH, Phillips PH. 1962. Absorption of colostral gammaglobulins and insulin by the newborn pig. *J. Anim. Sci.* 21:412–13
3. Azuma N, Hesaka E, Kaminogawa S,

Yamauchi K. 1989. Occurrence of high molecular weight EGF complexes in human milk. *Agric. Biol. Chem.* 53: 1043–50
4. Bagnell CA, Tashima L, Tsark W, Ali SM, McMurtry JP. 1990. Relaxin gene expression in the sow corpus luteum during the cycle, pregnancy and lactation. *Endocrinology* 126:2514–20
5. Ballard FJ, Tomas FM, Read LC, Knowles SE, Owens PC, et al. 1991. Effect of IGF-I and IGF analogs on growth during catabolic states in rats. In *Modern Concepts of Insulin-Like Growth Factors*, ed. EM Spencer, pp 617–27. New York: Elsevier
6. Barnard JA, Polk WH, Moses HL, Coffey RJ. 1991. Production of transforming growth factor-α by normal rat small intestine. *Am. J. Physiol.* 261: C994–1000
7. Barnard JA, Warwick GJ and Gold LI. 1993. Localization of transforming growth factor β isoforms in the normal murine small intestine and colon. *Gastroenterology* 105:67–73
8. Baumrucker CR, Blum JW. 1993. Secretion of insulin-like growth factors in milk and their effect on the neonate. *Livestock Prod. Sci.* 35:49–72
9. Baumrucker CR, Hadsell DL, Skaar TC, Campbell PG, Blum JW. 1992. Insulin-like growth factors (IGFs) and IGF binding proteins in mammary secretions: origins and implications in neonatal physiology. In *Mechanisms Regulating Lactation and Infant Nutrient Utilization*, eds. MF Picciano, B Lönnerdal, pp. 285–308. New York: Wiley-Liss
10. Baxter RC. 1990. Circulating levels and molecular distribution of the acid-labile (a) subunit of the high molecular weight insulin-like growth factor binding complex. *J. Clin. Endocrinol. Metab.* 70: 1347–53
11. Baxter RC, Zaltsman Z, Turtle JR. 1984. Immunoreactive somatomedin-C/insulin-like growth factor I and its binding protein in human milk. *J. Clin. Endocrinol. Metab.* 58:955–59
12. Beardmore JM, Lewis-Jones DI, Richards RC. 1983. Urogastrone and lactose concentrations in precolostrum, colostrum, and milk. *Pediatr. Res.* 17:825–28
13. Beardmore JM, Richards RC. 1983. Concentrations of epidermal growth factor in mouse milk throughout lactation. *J. Endocrinol.* 96: 287–92
14. Bradshaw RA. 1978. Nerve growth factor. *Annu. Rev. Biochem.* 47:191–216
15. Britton JR, George-Nascimento C, Koldovsky O. 1988. Luminal hydrolysis of recombinant human epidermal growth factor in the rat gastrointestinal tract: segmental and developmental differences. *Life Sci.* 43:1339–47
16. Britton JR, George-Nascimento C, Udall JN, Koldovsky O. 1989. Minimal hydrolysis of epidermal growth factor by gastric fluid of preterm infants. *Gut* 30: 327–32
17. Brown KD, Blakeley DM, Fleet IR, Hamon M, Heap RB. 1986. Kinetics of transfer of 125I-labelled epidermal growth factor from blood into mammary secretions of goats. *J. Endocrinol.* 109: 325–32
18. Brown CF, Teng CT, Pentecost BT, Di Augustine RP. 1989. Epidermal growth factor precursor in mouse lactating mammary gland alveolar cells. *Mol. Endocrinol.* 3:1077–83
19. Burrin DG, Shulman RJ, Reeds PJ, Davis TA, Gravitt KR. 1992. Porcine colostrum and milk stimulate visceral organ and skeletal muscle protein synthesis in neonatal piglets. *J. Nutr.* 122: 1205–13
20. Buts J-P, De Keyser N, Kolanowski J, Van Hoof F. 1990. Hormonal regulation of the rat small intestine: responsiveness of villus and crypt cells to insulin during the suckling period and unresponsiveness after weaning. *Pediatr. Res.* 27: 161–64
21. Carpenter G, Cohen S. 1990. Epidermal growth factor. *J. Biol. Chem.* 265:7709–12
22. Carpenter G Wahl MI. 1990. The epidermal growth factor family. In *Handbook of Experimental Pharmacology*, eds. MB Sporn, AB Roberts, 95:69–171. New York: Springer-Verlag
23. Chen S-S and Li Q. 1990. Transforming growth factor-β1 (TGF-β1) is a bifunctional immune regulator for mucosal IgA responses. *Cell. Immunol.* 128:353–61
24. Ciacci C, Lind SE and Podolsky DK. 1993. Transforming growth factor β regulation of migration in wounded rat intestinal epithelial monolayers. *Gastroenterology* 105:93–101
25. Clemmons DR. 1991. Insulin-like growth factor binding proteins: roles in regulating IGF physiology. *J. Dev. Phys.* 15:105–10
26. Collier RJ, Mill MA, Hildebrandt JR, Torkelson AR, Whilte TC, et al. 1991. Factors affecting insulin-like growth factor-I concentration in bovine milk. *J. Dairy Sci.* 74:2905–11
27. Connolly JM, Rose DP. 1988. Epidermal growth factor-like proteins in breast fluid and human milk. *Life Sci.* 42:1751–56
28. Corps AN, Blakeley DM, Carr J, Rees

LH, Brown KD. 1987. Synergistic stimulation of Swiss mouse 3T3 fibroblasts by epidermal growth factor and other factors in human mammary secretions. *J. Endocrinol.* 112:151–59
29. Corps AN, Brown KD, Rees LH, Carr J, Prosser CG. 1988. The insulin-like growth factor I content in human milk increases between early and full lactation. *J. Clin. Endocrinol. Metab.* 67:25–29
30. Daniel CW and Robinson SD. 1992. Regulation of mammary growth and function by TGF-β. *Mol. Reprod. Develop.* 32:145–51
31. Davis SR, Gluckman PD, Hart IC, Henderson HV. 1987. Effects of injecting growth hormone or thyroxine on milk production and blood plasma concentration of insulin-like growth factors I and II in dairy cows. *J. Endocrinol.* 114:17–24
32. Dehoff MH, Elgin RG, Collier RJ, Clemmons DR. 1988. Both type I and II insulin-like growth factor receptor binding increase during lactogenesis in bovine mammary tissue. *Endocrinology* 122:2412–17
33. Donovan SM, Hintz RL, Wilson DM, Rosenfeld RG. 1991. Insulin-like growth factors I and II and their binding proteins in rat milk. *Pediatr. Res.* 29:50–55
34. Donovan SM, Hintz RL, Rosenfeld RG. 1991. Insulin-like growth factors I and II and their binding proteins in human milk: effect of heat treatment on IGF and IGF binding protein stability. *J. Pediatr. Gastroenterol. Nutr.* 13:242–53
35. Donovan SM, Hintz RL, Rosenfeld RG. 1992. Sources of insulin-like growth factor-I and insulin-like growth factor binding proteins in rat milk. In *Mechanisms Regulating Lactation and Infant Nutrient Utilization*, eds. MF Picciano, B Lönnerdal, pp. 377–82. New York: Wiley-Liss
36. Donovan SM, McNeil LK, Jimenez-Flores R, Odle J. 1994. Insulin-like growth factors and IGF binding proteins in porcine serum and milk throughout lactation. *Pediatr. Res.* In press
37. Edery M, Pang K, Larson L, Colosi T, Nandi S. 1985. Epidermal growth factor receptor levels in mouse mammary glands in various physiological states. *Endocrinology* 117:405–11
38. Ellis LA, Picciano MF. 1992. Milk-borne hormones: regulators of development in neonates. *Nutr. Today* 27(5): 6–14
39. Fenton SE, Sheffield LG. 1991. Lactogenic hormones increase epidermal growth factor messenger RNA content of mouse mammary glands. *Biochem. Biophys. Res. Comm.* 181:1063–69
40. Fernandez-Moreno MD, Serrano-Rios M, Prieto JC. 1987. Identification of insulin-receptors in epithelial cells from duodenum jejunum, ileum, caecum, colon and rectum in the rat. *Diabetes Metab.* 13:135–39
41. Francis GL, Read LC, Ballard GJ, Bagley CH, Upton GH, et al. 1986. Purification and partial sequence analysis of insulin-like growth factor-I from bovine colostrum. *Biochem. J.* 233:207–13
42. Gale SM, Read LC, George-Nascimento C, Wallace JC, Ballard FJ. 1989. Is dietary epidermal growth factor absorbed by premature human infants? *Biol. Neonate* 55:104–10
43. Gentz J, Persson B, Kellum M, Bengtsson G, Thorell J. 1971. Effect of feeding on intravenous glucose tolerance and insulin response in piglets during the first day of life. *Life Sci.* 10:137–44
44. Gingerich RL, Gilbert WR, Comens RG, Gavin JR. 1987. Identification and characterization of insulin receptors in basolateral membranes of dog intestinal mucosa. *Diabetes* 36:1124–29
45. Grueters A, Lakshmanan J, Tarris R, Alm J, Fisher DA. 1985. Nerve growth factor in mouse milk during early lactation: lack of dependency on submandibular salivary glands. *Pediatr. Res.* 19:934–37
46. Grosvenor CA, Picciano MF, Baumrucker CR. 1993. Hormones and growth factors in milk. *Endocr. Rev.* 16:710–28
47. Grütter R, Blum JW. 1991. Insulin and glucose in neonatal calves after peroral insulin and intravenous glucose administration. *Reprod. Nutr. Dev.* 31: 389–97
48. Harness JR, Anderson RR. 1977. Effect of relaxin and somatotropin in combination with ovarian steroids on mammary glands in rats. *Biol. Reprod.* 17: 599–603
49. Heinz-Erian P, Kessler U, Funk B, Gais P, Kiess W. 1991. Identification and in situ localization of the insulin-like growth factor-II/mannose-6-phosphate (IGF-II/M6P) receptor in the rat gastrointestinal tract: comparision with the IGF-I receptor. *Endocrinology* 129: 1769–78
50. Hirata Y, Moore GW, Bertagna C, Orth DN. 1980. Plasma concentrations of immunoreactive human epidermal growth factor (urogastrone) in man. *J. Clin. Endocrinol. Metab.* 50:440–44
51. Hirsova D, Koldovsky O. 1969. On the question of the absorption of insulin from the gastrointestinal tract during

postnatal development. *Physiol. Bohemoslov.* 18:281–84
52. Hwang JJ, Lee AB, Fields PA, Haab LM, Mojonnier LE, Sherwood OD. 1991. Monoclonal antibodies specific for rat relaxin. V. Passive immunization with monoclonal antibodies throughout the second half of pregnancy disrupts development of the mammary apparatus and, hence, lactational performance in rats. *Endocrinology* 129:3034–42
53. Hwang JJ, Sherwood OD. 1988. Monoclonal antibodies specific for rat relaxin. III. Passive immunization with monoclonal antibodies throughout the second half of pregnancy reduces cervical growth and extensibility in intact rats. *Endocrinology* 123:2486–90
54. Iacopetta BJ, Grieu F, Horisberger M, Sunahara GI. 1992. Epidermal growth factor in human and bovine milk. *Acta Paediatr.* 81:287–91
55. Ichiba H, Kusuda S, Itagane Y, Fujita K, Issiki G. 1992. Measurement of growth promoting activity in human milk using a fetal small intestinal cell line. *Biol. Neonate* 61:47–53
56. Jaeger LA, Lamar CH, Bottoms GD, Cline TR. 1987. Growth-stimulating substances in porcine milk. *Am. J. Vet. Res.* 48:1531–33
57. Jin Y, Cox DA, Kenecht R, Raschdorf S, Cerletti N. 1991. Separation, purification and sequence identification of TGF-β1 and TGF-β2 from bovine milk. *J. Prot. Chem.* 10:565–75
58. Klagsbrun M. 1978. Human milk stimulates DNA synthesis and cellular proliferation in cultured fibroblasts. *Proc. Natl. Acad. Sci. USA* 75:5057–61
59. Kohno Y, Shiraki K, Mura T. 1991. The effect of human milk on DNA synthesis of neonatal rat hepatocytes in primary culture. *Pediatr. Res.* 29:251–55
60. Koldovsky O, Bedrick A, Rao R. 1991. Role of milk-borne prostaglandins and epidermal growth factor for the suckling mammal. *J. Am. Coll. Nutr.* 10:17–23
61. Koldovsky O, Philipps A, Rao RK, Schaudies P. 1992. Possible role of milk-borne peptide growth factors for the breast-fed infant. In *Regulatory Gut Peptides in Paediatric Gastroenterology and Nutrition*, ed P Heinz-Erian, J Deutsch, G Granditsch, pp. 150–69. Basel: Karger
62. Koldovsky O, Thornburg W. 1987. Hormones in milk. *J. Pediatr. Gastroenterol. Nutr.* 6:172–96
63. Konturek SJ, Brzozowski T, Majka J, Dembinski A, Slomiany A, Slomiany BL. 1992. Transforming growth factor alpha and epidermal growth factor in protection and healing of gastric mucosal injury. *Scand. J. Gastroenterol.* 27: 649–55
64. Kuenzi MJ, Sherwood OD. 1992. Monoclonal antibodies specific for rat relaxin. VII. Passive immunization with monoclonal antibodies throughout the second half of pregnancy prevents development of normal mammary nipple mor- phology and function in rats. *Endocrinology* 131:1841–47
65. Kulski JK, Hartmann PE. 1983. Milk insulin, GH and TSH: relationship to changes in milk lactose, glucose and protein during lactogenesis in women. *Endocrinol. Exper.* 17:317–26
66. Kurokowa M, Lynch K, Podolsky DK. 1987. Effects of growth factors on an intestinal epithelial cell line: transforming growth factor beta inhibits proliferation and stimulates differentiation. *Biochem. Biophys. Res. Comm.* 142:775–82
67. Laburthe M, Rouyer-Fessarrd C, Gammeltoff S. 1988. Receptors for insulin-like growth factors I and II in rat gastrointestinal epithelium. *Am. J. Physiol.* 254:G457–62
68. Lebenthal E, Lee PC, Heitlinger LA. 1983. Impact of development of the gastrointestinal tract on infant feeding. *J. Pediatr.* 102:1–9
69. Lee YA, Bryant-Greenwood GD, Mandel M, Greenwood FC. 1992. The complementary deoxyribonucleic acid sequence of guinea pig endometrial prorelaxin. *Endocrinology* 130:1165–72
70. Lecce JGG. 1973. Effect of dietary regimen on cessation of uptake by piglet intestinal epithelium (closure) and transport to the blood. *J. Nutr.* 103:751–53
71. Lindberg T. 1979. Protease inhibitors in human milk. *Pediatr. Res.* 13:969–75
72. Lippert TH, God B, Voelter W. 1981. Immunoreactive relaxin-like substance in milk. *IRCS Med. Sci.* 9:295
73. Maier R, Schmid P, Cox D, Bilbe G, McMaster GK. 1991. Localization of transforming growth factor-β1, -β2 and -β3 gene expression in bovine mammary gland. *Mol. Cell. Endocrinol.* 82:191–98
74. Malven PV, Head HH, Collier RJ, Buonomo FC. 1987. Periparturient changes in secretion and mammary uptake of insulin and in concentrations of insulin and insulin-like growth factors in milk of dairy cows. *J. Dairy. Sci.* 70:2254–65
75. Marti U, Burwen SJ, Jones AL. 1989. Biological effects of epidermal growth with emphasis on the gastrointestinal tract and liver: an update. *Hepatology* 9:126–38
76. Ménard D, Arsenault P. 1988. Epidermal

and neural growth factors in milk: effects of epidermal growth factor on the development of the gastrointestinal tract. In *Biology of Human Milk*, ed LA Hanson, pp. 105–20. New York: Vevey/Raven
77. Ménard D, Arsenault P, Pothier P. 1988. Biologic effects of epidermal growth factor in human fetal jejunum. *Gastroenterology* 94:656–63
78. Ménard D, Malo C, Calvert R. 1981. Insulin accelerates the development of intestinal brush border hydrolytic activities of suckling mice. *Dev. Biol.* 85: 150–55
79. Ménard D, Pothier P. 1991. Radioautographic localization of epidermal growth factor receptors in human fetal gut. *Gastroenterology* 101:640–49
80. Miettinen PJ. 1993. Transforming growth factor-β and epidermal growth factor expression in human fetal gastrointestinal tract. *Pediatr. Res.* 33:481–86
81. Moran JR, Courtney ME, Orth DN, Vaughan R, Coy S, et al. 1983. Epidermal growth factor in human milk; daily production and diurnal variation during early lactation in mothers delivering at term and at premature gestation. *J. Pediatr.* 103:402–5
82. Mortimore GE, Pösö AR. 1987. Intracellular protein catabolism and its control during nutrient deprivation and supply. *Annu. Rev. Nutr.* 7:539–64
83. Mroczkowski B, Reich M. 1993. Identification of biologically active epidermal growth factor precursor in human fluids and secretions. *Endocrinology* 132:417–25
84. Murphy RA, Saide JD, Blanchard MH, Young M. 1977. Nerve growth factor in mouse serum and saliva: role of the submandibular gland. *Proc. Natl. Acad. Sci. USA* 74:2330–33
85. Nagashima K, Itoh K, Kuroume T. 1990. Levels of insulin-like growth factors I in full- and preterm human milk in comparison to levels in cow's milk and in milk formulas *Biol. Neonate* 58:343–46
86. Nexø E, Lamberg SI, Hollenberg MD. 1981. Comparison of a receptor binding assay with a radioimmunoassay for measuring human epidermal growth factor-urogastrone in urine. *Scand. J. Clin. Lab. Invest.* 41:577–82
87. Okada M, Ohmura E, Kamiya Y, Murakami H, Onoda N, et al. 1991. Transforming growth factor (TGF)-β in human milk. *Life Sci.* 48:1151–56
88. Peaker M, Taylor E, Tashima L, Redman TL, Greenwood FC, Bryant-Greenwood GD. 1989. Relaxin dectected by immuno-cytochemistry and Northern analysis in the mammary gland of the guinea pig. *Endocrinology* 125:693–98
89. Pesonen L, Vinikka L, Koskimies A, Banks AR, Nicolson M, Perheentupa J. 1987. Size heterogeneity of epidermal growth factor in human body fluids. *Life Sci.* 40:2489–94
90. Petschow BW, Carter DL, Hutton GD. 1993. Influence of orally administered epidermal growth factor on normal and damaged intestinal mucosa in rats. *J. Pediatr. Gastroenterol. Nutr.* 17:49–58
91. Phillips AF, Rao R, McCracken D, Koldovsky O. 1990. Presence of insulin-like growth factor-I (IGF-1) in rat milk and the absorption of IGF-1 by the suckling rat. *Pediat. Res.* 27:49 (Abstr.)
92. Pierce AE, Risdall PC, Shaw B. 1964. Absorption of orally administered insulin by the newly born calf. *J. Physiol.* 171:203–15
93. Plaut K. 1993. Role of epidermal growth factor and transforming growth factors in mammary development and lactation. *J. Dairy Sci.* 76:1526–38
94. Prosser CG, Davis AJ, Fleet IR, Rees LH, Heap RB. 1987. Mechanism of transfer of IGF-I into milk. *J. Endocrinol.* 131:459–66
95. Prosser CG, Fleet IR. 1992. Secretion of insulin-like growth factor II into milk. *Biochem. Biophys. Res. Commun.* 183: 1230–37
96. Raaberg L, Nexø E, Tollund L, Poulsen SS, Christensen SB, Christensen MS. 1990. Epidermal growth factor reactivity in rat milk. *Regul. Pept.* 30:149–57
97. Rall LB, Scott J, Bell GI, Crawford JR, Penschow JD, et al. 1985. Mouse prepro-epidermal growth factor synthesis by the kidney and other tissues. *Nature* 313:228–31
98. Read LC, Ford WDA, Filsell OH, McNeil J, Ballard FJ. 1986. Is orally-derived epidermal growth factor beneficial following premature birth or intestinal resection? *Endocrinol. Exp.* 20: 199–207
99. Read LC, Francis GL, Wallace JC, Ballard FJ. 1985. Growth factor concentrations and growth promoting activity in human milk following premature birth. *J. Dev. Physiol.* 7:135–45
100. Read LC, Tomas FM, Howarth GS, Martin AA, Edson KJ, et al. 1992. Insulin-like growth factor-1 and its N-terminal modified analogues induce marked gut growth in dexamethasone treated rats. *J. Endocrinol.* 133:421–31
101. Read LC, Upton FM, Francis GL, Wallace JC, Dahlenberg GW, Ballard FJ. 1984. Changes in the growth-pro-

moting activity of human milk during lactation. *Pediatr. Res.* 18:133–39
102. Rechler MM, Nissley SP. 1991. Insulin-like growth factors. In *Peptide Growth Factors and Their Receptors* eds. MB Sporn, AB Roberts, LA Hanson, 1:263–368. New York: Springer-Verlag
103. Robinson SD, Roberts AB, Daniel CW. 1993. TGFβ supresses casein synthesis in mouse mammary explants and may play a role in controlling milk levels during pregnancy. *J. Cell. Biol.* 120: 245–51
104. Robinson SD, Silberstein GB, Roberts AB, Flanders KC, Daniel CW. 1991. Regulated expression and growth inhibitory effects of transforming growth factor-β isoforms in mouse mammary gland development. *Development* 113: 867–78
105. Ross M, Francis GL, Szabo L, Wallace JC, Ballard FJ. 1989. Insulin-like growth factor (IGF) binding proteins inhibit the biological activities of IGF-I and IGF-II but not des-(1–3) IGF-I, a potent IGF analogue, on growth hormone and IGF binding protein secretion form cultured rat anterior pituitary cells. *J. Endocrinol.* 130:93–99
106. Schams D, Eispanier R. 1991. Growth hormone, IGF-I and insulin in mammary gland secretions before and after parturition and possibility of transfer into the calf. *Endocr. Regul.* 25:139–43
107. Schober DA, Simmen FA, Hadsell DL, Baumrucker CR. 1990. Perinatal expression of type I IGF receptors in porcine small intestine. *Endocrinology* 126: 1125–32
108. Shamay A, Cohen N, Niwa M, Gertler A. 1988. Effects of insulin-like growth factor I on deoxyribonucleic acid synthesis and galactopoiesis in bovine undifferentiated and lactating mammary tissue. *Endocrinology* 123:804–9
109. Shimamoto GT, Byatt JC, Jennings MC, Comen-Keller PG, Collier RJ. 1992. Destripeptide insulin-like growth factor-I in milk form bovine somatotropin-treated cows. *Pediatr. Res.* 32: 296–300
110. Shimasaki S, Ling N. 1992. Identification and molecular characterization of insulin-like growth factor binding protein (IGFBP-1, -2, -3, -4, -5 and -6). *Prog. Growth Factor Res.* 3:243–66
111. Shulman RJ. 1990. Oral insulin increases small intestinal mass and disaccharidase activity in the newborn miniature pig. *Pediatr. Res.* 28:171–75
112. Shulman RJ, Tivey DR, Sunitha I, Dudley MA, Henning SJ. 1992. Effect of oral insulin on lactase activity, mRNA, and posttranscriptional processing in the newborn pig. *J. Pediatr. Gastroenterol. Nutr.* 1:166–72
113. Siminoski K, Gonella P, Bernanke J, Owen L, Neutra M, Murphy RA. 1986. Uptake and transepithelial transport of nerve growth factor in suckling rat ileum. *J. Cell. Biol.* 103:1979–90
114. Simmen FA, Cera KR, Maha DC. 1990. Stimulation by colostrum or mature milk of gastrointestinal tissue development in newborn pigs. *J. Anim. Sci.* 68:3596–603
115. Simmen FA, Simmen RCM, Reihnart G. 1988 Maternal and neonatal somatomedin-C/insulin-like growth factor-I (IGF-I) and IGF binding proteins during early lactation in the pig. *Dev. Biol.* 130:16–20
116. Skarr TC, Vega JR, Pyke SN, Baumrucker CR. 1991. Changes in insulin-like growth factor-binding proteins in bovine mammary secretions associated with pregnancy and parturition. *J. Endocrinol.* 131:127–33
117. Suikkari A-M. 1989. Insulin-like growth factor (IGF-I) and its low molecular weight binding protein in human milk. *Eur. J. Obstet. Gynecol.* 30:19–25
118. Svendsen LS, Weström BR, Svendsen J, Ohlsson B, Ekman R, Karlsson BW. 1986. Insulin involvement in intestinal macromolecular transmission and closure in neonatal pigs. *J. Pediatr. Gastroenterol. Nutr.* 5:299–304
119. Tavakkol A, Simmen FA, Simmen RCM. 1988. Porcine insulin-like growth factor-I (pIGF-I): complementary deoxyribonucleic acid cloning and uterine expression of messenger ribonucleic acid encoding evolutionarily conserved IGF-I peptides. *Mol. Endocrinol.* 2:674–81
120. Thornburg W, Matrisian L, Magun B, Koldovsky O. 1984. Gastrointestinal absorption of epidermal growth factor in suckling rats. *Am. J. Physiol.* 246:G80–85
121. Vega JR, Gibson CA, Skaar TC, Hadsell DL, Baumrucker CR. 1991. Insulin-like growth factor (IGF) and IGF binding proteins in serum and mammary secretions during the dry period and early lactation in dairy cows. *J. Anim. Sci.* 69: 2538–44
122. Widdowson EM, Colombo VE, Artavanis CA. 1976. Changes in the organs of pigs in response to feeding for the first 24 hours after birth. II. The digestive tract. *Biol. Neonate* 23:272–81
123. Wilson ME, Gordon TP, Chikazawa K, Gust D, Tanner JM, Rudman CG. 1991.

Effects of growth hormone on neonatal growth in nursing rhesus monkeys. *J. Clin. Endocrinol. Metab.* 72:1302–7

124. Wright CE, Gaull GE. 1983. Nerve growth factor is present in human milk. *Pediatr. Res.* 17:144 (Abstr.)

125. Zijlstra RT, Odle J, Hall WF, Petschow BW, Gelberg HB, Litov R. 1994. Effect of orally administered epidermal growth factor on intestinal recovery of neonatal pigs infected with rotavirus. *J. Pediatr. Gastroenterol. Nutr.* In press