

# Intestinal Effects of Milkborne Growth Factors in Neonates of Agricultural Importance<sup>1,2</sup>

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**ABSTRACT:** Reduction of postnatal morbidity and mortality of mammalian neonates poses a significant challenge to agricultural and medical sciences. Because nutritional insufficiency and diarrhea represent major stressors, an understanding of factors mediating postnatal growth and development of the gastrointestinal tract is essential. This review explores the role that milkborne growth factors may play in stimulating functional development of the neonatal intestine, with

emphasis on the porcine, bovine, and ovine species. Studies reporting milk concentrations and intestinal effects are reviewed, with emphasis on epidermal growth factor, insulin, and the insulin-like growth factors. Collectively, these studies suggest that milkborne growth factors may provide important regulatory signals to the neonatal intestine under both normal and pathophysiological states.

Key Words: Epidermal Growth Factor, Insulin, Insulin-Like Growth Factor, Neonates, Milk, Intestine

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## Introduction

Over 30 yr of basic and applied research in mammalian developmental biology have examined many stressors that impinge upon the neonate during the early postnatal period. Despite this knowledge, high morbidity and mortality of mammalian neonates remains a significant problem facing the agricultural and medical sciences. Notable among the adaptations that accompany birth are the dramatic growth and functional development of the gastrointestinal tract. Indeed, impairment of the timely adaptation and function of this organ contributes significantly to postnatal morbidity and mortality.

Stimulatory effects of colostrum and milk on growth and development of the intestine have long been recognized, and the putative role of milkborne growth factors in mediating this development has been the subject of considerable investigation (see Koldovsky et al., 1992; Baumrucker and Blum et al., 1993; Gros-

venor et al., 1993; Donovan and Odle, 1994 for recent reviews). These growth factors include a variety of proteins and peptides capable of stimulating cell growth and(or) expression of differentiated function. Most research has been conducted by investigators interested in the welfare of human infants and has employed predominantly rodent models. However, herein we will focus on research conducted with species of agricultural importance, emphasizing the porcine, bovine, and ovine neonate. In addition, because most research has focused on epidermal growth factor (**EGF**), insulin, IGF-I, and IGF-II, they will receive primary consideration.

## Postnatal Mortality

Neonatal mortality occurs at unacceptably high levels in all mammalian species. Recent estimates from the National Center for Health Statistics indicate that the rate of mortality among low-birth-weight (< 1.5 kg) human infants is 40% (NCHS, 1992), and the United States ranks 20th in infantile mortality among developed countries. Similarly, within agricultural species, recent surveys from the U.S. Department of Agriculture have estimated the mortality of heifer calves at 8.4% of animals born alive (USDA, 1993) and the loss of piglets at 15% (USDA, 1991). Current estimates of lamb mortality in the United States are not available, but rates ranging from 10 to 44% have been reported historically (Weiner et al.,

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Causes of Prewaning Mortality in the U.S. Swine Herd (1990)

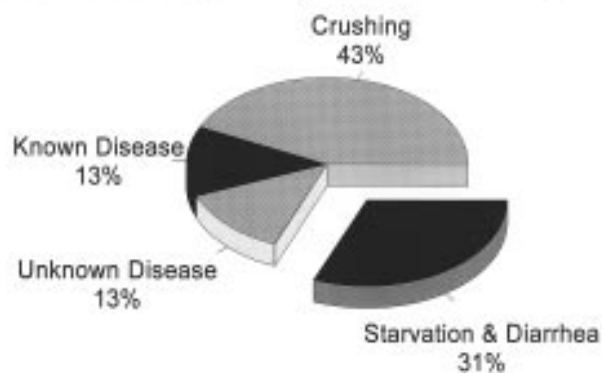


Figure 1. Among the various causes of piglet mortality, problems related to nutritional and gastrointestinal disturbance account for more than 30% of deaths. Adapted from the 1991 report of the National Animal Health Monitoring System's National Swine Survey (USDA, 1991).

1973). Unfortunately, despite continued advances in production technology, mortality rates have not improved appreciably.

In all production species, wide variation in mortality among farms (and among production cycles) stems from the underlying multifactorial etiology of neonatal mortality. Significant pig deaths are attributed to nutritional deficiency, hypothermia, low immunocompetence and disease resistance, and crushing by the dam. Retrospective surveys attempting to determine the relative importance of various stressors have characteristically identified aberrations of intestinal function as a major causative factor. In calves, mortality related to diarrhea constituted 52% of total deaths (USDA, 1993) and in piglets (USDA, 1991) starvation and diarrhea together accounted for  $\geq 30\%$  of deaths (Figure 1). Most deaths occur within the 1st wk after birth (e.g., 75% of piglet deaths). It may be more than coincidence that the intestine is the most rapidly developing tissue during this time interval.

#### *Early Postnatal Development of the Intestine*

Extensive literature regarding the gastrointestinal development of ruminant species is focused on the forestomach, but very little is available on the ontogeny of the small intestine (Roy, 1990). During the suckling period, relatively little development of the reticulorumen occurs because closure of the esophageal groove shunts milk directly to the omasum. Accordingly the small intestine represents the greatest proportion (ca. 60%) of gastrointestinal mass in suckling lambs (Lyford, 1988) and calves (Thivend et al., 1980). Using a flooding-dose of radiolabeled valine, Attaix and Arnal (1987) estimated that protein synthesis in the small intestine of 6-d-old suckling lambs represented 76% of that in the total

tract. Attaix and Meslin (1991) have further shown that epithelial cell migration rate from small intestinal crypts to villi was two- to threefold faster in suckling lambs at 1 wk than at 5 wk of age.

In the porcine neonate, Widdowson and coworkers (Widdowson and Crabb, 1976; Widdowson et al., 1976) documented the growth rates of several tissues during the first 24 h of life. Most notable was the impressive growth of the small intestine, pancreas, and kidney, the former increasing in mass by 61% in piglets that had suckled colostrum for 24 h compared with piglets fed water (Figure 2). A portion of the intestinal mass increase can be attributed to accumulation of colostrum immunoglobulin (IgG) within the mucosal cells; however, increases in intestinal length and a 40% increase in jejunal DNA content (Widdowson et al., 1976) suggested marked hyperplasia as well. The rapid growth was also associated with functional development in that total small intestinal lactase activity was increased by 52% and acid phosphatase was increased by 63% in the same 24-h period. Changes in lactase isoform abundance (Burrin et al., 1994) in newborn pigs fed milk or colostrum for 6 h compared to water-fed controls suggest alterations in the post-translational processing of lactase in response to feeding.

Several studies have compared intestinal growth and development of piglets fed colostrum vs mature milk. Radio-tracer experiments reported by Burrin et al. (1992) showed a 300% increase in jejunal protein synthesis rate in piglets fed colostrum or milk for 6 h after birth compared to water-fed controls. Their findings confirmed that intestinal protein accretion in colostrum-fed pigs was due in part to IgG uptake. They also observed a 33% increase in ileal protein synthesis rate in colostrum-fed pigs compared to those fed milk; however, this may have been due in part to the greater amounts of protein and energy in the colostrum (150% and 50% more, respectively) compared to that provided by the mature milk. In a second experiment (Burrin et al., 1995a) in which nutrient intake from colostrum and a cow-milk-based formula were comparable, colostrum accentuated skeletal muscle and jejunal protein synthesis, suggesting a role for non-nutritive component(s) in colostrum.

Others (Simmen et al., 1990a; Ulshen et al., 1991) have not demonstrated effects of colostrum beyond that observed in mature milk or milk-based formula. Simmen et al. (1990a) compared intestinal growth of newborn piglets fed equivalent amounts of dry matter from defatted colostrum or milk for 24 h. Pigs on both treatments increased intestinal mass (by 55 and 23% respectively) and [ $^3\text{H}$ ]thymidine incorporation per milligram of DNA (by 35 and 45%) compared to pigs fed a solution of 5% lactose; however, aside from increased mucosal uptake of IgG in colostrum-fed pigs, differences between colostrum-fed and milk-fed piglets were not detected. Similarly, Ulshen et al. (1991) observed equivalent small intestinal growth and

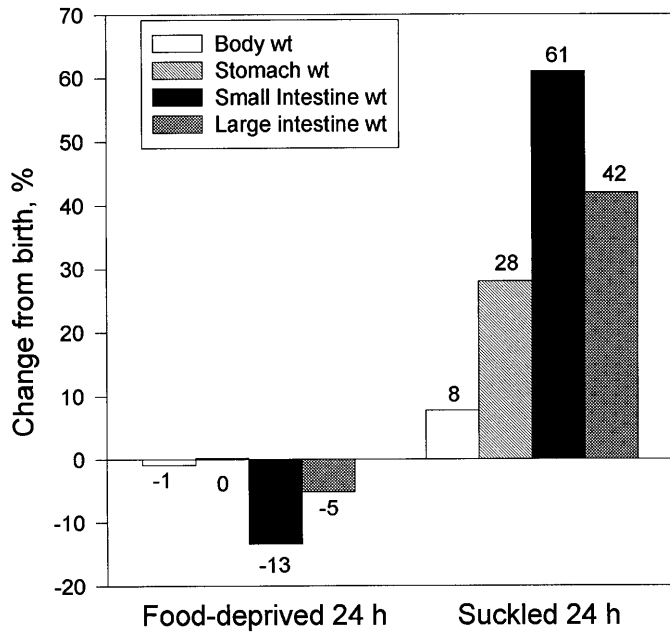


Figure 2. Gastrointestinal mass accretion in 24-h suckled piglets compared with food-deprived controls. Data are expressed as a percentage of values measured in equal-weight piglets at birth. Adapted (with permission of S. Karger AG, Basel Switzerland) from Widdowson et al., 1976).

ornithine decarboxylase activity in sow-nursed pigs compared to pigs reared artificially on a cow-milk-based formula.

Collectively, these studies illustrate the immediate and rapid nature of intestinal development during the early postnatal period. Several growth-promoting substances have been identified in milk (cf. Table 1), including EGF, insulin, IGF-I, and IGF-II. Due to their high concentrations in colostrum, and the presence of specific receptors for these factors within the intestine, these growth factors have been proposed to contribute to early postnatal gastrointestinal development (Zumkeller, 1992; Baumrucker and Blum, 1993; Donovan and Odle, 1994). However, growth-promoting effects of nutrient intake itself, mediated via endogenous insulin secretion or via the growth hormone-IGF axis, must also be considered. Regarding the latter, the reader is referred to other recent publications (Dauncey et al., 1994; Thissen et al., 1994; Straus, 1994; Burrin et al., 1995a; Jones and Clemmons, 1995). The following review examines the possible direct intestinal effects of milkborne bioactive peptides.

*Epidermal Growth Factor*

Epidermal growth factor (EGF) is a 6-kDa peptide composed of 53 amino acids (Carpenter and Wahl, 1990 for review). Pre-pro EGF contains 1,200 amino acids and is processed to form mature EGF. Peptide

homology is sufficient to allow EGF from a given species to elicit effects in other species. To induce cellular effects, EGF binds to a high-affinity cell membrane receptor, which contains an extracellular mitogen binding site and a cytoplasmic domain possessing tyrosine-kinase activity. Binding results in a second-messenger cascade that culminates in mitosis and(or) differentiation of the target cells. The EGF receptor also mediates effects of transforming growth factor alpha (TGF- $\alpha$ ) in mammalian tissues.

*Concentrations in Colostrum and Milk.* Milk EGF could be derived from either maternal circulation or from synthesis within the mammary gland itself. When the mammary gland of lactating goats was perfused with [<sup>125</sup>I]EGF, 83% of the administered dose was taken up, but only 3% appeared in milk (Brown et al., 1986). The presence of EGF mRNA in rodent mammary tissue and its modulation by lactogenic hormones (Fenton and Sheffield, 1991) suggest that local EGF synthesis may also contribute to milk EGF. When EGF was infused i.v. (500  $\mu$ g/d) into ewes for 22 d during gestation (Gow et al., 1991), the colostrum-EGF concentration was increased 10-fold (to 20  $\mu$ g/L), suggesting accumulation in the mammary gland during the prepartum period.

In most species, concentrations of EGF are highest in colostrum and decline rapidly thereafter (Table 1). Jaeger et al. (1987) reported EGF concentrations in porcine colostrum that were > 100-fold higher than those reported in other species. Whether this represents an idiosyncrasy of swine or variation attributable to assay methodology is not known. As reviewed previously (Donovan and Odle, 1994), assay methodology contributes significantly to the variation in published EGF concentrations. Indeed, using a homologous radioimmunoassay Vaughan et al. (1992) reported a much lower value (e.g., 5  $\mu$ g/L) for pig colostrum. Low levels of an EGF-related peptide were also detected and partially purified from porcine milk by Tan et al. (1990). Concentrations in bovine and ovine milk are close to those reported in humans (cf. Donovan and Odle, 1994).

*Intestinal Effects on the Neonate.* The ability of mammary secretions (especially colostrum) to stimulate the growth of cells in culture is unequivocal (Brown and Blakeley, 1983; Cera et al., 1987). However, for milkborne growth factors to elicit trophic effects on the intestine of the suckling neonate, they must survive the digestive process and arrive in sufficient concentration to bind to their intestinal receptors and thereby stimulate cell growth and(or) differentiation.

Because gastric secretion is attenuated and proteolytic digestion is incomplete in the newborn (Hartman et al., 1961), colostrum IgG and peptide growth factors can reach the small intestine intact. This is particularly true for EGF and IGF, which are acid-stable. In suckling rats, intestinal degradation of EGF was low, but increased 12-fold in weanling animals (Britton et

Table 1. Concentrations ( $\mu\text{g/L}$ ) of growth factors in milk of various agricultural species

Species	Colostrum <sup>a</sup>	Mature milk	Reference
Epidermal growth factor			
Porcine	1,500 $\pm$ 525 5 $\pm$ .9	160 to 240 NR <sup>b</sup>	Jaeger et al., 1987 Vaughan et al., 1992
Bovine	4 to 8	2	Iacopetta et al., 1992
Ovine	2 $\pm$ .3	<.8	Gow et al., 1991
IGF-I			
Porcine	39 $\pm$ 22 67 to 357	11.4 $\pm$ 1.4 7	Donovan et al., 1994 Simmen et al., 1988
Bovine	234 $\pm$ 27 226 $\pm$ 38 479 $\pm$ 13 150 to 300 <sup>c</sup> 192 to 312 NR NR	4.4 $\pm$ .9 24 $\pm$ 2 NR 35 <sup>c</sup> <5 4.3 $\pm$ .2 4.9 $\pm$ 1.4	Vega et al., 1991 Malven et al., 1987 Vacher et al., 1993 Campbell and Baumrucker, 1989 Lee et al., 1995 Zhao et al., 1994 Torkelson et al., 1988
Ovine	50 to 500	"Low"	Simmen et al., 1988
Caprine	NR NR NR	13 $\pm$ .8 11 14.5 $\pm$ 2.3	Prosser et al., 1990 Prosser et al., 1991a Prosser et al., 1991b
Equine	259 $\pm$ 10	11 $\pm$ 1	Hess-Dudan et al., 1994
IGF-II			
Porcine	82.3 $\pm$ 57.5	16.8 $\pm$ 5.6	Donovan et al., 1994
Bovine	217 $\pm$ 20 467 $\pm$ 46 113 to 187	2.9 $\pm$ 1 117 $\pm$ 10 25 to 32	Vega et al., 1991 Malven et al., 1987 Lee et al., 1995
Ovine	NR	NR	
Caprine	NR	106	Prosser et al., 1991a
Insulin			
Porcine	12.3 $\pm$ 3.3 <sup>c</sup>	1.6 to 3.3 <sup>c</sup>	Jaeger et al., 1987
Bovine	37 $\pm$ 14 21.2 $\pm$ 14.4 <sup>c</sup> 23.6 $\pm$ 1.4 6.2 <sup>c</sup> 327 $\pm$ 42	5.5 $\pm$ .6 NR NR .5 <sup>c</sup> 46 $\pm$ 19	Malven et al., 1987 Ballard et al., 1982 Vacher and Blum, 1993 Selbodzinski et al., 1986 Aranda et al., 1991
Ovine	144 $\pm$ 30	7.2 $\pm$ .5	Falconer et al., 1984
Equine	7.9 $\pm$ .6	.14 $\pm$ .01	Hess-Dudan et al., 1994

<sup>a</sup>Colostrum 1 to 4 d after birth; mature milk > 5 d after birth.

<sup>b</sup>Not reported.

<sup>c</sup>Converted to  $\mu\text{g/L}$  from the original published values using conversion factors of 7.175 pmol/mU and 5,743 g/mol of insulin, and 7,500 g/mol of IGF-I.

al., 1988). Similarly, Read et al. (1987) showed that following intragastric infusion of [<sup>125</sup>I]EGF into neonatal lambs, 70 to 90% reached the middle small intestine as immunologically intact EGF.

Receptors for EGF have been identified immunohistochemically throughout the luminal mucosa of 1- to 28-d-old piglets, from the esophagus to the ileum (Jaeger and Lamar, 1992). Receptors have also been identified in gastric parietal cells of mature pigs (Sjodin et al., 1992). Kelly et al. (1992) observed [<sup>125</sup>I]EGF binding to both apical and basolateral membranes of jejunal enterocytes from newborn and weaned pigs, suggesting that EGF may act at either luminal or serosal surfaces. Interestingly, binding was not detected in suckled pigs, suggesting that receptors were either occupied, blocked, and/or down-regulated by factor(s) present in milk.

Numerous studies have investigated the effects of EGF on intestinal growth and development (Malo and Ménard, 1982) and function (Bird et al., 1994) using rodent models (Koldovsky et al., 1992 for review). Fewer studies have been performed on species of agricultural importance. To our knowledge, with the exception of one pilot study in lambs (Gow and Moore, 1993), no data are available on ruminant species; however, several reports have examined effects in the piglet (Table 2). Subcutaneous injection of EGF (60  $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ ) into 3-d-old pigs for 3 d increased the specific activities of sucrase and maltase in the middle and distal small intestine and reduced lactase in the distal small intestine (James et al., 1987). This "maturation" of intestinal hydrolase activity may be mediated by changes in expression within older differentiated enterocytes as well as in newly formed cells in the crypts. In 21-d-old newly weaned pigs,

Table 2. Summary of studies investigating the role of growth factors in intestinal development of pigs and calves

Growth factor/ Animal model	Dose(s) used	Duration of study (days postpartum)	Reference
<b>Epidermal growth factor</b>			
Piglets	60 $\mu\text{g}\cdot\text{kg}\cdot\text{BW}^{-1}\cdot\text{d}^{-1}$ subcutaneously	3 to 6	James et al., 1987
Weanling pigs	372 $\mu\text{g}/\text{d}$	21 to 24	Jaeger et al., 1990
Piglets infected with rotavirus	500 to 1,000 $\mu\text{g}/\text{L}$ in sow milk replacer Ingested doses: 138 or 263 $\mu\text{g}\cdot\text{kg}\cdot\text{BW}^{-1}\cdot\text{d}^{-1}$	4 to 12	Zijlstra et al., 1994
<b>Insulin</b>			
Miniature piglets	85 U/L in sow milk replacer	2 to 8	Shulman, 1990; Shulman et al., 1992
<b>IGF-I</b>			
Calves	750 $\mu\text{g}/\text{L}$ in cow milk replacer	1 to 7	Baumrucker & Blum, 1994; Baumrucker et al., 1994a,b
Piglets	2,000 $\mu\text{g}/\text{L}$ in infant formula	1 to 2	Xu et al., 1994
Piglets	3,500 $\mu\text{g}\cdot\text{kg}\cdot\text{BW}^{-1}\cdot\text{d}^{-1}$ in sow milk replacer	1 to 4	Burrin et al., 1995b
Piglets	500 $\mu\text{g}/\text{L}$ in sow milk replacer Ingested dose: 200 $\mu\text{g}\cdot\text{kg}\cdot\text{BW}^{-1}\cdot\text{d}^{-1}$	1 to 14	Houle et al., 1995
<b>IGF-II</b>			
Piglets	2,000 $\mu\text{g}/\text{L}$ in infant formula	1 to 2	Xu et al., 1994

orally administered EGF (372  $\mu\text{g}/\text{d}$ ) also increased jejunal lactase (by 77%) and sucrase (by 97%) specific activities measured after 3 d of feeding (Jaeger et al., 1990). In culture, addition of EGF (500  $\mu\text{g}/\text{L}$ ) to the media of jejunal explants from neonatal pigs also increased incorporation of  $^{35}\text{S}$ -methionine into protein by twofold (Black and Ellinas, 1992).

Recently, we have examined the effects of oral EGF (0, 500, or 1,000  $\mu\text{g}/\text{L}$  of milk replacer) on the recovery of newborn piglets from rotaviral enteritis (Zijlstra et al., 1994). Supplemental EGF increased villus length and lactase specific activity (Figure 3) in a dose-response fashion when fed to virus-infected pigs for 8 d. At 500  $\mu\text{g}/\text{L}$ , effects were noted only in the proximal portions of the small intestine, whereas effects from the higher EGF level extended further down the tract. Kingsnorth et al. (1990) also reported increased tensile strength of gastric wounds in 20-kg pigs after 5 d of i.p. infusion of EGF ( $5 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ ). These results suggest that supplementation with EGF may aid in the recovery of traumatized gastric and intestinal tissues, but further studies are required to determine safe and effective upper limits. In addition, the comparative safety and efficacy of oral, as well as systemic, administration of EGF on gastrointestinal recovery need to be assessed.

#### *Insulin and Insulin-Like Growth Factors*

Insulin and the insulin-like growth factors (IGF-I and IGF-II) are members of the insulin family of peptides, which also includes relaxin. Insulin-like growth factor I and IGF-II are  $\sim 7.5$ -kDa single-chain

polypeptides that retain 70% amino acid homology with each other and a 50% homology with proinsulin (Rechler and Nissley, 1991). The IGF amino acid sequence is highly conserved across species, showing 100% identity among human, porcine, ovine, and bovine IGF-I and 96% homology between human and bovine IGF-II (Tavakkol et al., 1988).

Two cell membrane-associated IGF receptors have been identified and named the type I and type II receptors (Oh et al., 1993). The type I IGF receptor has a heterotetrameric structure, which is homologous to that of the insulin receptor. The type I receptor consists of two 135-kDa extracellular alpha-subunits, containing the ligand binding site, joined by disulfide bonding to two 90-kDa beta-subunits that contain the transmembrane domain, an ATP binding site, and a tyrosine kinase domain. The type I receptor has highest affinity for IGF-I, followed by IGF-II, and insulin. The type II receptor is a monomeric protein with an apparent  $M_r$  of 220 kDa under non-reducing conditions. The type II receptor shows highest affinity for IGF-II, then IGF-I, and does not bind insulin. Morgan et al. (1987) cloned and sequenced the cDNA and found that the type II receptor was homologous to the cation-independent mannose-6-phosphate receptor. The deduced protein has a long extracellular domain, consisting of approximately 15 repeated sequences of 147 residues each, followed by a 23 residue transmembrane domain and a 164 residue cytoplasmic domain. The cytoplasmic domain does not contain a tyrosine binding domain, nor does it contain any other recognizable signal transduction mechanism

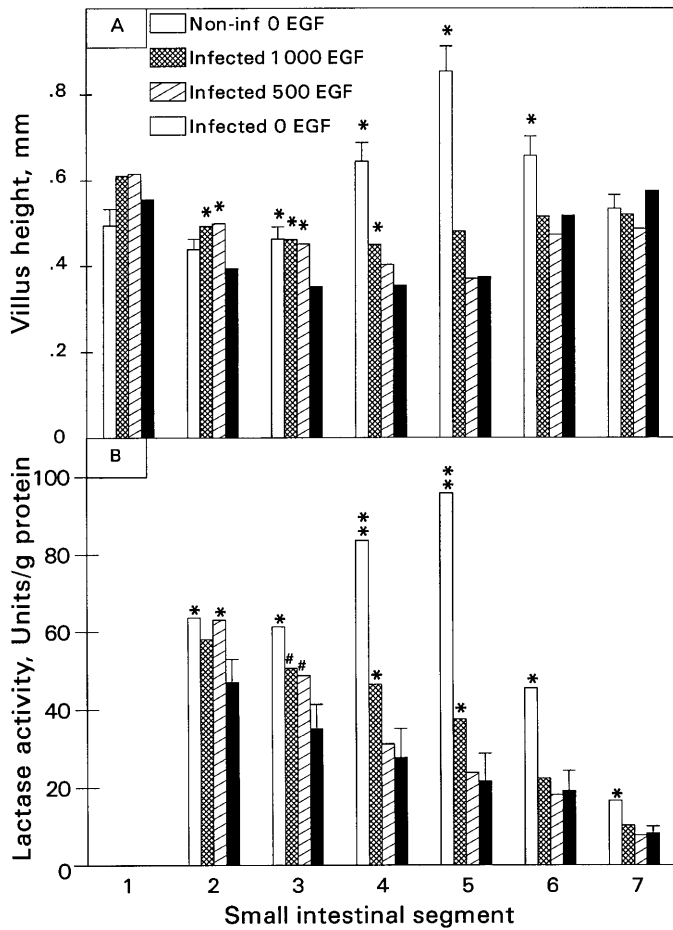


Figure 3. Effect of epidermal growth factor (EGF) fed in milk replacer at concentrations of 0, 500, and 1,000  $\mu\text{g}/\text{L}$  on small-intestinal villus height (panel A) and lactase specific activity (panel B) of piglets infected with rotavirus. Measurements were made 8 d following virus infection. Bars represent means at seven equidistant sites spanning from the proximal duodenum (segment 1) to the distal ileum (segment 7). \*Differs from infected, 0 EGF,  $P < .05$ . #Differs from infected, 0 EGF,  $P < .1$ . Reprinted (with permission) from Zijlstra et al., 1994.

(Oh et al., 1993). Recent studies have shown, however, that IGF-II binding to the type II receptor activates a calcium-permeable cation channel, perhaps through coupling to the guanine nucleotide binding protein ( $\text{Gi}_2$ ) (Nishimoto et al., 1989).

Unlike insulin and relaxin, IGF-I and IGF-II exist almost entirely (95 to 99%) in association with a family of structurally related binding proteins (IGFBP). Six genetically distinct IGFBP have been cloned and sequenced in the rat and human (Shimasaki and Ling, 1991). The amino acid sequences and gene structures of the IGFBP of production species have been less well characterized, with most work focusing on IGFBP-3 (Walton et al., 1989; Shimasaki et al., 1990; Spratt et al., 1991) and IGFBP-2 (Szabo et al., 1988; Walton et al., 1990). However, based on the porcine (McCusker et al.,

1985), bovine (McGuire et al., 1992), and ovine (Hodgkinson et al., 1989) serum IGFBP profiles and the presence of IGFBP-1 (Walton et al., 1990), IGFBP-4 (Walton et al., 1990; Shimasaki et al., 1991), and IGFBP-6 (Shimasaki et al., 1991) in porcine and ovine body fluids, the IGFBP seem to be well conserved across species.

A unique feature of IGFBP-3, the predominant serum IGFBP, is that it binds to an additional 85-kDa protein known as the acid-labile subunit (ALS) to form a 150-kDa IGF binding complex (Baxter, 1990). Unlike lower molecular weight IGFBP, such as IGFBP-1 and -2 (Bar et al., 1990), the 150-kDa complex cannot cross the vascular epithelium. Thus, the circulating half-life of IGF is extended from several minutes in the free state to 10 to 15 h when carried in the 150-kDa complex. In addition to serving as carrier proteins, IGFBP both positively and negatively affect the interaction of IGF with their target tissues (Clemmons, 1991).

**Concentrations in Colostrum and Milk.** Insulin, IGF-I, IGF-II, and relaxin have been reported in the milk of a variety of species (Table 1 and Donovan and Odle, 1994 for review). Comparing between species, the concentrations of growth factors are significantly higher in the colostrum of agricultural species (porcine, bovine) than in human or rat colostrum; however, species differences in IGF-I levels are no longer apparent in mature milk (Donovan and Odle, 1994 for review). For example, colostrum IGF-I concentrations in the human (Baxter et al., 1984) and rat (Donovan et al., 1991) are two- to threefold higher than those in mature milk, whereas bovine (Vega et al., 1991) and porcine (Simmen et al., 1988; Donovan et al., 1994) colostrum contain 10- to 500-fold higher levels of IGF-I than mature milk. Although the absolute concentrations of growth factors in colostrum vary between species, the overall profiles throughout lactation are similar among species, showing a marked decline during the transition from prepartum secretions and colostrum to mature milk (Vega et al., 1991; Donovan et al., 1994).

**Insulin.** Insulin concentrations have been measured in porcine (Jaeger et al., 1987) and bovine (Malven et al., 1987; Schams and Einspanier, 1991) colostrum and milk (Table 1). Insulin accumulates in the bovine mammary gland in late gestation, which is reflected in 3- to 10-fold higher concentrations of insulin in prepartum secretions and colostrum than in mature milk (Malven et al., 1987). By measuring arterio-venous differences, it was demonstrated that insulin was readily taken up from the maternal circulation by the lactating bovine mammary gland (3.94 U/d). Approximately 62% of the insulin taken up by the mammary gland appeared in milk in an immunoreactive form, suggesting an efficient mechanism of transport into milk (Malven et al., 1987).

**Insulin-Like Growth Factor I and Insulin-Like Growth Factor II.** Insulin-like growth factor I and IGF-II have been detected in the milk of all species

investigated (Table 1; for concentrations in other species see Donovan and Odle, 1994). In addition to native IGF-I, a truncated form of IGF-I (des[1-3]IGF-I) lacking the N-terminal Gly-Pro-Glu tripeptide was identified in bovine colostrum (Francis et al., 1986). Des-Insulin-like growth factor I binds poorly to IGFBP (Szabo et al., 1988) and has been shown to be a more potent stimulator of DNA synthesis in cultured cells than native IGF-I (Francis et al., 1988). Although des-IGF-I was reported to account for 50% of the IGF-I in colostrum (Francis et al., 1986), concentrations of des-IGF-I in mature milk were much lower (ca. 3% of total IGF-I) (Shimamoto et al., 1992). Whether or not des-IGF-I is found in the colostrum or milk of other species is unknown.

Within a species, IGF-I and IGF-II concentrations in milk are influenced by breed (Simmen et al., 1990b; Baumrucker and Blum, 1993), parity (Campbell and Baumrucker, 1989; Collier et al., 1991), and stage of lactation (Collier et al., 1991). Milk IGF-I concentration (Davis et al., 1987; Prosser et al., 1991b), but not IGF-II or des-IGF-I (Shimamoto et al., 1992), is increased by bovine somatotropin (bST) treatment. The increase in serum and milk IGF-I concentrations with bST treatment led researchers to hypothesize that IGF-I was mediating the action of bST in galactopoiesis (Davis et al., 1987; Prosser et al., 1991b). The role of IGF-I as a direct mediator of bST action within the mammary gland is not clear, however. For example, an elevation in serum IGF-I following GH administration does not guarantee an increase in milk production (Prosser et al., 1991b). In addition, it seems that the actions of IGF-I and GH on milk production differ depending on frequency of milking. If goats are milked at frequent intervals, the galactopoietic response to IGF-I is abolished (Prosser and Davis, 1992), whereas the response to bST is not (Davis et al., 1991). Finally, neither IGF-I nor IGF-II was able to reinstate lactation in rats treated with anti-somatotropin antibodies (Flint et al., 1992, 1994).

We have recently characterized the porcine serum and milk IGF and IGFBP profiles throughout lactation (Figure 4; Donovan et al., 1994). Swine colostrals IGF-II concentration exceeds that of IGF-I for the first 7 d postpartum, after which IGF-I and IGF-II levels are comparable. Interestingly, as milk IGF-II concentrations declined over the 1st wk postpartum, serum IGF-II levels progressively rose and continued to rise through d 28 postpartum. These data could be interpreted to indicate that porcine milk IGF-II was being derived from the maternal serum. This observation would be in agreement with tracer studies in which [<sup>125</sup>I]IGF-I (Prosser et al., 1987) or [<sup>125</sup>I]IGF-II (Prosser and Fleet, 1992) infused into the pudic artery of the mammary gland of lactating goats was secreted into milk. The exact mechanism(s) of transfer of IGF-I into milk remains to be determined (see

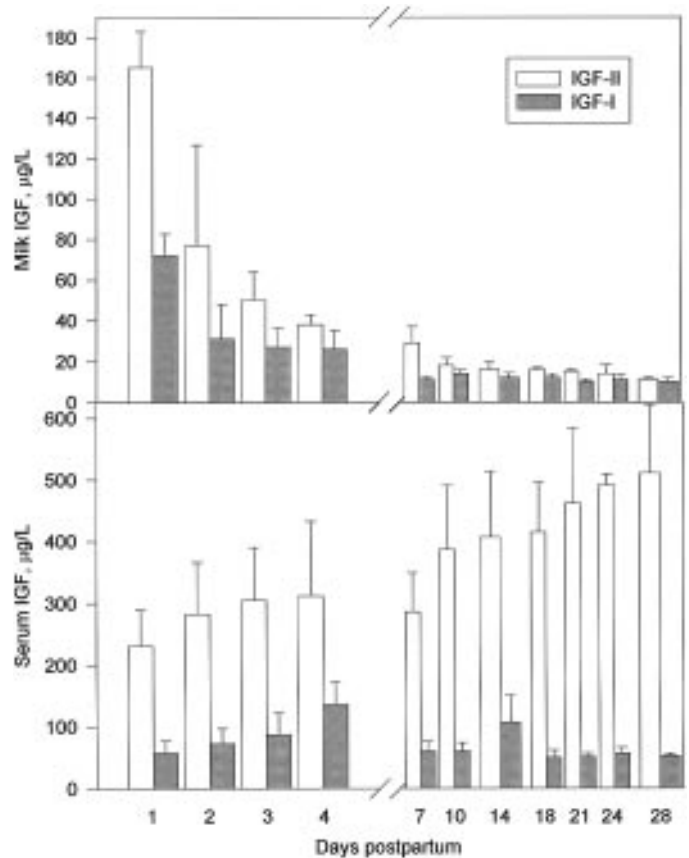


Figure 4. Lactational profiles of IGF-I and IGF-II in serum and mammary secretions of multiparous sows. Adapted (with permission) from Donovan et al., 1994.

Baumrucker and Blum, 1993 for discussion); however, the presence of type I and type II IGF receptors in mammary tissue (Dehoff et al., 1988; Hadsell et al., 1990) may function to sequester IGF from the maternal circulation. Although both IGF-I and -II seem to be derived from maternal blood, the mechanism of transport seems to differ for the two peptides. Inclusion of unlabeled IGF-I in the infusate reduced the specific activity of [<sup>125</sup>I]IGF-I in milk, suggesting that the IGF-I transport mechanism is competitive and saturable (Prosser et al., 1987). In contrast, the presence of either unlabeled IGF-II or IGF-I in the infusate did not lower the specific activity of [<sup>125</sup>I]IGF-II in milk (Prosser and Fleet, 1992). The authors concluded that both IGF-I and -II were transported transcellularly into milk; however, IGF-I was transported via a specific route, whereas IGF-II was transported nonspecifically. Four of the six known IGFBP have been detected in milk. Under native conditions, IGFBP can be separated into high (150 kDa) or low (< 50 kDa) molecular weight fractions by size exclusion chromatography. The 150-kDa peak consists of IGFBP-3 complexed with IGF-I and the 85-kDa acid-labile subunit (ALS), so named due to the fact that the 150-kDa peak can be dissociated at low pH (Baxter, 1990). The only physiological fluids

in which the 150-kDa IGFBP complex has been reported are serum and the milk (Baxter et al., 1984; Simmen et al., 1988). Using [ $^{125}$ I]IGF-I and [ $^{125}$ I]IGFBP-3 injected into lactating rats, we have recently determined that both IGF-I and IGFBP-3 are transported from maternal serum into milk (Donovan et al., 1995). By comparing the percentages of [ $^{125}$ I]IGFBP-3 in the 150-kDa peak (72% in serum vs 24% in milk) and 50-kDa peak (11% in serum vs 63% in milk), it seems that although IGFBP-3 is transported from serum into milk, the amount of ALS, present either within the mammary gland or in milk, is limiting the formation of the 150-kDa complex (Donovan et al., 1995).

The < 50-kDa IGFBP peak is heterogeneous, consisting of one or more of the other five IGFBP. Using the western ligand blotting technique (Hoslenopp et al., 1986), IGFBP-3, IGFBP-2, IGFBP-1, and IGFBP-4 have been identified in milk. The IGFBP present in milk and their relative concentrations are species-specific (Donovan and Odle, 1994 for review). How IGFBP in milk may modulate the action of IGF-I and IGF-II within the neonatal intestine is unknown. It is possible that their association with IGFBP may help protect IGF-I and IGF-II from digestion; however, the IGFBP may also inhibit their interaction with IGF receptors within the intestine.

*Intestinal Effects on the Neonate.* Insulin receptors (Fernandez-Moreno et al., 1987) and type I and type II IGF receptors (Schober et al., 1990; Young et al., 1990; Baumrucker et al., 1994b) have been detected along the entire length of the neonatal intestine. Autoradiography of rat jejunal segments incubated with [ $^{125}$ I]IGF-I demonstrated receptor binding from the submucosa to the villi; however, the density of receptors was five times higher on the proliferative crypt cells than on the cells located on the tips of the villi (Young et al., 1990). In the piglet, binding of [ $^{125}$ I]IGF-I to intestinal receptors was highest at birth, declined at 3 and 5 d postpartum, but recovered by 21 d postpartum (Schober et al., 1990). Recently, Baumrucker et al. (1994b) reported that oral administration of IGF-I to newborn dairy calves for 7 d increased IGF-I binding to intestinal microsomal membranes isolated from the calves, suggesting that intestinal IGF-I receptors are responsive to luminal IGF-I exposure.

The stability of orally administered insulin and IGF-I to digestion and their potential influence on circulating levels of these hormones has been recently addressed. Feeding of colostrum to piglets (Burrin et al., 1992) or calves (Schams and Einspanier, 1991) resulted in two- to fourfold higher serum insulin concentrations compared to milk-fed animals; however, whether this difference arose from absorption of colostrum insulin or enhanced endogenous secretion of insulin by colostrum-fed animals remains equivocal. Although early studies showed that oral administration of pharmacological levels of insulin to

the piglet (Asplund et al., 1962) or calf (Pierce et al., 1964) resulted in hypoglycemia, more recent studies in which insulin was added to milk replacer (Shulman, 1990) or given orally immediately prior to feeding colostrum (Grütter and Blum, 1991) did not effect serum insulin or glucose concentrations. Recent support for endogenous production as the major source of serum insulin came from the observation that c-peptide excretion was higher in colostrum-fed than in milk-fed piglets (Burrin et al., 1995a).

Three studies have investigated the ability of the neonatal calf (Baumrucker et al., 1992; Lee et al., 1995) and piglet (Donovan et al., 1996) to absorb orally administered [ $^{125}$ I]IGF-I. Baumrucker et al. (1992) assessed the timing and degree of IGF-I absorption in newborn calves by orally administering 160  $\mu$ Ci of [ $^{125}$ I]IGF-I within 4 h of birth and taking repeated blood samples for approximately 22 h after administration. The [ $^{125}$ I]IGF-I was added to 1 L of bovine colostrum, which contained 350  $\mu$ g/L of endogenous IGF-I and IGFBP. When serum from the neonatal calves was pooled over the first 5 h of the study and subjected to acid chromatography, approximately 12% of the pooled peak was immunoprecipitable with an antibody to IGF-I (Baumrucker et al., 1992). Lee et al. (1995) monitored plasma IGF-I and II, cortisol, and growth hormone concentrations in calves following ingestion of either colostrum (containing 192 to 312  $\mu$ g/L IGF-I), milk (containing < 5  $\mu$ g/L IGF-I), or milk replacer. Although transient postprandial fluctuations in hormone concentrations were observed, no diet effects were detected. In piglets, the degree of IGF-I absorption was assessed by fitting colostrum-deprived newborn piglets with arterial and portal catheters within 2 h of birth (Donovan et al., 1996). Piglets were orally gavaged with 25  $\mu$ Ci of [ $^{125}$ I]IGF-I (specific activity 200  $\mu$ Ci/ $\mu$ g) in milk replacer, which was devoid of immunoreactive IGF-I and IGFBP, and serial blood samples were obtained for 4 h after administration. Radioactivity in both portal and arterial blood was detectable within 15 min and was significantly higher in portal than in arterial samples until 60 min after administration. Both total radioactive counts and immunoprecipitable counts reached a plateau by 60 min and were maintained throughout the 4-h study. Approximately 5% of the radioactive counts in serum were immunoprecipitable with an antibody to IGF-I. However, when calculations were performed to determine the potential contribution of the absorbed [ $^{125}$ I]IGF-I to the serum IGF-I pool (~1,350 ng), it was determined that the absorbed [ $^{125}$ I]IGF-I was contributing less than .1% of the total pool. These results suggest that in the immediate postpartum period small quantities of orally administered insulin and IGF-I may be absorbed by the calf and piglet; however, the contribution to the circulating pools is most likely negligible.

Since 1990, several studies have investigated the effect of oral administration of insulin, IGF-I, or IGF-II on growth, serum hormone concentrations, and intestinal development of calves and piglets (Table 2). All of these studies have investigated the role of one specific growth factor by adding it to a milk replacer that is devoid of bioactive components. Although this experimental approach provides a well-controlled system to assess the effects of a single growth factor, it represents a highly simplified picture of the potential synergistic and antagonistic interactions between growth factors and IGFBP that are present in colostrum and milk. In addition, the doses of growth factors used in these studies represent the highest end of physiological levels up to clearly pharmacological doses. Also, with the exception of the 14-d study of Houle et al. (1995), all studies have been relatively short-term (1 to 7 d).

**Insulin.** Enterally and systemically administered insulin has been shown in numerous rodent studies to accelerate gastrointestinal growth and maturation (Donovan and Odle, 1994 for review). Recently, the effect of milkborne insulin was studied in neonatal miniature piglets consuming sow milk replacer with or without supplemental porcine insulin (85 U/L) (Shulman, 1990). This dose is approximately 280 times greater than the insulin concentration measured in porcine colostrum of .3 U/L (Jaeger et al., 1987). Piglets were studied for 7 d from d 2 to 8 postpartum. Formula intake, weight gain, and serum insulin, glucose, or cortisol levels were not significantly different between treatment groups. However, piglets consuming milk replacer with insulin (mean intake 16 U/d) had increased ileal mucosal weight, protein, RNA and DNA content, and lactase and maltase activities compared to piglets receiving milk replacer alone. A subsequent study was performed to determine the mechanism for the increased ileal lactase activity in piglets receiving milk replacer containing 85 U/L of insulin (Shulman et al., 1992). Although ileal lactase activity was elevated, no significant increase in lactase mRNA expression was observed. In addition, no differences in the relative proportions of the 207-, 210-, and 230-kDa precursors of the mature 160-kDa enzyme were seen. Further work is needed to determine the underlying mechanism of insulin's action on lactase activity because it does not seem to be occurring either at the level of transcription or during post-translational processing of the enzyme.

**Insulin-Like Growth Factors I and II.** Four studies have investigated the role of orally administered IGF-I or IGF-II in neonatal calves (Baumrucker and Blum, 1994; Baumrucker et al., 1994a,b) or piglets (Xu et al., 1994; Burrin et al., 1995b; Houle et al., 1995) (Table 2). In the first study, three groups of newborn dairy calves were fed 1) milk replacer, 2) milk replacer containing 750  $\mu\text{g/L}$  of recombinant human IGF-I (milk replacer+IGF-I); or 3) pooled bovine colostrum for the first four feedings, then milk

replacer for the remainder of the study (Baumrucker and Blum 1994; Baumrucker et al., 1994a,b). The pooled colostrum contained 497  $\mu\text{g/L}$  of IGF-I and 393.3  $\mu\text{g/L}$  of IGF-II. To assess gut absorptive development, D-xylose (.5 mg/kg BW) and gamma-glutamyl transferase (5,000 U) were added to the milk replacer diet at the third feeding. On d 7 postpartum, no differences in BW, small or large intestinal length (Baumrucker et al., 1994b), or serum IGFBP profiles (Skaar et al., 1994) were observed between the groups. Serum hormones were not consistently affected by any of the treatments, although several transient effects were observed (Baumrucker and Blum, 1994). For the first 8 h, serum prolactin concentrations were approximately two- to threefold higher in calves receiving the milk replacer+IGF-I compared to the other groups. Effects on serum insulin and IGF-I were not apparent until 4 d into the study, when serum IGF-I levels were elevated in the milk-replacer+IGF-I group, and serum insulin was highest in the group receiving milk replacer alone. These results are intriguing because they suggest that orally administered IGF may modulate the secretion of other hormones, potentially in an endocrine manner (Baumrucker and Blum, 1994). Receptor-binding studies with intestinal microsomal membranes demonstrated 50% higher [ $^{125}\text{I}$ ]IGF-I binding capacity in the membranes from calves fed milk replacer+IGF-I than calves fed colostrum or milk replacer alone (Baumrucker et al., 1994b). When [ $^3\text{H}$ ]thymidine incorporation into intestinal explants was assessed in vitro, incorporation per microgram of DNA was approximately twofold higher in tissue from the formula+IGF group than the other two groups (Baumrucker et al., 1994b). The ileum was more sensitive to the action of oral IGF-I for both type I receptor binding and thymidine incorporation than was the duodenum or the jejunum. The authors speculated that up-regulation of the type I receptor may be responsible for the enhanced thymidine incorporation in the intestinal explants from calves that were fed milk replacer+IGF-I. Finally, the effect of ingestion of milk replacer+IGF-I on blood protein profiles and gut absorptive capacity was assessed (Baumrucker et al., 1994a). No differences in serum total protein, albumin, or globulin concentrations were observed between the groups receiving milk replacer with or without IGF-I. No effect on gamma-glutamyl-transferase absorption was detected, indicating that the rate of gut closure was not influenced by oral IGF-I. Oral IGF-I may affect either the development or activity of intestinal sugar transporters, because pharmacokinetic analysis of D-xylose absorption showed that calves fed milk replacer absorbed 17.9% of the dose, whereas calves fed milk replacer+IGF-I only absorbed 12.5% (Baumrucker et al., 1994a).

Three studies in which IGF-I or IGF-II was orally administered to the neonatal piglet have been reported recently (Xu et al., 1994, Burrin et al.,

1995b; Houle et al., 1995) (Table 2). In the first study, newborn colostrum-deprived piglets were fed a human infant formula supplemented with either 2,000  $\mu\text{g/L}$  recombinant human IGF-I or 2,000  $\mu\text{g/L}$  recombinant human IGF-II for the first 24 h postpartum. Neither IGF-I nor IGF-II significantly affected weight gain compared to formula alone. Treatment with IGF-I or IGF-II failed to show any significant effect on the weight of the esophagus, stomach, small intestine, large intestine, mandibular glands, kidneys, or spleen. However, compared to that of piglets fed formula alone, pancreas weight was increased 17% and 12% by oral IGF-I and IGF-II, respectively. Tissue protein, DNA and RNA content of the small intestinal mucosa, stomach, pancreas, liver, and spleen were assessed and demonstrated that oral IGF-I or IGF-II increased the DNA content of the stomach and the DNA and RNA content of the pancreas compared to formula alone. Although no differences in histomorphological indices (villus height, crypt depth, muscularis thickness, etc.) were observed in response to oral IGF, cell proliferation in the small intestinal crypts was stimulated. The number of cells labeled *in vivo* with BrdU, an index of cellular mitotic activity, was increased 30% and 23% by IGF-I and IGF-II, respectively, suggesting that if the treatment had continued longer than 24 h, an effect on intestinal histomorphology may have been observed. A limitation of this study was that piglets were fed a human infant formula, which due to its relatively low nutrient density provided less energy and protein than would have been provided by a sow milk replacer and only 50% of the nutrient intake that would be provided by an equal volume of colostrum. The authors concluded that the low nutrient intake may have limited the response of animals to the IGF-I and -II.

In the final two studies, recombinant human IGF-I was added to a sow milk replacer and fed to colostrum-deprived piglets for either 4 (Burrin et al., 1995b) or 14 d postpartum (Houle et al., 1995). In the first study, IGF-I was provided at a dose of 3,500  $\mu\text{g}\cdot\text{kg}\cdot\text{BW}^{-1}\cdot\text{d}^{-1}$  (Burrin et al., 1995b), whereas in the second study IGF-I was added to sow milk replacer at a dose of 500  $\mu\text{g/L}$  (Houle et al., 1995), which is within the range measured in porcine colostrum (Simmen et al., 1988; Donovan et al., 1994). Piglets receiving the IGF-I-supplemented formula consumed approximately 200  $\mu\text{g}\cdot\text{kg}\cdot\text{BW}^{-1}\cdot\text{d}^{-1}$  (Houle et al., 1995). Consistent with previous studies, two studies (Baumrucker and Blum, 1994; Xu et al., 1994) indicated that weight gain and final BW were unaffected by oral IGF-I administration. Serum IGF-I (Burrin et al., 1995b; Houle et al., 1995), serum IGF-II (Houle et al., 1995), or serum IGF-BP profiles (Houle et al., 1995) were also unaffected by oral IGF-I intake, supporting our previous study that suggested that absorbed [ $^{125}\text{I}$ ]IGF-I made a negligible contribution to serum IGF-I (Donovan et al., 1996). At a dose of 3,500  $\mu\text{g}\cdot\text{kg}\cdot\text{BW}^{-1}\cdot\text{d}^{-1}$ , small intestinal weight

(g/kg BW), protein (mg/kg BW), and DNA (mg/kg BW) content were increased by 28%, 34%, and 53%, respectively, compared to the group receiving milk replacer alone (Burrin et al., 1995b). In contrast, no significant differences in intestinal weight (g/kg), length (cm/kg), or protein content (mg/g tissue) were observed when piglets were consuming IGF-I at a dose of 200  $\mu\text{g}\cdot\text{kg}\cdot\text{BW}^{-1}\cdot\text{d}^{-1}$ . Although this lower dose of IGF-I did not increase total intestinal weight, significant differences in villus height and digestive enzyme activity were observed between the groups. Villus height in the distal ileum was 40 to 60% greater in piglets consuming 200  $\mu\text{g}\cdot\text{kg}\cdot\text{BW}^{-1}\cdot\text{d}^{-1}$  than in control piglets. In addition, the specific activities of sucrase in the jejunum and lactase in the jejunum and proximal ileum were two- to fourfold higher in piglets receiving oral IGF-I. Despite local effects within the intestine, IGF-I did not seem to be acting systemically, as evidenced by the lack of effect on circulating levels of IGF-I (Burrin et al., 1995b; Houle et al., 1995) or tissue weights (spleen, kidney, liver, heart, lung, pancreas, stomach, and colon) (Houle et al., 1995).

Taken together, several conclusions can be drawn from these studies. First, orally administered IGF-I or IGF-II seems to survive digestion and exert local effects within the intestine. Orally administered IGF-I does not influence circulating concentrations of IGF-I (Baumrucker et al., 1994b; Burrin et al., 1995b; Houle et al., 1995; Lee et al., 1995) or IGF-II (Houle et al., 1995), even at pharmacological doses, suggesting that it is not absorbed to a significant extent. The lack of effect on overall body weight or tissue weights observed after consumption of colostrum levels of IGF-I (500  $\mu\text{g/L}$ ) for 14 d suggests that orally administered IGF-I at a high physiological dose is not a major regulator of neonatal growth (Houle et al., 1995). Although a pharmacologic dose of IGF-I or IGF-II (2,000  $\mu\text{g/L}$ ) increased pancreatic growth in the first 24 h of life (Xu et al., 1994), the physiological relevance of this finding is questionable. With respect to the intestine, results thus far suggest that the magnitude of the response is affected both by the dose administered and the duration of the exposure. Ingestion of 200  $\mu\text{g}\cdot\text{kg}\cdot\text{BW}^{-1}\cdot\text{d}^{-1}$  for 14 d increased ileal growth and sucrase and lactase activities in piglets without significantly increasing small intestinal weight, length, or protein content (Houle et al., 1995). However, ingestion of a dose of IGF-I that was 70 times higher (3,500  $\mu\text{g}\cdot\text{kg}\cdot\text{BW}^{-1}\cdot\text{d}^{-1}$ ) increased the weight and protein and DNA content of the small intestine (Burrin et al., 1995b). Ingestion of formula containing 2,000  $\mu\text{g/L}$  of IGF-I or IGF-II increased proliferative activity in the crypts (BrdU labeling); however, the study duration was too short (24 h) to detect any differences in intestinal histology (Xu et al., 1994). The important observation from this study was that IGF-I and to a lesser extent IGF-II were mitogenic in the neonatal intestine. Results of the study in calves suggest that the effects of IGF-I in the

neonatal intestine are due in part to an increase in cellular proliferation ( $^3\text{H}$ thymidine incorporation), and this effect may be mediated by an up-regulation of the type I IGF receptor in response to oral IGF-I intake (Baumrucker and Blum, 1994). The regional distribution of IGF receptors within the intestine supports this hypothesis. Insulin-like growth factor I receptors are highest in the crypt cells (Young et al., 1990), and several studies have shown enhanced proliferation in the crypts in response to oral IGF-I (Baumrucker et al., 1994b; Xu et al., 1994). In addition, IGF receptor binding is highest in the ileum (Schober et al., 1990; Young et al., 1990), and indeed the ileum was found to be most responsive region of the intestine to oral IGF-I in terms of villus growth (Houle et al., 1995),  $^3\text{H}$ thymidine incorporation, and type I receptor binding (Baumrucker et al., 1994b). Interestingly, the ileum was also most responsive to orally administered insulin (Shulman, 1990), suggesting the potential for a common mechanism of action for insulin acting through the insulin and IGF-I through the type I IGF receptor.

### Concluding Remarks

These initial studies collectively demonstrate a possible role for milkborne growth factors in the postnatal growth and development of the neonatal intestinal tract that may be important for several agricultural species. Unfortunately, the studies reported to date have been fairly descriptive, and very little is known about the underlying mechanism(s) by which the growth-factor effects are mediated. Clearly, future research should focus on the mechanism of action and should explore possible synergy (or antagonism) between different growth factors. Are effects of EGF and IGF additive? Are the mitogenic effects of IGF-I and IGF-II mediated exclusively via the type-I receptor? Does increased luminal exposure to growth factors down-regulate the corresponding intestinal receptors? Furthermore, interactions with various nutrients should be explored. How are responses to growth factors impacted by malnutrition? Increased knowledge in these areas may help in the formulation of strategies/therapies to assist the development of the mammalian neonate and facilitate successful transition to extrauterine life.

### Implications

The studies examined in this review show that growth factors (especially epidermal growth factor and insulin-like growth factor I) are present in colostrum and milk of agriculturally important species (i.e., porcine, bovine, ovine, caprine, and equine) at physiologically active concentrations. Evidence thus far suggests that the growth factors may survive

digestion, bind to intestinal receptors, and stimulate growth, maturation, and(or) functional development of the intestinal tract. In addition to playing a role in normal intestinal growth and development, future applications might involve prophylactic administration of growth factors to speed recovery from gastrointestinal trauma. Equally attractive is the future possibility of enhancing milk concentrations of bioactive peptides using transgenic methodology (Hadsell et al., 1996).

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