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THE EFFECTS OF DIET AND FEEDING ON SMALL INTESTINAL DEVELOPMENT IN PIGLETS DURING THE FIRST 24 HOURS AFTER BIRTH

A thesis presented in partial fulfilment of the requirements
for the degree of Doctor of Philosophy
in Physiology and Anatomy at
Massey University

Prapaporn Tungthanathanich

1994

Nothing in the world is perfect
In accepting with understanding,
there is peace in the heart.
No one in the world is perfect
In forgiveness with compassion,
there is peace in the heart.

THE EFFECTS OF DIET AND FEEDING ON SMALL INTESTINAL DEVELOPMENT IN PIGLETS DURING THE FIRST 24 HOURS AFTER BIRTH

VOLUME I

TEXT

(Volume II contains the Figures and Appendices)

ABSTRACT

To study the effects of feeding and diet on postnatal development of the small intestine in newborn piglets during the time 0 - 24 hours after birth, three studies were conducted:

1. Unsuckled newborn piglets were fed from a bottle with colostrum or milk from either sows or cows, infant formula, or water. After 24 hours intestinal development was compared with that in piglets at birth and others naturally suckled. Sow colostrum caused greater increases in weight and length of the small intestine than did any of the other diets. The increases were due to mucosal cell swelling caused by cellular protein accumulation, hyperplasia and, in the duodenum, hypertrophy. Feeding sow colostrum increased mucosal lactase activity. Cow colostrum caused decreases in mucosal RNA levels. Increases in the DNA content of the intestinal mucosa occurred in all groups, including the water fed group. Colostrum feeding also enhanced pancreatic growth and feeding infant formula increased liver weight.

2. The effects of enteral feeding on small intestinal development were investigated by feeding nutrient solution to unsuckled newborn piglets by orogastric tube or parenterally. Both groups after 24 hours had greater intestinal development than did the piglets at birth. The development was most pronounced in the duodenum and lower ileum. Apart from a greater small intestinal length in the orogastrically fed piglets there were no significant differences between the orogastrically and parenterally fed groups.

3. To investigate the effects of sucking *per se* on small intestinal development, groups of unsuckled piglets were fed for 24 hours with either sow colostrum or infant formula by orogastric tube or being allowed to suck from a bottle. Sucking did not affect intestinal development whereas colostrum, regardless of how it was fed, had significantly greater effects on intestinal development than did infant formula. For the colostrum fed piglets the intestinal length, tissue weight, circumference, wall thickness, villous height and width, RNA content, protein:DNA ratio and RNA:DNA ratio were all significantly greater than for those fed infant formula. In the duodenum the estimated cell migration rate was faster and mucosal cell replacement time was shorter than in

other parts of the small intestine, regardless of the diet fed. The greater villous height in the piglets fed sow colostrum was most likely due to the combined effects of cellular swelling and an increase in the number of villous cells.

These results indicate that (a) sow colostrum causes cellular swelling related to colostral protein accumulation, cell hyperplasia and, in the duodenum, hypertrophy, (b) there is a basal rate of mucosal cell division which contributes to mucosal growth regardless of diet and method of feeding, (c) the duodenum exhibits a greater growth and sensitivity to the trophic effects of colostrum compared to other parts of the small intestine, (d) feeding cow colostrum to newborn piglets causes a pronounced decrease in mucosal RNA content and (e) diets affect postnatal development of the small intestine whereas the route or method of feeding has no significant effects on small intestinal development in piglets during the first 24 hours after birth.

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LIST OF ABBREVIATIONS

Abbreviation		
B	=	at birth
BrdU	=	5-bromo-2'-deoxyuridine
BSA	=	bovine serum albumin
bw	=	body weight
^{o}C	=	degree Celcius
CC	=	cow colostrum
CCK	=	cholecystokinin
CD	=	crypt depth
CI	=	confidence interval
CM	=	cow milk
CMR	=	cell migration rate
cm	=	centimetre
$CoCl_2$	=	cobalt chloride
conc.	=	concentration
cont.	=	content
contd.	=	continued
$CuSO_4.5H_2O$	=	copper sulfate pentahydrate
CW	=	cell width
CWP	=	percentage increase in villous width
DAB	=	diaminobenzine
DNA	=	deoxy ribonucleic acid
DUO	=	duodenum
EGF	=	epidermal growth factor
Fig(s).	=	figure(s)
g	=	gramme
g	=	gravity
GIP	=	gastric inhibitory polypeptide
hr(s)	=	hour(s)
H_2O	=	water
H_2O_2	=	hydrogen peroxide
I.D.	=	inner diameter
IF	=	infant formula
Ig	=	immunoglobulin
IGF	=	insulin-like growth factor

ILE	=	ileum
JEJ	=	jejunum
kg	=	kilogramme
KH_2PO_4	=	potassium phosphate
kJ	=	kilojoule
KOH	=	potassium hydroxide
L	=	litre
LOI	=	lower ileum
LOJ	=	lower jejunum
mg	=	milligramme
min(s)	=	minute(s)
ml	=	millilitre
mm	=	millimetre
mmol	=	millimole
mol	=	mole
mOsm	=	milliosmole
MUC	=	mucosa
MUS	=	muscle
N	=	normality
NaCl	=	sodium chloride
Na_2CO_3	=	sodium carbonate
Na_2HPO_4	=	disodium phosphate
NaOH	=	sodium hydroxide
NiCl	=	nickel chloride
nm	=	nanometre
NS	=	naturally suckled
N.S.	=	no statistically significant difference
OD	=	optical density
O.D.	=	outer diameter
OGF	=	orogastric feeding
PBS	=	phosphate buffer saline
PP	=	pancreatic polypeptide
%	=	percent
RMD	=	relative migration distance
RNA	=	ribonucleic acid
RT	=	replacement time

S	=	sucking
SC	=	sow colostrum
S.D.	=	standard deviation
S.E.	=	standard error
SI	=	small intestine
SM	=	sow milk
sq.um	=	square micrometre
TGO	=	Tris-glucose-oxidase
TPN	=	total parenteral nutrition
µg	=	microgramme
UPI	=	upper ileum
UPJ	=	upper jejunum
µm	=	micrometer
µmol	=	micromole
VH	=	villous height
VHP	=	percentage increase in villous height
vs	=	versus
W/W	=	weight by weight

ANIMAL ETHICS APPROVAL

The protocols for using live animals for the experiments described in this thesis have been approved by the Massey University Animal Ethics Commitee.

CHAPTER 1
INTRODUCTION

One of the major determinants of the successful transition from fetal to extrauterine life in mammals is the ability of the individual at birth to obtain its nutrient requirements via its own gastrointestinal tract rather than via the placenta. The gastrointestinal tract must be developed sufficiently to enable the newborn to make co-ordinated swallowing movements, to transport digesta in an orderly fashion along the gut, to digest substrate and absorb nutrients through the gut wall and to excrete the waste. Thus, the state of maturation of the gastrointestinal tract largely determines whether the transition from intrauterine to extrauterine life will be successful or not (Trahair & Harding, 1987).

According to Lebenthal & Lee (1983) postnatal development of the gastrointestinal tract, like other organs, results from the interaction of 4 major determinants; namely genetic endowment, the animal's biological clock, physiological regulatory mechanisms and environmental influences. The interaction between the genetic endowment and the biological clock results in a preprogrammed pattern of development. The expression of this predetermined pattern of development is accomplished by way of the physiological regulatory mechanisms which may include endogenous hormonal, neural and paracrine reflexes. The full expression of the preprogrammed development and the endogenous regulatory mechanism requires the presence of an optimal environment which is important for normal development. Based on this scheme the environmental influences appear to be the only one determinant that we are able to easily manipulate so as to beneficially, or otherwise, alter development of the gastrointestinal tract during the postnatal period. One of the environmental factors is the influence of diet which may alter both morphological and functional development of the gastrointestinal tract, particularly during the immediate postnatal period.

The present study focuses on some of the environmental factors which potentially affect postnatal development of the small intestine in the newborn piglet during the time 0 - 24 hours after birth for several reasons. Firstly the small intestine is the major site

for the digestion and absorption of most nutrients ingested by monogastric animals. Environmental factors affecting small intestinal development will affect the supply of nutrients to the internal organs during a time when the animal as a whole experiences a period of rapid growth and development. Secondly the small intestine itself has been reported to undergo rapid growth and development during the first 24 hours after birth (Widdowson *et al.*, 1976). Factors affecting the development of the intestine during this period will be expected to markedly alter the functional and morphological characteristics of this part of the gut. Thirdly the pig is an important food animal world-wide and especially in Thailand and many other South East Asian countries. Knowledge gained about the environmental factors affecting postnatal development of the pig small intestine may have direct application to the food production industries. Fourthly the pig is physiologically similar to humans in many respects and is used widely as a model of human physiological processes (Pond & Houpt, 1978). The piglet appears to be remarkably closer to the human than any other species used in the field of experimental paediatrics (Glauser, 1966; Miller & Ullrey, 1987; Moughan *et al.*, 1992). Part of the attraction of the newborn piglet for studies of neonatal development is its convenient size and the size of the litters; the latter facilitates the conduct of paired experiments with minimum interference from variations in environmental, gestational and genetic influences (Glauser, 1966).

The experiments reported here were carried out to determine the effects of (1) different diets, (2) the route of feeding (enteral and parenteral) and (3) the method of feeding (intragastric and sucking) on the morphological and functional development of the small intestine in piglets during the first 24 hours after birth.

CHAPTER 2
LITERATURE REVIEW

The small intestine begins to develop early in fetal life and by birth is capable of digesting and absorbing nutrients from ingested colostrum and milk. It is also temporarily capable of absorbing intact macromolecules, including maternal antibodies. High levels of some enzymes are present in the newborn, notably lactase, so that lactose, the principle carbohydrate in milk, can be utilized.

In the neonatal period, particularly in the first 24 hours, the small intestine grows dramatically, at a faster rate even than the body as a whole, and the digestive capacity increases along with this physical growth. The causes of this growth and development have not been fully elucidated, but are known to be strongly influenced by the commencement of enteral feeding.

In this chapter, the morphological and functional development of the small intestine, both before and after birth, and particularly the factors affecting this development will be reviewed.

2.1 MORPHOLOGICAL DEVELOPMENT OF THE SMALL INTESTINE

The morphological development follows a common pattern during the gestation period in mammals although the onset and duration of the different phases varies between the species. According to the degree of maturity at birth, mammals can be divided into long-gestation (precocious) species, in which the newborns are relatively mature, and short-gestation (altricial) species in which the small intestine is less developed at birth. Major changes in gastrointestinal structure occur postnatally in the short-gestation species, such as the rat and mouse, whereas in the long-gestation species, such as the pig, human and sheep, most development occurs *in utero* (Weaver & Walker, 1988). Even among the precocious species the degree of small intestinal maturity at birth differs, with the human infant having more advanced development, of both structure and function, than other species (see Section 2.1.1.2).

2.1.1 Prenatal Development

The digestive tract of the mammal is derived from the gut tube which consists of the foregut, midgut and the hindgut. Except for the descending duodenum, which is formed from the foregut, all parts of the small intestine develop from the midgut (Noden & De Lahunta, 1985). The formation and maturation of the small intestine during the embryonic and fetal periods proceed through several general phases: (a) organogenesis leading to the formation of the intestinal tube by cylindrical folding of the embryonic endoderm and the associated mesenchyme; (b) general growth in length, width and mass of the intestinal tube; (c) formation of previllous ridges and reshaping into villi and crypts, coincident with a change from a stratified to a simple columnar epithelium; (d) proliferation and differentiation of cellular elements in the wall and the establishment of specific enzyme patterns (Klein & McKenzie, 1983a).

2.1.1.1 Organogenesis and Early Growth

The small intestine originates from the folding into a gut tube of the splanchnopleure, which is composed of two layers - the endoderm and a meroblastic mesenchymal layer. The epithelial lining and glandular epithelium of the intestine are derived from the endoderm, whereas the muscular, vascular, connective tissue and serosal elements arise from the mesodermal layer (Deren, 1968). Early fetal development is influenced by epithelial-mesenchymal interactions which are important for the development of previllous ridges (Sbarbati & Strackee, 1980) and villi (Mathan *et al.*, 1972; Mathan *et al.*, 1976). Xenoplastic grafting of a combination of mesenchyme and epithelium from the fetal chick and rat demonstrated that the epithelium is the determinant of biochemical differentiation, while the mesenchyme is more closely associated with the control of growth and morphological development (Kedinger *et al.*, 1981).

After formation of the gut tube, the small intestinal epithelium changes from a single layer of undifferentiated cells to a stratified epithelium (Shawdunn, 1967; Trier & Moxey, 1979), and the whole structure increases in length and weight.

2.1.1.2 Morphogenesis of Villi and Crypts

The architecture of the small intestinal wall then begins its transformation into the complex structure present in the late fetus and in postnatal life. The same developmental pattern occurs, but at different times during gestation and for different durations in the various species (Table 2.1) (Deren *et al.*, 1965; Shawdunn, 1967; Grand *et al.*, 1976; Toofanian, 1976a,b; Trier & Moxey, 1979; Trahair & Robinson, 1986a).

Table 2.1 The approximate timing of fetal small intestinal development in humans, cattle, sheep, rabbits and rats.

STAGE OF DEVELOPMENT	ESTIMATED GESTATIONAL AGE (days)				
	Human	Cattle	Sheep	Rabbit	Rat
Gestation period	270	282	148	32	22
Single cell layer lining	42 - 56	-	24 - 26	-	13
Stratified epithelium	56 - 70	-	26 - 39	-	15 - 18
Villi start to develop	49 - 56	30	39	23 - 25	18
Crypts start to develop	70 - 84	110	56	23 - 25	after birth

(Data adapted from Deren *et al.*, 1965; Shawdunn, 1967; Grand *et al.*, 1976; Toofanian, 1976a,b; Trier & Moxey, 1979; Trahair & Robinson, 1986a).

Whereas villus formation begins early in the long-gestation species (during the first trimester), in the short-gestation species it begins in the last trimester. The principal features of the sequence of development of the villi are shown in Figs. 2.1 and 2.2. In Fig 2.1, the development of previllous ridges as outgrowths of the epithelium followed by the transition to crypts and villi is shown diagrammatically. The previllous ridges are covered by a stratified epithelium which undergoes degenerative changes in the more superficial layers causing cells to slough into the intestinal lumen so that the degree of stratification decreases. Aggregates of mesenchyme invaginate into the base of the epithelium to form the cores of the villi. The formation of secondary lumina and subsequent enlargement and fusion with one another and the main lumen of the intestine (Fig. 2.2), results in the formation of short villi lined by a single layer of columnar

epithelium (Mathan *et al.*, 1976; Trier & Moxey, 1979). Finally, only the lining of the intervillous area remains stratified (Toofanian, 1976b; Moxey & Trier, 1978) (Fig. 2.1).

The pattern of mitotic activity changes simultaneously with the development of previllous ridges (Klein & McKenzie, 1983a) (Fig. 2.1). During the period leading to the formation of previllous ridges and intervillous areas, the distribution of mitotic activity in the epithelium is fairly uniform. Later, cell proliferation is restricted to the bottom of the intervillous spaces (Hermos *et al.*, 1971; Trier & Moxey, 1979).

Crypt development follows the formation of villi. In the short-gestation species, the intestinal mucosa is fully differentiated, with fully developed microvilli, shortly before birth but no crypts are present (Shawdunn, 1967; Hermos *et al.*, 1971) until after birth (Hermos *et al.*, 1971). In contrast, crypts develop early in the long-gestation species. At first, they appear as solid cords of epithelial cells with a cytoplasm darker than that in villus epithelium and extend from the base of adjacent villi into the underlying mesenchyme (Toofanian, 1976a,b; Moxey & Trier, 1978). Later, small lumina develop and the developing crypts are lined by simple columnar undifferentiated cells (Moxey & Trier, 1978). Crypt development proceeds in a proximal to distal direction in the same way as did the villi (Colony, 1983).

2.1.1.3 Cell Differentiation and Migration

In the long-gestation species, cell differentiation, which is characterized by the appearance of a wide variety of distinctive cell types, overlaps with the morphogenesis of villi and crypts (Colony, 1983). In the fetal gut, differentiated cells arise from a single precursor in accordance with the unitarian stem cell theory proposed by Cheng & Le Blond (1974). Stem cells located near the base of the crypts proliferate providing replacements for dying or lost cells in continuously renewing populations. When a stem cell divides, it forms two daughter cells - one will remain identical to the stem cell while the other cell will differentiate into a specialized enterocyte. Progeny of the stem cells differentiate into the four mature cell types - paneth cells, enteroendocrine (argentaffin) cells, goblet cells and villous columnar cells. Most of the presumptive

enterocytes and goblet and enteroendocrine cells move upward to enter the functional villous compartment. Some of these cells and all the paneth cells move downward as they differentiate (Bjerknes & Cheng, 1981).

The morphology of the enterocytes changes dramatically throughout gestation, and maturation gradients from the villous base to the villous tip are observed at all ages (Trahair *et al.*, 1986b,c). A gradual and progressive change in the structure of the epithelial cells occurs along the length of the small intestine from the duodenum to the ileum (Hardy *et al.*, 1971). Initially, the columnar cells in the proximal and distal intestine are morphologically similar but later, differentiation of the intracellular organelles results in distinct regional differences (Colony, 1983; Trahair *et al.*, 1986b, c). The cells which line the newly formed villi are characterized by short regular microvilli, large supra- and infranuclear glycogen deposits, a moderately well developed Golgi complex and the presence of lysosomal elements and vesicles in the apical cytoplasm. Several of these characteristics have functional correlates, e.g. the development of a highly organized microvillus border is associated with the appearance of many brush border enzymes.

An apical tubular system develops in the enterocytes before birth (Hardy *et al.*, 1971; Moxey & Trier, 1978, 1979). In the fetal pig, the apical tubular system is present for up to 4 weeks before birth and is still present for more than 6 days after birth (Kenworthy *et al.*, 1967) by which time normal macromolecular absorption has ceased (Hardy *et al.*, 1971). The presence of an apical tubular system partially correlates with the ability to absorb macromolecules (Hardy *et al.*, 1971) because the endocytosis of macromolecules from the intestinal lumen into the tubules and vesicles of the apical tubular system is the first step in macromolecular absorption (Clark, 1959; Kraehenbuhl & Campiche, 1969; Hardy *et al.*, 1971). In the piglet, macromolecular absorption consists first of endocytosis of the macromolecules into the cell, followed by transmission into the circulation (see Section 2.2.2). Histological studies in suckled newborn piglets have shown that the absorbed material is taken up within the apical cytoplasm as small droplets which then coalesce to form large globules and that these materials also appear in the lymphatic and blood vessels of the villus (Comline *et al.*,

1953; Payne & Marsh, 1962a,b). Unstained vacuoles in the epithelial cells have been reported in fetal piglets 2 to 3 weeks before birth and in unsuckled neonatal piglets (Comline *et al.*, 1953), whereas in fully weaned piglets there are no vacuoles (Hardy *et al.*, 1971). These observations support a connection between the presence of an apical tubular system and the ability of the cell to take in macromolecules.

Vacuoles are present in the enterocytes in the fetus and for some time after cessation of macromolecular absorption. Macromolecular absorption in the postnatal period has also been correlated with the presence of vacuoles in the enterocytes. Hardy *et al.* (1971) studied the histological structure of the small intestine in the fetal pig (73 days gestation to term) and found that cells usually contained one large unstained vacuole which was different from the stained vacuoles found in newborn piglets suckled for 24 hours. They reported vacuolation of enterocytes in the terminal ileum and that the position in the intestine at which obvious vacuolation started varied with age. It was confined to the last 10% of the small intestine at 73 days gestation and just before term, whereas at 93 and 101 days gestation it was more extensive and occupied the terminal 30%. In the 93-day and 101-day fetus, the vacuoles in the most proximal part of the vacuolated ileum were sometimes below the nucleus. This reversal of the position relative to that of the nucleus usually occurs near the apex of a villus, the cells nearer the base having vacuoles in the cell apex (Hardy *et al.*, 1971). Xu *et al.* (1992) also reported that in the newborn piglet suckled for 24 hours there is a basal location of the epithelial cell nuclei in the distal ileum, which they suggested was related to protein absorption being in its early stages.

There is a complex relationship between crypt cell proliferation, cell migration, shedding of cells at the villous tips and growth of the crypts and villi which causes researchers to assess differently the rates of cell migration and cell renewal time in the fetus (Trahair *et al.*, 1986b). The significance of cell proliferation and migration studies cannot be fully assessed unless the rate of growth of the various compartments, the cell density and morphology, and especially changes in the crypt-villus architecture are known (Trahair *et al.*, 1986b). The migration rate of enterocytes in the ovine fetus increased with age, but was slower than in the adult, and the greater increase was in the

proximal region (Trahair *et al.*, 1986b). In the 136 day-old-fetus, the migration rates were 0.23 and 0.76 mm/day for the proximal and distal intestine whereas the rates of villous height increase were 0.007 and 0.012 mm/day, respectively, therefore the migration rates were between 3 - 16 times greater than the villous growth rate and the estimated cell renewal time ranged from 8 - 20 days (Trahair *et al.*, 1986b). In contrast, Smeaton & Simpson-Morgan (1985) suggested that little or no renewal of the epithelium occurred in the fetal sheep intestine. Trahair *et al.* (1986b) could not offer a reason for this discrepancy. There appeared to be no difference in the techniques used in the two studies, although the animals Trahair studied were cross-breeds (Corriedale-Merino) whereas those in Smeaton & Simpson-Morgan's work were pure bred (Merino), which is unlikely to be of significance. Whether or not the villous cells are replaced in the fetus, or whether only villous elongation occurs, these studies are in agreement that the cell proliferation rate and renewal time in the fetus are slower than those in the newborn lamb.

Finally, the fetal small intestine exhibits two gradients of maturation. First, the enterocytes at the tip of the villi are more mature than those at the base (Moxey & Trier, 1979; Trahair & Robinson, 1986a). Secondly, the proximal region of the small intestine matures earlier than the distal region (Deren, 1968; Toofanian, 1976b; Moxey & Trier, 1979; Trahair & Robinson, 1986a; Trahair *et al.*, 1986a).

2.1.1.4 Factors Affecting Prenatal Development

Prenatal development of the small intestine proceeds along a predestined path, but requires the normal expression of hormones in the fetus. Support for the importance of intrinsically controlled development comes from experiments such as the transplantation of fetal small intestinal tissue into the kidney capsule of adult rats and mice; although the normal biochemical and structural development occurred, cell proliferation was reduced (Ferguson & Parrott, 1972; Ferguson *et al.*, 1973; Jolma *et al.*, 1980; Montgomery *et al.*, 1981). The specific triggers for small intestinal growth and development and how their effects are mediated are not clear. Intrinsic changes in the levels of both fetal systemic and gastrointestinal hormones will occur spontaneously.

The stimulatory effect of fetal sucking and swallowing of amniotic fluid may also regulate small intestinal growth since there are substances present in amniotic fluid which have been implicated in stimulating gut growth (Klein & McKenzie, 1983b).

It appears that either hypophyseal hormones or hormones secreted by tissues under hypophyseal control, particularly the adrenal cortex, have an influence on cell proliferation in the fetal intestine (Klein & McKenzie, 1983b). The development of previllous ridges and villi and the height of individual epithelial cells are severely decreased (Hinni & Watterson, 1963) and the developmental change in the pattern of mitotic activity are arrested (Bellware & Betz, 1970) in the chick embryo by hypophysectomy. Adrenocortical secretions have been implicated as regulators of developmental changes in the fetus, both *in vivo* and *in vitro* (Moog & Richardson, 1955; Moog & Ortiz, 1960; Hayes, 1965a,b). Cortisone accelerates villus maturation (Hayes, 1965a) and induces enzyme activity (Hayes, 1965b) in the embryonic chick duodenum *in vitro*.

Trahair and his colleagues have performed a number of studies in fetal sheep involving bilateral adrenalectomy, hypophysectomy or administration of exogenous cortisol which support the importance of cortisol in the development of the small intestine (for review: see Trahair & Harding, 1987). Bilateral adrenalectomy reduced small intestinal mucosal growth, decreased the migration rate and increased the estimated renewal time of enterocytes, but had no effect on enterocyte morphology, cellular differentiation or maturation (Trahair *et al.*, 1987a). Exogenous cortisol increased the migration rate and decreased the renewal time of enterocytes in the fetal sheep, but there was no alteration to enterocyte morphology (Trahair *et al.*, 1987b). Trahair *et al.* (1986b) observed increases in cell proliferation and cell migration rate in fetal sheep a few days before birth, in contrast to the reported decline in mitotic activity of intestinal enterocytes a few days before birth in fetal mice (O'Connor, 1966) and rats (Mathan *et al.*, 1972). Trahair *et al.* (1987b) suggested that the increases in cell proliferation and migration rate in the fetal sheep were related to the prepartum cortisol surge of the fetus (Bassett & Thornburn, 1969; Nathanielsz *et al.*, 1972). The prepartum cortisol surge has also been reported in the fetal human (Murphy *et al.*, 1975), cattle

(Comline *et al.*, 1974), goat (Currie & Thornburn, 1977) and pig (Randall, 1983), possibly in response to stress *in utero* but due mainly to the mechanism triggering labour. Whether accelerated small intestinal growth is related to the prepartum surge in cortisol in the fetus of other species in the same way as in the fetal sheep is still unknown, since excluding the sheep, there have been no studies in long-gestation species which correlate the prepartum cortisol surge with the prenatal growth of the small intestine.

Endogenous intestinal hormones secreted by enteroendocrine cells have been suggested to play a role in the control of proliferative activity and morphological development in the fetal intestine as these cells appear immediately preceding previllous ridge formation and attendant changes in cell proliferation (Monesi, 1960; Larsson *et al.*, 1977; Calvert, 1978; Larsson & Jorgensen, 1978) and their distribution alters during gestation (Larsson, 1980; Buchan *et al.*, 1981). This raises the possibility that gut hormones may act as a local inducing agent in the fetus (Aynsley-Green, 1983).

Amniotic fluid contains a number of hormones, growth factors and substances, including cortisol (Carson *et al.*, 1979), epidermal growth factor (EGF) (Barka *et al.*, 1978) and insulin-like growth factors (somatomedin) (Bala *et al.*, 1978) which may affect development *in utero*. Cortisol and EGF in particular may be involved in small intestinal differentiation (Klein & McKenzie, 1983b). Simultaneous administration of EGF into the amniotic fluid and the fetal abdominal cavity of monkeys to expose the fetal gut to EGF from both the luminal (via swallowed amniotic fluid) and systemic sides resulted in a marked increase in the wet weight, DNA, protein content and enzyme activities of the small intestine (Read *et al.*, 1989). A number of studies have also demonstrated the trophic effects of amniotic fluid on development of small intestinal tissue *in vitro* (Calvert, 1981; Calvert *et al.*, 1983).

By the end of gestation, the small intestine has developed the structures and enzymes which enable the newborn to obtain nutrients from colostrum and milk during the suckling period. Further growth and maturation of the intestine is required to allow the young animal to be weaned on to the solid diet of the adults of the species.

2.1.2 Postnatal Development

During the first 24 hours after birth the intestine undergoes rapid increases in both length and weight in newborn suckled piglets (Widdowson *et al.*, 1976; Widdowson, 1984), rabbits (Hall & Widdowson, 1979), rats (Berseth *et al.*, 1983; Berseth, 1987a; Simmen *et al.*, 1990a) and dogs (Heird *et al.*, 1984). Growth of the small intestine in the early postnatal period results in increased villous height and crypt depth and increased numbers of villi per cross-sectional area, cells per crypt, cells per villus and crypts per villus (O'Connor, 1966; Clarke, 1972; Yeh, 1977; Smith & Jarvis, 1978; Smith & Peacock, 1980). Since the degree of maturity of the digestive tract is very different between the short- and long-gestation species, small intestinal development in the immediate postnatal period is different in the two groups.

In the short-gestation species, such as the rat, the small intestine is very immature at birth, with dividing cells being present along the entire length of the villus and no shedding of cells at the tip (Hermos *et al.*, 1971). Crypts develop at the extreme base of the villi shortly after birth (Hermos *et al.*, 1971). Restriction of cell proliferation to the crypts occurs during the seventh day after birth (Koldovsky *et al.*, 1966) but adult patterns of cell proliferation and differentiation are not established until 21 days after birth (Hermos *et al.*, 1971). Small intestinal growth in the neonatal rat results in an increase in the villous height, crypt depth and crypt:villus ratio in the small intestine (Yeh, 1977). During the suckling period, cell production contributes partially to cell renewal, but mostly to growth within the crypt (Altman & Enesco, 1967). The rate of cellular proliferation and division proceeds much more slowly in the suckled than in the weaned animal (Koldovsky *et al.*, 1966; Klein, 1977). Cellular migration, as measured by the transit of labelled cells from the crypt to the villus, is fourfold greater in 28-day-old or adult rats compared with suckled rats (Koldovsky *et al.*, 1966). The rapid increase in cell proliferation at weaning (during the third to the fourth week after birth) is associated with adaptation of the rat to an adult feeding regimen, and correlates with the replacement of immature vacuolated ileal enterocytes by new adult-like cells and cessation of macromolecular absorption (Klein & McKenzie, 1983a).

Even among the long-gestation species, the pattern of postnatal development of the small intestine differs from species to species. The guinea pig, horse and human neonate have comparatively mature intestines at birth while those of the lamb and piglet are relatively less developed (Weaver, 1986).

2.1.2.1 Growth of the Small Intestine in Newborn Piglets

The small intestine of the piglet grows very rapidly during the first 24 hours after birth (Widdowson *et al.*, 1976; Widdowson, 1984; Xu *et al.*, 1992). The duodenum gains 42% of its weight at birth on the first day and five times its weight at birth by the tenth day. The growth pattern of the jejunum and ileum are similar, both gaining four times their weight at birth by the tenth day. The mucosa grows particularly rapidly in the first 24 hours when the birth weight is doubled in both the jejunum and ileum. Both the jejunal and ileal length also grow rapidly during the first 24 hours, increasing by 27% and 24% their length at birth respectively (Widdowson *et al.*, 1976; Widdowson, 1984). Xu *et al.* (1992) reported a 78% increase in small intestinal weight, a 24% increase in length and a 15% increase in diameter during the first day after birth. Smith & Jarvis (1978) also reported the rapid growth of the small intestine in piglets including an 80% increase in length, a 30% increase in diameter, and a doubling of surface area between birth and 10 days. All parts of the small intestine grow proportionately much more rapidly than the body as a whole, especially during the first 24 hours, the growth of the jejunum and ileum being particularly remarkable.

2.1.2.2 Growth of the Small Intestinal Mucosa in Newborn Piglets

The increase in weight was more pronounced in the mucosa than in the muscle (Widdowson *et al.*, 1976). In the first 24 hours, there were reported increases in weight of the mucosa of 73% in the duodenum, 104% in the jejunum and 124% in the ileum (Xu *et al.*, 1992). This is reflected in the increased amount of DNA and protein in the whole jejunal mucosa, although the increase in DNA is proportionally less than that in the protein (Widdowson, 1984). Increases in total mucosal DNA content of the small intestine are about 55% and 116% by the first and the third day after birth (Xu *et al.*,

1992).

The marked increase in the weight of the mucosa in the first day after birth is caused by both tissue growth and by accumulation of colostral protein in the enterocytes. The contribution of the latter process has recently been emphasized by Burrin *et al.* (1992) and Xu *et al.* (1992). Tissue growth is very marked in the duodenum and less in more distal parts of the small intestine. This is supported by the 96% increase in mucosal DNA content in the duodenum but a progressively declining increase along the tract to only 30% in the ileum (Xu *et al.*, 1992). On the other hand, protein accumulation is greater in the distal small intestine which is the active segment for macromolecular absorption (Pierce & Smith, 1967; Clarke & Hardy, 1971). The mucosal protein:DNA ratios in the jejunum and ileum increase markedly during the first day and return to the birth values by the third day, whereas in the duodenum the mucosal protein:DNA ratio did not change from birth to either days 1 or 3 (Xu *et al.*, 1992). These findings are consistent with the greater increase in mucosal protein than DNA in the jejunum reported by Widdowson (1984). Burrin *et al.* (1992) concluded that the major contribution to the protein accretion in the jejunum and ileum in newborn piglets fed sow colostrum is the absorption and retention of ingested immunoglobulins rather than synthesis of protein by the cells. Xu *et al.* (1992) suggested that the mucosal cell swelling in the distal small intestine of newborn suckled piglets is related to colostral protein retention.

The enterocytes in the intestinal mucosa of the fetal pig contain empty vacuoles which increase in number and become full of protein shortly after birth (Hardy *et al.*, 1971). About 70 - 80 % of all villous cells in suckled, newborn piglets become protein vacuolated within 2 hours after birth. Vacuoles disappear from the cells in the proximal small intestine within 48 hours, while in the middle and distal parts they generally decrease between 48 - 72 hours and disappear by 4 days after birth (Smith & Jarvis, 1978).

Elongation of the villi occurs during the neonatal period, although the two early studies in newborn piglets produced very different data. On the one hand, Smith &

Jarvis (1978) observed increases in villous height, of 29%, 50% and 75% in the proximal, middle and distal portions of the small intestine respectively, during the first ten days after birth. In the newborn piglet, most villi were of similar length, although some were shorter, but by 9 days there were two populations of villi, one very long and one very short. During the first 7 - 10 days, mitosis was confined to the crypts, newly formed cells migrated on to the villi and there was virtually no villous cell extrusion, resulting in an increase in villous height. The crypt depth remained unchanged and the cell migration rate appeared to be constant. There was no noticeable difference in the speed of migration between different regions of the small intestine and the estimated time taken for complete cell replacement in the piglet was approximately 19 days (Smith & Jarvis, 1978). These findings appear to be in conflict with the data of Moon (1971) who reported a decrease in crypt depth of about 50% during the first 3 weeks after birth in the pig and also a decrease in villous height in the proximal jejunum. In addition, the epithelial cell migration rate at 1 day of age gave a calculated cell replacement time of 9 - 10 days in the proximal regions and 7 - 10 days in the more distal portions, whereas these were shortened to 2 - 4 days at 3 weeks of age. However, a study in the rat reporting increases in villous height in the duodenum, jejunum and ileum during 6 - 22 days after birth (Yeh, 1977) supports the findings of Smith & Jarvis (1978).

The reason for these conflicting results in the crypt depth and villous height between the two studies may be due to different changes occurring in the proximal and distal small intestine and to the ages at which the piglets were studied. Xu *et al.* (1992) reported increases in villous height in the duodenum of 53% at 1 day and 83% by 3 days of age, whereas in the rest of the small intestine the villous height increased by 33 - 90% during the first day and then declined to 11 - 46% in the following two days. The crypt depth generally showed a progressive increase with age during the first 3 days (Xu *et al.*, 1992). These measurements of villous height are in accord with those reported by Smith & Jarvis but crypt depth changes were different from those described by both Smith & Jarvis and Moon. Klein & McKenzie (1983a) suggested the discrepancy between the latter two studies may be the result of the pigs used by Smith & Jarvis being free of intestinal microorganism infection while those used by Moon were not, and that the infection might result in villous atrophy and decreased crypt cell

production. This explanation seems to be partly incorrect as infection usually results in increased crypt cell production in response to the loss of villous cells (Hughes, 1984). Smith & Jarvis's work seems to be reliable as it is partly supported by the report of Xu *et al.* (1992).

Another possible reason for the discrepancy is the way villous height is measured since there are two populations of villi (the short and long villi) in the small intestine of newborn piglets from 9 days of age (Smith & Jarvis, 1978). In addition the plane of cutting the tissue samples affects the measurement since different planes can cause differences in villous height (Smith & Jarvis, 1978).

The discrepancies in the cell replacement time between the two studies could be due to the difference in age of the oldest animals studied (10 and 21 days). As well, it can be difficult to determine the cell replacement rate using the leading labelled cell as an indicator since there are indications of cell mixing on the villi rather than linear cell replacement. Smith & Jarvis (1978) offer two hypotheses for this: (a) unequal contributions of cells by several crypts supplying cells to a single villus, leading to an asymmetric removal of fetal-type enterocytes or (b) vacuolated and nonvacuolated cells providing varied resistance to cells emerging from the crypts, resulting in different migration routes and incomplete removal of fetal-type cells. Since new crypts and villi are also forming postnatally, crypt cells must occasionally move laterally instead of into the villous compartment (Smith & Jarvis, 1978). Therefore, the absolute renewal time in the newborn piglet might be incorrectly estimated if it were determined on the assumption that the leading labelled cells indicate the degree of total cellular renewal (Smith & Jarvis, 1978).

During the first 24 hours after birth, the small intestine of the piglet grows at a very rapid rate compared with the rate at 10 days after birth. Part of the mucosal weight increase during the first 24 hours is caused by colostral protein absorption. Crypt cell production results in migration of cells on to the villi and an increase in villous height as there is probably little cell extrusion during the first week after birth.

2.2 FUNCTIONAL DEVELOPMENT OF THE SMALL INTESTINE

Functional development of the small intestine, which occurs simultaneously with morphological development, includes changes in enzymatic activities which relate to the dietary components of each species (Corring *et al.*, 1982; Kretchmer, 1985; Henning, 1986) and cessation of the macromolecular absorption which occurs only for a short period after birth (Patt, 1977).

2.2.1 Enzyme Development

The diet of newborn animals gradually changes between birth and weaning from milk to solid food, which results in a decrease in dietary fat, no appreciable change in dietary protein but a remarkable change in both quality and quantity of dietary carbohydrates (Kretchmer, 1985). There are correspondingly marked alterations in lactase, sucrase and maltase activities in the small intestine during this period (Kretchmer, 1985) whereas peptidase is generally very low during the whole suckling period (Corring *et al.*, 1982).

2.2.1.1 Lactase

As lactose is the major carbohydrate in the milk of most mammals (Pierse *et al.*, 1988), high lactase activity is found in the intestinal mucosa of suckled animals (Deren, 1968; Kretchmer, 1971). In most species, including the cat, rabbit and guinea pig, lactase levels increase markedly in the last few days of gestation, remain high during the suckling period, then undergo a postweaning decline to adult levels (Henning & Kretchmer, 1973). In the rat, lactase is demonstrable on day 18 of gestation, reaches its maximum during the first week after birth, then declines to reach adult levels by the end of the fourth week (Doell & Kretchmer, 1962).

In the pig, the earliest reported gestational age at which lactase is present is at 30 days, and in 30, 51, 72 and 93 day-old fetuses lactase activity was significantly lower than at birth (Spraque *et al.*, 1963). After birth, lactase activity increases to its

maximum at 10 - 20 days and declines rapidly to minimal levels at 4 - 5 weeks (Bailey *et al.*, 1956; Hartman *et al.*, 1961; Manners & Stevens, 1972). Aumaitre & Corring (1978) reported the peak lactase activity to be at 1 week after birth, with a large variation between individual animals. The time of maximum lactase activity is known to be different in some breeds of pigs (Ekstrom *et al.*, 1975) but to differ little with diet (Manners & Stevens, 1972).

Greater lactase activity is found in the cranial and middle sections of the jejuno-ileum in the fetal pig (Spraque *et al.*, 1963). In newborn piglets, the peak lactase activity occurred in the proximal one-third of the small intestine, i.e. jejunum (Manners & Stevens, 1972; Ekstrom *et al.*, 1975; Aumaitre & Corring, 1978; Shulman *et al.*, 1988), and the lowest activity occurred in the ileum (Shulman *et al.*, 1988). Lactase activity declines significantly with postnatal age in all regions of the small intestine (Shulman *et al.*, 1988).

2.2.1.2 Sucrase and Maltase

Sucrase and maltase, intestinal hydrolases involved in the digestion of carbohydrate components of solid food, are generally absent or low at birth and gradually increase during the suckling period (Corring *et al.*, 1982; Henning, 1986). In the newborn piglet, sucrase and maltase activities rise steadily from negligible levels at birth to significant levels at 1 to 2 weeks and undergo further changes with age before weaning (Bailey *et al.*, 1956). Both enzymes are present in all segments of the small intestine at 1 week of age (Manners & Stevens, 1972; Shulman *et al.*, 1988). Sucrase activity is highest in the jejunum and lowest in the duodenum, whereas maltase activity is highest in the jejunum, but more evenly distributed along the small intestine than is that of sucrase (Shulman *et al.*, 1988). Sucrase and maltase gradually increase to adult levels by 7-8 weeks of age (Bailey *et al.*, 1956; Walker, 1959; Hartman *et al.*, 1961; Manners & Stevens, 1972; Aumaitre & Corring, 1978; Shulman *et al.*, 1988). The reported rate of maturation of intestinal enzymes varies considerably, probably due to the wide variety of strains of pigs used by different researchers (Shulman *et al.*, 1988).

In humans, the levels of sucrase and maltase in the young fetus are comparable to those in the mature intestine at 2 - 11 months of age (Dahlqvist & Lindberg, 1965; Antonowicz *et al.*, 1974; Grand *et al.*, 1976), although there is a marked increase just before birth (Antonowicz *et al.*, 1974; Grand *et al.*, 1976). In the rat, there are very different patterns of development of sucrase and maltase activities which begin to rise only in the suckling period (Grand *et al.*, 1976). Maltase activity is low during the first 2 postnatal weeks, then undergoes a 5- to 10-fold increase during the next two weeks (Rubino *et al.*, 1964; Reddy & Wostmann, 1966), whereas sucrase activity is undetectable in the intestine during the first and second postnatal weeks, appears on approximately day 16 and rises rapidly and reaches adult levels by the end of the fourth week (Doell & Kretchmer, 1964; Rubino *et al.*, 1964, Reddy & Wostmann, 1966).

2.2.1.3 Peptidase

The proteolytic activity in the small intestine of newborn piglets is low at birth then increases during the first two postnatal months (Hartman *et al.*, 1961). Histological studies on pigs have shown that the development of intestinal peptidase activity coincides with the development of brush border structures in the small intestinal mucosa (Lindberg & Karlsson, 1970). Similar results have been obtained using fetal rats (Lindberg & Owman, 1966) and human fetuses (Lindberg, 1966).

2.2.2 Macromolecular Absorption

The piglet acquires passive immunity from maternal plasma principally by intestinal absorption of antibodies from colostrum (Brambell, 1970; Patt, 1977). The neonatal small intestinal cells are capable of ingesting macromolecules by an endocytotic mechanism. When a macromolecule comes in contact with the cell membrane, invagination occurs and small vesicles are formed. The process of uptake is energy-dependent, since invagination can be inhibited by inhibitors of both glycolysis and oxidative phosphorylation. Energy is presumably required for the resynthesis of cell membrane to replace that utilized by invagination (Walker, 1981). The transmission of protein from colostrum into the blood of the neonatal pig is not an entirely non-selective

process, as proteins with different molecular weights and structure are absorbed at different rates and in different amounts (Carlsson *et al.*, 1980). There is a correlation between the molecular weight of the fed proteins and the time at which the maximum concentration is reached in the blood of the newborn piglet (Carlsson *et al.*, 1980).

The capacity of the newborn piglet to absorb intact antibodies from the gut contents is transitory; if colostrum is not fed within a specific period of time after birth (approximately 24 - 36 hours), then antibodies will be digested instead of being transmitted and only their degradation products will reach the circulation (Brambell, 1970). During the period before closure, the γ-globulin levels in the serum of the neonate increase from virtually undetectable amounts to adult levels, and the concomitant marked proteinuria supports the occurrence of enhanced macromolecular transport (Walker, 1981) since the proteinuria is transient and rapidly declines coincidently with the cessation of macromolecular absorption (Loh *et al.*, 1972). The low peptic activity in the stomach (Hartman *et al.*, 1961; Corring *et al.*, 1982) and the buffering action of the colostrum protect the macromolecular proteins from gastric digestion (Morris, 1968).

Sow colostrum enhanced intestinal transmission of macromolecules during the preclosure period more than did equal amounts of bovine colostrum, while commercial milk replacer had no apparent effect (Westrom *et al.*, 1985). Sow colostrum trypsin inhibitor reduces intestinal proteolytic activity and leads to an increased intestinal protein content and availability of the protein for transmission (Carlsson *et al.*, 1980; Westrom *et al.*, 1985). The immunoglobulin G (IgG) and immunoglobulin A (IgA) levels in newborn suckled piglets are influenced not only by the immunoglobulin concentrations in maternal colostrum but also by the concentration of sow colostrum trypsin inhibitor (Jensen & Pedersen, 1979). The high protein content of colostrum enhances macromolecular transmission in the piglet (Burton & Smith, 1977; Westrom *et al.*, 1985) by merely acting as a protein solution that non-specifically stimulates endocytosis by the intestinal epithelial cells (Burton & Smith, 1977). However, Westrom *et al.* (1985) suggested that the protein content *per se* cannot be the only factor in colostrum that enhances transmission since feeding model proteins to represent the macromolecular

protein content of colostrum is not as effective as colostrum in promoting transfer, and that colostrum, in addition to the high protein content and sow colostrum trypsin inhibitor, contains other factors that affect the efficiency of transmission.

2.2.3 Cessation of Macromolecular Absorption (Closure)

There are considerable differences between species in the duration of post-partum intestinal permeability to antibodies (Patt, 1977). In the pig, ruminant, cat and dog, the cessation of intestinal absorption of antibodies, termed 'closure' (Lecce & Morgan, 1962), occurs at about 24 - 36 hours after birth, while in the mouse, rat and rabbit the process is delayed and occurs at about 16 - 17, 16 - 22 and 23 - 24 days of age respectively (Clarke & Hardy, 1969; Brambell, 1970; Rundell & Lecce, 1972; Yeh & Moog, 1974). Clarke & Hardy (1971) described closure as the cessation of two processes, the uptake of macromolecules into the epithelial cells via endocytosis and further transmission of the internalized material into the blood and lymphatic systems. Lecce (1973) defined it as the cessation of uptake into the cell, which requires approximately 3 weeks to be complete in the piglet. It is the cessation of transmission, which in the piglet occurs at 18 - 36 hours after birth, which is actually of the most interest immunologically, nutritionally and physiologically (Svendsen *et al.*, 1986).

The mechanism of closure appears to differ between species (Clarke & Hardy, 1969; Rundell & Lecce, 1972; Patt, 1977). Closure results from a replacement of the vacuolated cells by non-absorptive cells produced by the crypts in the rat (Klein & McKenzies, 1983a; Patt, 1977) and in the sheep (Smeaton & Simpson-Morgan, 1985; Trahair & Robinson, 1986b). In some species, including the rabbit, hamster, guinea pig and pig, cell turnover and closure appear to be partially independent (Clarke & Hardy, 1971; Rundell & Lecce, 1972; Lecce, 1973). In the pig, jejunal-ileal epithelial cells were not extruded at 2 or 3 days of age, but remained on the villi for 7 - 10 days, which was considerably beyond the actual onset of closure (Moon, 1971). This suggested that closure in piglets may result from changes in the properties of the intestinal epithelium *in situ* rather than from a replacement of its cells (Patt, 1977). Piglet intestine can absorb, but not transport, macromolecules for several days following closure (Clarke &

Hardy, 1971; Lecce, 1973). It is possible that in the neonatal rat the enterocytes remain on the villi for several days post-partum (18 - 21 days) (Clarke & Hardy, 1969) and that they retain their ability to internalize macromolecules throughout their life-span (Clarke & Hardy, 1969), whereas the epithelial cells of the piglet probably lose their ability to transport absorbed macromolecules into the circulation some time before they are shed from the villi (Patt, 1977). A study in neonatal piglets of transposed intestinal segments showed that cessation of macromolecular absorption occurs in a sequential pattern from the duodenum to the ileum (Leary & Lecce, 1976).

In summary, functional development of the small intestine in newborn piglets includes 2 major occurrences: cessation of macromolecular absorption at about 24 - 36 hours after birth and changes in enzyme activities corresponding with changes of dietary constituents, particularly carbohydrates. Lactase activity is high at birth, continues to increase to a maximum at 10 - 20 days and gradually declines to its minimum at 4 - 5 weeks. Sucrase and maltase are very low at birth and increase to significant levels in 1 - 2 weeks.

2.3 FACTORS AFFECTING POSTNATAL DEVELOPMENT OF THE SMALL INTESTINE

Small intestinal development in the newborn is not only regulated by systemic hormones (cortisol, thyroxine and insulin) as is the development of other organs, but it is also affected by the ingestion and presence of food within the digestive tract. A large body of experimental evidence strongly indicates that the principal stimuli for small intestinal growth are luminal factors (reviewed by Levine, 1991), which include the nutrients themselves, ingested trophic factors and the release of hormones and other endogenous secretions that have been reported to stimulate intestinal growth (reviewed by Johnson, 1988).

2.3.1 Systemic Hormones

Spontaneous changes in the hormone balance which occur at specific ages in

each species have been suggested to be the main factor involved in the maturation of the digestive system (Yeh & Moog, 1975b) and the development of digestive enzymes (Corring *et al.*, 1982). The hypophyseal-adrenal axis is principally involved in the rat (Yeh & Moog, 1975b) since hypophysectomy causes reductions in both morphological and functional growth of the small intestine (Yeh & Moog, 1975a; Moog & Yeh, 1979; Bastie *et al.*, 1982; Yeh & Moog, 1984), which can be restored to various degrees by cortisone or thyroxine but not by growth hormone or prolactin (Yeh & Moog, 1975b). There appears to be a complex synergism of action between insulin, cortisone and thyroxine on the functional maturation and mitotic activity, at least in the suckled mouse (Malo & Menard, 1983).

2.3.1.1 Glucocorticoid Hormones

The role of the pituitary-adrenal axis in the prenatal development of the small intestine has been described earlier (see Section 2.1.1.4) and these hormones continue in the neonatal period to be involved in the regulation of morphological and functional development (Moog, 1979). A rise in plasma corticosterone precedes the enzymatic changes at weaning in the rat by approximately 48 hours (Henning, 1978), suggesting corticosterone regulates intestinal enzyme development (Henning, 1985, 1986, 1987). Adrenalectomy in neonatal rats results in shorter villi, shallower crypts, and a lower mitotic index (Herbst & Koldovsky, 1972) and cortisone treatment after neonatal adrenalectomy restores the normal mitotic index and villous and crypt morphometric parameters (Yeh & Moog, 1977). The normal rate of the developmental increase in sucrase and maltase activities and decrease in lactase activity in the rat were depressed but not abolished by adrenalectomy (Henning, 1984), and these changes can be restored by administration of cortisone (Martin & Henning, 1984). In addition, the use of organ culture convincingly demonstrated the direct action of glucocorticoids on cultured intestinal explants from neonatal rats and mice by eliciting a premature appearance of sucrase and a precocious increase in the other brush border hydrolytic activities (Simon *et al.*, 1982; Arsenault & Menard, 1984a)

A prepartum surge in circulating cortisol levels occurs in fetal sheep (Bassett & Thornburn, 1969; Nathanielsz *et al.*, 1972), goats (Currie & Thornburn, 1977), pigs (Randall, 1983), cattle (Comline *et al.*, 1974) and humans (Murphy *et al.*, 1975). The resulting high level of cortisol in the newborn lamb has been reported to induce postnatal development of the small intestine in the sheep (Trahair *et al.*, 1987b). The prepartum cortisol surge in the pig (Randall, 1983), may also be a factor in the rapid postnatal growth of the small intestine seen in the newborn piglet. Studies in the rat suggest that the small intestine of the newborn is sensitive to glucocorticoids only during a particular period (14 - 17 days of age) (Henning & Sims, 1979; Henning, 1986). Unless it is demonstrated that the tissues are responsive to cortisol only for a short period in piglets, then it is possible that the high levels of cortisol and rapid growth of the small intestine in the early postnatal period might only occur coincidently and not be causally linked.

2.3.1.2 Thyroid Hormones

The suppressive effects of thyroidectomy and hypothyroidism on morphological and functional development of the small intestine have been demonstrated in the newborn rat (Yeh & Moog, 1974, 1977; Koldovsky *et al.*, 1975; Henning, 1978) and these effects can be reversed by thyroxine treatment (Yeh & Moog, 1977). Administration of thyroxine or triiodothyronine causes a precocious decline in jejunal lactase (Paul & Flatz, 1983) and an increase in jejunal sucrase and maltase activities (Jumawan & Koldovsky, 1978). The suppressive effect of hypothyroidism on the development of sucrase and maltase can be reversed just as effectively by glucocorticoid administration as by thyroxine treatment (Yeh & Moog, 1977; Martin & Henning, 1982), and there is no stimulation of sucrase and maltase when thyroxine is administered unless serum corticosterone is also allowed to increase (Martin & Henning, 1982). This suggests that the effects of thyroid manipulation are at least partly dependent on the accompanying changes of serum corticosterone (Henning, 1985, 1986, 1987), however, the study of Menard & Calvert (1991) suggests there is a direct effect of thyroxine on sucrase development.

2.3.1.3 Insulin

Several lines of evidence support a role for endogenous insulin in the regulation of intestinal development (Menard & Calvert, 1991). The circulating insulin concentration increases steadily in the rat between 10 and 25 days after birth (Blazquez *et al.*, 1970). Intestinal epithelial cells possess receptors for insulin (Bergeron *et al.*, 1980; Forgue-Lafitte *et al.*, 1980; Reinprecht,1980) which have been identified by autoradiographic techniques *in vivo* on both villous and crypt cells (Bergeron *et al.*, 1980). Exogenous insulin caused a dose-dependent precocious appearance of sucrase activity, increased maltase and lactase activities and accelerated the migration rate of enterocytes on the villus (Blazquez *et al.*, 1970, 1975; Menard & Malo, 1979; Menard *et al.*, 1981). These effects are not secondary to insulin-induced elevation of endogenous glucocorticoid hormones since the same phenomena are observed when insulin is added alone to serum-free cultured intestinal explants (Menard & Malo, 1981; Arsenault & Menard, 1984b).

2.3.2 Growth Promoting Substances in the Colostrum and Milk

The rapid growth of the small intestine in the neonatal period, particularly in the first day after birth, in young being suckled on natural milk suggests the importance of milk constituents in promoting this growth. During the first 24 hours after birth, the small intestine of the newborn undergoes rapid increases in both weight and length in pigs (Widdowson *et al.*, 1976), rabbits (Hall & Widdowson, 1979), rats (Berseth *et al.*, 1983; Berseth, 1987a; Simmen *et al.*, 1990a) and dogs (Heird *et al.*, 1984) when they are fed colostrum but not when they are maintained only on water (Widdowson *et al.*, 1976; Hall & Widdowson, 1979). The small intestine of piglets fed sow colostrum had a greater increase in weight, size and DNA content compared with piglets fed water during the first 24 hours postpartum (Widdowson *et al.*, 1976). When the caloric intake and method of feeding are controlled, natural milk enhances neonatal intestinal growth more than artificial formula (Berseth, 1987a). These observations strongly suggest the existence of trophic effects of colostrum and milk on postnatal development of the small intestine.

As well as the small intestine, the accessory organs of the gut, particularly the pancreas, have their growth enhanced by colostrum feeding. The pancreas of newborn piglets grew extremely rapidly during the first 24 hours after birth in response to suckling (Widdowson & Crabb, 1976). The liver weight also increased as a result of colostrum feeding, which was suggested to result from an increase in protein, fat and water content, despite a decrease in carbohydrate content (Widdowson & Crabb, 1976).

The trophic effects of colostrum may only manifest themselves during a critical period of intestinal development (Sheard & Walker, 1988) since in guinea pigs there was a significant difference between the effects of suckling and artificial feeding on small intestinal growth during the first 24 hours after birth in guinea pigs delivered 5 days prematurely but not in full term animals (Thompson *et al.*, 1986). This may also occur in the pig since there were no significant differences of the mucosal mass, intestinal length and circumference between suckled and artificially fed newborn piglets at 2, 8 and 15 days of age (Ulshen *et al.*, 1991), despite the demonstration of greater growth-promoting activity of porcine colostrum than artificial diet *in vitro* on murine Swiss 3T3 cells (Ulshen *et al.*, 1991).

Colostrum and milk contain hormones and trophic factors which have been shown to stimulate postnatal growth of the small intestine in the newborn. These substances includes the hormones corticosterone, thyroxine and insulin (Koldovsky, 1980; Hazum, 1983; Read *et al.*, 1984; Yeh, 1984; Jaeger *et al.*, 1987; Westrom *et al.*, 1987; Koldovsky *et al.*, 1988; Polk, 1992), growth factors (Baxter *et al.*, 1984; Cera *et al.*, 1987; Jaeger *et al.*, 1987; Read, 1988; Simmen *et al.*, 1990b) and nutrients such as carbohydrates, lipids, proteins and amino acids (Aumaitre & Seve, 1978; Blanc, 1981; Oftedal, 1984; Klobasa *et al.*, 1987; Pierse *et al.*, 1988; Kelly *et al.*, 1991; Jensen & Jensen, 1992).

2.3.2.1 Hormones

Corticosterone, thyroxine and insulin are present in colostrum and milk (Koldovsky, 1980; Hazum, 1983; Read *et al.*, 1984; Yeh, 1984; Jaeger *et al.*, 1987;

Westrom *et al.*, 1987; Koldovsky *et al.*, 1988; Polk, 1992) and all have been shown to have trophic effects on small intestinal tissue (see Section 2.3.1). Whether or not milk hormones have a physiological role in the suckled neonate has not been established.

Porcine colostrum contains high concentrations of insulin, although it declines after 72 hours of lactation (Westrom *et al.*, 1987). The concentration of insulin in sow colostrum (411 µU/ml) at birth is approximately 80 times greater than that in serum (5 µU/ml) (Westrom *et al.*, 1987), suggesting that this colostral hormone may take part in promoting functional development of the small intestine in piglets. Oral insulin stimulated an increase in ileal mass and disaccharidase activity in the newborn miniature pig without apparent concomitant changes in serum glucose, insulin or cortisol (Shulman, 1990). In a later study, orally administered insulin had no effect on the level of ileal lactase mRNA or on the relative proportion of lactase precursor proteins, suggesting that the enhancing effect of oral insulin on lactase activity in the newborn piglet is not regulated at the level of translation and postranslation (Shulman *et al.*, 1992).

2.3.2.2 Epidermal Growth Factor (EGF)

Human milk was found to contain a mitogenic factor that stimulated DNA synthesis and cellular proliferation in the mouse and human fibroblasts (Klagsbrun, 1978). Since the mitogenic activity could be decreased by including antibody to EGF in the culture media (Carpenter, 1980), this factor has been characterized as EGF (Carpenter, 1980; Shing & Klagbrun, 1984). EGF is present in the milk of a number of mammalian species including humans (Tapper *et al.*, 1979; Moran *et al.*, 1983; Read *et al.*, 1984), cattle (Steimer *et al.*, 1981), rats (Berseth, 1987b; Schaudies *et al.*, 1990), mice (Grueters *et al.*, 1985) and pigs (Jaeger *et al.*, 1987). The concentration of EGF falls as a function of the duration of lactation, but in humans it remains 10 to 100 fold higher than the plasma EGF concentration (Polk, 1992).

EGF is resistant to digestion by acid and proteolytic enzymes and the entire molecule has been demonstrated to pass through the enterocyte immunologically and

functionally intact (Thornburg *et al.*, 1984). The intestine is the primary site of gastrointestinal absorption of EGF, the absorption from the stomach being negligible (Thornburg *et al.*, 1986). Receptors for EGF are widely distributed in the intestinal mucosa and submucosa of the newborn piglet, and *in vitro* autoradiography has demonstrated EGF receptors on both the microvillar and basolateral surfaces of the enterocytes, suggesting that luminal EGF could influence developmental processes in the intestine either directly or indirectly (Kelly *et al.*, 1992). In the suckled rat, the degradation and transfer of EGF were higher in the jejunum than in the ileum, whereas in the weanling and adult rat, there was no difference in the degradation and transfer in the jejunum and ileum (Rao *et al.*, 1986).

The trophic effects of EGF on the small intestine have been linked to its stimulation of polyamine synthesis by increasing the activity of the rate-limiting enzyme ornithine decarboxylase (Feldman *et al.*, 1978; Ulshen *et al.*, 1986) a rate limiting-step enzyme for biosynthesis of polyamines (Luk & Baylin, 1983) which have been associated with intestinal cell growth and differentiation (Conteas *et al.*, 1991). EGF increased the mucosal DNA levels in the distal small intestine in suckled rabbits when given either intraperitoneally or orogastrically, indicating that both systemically and orally administered EGF may regulate growth and postnatal maturation of the gastrointestinal tract (O'Loughlin *et al.*, 1985). Long term effects of EGF were demonstrated in newborn rats fed with artificial formula containing high levels of EGF from birth to 36 hours of age. Although the rats were returned to natural suckling, the increased DNA synthesis and content in the small intestine and weight of the intestine and its RNA and DNA content were sustained until the rats were euthanased on day 5 (Berseth, 1987b).

EGF is capable of influencing disaccharidase activities in the small intestine. Discrepancies exist in the reported biological effects of EGF on proliferation and differentiation of small intestinal mucosal cells and on enzyme activities in suckled animals because of the variation in dosages, routes of administration and species of experimental animals used in different studies (Menard & Arsenault, 1988), however, all studies agree that EGF has a stimulating effect on the enzyme activities of the small

intestine. EGF administered orogastrically induces precocious maturation of intestinal brush border disaccharidase activities in suckled rabbits (O'Loughlin *et al.*, 1985). Injection of EGF into 3-day-old piglets increased the intestinal sucrase and maltase activities, and these increases were confined to the mid- and distal regions of the small intestine (James *et al.*, 1987). Orally administered EGF significantly increased jejunal lactase and sucrase activity in 3-day postweaning piglets (Jaeger *et al.*, 1990).

2.3.2.3 Insulin-like Growth Factors (IGFs)

Insulin-like growth factors (IGFs), single chain polypeptide mitogens structurally similar to insulin, exert insulin-like activity (Zapf *et al.*, 1978) and are capable of stimulating DNA synthesis and mitosis in a variety of cell types, including human fibroblasts, fetal rat hepatocytes and rat myoblasts (Reddan & Dziedzic, 1982). IGF-I and IGF-II are potent inducers of the growth-related enzyme ornithine decarboxylase in gut-derived cells (Olanrewaju *et al.*, 1992) and stimulate growth of a cell line derived from rat jejunal crypts (Park *et al.*, 1990).

The presence of IGFs and their binding proteins has been demonstrated in human, bovine, goat, pig and rat milk (Zumkeller, 1992). The levels of both IGF-I and IGF-II are considerably higher in colostrum than in mature milk (Baxter *et al.*, 1984; Campbell & Baumrucker, 1989; Donovan *et al.*, 1991). There is strong circumstantial evidence that milk IGFs exert their mitogenic effects on the small intestine as a result of binding to specific receptors. High levels of IGF-I have been demonstrated in porcine colostrum (Simmen *et al.*, 1988), while IGF-I binding in the developing porcine small intestine was maximal on the first postpartum day (Schober *et al.*, 1990). The tissue IGF-I content varied with age and the part of the small intestine, being highest in the proximal segment of the small intestine on day 3 postpartum but greatest in the middle segment on day 21 (Schober *et al.*, 1990). Since intestinal IGF-I mRNA content did not change over this period, the increased amounts of IGF are believed to result from uptake from milk rather than from tissue segments (Zumkeller, 1992).

IGF-II administered orally and IGF-I administered both orally and parenterally to suckled rats stimulated jejunal brush border enzymes, but had no effect on gut weight (Young *et al.*, 1990), whereas intraileal infusion of IGF-I to adult rats significantly increased the mucosal wet weight, total mucosal RNA and DNA and protein content compared with control rats (Olanrewaju *et al.*, 1992). The differences in dose, route of administration and age of the rats may account for these discrepancies. After partial gut resection in adult rats, treatment with IGF-I and its analogues caused a significant weight increase in the stomach, duodenum and large intestine (Lemmy *et al.*, 1991; Read *et al.*, 1991) particularly in the mucosa and muscle (Read *et al.*, 1991).

2.3.2.4 Putative Growth Factors

Colostrum and milk contain other substances with reported trophic effects on the gut. Bombesin is a tetradecapeptide isolated from amphibian skin and in mammals the bombesin-related peptide, gastrin releasing peptide (GRP), is produced by intrinsic enteric neurons (Walsh, 1989). High concentrations of bombesin-like immunoreactivity have been detected in the colostrum of pigs (Westrom *et al.*, 1987), cows (Jahnke & Lazarus, 1984) and humans (Berseth *et al.*, 1990). This milk peptide may be important for growth and maturation of the gastrointestinal tract in neonates (Berseth *et al.*, 1990), although its trophic effects have been found to be considerable in stomach, pancreas and large intestine, but only slight in the small intestine (Puccio & Lehy, 1989). Bombesin significantly increased only the weight of the intestine, but had no effect on the duodenal mucosal height in 7-day-old sucking rats (Lehy *et al.* 1986).

Prostaglandins (PGs) are present in fresh human milk (Lucas & Mitchell, 1980; Reid *et al.*, 1980) and $PGF_{2\alpha}$ in bovine milk (Manns, 1975). Prostaglandins are effective in promoting healing of peptic ulcers and in protecting the gastrointestinal mucosa against experimentally induced ulcers (Materia *et al.*, 1984). Their cytoprotective effect in the gastrointestinal tract may be related to their stability and lack of degradation in milk and gastric juice (Bedrick *et al.*, 1989). Prostaglandin E_2 is a regulator of cell kinetics in the gastrointestinal epithelium due to its dual actions on cell proliferation and on the regulation of epithelial cell losses (Uribe *et al.*, 1992).

Spermidine is one of the polyamines present in the cells which play important roles in a variety of cellular processes, especially those associated with growth and differentiation (Conteas *et al.*, 1991). The presence of high levels of spermidine in porcine milk and in the intestinal tissue of suckled piglets, has led to the suggestion that milk spermidine is taken up from the intestinal lumen and is involved in potentiating intestinal differentiation during the later part of the suckling period (Kelly *et al.*, 1991).

2.3.3 Local Nutrition

Local nutrition has been defined as the direct nutritional effect of absorbed products of digestion on the absorbing cells before the nutrients circulate systemically (reviewed by Johnson, 1988). The importance of luminal nutrients in maintaining the normal mass and function of the small intestine is evident from the greater small intestinal length, weight and villous height and surface area during oral feeding (Meurling & Roo, 1981; Raul *et al.*, 1987; Shulman, 1988) whereas parenteral feeding decreased the mucosal mass, protein and DNA content (Cameron *et al.*, 1974; Levine *et al.*, 1974; Hughes & Dowling, 1980; Hughes & Ducker, 1982; Popp & Wagner, 1984), epithelial cell proliferation (Eastwood, 1977) and enzyme activity (Levine *et al.*, 1974; Hughes & Dowling, 1980; Hughes & Ducker, 1982). While these experiments show that enteral feeding is necessary for the maintenance of normal intestinal mass and function, they cannot clearly distinguish between the local effects on the absorbing cells of specific substances, the effect of the work-load (see Section 2.3.4) or physiological responses related to ingestion of food or its presence in the digestive tract.

Stimulation of gut growth by specific local nutrition has been tested by the infusion of substances into by-passed loops (reviewed by Johnson, 1988) and by intragastric infusion in parenterally nourished rats (reviewed by Levine, 1991).
Each of the three principal nutrients, carbohydrates, protein and fat, tested in this way have been shown to stimulate growth of the small intestine.

2.3.3.1 Carbohydrates

Carbohydrates stimulate small intestinal growth. Mice raised on a high carbohydrate diet had approximately 15% greater proximal intestinal mass than mice raised on a carbohydrate-free ration (Karasov *et al.*, 1985). Investigation of the relative potency of various sugars in stimulating mucosal growth revealed that disaccharides such as lactose are more trophic to the intestine than monosaccharides such as glucose, galactose and fructose (Weser *et al.*, 1986). Infusion of dextrose intragastrically or into the mid-ileum causes profound gut hyperplasia (Spector *et al.*, 1977) and there is a linear correlation between the quantity of dextrose infused into the gut and the mucosal growth (Weser *et al.*, 1985).

The sugars had to be absorbed to have trophic effects, since the infusion of a glucosidase inhibitor blunted or shifted the trophic effects of disaccharides further down the gut (Weser *et al.*, 1986). Furthermore, mannitol, a non-absorbable sugar, is ineffective in producing gut growth (Spector *et al.*, 1977) whereas the infusion of 3-O-methyl glucose promotes gut growth (Richter *et al.*, 1983).

Experiments which involve multiple variables in the diet have shown that when a high concentration of one component is added, the uptake of another invariably decreases. In animals switched from high carbohydrate feeding to a carbohydrate-free diet, a rapid decrease in glucose uptake and a concomitant increase in amino acid uptake occurred on the carbohydrate-free, high protein diet (Karasov & Diamond, 1983; Karasov *et al.*, 1985).

2.3.3.2 Amino acids

Amino acids in low concentrations stimulate small intestinal mucosal growth to a greater degree than either isotonic saline or isocaloric dextrose when infused intraluminally into the mid-jejunum of parenterally nourished rats (Spector *et al.*, 1977). Each amino acid had its own trophic effect, e.g. histidine was a better stimulator of mucosal growth than valine or glycine in both the jejunum and ileum, whereas glycine

caused hyperplasia in the ileum (Spector *et al.*, 1981). The mechanism by which amino acids cause mucosal growth is unknown, but the infusion of some amino acids increases ornithine decarboxylase (Jain *et al.*, 1987).

Glutamine has been introduced into 'total parenteral nutrition' solutions since it is as an important substrate for increasing protein synthesis in the intestinal mucosa in the rat (Grant & Snyder, 1988; Yoshida *et al.*, 1992) and for preventing deterioration of gut permeability and preserving mucosal structures in humans (van der Hulst *et al.*, 1993). However, parenteral glutamine or glutamic acid supplemented at physiological doses did not affect small intestinal growth in piglets (Burrin *et al.*, 1991). Furthermore, glutamine fed intraluminally exhibits no significantly different trophic effect from aminoisobutyric acid, a nonmetabolizable amino acid (Levine, 1986).

2.3.3.3 Lipids

Lipid has a relatively modest effect on mucosal growth. Feeding a high fat diet to rats resulted in a greater mucosal weight (Morin *et al.*, 1980) and greater villous height in the distal jejunum (Balint *et al.*, 1980), but there was no significant difference in DNA content, suggesting that a high fat diet produces intestinal hypertrophy (Balint *et al.*, 1980). The opposite result has also been reported, in that a high fat diet in rats decreased the jejunal mucosal weight and produced mucosal atrophy in the distal ileum (Jacobs, 1983) and Sircar *et al.* (1983) found that there was no relationship between the fat content of the diet and parameters of intestinal growth in the rat. Although the mechanism by which fat increases gut mass is unknown (Levine, 1991), feeding a high fat diet improves fat absorption and the mucosal fat esterification capacity even though the total mucosal mass may not increase markedly (Balint *et al.*, 1980; Kotler *et al.*, 1980).

2.3.4 Work-load Hypothesis

Clarke (1977) proposed the 'work-load hypothesis' that the functional work load influences the growth of the gut mucosa: active absorptive or secretory work requires

cell replacement, irrespective of the nutrient value of the absorbed material. This hypothesis was based on the observation that distilled water produced approximately 10% greater villous height and crypt depth in an infused upper small intestinal loop compared with a control loop that did not receive an infusion. Further supporting the hypothesis is the observation that the mid-jejunal mass of parenterally fed rats was significantly greater after luminal infusion of isotonic saline (Richter *et al.*, 1983; Levine, 1986). Furthermore, when isolated loops were infused with different sugars and saline, it was observed that sodium chloride, galactose and glucose all produced a modest but nonsignificant increase in villous height and inconsistent increases in the number of crypt metaphases (Clarke, 1977). However, other researchers have been unable to obtain similar results to those of Clarke. Menge *et al.* (1975) found that only glucose increased the villous height whereas nonmetabolizable sugar and electrolytes had no effect. The effect of the work-load component on growth of small intestinal mucosa therefore remains questionable.

2.3.5 Gastrointestinal Hormones

A number of studies indicated that the release of gastrointestinal hormones in response to feeding is a key link between the presence of food in the gut and the postnatal development of the gastrointestinal tract (Aynsley-Green *et al.*, 1981; Aynsley-Green, 1983; Lucas *et al.*, 1981a, 1982a). Among these gut peptides gastrin, enteroglucagon and CCK have been suggested as having important roles in the postnatal growth of the small intestine (Aynsley-Green, 1989).

Gastrointestinal hormones or gut peptides have been identified, together with their corresponding cell lines, in human fetal gut from 6 to 10 weeks post-conception and gut tissue concentrations of most of these peptides rise during gestation to values at least as high as those in adults (Buchan *et al.*, 1981). These hormones are present in the human fetal circulation or amniotic fluid as early as 18 to 21 weeks gestation (Aynsley-Green, 1985). The gut endocrine system is well developed before birth in humans (Lucas, 1989), and most of the hormones have concentrations in venous cord blood at birth after normal delivery which are similar to those seen in healthy fasting

adults (Lucas, 1989).

2.3.5.1 Gastrin

Gastrin, a trophic hormone released from the antrum of the stomach in response to the presence of food (Korman *et al.*, 1971), stimulates DNA synthesis in the small intestinal mucosa *in vivo* and *in vitro*, and has trophic actions in several regions of the gastrointestinal tract (Johnson, 1976).

Many studies involving the trophic effects of gastrin have been done at pharmacological levels in the mature small intestine therefore do not necessarily support a role for gastrin in the postnatal development of the small intestine. The antral gastrin concentration in the rat increases dramatically during weaning in the third week after birth when major developmental changes in gut structure occur, suggesting there may be a relationship between gastrin and the intestinal development at weaning (Lichtenberger and Johnson, 1974). However, no gastrin receptors are present in the oxyntic gland mucosa of the rat before weaning and both gastrin receptors and biological sensitivity to gastrin appear shortly after the start of weaning (Takeuchi *et al.*, 1981). Gastrin injected intraperitoneally (500 µg/kg/day) for 2 weeks did not affect the development of the small intestine in the newborn rabbit (Zahavi *et al.*, 1984), and both gastrin (10 - 40 µg/kg) and pentagastrin (100 - 1000 µg/kg) injected intraperitoneally did not stimulate small intestinal cell proliferation in the rat (Fatemi et al,1984). These observations suggests that gastrin may not be involved in small intestinal growth in the early postnatal period until weaning, at least in these species.

Avila *et al.* (1989) examined the role of endogenous gastrin in the development of the small intestine in the sheep fetus by removing the gastric antrum which is the major source of endogenous gastrin. They found that there were significant correlations between the plasma gastrin concentration and the villus density and the crypt-villus ratio in the proximal small intestine but the villus height, crypt depth and villus cell migration rate in the small intestine were not affected. They concluded that gastrin appeared to have a regulatory or trophic role on the gut mucosa in antrectomized sheep, however,

gastrin seemed to have no effect during normal development, suggesting either that antrectomy removed a factor which is inhibitory or regulatory for gastrin or that the remaining forms of circulating gastrin have a different effect on the mucosa from antral gastrin (Avila *et al.*, 1989).

2.3.5.2 Enteroglucagon

Enteroglucagon, also known as glicentin, is released from a specific endocrine cell in the mucosa of the distal ileum and large intestine and has a molecular sequence containing the entire amino acid sequence of pancreatic glucagon (Bataille,1989). It was suggested as a trophic factor for small intestinal mucosa because a patient with an enteroglucagon-producing renal tumour had massive villus hypertrophy of the small intestine (Gleeson *et al.*, 1971). In contrast, pancreatic glucagon had no effect in inducing early villous and crypt cell enzyme activity in suckled rats, and in adaptation of the small intestinal mucosa which occurs at weaning (Buts *et al.*, 1983). Although there are correlations between plasma enteroglucagon levels and growth in the small intestine (Bloom & Polak, 1982; Goodlad *et al.*, 1983), there is little direct evidence that enteroglucagon is indeed a trophic substance which stimulates small intestinal mucosal growth (Johnson, 1989).

2.3.5.3 Cholecystokinin (CCK)

Cholecystokinin has a well documented trophic effect on the pancreas (Rehfeld, 1989). It has been suggested that CCK is important in the postnatal growth of the small intestine (Aynsley-Green, 1989). Intravenous CCK partially stimulated mucosal growth after jejunal resection in rats nourished by total parenteral nutrition (TPN) since these rats had comparable mucosal growth to rats fed intragastrically (Weser & Tawil, 1978). However, CCK did not affect the ontogenic development of the small intestine in newborn rabbits (Zahavi *et al.* 1984). It is possible that the trophic effect of CCK may have been secondary to pancreatico-biliary secretion (see Section 2.3.6).

2.3.5.4 Other Peptides

A number of other peptides have been postulated to have effects on postnatal development of the gut because their concentrations increase at a time when there is accelerated gut growth, and others because of their effects on gut motility and secretion (Lucas, 1989). These include motilin (Lucas, 1989), neurotensin (Blackburn *et al.*, 1980), gastric inhibitory polypeptide (GIP) (Lucas *et al.*, 1980d), substance P (Holzer *et al.*, 1981) bombesin-like peptides (Lehy *et al.*, 1986; Puccio & Lehy, 1989) and secretin (Aynsley-Green *et al.*, 1981).

2.3.6 Pancreatico-biliary Secretion

The combined pancreatic and bile secretions have been shown to be trophic for the small intestine under a variety of conditions. Either the pancreatic juice or the bile is able to produce intestinal hyperplasia since transplantation of the pancreatic ampulla or the bile duct to the distal intestine significantly increased the villous size index near the implantation site (Altmann, 1971). Furthermore, diverting bile from the proximal small intestine reduced the proliferative activity and ^{14}C thymidine incorporation into DNA, and these changes could be reversed by the infusion of sodium taurocholate (Roy *et al.*, 1975), suggesting the trophic effect of biliary secretion. A number of studies involving intestinal adaptation after small bowel resection also support a trophic effect for pancreatico-biliary secretions (Dowling & Booth, 1967; Feldman *et al*, 1973; Dowling *et al.*, 1974; Feldman *et al.*, 1976; Levine *et al.*, 1976; Morin *et al.*, 1978; Shin *et al.*, 1980; Postuma *et al.*, 1983; Biasco *et al.*, 1984; Ford *et al.*, 1984).

Studies on rats suggested that secretin and CCK indirectly affected the small intestinal mucosa by stimualting pancreatico-biliary secretion. A daily infusion of secretin and CCK, in sufficient amounts to stimulate pancreatic secretion maintained the intestinal mass and sugar absorption in the dogs fed parenterally for 6 weeks at levels comparable to those in control dogs (Hughes *et al.*, 1978). In rats, secretin plus CCK-octapeptide significantly increased the mass of the intestinal mucosa (Weser *et al.*, 1977) this effect was due to the hormones stimulating pancreatic and biliary secretions, and

not to direct effects on intestinal receptors since secretin, CCK-octapeptide, or both did not produce mucosal hyperplasia in the bypassed jejunal loop in parenterally nourished rats, compared with that in the segment in continuity with the luminal contents (Fine *et al.*, 1983).

2.4 FEEDING AND SMALL INTESTINAL DEVELOPMENT

Major changes in metabolism and gastrointestinal function must occur soon after birth to enable the newborn to acquire nutrients via suckling rather than placental transfer (Aynsley-Green, 1983). Natural suckling results in marked structural and functional changes to the gastrointestinal tract which are not seen in newborn animals prevented from suckling (Lichtenberger & Johnson, 1974; Widdowson, 1985). Acute changes in circulating insulin and glucagon levels, together with other metabolic hormones, to maintain normoglycemia occur after the first and subsequent episodes of suckling (Aynsley-Green *et al.*, 1977; Aynsley-Green, 1983), but not in infants receiving intravenous feeding (Lucas *et al.*,1980a, 1983). These concomitant changes in circulating hormones and in gut development suggest the importance of enteral feeding, including the feeding mechanism itself, for normal postnatal development of the gut (see Sections 2.3.2 - 2.3.6).

The importance of luminal nutrients for maintaining normal mass and function of the mature small intestine has been demonstrated in several studies comparing enteral and parenteral feeding. Oral feeding increased the small intestinal length, weight and villous height and surface area (Meurling & Roo, 1981; Raul *et al.*, 1987; Shulman, 1988) whereas parenteral feeding decreased the mucosal mass, protein and DNA content (Cameron *et al.*, 1974; Levine *et al.*, 1974; Hughes & Dowling, 1980; Hughes & Ducker, 1982; Popp & Wagner, 1984;), epithelial cell proliferation (Eastwood, 1977) and enzyme activity (Levine *et al.*, 1974; Hughes & Dowling, 1980; Hughes & Ducker, 1982). Comparison of parenteral and enteral feeding demonstrated that enteral feeding after partial small intestinal resection resulted in increases in the length and circumference of the residual intestine (Shin *et al.*, 1980; Ford *et al.*, 1984), increases in the number of villi, the villous height and width and the crypt depth (Feldman *et al.*,

1976; Shin *et al.*, 1980; Biasco *et al.*, 1984) and an increase in absorptive function due to hyperplasia of the villi (Morin *et al.*, 1978). The effects of intraluminal nutrition upon small intestinal structure and function have also been demonstrated using a 'by-passed intestinal loop' in rats. Mucosal hyperplasia and hypertrophy and increased enzyme activities (Gleeson *et al.*, 1972a,b; Castro *et al.*, 1975) and increased glucose absorption (Gleeson *et al.*, 1972a; Kotler *et al.*, 1981) occurred in the re-established intestine compared with the intestinal loop. Also mucosal hyperplasia and hypertrophy and increased enzyme activities were seen in the by-passed intestine of orally fed animals compared with those parenterally fed indicating digesta in the re-established intestine affected growth of the by-passed intestinal loop (Dworkin *et al.*, 1976; Adams *et al.*, 1978). These observations suggest an important role for the route of feeding on small intestinal structure and function.

The method of oral feeding has been shown to have marked effects on the subsequent pattern of gastrointestinal hormone release (Aynsley-Green, 1983). Bolus feeding combined with nonnutritive sucking in preterm infants reduced circulating somatostatin levels and increased gastrin levels (Widstrom *et al.*, 1988). Nonnutritive sucking alone in both term and preterm infants caused a significant increase in circulating insulin levels within 45 seconds of the start of sucking (Marchini *et al.*, 1987), and breast feeding in 3-day-old infants caused a significant increase in circulating gastrin levels within 10 minutes of the start of feeding (Marchini *et al.*, 1992). Also a significant increase of circulating CCK was demonstrated immediately after breast feeding in 4-day-old infants (Uvnas-Moberg *et al.*, 1993). The effects of nonnutritive sucking or breast feeding in releasing gastrointestinal hormones may be due to stimulation of the vagus nerves (Marchini *et al.*, 1987; Widstrom *et al.*, 1988; Uvnas-Moberg, 1989). If these gastrointestinal hormones are released in response to sucking, sucking itself could stimulate growth and maturation of the gut (Uvnas-Moberg, 1989).

Oral feeding by means of sucking may influence postnatal development of the small intestine via vagal reflexes during the cephalic phase of digestion (Uvnas-Moberg, 1989). In mature animals stimuli acting in the region of the head, such as the sight, smell, taste, thought and chewing of palatable food, induce gastric acid and pepsinogen

secretion and the subsequent release of other secretions from the intestine and other accessory organs of the digestive system (Debas, 1987). The sucking mechanism is thought to activate sensory nerves in the oral mucosa, resulting in vagal stimulation (Uvnas-Moberg, 1989) which induces the releases of several gastrointestinal hormones (Marchini *et al.*, 1987; Widstrom *et al.*, 1988; Uvnas-Moberg, 1989).

Some gastrointestinal responses to feeding formula-based diets were modified by the method of administering the diet, i.e. orally or intragastrically. Intragastric feeding stimulates greater pancreatic activity than oral feeding of isocaloric amounts of the same formula in the rat (Young *et al.*, 1982). Two possibilities were suggested for the differences between the two method of feeding : (a) nutrients delivered orally may stimulate humoral or neural responses differently from when these same nutrients are delivered directly into the stomach and (b) when nutrients are delivered by continuous intragastric infusion, gastric secretions may be reduced and gastric emptying patterns may be altered (Young *et al.*, 1982).

Similarly, there are differences in the circulating hormone levels of infants receiving a regular bolus of milk from birth compared with those receiving the same volume continuously into the stomach. The infusion-fed babies had higher insulin, GIP and gastrin levels than the bolus fed infants (Aynsley-Green *et al.*, 1982). Bolus feeding causes gastric distension altering the motility of the gut and requires the newborn to maintain metabolic homeostasis during alternating periods of feeding and fasting, whereas in continuous feeding only small amounts of food are introduced into the stomach during each hour. This causes the bolus-fed infant to experience major cyclic changes in hormones and metabolites that are not seen in the steady state circumstances of the continuously fed infant. (Aynsley-Green *et al.*, 1982).

Thus, enteral feeding undoubtedly stimulates gut function and maintains its structure. The relative importance of the different factors, however, is difficult to determine.

2.5 PRESENT STUDIES

From the foregoing discussion it is apparent that in addition to the effects of genetic endowment and the biological clock, growth and development of the small intestine in the newborn involves a complex interaction on the tissue between the animal's physiological regulatory mechanism, in particular systemic hormones and gut peptides, and ingested colostrum and milk. Both of these fluids contain nutrients and growth factors which can be shown to stimulate intestinal growth. Feeding methods *per se* may also influence intestinal growth by vagal mechanisms inducing hormonal release. It is not a simple task to evaluate the relative importance of each of these factors.

The objective of the present study was to investigate the effects of different colostrum/milk diets, intraluminal diets and oral feeding *per se* on postnatal development of the small intestine in piglets during the first 24 hours after birth. Three experiments were conducted as follows: (a) a study of the effects of feeding different diets, i.e. colostrum, milk or infant formula, compared with water feeding and natural suckling (Chapter 3), (b) a study of the effects of intraluminal nutrition by feeding a nutrient solution parenterally or orogastrically (Chapter 4) and (c) a study of the effects of sucking by feeding colostrum or infant formula by either bottle sucking or orogastric tube feeding (Chapter 5). The direct implications of the findings are discussed in each chapter, and more general issues are addressed in the General Discussion (Chapter 6).

CHAPTER 3

THE EFFECTS OF COLOSTRUM AND MILK ON POSTNATAL DEVELOPMENT OF THE SMALL INTESTINE IN PIGLETS DURING THE FIRST 24 HOURS AFTER BIRTH

3.1 INTRODUCTION

During the first 24 hours after birth the intestine undergoes rapid increases in both length and weight in newborn suckled piglets (Widdowson *et al.*, 1976; Widdowson, 1984), rabbits (Hall & Widdowson, 1979), rats (Berseth *et al.*, 1983; Berseth, 1987a; Simmen *et al.*, 1990a) and dogs (Heird *et al.*, 1984). These increases are smaller when the newborns are fed artificial milk formula (Heird *et al.*, 1984; Berseth, 1987a) and absent when they are maintained on water only (Widdowson *et al.*, 1976; Hall & Widdowson, 1979; Burrin *et al.*, 1992) indicating that colostrum and milk contain substances necessary for normal intestinal development in newborn animals.

Substances shown to be present in colostrum or natural milk, which either stimulate or facilitate intestinal development include epidermal growth factor (EGF) and insulin-like growth factors (IGF-I and IGF-II) (see Section 2.3.2), the hormones corticosterone, insulin and thyroxine (Koldovsky, 1980; Hazum, 1983; Read *et al.*, 1984; Yeh, 1984; Jaeger *et al.*, 1987; Westrom *et al.*, 1987; Koldovsky *et al.*, 1988; Polk, 1992) and a range of metabolites such as carbohydrates, lipids, proteins and amino acids (Aumaitre & Seve, 1978; Blanc, 1981; Oftedal, 1984; Klobasa *et al.*, 1987; Pierse *et al.*, 1988; Kelly *et al.*, 1991; Jensen & Jensen, 1992). High levels of EGF and IGF-I have been demonstrated in sow colostrum and milk (Jaeger *et al.*, 1987; Simmen *et al.*, 1988; Simmen *et al.*, 1990b), however their concentrations decline as lactation progresses (Baxter *et al.*, 1984; Cera *et al.*, 1987; Jaeger *et al.*, 1987; Read, 1988; Simmen *et al.*, 1990b). These growth factors have been reported to stimulate gastrointestinal development in several species including pigs, rabbits and rats (for reviews see: Berseth, 1987b; Thornburg & Koldovsky, 1987; Menard & Arsenault, 1988; Zumkeller, 1992).

Among the hormonal modulators of intestinal development found in milk, corticosterone has been extensively studied with regard to its possible stimulatory effects on intestinal enzyme development (for reviews see Moog, 1981; Henning, 1987). Thyroxine apparently enhances intestinal enzyme development (Henning, 1985) but its effect is believed to be secondary to stimulation by corticosterone (D'Agostino & Henning, 1982; Martin & Henning, 1982). However Yeh *et al.* (1988) reported that the administration of thyroxine to intact or adrenalectomized rats induced a precocious expression of sucrase-isomaltase protein without any increase in serum corticosterone, suggesting a direct effect of thyroxine on small intestinal enzyme development. Insulin acting alone (Menard *et al.*, 1981) or synergistically with thyroxine (Malo & Menard, 1983) has been shown to stimulate differentiation and proliferation of intestinal mucosal cells from suckling mice. These effects cannot be secondary to insulin induced elevation of endogenous glucocorticoid hormones since the same phenomena are observed when insulin is added alone to serum-free cultured intestinal explants (Menard & Malo, 1981; Arsenault & Menard, 1984b). Further details of the effects of these hormones on postnatal development of the small intestine have been presented in Section 2.3.1.

Carbohydrates, proteins and lipids facilitate growth of the gut. Disaccharides, including lactose, increase intestinal mass compared to saline or monosaccharides such as glucose, galactose and fructose (Weser *et al*, 1986). Although the mechanism by which amino acids cause mucosal growth is unknown, studies in parenterally nourished rats have demonstrated that the infusion of low concentrations of amino acids stimulates mucosal growth to a greater degree than either isocaloric dextrose or isotonic saline (Specter *et al.*, 1977). Other studies have revealed the importance of glutamine in total parenteral nutrition for increasing protein synthesis in the intestinal mucosa in rats (Grant & Snyder, 1988; Yoshida *et al.*, 1992), and for preventing deterioration of gut permeability and preserving mucosal structure in adult humans fed parenterally (van der Hulst *et al.*, 1993). Lipid has a relatively modest effect on mucosal growth: a high-fat diet increases mucosal weight (Morin *et al.*, 1980) and villous width, without a change in DNA content, suggesting that a high-fat diet produces intestinal hypertrophy (Balint *et al.*, 1980). However, Jenkins & Thompson (1989) reported a trophic effect of fatty

acid on the small intestinal mucosa of rats, increasing mucosal weight and protein content, crypt cell production rate and total mucosal DNA in the middle small intestine. Further details of the conflicting effects of lipid on small intestinal growth reported by other workers have been presented in Section 2.3.3.3.

It is against this background that the present experiment was conducted to investigate the effect of different milk diets on postnatal development of the small intestine in newborn piglets during the first 24 hours after birth. Specifically the experiment was designed first to determine normal intestinal development during the first 24 hours by comparing piglets at birth with naturally suckled piglets, and secondly to determine the effects on intestinal development of different milk diets. The second objective was achieved by comparing groups of piglets removed from the sow before sucking and bottle-fed colostrum or milk from the same or a different species, or fed artificial milk (infant formula) or fed only water for 24 hours.

At the time these experiments were planned the general presumption was that growth factors in the milk diet were mainly responsible for the early postnatal increase in small intestinal weight of newborn animals. Hence, colostrums and milks of different types were fed to further clarify the possible roles of the diet in increasing small intestinal weight during the first 24 hours. However, studies conducted simultaneously have shown that mucosal cell swelling related to colostral protein retention occurs in suckled newborn piglets (Burrin *et al.*, 1992; Xu *et al.*, 1992), suggesting the possibility that the greater increases in small intestinal weight are due in part at least to transient cell swelling and not to growth. Attention was therefore also given to this phenomenon when considering the implications of the present results.

3.2 MATERIALS AND METHODS

3.2.1. Animal Preparation

Forty Large White piglets from the Pig Research Centre, Massey University were separated from the sows and prevented from sucking at birth. They were dried before being weighed on an electric digital balance (Mettler PM 15-N, Switzerland). Their body weights were within 1 S.D. of the average birth weight (1.31 $\pm$ 0.32 kg) of 294 piglets born at the piggery during the period January to March 1990, and their gestational age was between 113 to 115 days. They were randomly assigned to one of 8 groups (5 piglets per group). There were two control groups consisting either of piglets killed at birth (B) or naturally suckled (NS) piglets left with their sows for 24 hours before euthanasia. The piglets in the remaining groups were transported to the Department of Physiology & Anatomy, Massey University and fed by bottle with either sow colostrum (SC), cow colostrum (CC), sow milk (SM), cow milk (CM), a human infant formula (IF) or water (H_2O) for 24 hours. The sources and compositions of these feeds are noted below (see Section 3.2.2). Because of the large number of groups it was not possible to obtain enough piglets of a uniform body weight from the same litter to assign one piglet to each group. Therefore paired comparisons were not attempted. Instead care was taken to ensure that piglets from the same litter were not assigned to the same group.

An umbilical artery of each piglet in the bottle fed groups was cannulated with a sterile intravenous cannula (3FG, length 300 mm, Portex, England) before the vessel became constricted and 30 mg/kg bw 5-bromo-2'-deoxyuridine (BrdU) (Sigma, USA), 10 mg/ml 0.9% NaCl was injected via this cannula for the labelling of dividing mucosal cells and subsequent measurement of mucosal cell proliferation and migration (see Section 3.2.4.2). The cannula was then pulled out and the umbilical cord tied, cut and cleaned with 70% alcohol. The piglets were kept in open-topped plastic bins (40 cm wide, 62.5 cm long, 37 cm high; 2 - 3 piglets per bin) where the temperature was maintained between 32 - 38 °C by a heating lamp, suspended above the bin. They were fed on 8 occasions according to the schedule shown in Table 3.1., but the piglets in the

* This schedule was chosen for the following reasons. Firstly the total volume of the feeds was approximately that consumed by naturally suckled piglets during the first 24 hours after birth (Fraser & Rushen, 1992). Secondly as there is no reliable data for the frequency and volumes of individual feeds consumed by piglets during the first few days after birth (Pettigrew *et al.*, 1985) the feeding regime chosen for this experiment was that considered necessary to maintain normoglycaemia during the experimental period and to achieve satiety at the selected feeding times.

Fraser, D. & Rushen, J. (1992). Colostrum intake by newborn piglets. *Can. J. Anim. Sci.*, 72, 1-13.

Pettigrew, J. E., Cornelius, S. G., Moser, R. L. & Sower, A. F. (1985). A comparison of isotope dilution and weigh-sucker-weigh methods for estimating milk intake by pigs. *Can. J. Anim. Sci.*, 65, 989-992.

H_2O group accepted only about one-third to one-half of the water offered.

Table 3.1 Feeding times and the volumes of the feeds given to the piglets in the bottle fed groups.

FEED	TIME AFTER BIRTH (hrs)	VOLUME (ml/kg bw)
1st	2	20
2nd	4	20
3rd	6	20
4th	8	20
5th	10	20
6th	14	40
7th	18	40
8th	22	40

3.2.2. Feed Preparation

Sow colostrum was collected by hand milking during farrowing induced let-down and sow milk (7 - 10 days after farrowing) was collected by using the technique of Westrom *et al* (1987). Cow colostrum (within 3 days of calving) and cow milk were collected from cows on a Massey University dairy farm. Each type of feed was pooled, dispensed into small bottles (80 ml) and stored frozen at -20 °C. It was thawed and warmed to approximate body temperature in a water bath before feeding. Human infant formula (12% W/W) was freshly prepared using milk powder (SMA, John Wyeth & Brother, NZ) and warm boiled water. Distilled water was also warmed to body temperature before feeding. Protein, fat and lactose concentrations of the liquid foods (Table 3.2) were determined by the NZ Dairy Research Institute, Palmerston North, and the nutrient consumption of each group was calculated (Table 3.3). Protein content was estimated by multiplying the difference between the non-protein and total nitrogen concentrations by 6.38. The total nitrogen was determined by the Kjeldahl method (Rowland, 1938) using a Kjel-Foss automatic 16200, A/S N nitrogen analyzer (Foss Electric, Hillerod, Denmark). The non-protein nitrogen was determined by precipitating the protein in each sample with 12% trichloroacetic acid, followed by analysis of the filtrate nitrogen by the Kjeldahl method as described for the analysis of total nitrogen. The fat content was determined gravimetrically by extraction of the fat with an

ammonical alcohol and ether solution according to the Rose-Gottlieb procedure (International Dairy Federation, 1983). Lactose content was determined on an autoanalyzer system (Chem Laboratory Instrument Ltd., Hornchurch, England) using the ferricyanide reduction method (Technicon Method 120, 1973). Yellow ferricyanide was reduced by the lactose to give ferrocyanide which was measured by colorimeter at 420 nm.

Table 3.2 The nutritional value (%W/W) of the liquid feeds given to the piglets in the bottle fed groups (SC = sow colostrum; CC = cow colostrum; SM = sow milk; CM = cow milk; IF = infant formula).

(% W/W)	SC	CC	SM	CM	IF
Protein	18.07	10.41	3.64	3.32	1.53
Lipid	5.92	2.21	6.37	4.66	3.12
Lactose	2.45	3.30	5.78	4.96	6.57

Table 3.3 Nutrient consumption of the piglets in the bottle fed groups (SC = sow colostrum; CC = cow colostrum; SM = sow milk; CM = cow milk; IF = infant formula).

CONSTITUENTS	SC	CC	SM	CM	IF
Protein[1] (g/kg bw)	39.75	22.90	8.01	7.30	3.37
Lipid[2] (g/kg bw)	12.80	4.86	14.01	10.25	6.86
Lactose[3] (g/kg bw)	5.39	7.26	12.72	10.91	14.45
Total energy (kJ/kg bw)	1274	708	901	712	573
Usable energy[4] (kJ/kg bw)	591	314	764	586	515

[1] Protein = 17.2 kJ./g.
[2] Lipid = 38.9 kJ./g.
[3] Lactose = 17.2 kJ./g.
[4] Usable energy is the energy which is produced by metabolism of lactose and lipid; during the first 24 hours the piglet can metabolize protein only at a very low rate (Mellor & Cockburn,1986).

3.2.3. Specimen Collection

All piglets were weighed and euthanased by intracardiac injection of sodium pentobarbitone (Pentobarb 500, South Island Chemicals, NZ). The whole of the gastrointestinal tract was removed and dissected on ice according to the method of Widdowson *et al.* (1976). The small intestine was separated from the stomach and large intestine and divided into two parts at the junction of the duodenum and jejunum (the point where the duodenum emerges after passing under a loop in the coil of the colon). The lower part was cut into two equal lengths, the upper half was designated jejunum and the lower half ileum. The length of each section was measured with a metre ruler while the tract was suspended vertically. Both the jejunum and ileum were cut again into two equal lengths. In this way, the small intestine was divided into five parts: duodenum, upper jejunum, lower jejunum, upper ileum and lower ileum. A tissue sample about 1 cm long was taken from the middle of each part and fixed in Bouin's fluid (see Section 3.2.5.1) for histological analyses. The remaining tract was then emptied and opened longitudinally. The mucosa of each part was scraped off with the sharp edge of a microscope slide. The remaining muscle and the mucosa were weighed separately on an electric digital balance (Mettler AE166, Delta Range, Switzerland). A mucosal specimen was collected and stored frozen at -20 °C for biochemical analyses. The liver was weighed and a tissue sample collected and fixed in Bouin's fluid for histological examination.

Because of the relatively short length of the duodenum, the 1 cm tissue sample collected for histological study represented a substantial part of the total duodenal mucosal and muscular weights. Therefore the entire duodenum was weighed before sample collection and this value was used to calculate the total weight of mucosa and total weight of muscle according to the equation:

Total mucosal weight

$$= \frac{\text{Total duodenal weight before sampling}}{\text{Total duodenal weight after sampling}} \times \text{Mucosal weight after sampling}$$

Total muscular weight

$$= \frac{\text{Total duodenal weight before sampling}}{\text{Total duodenal weight after sampling}} \times \text{Muscular weight after sampling}$$

3.2.4. Specimen Analyses

3.2.4.1 Histological Measurement

The tissue was fixed in Bouin's fluid for 16 - 24 hours, then processed with an automatic tissue processor (SE400, Shandon, England) and embedded in wax using an embedding machine (Tissue-Tek, Miles Scientific, England). Cross sections (6 μm thick) were mounted, dewaxed and stained with Alcian Blue, Celestian Blue, Hematoxylin and van Gieson. An image of each section was projected on to a digitizer pad (Sketch Master LCD-1218, GTCO Corporation, USA) and the total wall thickness, muscular thickness, submucosal thickness, crypt depth, the villous height and width and the intestinal circumference were measured using a computer (Morse 386SX) and the Sigma Scan Program (Jandel Scientific, USA).

A single cross-section from each specimen was divided into 12 sectors and the value for each parameter calculated by averaging the measurements from all sectors, except for the circumference which was measured only once from each section. The validity of using only one cross-section from each specimen was tested by repeating the measurements on 5 cross-sections (no.'s 1, 11, 21, 31 and 41) from each of 5 tissue samples (duodenum, upper jejunum, lower jejunum, upper ileum and lower ileum) from a 24-hour-old suckled piglet, and statistically comparing the results to those obtained by measuring a single cross-section from each tissue sample. In each case the results obtained by measuring a single cross-section were within the 95% confidence limits of the results obtained by measuring multiple cross-sections. The data for this validation are shown in Appendix 3.1.

3.2.4.2 Measurement of Mucosal Cell Proliferation and Migration Distance

Mucosal cell proliferation and migration was determined for all the bottle fed piglets, but not those in the B and NS groups which were not injected with BrdU. All measurements were made after labelling the cells containing BrdU using the immunocytochemical method described by Holle & Birtles (1990). Tissue specimens were processed and embedded in wax as described in Section 3.2.4.1. All sections (4 μm thick) were mounted on glass slides and deparaffinized in two changes of xylene and rehydrated in a graded series of ethanol washes to water. The slides were then rinsed in 0.01 M phosphate buffered saline (PBS) pH 7.4 (see Section 3.2.5.2), treated with 1% bovine serum albumin (BSA) (Sigma, USA) in PBS for 5 minutes and incubated with mouse anti-BrdU antibody (Amersham International, UK) diluted 1:100 in 1% BSA in PBS for 60 minutes at room temperature in a humidified chamber. After incubation, all slides were washed three times in PBS, for 1 minute each time, and incubated for 30 minutes under the same conditions as described above with biotinylated anti-mouse IgG (Amersham International, UK) diluted 1:200 in 1% BSA in PBS. The slides were washed three times in PBS and incubated again with streptavidin-biotinylated horseradish peroxidase complex (Amersham International, UK) diluted 1:300 in 1% BSA in PBS for 20 minutes. After another three washes in PBS, the slides were incubated for 4 minutes with diaminobenzine solution (DAB) (see Section 3.2.5.3). The slides were then washed in water, counterstained with 1% Eosin for 15 seconds, dehydrated through graded ethanol, cleared in xylene and mounted. The labelled dividing cells were identified by the black-red colour of their nuclei. All dividing cells from 12 crypts randomly chosen were counted and the areas of those crypts measured. The number of cells per crypt area was calculated by dividing the total number of cells counted by the total area of the crypts. The distance between the base of the crypt and the labelled cell which had migrated farthest up the villus was measured. This distance was not the true migration distance as the precise starting point of migration was not located. Thus assuming that every cell started migrating from the same level i.e. at the base of the crypt, this distance could be compared between groups and was defined as the *relative migration distance*. The digitizer pad and computer program described in Section 3.2.4.1. was used to count the labelled cells and measure the relative migration

distances.

3.2.4.3 Biochemical Analyses of Mucosal Specimens

A mucosal sample (100 mg) was homogenized with 5 ml cold 0.9% saline for 90 seconds in an ultrasonic disintegrator (Soniprep 150, MSE Scientific Instrument, England) with an exponential microprobe amplitude of 8 microns. The homogenate was then divided and duplicate samples were analyzed for lactase, protein, DNA and RNA concentrations according to the methods described below.

3.2.4.3.1 *Lactase Analysis*

As sucrase and maltase activities are either absent or present at very low levels in the newborn piglet (Bailey *et al*, 1956; Walker, 1959; Dahlqvist, 1961; Hartman *et al*, 1961; Manners & Stevens,1972; Shu *et al*., 1992), only lactase activity was examined. The lactase activity was assayed using the method described by Dahlqvist (1964) as modified by Manners & Stevens (1972). Briefly, a mixture of 0.1 ml tissue homogenate (dilution 1:200 in 0.9% NaCl), 0.2 ml phosphate buffer (Sigma, USA) 0.1 M pH 6.0 and 0.2 ml lactose (Sigma, USA) was incubated at 37 °C for 20 minutes. The hydrolysis reaction was terminated by submerging the tubes in boiling water for 2 minutes. The blank for each sample was prepared in the same way as the sample but the reaction was terminated immediately without any incubation. Lactase activity was indicated by the amount of glucose produced during the incubation period. The amount of glucose in the sample and blank were determined by adding 2 ml Tris-glucose-oxidase reagent (TGO) (see Section 3.2.5.4) and incubating again at 37 °C for 60 minutes. The optical densities of the sample and blank were measured using a spectrophotometer (SP6-550 UV/VIS, Pye Unicam, England) at wavelength 436 nm. The results were compared with a standard curve of glucose concentration (Sigma, USA) ranging from 0 to 1 mmol/L (0, 0.25, 0.50, and 0.75). The lactase activity of each sample was expressed as µmol disaccharide hydrolysed per g wet tissue per min. This was calculated as shown below.

Lactase activity = (S-B) x 50 µmol/g.min

S = glucose concentration of each sample.

B = glucose concentration of blank of each sample.

3.2.4.3.2 *Protein Analysis*

The protein concentration was determined by the method described by Lowry *et al* (1951). The tissue homogenate (0.2 ml, dilution 1:200 in 0.9% NaCl) was mixed with 2 ml alkaline copper solution (see Section 3.2.5.5) and left standing for 10 minutes. It was then mixed with 0.2 ml diluted Folin reagent (see Section 3.2.5.6) and left standing for 30 minutes. The solutions for the standard curve were prepared in the same way as the samples by using bovine serum albumin (BSA) (Fraction V, Sigma, USA) at concentrations of 0, 125, 250, 500, 750 and 1000 µg/ml. The optical density of the standard solutions and samples was measured with a spectrophotometer at the wavelength of 500 nm. Protein concentration of each sample was expressed as mg/g wet tissue.

3.2.4.3.3 *RNA and DNA Analyses*

RNA and DNA were extracted by the method of Johnson & Chandler (1973). A 2 ml sample of tissue homogenate (dilution 1:100 in 0.9% NaCl) was mixed with 1 ml perchloric acid solution (May & Bager, England) 0.6 N and left standing in ice for 10 minutes. The mixture was then centrifuged (TJ-6 Centrifuge with TJ-R Refrigeration Unit, Beckman, USA) at 500*g* and 4 °C for 10 minutes. The supernatant was discarded and the precipitate was twice washed with 2 ml perchloric acid 0.2 N. The precipitate was then dissolved in 2 ml KOH (BDH Chemicals, England) 0.3 N and incubated at 37 °C for 60 minutes. After incubation, it was mixed with 1 ml perchloric acid 1.2 N and left standing in ice for 10 minutes, before centrifuging at 500*g* for 15 minutes. The supernatant was collected and the precipitate washed with 2 ml perchloric acid 0.2 N. The washings and the supernatant were then pooled for RNA measurement. RNA concentration was estimated according to the method described by Fleck & Begg (1965). The optical density of the RNA extracts was measured (see Section 3.2.4.3.1) at the

wavelengths of 232 nm and 260 nm. The RNA concentration was calculated using the equation:

RNA concentration (mg/g wet tissue)

$= [((3.40 \times OD_{260\ nm}) - (1.44 \times OD_{232\ nm}))/ 0.068] / 4$

The remaining precipitate from the RNA extracts was dissolved in 4 ml perchloric acid 1.0 N and left standing in boiling water for 10 minutes. The mixture was then centrifuged at 500*g* for 20 minutes and the supernatant collected for DNA analysis. The DNA concentration was analyzed with diphenylamine reaction according to the method described by Burton (1956) as modified by Giles & Myers (1965). If a sample had a high concentration of DNA, the DNA extract was diluted with perchloric acid 1.0 N to make the concentration between 5 and 40 µg/ml. The standard solutions were prepared by diluting standard DNA (Herring sperm, Boehringer Mannheim, Germany) with perchloric acid 1.0 N to concentrations ranging from 0 to 40 µg/ml (0, 10, 20, 30 and 40). One ml of each standard solution or each tissue sample was mixed with 1 ml diphenylamine (Sigma, USA) 4% in glacial acetic acid (BDH Chemicals, England) and 0.1 ml aqueous acetaldehyde (BDH Chemicals, England) 1 mg/ml. The mixture was incubated at 30 °C for 16 - 20 hours. The optical density of the mixture was measured using a spectrophotometer (see section 3.2.4.3.1) at wavelength 595 nm. and compared with the standard curve concentration. The DNA concentration of each sample was expressed as mg/g wet tissue.

3.2.5. Reagent Preparation

All chemicals used to prepare the following solution were analar grade.

3.2.5.1 Bouin's Fluid

Bouin's fluid was prepared by mixing 75 ml saturated aqueous solution of picric acid (BDH Chemicals, England), 25 ml formalin (40% formaldehyde)(BDH Chemicals, England) and 5 ml glacial acetic acid (BDH Chemicals, England).

3.2.5.2 Phosphate Buffered Saline (PBS)

PBS was prepared by dissolving 1.09 g KH_2PO_4 (BDH Chemicals, England), 4.54 g Na_2HPO_4 (May & Baker, England) and 36 g NaCl (May & Baker, England) in 4 litres of distilled water.

3.2.5.3 Diaminobenzine Solution (DAB solution)

This solution was prepared within 30 minutes of use by dissolving 5 mg of diaminobenzine tetrahydrochloride dihydrate (Aldrich Chemical Company, USA) in 10 ml PBS and adding 12 µl H_2O_2 solution (BDH Chemicals, England) and one drop of cobalt-nickel formulation (1% $CoCl_2$ and 1% $NiCl_2$ in distilled water).

3.2.5.4 Tris-Glucose-Oxidase Reagent (TGO reagent)

The TGO reagent was prepared by mixing 5.58 mg of glucose oxidase (Sigma, USA) in 50 ml Tris buffer (Sigma, USA) 0.5 N, pH 7.0. The mixture was shaken for 30 seconds before adding 0.5 ml peroxidase solution (Serva, USA) 1 mg/ml H_2O, 1 ml 3,3' dimethoxy benzidine solution (O-dianisidine) (Sigma, USA), 10 mg/ml 95% ethanol and 1 ml of detergent solution (Triton X-100, Rohm and Hass, NZ) diluted 1:4 in 95% ethanol. The mixture was then mixed with Tris buffer to make the volume up to 100 ml.

3.2.5.5 Alkaline Copper Solution

Alkaline copper solution was prepared by mixing 50 ml Na_2CO_3 (May & Baker, England) 2% in NaOH (BDH Chemicals, England) 0.1 N and 1 ml of $CuSO_4.5H_2O$ (May & Baker, England) 0.5% in 1% K-Na tartrate (BDH Chemicals, England). This solution was made up fresh before use.

3.2.5.6 Diluted Folin Reagent

Diluted Folin reagent was prepared by diluting 2 N Folin-Ciocalteu's phenol reagent (Sigma, USA) 1:1 with glass distilled H_2O to give the final concentration of 1 N in acid.

3.2.6 Statistical Analyses

As the variation in body weight of the piglets at the beginning of the experiment could have affected the outcome of the experiment, all data were subjected to analysis of covariance using body weight at birth as a covariate. The following pre-planned comparisons were made to determine the effect of diet on small intestinal development.

(a) A comparison between colostrum and milk (with 3 contrasts):
SC+CC vs SM+CM
SC vs SM
CC vs CM
(b) A comparison between species (with 3 contrasts):
SC+SM vs CC+CM
SC vs CC
SM vs CM
(c) A comparison between natural and artificial milk:
SM+CM vs IF
(d) A comparison between colostrum/milk and water:
SC+CC+SM+CM+IF vs H_2O
(e) A comparison between 0 hr and water feeding:
B vs H_2O
(f) A comparison between natural sucking and bottle feeding of sow colostrum:
NS vs SC
(g) A comparison between 0 hr and 24 hrs:
B vs NS+SC+CC+SM+CM+IF+H_2O

In addition to these pre-planned comparisons, each group was compared to each of the other 7 groups. The level of significance of the pre-planned comparisons is shown below the legend of each figure, whereas that of the individual comparisons is shown in the text.

All data were analyzed by computer using the SAS and MINITAB programs. The level of significance is expressed as * = $P<0.05$, ** = $P<0.01$ and *** = $P<0.001$. The means and S.D.'s for the results presented in this chapter are given in Appendices 3.2 - 3.5.

3.3 RESULTS

The body weights of the naturally suckled piglets increased during the first 24 hours. These increases were accompanied by increases in small intestinal length and weight, mucosal DNA, RNA and protein content, and the height and width of the villi. Lactase activity also increased. When comparison was made between the groups fed different diets by bottle, those piglets fed colostrum, especially sow colostrum, had the greatest increases in small intestinal weight and length. Furthermore colostrum fed piglets generally exhibited greater increases in most other parameters of small intestinal growth than milk fed piglets. The detailed results are presented below.

3.3.1 Body Weight Change, and Liver and Pancreatic Weights

The body weights of the piglets fed colostrum and those naturally suckled were significantly ($P<0.001$ and $P<0.01$ respectively) greater than the body weights of the piglets at birth (Fig. 3.1). When the effects of the different diets on body weight gain were compared, the piglets fed colostrum had greater increases in body weight than did those fed milk, and those fed sow colostrum had greater increases in body weight than did those fed cow colostrum. When comparison was made between the effects of the colostrum plus milk from sow or cow, the sow colostrum and sow milk fed groups combined had greater increases in body weight than did those fed cow colostrum and cow milk combined. The body weights of the piglets in the H_2O group were significantly less than those of the piglets in the other feeding groups combined.

The weight of the liver did not change significantly from birth in the piglets of the NS group (Fig. 3.1). Also there was no significant difference in the liver weight between the B group and the SC, CC, SM or CM groups. The IF group had the highest liver weight which was significantly ($P<0.01$ to $P<0.05$) higher than those of the other groups when compared to each group separately or when compared to the SM and CM groups combined. By contrast in the H_2O group, the liver weight decreased significantly from birth and in relation to the other bottle fed groups combined.

The pancreatic weight for all of the 24-hour groups combined was significantly higher than that of the B group (Fig. 3.1). The increase in pancreatic weight was more pronounced in the piglets fed sow colostrum and those in the NS group, than in other bottle fed groups. Also the two colostrum fed groups combined had significantly higher pancreatic weights than did the combined milk fed groups. The piglets in the H_2O fed group appeared to have the lowest pancreatic weight among the bottle fed groups.

3.3.2 Small Intestinal Length and Weight

The total length of the small intestine and the lengths of the individual segments were all significantly greater in the 24-hour groups combined compared to the B group (Fig. 3.2). The piglets fed sow colostrum had greater small intestinal lengths than did those fed sow milk, and the group fed sow milk plus that fed cow milk had a greater small intestinal length than did the group fed infant formula. These effects of colostrum and milk were seen in both the jejunum and ileum, but not in the duodenum. The water fed piglets had the shortest small intestine of all the bottle fed piglets.

The total weight of the intact small intestine and the total mucosal weight were significantly greater in the 24-hour groups combined than in the B group (Fig. 3.3). Only the colostrum fed groups and NS group, but not those of the SM, CM or IF groups, had an intact weight and mucosal weight of the small intestine significantly ($P<0.001$ to $P<0.01$) greater than those of the B group. Colostrum feeding resulted in significantly greater intestinal tissue weights than did milk feeding, and sow colostrum was more effective than cow colostrum. This was true for the intact, mucosal and muscular weights of small intestine. Furthermore piglets fed sow colostrum from a bottle had greater intact and mucosal intestinal weights than did naturally suckled piglets. Intestinal tissue weights for the IF group were not significantly different from those for the SM or CM groups. Nor were there any differences in intestinal tissue weights between the SM and the CM groups. The intact, mucosal and muscular small intestinal weights for the H_2O group were comparable to those of the B group and significantly less than those for all the other bottle fed groups combined.

These effects of diet on intestinal tissue weights were seen throughout the entire length of the small intestine but were most pronounced in the jejunum and ileum (Fig. 3.4). The greater intestinal weights in the colostrum fed and naturally suckled groups were due to greater mucosal weights (Fig. 3.5) and to a lesser extent, muscular weights (Fig 3.6).

3.3.3 Microscopic Structures of the Small Intestine

Small intestinal circumference in the jejunum and upper ileum increased during the first 24 hrs after birth in the NS group and all of the bottle fed groups combined (Fig. 3.7). These increases were more pronounced in the colostrum fed piglets compared to the other bottle fed piglets. Thus the circumference of the upper ileum was significantly greater in the piglets given colostrum compared to those given milk. The piglets fed sow colostrum had greater intestinal circumferences in the lower jejunum and ileum than did those naturally suckled. The group fed infant formula had a significantly greater duodenal circumference than did those fed either sow or cow milk. The jejunal and upper ileal circumferences were smallest in the H_2O group compared to the other bottle fed groups.

Colostrum feeding significantly increased wall thickness in both the jejunum and ileum, but not in the duodenum, when compared to milk feeding (Fig. 3.8). Similarly, increases in wall thickness in the ileum were more pronounced when the piglets were fed with sow colostrum or milk than with cow colostrum or milk. When all 24-hour groups were combined, there was no statistically significant difference in wall thickness between the piglets at birth and those at 24-hours except in the lower ileum. However, both the NS and SC groups had greater wall thicknesses in the upper and lower ileum ($P<0.001$ to $P<0.05$) compared to each of the other groups.

It is instructive to consider the contribution of the different components of the intestinal wall to these changes.

Submucosal (Fig.3.9) and muscular (Fig. 3.10) thicknesses appeared to be less affected by feeding the different diets than was overall wall thickness. The submucosa of the duodenum and lower jejunum was significantly thicker in the H_2O group compared to all of the other bottle fed groups combined, and the muscular thickness of the duodenum in the H_2O group was thicker than those in the other bottle fed groups combined. Furthermore, the muscular thickness in the ileum of the colostrum fed groups combined was thinner than those of the milk fed groups combined.

The villi (Fig. 3.11) in the duodenum and lower ileum were significantly higher in all of the 24-hour groups combined compared to the B group. Colostrum fed groups had significantly greater villous heights in the jejunum and ileum, but not in the duodenum, when compared to milk feeding. The sow colostrum fed group had significantly higher villi than did the cow colostrum group and the SC and SM groups combined had significantly higher villi than the CC and CM groups combined.

Villous width (Fig. 3.12) in the upper and lower jejunum and upper ileum were increased significantly in all of the 24-hour groups combined compared to the B group. Only in the ileum was the villous width in the piglets fed colostrum significantly greater than in the piglets fed milk, and sow colostrum feeding caused significantly greater villous width than did cow colostrum feeding. The H_2O group had significantly thinner villi in the jejunum and upper ileum than did all the other bottle fed groups combined.

The duodenal crypts of all the 24-hour groups combined tended to be deeper than those of the B group (Fig. 3.13), but generally crypt depth was not affected by diet with the exception of the H_2O group which had shallower crypts than all the other bottle fed groups combined.

Microscopic examination of the mucosae of the colostrum fed groups (Figs. 3.14A,B,C,D), especially the SC group, revealed swollen epithelial cells along the villi particularly in the jejunum and ileum. These cells contained large vacuoles. The nuclei were mostly located close to the apex of the cells in the jejunum and upper ileum and close to the base of the cells in the lower ileum. In the SM, CM and IF groups, the

mucosal cells appeared to contain smaller vacuoles compared to those in the mucosal cells of the colostrum fed piglets. In these milk fed piglets, apical and basal locations of the nuclei were seen in the upper ileum (Figs. 3.15A,B,C) whereas in the lower ileum the nuclei were located basally (Figs. 3.16A,B,C). In the B and H_2O groups, there were no clearly visible vacuoles, and the nuclei of the cells in the lower ileum in the B group (Fig. 3.17A) were mainly positioned in the middle of the cells whereas the nuclei in the lower ileum in the H_2O group (Fig. 3.17B) were located at the base of the cells.

3.3.4 Number of Dividing Cells and Cell Migration Rate

Compared to all the bottle fed piglets, those fed water generally had a high number of labelled dividing cells per crypt area in the upper ileum (Fig. 3.18). There were more labelled dividing cells per crypt area in the upper jejunum of piglets fed cow milk compared to those fed sow milk. This was due to smaller total crypt areas (Fig. 3.19) in the cow milk fed group rather than a greater total number of dividing cells (Fig. 3.20). In the upper ileum of the piglets fed water, however, the greater number of labelled dividing cells per crypt area was due to the greater total number of dividing cells rather than a smaller total crypt area.

Relative cell migration distance was greatest in the sow colostrum fed piglets. This was evident in the jejunum and ileum but not the duodenum (Fig. 3.21). The relative migration distance was significantly greater in the lower jejunum and upper ileum of the two colostrum fed groups combined compared to the two milk fed groups combined, and sow colostrum had a greater effect on relative cell migration distance than did either sow milk or cow colostrum. With the exception of the duodenum, the H_2O group had a shorter relative migration distance than all of the other bottle fed groups combined.

3.3.5 DNA, RNA and RNA:DNA Ratio in the Small Intestinal Mucosa

During the first 24 hrs the mucosal DNA content increased significantly throughout the length of the small intestine in all groups combined (Fig. 3.22). The

increases were most pronounced in the piglets fed either sow colostrum or infant formula. Sow colostrum fed piglets had a higher DNA content in both the upper and lower jejunum than did sow milk fed piglets. The piglets fed infant formula had more DNA in the lower jejunum and upper ileum compared to sow and cow milk fed piglets. Significant increases from birth in DNA content were also seen in the piglets fed water, especially in the jejunum, but not in the ileum.

Of particular interest was the effect of feeding on DNA concentrations which was different from that on DNA content. Whereas there was no statistically significant difference in DNA concentrations between the piglets in the B group and all the 24-hour groups when combined, colostrum feeding decreased mucosal DNA concentrations (Fig. 3.23). Thus the DNA concentration in the two colostrum fed groups combined was significantly less than that of the two milk fed groups combined in each of the 5 parts of the small intestine. Furthermore there were significantly lower DNA concentrations throughout the length of the small intestine in the piglets fed sow colostrum compared to those fed sow milk. Also those fed cow colostrum had lower DNA concentrations than those fed cow milk, except in the lower ileum.

Duodenal DNA concentration in the group fed infant formula was significantly higher than that in the two milk fed groups combined. The concentration of DNA in the H_2O group was significantly higher throughout the intestine than the B group. Also the H_2O group had significantly higher mucosal DNA concentrations in the duodenum and jejunum than did the combined feeding groups.

The total RNA content of the intestinal mucosa in the combined 24-hour groups was significantly higher than that of the B group in all parts of the small intestine except the lower ileum (Fig. 3.24). These increased levels of mucosal RNA were most pronounced in the piglets fed sow colostrum. Total duodenal and jejunal RNA levels for this group were significantly higher than those for the sow milk fed group. The total RNA content of the intestinal mucosa in the SC group was not significantly different from that in the NS group except for the duodenum where the NS group had a lower RNA content.

The mucosal RNA content was very low in the piglets fed cow colostrum. In this group the RNA content was significantly lower throughout the intestine compared to the group fed sow colostrum and significantly lower in the jejunum and ileum compared to the cow milk fed group. Also the RNA contents of the jejunum and upper ileum were lower in the groups fed cow colostrum and cow milk combined than in the groups fed sow colostrum and sow milk combined. This difference was largely due to the very low mucosal RNA content in the cow colostrum fed piglets together with the high mucosal RNA content in the sow colostrum fed piglets.

The effects of feeding on RNA concentrations were similar in many respects to the effects on DNA concentrations. Whereas there was no significant difference for RNA concentration between the B group and all the 24-hour groups combined, the concentrations of RNA in the intestinal mucosa of the colostrum fed groups combined were significantly lower than those of the milk fed groups combined in both the jejunum and ileum, but not in the duodenum (Fig. 3.25). Also the lower jejunal and ileal RNA concentrations were significantly less in the piglets fed sow colostrum than in the piglets fed sow milk.

The mucosal RNA concentration, like the RNA content, was very low in the piglets fed cow colostrum. In this group, mucosal RNA concentrations were significantly lower in both the jejunum and ileum compared to the cow milk fed group, and in the jejunum compared to the sow colostrum fed group. The H_2O group had significantly higher mucosal RNA concentrations in the lower jejunum and the upper ileum than did the combined feeding groups. This was most likely due to the relatively low RNA concentrations in the NS, SC and CC groups.

The RNA:DNA ratios in the jejunum and upper ileum were significantly higher in the B group compared to the combined 24-hour groups (Fig. 3.26). This was due in part to the low RNA:DNA ratio in the CC group. Since the RNA content in the jejunum and ileum of the CC group was very low, the RNA:DNA ratio for this group was also low. The SC group had the highest RNA:DNA ratio in the duodenum, and this caused a significant difference in the RNA:DNA ratio between the colostrum and milk

fed groups and between sow and cow. On the other hand, in the jejunum and ileum the RNA:DNA ratio of the SC and SM groups combined was significantly higher than that of the CC and CM groups combined because of the lower RNA:DNA ratio for the CC group. Also the RNA:DNA ratios in the upper and lower jejunum of the water fed group were significantly lower than those of the B group.

3.3.6. Protein and Protein:DNA Ratio in the Small Intestinal Mucosa

Whereas there were noticeable differences between the concentration and the content of both RNA and DNA, the protein concentration (Fig. 3.27), protein content (Fig. 3.28) and protein:DNA ratio (Fig. 3.29) exhibited parallel patterns which clearly indicated the higher mucosal protein in the colostrum fed groups than in the milk fed groups. The mucosal protein concentrations in the jejunum and ileum of the combined 24-hour groups were significantly higher than the B group, and those of the combined fed groups were also significantly higher than the H_2O group. There were no significant differences in mucosal protein concentrations throughout the small intestine between the B group and the SM, CM, IF or H_2O groups when compared individually. The colostrum fed groups combined had higher mucosal protein concentrations than did the milk fed groups combined in all parts of the small intestine. Also the SC group had significantly ($P<0.001$) higher mucosal protein concentrations than that of every group when compared individually, particularly in the lower jejunum and the ileum. Because of the high mucosal protein concentration in the SC group, the SC and SM groups combined also had significantly higher mucosal protein concentration than those of the CC and CM groups combined in the lower jejunum and the ileum.

The mucosal protein content of the combined 24-hour groups was significantly higher in each part of the small intestine compared to the B group, and those of the combined fed groups were also significantly higher than in the H_2O group. There was no significant difference in mucosal protein content in the jejunum and ileum between the B group and the SM, CM, IF or H_2O groups when compared individually. The SC group had a significantly ($P<0.001$ to $P<0.01$) higher mucosal protein content than that of every group when compared individually. With the exception of the NS group, this

was evident throughout the length of the intestine. The mucosal protein content of the SC group was significantly ($P<0.01$ to $P<0.05$) higher than the NS group in all parts except in the upper jejunum. The high mucosal protein content in the SC group undoubtedly contributed to the large significant differences between the colostrum and milk fed groups, and between the SC + SM and CC + CM groups.

There was no significant difference between the protein:DNA rations for the combined 24-hour fed groups and the B group. The combined fed groups had higher protein:DNA ratios than did the water fed group in all parts except the lower ileum. There were no significant differences between the mucosal protein:DNA ratios for the B group and the SM, CM, IF or H_2O groups when compared individually, in all parts except the duodenum; the B group had significantly ($P<0.05$ and $P<0.01$ respectively) higher protein:DNA ratio in the duodenum than did the CM and IF groups. The high protein:DNA ratio in the SC group caused the difference in the comparison between the colostrum and milk fed groups, and between sow and cow. There were no significant differences between the protein:DNA ratios for the SC group and the NS group, except in the duodenum where it was higher in the SC group.

3.3.7 Lactase Activity

The total lactase activity showed no significant difference between the 24-hour groups combined and the B group except in the lower jejunum (Fig. 3.30). The total lactase activity of the piglets naturally suckled was significantly ($P<0.01$ to $P<0.05$) increased from birth in all parts except the duodenum. The colostrum fed groups combined had significantly higher total lactase activity than that of the milk fed groups combined in the duodenum and upper jejunum. The total lactase activity of the IF group was significantly higher than the milk fed groups combined only in the upper ileum.

There was no significant difference between the lactase activity per mucosal weight for the B group and the 24-hour groups combined (Fig. 3.31). Feeding different diets appeared to have greater effect on lactase activity per mucosal weight than on total

lactase activity as there were more significant differences between groups for the lactase activity per mucosal weight than there were for the total lactase activity. Lactase activity per mucosal weight of the water fed group was significantly higher than that of the other fed groups combined in all parts except for the lower ileum. The IF group exhibited significantly higher lactase activity than the SM and CM groups combined in the jejunum and upper ileum. The colostrum fed groups combined had significantly higher lactase activity than did the milk fed groups combined only in the duodenum.

Lactase activity per DNA weight (i.e. cellular lactase activity) exhibited no significant difference between the B group and the 24-hour groups combined (Fig. 3.32). Feeding different diets by bottle significantly affected cellular lactase activity only in the duodenum. The SC group had significantly ($P<0.001$ to $P<0.05$) higher cellular lactase activity in the duodenum when compared to every group individually. The high duodenal lactase activity in the SC group caused the significant difference in the comparison between the colostrum feeding and the milk feeding, and between the SC + SM and CC + CM groups. In the upper and lower ileum, however the NS group exhibited significantly higher cellular lactase activity than the SC group.

3.4 DISCUSSION

The major findings of this study are as follows. During the first 24 hours after birth, the small intestine of newborn piglets fed sow colostrum exhibited greater increases in both weight and length compared to those fed other diets. Cell swelling, hyperplasia and in the duodenum, hypertrophy, contributed to the greater increases. Colostrum feeding enhanced lactase activity and feeding cow colostrum, but not sow colostrum, decreased mucosal RNA. Piglets fed sow colostrum from bottles exhibited greater intestinal growth than those naturally suckled. Of interest is an increase from birth in the DNA content of the intestinal mucosa of the piglets in all groups, including the water fed group. Colostrum feeding also enhanced pancreatic growth and feeding infant formula increased liver weight.

3.4.1 Effects of Sow Colostrum and Milk on Mucosal Weight and Protein Accumulation

During the first 24 hours the piglets fed colostrum had greater increases in both the length and weight of the small intestine compared to milk fed piglets, and those fed sow colostrum had the greatest increases (Figs. 3.2, 3.3). The major increase in intestinal weight was largely due to an increase in mucosal mass (Fig. 3.5) and this corresponded to increases in villous height and villous width (Figs. 3.11, 3.12). The increases in the mucosal mass could have been due to increases in cell size or cell number, or both.

There are two possible causes for an increase in cell size; cell swelling and cell growth. Xu *et al.* (1992) have presented evidence for transient cell swelling related to protein retention in the small intestine of piglets suckled naturally during the first 72 hours after birth. Cell swelling reached its peak at 24 hours and had disappeared by 72 hours. In the present study, mucosal cell swelling occurred, but only in the colostrum fed piglets (Figs. 3.14 - 3.17). Microscopic examination of the intestinal mucosa showed swollen cells, with large vacuoles in their cytoplasm, along the villi in the jejunum and ileum, particularly in the groups fed sow colostrum. These cells differed

from the smaller villous cells, which were without large vacuoles, in the mucosae of the piglets fed either sow or cow milk or those fed infant formula. Xu *et al.* (1992) reported that the epithelial cell nuclei were located at the apex of the cell in the jejunum and upper ileum, and at the base of the cell in the lower ileum in suckling piglets. Similar positions of nuclei to those reported by Xu *et al.* (1992) were also found in the present piglets fed colostrum. Furthermore, the position of nuclei close to the base of the cells was exhibited in the lower ileum of the piglets in all feeding groups, including the H_2O group (Fig. 3.17). The proximity of the nucleus to the base depended on the size of vacuole in the cell, the larger the vacuole the closer the nucleus to the base.

The presence of the nuclei in the base of the cells in the lower ileum may not be entirely due to the stage of absorption of colostral protein as suggested by Xu *et al.* (1992), as it occurs in fetal piglets (Hardy *et al.*, 1971) and newborn piglets fed water (Fig. 3.17B). Also Hardy *et al.* (1971) reported the location of epithelial cell nuclei in the terminal ileum of fetal and newborn unsuckled piglets was the same as that seen in suckled piglets. The basal location of nuclei in the distal ileum is also found in 3 to 15 day-old piglets fed infant formula from birth (D. Shu, H.V. Simpson, R-J. Xu, D.J. Mellor, G.W. Reynolds & R.B. Marshall; unpublished observation).

There is evidence that the protein content of the diet is a major determinant of protein accumulation in the small intestinal mucosa during the first 24 hours. Colostrum normally contains more protein than does milk. In the present study the piglets fed sow colostrum received approximately 5 times more protein than did those fed sow milk (39.75 vs 8.01 g/kg). Furthermore, the mucosal protein concentration and content and the protein:DNA ratio (Figs. 3.27 - 3.29) were all significantly higher in the colostrum fed piglets compared to the milk fed animals, particularly in the jejunum and ileum where active macromolecular absorption occurs in newborn piglets (Pierce & Smith, 1967; Clarke & Hardy, 1971). Also there was a positive correlation ($P<0.01$) between the protein concentrations of the diets and the protein contents of each part of the small intestine.

However, protein within mucosal cells is composed of protein taken up from exogenous sources and protein synthesized within the cells themselves. Although the present methods could not distinguish between protein synthesis and protein uptake in the small intestine, the method of Burrin *et al.*(1992) did. A review of Burrin's work revealed several important points. Firstly, the net increase in the small intestinal (jejunal and ileal) protein content in milk fed piglets, obtained by comparing water fed and milk fed groups, was similar to the net increase in protein synthesis determined dynamically using labelled amino acids (Burrin *et al.*, 1992). Thus the net increase in small intestinal protein content in the milk fed group in the present experiment must have been virtually entirely due to *de novo* synthesis of protein, and the contribution of the retention of exogenous protein in these milk fed piglets would therefore have been virtually zero. Secondly, 83% of the increase from birth in mucosal protein content in the colostrum fed piglets was due to the retention of exogenous protein and 17% was due to the *de novo* synthesis which was higher (by 9%) in colostrum fed than in milk fed piglets (Burrin *et al.*, 1992). Burrin *et al.* (1992) concluded that the increased rate of intestinal protein accretion in colostrum fed piglets was largely due to absorption and retention of colostral protein. Similarly, Patureau-Mirand *et al.* (1990) found that in newborn colostrum fed lambs, the increase in intestinal protein content resulted primarily from the retention of colostral protein in the mucosal cells. Therefore it is likely that most of the increase in mucosal protein content in the piglets fed colostrum in the present study would be due to exogenous protein retention, rather than protein synthesis.

3.4.2 Effects of Sow Colostrum and Milk on Small Intestinal Growth

In general, growth of a tissue can be classified into hyperplasia and hypertrophy. The former is defined by the increase in cell number and is normally indicated by the increase in DNA content, the latter is defined by the increase in cell size, i.e. cell growth (Corring *et al.*, 1982). There are several indicators of cellular hypertrophy, some of which are fresh weight:DNA, protein:DNA and RNA:DNA ratios (Winick et al, 1972; Corring *et al.*, 1982). Under certain circumstances some of these ratios may not be suitable indicators of hypertrophic growth. For example in the present experiment cell swelling related to colostral protein accumulation increased both cell size (as

demonstrated by photomicrographs of the villi) and protein:DNA ratio, therefore in newborn piglets increased cell size and protein:DNA ratio are not accurate indicators of hypertrophic growth. Similarly, the mucosal wet weight:DNA ratio is presumably affected by the mass of protein accumulated within the mucosal cells. In the present study therefore RNA:DNA ratio was chosen as an appropriate indicator of hypertrophic growth.

Based on the RNA:DNA ratios obtained in the present experiment, it appears that cell swelling, not cell growth, is the major contributor to the greater mucosal cell size in the colostrum fed piglets compared to the milk fed piglets. Evidence for the lack of cell growth is provided by the absence of an increase from birth in the RNA:DNA ratios in the combined groups (Fig. 3.26). Only in the duodenum did the RNA:DNA ratio increase significantly and this was mainly due to a large increase in the duodenal RNA:DNA ratio in the SC group. As the mucosal DNA content (Fig. 3.22) and RNA content (Fig. 3.24) increased in parallel in the piglets fed sow colostrum, mucosal growth will have been due primarily to an increase in cell number (hyperplasia) and not to growth of the individual cells (hypertrophy). This hyperplastic growth was apparently accompanied by an increase in protein synthesis. Burrin *et al.* (1992) reported that jejunal protein synthesis was nearly 300% greater in piglets fed either milk or colostrum than in piglets fed water, and jejunal protein synthesis was 9% more in colostrum fed than in milk fed piglets. Similarly intestinal protein synthesis in lambs fed colostrum during the first 24 hours was more active than that of milk fed animals (Patureau-Mirand *et al.*, 1990).

There is a discrepancy between the mitotic index of crypt cells (BrdU labelling) and the hyperplasia index (DNA content) of the small intestinal mucosa. Colostrum feeding did not increase the number of BrdU labelled dividing cells per crypt area (Fig. 3.18), indicating mucosal cell proliferation might not have been affected by colostrum. On the other hand, the mucosal DNA content (Fig. 3.22) increased significantly in the colostrum fed piglets indicating an increased number of mucosal cells. This apparent anomaly may have been due to a combination of several factors. Firstly, a single injection of BrdU was given at birth, hence most dividing cells would have been

labelled during the early stages of the experiment when feeding had just begun, and the amount of BrdU within each piglet would have decreased rapidly with time (V.J. Grant, M.J. Birtles, P.A. Clifton & D.N. Palmer; unpublished observation) so that no cells would have been labelled during the later stages of the experiment. Secondly, during the first 24 hours the dividing cells in the crypts which had been labelled would have migrated out of the crypt and onto the villi, especially in the sow colostrum fed piglets which had faster migration rates. Thirdly, the larger crypt size in the colostrum fed groups gave larger total crypt areas, resulting in a lower number of labelled dividing cells per crypt area. In view of these considerations the mucosal DNA content appears likely to be a more reliable index of mucosal growth than the results of BrdU labelling of dividing cells using the present timing of BrdU administration. It may still be concluded therefore that colostrum feeding stimulated mucosal cell proliferation more than did the other diets.

Although the RNA contents (Fig. 3.24) in the colostrum fed groups combined were higher than those of the milk fed groups combined, particularly in the jejunum and ileum, the mucosal RNA concentration (Fig. 3.25) was lower. The lower RNA concentration was most likely due to the large amount of protein in the mucosal cells diluting the RNA, but not affecting its total content. The lower concentration of DNA (Fig. 3.23) in the mucosa of colostrum fed piglets can also be explained by the high protein content of the cells in the same way as occurred with the RNA concentration.

The length of the small intestine (Fig. 3.2) was greatest in the piglets fed colostrum. This greater increase in the length could be due to a combination of factors, including the increase of cell number (hyperplasia), the increase of cell size (cell swelling) and the presence of digesta in the lumen of the gut during the measurement of intestinal length. The hyperplasia is suggested by the comparatively greater increases in mucosal DNA contents (Fig. 3.22) in the sow colostrum fed piglets. Whether or not the greater increase in small intestinal length was partly due to cell swelling remains an open question. Since cell swelling occurred at the villous site, and not in the crypts, the increased volume of the villi due to cell swelling could freely occupy the space in the intestinal lumen without affecting intestinal length.

It is likely that the amount of digesta remaining in the intestine varied between the groups as the nature of the intestinal contents and the rate of gastric emptying are known to affect intestinal transit times (Malagelada & Azpiroz, 1989). Products of digestion of fat, protein, and carbohydrate have been shown to slow gastric emptying when instilled directly into the intestine (Cooke, 1977). Also increasing the viscosity of liquid meals is associated with a progressive decrease in gastric emptying rate (Russell & Bass, 1985). Colostrum has a high protein content and is noticeably more viscous than milk. This may have delayed both gastric emptying and intestinal transit time in the colostrum fed piglets resulting in increased retention of intestinal contents and causing stretch of the intestine during the measurement of its length. No record was made of the volume of the intestinal contents remaining at post-mortem.

The intestinal length of the group fed infant formula increased less than did the lengths of the combined natural milk fed groups. The difference was not apparently due to a lower intestinal growth as the DNA content (Fig. 3.22) in the jejunum and ileum of the IF group was in fact significantly higher than that of the groups fed natural milks. Also, the DNA concentration (Fig. 3.23) in the duodenum of the IF group was significantly higher than in the combined milk fed groups. Furthermore, there was a greater increase in duodenal wall thickness (Fig. 3.8) and circumference (Fig. 3.7) in the infant formula fed piglets compared to the natural milk fed groups. A possible explanation for the greater length of the small intestine in the natural milk fed animals is the effect of the remaining ingesta in the small intestinal lumen during the measurement process. Since sow and cow milk had higher percentages of both protein and lipid, intestinal transit times may have been greater in the piglets fed those diets compared to the piglets fed infant formula, and the milk remaining in the small intestinal tract may have caused stretching of the intestine while it was suspended vertically for measurement.

The increased growth of the small intestine of newborn piglets fed colostrum during the first 24 hours in the present study is in accord with the results of previous studies reporting the trophic effects of colostrum in newborn piglets (Widdowson *et al.*, 1976; Simmen *et al.*, 1990a; Burrin *et al.*, 1992; Reinhart *et al.*, 1992), puppies (Heird

et al., 1984), rats (Berseth *et al.*, 1983; Berseth, 1987a) and lambs (Patureau-Mirand *et al.*, 1990). Several lines of evidence, as well as the present results, indicate that growth promoting substances in the colostrum, for the piglet possibly EGF and IGF-I, enhance small intestinal development. Firstly, the presence of fibroblast and epithelial cell growth promoting molecules has been demonstrated in sow colostrum by Cera *et al.* (1987) and Simmen *et al.* (1988). Batley & Fletcher (1992) using intestinal epithelial cells *in vitro*, found that the growth promoting activity of sow colostrum declines with time of lactation, but is still high at 21 days of lactation. Other studies have shown that sow colostrum contains high concentrations of EGF and IGF-I (Jaeger *et al.*, 1987; Simmen *et al.*, 1988; Simmen *et al.*, 1990b) which enhance gut development (Johnson, 1988; Menard & Arsenault, 1988; Read, 1988; Read *et al.*, 1991; Zumkeller, 1992). Secondly, EGF receptors are widely distributed in the intestinal mucosa of newborn piglets, suggesting a functional role for EGF during postnatal development (Kelly *et al.*, 1992). These receptors are located on the apical membranes of the enterocytes of newborn piglets, indicating that luminal EGF has a direct physiological effect on the intestinal epithelium (Kelly *et al.*, 1992). IGF-I receptors have been detected in the developing small intestine of neonatal piglets (Schober *et al.*, 1990). Binding of IGF-I in the small intestine is maximal during the first postnatal day, then declines but returns to a comparable maximal level again on the twenty-first postnatal day (Schober *et al.*, 1990). The presence of IGF-I receptors in the intestinal epithelium of newborn piglets and the high concentration of IGF-I in sow colostrum (Schober *et al.*, 1990) indicate that colostral IGFs, particularly IGF-I, may play a role in the development and differentiation of the small intestinal epithelia through these receptors. Thirdly, there have been several studies of the effects of EGF and IGFs on gut growth both *in vitro* and *in vivo* (see Section 2.3.2).

3.4.3 Effects of Sow Colostrum and Milk on Mucosal Lactase Activity

Common indices of functional development of the small intestine are enzyme activities. The present use of lactase activity as one index indicated that colostrum enhanced functional development of the small intestine. The higher lactase activity may have been due to the effect of growth promoting substances in the colostrum, possibly

EGF and insulin. EGF has been reported to affect the development of mucosal enzymes in the small intestine of piglets. James *et al.* (1987) reported that injection of EGF into 3-day-old piglets increased intestinal sucrase and maltase activities. These increases were confined to the middle and distal regions of the small intestine. Jaeger *et al.* (1990) investigated the effect of orally administered EGF on the jejunal mucosa of weaned pigs and found that EGF significantly increased jejunal lactase and sucrase activity by 3 days postweaning. However, the effect of EGF on intestinal lactase activity in newborn sucking piglets has not been investigated.

Insulin given orally to 2-day-old miniature piglets for 6 days has been reported to increase intestinal lactase activity and ileal mass (Shulman, 1990). Interestingly in a later study orally administered insulin had no effect on the level of ileal lactase mRNA or on the relative proportion of lactase precursor proteins, suggesting that the enhancing effect of oral insulin on lactase activity in the newborn piglet is not regulated at the level of translation and postranslation (Shulman *et al.*, 1992). In another study, by Menard et al. (1981), daily insulin injections for 3 days increased intestinal brush border lactase activity in suckling mice. Thus insulin administered either orally or parenterally may stimulate intestinal lactase activity. The concentration of insulin in sow colostrum (411 μU/ml) at birth is approximately 80 times greater than that in serum (5 μU/ml) (Westrom *et al.*, 1987), suggesting this colostral hormone may take part in promoting functional development of small intestine in the piglets.

Increasing the frequency of feeding in adult sheep causes an increase in the frequency of insulin release (Bassett, 1974a). Hence the greater cellular lactase activity (Fig. 3.32) in the naturally suckled piglets compared to those fed sow colostrum by bottle may have resulted from the greater frequency of endogenous insulin release in the suckled piglets due to the multiple feeding periods during natural suckling. Sows normally nurse their piglets more than 24 times in 24 hours but only for a few minutes each time (Pond & Maner, 1984). Originally it was thought that the only mechanism controlling insulin release was the direct feedback effect of blood glucose levels on pancreatic insulin secretion. However, two phases of insulin release following sucking have been demonstrated in 1 to 3 month-old lambs (Bassett,1974b) and in the 7-day-old

lambs and 1-day-old rabbits (Porter & Bassett, 1979); the first release occurs within 5 minutes of the finish of sucking and the second release 60 to 120 minutes after the finish of feeding. Cholinergic mechanisms, possibly as part of a 'cephalic phase' of insulin release, are thought to be responsible for the early insulin response to feeding, which is independent of absorption of nutrients (Morgan *et al.*, 1988). This is supported by experiments demonstrating that atropine administration before feeding abolished the early phase of insulin release (Bloom *et al.*, 1978; Porter & Bassett, 1979). It is well known that oral glucose is more effective in stimulating insulin secretion than intravenous glucose given in amounts sufficient to produce similar degrees of arterial hyperglycaemia (Elrick *et al.*, 1964; McIntyre *et al.*, 1964), and that nutrients taken by mouth stimulate the secretion of one or more gut hormones such as cholecystokinin (CCK), gastrin and especially gastric inhibitory polypeptide (GIP) which in turn stimulate insulin release (for a review see Morgan *et al.*, 1988). Thus the greater the frequency of feeding, as would have occurred in the present naturally suckled piglets, the more likely that such insulin release would have occurred, and hence the greater the intestinal lactase activity.

The higher lactase activity in the group fed infant formula compared to those fed milk may also be due to increased insulin release. The infant formula contained the highest percentage of lactose compared to either colostrum or milk, and hence would be expected to cause higher blood glucose levels than any of the other diets as occurs in the newborn lambs (Mellor, 1987), so that more insulin would be secreted. Also the higher percentage of lactose in the infant formula may have provided a greater stimulus to GIP release. This intestinal hormone is secreted in response to intraluminal glucose (Ponter *et al.*, 1990) and provides a strong stimulus to pancreatic insulin release (see Morgan, 1992).

Dietary lactose itself, especially the high content in the infant formula may have affected intestinal lactase activity directly. Giving suckling rats disaccharides apparently induces the synthesis of the corresponding hydrolytic enzymes (Raul *et al.*, 1978) and lactose reportedly acts as a substrate inducer on lactase (Goldstein *et al.*, 1971). Whether this also occurred in newborn piglets remains to be established.

However, there are other growth promoting substances such as cortisol and thyroxine which are known to be in the colostrum of other species (see Section 2.3.2) and which have been demonstrated to stimulate both structural and functional development of the small intestine (see Section 2.3.1), but their levels in sow colostrum have not apparently been measured.

As was shown for the RNA and DNA concentrations, the comparatively low lactase activity per mucosal weight (Fig. 3.31) in the piglets fed sow colostrum was most likely due to the large amount of protein in the mucosal cells diluting the lactase activity per mucosal weight, but not affecting the total lactase activity. This is in accord with the observation that the total lactase activity of the piglets in this group was generally higher than those of the piglets fed other diets. The possible causes of the relatively high lactase activity in the piglets fed water are discussed below.

3.4.4 Comparative Effects of the Sow and Cow Colostrums on the Mucosal RNA

Both sow and cow colostrum enhanced the structural and functional development of the small intestine, but there were several noteworthy differences between the effects of sow and cow colostrum. The piglets fed cow colostrum had the lowest RNA content and RNA:DNA ratio (Figs. 3.24 and 3.26), reflecting either a reduced rate of RNA synthesis or increased rate of RNA degradation, or both. Which of the three types of RNA (messenger RNA, ribosomal RNA and transfer RNA) is affected is unknown. Although the mucosal RNA:DNA ratio was low, the villous cells in the piglets fed cow colostrum appeared normal (Fig. 3.14D) and exhibited swelling due to the effect of colostral protein uptake. The cow colostrum used in the present study contained 10.4% protein which is approximately three times the amount of protein in sow or cow milk. The present experiment indicated that large amounts of heterologous protein were absorbed into the mucosal cells during the first 24 hours (Figs. 3.27 - 3.29). The effect, if any, of heterologous proteins on epithelial cell RNA is not known. However it is of interest to note that in an independent study conducted in this laboratory, newborn piglets fed bovine lactoferrin 5%(W/V) orally for 5 days in an infant formula identical to the one used in this study exhibited significant decreases in RNA content and

RNA:DNA ratios in the jejunum and ileum (D. Shu, H.V. Simpson, R-J. Xu, D.J. Mellor, G.W. Reynolds & R.B. Marshall; unpublished observations). The precise details of this effect of feeding heterologous protein on the mucosal RNA remain to be clarified.

3.4.5 Comparative Effects of Natural Suckling and Bottle Feeding of Sow Colostrum

The piglets fed sow colostrum by bottle exhibited greater increases in small intestinal weight (Fig. 3.3) and body weight gain (Fig. 3.1) compared to those naturally suckled. There are two important differences between these two groups which may account for the greater increases in the bottle fed group: the levels of growth promoting substances in the colostrum and the colostrum intake. Firstly, the pooled colostrum collected from farrowing sows would have had consistently high levels of growth promoting substances, whereas the levels of these substances in colostrum suckled from the sow gradually declines with the length of the lactation period (Baxter *et al.*, 1984; Cera *et al.*, 1987; Jaeger *et al.*, 1987; Read, 1988; Simmen *et al.*, 1990b). Secondly, the piglets naturally suckled might have obtained less colostrum than the SC group as when piglets are suckled they compete with their littermates for the available nipples and the sow usually nurses the piglets for only a few minutes at a time but more often (Pond & Maner, 1984).

3.4.6 Water Feeding and Small Intestinal Development

In the present experiment, although the H_2O group obtained no exogenous nutrients and the body weight decreased, the mucosal DNA content (Fig. 3.22) was significantly higher than in the group at birth, indicating that mucosal cell division continues during the first 24 hours after birth despite the absence of an exogenous source of energy, presumably due to a 'basal' rate of cell division. According to the work-load hypothesis proposed by Clarke (1977) (see Section 2.3.4) this 'basal' cell division may be causally linked to the absorption of the ingested water. Alternatively it might be due to an obligatory low rate of cell division or the stimulation from other

trophic factors, possibly salivary EGF (Johnson, 1988, 1989) or other endogenous systemic hormones, or to the provision of endogenous nutrients, or to any combination of these possibilities. Despite a 'basal' rate of cell division mucosal weights for the H_2O group were lower than for all the other bottle fed groups combined. Also the lower crypt depth, villous height and wall thickness, and the shorter relative migration distance in the group fed H_2O compared to the colostrum and milk fed groups combined presumably reflected a low rate of cell proliferation in the piglets fed H_2O.

The smaller increase in intestinal length (Fig. 3.2) in the water fed piglets compared to the other fed groups indicates the importance of nutrients in the development of the small intestine. McManus & Isselbacher (1970) reported a greater weight and DNA content of the small intestine in fed mature rats compared to rats fasted for 24 hours, whereas the small intestinal length and circumference, and the body weight showed no significant differences between the two groups. The difference between the results for small intestinal length seen in their experiment and the present experiment could be due to the different species and ages of the animals studied; McManus & Isselbacher (1970) used mature rats whereas newborn piglets were used for the present study. Despite these differences both studies emphasized the importance of exogenous nutrients for small intestinal growth.

The H_2O group had a higher lactase activity per mucosal weight (Fig. 3.31) compared to fed groups. This was possibly due to the large amount of protein in the mucosal cells of colostrum fed piglets diluting and lowering the lactase activity per mucosal weight in those groups. However, total and cellular lactase activity (Figs. 3.30 and 3.32) of the water fed group were also high. This might be due to the continued production of lactase in the mucosal cells during cell migration along the villi despite the absence of a luminal substrate. Furthermore, the H_2O group demonstrated the lowest relative migration distance (Fig. 3.21) which indicated a slower rate of cell migration and cell shedding, increasing the period for cellular lactase production, but this might be offset by the short villous height in this group.

3.4.7 Effects of Sow Colostrum and Milk on the Pancreas

Apart from the intestinal tract, colostrum also promotes growth of other visceral organs (Widdowson & Crabb, 1976). In the present study the colostrum fed piglets had a higher pancreatic weight (Fig. 3.1) compared to milk fed piglets. This corresponds to the results obtained by Widdowson & Crabb (1976) who reported that the pancreas of newborn piglets increased in weight more rapidly in response to feeding during the first 24 hours after birth than any other organ, but there was no significant change in the amount of DNA. Burrin *et al.* (1992) demonstrated a higher rate of protein synthesis in the pancreas of piglets fed colostrum compared to milk fed and water fed piglets, suggesting that the weight increase was, at least, partly due to cell growth.

Growth promoting substances in the colostrum may have stimulated, at least partly, this pancreatic growth. IGF-I or IGF-II given orally with infant formula to piglets during the first 24 hours after birth stimulate pancreatic growth (R-J. Xu, D.J. Mellor, M.J. Birtles, B.H. Brier & P.D. Gluckman; unpublished observations). Bombesin has a trophic effect on the pancreas in neonatal rabbits (Karkashan *et al.*, 1992) and in suckling rats (Borysewicz *et al.*, 1992). High concentrations of bombesin-like peptides detected in sow colostrum at farrowing are sustained for at least 72 hours after birth (Westrom *et al.*, 1987). These observations indicate that colostral IGFs and bombesin may have a role in the regulation of pancreatic growth.

High concentrations of trypsin inhibitor and protein in colostrum may also promote pancreatic growth. The chronic feeding of trypsin inhibitor has been reported to increase pancreatic growth in rats and mice (Schneeman *et al.*, 1977; Roy & Schneeman, 1981). Green & Lyman (1972) have hypothesised that trypsin inhibitor prevented the feedback inhibition of CCK release by trypsin and chymotrypsin, thus leading to increased serum levels of CCK. There have been several studies showing the trophic effects of CCK on pancreatic growth in rats (Barrowman & Mayston, 1974; Mainz *et al.*, 1973; Brants & Morrisset, 1976; Ihse *et al.*, 1976; Petersen *et al.*, 1978). Also high protein diets have been shown to induce pancreatic growth in rats. This effect is thought to be mediated by CCK released in response to the increased protein intake

(Morisset *et al.*, 1992). However, further investigation is required to clarify the effect of CCK on pancreatic growth in the pig, as Struthers *et al.* (1983) reported that pancreatic growth did not increase in pigs either fed trypsin inhibitor or given CCK for 14 days compared to the controls. Also Pierzynowski *et al.* (1990) reported that basal pancreatic function of the young pig is low, and intravenous CCK or secretin does not stimulate pancreatic exocrine function during the first 2 weeks of life.

3.4.8 Effects of Diets on the Liver

The high liver weight (Fig. 3.1) in the group fed infant formula during the first 24 hours after birth could have been due partly to less glycolysis in the liver. The carbohydrate utilized in the newborn piglets during the first 24 hours is initially obtained from stored glycogen and subsequently from ingested colostrum/milk (Mellor & Cockburn, 1986). Available energy from liver glycogen in the newborn piglet is 43 kJ/kg bw or 2.5 g glycogen/kg bw (Mellor & Cockburn, 1986) which is approximately 3.75 g of glycogen for each piglet in the present study. By comparison, there was a 15 g loss of liver weight in the water fed piglets. The reason for the loss of the remaining 11.25 g is not known. It is of interest to note that in the present study there was a positive correlation ($P<0.01$) between the liver weight change per body weight and lactose concentration of the diets, indicating that dietary lactose may help maintain liver weight. The precise mechanism of this effect is unknown.

3.4.9 Conclusions

During the first 24 hours after birth the small intestine of piglets fed sow colostrum exhibited greater increases in both weight and length compared to other fed groups. The greater increases were associated with cell swelling related to colostral protein accumulation and to hypertrophy and hyperplasia which were possibly caused by colostral growth promoting substances. The greater small intestinal weight in the piglets fed sow colostrum compared to those naturally suckled was possibly due to the higher levels of growth promoting substances in the pooled colostrum, and to the lower intake of colostral growth promoting substances in the naturally suckled piglets due to

the transition from colostrum to milk in the first 24 hours and to possibly lower colostrum/milk intake through littermate competition. The enhanced lactase activity in the piglets fed sow colostrum was most likely caused by colostral growth promoting substances, e.g. insulin. In the naturally suckled piglets the higher lactase activity may have resulted from a greater frequency of feeding inducing more endogenous insulin release. The high lactase activity in the piglets fed infant formula was possibly due to higher insulin release caused by ingested infant formula with high lactose concentration compared to other diets. Feeding cow colostrum decreased mucosal RNA content, which was possibly due to the larger amount of heterogenous protein absorbed into mucosal cells, causing an unknown effects on RNA metabolism. The increase of mucosal DNA content in all groups, including water fed piglets, indicated that there is a basal rate of cell division during the first 24 hours after birth regardless of external sources of energy and types of diets. However nutrients are important for small intestinal development as revealed by the shortest small intestinal length in the piglets fed water. Apart from its effects on the small intestine, colostrum promotes pancreatic growth, whereas feeding infant formula with a high lactose concentration promotes liver growth and may help to maintain stored liver glycogen by providing more exogenous carbohydrate.

In the following chapter an experiment to determine the effects of the route of feeding (parenteral versus enteral) and deprivation of colostral growth promoting substances on small intestinal development are described and discussed.

CHAPTER 4

THE EFFECTS OF INTRALUMINAL NUTRITION AND TOTAL PARENTERAL NUTRITION ON POSTNATAL DEVELOPMENT OF THE SMALL INTESTINE IN PIGLETS DURING THE FIRST 24 HOURS AFTER BIRTH

4.1 INTRODUCTION

In addition to the several growth factors and hormones present in colostrum, ingested metabolites including carbohydrates, proteins and lipids have been reported to enhance growth of the gut (see details in Chapter 2). The importance of colostral growth factors has been discussed in the previous chapter.

The importance of luminal nutrients for maintaining normal mass and function of the mature small intestine has been demonstrated in several studies, most of which used parenteral feeding as a tool to assess the effect of intraluminal nutrition; oral feeding increased small intestinal length, weight and villous height and surface area (Meurling & Roo, 1981; Raul *et al.*, 1987; Shulman, 1988) whereas parenteral feeding decreased mucosal mass, protein and DNA (Cameron *et al.*, 1974; Levine *et al.*, 1974; Hughes & Dowling, 1980; Hughes & Ducker, 1982; Popp & Wagner, 1984;), epithelial cell proliferation (Eastwood, 1977) and enzyme activity (Levine *et al.*, 1974; Hughes & Dowling, 1980; Hughes & Ducker, 1982).

Furthermore, studies of intestinal adaptation to resection when parenteral and enteral feeding were used postoperatively provide further support for the idea that luminal nutrition stimulates intestinal development. In these circumstances enteral feeding apparently results in (1) increases in the length and circumference of the residual intestine (Shin *et al.*, 1980; Ford *et al.*, 1984), (2) mucosal hyperplasia (Levine *et al.*, 1976; Biasco *et al.*, 1984) and increases in the number of villi, villous height and width and crypt depth (Feldman *et al.*, 1976; Shin *et al.*, 1980; Biasco *et al.*, 1984) and (3) an increase in glucose absorptive function due to hyperplasia of the villi (Morin *et al.*, 1978). Furthermore, a number of 'by-passed loop' experiments designed to test the influence of intraluminal nutrition upon small intestinal structure and function in rats

demonstrated mucosal hyperplasia and hypertrophy and increased enzyme activities (Gleeson *et al.*, 1972a,b; Castro *et al.*, 1975) and glucose absorption (Gleeson *et al.*, 1972a; Kotler *et al.*, 1981) in the continued intestine compared to the by-passed intestinal segment. Also mucosal hyperplasia and hypertrophy and increased enzyme activities were demonstrated in the by-passed intestine of orally fed compared to parenterally fed animals (Dworkin *et al.*, 1976; Adams *et al.*, 1978). These observations suggest that direct effects of intraluminal nutrition, including physiological responses related to food ingestion, are important in maintaining small intestinal structure and function.

There have been a limitted number of studies of the effects of total parenteral nutrition on gastrointestinal development using the piglet as an experimental model (Goldstein *et al.*, 1985; Morgan *et al.*, 1987), but none on piglets aged 0-24 hours. Accordingly, the present experiment was conducted to study the effect of (a) metabolites alone, excluding colostral growth promoting substances and (b) intraluminal nutrition compared to total parenteral nutrition, on postnatal development of the small intestine in piglets during the first 24 hours after birth.

4.2 MATERIALS AND METHODS

4.2.1. Animal Preparation

Ten Large White piglets were obtained from the Pig Research Centre, Massey University as described in Section 3.2.1. Two piglets from each of five litters were separated into two groups; one group was fed by orogastric tube (orogastric feeding - OGF) and the other parenterally (total parenteral nutrition - TPN).

The newborn piglet normally gets its immunity from colostrum during the first 24 hrs of life (Pond & Maner, 1984), but in the present experiment the piglets were prevented from sucking from the sow and as a consequence were regarded as being immuno-incompetent. Umbilical arterial cannulation was used in preference to surgical cannulation of deeper blood vessels in order to avoid stress of surgery and to minimize the possibility of wound infection in immuno-incompetent animals.

At birth one of the umbilical arteries of each piglet was cannulated with a sterile cannula (3 FG, length 30 mm; Portex, England) and a 2 ml sample of blood collected for blood glucose determination. All blood samples were place on ice to clot. The serum was then separated by centrifugation at 500*g* for 15 mins. and stored frozen at -20 °C.

After collecting the blood sample, five-bromo-2'-deoxyuridine (BrdU) (Sigma, USA) was injected to label dividing mucosal cells for the subsequent measurement of mucosal cell proliferation and migration (as described in section 3.2.1). The BrdU was dissolved in 0.9% sterile saline to a concentration of 10 mg/ml before being injected. It was given to 2 of the 5 pairs of piglets as a single injection (30 mg/kg.bw) via the umbilical cannula. In order to increase the number of dividing cells labelled with BrdU, the remaining 3 pairs of piglets were given BrdU by repeated intraperitoneal injections (30 mg/kg.bw) at 1 and 2 hrs and at 22 and 23 hrs after birth.

After the BrdU was given to the 2 pairs of piglets via the umbilical cannula, the cannula was removed from piglets in both groups and the cord of the piglets in the OGF group was tied, cut and swabbed clean with 70% alcohol. A second longer cannula was then inserted into the other umbilical artery of the piglets in the TPN group for the infusion of the TPN solution. Two of the piglets were cannulated using a double-lumen polyethylene tube (minor I.D. 0.41 mm, O.D. 1 mm, major I.D. 0.75 mm, length 900 mm; Dural, Australia), and the remaining three piglets using a single-lumen polyethylene tube (I.D. 0.97 mm, O.D. 1.27 mm, length 450 mm; Dural, Australia). All cannulations were carried out using full aseptic procedures. Each cannula was fixed to the body of the piglet by adhesive tape (Leukoplast, Beiersdorf, Germany) and connected to packages of nutrient solution by a sterile disposable three-way tap and polyethylene tubes (I.D. 1.0 mm, O.D. 2.0 mm, length 200 cm; Dural, Australia).

Each pair of piglets was kept in the same crate but the individuals were separated from each other by a perspex partition (each cell; 22 cm wide, 38 cm long, 46 cm high). The temperature in the crate was maintained between 32 - 38 °C by a heating lamp suspended above the crate. All piglets in both groups were given the same nutrient solution with an equivalent nutritional value (Table 4.1). A total volume of 250 ml was infused into each piglet in the TPN group, whereas distilled water (20 - 80 ml) was added to the solution fed to the OGF group to make up the total volume fed to 220 ml/kg bw, which was within the range of colostrum intake of naturally suckled piglets (Le Dividich & Noblet, 1981). The solution was then divided into eight meals (Table 4.2) and given to the piglets by an orogastric tube (Feeding Tube, Pharmaseal, USA) introduced into the stomach via the oesophagus before and removed after each meal. The nutritional composition of the nutrient solution and the energy provided to the piglets are shown in Table 4.3.

Table 4.1 Composition of the nutrient solution given to the piglets by total parenteral nutrition (TPN) and by orogastric feeding (OGF).

CONSTITUENTS	TPN	OGF
Dextrose[1]	20 ml/kg bw	20 ml/kg bw
10% Amino acid[2]	50 ml/kg bw	50 ml/kg bw
20% Fatty acid[3]	30 ml/kg bw	30 ml/kg bw
0.9% Sodium chloride[4]	add to make up the total volume to 250 ml.	add to make up the total volume to 250 ml.
Distilled water	none	add to make up the total feeding volume 220 ml/kg bw per 24 hrs.

(Osmolality varied between 770-930 mOsm/L because of the different amounts of 0.9% sodium chloride solution required to make the total volume up to 250 ml for the TPN group)

[1] Dextrose 50%, Travenol, Australia
[2] Synthamine 17 with electrolytes, Travenol, Australia
[3] Intralipid 20%, KabiVitrumAB, Sweden
[4] Travenol, Australia

Table 4.2 Feeding times and volumes of nutrient solution given to the piglets by orogastric feeding (OGF).

FEED	TIME AFTER BIRTH (hrs)	VOLUME (ml/kg bw)
1st	2	20
2nd	4	20
3rd	6	20
4th	8	20
5th	10	40
6th	14	40
7th	18	40
8th	22	20

Table 4.3 Nutrient and energy intake of the piglets given nutrient solution by total parenteral nutrition (TPN) and by orgastric feeding (OGF). The energy intakes were the same in both groups.

CONSTITUENTS	INTAKE	
	g/kg bw	kJ/kg bw
Dextrose[1]	10	157
Fatty acid[2]	6	252
Amino acid[3]	5	86
Total energy	-	495
Usable energy[4]	-	409

[1] Dextrose 50%, Travenol, Australia (15.72 kJ/g dextrose)
[2] Synthamine 17 with electrolytes, Travenol, Australia (42 kJ/g fatty acid)
[3] Intralipid 20%, KabiVitrumAB, Sweden (17.2 kJ/g amino acid)
[4] Usable energy is the energy which is produced by metabolism of lactose and lipid; during the first 24 hours the piglet can metabolize protein only at a very low rate (Mellor & Cockburn,1986).

To avoid breaking of the fatty acid emulsion and the entry of large fat droplets into the blood stream, before being infused the fatty acid solution was not mixed within the same infusion pack containing the dextrose, amino acids and electrolytes. Instead the two solutions were either infused separately, as was the case for those piglets cannulated with a double lumen cannula, or allowed to mix within the infusion cannula when infused into the piglets with a single lumen cannula. The two solutions were infused continuously at a combined rate of 11.5 ml/hr by a multichannel peristaltic pump (Peristaltic Pump P-3, Pharmacia, Sweden).

Since the composition of sow milk changes rapidly during the first few days after farrowing (Pond & Maner,1984), the nutrient solution was initially formulated to imitate the average nutritional value of sow milk during the first 3 days after parturition. This made a solution having a high protein content and low lipid and carbohydrate contents, which was found by calculation to provide insufficient carbohydrate and lipid to meet

the energy needs of newborn piglets (Mellor & Cockburn,1986). Therefore, the nutrient solution was reformulated in order to correct these deficits, taking account of the volumes of colostrum/milk usually consumed by naturally suckled piglets during the first 24 hrs (see Appendix 4.1).

4.2.2 Specimen Collection

After 24 hours, all piglets were anaesthetized by intraperitoneal injection of 1 ml pentobarbitone sodium 500 mg/ml (Pentobarb 500, South Island Chemicals, NZ) diluted 1:5 in 0.9% NaCl, and weighed. A 2 ml blood sample was collected for blood glucose determination by cardiac puncture before euthanasia by intracardiac injection of pentobarbitone sodium. The whole of the gastrointestinal tract was removed and dissected and the tissue specimens collected as described in Section 3.2.3.

4.2.3 Specimen Analyses

The tissues were weighed, measured and analyzed for DNA, RNA, protein and lactase contents, and examined microscopically and mucosal cell proliferation and migration measured as described in Section 3.2.4. The serum glucose concentrations in the blood samples collected from the piglets at birth and before euthanasia were determined using a commercially available enzymatic UV test with hexokinase (Uni-Kit II Glucose HK, Roche, Switzerland). These assays were done by technicians in the Department of Veterinary Pathology and Public Health, Massey University.

4.2.4 Statistical Analyses

As the period of the present experiment overlapped with the previous experiment, and the piglets for both experiments were selected using the same criteria and from the same piggery, the data for the piglets examined at birth (B) in the previous experiment are included here for comparison with the TPN and OGF groups. It is acknowledged that ideally piglets from the same litters should have been used as control animals sampled at birth. However, with the experimental justification noted above and to

minimise the ethical cost of the experiment, it was decided to use data from the B group animals studied previously (Chapter 2). The following comparisons were made to determine the effect of metabolites and intraluminal nutrition on small intestinal development.

(a) A comparison between the control piglets examined at birth and those given metabolites for 24 hours:
(B vs OGF+TPN)
(b) A comparison between the controls and those fed enterally:
(B vs OGF)
(c) A comparison between the controls and those fed parenterally:
(B vs TPN)
(d) A comparison between enterally and parenterally fed piglets:
(OGF vs TPN).

As the variation in body weight of the piglets at the beginning of the experiment could have affected the outcome of the experiment, all data were subjected to analysis of covariance using body weight at birth as a covariate. All data were analyzed by computer using the SAS and MINITAB programs. The level of significance is expressed as * = $P<0.05$, ** = $P<0.01$ and *** = $P<0.001$. The means and S.D.'s for the results for the OGF and TPN groups are given in Appendices 4.2 - 4.5, and for the B group in Appendices 3.2 - 3.5.

4.3 RESULTS

Feeding nutrient solution for first 24 hours slightly enhanced small intestinal growth in both the TPN and OGF groups as compared to the B group. The effect was most pronounced in the duodenum and lower ileum. Apart from a greater small intestinal length in the orogastrically fed compared to the parenterally fed piglets, there were no major differences between the TPN and OGF groups.

4.3.1 Body Weight Change, and Liver and Pancreatic Weights

The piglets in both the OGF and TPN groups significantly gained body weight during the first 24 hours (Fig. 4.1). The small negative value in the B group, which was nevertheless not significantly different from zero, was due to the effect of statistical analysis of covariance, using birth weight as a covariate. Liver and pancreatic weights showed no significant differences between groups (Fig. 4.1).

4.3.2 Small Intestinal Length and Weight

Orogastric feeding was associated with increased small intestinal length, but not weight. The total length of the small intestine, and the jejunal and ileal lengths in the OGF group were significantly longer than those of both the TPN group and the control (B) group (Fig. 4.2). Both the OGF and TPN groups had significantly longer duodena than the B group. The total weights of the intact small intestine and the intestinal mucosa and muscle were not significantly different among the three groups (Fig. 4.3). However the intact (Fig. 4.4) and mucosal (Fig. 4.5) weights for the duodenum in the OGF and TPN groups were significantly higher than those in the B group. There were no significant differences among the three groups in the muscular weights of the different regions of the small intestine (Fig. 4.6).

4.3.3 Microscopic Structures of the Small Intestine

Changes to the microscopic structures of the small intestine were consistent with

there having been intestinal growth in both groups given nutrient solution for the first 24 hours, particularly in the duodenum and lower ileum. The duodenal wall thickness (Fig. 4.7) of the OGF group and the duodenal crypt depth (Fig. 4.8) of the TPN group were significantly higher than the B group. The circumference in the lower ileum of the two groups combined was significantly smaller than in the B group (Fig. 4.9) whereas the lower ileum of the OGF and TPN groups had significantly thicker walls (Fig. 4.7) and muscular layers (Fig. 4.10) than in the B group. The heights of the villi in the duodenum were significantly greater in the OGF and TPN groups compared to the B group (Fig. 4.11).

When comparisons were made between the OGF and TPN groups, orogastric feeding had little effect on the microscopic appearance of the small intestine compared to parenteral feeding (Figs. 4.12 and 4.13). While there were no differences in epithelial cell height, nuclear shape and position, and cytoplasmic consistency between the two groups, there was a higher ratio of goblet cells to columnar absorptive cells, and the villi appeared more compact in the ileum of the TPN group. Also the villous width in the upper jejunum was significantly greater in the OGF group compared to the TPN group (Fig. 4.14).

On the other hand, the villi in the B group were significantly wider than those of the OGF and TPN groups in the jejunum and ileum (Fig. 4.14), and the duodenal submucosa of the B group was significantly thicker than that of the OGF group (Fig. 4.15).

4.3.4 Mucosal Cell Proliferation and Migration Rate

The numbers of labelled dividing cells per crypt area (Fig. 4.16) and the relative migration distances (Fig. 4.17) of the mucosal cells were similar for both the OGF and TPN groups. Increasing the number of injections of BrdU from a single injection at birth to multiple injections, at 1 and 2 hrs and at 22 and 23 hrs, had no detectable effect on the number of dividing cells labelled.

4.3.5 Mucosal DNA, RNA and Protein

Both the OGF and TPN groups had significantly higher mucosal DNA concentrations (Fig. 4.18) and contents (Fig. 4.19) compared to the B group. There were no significant differences in the concentrations of RNA (Fig. 4.20), whereas in the duodenum the RNA contents (Fig. 4.21) of the TPN and OGF groups combined and the OGF group alone were significantly higher than in the B group. The RNA:DNA ratio for the jejunum in the B group was significantly higher than in the TPN group, and in the combined OGF and TPN groups (Fig. 4.22). Mucosal protein concentrations (Fig. 4.23) in the lower ileum of the TPN group and the TPN and OGF groups combined were significantly higher than in the B group. Also the mucosal protein contents (Fig. 4.24) in the duodenum of the OGF group, TPN group and both groups when combined were significantly higher than in the B group, whereas the protein:DNA ratios (Fig. 4.25) in the B group were higher than those in the OGF and TPN groups, especially in the duodenum and upper jejunum. There was no detectable effect of orogastric feeding compared to total parenteral nutrition on the mucosal RNA, DNA and protein content or the RNA:DNA and protein:DNA ratios.

4.3.6 Lactase Activity and Serum Glucose

There were no significant differences between groups for the lactase activity per mucosal weight (Fig. 4.26) and for the total lactase activity (Fig. 4.27), but cellular lactase activity, i.e. lactase activity per mg of DNA, (Fig. 4.28) in the duodenum, lower jejunum and upper ileum for the B group was significantly higher than for either the OGF or the TPN groups. The serum glucose concentrations at 24 hours and the difference in glucose levels between birth and 24 hours were not different for the OGF and TPN groups (Fig. 4.29).

4.4 DISCUSSION

Feeding nutrient solution either orogastrically or parenterally slightly enhanced small intestinal growth of the newborn piglets during the first 24 hours after birth. The effect was more pronounced in the duodenum and lower ileum. By contrast the route of feeding had no apparent effect on other parameters except on small intestinal length which increased in the orogastrically fed piglets compared to those fed parenterally, possibly due to physiological elongation as opposed to growth.

4.4.1 Comparisons of Regional Growth of the Small Intestine Related to Diets and Route of Feeding

There were increases between birth (group B) and 24 hours in several parameters which, at first sight, suggested that intestinal growth had been stimulated by the OGF and TPN treatments. There were increases in small intestinal length (OGF only) and the DNA content of the intestinal mucosa. The duodenal weight and duodenal RNA and protein contents also increased in both groups during the first 24 hours, but the jejunal RNA:DNA ratio and the protein:DNA ratios in the duodenum, upper jejunum and upper ileum decreased in both TPN and OGF groups. These decreases were presumably due to the increases in the DNA content of each of these regions of the small intestine. Furthermore, villous height, crypt depth and wall and muscular thickness in the duodenum and lower ileum increased in both the OGF and TPN groups.

However, a comparison of the present OGF and TPN results with those from the water fed (H_2O) and naturally suckled (NS) piglets presented in Chapter 3, suggests that the explanation may not be quite so straightforward. The percentage changes in several of the growth parameters during the first 24 hours among groups fed nutrient solution (OGF and TPN), water (H_2O) and those which were naturally suckled (NS) have therefore been compared in Table 4.4. When the general magnitudes of the percentage changes were of the same order, effects in the different groups were considered to be similar, and vice versa.

TABLE 4.4 The estimated percentage increases from birth in small intestinal length, weight, DNA and RNA contents and RNA:DNA ratio of the piglets given nutrient solution by orogastric feeding (OGF) or by total parenteral nutrition (TPN), fed water by bottle (H_2O) or naturally suckled (NS). Data for the H_2O and NS groups were derived from Chapter 3.

	ESTIMATED PERCENTAGE INCREASE FROM BIRTH (B) [1]			
	OGF	TPN	H_2O	NS
SI length :-				
Total	15	3	-4	23
Duodenum	37	25	15	27
Jejunum	14	3	-5	22
Ileum	14	3	-5	22
SI weight :-				
Total intact	5	-2	-1	55
Total mucosa	7	-3	2	75
Mucosal weight:-				
Duodenum	64	55	25	73
Up. jejunum	19	2	7	77
Lo. jejunum	5	2	8	87
Up. ileum	2	-8	2	76
Lo. ileum	-3	-15	-13	60
DNA content :-				
Duodenum	115	119	75	78
Up. jejunum	44	107	88	61
Lo. jejunum	24	73	57	46
Up. ileum	59	43	56	39
Lo. ileum	16	53	21	21
RNA content :-				
Duodenum	65	40	64	111
Up. jejunum	11	12	22	88
Lo. jejunum	-9	6	20	59
Up. ileum	-1	12	25	34
Lo. ileum	-7	6	3	35
RNA:DNA ratio :-				
Duodenum	-28	-38	-6	11
Up. jejunum	-28	-46	-39	13
Lo. jejunum	-31	-40	-29	4
Up. ileum	-43	-31	-27	-9
Lo. ileum	-25	-37	-22	6

[1] Estimated percentage increase = $\frac{(\text{group}) - (B)}{(B)} \times 100$ %

This comparison revealed five noteworthy points:

(a) There were little or no changes in the length of the whole small intestine in the TPN and H_2O groups, whereas there were detectable increases in length of the same order of magnitude in the OGF and NS groups.

(b) When compared to all other parts of the small intestine, the duodenum generally showed greater percentage increases from birth (group B) in most parameters.

(c) Although the weight of the duodenal mucosa increased proportionately from birth by about the same amounts in each of the four groups, the mucosal weight increases in the rest of the small intestine were proportionately much less in the OGF, TPN and H_2O groups than in the NS group.

(d) The percentage increases in mucosal DNA content in all four groups were generally of the same order of magnitude in the corresponding parts of the small intestine.

(e) The mucosal RNA:DNA ratios in the small intestine generally increased in the NS group but decreased in the OGF, TPN and H_2O groups.

These five points will now be considered in detail.

(a) There was little or no change in the length of the whole small intestine in the TPN and H_2O groups, whereas there were detectable increases in length of the same order of magnitude in the OGF and NS groups (Table 4.4).

The greater length in the enterally fed than in the parenterally fed piglets may have been due to (1) ingesta remaining in the intestine at post-mortem and causing physical stretch of the intestine during the measurement process, (2) physiological elongation caused by the combined effects of the intraluminal contents and the viscoelastic properties of the small intestinal smooth muscle (Meiss, 1989) or (3) growth. The effect of physical stretch has been discussed previously (see Section 3.4.2) when considering the effects of diet on intestinal length. In the present experiment, if sufficient fluid to cause stretch remained within the intestine at post-mortem, it would have been detected when it dripped from the cut end of the intestine when the intestine was suspended vertically while it was being measured. No fluid was seen to drip from

the intestine. Moreover, the length was about 10% greater which was considered to be too large to be caused by physical stretch. If the greater small intestinal length had been due to stretch of the intestine, there should have been a difference in small intestinal wall thickness between enterally and parenterally fed piglets. But no such difference was evident. This might be due to the loss of tone of the intestinal smooth muscle when the animals were euthanased (Schofield, 1968). Nevertheless, the weight of evidence suggests that the greater length of the small intestine in the OGF group was more likely to be due to physiological elongation or to actual growth than to physical stretch.

The concept of physiological elongation arises from previous studies showing that the presence and the texture of the food in the small intestine can influence its length. Morgan *et al.* (1987) and Goldstein *et al.* (1985) studied the effect of TPN on gastrointestinal development in 3-day-old piglets and 6-week-old piglets, respectively, for 3 weeks. Goldstein *et al.* (1985) found that the distal small intestine in the TPN animals was 16% shorter than that of animals given the same formula by intragastric infusion, and 35% shorter than that in animals fed solid food. Also Morgan *et al.* (1987) found that the proximal small intestine in TPN piglets was only 73% of the length of the proximal small intestine of intragastrically fed piglets and 65% of the length in naturally suckled piglets. Koga *et al.* (1975) concluded for puppies that the passage of diet through the small intestine is essential for an increase in its length to occur. Other studies (Castillo *et al.*, 1988, 1990) have shown that the intestine is shorter in TPN fed rats compared to that of rats fed intragastrically. However the techniques used for measuring small intestinal length in these previous studies also create uncertainties in addition to that expressed above regarding the cause of any increases in length: Castillo *et al.* (1988, 1990) attached a standard 10-g weight to the intestine to provide traction during measurement whereas Goldstein *et al.* (1985) and Morgan *et al.* (1987) measured along the mesenteric border of the unstretched bowel from the pylorus to the anus, which may have limited the accuracy of their measurements.

In the present experiment although there was a significantly greater increase in small intestinal length in the enterally fed than in the parenterally fed piglets, there was no difference in the weight or mucosal DNA content of the small intestine.

Furthermore, both mucosal RNA contents and mucosal RNA:DNA ratios of the two groups showed no significant differences in all parts of the small intestine. These results therefore support the view that the greater length in the enterally fed piglets was due to physiological elongation, rather than growth.

It is of interest to compare the percentage increase in small intestinal length during the first 24 hours in the piglets fed nutrient solution enterally with the increases in the piglets fed sow milk, cow milk, infant formula and water as part of the previous experiment (Table 4.5). The percentage increases in small intestinal length in the piglets enterally fed nutrient solution was similar to that in piglets fed sow milk or cow milk but greater than that in piglets fed infant formula. In the piglets fed water, the intestinal length appeared to have decreased. These results indicated that intestinal length increases in piglets enterally fed nutrient solutions were comparable to that seen in piglets fed natural milk during the first 24 hours after birth.

TABLE 4.5 The percentage increases from birth in small intestinal length in the piglets given nutrient solution by orogastric feeding (OGF) and fed by bottle with sow milk (SM), cow milk (CM), infant formula (IF) and water (H_2O). Data for the SM, CM, IF and H_2O groups were derived from Chapter 3.

	ESTIMATED PERCENTAGE INCREASE FROM BIRTH (B) [1]				
	OGF	SM	CM	IF	H_2O
Total length	15	11	18	5	-4
Duodenum	37	25	36	19	15
Jejunum	14	10	17	4	-5
Ileum	14	10	17	4	-5

[1] Estimated percentage increase = $\frac{(\text{group}) - (B)}{(B)} \times 100\ \%$

(b) When compared to all other parts of the small intestine, the duodenum generally showed greater percentage increases from birth (group B) in all parameters (Table 4.4).

Except for mucosal weight in the NS group, the percentage increases from birth in length, mucosal weight, and in mucosal DNA and RNA contents, and RNA:DNA ratio were generally greater in the duodenum than in the rest of the small intestine (Table 4.4). Furthermore, in the duodenum of the OGF and TPN groups, there were significant increases from birth in crypt depth, villous height, wall thickness and protein content which were not seen elsewhere in the small intestine. As these effects on the duodenum apparently occurred whether nutrient entered the gut (OGF and NS groups) or not (TPN and H_2O groups), these duodenal changes presumably occur as a consequence of endogenous mechanisms. There are several possible explanations. (1) The duodenum may exhibit an obligatory basal developmental pattern which is independent of diet and other influences, a possibility which is difficult to test using the present experimental procedures. (2) Salivary EGF could have entered the duodenum after being swallowed (Johnson, 1988, 1989) by both the enterally and parenterally fed animals. If so, the duodenal effects suggested that insufficient EGF is produced for it to act further down the gut or that it does not reach more distal regions of the small intestine. The further possible explanation that the more distal regions of the gut are unresponsive to EGF seems to be unlikely because EGF is known to act on all parts of the small intestine (O'Loughlin *et al.*, 1985; James *et al.*, 1987; Jaeger *et al.*, 1990). (3) Another possibility is an effect of biliary and pancreatic secretions which have been reported to cause mucosal hyperplasia in the ileum of pancreatico-biliary diversion rats (Hughes, 1984; Johnson, 1988; Williamson & Malt, 1980). Such an effect may be expected particularly in the enterally fed piglets, as luminal nutrients trigger the release of pancreatico-biliary secretions (Nakajima & Magee, 1970; Hamilton *et al.*, 1971; Towne *et al.*, 1973; Hughes, 1984) which, in addition to the food itself, could have trophic effects on the intestinal mucosa (Hughes, 1984). Much of the evidence for the trophic effect of these secretions comes from experimental animals in which pancreatico-biliary secretions were diverted into the ileum causing an increase in villus size in rats (Altmann & Leblond, 1970; Altmann, 1971; Weser *et al.*, 1977; Williamson *et al.*, 1977;

1978a,b).

(c) Although the weight of the duodenal mucosae increased proportionately from birth by about the same amounts in all four groups, the mucosal weight increases in the rest of the small intestine were proportionately much less in the OGF, TPN and H_2O groups than in the NS group (Table 4.4).

These observations are in accord with the results in Chapter 3 demonstrating there are greater increases of mucosal weight, particularly in the jejunum and ileum, in piglets fed colostrum than in those fed other milks. As argued previously (Chapter 3) these effects were presumably due to the effects of accumulation of colostral protein in the enterocytes of the jejunum and ileum which are reported to be active for macromolecular absorption in the newborn piglets (Pierce & Smith, 1967; Clarke & Hardy, 1971). The greater percentage increases of the mucosal weight in the jejunum and ileum of the NS group compared to the other three groups which obtained no colostrum, should therefore be largely due to the mass of colostral protein accumulated in the mucosal cells (Xu *et al.*, 1992). The absence of this group difference with respect to the duodenum was presumably due to the fact that there was little or no colostral protein accumulation in the duodenal mucosa (Figs. 3.29 and 4.25; Xu *et al.*, 1992).

(d) the percentage increases in mucosal DNA content in all four groups were generally of the same order of magnitude in corresponding parts of the small intestine (Table 4.4).

This is an especially interesting observation because it suggests that during the first 24 hours after birth there is a minimum rate of cell division in the small intestinal mucosa (indicated by the DNA content) which is usually independent of dietary influences; other features like length, mucosal weight, RNA content and to a lesser extent RNA:DNA ratio are apparently affected by diets. On the other hand, in the unusual circumstances of feeding pooled sow colostrum or infant formula throughout the first 24 hours mucosal cell division was apparently accelerated as indicated by the higher DNA contents in the mucosae from those groups (Fig. 3.22). The pooled sow colostrum

would have provided higher inputs of growth promoting substances than would the colostrum/milk suckled from the sow because of the natural changes in colostrum /milk composition which occurs after birth (Baxter *et al.*, 1984; Cera *et al.*, 1987; Jaeger *et al.*, 1987; Read, 1988; Simmen *et al.*, 1990b), and infant formula fortified with lactose and containing unidentified potential growth promoting substances could have induced more cell division in the piglet than other diets.

(e) The mucosal RNA:DNA ratios in the small intestine generally increased in the NS group but decreased in the OGF, TPN and H_2O groups (Table 4.4).

The NS group was the only one to exhibit increases in the mucosal RNA:DNA ratios, the other three groups (OGF, TPN and H_2O) exhibited decreases. Since an increase in mucosal RNA:DNA ratio is an indicator of hypertrophic growth of the mucosa, these observations indicate that neither orogastric nor parenteral feeding of nutrient solution cause enterocyte hypertrophy. In contrast, colostrum received during natural suckling appears to be an effective, although weak, stimulus to mucosal cell hypertrophy, in addition to causing cell swelling.

4.4.2 Comparative Effects of TPN and OGF on Small Intestinal Growth

Feeding nutrient solution enterally compared to parenterally caused no significant differences in growth parameters such as intestinal weight, DNA, RNA and protein content and RNA:DNA and protein:DNA ratios. These observations are different from those in previous reports of trophic effects on the gut of enteral feeding: (1) small intestinal weight is significantly lower in parenterally fed piglets (Goldstein *et al.*, 1985; Morgan *et al.*, 1987) and rabbits (Gall *et al.*, 1987) than in enterally fed animals, particularly in the proximal small intestine; (2) rats maintained by TPN for 8 - 10 days have lower intestinal mucosal weights than do intragastrically fed animals (Castillo *et al.*, 1988, 1990); and (3) the mucosal protein and DNA contents are less in rabbits (Gall *et al.*, 1987) and rats (Castillo *et al.*, 1988, 1990) given TPN compared to others fed by intragastric infusion. The effect of enteral feeding on the gastrointestinal tract in newborn animals is different from that in mature animals. Enteral feeding promotes

small intestinal development in newborn animals (Koga *et al.*, 1975; Goldstein *et al.*, 1985; Gall *et al.*, 1987; Morgan *et al.*, 1987; Castillo *et al.*, 1988, 1990; Shulman, 1988), whereas several studies in mature animals have shown that enteral feeding is required to prevent decreases in the mass and normal functions of the small intestine (Cameron *et al.*, 1974; Levine *et al.*, 1974; Eastwood, 1977; Spector *et al.*, 1977; Hughes & Dowling, 1980; Meurling & Roo, 1981; Hughes & Ducker, 1982; Weser *et al.*, 1982a,b; Popp & Wagner, 1984; Weser *et al.*, 1985; Raul *et al.*, 1987; Shulman, 1988).

Furthermore, while previous studies have shown marked reductions in mucosal thickness, villous height and crypt depth (especially in the proximal small intestine) in TPN animals compared to intragastrically fed animals (Castillo *et al.*, 1988; 1990; Eastwood, 1977; Goldstein *et al.*, 1985; Koga *et al.*, 1975; Morgan *et al.*, 1987), the present study showed no significant differences in the histological structure of the small intestine between the TPN and OGF groups. These inconsistencies could be due to variations in age and to species differences and, especially to the duration of the experimental period, which was longer in most previous studies. The present study appears to be the first to use unsuckled piglets 0 - 24 hours old.

However, the more compact villi and the greater number of goblet cells in the TPN group compared to the OGF group found here are in accord with the reports of Cameron *et al.* (1974) and Heitman *et al.* (1980) who reported similar effects of TPN on morphology of the small intestine in mature rats. And the greater villous width in the OGF group compared to the TPN group possibly related to the uptake of nutrient solution along the villi.

During the present experiment, a 20%, instead of the usual 10%, fatty acid suspension was accidentally used to prepare the nutrient solution and was fed to four pairs of piglets using the same procedure as described in Section 4.2. Although the detailed results are not presented here, it is of interest to note that the results for the small intestinal parameters for these piglets were similar to those fed the present nutrient solution with 10% fatty acid. The liver weight, however, was significantly ($P<0.01$)

higher in the piglets enterally fed 20% fatty acid compared to those parenterally fed 20% fatty acid. Furthermore, when comparing the effects of the combined parenterally and enterally fed groups given either 20% or 10% fatty acid, feeding the high fatty acid suspension resulted in greater villous width ($P<0.001$ to $P<0.05$), mucosal RNA concentration ($P<0.01$ to $P<0.05$) and RNA:DNA ratio ($P<0.01$ to $P<0.05$). Jenkins & Thompson (1989) studied the trophic effect of fatty acids fed enterally on small intestinal mucosa and found that rats fed long-chain-fatty acids, instead of glucose, had greater increases in gut weight, mucosal weight and mucosal protein in all parts of the small intestine. Total mucosal DNA was also significantly increased in the middle small intestine, together with an increased crypt cell production rate (Jenkins & Thompson, 1989). The mechanism by which fat increases gut mass is unknown, however the texture of the diet appears to be involved (Jenkins & Thompson, 1989). The presence of fat in a mixed diet has a relatively modest effect on mucosal growth (Levine, 1991). Balint *et al.* (1980) compared the effects of feeding a diet containing 4% fat with a high-fat diet (45%) and found that the villous height in the distal jejunum was greater in the high-fat group, without a significant difference in DNA, suggesting that a high-fat diet produced intestinal hypertrophy, whereas Sircar *et al.*, (1983) reported no relationship between the fat content of the diet and parameters of intestinal growth.

4.4.3 The Intestinal Wall of the Lower ileum

The apparently greater wall and especially muscular thicknesses in the lower ileum of the TPN and OGF piglets compared to the piglets at birth could be due to distension of the intestinal tract by meconium in the intestinal lumen of the piglets sampled at birth (B). Distension of the ileum due to obstruction is known to cause thinning of the intestinal wall (Gabella, 1989). In the present experiment there was no evidence of obstruction, however distension of the ileum by meconium would tend to decrease its wall and muscular thickness in the piglets at birth. Conversely the wall and muscular thicknesses in the ileum of the piglets at 24 hrs, particularly in the TPN group, would tend to be thicker because, by this time, the meconium would have been expelled. Supporting this idea is the observation that the circumference in the lower ileum was smallest in the piglets at 24 hrs.

Another possible explanation for the greater wall and muscular thicknesses in the ileum is an effect on intestinal growth of the muscular contractions required to expel the meconium from the lower ileum during the first 24 hrs. Also, food entering the stomach of enterally fed piglets would stimulate, via the gastroileal reflex, contractions of the lower ileum (Kosterlitz, 1968; Roman & Gonella, 1987). Regular contractions cause hypertrophy of skeletal muscle (Goldspink, 1983; Saltin & Gollnick, 1983), but the effect of contractions of the gut on intestinal smooth muscle growth during the first 24 hrs after birth remain to be determined.

4.4.4 Conclusions

Feeding nutrient solution either orogastrically or parenterally slightly enhanced hyperplastic (but not hypertrophic) growth of the small intestine of the piglets during the first 24 hours after birth. The effect was more pronounced in the duodenum than other parts of the small intestine. This was possibly due to an obligatory basal development pattern, specifically in this region, or to effects on the duodenum of salivary EGF and pancreatico-biliary secretions. Also the lower ileum showed greater wall and muscular thicknesses in the piglets fed nutrient solution compared to those in piglets at birth. This could have resulted from distension of the distal small intestinal tract by the existence of meconium in the fetal piglet until birth or to contractions of small intestinal muscle to expel meconium during the first 24 hours after birth. The route of feeding had no effects on other growth parameters, except the small intestinal length which was greater in the piglets fed orogastrically. This is likely to be due to physiological elongation rather than growth.

In the following chapter the results of an experiment to investigate the effects of sucking on small intestinal development are presented and discussed.

CHAPTER 5

EFFECTS OF SUCKING AND OROGASTRIC FEEDING ON POSTNATAL DEVELOPMENT OF THE SMALL INTESTINE IN PIGLETS DURING THE FIRST 24 HOURS AFTER BIRTH

5.1 INTRODUCTION

Previous studies have shown that newborn animals naturally suckled gain weight more rapidly than do those fed by orogastric tube (Miller & Dymsza, 1963; Satinoff & Stanley, 1963). Newborn rats fed by orogastric tube without sucking opportunities exhibit abdominal distension, poor gastric emptying, decreased gastrointestinal motility and growth retardation (Miller & Dymsza, 1963). In ruminants, continuous sucking is an important stimulus for initiating oral lipase secretion (Ramsey *et al.*, 1956). Intermittent changes in pressure during sucking contribute to the stimulation of oral lipase secretion, which facilitates and improves small intestinal lipid absorption (Hamosh, 1979). These previous studies suggest a beneficial effect of sucking *per se* in newborn animals.

Sucking *per se* might influence postnatal development of the small intestine by way of the cephalic phase of digestion which is mediated entirely by the vagus nerves (Hirschowitz, 1989). The cephalic phase is represented by those motile and secretory activities of the gastrointestinal tract which are evoked by stimuli acting in the region of the head, such as the sight, smell, taste, expectation and chewing of palatable food (Debas, 1987). Sucking as part of the cephalic phase, possibly stimulates the vagal nerves by activating sensory nerves in the oral mucosa (Uvnas-Moberg, 1989), thereby causing the release of gastrointestinal hormones (Marchini *et al.*, 1987; Widstrom *et al.*, 1988; Uvnas-Moberg, 1989). Postprandial changes in plasma motilin, gastrin and pancreatic polypeptide concentrations are in part dependent upon intact vagal pathways (Chung *et al.*, 1992). Thus, if such gastrointestinal hormones are released in response to sucking, sucking could stimulate growth and maturation of the gastrointestinal tract (Uvnas-Moberg, 1989).

There have apparently been no studies of the cephalic phase of digestion in the newborn. It may be speculated, however, that sucking is a strong stimulus of the cephalic phase of digestion in the newborn as the act of sucking involves large pressure changes within the oral cavity (Titchen & Newhook, 1975). Alternatively, as newborn animals have no prior experience of food, at first the expectation of receiving food may be a weak stimulus for the cephalic phase. Also stimuli from chewing solid food may be less or none as milk is the only food in the newborn. However, olfactory, visual, thermal and chemical stimuli are considered to be important in the initiation of sucking behaviour in newborn ruminants as well as in other species (Titchen & Newhook, 1975).

Non-nutritive sucking in both term and preterm infants causes a significant increase in circulating insulin levels within 45 seconds of the start of sucking (Marchini *et al.*, 1987). Breast feeding causes a significant increase in circulating gastrin levels in 3-day-old infants within 5 - 10 minutes of the start of feeding (Marchini *et al.*, 1992). Somatostatin levels are unaffected by non-nutritive sucking (Marchini *et al.*, 1987) or breast feeding (Marchini *et al.*, 1992) but are significantly reduced when non-nutritive sucking is combined with bolus feeding (Widstrom *et al.*, 1988). Plasma cholecystokinin (CCK) levels in 4 day-old newborn infants increase almost immediately after breast feeding begins, but then decline after about 10 minutes, followed by a secondary rise 30 - 60 minutes after feeding (Uvnas-Moberg *et al.*, 1993). The first peak may be due to a sucking related activation of vagal nerves while the second may be due to an effect on the duodenal CCK-producing cells of food in the duodenum (Uvnas-Moberg *et al.*, 1993).

These studies indicate that gastrointestinal hormones may be released in response to sucking. However, there have apparently been no studies of the effect of sucking *per se* on postnatal development of the gastrointestinal tract in neonates. Therefore, the primary objective of the present study was to investigate the effects of sucking on postnatal development of the small intestine in newborn piglets during the first 24 hours.

Several studies indicate that the composition of the food influences the pattern of gut hormone release in response to feeding in newborns (Aynsley-Green *et al.*, 1979;

Lucas *et al.*, 1980c,1981b). Thus newborn infants fed breast milk have significantly higher circulating enteroglucagon, but not gastrin, insulin, pancreatic glucagon or gastric inhibitory polypeptide (GIP) levels than do infants fed 10% dextrose (Aynsley-Green *et al.*, 1979), and infants fed cow's milk formula on the sixth postnatal day have significantly greater insulin and GIP responses to feeding than do breast fed infants (Lucas *et al.*, 1981b). Also there are significant differences in plasma GIP and pancreatic polypeptide (PP) concentrations and plasma amino acid profiles between premature infants fed with human milk and milk formula, with higher GIP levels in the infants fed milk formula and higher PP levels in the breast fed infants (Calvert *et al.*, 1985). Therefore the present experiment was also designed to study interactions between the effect of sucking and the two different diets, sow colostrum and infant formula.

5.2 MATERIALS & METHODS

5.2.1 Animal Preparation

Twenty unsuckled Large White newborn piglets were selected on the basis of body weight as described previously (see Section 3.2.1). Four piglets from each of 5 sows were randomly assigned to one of 4 treatment groups. The first 2 groups, SC-S (sow colostrum-sucking) and SC-OGF (sow colostrum-orogastric feeding), were fed sow colostrum by bottle or orogastric tube (Feeding tube, Pharmaseal, USA) respectively. The third and forth groups, IF-S (infant formula-sucking) and IF-OGF (infant formula-orogastric feeding), were fed infant formula, the former by bottle and the latter by orogastric tube. Four piglets, one from each group, were kept together in an open-topped plastic bin (40 cm wide, 62.5 cm long, 37.5 cm high) where the temperature was maintained between 32 - 38°C by suspending a heating lamp above the crate. Sow colostrum and infant formula with the same total volume (220 ml/kg bw) were prepared (see Section 3.2.2) and fed to the piglets according to the schedule shown in Table 5.1. The nutritional values of the diets are shown in Table 5.2, and the nutrient and energy intakes are shown in Table 5.3. In order to study mucosal cell proliferation and migration BrdU was administered using the 4-dose regime described in Section 4.2.1.

5.2.2 Specimen Collection and Analyses

After 24 hours the piglets were euthanased (see Section 4.2.2) and the gastrointestinal tract was dissected and tissue samples were collected as described in Section 3.2.3. The histological measurements, biochemical analyses, and mucosal cell proliferation and migration estimates were all conducted as previously described (see Sections 3.2.4).

Table 5.1 Feeding times and volumes of the feeds given to the piglets fed sow colostrum (SC) or infant formula (IF) by bottle sucking (S) or by orogastric feeding (OGF).

FEED	TIME AFTER BIRTH (hrs)	VOLUME (ml/kg bw)
1st	2	20
2nd	4	20
3rd	6	20
4th	8	20
5th	10	40
6th	14	40
7th	18	40
8th	22	20

Table 5.2 The nutritional value (% W/W) of the sow colostrum and infant formula.

CONSTITUENTS	NUTRITIONAL VALUE (%W/W)	
	SOW COLOSTRUM	INFANT FORMULA
Lactose	2.41	6.57
Lipid	6.28	3.12
Protein	17.35	1.53

Table 5.3 Nutrient and energy intake of the piglets given either sow colostrum (SC) or infant formula (IF) by bottle sucking (S) or orogastric feeding (OGF).

CONSTITUENTS	INTAKE	
	SC-S & SC-OGF	IF-S & IF-OGF
Protein (g/kg bw)[1]	38.17	3.37
Lipid (g/kg bw)[2]	13.82	6.86
Lactose (g/kg bw)[3]	5.30	14.45
Total energy (kJ/kg bw)	1285	573
Usable energy (kJ/kg bw)[4]	629	515

[1] Protein = 17.2 kJ/g
[2] Lipid = 38.9 kJ/g
[3] Lactose = 17.2 kJ/g
[4] Usable energy is the energy produced by metabolism of lactose and lipid; during the first 24 hours piglets can metabolize protein only at a very low rate (Mellor & Cockburn,1986)

5.2.3 Statistical Analysis

The following statistical comparisons were made.

(a) A comparison between bottle sucking and orogastric tube feeding:
(SC-S + IF-S vs SC-OGF + IF-OGF)
(b) A comparison between bottle sucking and orogastric tube feeding of sow colostrum:
(SC-S vs SC-OGF)
(c) A comparison between bottle sucking and orogastric tube feeding of infant formula:
(IF-S vs IF-OGF)
(d) A comparison between sow colostrum and infant formula:
(SC-S + SC-OGF vs IF-S + IF-OGF)
(e) A comparison between bottle sucking of sow colostrum and infant formula:
(SC-S vs IF-S)
(f) A comparison between orogastric tube feeding of sow colostrum and infant formula:
(SC-OGF vs IF-OGF)

In addition, comparisons were made between individual groups. As in the previous experiments the variation in body weight of the piglets at the beginning of the experiment could have affected the outcome of the experiment, therefore all data were subjected to analysis of covariance using body weight at birth as a covariate. All data were analyzed by computer using the SAS and MINITAB programs. The level of significance is expressed as * = $P<0.05$, ** = $P<0.01$ and *** = $P<0.001$. The means and S.D.'s for the results presented in this chapter are given in appendices 5.1 - 5.4. The results for the B and NS groups from the first experiment (Chapter 3) have been included in the graphs for visual comparison; they were not included in the statistical analysis.

5.3 RESULTS

There were no statistically significant differences between the effects of sucking and orogastric tube feeding with either diet on small intestinal development during the first 24 hours, whereas the diet (colostrum or infant formula) had significantly different effects on almost all parameters of growth. Consequently, the significant differences between the SC-OGF and IF-S groups and between the SC-S and IF-OGF groups were likely to be due to differences between the diets rather than to the methods of feeding. Therefore when presenting the results below no distinction has been made between the methods of feeding.

5.3.1. Body Weight Change, and Liver and Pancreatic Weights

The groups fed sow colostrum demonstrated significantly greater increases in body weight compared to the groups fed infant formula (Fig. 5.1). Conversely, the liver weights (Fig. 5.1) for the two infant formula fed groups combined were significantly higher than those of the colostrum fed groups combined; the IF-OGF group had the highest liver weight which was significantly higher than the SC-S group. There were no significant differences between the pancreatic weights of all groups (Fig. 5.1).

5.3.2. Small Intestinal Length and Weight

Sow colostrum feeding resulted in a significantly greater total intestinal length, and jejunal and ileal lengths, compared to infant formula feeding whereas there were no significant differences among all groups for the duodenal length (Fig. 5.2).

The total weights of the intact small intestine and small intestinal mucosa and muscle of the colostrum fed groups were significantly higher than those of the infant formula fed groups (Fig. 5.3). Sow colostrum feeding significantly increased the weight of each of the 5 parts of the intact small intestine compared to infant formula feeding (Fig. 5.4). Similar results were obtained for the mucosal weight of the upper and lower jejunum and ileum, but not the duodenum (Fig. 5.5). Differences between the effects

of sow colostrum and infant formula feeding on the muscular weight were less pronounced than those observed for the mucosal weight, and were significant except in the upper ileum (Fig. 5.6).

5.3.3 Microscopic Structures of the Small Intestine

The diet, but not the method of feeding, significantly affected almost all the microscopic measurements, including the circumference, wall thickness, villous height and width and crypt depth. The muscular and submucosal thicknesses were not affected significantly.

Colostrum feeding significantly increased the circumferences of the upper and lower jejunum and ileum, but not the duodenum, when compared to feeding of infant formula (Fig. 5.7). Wall thickness (Fig. 5.8) and villous height (Fig. 5.9) in piglets fed colostrum were significantly greater in all parts of small intestine than those in the piglets fed infant formula. The villous width in the piglets fed colostrum was significantly greater than in those fed infant formula in the jejunum and ileum, but not in the duodenum (Fig. 5.10). In contrast, there were no significant differences between colostrum and infant formula feeding in crypt depth, except for the duodenum where the two colostrum fed groups combined had significantly greater crypt depths than did the two infant formula fed groups combined (Fig. 5.11). Colostrum feeding had no significant effects on muscular thickness (Fig. 5.12) and submucosal thickness (Fig. 5.13) compared to the feeding of infant formula.

Microscopic examination of the mucosae revealed swollen mucosal cells containing large vacuoles in the lower jejunum, and upper and lower ileum, in the piglets fed colostrum whereas the mucosal cells in the piglets fed infant formula were less swollen and contained smaller vacuoles (Figs. 5.14 and 5.15).

5.3.4 Mucosal Cell Proliferation and Migration Rate

There was no apparent difference between the effects of the diets on mucosal cell

proliferation as there was no significant difference in the number of labelled dividing cells per crypt area (Fig. 5.16). The piglets orogastrically fed colostrum had significantly longer relative migration distances in the duodenum than those fed infant formula by bottle (Fig. 5.17).

5.3.5 DNA, RNA and Protein in the Small Intestinal Mucosa

Since mucosal DNA and RNA concentrations (Figs. 5.18 and 5.19) can be affected by the high protein content of the mucosa due to intracellular protein retention as discussed in Section 3.4.2, only the results from the estimation of total DNA and RNA contents will be described here and used for interpretation. The mucosal DNA contents (Fig. 5.20) showed no significant differences at all, whereas the mucosal RNA contents (Fig. 5.21) of the piglets fed colostrum were significantly higher than those in the piglets fed infant formula in the jejunum and ileum, but not the duodenum. Both mucosal protein concentration and content were affected by colostrum feeding. Colostrum fed groups had significantly higher mucosal protein concentration (Fig. 5.22) and content (Fig. 5.23) than did those fed infant formula in all parts of the small intestine.

Since there were no significant differences in the mucosal DNA contents, the means for the protein:DNA and RNA:DNA ratios were influenced directly by protein and RNA contents respectively. Thus the protein:DNA (Fig. 5.24) and RNA:DNA (Fig. 5.25) ratios for the colostrum fed groups were significantly higher than those for the piglets fed infant formula, in all parts of the small intestine except for the RNA:DNA ratio in the duodenum.

5.3.6 Lactase Activity

Similarly, since the mucosal lactase activity per g wet tissue (Fig. 5.26) was affected by the intracellular protein retention of mucosal cells as discussed in Section 3.4.3, only the total lactase activity and lactase activity per DNA weight will be presented here and used for interpretation. The total lactase activity (Fig. 5.27) of all

groups showed a physiologically normal distribution along the small intestine (Manner & Stevens, 1972; Aumaitre & Corring, 1978), with high activity in the middle parts and low in the duodenum and lower ileum. The total lactase activity showed no significant difference between colostrum and infant formula feeding in all parts of the small intestine, except in the duodenum where the two colostrum fed groups combined had a significantly higher total lactase activity than did the two infant formula fed groups combined. The lactase activities per mg DNA, i.e. cellular lactase activities, in the upper and lower ileum of the piglets fed infant formula were significantly higher than those of the piglets fed colostrum (Fig. 5.28).

5.4 DISCUSSION

The present study is apparently the first to evaluate the effects of sucking *per se* on small intestinal development during the first 24 hours after birth. Sucking *per se* appeared to have no obvious effects during this period. On the other hand, compared to the feeding of infant formula, feeding colostrum caused significantly greater effects on small intestinal growth, as reported in Chapter 3, but the present experiment used a more sensitive model than before because piglets from the same litters were equally represented in each group. The present experiment provided in addition an opportunity to evaluate the dynamics of cell proliferation in the small intestinal mucosa, involving as it does cell division in the crypts, cell migration along the villi and cell shedding from the villous tips.

5.4.1 Effects of Sucking

In the present study the possible interactive effects on small intestinal development of sucking *per se* and diet were studied by including groups of piglets given two separate diets, sow colostrum and infant formula, by two different methods, sucking and orogastric tube. Colostrum was chosen as one of the diets to allow observation of possible interactive effects between sucking and colostral growth promoting substances. The nutrient solution used in the previous study (Chapter 4) would have been a better control diet than the infant formula (processed and fortified bovine milk), because its composition can be controlled to exclude unknown natural constituents which might have trophic effects on the gut, but the piglets refused to suck the nutrient solution.

No apparent effects of sucking on postnatal development of the small intestine in the present newborn piglets were demonstrated during the first 24 hours as no significant differences were observed between the small intestinal features in the bottle fed and tube fed piglets. This may have been due to the period of study being too short to detect an effect and/or to the low frequency of feeding during the present experimental period. Although sucking may stimulate the release of gut hormones by

vagal stimuli (Uvnas-Moberg, 1989), and these hormones can induce small intestinal development (see Section 2.3.5), their effects may be cumulative and take longer than 24 hours to become detectable. Also under normal conditions a sow will nurse her piglets every hour, or even more frequently, so that the piglets can be fed more than 24 times during the first 24 hours (Pond & Maner, 1984). In the present experiment the piglets were fed 8 times during the 24-hour experimental period.

5.4.2 Effects of Colostrum and Infant Formula Feeding

The differences in intestinal development between the sow colostrum and infant formula fed piglets largely paralleled those described in Chapter 3. Although the piglets fed colostrum generally had greater small intestinal weights (Figs. 5.3 - 5.6) than did those fed infant formula, there was no difference in the mucosal DNA content (Fig. 5.20). However mucosal DNA content for each of the groups was noticeably greater than for the piglets at birth. Although the piglets fed colostrum had a greater mucosal RNA content (Fig. 5.21) and RNA:DNA ratios (Fig. 5.25) than did those fed infant formula, both groups appeared to have lower RNA:DNA ratios compared to the piglets at birth. These observations indicate that both sow colostrum and infant formula feeding increase mucosal cell number (hyperplasia), but neither stimulates cell growth (hypertrophy). This latter observation conflicts in part with the results for the experiment described in Chapter 3. In that experiment feeding caused duodenal hypertrophy as evidenced by an increase from birth in the duodenal RNA:DNA ratio. The reason for this conflict is not readily apparent but may be due to the relatively low RNA concentrations recorded for the present experiment (see Appendix 5.3) compared to those for the two previous experiments (see Appendices 3.4 and 4.4); the experiment in Chapter 3 included the piglets sampled at birth i.e. the B group. The differences between the RNA concentrations is most likely due to the samples from the three experiments having been assayed in different batches.

The greater total lactase activity (Fig. 5.27) in the duodenum of the piglets fed sow colostrum presumably was due to substances in colostrum, e.g. insulin which as discussed in Section 3.4.3. has been shown to increase enterocyte lactase activity in

some species. Conversely, cellular lactase activities in the upper and lower ileum of the piglets fed infant formula were significantly higher than in the piglets fed sow colostrum. The reasons for this are not known as there were no significant effects of diet on the total lactase activities and DNA contents in either region of the ileum. It is noted that the lactase activities for the groups in the present experiment were lower than those for the NS and B groups (Figs. 5.26 - 5.28). This was possibly due to the fact that the samples were assayed in different batches by different people.

5.4.3 Mucosal Cell Dynamics in the Duodenum and Elsewhere in the Small Intestine

The changes to the microscopic structures of the small intestine reported in the present experiment reflected the trophic effects of sow colostrum and the increases in cell size caused by cell swelling as described above. Colostrum feeding increased wall thickness (Fig. 5.8) and villous height (Fig. 5.9) in all parts of the small intestine, whereas increases in villous width (Fig. 5.10) and small intestinal circumference (Fig. 5.7) were confined to the jejunum and ileum and were absent from the duodenum where less macromolecular absorption occurs (Lecce, 1973; Leary & Lecce, 1976). Of interest is the present observation that the relative migration distance, crypt depths and muscular and submucosal thicknesses were greatest in the duodenum compared to other parts of the small intestine, regardless of diet or method of feeding. Therefore, estimates of the cell migration rates and replacement times were made to compare the mucosal cell dynamics in the duodenum with other parts of the small intestine.

The estimated cell migration rate (the proportion of villous height occupied by new enterocytes during the first 24 hours) was calculated by dividing the difference between the relative migration distance and the crypt depth by the villous height, then multiplying by 100 to obtain a percentage. The estimated replacement time (number of days required for enterocytes to migrate from the crypt bases to the villous tips at a fixed migration rate) was calculated by dividing the total length of the crypt depth plus the villous height by the relative migration distance. This calculation did not take into account the effects on the replacement time of cell mixing or lateral movement of the

cells emerging from the crypts (see Section 2.1.2.2). Comparison of the estimated cell migration rates and replacement times at 24 hours in the piglets fed sow colostrum (Table 5.4) and in the piglets fed infant formula (Table 5.5) demonstrated faster rates (2 - 4 times faster) and shorter replacement times (0.4 - 0.6 times shorter) in the duodenum than in the other parts of the small intestine, regardless of diet fed. The shorter replacement time in the duodenum was evidently due to the combined effects of the greater relative migration distance (Fig. 5.17) and shorter total distance (crypt depth plus villous height) (Figs. 5.9 and 5.11) in the duodenum than in the jejunum and ileum. The results for the replacement times at 24 hours in the present study (about 5 days for the duodenum and about 8 - 14 days elsewhere) are different from those of Moon (1971) who reported replacement times (at 24 hours) of 9 - 10 days in the duodenum, upper jejunum and lower jejunum, and times in the upper and lower ileum of 7 - 10 days. The discrepancy between these results could be due to the different methods used; Moon (1971) used autoradiography with [^{3}H]thymidine labelling and the piglets were naturally suckled.

The observation that crypt depth in the duodenum was greater in the colostrum fed piglets compared to those fed infant formula is of interest. The results presented in Chapter 3 show increases in both duodenal crypt depth (Fig. 3.13) and duodenal villous height (Fig. 3.11) during the first 24 hours in the piglets fed sow colostrum whereas previous studies have shown that crypt depth in piglets either remains unchange during the first 10 days after birth (Smith & Jarvis, 1978) or may actually decrease during the first 3 weeks after birth (Moon, 1971). In rats there are concomitant increases in crypt depth and villous height in the duodenum, jejunum and ileum during postnatal days 6 to 22 (Yeh, 1977). The results of the experiment described in Chapter 3 indicate that in piglets during the first 24 hours, mucosal growth in the duodenum at least is characterised by concomitant increases in both villous height and crypt depth. The present results indicate that the increase in crypt depth at least may be due to some factor(s) within colostrum.

Table 5.4 Estimated cell migration rates (CMR) and replacement times (RT) of epithelial cells in the small intestine of newborn piglets fed *sow colostrum* for 24 hrs (n = 10).

PARAMETERS	DUO	UPJ	LOJ	UPI	LOI
RMD (µm)[1]	171	119	103	101	86
CD (µm)[2]	118	82	77	74	70
VH (µm)[3]	799	1201	1194	1251	965
CMR (%)[4]	6.5	3.0	2.2	2.3	2.0
RT (days)[5]	5.5	10.9	12.5	13.6	12.8

[1] Relative migration distance
[2] Crypt depth
[3] Villous height
[4] $CMR = \frac{(RMD-CD)}{VH} \times 100$
[5] $RT = \frac{(CD+VH)}{RMD}$

Table 5.5 Estimated cell migration rates (CMR) and replacement times (RT) of epithelial cells in the small intestine of newborn piglets fed *infant formula* for 24 hrs (n = 10).

PARAMETERS	DUO	UPJ	LOJ	UPI	LOI
RMD (µm)[1]	148	106	97	90	87
CD (µm)[2]	103	82	74	69	73
VH (µm)[3]	586	783	780	807	576
CMR (%)[4]	9.1	3.0	2.8	2.8	2.4
RT (days)[5]	4.9	8.4	10.2	10.2	11.6

[1] Relative migration distance
[2] Crypt depth
[3] Villous height
[4] $CMR = \frac{(RMD-CD)}{VH} \times 100$
[5] $RT = \frac{(CD+VH)}{RMD}$

5.4.4 Mucosal Cell Shedding in the Piglets Fed Colostrum and Infant Formula

Four observations made in the present study allowed the effects of the different diets on cell shedding to be explored. Firstly, crypt depth (Fig. 5.11) varied in different parts of the small intestine but diet generally had no effect on crypt depth, except in the duodenum where the crypts were slightly deeper in the colostrum fed piglets. Secondly, villous height (Fig. 5.9) was greater in the piglets fed sow colostrum than in those fed infant formula. Thirdly, the relative migration distances (Fig. 5.17) of cells upwards from the base of the crypts during the first 24 hours were similar for both diets, again except for the duodenum where migration distances were greater in the group fed colostrum by orogastric tube. Fourthly, mucosal cell swelling apparently associated with retention of colostral protein (Chapter 3) was greater in piglets fed sow colostrum than in those fed infant formula (Fig. 5.15). These observations give rise to 2 questions. Firstly, what caused the greater villous height in the piglets fed sow colostrum - cell swelling or an increase in cell number, or both? Secondly, what do these observations reveal about cell shedding from the villous tips?

The relative contributions of cell swelling and increases in cell number to the greater villous height in the colostrum fed piglets compared to the infant formula fed piglets were explored in the following way. The percentage increases in villous cell width, as an indicator of cell swelling, were compared to the percentage increases in villous height in every part of the small intestine (see Table 5.6). If the percentage increases in cell width and villous height were similar, then the greater villous height in the piglets fed colostrum would have been mainly due to cell swelling. On the other hand, if the percentage increases in villous height were greater than those of cell width then the greater villous height would have been due to the combined effects of a greater cell number along the villi and cell swelling. The percentage increase in cell width was estimated by dividing the difference between the cell widths in piglets fed sow colostrum and infant formula by the cell width of the piglets fed infant formula, then multiplying by 100 to obtain a percentage. The estimated percentage increase of villous height was calculated in the same way as for the cell width. That method was justified because the villous height of the piglets fed infant formula were similar to those of the

Table 5.6 Comparison of the estimated percentage increases in intestinal cell width (CWP) and villous height (VHP) between the piglets fed sow colostrum (SC) and infant formula (IF) for 24 hrs (n = 10 per group).

PARAMETERS	DUO	UPJ	LOJ	UPI	LOI
CW (μm per 100 cells)[1] :-					
SC	717	899	966	1034	891
IF	665	750	791	768	648
CWP (%) [2]	8	20	22	35	37
VH (μm)[3] :-					
SC	799	1201	1194	1252	969
IF	586	784	870	807	576
VHP (%)[4]	36	53	37	55	68

[1] Cell width

[2] CWP = $\frac{(SC-IF)}{IF} \times 100$ (of cell width)

[3] Villous height

[4] VHP = $\frac{(SC-IF)}{IF} \times 100$ (of villous height)

Table 5.7 Comparison of the increases of villous height with the relative migration distance of epithelial cells in the small intestine of newborn piglets fed sow colostrum for 24 hrs.

PARAMETERS (μm)	DUO	UPJ	LOJ	UPI	LOI
VH[1] :-					
B[2]	445	897	809	882	565
SC[3]	799	1201	1194	1251	965
VH increase	354	404	385	369	400
RMD[4]	171	119	103	101	86

[1] Villous height

[2] Piglets at birth (n = 5 from Chapter 3)

[3] Piglets fed sow colostrum for 24 hrs. (n = 10)

[4] Relative migration distance

piglets at birth, little or no mucosal cell swelling apparently occurs in the piglet fed infant formula (Chapter 3), and the piglets fed sow colostrum and infant formula in the present experiment were from the same litters unlike the piglets studied at birth.

As shown in Table 5.6, the mucosal cells and villi in the duodenum were 8% wider and 36% longer, respectively, in the piglets fed sow colostrum compared to those fed infant formula. Therefore cell swelling can account for about 22% (8/36 x 100) of the increase in villous height in the duodenum of piglets fed colostrum whereas the rest could be due to a greater number of cells along the villi. Similarly, the data in Table 5.6 revealed that in the lower jejunum, upper ileum and lower ileum of the piglets fed colostrum, 54 - 64% of the greater villous height can be accounted for by cell swelling, and the rest could be due to an increase in the number of villous cells. These observations correspond with the fact that the jejunum and ileum are sites of active macromolecular absorption (Pierce & Smith, 1967; Clarke & Hardy, 1971) and the microscopic appearance of the mucosae in both these regions of the intestine in the piglets fed sow colostrum clearly demonstrated cell swelling, unlike the duodenum (Fig. 5.15).

The second question regarding the extent of cell shedding from the tips of the villi is considered as follows. First, there were no increases in villous height (Fig. 5.9) or in crypt depth (Fig. 5.11) from birth in the piglets fed infant formula, and the mucosal cells migrated upwards towards the tips of the villi as indicated by relative migration distance (Fig. 5.17). Similar results (Figs. 3.11, 3.13 and 3.21 respectively) were also found in the experiment described in Chapter 3. Based on the assumption that the number of cells on the villi depends upon two opposing factors - cell production in the crypts and cell extrusion from the villous tips (Klein & McKenzie, 1983b) - these observations indicate that cell shedding from the tips of the villi must have occurred in the piglets fed infant formula during the first 24 hours. However it could be argued that the size of the villous cells in the piglets fed infant formula might have been smaller than that in the piglets at birth, so that although new cells migrated up towards the tips of the villi, there was no change in overall villous height, and no cells were extruded from the tips of the villi. The microscopic appearance of the villous cells in the piglet

fed infant formula (Figs. 3.15C and 3.16C) and the villous cells in the piglet at birth (Fig. 3.17A) do not support this argument, as there appeared to be little or no difference in cell size between the two groups.

In the experiment described in Chapter 3, there were increases in villous height in the jejunum and ileum of the piglets fed sow colostrum during the first 24 hours (Fig. 3.11) but there was no change from birth in the villous height for the piglets fed infant formula. Thus in the present experiment the differences between the sow colostrum and infant formula fed piglets in the height of the villi in these regions of the small intestine are representative of the increases in villous height from birth in piglets fed sow colostrum. According to the information presented in Tables 5.6, 54 - 64% of the increases in the height of the villi in the jejunum and ileum can be accounted for by cell swelling; the remaining 34 - 46% can be accounted for by the increase in the number of cells along the villi. According to the results for the relative migration distance (Table 5.7) the migration of new cells up the villi from the crypts is only 20 - 29% of the increase in villous height from birth in colostrum fed piglets. Despite this apparent anomaly, these data when considered together indicated there was little or no shedding of cells from the villous tips in the piglets fed sow colostrum. Because this conclusion is based on the comparison of data from separate experiments it needs to be confirmed by a specific study of mucosal cell dynamics in newborn piglets fed colostrum, possibly using electronmicroscopic techniques. It is noteworthy, however, that the present conclusion is in accord with the observations that there is virtually no villous cell extrusion from villous tips in newborn piglets during the first week after birth (Smith & Jarvis, 1978).

If the above conclusion is true, the shedding of cells from the tips of the villi in the piglets fed infant formula, and the likely absence of such shedding in the piglets fed sow colostrum could be due to one or both of the following: the presence in the infant formula of constituents which stimulate cell shedding or the presence in the sow colostrum of constituents which inhibit cell shedding. Both of these possibilities merit further investigation. Whether early cell shedding from the villi and short replacement times in newborn piglets fed infant formula would disturb the normal growth pattern and

functions of the small intestine requires study, as previous studies on rats revealed that intestinal enzyme activity increases in direct relation to the differentiation and development of subcellular organelles during enterocyte migration (DeBoth & Plaisier, 1974; DeBoth *et al.*, 1974).

5.4.5 Conclusions

Sucking *per se* showed no obvious effects on small intestinal growth during the first 24 hours after birth, which might have been due to the short period of the experiment or to a lower than normal frequency of feeding. On the other hand, feeding colostrum caused significantly greater effects on small intestinal development compared to feeding infant formula. The estimated cell migration rate was faster and mucosal cell replacement time was shorter in the duodenum than in other parts of the small intestine, regardless of diet fed. The greater villous height in the piglets fed sow colostrum compared to those fed infant formula was apparently due to the combined effects of cell swelling and an increase of the number of cells along the villi. Calculations which revealed that cell shedding from the tips of villi occurred in the piglets fed infant formula but not in those fed sow colostrum, indicate either that constituents in infant formula may stimulate cell shedding or that constituents in sow colostrum may inhibit cell shedding, or both.

CHAPTER 6
GENERAL DISCUSSION

6.1 COMPONENTS OF SMALL INTESTINAL GROWTH

At the time the present experiments were planned, the first 24 hours after birth was considered to be a period of marked small intestinal growth, as indicated by 100% increases from birth in the weight of the small intestinal mucosa (Widdowson *et al.*, 1976; Widdowson, 1984). Furthermore, the growth was thought to be primarily due to the effects of colostral/milk-borne growth factors (Hall & Widdowson, 1979; Berseth *et al.*, 1983; Berseth, 1987a; Simmen *et al.*, 1990a). Therefore, the first 24 hours after birth were chosen for study as a period when the small intestine was likely to be particularly sensitive to stimulation by growth factors.

Work in this thesis, especially that reported in Chapter 3, and concurrent studies in the same laboratory (Xu *et al.*, 1992) and elsewhere (Burrin *et al.*, 1992) have led to a significant modification of these ideas. That is because the increase in mucosal weight in the small intestine, which the present work (Chapter 3) and the concurrent work (Xu *et al.*, 1992) confirmed had a magnitude of about 100% during the first 24 hours after birth, has now been shown to have three likely components: cellular swelling, hyperplasia and possibly hypertrophy.

The first component is cellular swelling associated with retention of colostral/milk proteins, especially in the jejunum and ileum. The histological appearance of the enterocytes themselves at 24 hours after birth indicated cell swelling as opposed to growth (Chapter 3; Xu *et al.*, 1992), a swelling which was not evident at birth (Chapter 3; Xu *et al.*, 1992) or at 72 hours after birth (Xu *et al.*, 1992). That swelling was present only when the mucosal protein concentrations were high: (1) in the present study swelling was evident at 24 hours only in those piglets fed high protein liquid foods (colostrum) but not in those fed foods with lower protein concentrations (milks); (2) swelling was also observed in naturally suckled piglets only at 24 hours when mucosal protein concentrations were high (Chapter 3; Xu *et al.*, 1992), but not at 72

hours when the concentrations had returned to birth values (Xu *et al.*, 1992). Furthermore, the calculations in Chapter 5 revealed that swelling might have contributed as much as 50% to the increase in villous height in the jejunum and ileum, which are the sites of active macromolecular absorption in the newborn piglet (Pierce & Smith, 1967; Clarke & Hardy, 1971). This result also suggests that there was a large contribution of cell swelling to the increase of mucosal weight.

The second component of the mucosal weight increase is hyperplasia as identified in the present work (Chapter 3) and by Xu *et al.* (1992). The hyperplasia was indicated by the percentage increase (approximately 55%) in the mucosal DNA content during the first 24 hours, which Xu *et al.* (1992) attributed to actions of colostrum/milk derived growth factors. However, Xu *et al.* (1992) studied only naturally suckled piglets so that their experimental design could not distinguish between colostrum/milk-induced hyperplasia and any inherent mucosal cell division which might have occurred independently of intakes of liquid foods. The experimental design used in Chapter 3 could make this distinction because it employed feeding piglets with a range of diets, including colostrum, milk and water alone. As outlined in Chapters 3 and 4, mucosal cell hyperplasia during the first 24 hours after birth was found to occur independently of the diet in those piglets fed sow milk, cow colostrum, cow milk and water, and the level of that hyperplasia was similar to that found in naturally suckled piglets (see also Xu *et al.*, 1992). It is particularly striking that the general magnitudes of the increases in the mucosal DNA contents in the different segments of the small intestine were similar in water fed, milk fed and naturally suckled piglets (Chapter 3), and in those piglets given nutrient solution enterally or parenterally (Chapter 4). These observations indicate some form of obligatory hyperplasia which is independent of the intakes both of exogenous growth factors and of nutrients. However, the present results (Chapter 3) provide evidence that this obligatory hyperplasia can be accelerated during the first 24 hours by some diets, because the piglets fed pooled sow colostrum and those fed infant formula exhibited significantly greater mucosal DNA contents at 24 hours than did any other groups. As metabolites alone, given enterally or parenterally (Chapter 4), did not increase DNA contents above those found in water fed piglets (Chapter 3 and 4), the stimulation of hyperplasia both by pooled sow colostrum and by the infant formula was

probably due to growth promoting substances which in colostrum could include growth factors such as EGF (Jaeger *et al.*, 1987) and IGF-I (Simmen *et al.*, 1988), and hormones such as insulin (Jaeger *et al.*, 1987), but which in infant formula remain to be identified.

The third component which could have contributed to the mucosal weight increase is hypertrophy, i.e. cellular growth. However any hypertrophy appeared to be confined to the duodenum. The RNA:DNA ratio used as an indicator of cellular growth increased during the first 24 hours after birth only in the duodenum and only in the piglets fed sow colostrum (Chapter 3), whereas this ratio decreased in the rest of the small intestine.

It may be concluded therefore that the increase in the mass of the intestinal mucosa during the first 24 hours is to a large extent due to mucosal cell swelling which could mask the trophic effects of colostral/milk-borne growth factors. Despite this, it is apparent from the present experiment that pooled sow colostrum with extra growth factors and fortified diets such as infant formula accelerate hyperplasia of the small intestine (Chapter 3). These observations raise several interesting questions which are worth further investigation, namely: (a) what are the factors or substances in sow colostrum or infant formula that induce small intestinal growth, (b) what is the effective dose of those substances and (c) does the route of administration influence the effectiveness of those substances?

The experimental techniques developed for the experiment described in Chapter 4 may form the basis of a useful model for the study of these questions since they allow the study of intestinal development in the absence of colostral protein and growth promoting substances. The piglets in that experiment were prevented from sucking to eliminate the effects of milk growth factors and the TPN was used to completely exclude exogenous luminal nutrients from the intestine while maintaining the nutritional status of the piglets at what was calculated to be a normal level. The same TPN solution with equivalent caloric value was given to the matched enterally fed piglets to minimize variation in the nutritional status between the two groups. The DNA and

morphometric results for that experiment indicate there is no difference in small intestinal growth between piglets fed nutrient solution parenterally or enterally for the first 24 hours after birth. In this respect the results of the present study provide valuable information for future studies of the enteral and parenteral actions of growth promoting substances affecting small intestinal development in neonatal pigs. The OGF model can be used separately to study the effects of growth factors, and both the TPN and the OGF models used together would be suitable for studying the effects of route of administration, by fortifying nutrient solution with growth factors. However in order to study the effects of growth factors on postnatal development using these experimental models, it would be advisable to extend the period of study beyond 24 hours for several days.

For the longer experimental period, there are two things which have to be considered. Firstly, it is possible that under these new conditions a difference between the parenteral and enteral feeding on small intestinal growth might have become obvious. Secondly, as newborn piglets deprived of colostrum are immuno-incompetent pretreatment with antibiotics to prevent bacterial infection is recommended. Therefore the different routes of feeding between parenteral and enteral at the period beyond 24 hours as well as effects of antibiotic treatment on intestinal growth would have to be assessed.

6.2 UNIQUE FEATURES OF THE DUODENUM

A striking outcome of all three experiments (Chapters 3, 4 and 5), not apparently reported before, was that the duodenum exhibited unique developmental features during the first 24 hours after birth. These features can be classified into 2 components: a greater development and a greater sensitivity to trophic substances, than in other parts of the small intestine.

In the piglets fed nutrient solution either parenterally or enterally for 24 hours (Chapter 4), there were significant increases in intact weight and mucosal weight of the duodenum, whereas there were no corresponding increases in other parts of the small

intestine. Significant increases in mucosal RNA and protein contents were also confined to the duodenum. These observations indicated greater cell growth in this part than in other parts of the small intestine, regardless of feeding routes. Furthermore, at the microscopic level the duodenal structures also had unique features: (1) piglets fed nutrient solution either parenterally or enterally for 24 hours exhibited significant increases in wall thickness, villous height and crypt depth only in the duodenum, regardless of feeding routes (Chapter 4); (2) compared to piglets at birth the piglets in the combined 24-hour-groups exhibited a significant increase of crypt depth only in the duodenum (Chapter 3); (3) greater relative migration distances of enterocytes and crypt depths were seen in the duodenum than in the rest of small intestine, regardless of the diet or method of feeding (Chapter 5); and (4) both in piglets fed sow colostrum and in those fed infant formula, the estimated cell migration rate was faster and the estimated cell replacement time was shorter in the duodenum than in other parts of the small intestine. All of these observations lead to the conclusion that during the first 24 hours after birth the duodenum exhibits unique developmental features which are independent of diet, route of feeding (enteral or parenteral) and method of feeding (sucking or orogastric tube feeding).

In addition to a faster growth rate, the duodenum exhibited a greater sensitivity to the trophic effects of colostral constituents. Piglets fed sow colostrum for 24 hours (Chapter 3) had significantly increased mucosal RNA:DNA ratios in the duodenum whereas the rest of the intestine showed no significant changes. When the effects of feeding sow colostrum were compared with those of infant formula (Chapter 5), significantly greater crypt depths and greater relative migration distances were found in the piglets fed sow colostrum but only in the duodenum.

A number of possible factors which may induce extra growth in the duodenum have been discussed previously in Section 4.4.1 (i.e. an obligatory basal development pattern or salivary EGF and/or pancreatico-biliary secretions). Three more possible contributory factors will now be considered. They are (1) the embryonic origin of the duodenum, (2) the location of the duodenum in the gastrointestinal tract and (3) the pH of the duodenal contents.

As the embryological origin of the duodenum is the foregut, whereas the jejunum and ileum develop from the mid gut (Noden & De Lahunta, 1985), the duodenum could have a developmental pattern which is different from that of the rest of the small intestine. The location of the duodenum at the proximal end of the small intestine means that it is the first part of the intestine to be exposed to salivary EGF and pancreatico-biliary secretions which have been reported to be trophic to intestinal tissue (see Sections 2.3.2.2 and 2.3.6 respectively), and the first to be exposed to ingested nutrients. The duodenum would therefore be expected to be exposed to high concentrations of potential growth promoters which could result in a more rapid growth of it compared to the other parts of the small intestine. In addition to those growth promoters, the duodenum is also the first part of the intestine to be exposed to the low pH of stomach contents entering the small intestine (pH 2.6 - 6.1 in newborn piglets during the first 24 hours; Xu & Cranwell, 1990). As both the stomach and the duodenum develop from the foregut (Noden & De Lahunta, 1985), this might confer on both of them a common capability of dealing with acidic contents, unlike the jejunum and ileum which have a different origin. This inherent capability might therefore be related to the embryonic origin of the stomach and duodenum. It is possible that exposure to corrosive contents from the stomach could induce more cell shedding from the tips of the duodenal villi. In order to cope with large extrusions of villous cells, the mucosal cells of the duodenum would need to have faster migration rates, shorter replacement times and/or greater proliferation rate in the crypts. These conclusions are supported by the evidence presented in the preceding chapters of this thesis.

6.3 PARTICULAR RESPONSES TO FEEDING COW COLOSTRUM AND FEEDING INFANT FORMULA

Feeding cow colostrum to newborn piglets during the first 24 hours resulted in pronounced decreases in the mucosal RNA concentration, RNA content and RNA:DNA ratio (Chapter 3). Independent work in this laboratory has shown that bovine lactoferrin fed orally to newborn piglets for 5 days decreased the RNA content and RNA:DNA ratio in the intestinal mucosa (D. Shu, H.V. Simpson, R-J. Xu, D.J. Mellor, G.W. Reynolds & R.B. Marshall; unpublished observations). It is of interest to note that

antigenic activity of cow's milk which induces hypersensitivity reactions is confined to the protein component, and neither milk fat nor lactose is antigenic (Ferguson & Watret, 1988). Whether the decrease in the RNA content in the piglets fed cow colostrum in the present experiment was due to lactoferrin only or to some other heterologous protein remains to be determined. In the present study the piglets fed cow milk (heterologous protein at a lower concentration) and those fed sow colostrum (homologous protein at a higher concentration) showed no decreases of RNA content, therefore the effect of cow colostrum might have been due to its high concentration of heterologous protein. A number of studies of cow milk allergy have been conducted (see Ferguson & Watret, 1988), some have been concerned with hypersensitivity reactions of the intestinal mucosa (see Ferguson, 1987), but there have apparently been no studies of the effect of cow colostrum/milk allergens on mucosal RNA content.

Although feeding cow milk, in contrast to cow colostrum, did not decrease mucosal RNA content in the newborn piglets, its effect may be different in other species such as the human. The protein concentration of unprocessed cow milk (about 33 g/L) is more than three times that of human milk, and processed cow milk (infant formula), which is most commonly used for term infants, contains 15 g/L of cow milk protein (Savilahti & Kuitunen, 1992). In view of the present results, the effect, if any, of these relatively high concentrations of heterologous protein on the RNA content of the small intestinal mucosa in the human infant deserves further investigation. It is interesting to note that in the piglet the effect of feeding heterologous protein on mucosal RNA content continues at least until the fifth day of age (D. Shu, H.V. Simpson, R-J. Xu, D.J. Mellor, G.W. Reynolds & R.B. Marshall; unpublished observation), although by this time macromolecular absorption has already ceased (Pond & Maner, 1984). It can be speculated that if cow milk decreases mucosal RNA content of the infant small intestine, the effect might be age dependent and disappear within a short period after birth when the intestine is mature and can fully inhibit the passage of some specific protein in the milk. Whether or not such decreases in mucosal RNA content, even for a brief period of time, influence later postnatal development of the small intestine remains an open question.

Feeding infant formula, which has a high concentration of lactose, provides more glucose than does natural milk, and subsequently helps prevent hypoglycaemia and sustain glycogen stores during the immediate postnatal period (Mellor & Cockburn, 1986). The large amount of glucose resulting from lactose digestion would also be expected to cause more endogenous insulin release (Mellor, 1987) and thereby induce more lactase activity in the piglets fed infant formula compared to milk fed piglets (Chapter 3). The mucosal DNA content of the piglets fed infant formula during the first 24 hours was comparable to that in the piglets fed sow colostrum (Chapter 5) and significantly greater than that in the piglets fed sow milk or cow milk (Chapter 3). Thus although colostrum has a physiologically significant effect on postnatal growth of the small intestine in newborn animals, feeding infant formula as a replacement of natural milk gives a comparable outcome in small intestinal growth in these respects.

It may be concluded from the present observations that feeding infant formula can have a beneficial effect on small intestinal development in newborn animals, however the potential effects of the heterologous constituents in the infant formula on mucosal cell RNA content have to be considered. In this regard, feeding infant formula may be an adequate short-term replacement for colostrum (if colostrum is unavailable), however natural milk from the homologous species is a more suitable long-term substitute. It should be noted that the benefits of homologous colostrum/milk antibody in providing passive immunity immediately after birth and in providing continuing protection against gastrointestinal pathogens would be lost if natural colostrum/milk was not fed (Patt, 1977).

6.4 THE TPN EXPERIMENT AND ITS IMPLICATIONS

Generally the results of the TPN experiment described in Chapter 4 showed no beneficial effect of enteral compared to parenteral feeding on postnatal development of the small intestine. This does not accord with the work of Aynsley-Green and colleagues who introduced and largely developed the hypothesis that enteral feeding in newborn infants stimulates metabolic and postnatal gastrointestinal adaptation to extrauterine nutrition through the release of gut peptides (see Aynsley-Green, 1983,

1985, 1989). Furthermore it does not support the idea prevalent in hospital neonatal units that enteral feeding is important for gut development in both preterm and full term babies (Schandler, 1988). There are two possible causes for this apparent discrepancy. Firstly, it is possible that during the first 24 hours enteral feeding has no apparent effect on postnatal growth of the small intestine whereas in the neonatal unit the infants are generally fed enterally for longer periods (Schandler, 1988). Thus if the present experiment had been continued for a longer period, beneficial effects of enteral feeding on small intestinal development may have become apparent. Secondly enteral feeding of nutrient solution in the form of an elemental diet is not effective in protecting endogenous growth factors for example salivary EGF, from digestion by luminal protease. Feeding an elemental diet either parenterally or enterally has previously been shown to be associated with intestinal atrophy, and this atrophy can be prevented by enteral feeding of whole protein rather than the constituent amino acids (Playford *et al.*, 1993). An *in vitro* study has demonstrated that luminal growth factors in the intestine can be digested by proteases, but this degradation can be prevented by the presence of food proteins, probably via occupation of the active sites of the enzymes (Playford *et al.*, 1993). Therefore, it is possible that whereas food protein prevents digestion of luminal growth factors, the nutrient solution used in the present experiment does not. Thus, the nutrient solution used here might cause different results from those in newborn infants in hospital neonatal units where infants are generally fed human milk (Schandler, 1988).

Substantial changes in plasma levels of a wide range of gut peptides are induced by oral feeding in newborn humans, and these changes do not occur in infants deprived of enteral feeding (Lucas *et al.*, 1980a,b,1985; also see Section 2.4). It would be of interest to determine whether enteral feeding induces similar changes in circulating gut peptide levels in newborn piglets. Such determinations were beyond the scope of the present study. Studies of circulating gut peptide levels should be carried out in parallel with future investigations of factors controlling gut development in neonatal piglets.

In the TPN experiment described in Chapter 4, hypertonic nutrient solution (770-930 mOsm/L) was infused into newborn piglets during the first 24 hours. The methods

used for the infusion of nutrient solution were carefully chosen to help the piglets cope with the hypertonic solution. Nutrient solution was infused through a cannula into an umbilical artery rather than an umbilical vein to eliminate the risk of portal venous congestion (Touloukian *et al.*, 1973) and physical damage to the liver by the catheter tip and the hypertonic solution. Also care was taken when cannulating the artery to position the tip of the cannula in the abdominal aorta, so that the hypertonic solution was diluted rapidly when it entered the circulation. The TPN piglets apparently experienced no difficulties adapting to the infusion of hypertonic solution during the first 24 hours after birth, because the nutrient solution was infused at a slow rate (11.25 ml/hr) and its constituents would have been removed from the circulation and processed metabolically. During the first 24 hours the newborn piglet has a high metabolic rate to maintain body temperature (Pond & Maner, 1984; Mellor & Cockburn, 1986). The nutrient solution provided approximately 409 kJ/kg bw of energy (see Table 4.3) while the piglets required approximately 227 kJ/kg bw (see Appendix 4.1), so that more than 50% of nutrient solution energy would have been utilized within 24 hours. The excess would have been stored as glycogen or fat after metabolic conversion or incorporated into body tissues (Mellor & Cockburn, 1986). Moreover the complete metabolism of utilized nutrients will produce carbon dioxide and water, and this metabolic water would act to reduce the osmolality. This is confirmed by the serum osmolality of 283 mOsm/L in the TPN piglets after infusion of nutrient solution for 24 hours. In the OGF piglets enteral feeding of hypertonic nutrient solution theoretically could have caused the movement of water into the intestinal lumen. Such a water flux would decrease the osmolality in the lumen. Later the nutrient constituents (digested or not) of the nutrient solution would be absorbed into the cells of the intestinal mucosa and circulation.

6.5 COMPARATIVE EFFECT OF BrdU INJECTION METHODS ON MEASUREMENT OF CELL PROLIFERATION IN THE CRYPTS AND ON MEASUREMENT OF RELATIVE MIGRATION DISTANCE

In the present study BrdU was used to determine (1) cell proliferation in the crypts and (2) relative migration distance of the mucosal cells. BrdU injected at the end of the treatment (1 and 2 hours before euthanasia) would give a suitable index of *cell*

proliferation at that time whereas BrdU injected at the beginning of the treatment (soon after birth) would be a suitable marker for *cell migration distance* during the period between injection and death.

In the experiment described in Chapter 3, a single dose of BrdU was injected intravenously at birth, before the start of feeding. A recent study on the clearance rate for BrdU (V.J. Grant, M.J. Birtles, P.A. Clifton & D.N. Palmer; unpublished observation) has shown that BrdU is metabolized very rapidly by the liver and is likely to be removed from the circulation after one pass through the liver. Therefore a single dose of BrdU at birth would have been inadequate for maintaining circulating levels of BrdU sufficiently high for labelling cells which divided during the middle and later stages of the experiment. Thus the present results for the mitotic index (BrdU labelling) may not directly reflect changes in crypt cell proliferation due to the effects of the diets. This may have caused the discrepancy observed between the mitotic index of crypt cells (BrdU labelling) and the hyperplastic index (DNA content) as explained in Section 3.4.2.

Because of the emergence of the limitations described above, the method of BrdU injection was changed in the experiments outlined in Chapters 4 and 5. BrdU was injected intraperitoneally at 1 and 2, and at 22 and 23 hours after birth. The number of doses was increased in an attempt to maintain high circulating levels of BrdU for a prolonged period so that most if not all of the dividing cells would be labelled with BrdU. The intraperitoneal route of administration was chosen because of the difficulty of injecting into superficial blood vessels in piglets. The times of the injections at 1 and 2 hours before euthanasia (i.e. 22 and 23 hours after birth) were chosen to label the crypt cells dividing at the end of the treatment period. Despite these changes there were no significant differences in mitotic indices between the OGF and TPN groups (Chapter 4) and between the piglets fed sow colostrum and those fed infant formula either by sucking or by orogastric feeding (Chapter 5). These results suggest that either there is no difference between the effects of the different treatments on the mitotic index in the crypts, or that the BrdU technique for labelling dividing cells did not detect differences between the treatments. The first explanation is the most plausible as in the experiments described in Chapters 4 and 5 the mitotic index results (BrdU labelling) corresponded

with the results for the hyperplastic index (DNA content). Another possible explanation is the toxic effects of BrdU which has been reported to inhibit or change the pattern of cell proliferation in the small intestine (Weghorst *et al.*, 1991; V.J. Grant, M.J. Birtles, P.A. Clifton & D.N. Palmer, unpublished observation). Whether or not this affected the present results is not known.

There were significantly greater relative migration distances in the jejunum and ileum of the piglet fed sow colostrum compared to other groups in the experiment described in Chapter 3 (using a single dose of BrdU injected intravenously at birth) whereas the results of the experiment described in Chapter 5 (using 4 doses of BrdU injected intraperitoneally) indicated that the relative migration distances in the jejunum and ileum in the colostrum fed piglets were not significantly different from those fed infant formula. According to these observations it is possible that a single intravenous injection of BrdU before the start of feeding might be more appropriate than the other method, as an early time of injection may provide more time for villous cells to migrate and exhibit the different effects between treatments. Another possibility is that BrdU has been reported to be toxic to cell proliferation, it is not known whether or not it affected villous cell migration and the present results.

6.6 CONCLUSIONS

Work described in this thesis on small intestinal development in piglets during the first 24 hours after birth has demonstrated several phenomena which are noteworthy and can be summarised as follows.

(1) The greater increase of small intestinal weight in the piglets fed sow colostrum compared to those in the piglets fed other diets is caused by cellular swelling related to colostral protein accumulation, cell hyperplasia and possibly cell hypertrophy.

(2) During the first 24 hours after birth basal cell division in the small intestinal mucosa occurs independently of food intake and routes or methods of feeding, however the rate of cell division can be accelerated by some as yet undefined constituents in sow colostrum or infant formula.

(3) The duodenum exhibits unique features which can be classified into 2 major

components: a greater growth and a greater sensitivity to the trophic effect of sow colostrum, compared to other parts of the small intestine. These features are possibly due to an obligatory developmental pattern of the tissue itself, the trophic effects on the duodenum of nutrients, salivary EGF and/or pancreatico-biliary secretions, and/or the adaptation of duodenal mucosa on exposure to acidic contents from the stomach.

(4) Feeding cow colostrum to newborn piglets during the first 24 hours causes a pronounced decrease in mucosal RNA content with unknown effects.

(5) Diets influence postnatal development of the small intestine whereas the route (enteral or parenteral) or the method (sucking or orogastric tube feeding) of feeding had no significant effects on small intestinal development in piglets during the first 24 hours after birth.

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