National Library of Canada
Acquisitions and Bibliographic Services Branch
395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada
Direction des acquisitions et des services bibliographiques
395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Canada

EFFECTS OF ORALLY ADMINISTERED SPERMIDINE ON ABSORPTIVE ENZYME AND NUTRIENT TRANSPORTER GENE EXPRESSION IN THE RAT SMALL INTESTINE DURING POSTNATAL DEVELOPMENT

by

Lynne E. Searles

School of Dietetics and Human Nutrition
MacDonald College
McGill University, Montreal

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements of the degree of Master of Science.

July, 1995

ISBN 0-612-08052-8

TITLE SHORTENED VERSION:

Effects of spermidine on enzyme and SGLT1 gene expression in the rat.

To my parents
Sylvan and William Searles

ACKNOWLEDGEMENTS

My deepest gratitude is expressed to my supervisors Dr. Kristine Koski and Dr. Gary Wild who provided me with guidance and encouragement during this research project. Thanks is extended to Dr. Gary Wild who introduced me to the exciting fields of cell and molecular biology. Many thanks to Dr. Alan Thomson for his helpful discussions and support.

My sincere thanks to the departments of Anatomy and Cell Biology of McGill University and the McGill University Medical Clinic for permitting me the use of laboratory facilities necessary to bring this project to completion. Many thanks to staff from the department of Dietetics and Human Nutrition of McGill University for all their help and support.

A special thanks is expressed to the McGill Inflammatory Bowel Disease Research Group for scholarship support. I am grateful to Dr. Alan Thomson and Dr. Gary Wild for financial support during my graduate studies.

Thanks are extended to Dr. Richard Hemming and Dr. Julio Faria for their valuable advice, technical support and constant encouragement. I express a special thanks to Monica Keelan for her advice, technical help and encouragement towards the completion of this thesis.

Finally, I wish to express my great appreciation to family and friends for their love, support, encouragement, and understanding throughout this work.

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

TABLE OF CONTENTS CONTINUED

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES CONTINUED

LIST OF FIGURES CONTINUED

LIST OF FIGURES CONTINUED

LIST OF ABREVIATIONS

ACTH	adrenocorticotropic hormone
ATP	adenosine triphosphate
BLOTTO	bovine lacto transfer technique optimizer
BLM	basolateral membrane
BSA	bovine serum albumin
cDNA	complementary deoxyribonucleic acid
DEPC	diethylpyrocarbonate
DFMO	α-difluoromethylornithine
EGF	epidermal growth factor
GLUT2	facilitative glucose transporter
GLUT5	facilitative fructose transporter
K^+	potassium ion
kb	kilobase
kDa	kilodaltons
mRNA	messenger ribonucleic acid
Na^+	sodium ion
Na^+K^+ ATPase	sodium - potassium - adenosinetriphosphatase
M_r	apparent molecular weight
MVM	microvillous membrane
ODC	ornithine decarboxylase
RNase	ribonuclease
rRNA	ribosomal ribonucleic acid
SDS	sodium dodecyl sulfate
SGLT1	sodium-glucose co-transporter
SI	sucrase-isomaltase
SSC	saline sodium citrate
TTBS	Tris-TWEEN buffered saline

Abstract

The developmental profiles of mRNA and protein expression for ornithine decarboxylase (ODC), the Na^+-dependent glucose co-transporter (SGLT1), sucrase isomaltase (SI), and the Na^+K^+ ATPase α_1 and β_1 subunit isoforms in the postnatal rat small intestine, as well as the effects of exogenous spermidine on their precocious development, were examined. Postnatal age had a significant effect with all enzymes and the nutrient transporter maturing around weaning. Consecutive exposure to exogenous spermidine during suckling precociously induced ODC mRNA, SI protein, and SGLT1 gene expression in the proximal and distal small intestine. Levels of Na^+K^+ ATPase α_1 and β_1 subunit isoform mRNA were precociously induced in the proximal small intestine only. These findings show that exposure to exogenous spermidine can promote precocious alterations in intestinal enzyme and nutrient transporter expression; however, it appears that spermidine must be continuously supplied for these alterations to persist in suckling rats.

Résumé

Nous avons procédé à l'analyse des enzymes ornithine decarboxylase (ODC), sucrase-isomaltase (SI), la Na^+K^+ ATPase (les isoformes α_1 et β_1), et le transporteur SGLT1 au niveau de l'ARN et l'expression de ces protéines au cours du développement de l'intestin grêle du jeune rat, et après l'administration de la spermidine. Le niveau de l'ARN et de l'expression de ces proteines ont augmentés avec l'âge et ont demontrés un accroissement associer avec le sevrage. L'administration consécutif de la spermidine a stimulé prématurément la transcription des gènes de ODC, SGLT1 et SI dans les portions proximal et distal de l'intestin grêle du rat néonatale. Dans le cas des isoformes α_1 et β_1 de la Na^+K^+ ATPase la transcription prématurée a été observer uniquement dans la portion proximal de l'intestin grêle. Ces résultats démontre que l'administration de la spermidine doit être continuelle pour maintenir l'expression précoce de la transcription des gènes pour les enzymes intestinales et le transporteur de glucose que nous avons etudiés dans ce travail.

REVIEW OF LITERATURE

INTRODUCTION

Ontogeny of the Postnatal Small Intestine

The endpoints of development in the small intestine are associated with the acquisition of a mature profile of digestive enzymes and transport mechanisms which facilitate nutrient absorption. These endpoints have been examined extensively in the rodent. In the postnatal rat, weaning is associated with the establishment of jejunoileal gradients of intestinal morphology and function that are characteristic of the adult phenotype [Henning, 1985; Castillo et al., 1992].

During the third postnatal week, a characteristic proliferation and differentiation of epithelial cells takes place that results in morphological and functional changes that are peculiar to the mature small intestine [Henning, 1987]. The small intestine of the postnatal rat exhibits enzyme activities that are typically abundant at birth but subsequently decline with weaning (ie. lactase, ß-galactosidase), or activities that are absent, or low at birth and later appear or surge at weaning (ie. sucrase and maltase respectively) to rapidly reach, and in some cases, overshoot adult levels [Henning, 1987; Toloza and Diamond, 1992]. As weaning is approached, the high lipid permeability and typical endocytic capacity characteristic of the suckling rat small intestinal mucosa declines leading to physiological closure to the uptake of macromolecules [Weaver and Walker, 1989; Meddings and Theisen, 1989]. Within this time frame, the enterocyte life span is reduced from 7-10 days (immature pattern) to approximately 2-3 days (adult pattern) and is explained by an increase in cellular proliferation and migration rates along the villus axis [Tsuboi, 1981]. Nutrient uptake is altered at weaning, with apparent increases in the intestinal uptake of nutrients such as glucose, fructose, and lysine, and concomitant reductions in the uptake of proline and leucine [Toloza and Diamond, 1992].

The developmental changes associated with weaning are

generally well characterized, however, the factors regulating the adaptive changes in intestinal form and function during the postnatal period remain unclear. Much research in this area has focused on factors such as surges in circulating hormones and qualitative changes in dietary constituents, which are known to undergo developmental changes just prior to, or at the time of intestinal maturation. Lebanthal (1989) proposed that the processes regulating intestinal maturation may be influenced by interrelated mechanisms such as genotype, intrinsic timing, and endogenous regulatory mechanisms, as well as environmental factors. This would suggest that internal (ie. genetically hard-wired timing mechanisms) and/or external signals (ie. nutrients) regulate age- and region-specific postnatal intestinal maturational processes [Buddington, 1994].

There is a growing body of experimental evidence which suggests that polyamines may play a critical role as intracellular messengers in the regulation of gastrointestinal mucosal growth. The observation that intestinal ornithine decarboxylase (ODC) activity and mucosal polyamine concentrations are markedly increased during weaning suggests a regulatory role for polyamines in postnatal intestinal maturation [Luk et al., 1980]. Given the concentrations of these substances in rat colostrum, mature rat milk and rat chow, ingested polyamines may modulate the adaptive changes which take place in the postnatal rat small intestine around weaning [Romain et al., 1992]. While the induction of a precocious maturation of rat small intestinal morphology and function following the administration of exogenous polyamines has been documented [Dufour et al., 1988; Georges et al., 1990; Wild et al., 1993;1994; Kaouass et al., 1994], it remains to be determined whether polyamines are natural "triggers" of postnatal intestinal maturation.

These initial observations form the framework to further examine the role of polyamines in the ontogeny of carbohydrate

absorption in the postnatal rat small intestine. The aim of the present review is to summarize what is known of the factors influencing carbohydrate absorption during development in the postnatal rat small intestine. To this end we will focus on the functional expression of the Na^+/glucose cotransporter (SGLT1) in the postnatal rat, and other apical and basolateral membrane markers of intestinal maturation (ie. sucrase-isomaltase and Na^+K^+ ATPase respectively).

THE INTESTINAL ABSORPTION OF SUGARS

In the mature omnivore, the digestion of dietary carbohydrate occurs predominantly in the small intestine and is mediated by hydrolytic enzymes released from the pancreas as well as by carbohydrases, such as sucrase, maltase, and lactase, present on the microvillus membrane (MVM) of mature enterocytes [Levin, 1994]. The final products of carbohydrate digestion, primarily D-glucose, D-galactose, and D-fructose, are absorbed into the body by enterocytes lining the upper third of the intestinal villi [Hediger and Rhoads, 1994]. Sugar assimilation can occur along the entire length of the small intestine, however, most monosaccharides are absorbed before the chyme reaches the ileum [Wright, 1993].

The lipid bilayer which forms the plasma membrane of the cell is impermeable to polar molecules. For monosaccharide absorption to proceed, a two-step process involving a carrier mediated transcellular transport of sugars occurs. D-glucose and D-galactose are actively absorbed across the MVM of mature enterocytes by an integral membrane protein, the Na^+/glucose cotransporter (SGLT1) [Crane, 1977; Hediger et al., 1989]. This symport couples sugar transport to intracellular sodium and electrical gradients [Crane, 1962]. The SGLT1 cotransports two Na^+ for each molecule of glucose/galactose [Hediger and Rhoads, 1994], and the luminal to cytosol sodium gradient which drives sodium-dependent transport is established by the activity of the Na^+/K^+ ATPase located on the

basolateral membrane (BLM) of the cell [Crane, 1977; Horisberger, 1991]. By contrast, D-fructose absorption appears to be independent of sodium transport and is mediated by a facilitative transporter specific for fructose absorption (GLUT5) [Kayano et al., 1990; Thorens, 1993]. GLUT5 has been localized to the MVM of fully differentiated enterocytes occupying the upper half of the small intestinal villus [Davidson et al., 1992]. The exit of monosaccharides from the enterocyte down their concentration gradients into the interstitium occurs via a facilitative sugar transporter (GLUT2) which is localized to the BLM of fully differentiated absorptive cells [Thorens et al., 1990]. GLUT2 may mediate the transport of D-glucose, D-galactose, and D-fructose out of the mature enterocyte [Thorens, 1993]. The transcellular monosaccharide transporters are illustrated in Figure 1.

SUCRASE-ISOMALTASE

Physiological Significance

Sucrase and isomaltase are two distinct disaccharidases that are essential for the terminal digestion of dietary carbohydrate. The hydrolysis of sucrose into glucose and fructose is achieved by sucrase, while isomaltase generates two glucose molecules from the hydrolysis of isomaltose [Hoffman and Chang, 1993]. In humans, sucrase-isomaltase activities develop early in gestation with adult levels of activity present at birth [Buddington, 1994]. In altricial species, such as the rat, both sucrase and isomaltase activities are undetectable until approximately postnatal day 17 with adult levels of activity reached by the end of weaning [Henning, 1981]. The low levels of maltase activity detected in the suckling rat small intestine increase dramatically with weaning [Tsuboi et al., 1979; Henning, 1981]. Given the distinctive developmental curve for sucrase-isomaltase, this enzyme complex has been extensively studied as an apical marker of intestinal development in the postnatal rat.

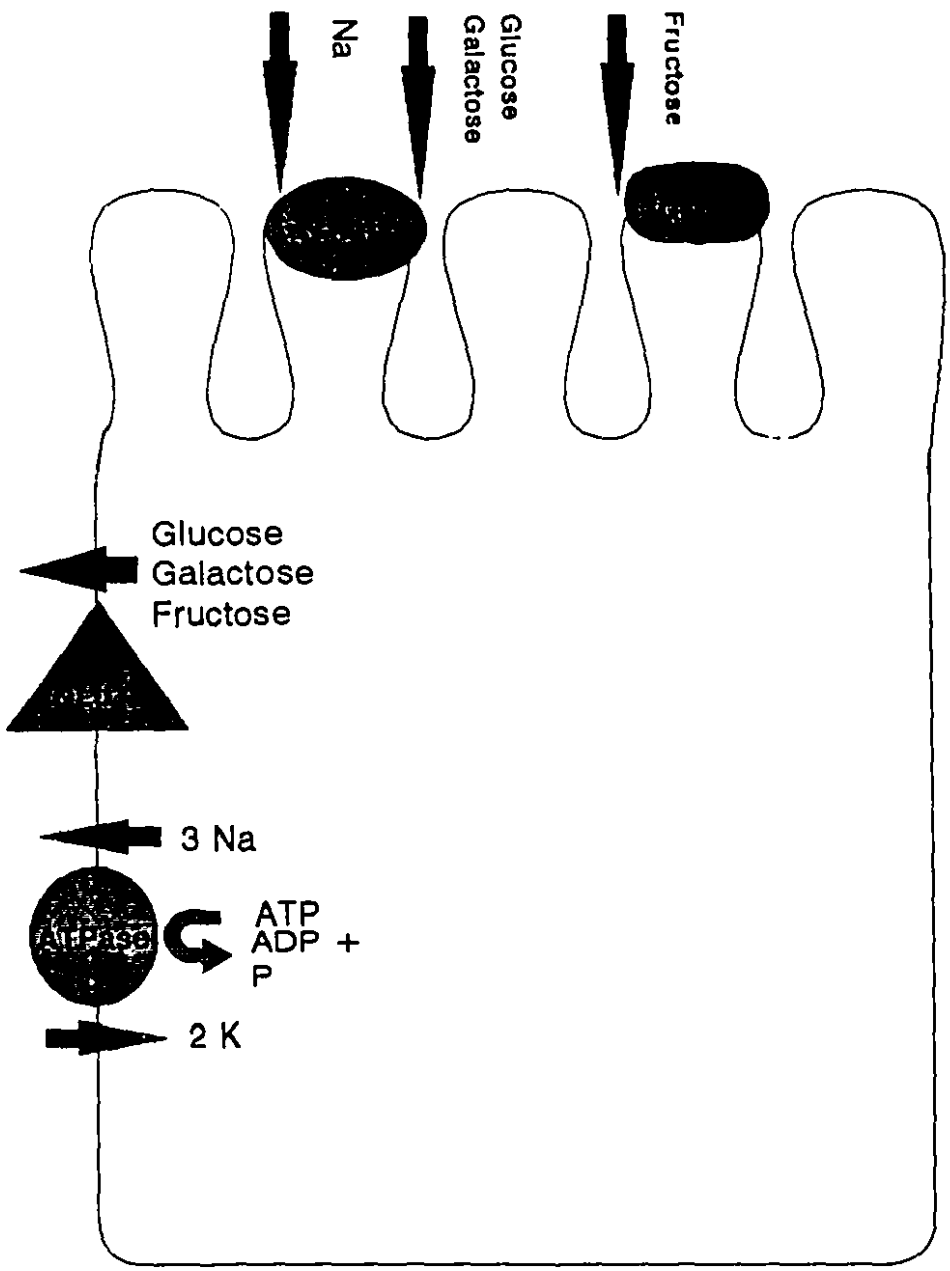


Figure 1: The carrier-mediated transcellular transport of monosaccharides.

Cell and Molecular Biology

The sucrase-isomaltase complex (SI) is synthesized as a single polypeptide from an mRNA of approximately 6.0 kb [Hunziker et al., 1986]. The SI polypeptide is heavily glycosylated in the endoplasmic reticulum and Golgi apparatus prior to transport by smooth vesicles to the apical portion of the enterocyte, where the mature glycoprotein is inserted into the MVM [Lorenzsonn et al., 1987; Levin, 1994]. Luminal (pancreatic) proteases cleave the SI protein complex, separating it into two functional units: sucrase and isomaltase. Once inserted into the MVM, sucrase is noncovalently bound to isomaltase, which is anchored to the cell membrane by a small length of hydrophobic amino acids near the N-terminus [Hunziker et al., 1986; Hoffman and Chang, 1993].

The high mannose form of SI (M_r 210 kDa) is considered immature given its lower specific activity as compared to other forms of SI [Lorenzsonn et al., 1987; Naim et al., 1988]. Further processing of immature SI in the Golgi produces the mature, complexly glycosylated form of SI (M_r 217 kDa) possessing full enzymatic activity [Lorenzsonn et al., 1989; Hoffman and Chang, 1993]. Once inserted in the MVM, cleavage of SI by pancreatic hydrolases yields isomaltase (M_r 140 kDa) and sucrase (M_r 120 kDa).

Sucrase-Isomaltase Expression

SI activity appears to increase progressively along the crypt-villus axis during enterocyte differentiation. Little or no activity can be detected in villus crypts, and maximal SI activity is present mid-villus [Hoffman and Chang, 1993]. Using a panel of monoclonal antibodies raised to human SI, Beaulieu et al. (1989) demonstrated SI protein in both crypt and villus cells. Thus, the authors suggested that posttranslational mechanisms control the differential expression of SI in human jejunum crypt and villus cells

[Beaulieu et al., 1989]. However, subsequent studies in both human and rat small intestine detected SI mRNA at the crypt-villus junction, suggesting that SI gene expression is regulated primarily by the levels of SI mRNA accumulated in enterocytes as they emerge from the crypts [Traber, 1990; Traber et al., 1992]. Using RNase protection assays, recent work has demonstrated increased transcriptional rates for SI in the postnatal rat small intestine during development [Krasinski et al., 1994]. Given the close correlation found between SI mRNA, protein levels, and sucrase specific activities in respective regions of the postnatal small intestine, Krasinski et al. (1994) suggest that the developmental and horizontal regulation of SI biosynthesis is determined primarily by gene transcription. The relative contributions of transcriptional and posttranscriptional mechanisms as determinants of SI gene expression require further analysis.

In the mature rat small intestine the greatest abundance of SI mRNA is seen in the distal jejunum, which is paralleled by maximal sucrase activity in this region [Leeper and Henning, 1990; Hoffman and Chang, 1993]. In decreasing order of magnitude, SI mRNA abundance has also been detected in the proximal jejunum, the proximal ileum, the duodenum, and the distal ileum [Leeper and Henning, 1990]. In contrast to the jejunum, sucrase activity does not necessarily correspond to SI mRNA content in regions of the duodenum and ileum and this argues in favour of the post-transcriptional control of regional enzyme activity [Hoffman and Chang, 1993]. During small intestinal ontogeny in the rat, SI mRNA is first detectable in the jejunum at postnatal day 14 at levels corresponding to sucrase activity in this region [Krasinski et al., 1994]. Subsequent developmental increases in SI mRNA abundance have been found to accompany increased enzyme activity [Krasinski et al., 1994], both of which plateau at postnatal day 24 [Leeper and Henning, 1990].

Regulatory Control in the Small Intestine

The regulation of sucrase-isomaltase in the developing small intestine of the postnatal rat is reviewed elsewhere (see Regulatory Mechanisms of Ontogeny).

It is generally accepted that sucrase and isomaltase activities can be rapidly altered in response to dietary manipulation. Studies have demonstrated increases in SI activity following high carbohydrate diets [Broyart et al., 1990] and refeeding [Holt and Yeh, 1992], and decreased enzyme activity in response to low carbohydrate diets [Broyart et al., 1990] or fasting [Holt and Yeh, 1992]. In adult rats, decreases in SI activity and SI protein have been shown to change in proportion following short periods of starvation and refeeding [Holt and Yeh, 1992]. Similarly, Broyart et al. (1990) have demonstrated rapid increases in SI mRNA following sucrose force feeding compared to rats fed a carbohydrate-free diet as early as three hours after the onset of feeding. Together, these findings suggest that increased SI activity following refeeding and sucrose force feeding are accounted for by de novo synthesis of the enzyme [Broyart et al., 1990; Holt and Yeh, 1992; Levin, 1994].

The effects of the polyamines in the developmental regulation of SI in the postnatal small intestine are reviewed elsewhere (see Polyamines).

THE Na^+K^+ ATPase

Physiological Significance

The Na^+K^+ ATPase is a membrane-bound enzyme that is present in virtually all mammalian cells. This enzyme establishes and maintains the sodium (Na^+) and potassium (K^+) electrochemical gradients across the cell membrane by coupling the free energy contained within adenosine triphosphate (ATP) to the translocation of Na^+ and K^+ [Horisberger et al., 1991]. These electrochemical gradients are created as the Na^+K^+ ATPase pump drives 3 Na^+ out of the cell and 2 K^+ into the cell at the

expense of 1 molecule of ATP [Schultz and Hudson, 1991].

In the small intestine, the Na^+K^+ ATPase restricted to the enterocyte BLM maintains the transcellular Na^+ gradient that is required for the activity of Na^+-dependent nutrient cotransporters located on the enterocyte MVM [Schultz and Hudson, 1991; Wright, 1993]. SGLT1, for example, exploits the luminal to cytosol sodium gradient established by Na^+K^+ ATPase activity for the active transport of luminal glucose and galactose into the enterocyte [Crane, 1977; Hediger and Rhoads, 1994].

Cell and Molecular Biology

The Na^+K^+ ATPase heterodimer consists of an α catalytic subunit (M_r 110 kDa) and a highly glycosylated ß subunit (M_r 55 kDa) [Horisberger et al., 1991]. The α subunit is the catalytic unit of the Na^+K^+ ATPase featuring a Na^+-binding site on the extracellular domain of the enzyme, and both K^+- and ATP-binding sites on the cytosolic domain of the enzyme [Horisberger et al., 1991]. The ß subunit appears to facilitate the assembly, transport and insertion of the mature enzyme complex to the plasma membrane [Horisberger et al., 1991].

The Na^+K^+ ATPase α and ß subunit isoforms are encoded by unique mRNA sequences, translated independently, and are assembled to form the Na^+K^+ ATPase α-ß complex in the endoplasmic reticulum. Posttranslational modifications to the high-mannose glycosylated complex occur in the Golgi apparatus prior to BLM targeting and insertion of the functional protein complex [Fambough et al., 1991].

To date, three α subunit (α_1, α_2, α_3) and two ß subunit ($ß_1$, $ß_2$) isoforms have been described [Horisberger et al., 1991; Zemelman et al., 1992]. The cDNAs encoding the Na^+K^+ ATPase α- and ß- subunit isoforms have been isolated and characterized, and from determinations of structural heterogeneity it would appear that this enzyme belongs to a

multigene family [Lingrel et al., 1990]. The α and ß subunit isoforms exhibit tissue-specific expression and are subject to developmental regulation [Orlowski and Lingrel, 1988].

Na^+K^+ ATPase Expression

To date, few studies have examined Na^+K^+ ATPase expression in the small intestine. In the adult mammalian small intestine only the α_1 and $ß_1$ subunit isoforms are expressed [Zemelman et al., 1992; Giannella et al., 1993; Sauriol, 1993; Wild et al., 1994]. In adult rats, a proximal to distal gradient of Na^+K^+ ATPase activity has been described [Charney et al., 1974; Murray and Wild, 1980]. Similarly, longitudinal gradients for both immunodetectable α_1 and $ß_1$ isoform proteins [Giannella et al., 1993; Wild et al., 1994] and mRNA encoding the Na^+K^+ ATPase α_1 and $ß_1$ isoforms have been demonstrated in rat small intestine [Gianella et al., 1993; Wild et al., 1994]. In the jejunum and ileum, crypt to villus increases in both α_1 and $ß_1$ subunit isoform mRNA abundance have been shown to correlate with observed Na^+K^+ ATPase activity along the crypt-villus axis in these intestinal regions [Gianella et al., 1993; Wild and Murray, 1992].

Little is known of the relationship between the levels of Na^+K^+ ATPase and the processes associated with intestinal transport during postnatal development. Compared to the adult, the proximal small intestine of the newborn rabbit exhibits an increased permeability and decreased capacity for active Na^+ transport, Na^+ coupled glucose absorption, and Na^+K^+ ATPase activity [Shepard et al., 1980]. During the third postnatal week, the rat colon demonstrates increased levels of Na^+K^+ ATPase activity paralleled by an increased abundance of α_1 and $ß_1$ mRNA [Fuller and Verity, 1990]. Similar developmental and regional patterns of Na^+K^+ ATPase activity [Sauriol, 1993] as well as α_1 and $ß_1$ mRNA abundance have been described in the postnatal rat small intestine [Zemelman et al., 1992].

Regulatory Control in the Small Intestine

Little is known regarding the regulatory mechanisms influencing Na^+K^+ ATPase isotype gene expression in the small intestine. Significant increases in Na^+K^+ ATPase α_1 and β_1 subunit isoform mRNA, protein levels, and enzyme activity have been described in the suckling rat small intestine following treatment with exogenous glucocorticoids compared to controls [Zemelman et al., 1992]. Precocious increases in Na^+K^+ ATPase activity have been demonstrated in the small intestine following the administration of polyamines [Wild et al., 1993]. Further study is required to determine the developmental and mechanistic basis of Na^+K^+ ATPase gene expression in the postnatal small intestine.

THE INTESTINAL Na^+/GLUCOSE COTRANSPORTER (SGLT1)

Physiological Significance

The Na^+/glucose cotransporter (SGLT1) is essential to the accumulation of D-glucose and D-galactose within the enterocyte. A molecular defect in SGLT1 is the origin of a rare autosomal recessive disease called glucose/galactose malabsorption syndrome [Turk et al., 1991], which presents with an acute, potentially fatal watery diarrhoea [Desjeux, 1989]. The severe diarrhoea resolves within one hour following the removal of glucose, galactose, and lactose sources from the diet [Desjeux, 1989], the absorption of fructose and xylose is normal [Turk et al., 1991].

The presence of intraluminal sugar is stimulatory to salt and fluid absorption [Crane, 1977]. SGLT1 translocates two Na^+ across the MVM with each glucose/galactose molecule transported into the enterocyte [Hediger and Rhoads, 1994]. The Na^+/K^+ ATPase located on the enterocyte BLM extrudes the intracellular Na^+ into lateral intercellular spaces thereby creating local osmotic gradients which drive fluid absorption [Horisberger, 1991]. Together, the sodium-dependent cotransport of nutrients and ATPase activity form the basis of

oral rehydration therapy whereby fluid losses, incurred by intestinal secretory states, such as cholera, can be replaced by oral glucose electrolyte solutions [Wright, 1993].

Molecular Biology

The cDNAs encoding SGLT1 in both rabbit and human intestine, as well as that found in rat kidney, have been cloned, sequenced, and expressed [Hediger et al., 1987a; 1989; 1991; Lee et al., 1994]. SGLT1 has been identified as a 72-75 kDa polypeptide using a monoclonal anti-SGLT1 antibody [Schmidt et al., 1983; Peerce and Clarke, 1990]. A novel expression cloning system using oocytes was used to determine the size of the mRNA encoding the SGLT1 polypeptide. When poly(A)+RNA isolated from rabbit small intestine was injected into Xenopus laevis oocytes a phlorizin-sensitive, sodium dependent uptake of α-methyl-D-glucopyranoside was demonstrated by the oocytes [Hediger et al., 1987b]. The mRNA encoding SGLT1 was determined to be a 2.3 kb fraction [Hediger et al., 1987a; 1987b]. Subsequent work has shown that when SGLT1 cDNA is expressed in Xenopus laevis oocytes, COS-7 cells, baculovirus-infected sf9 cells or Hela cells, the protein expressed in these systems demonstrates a sodium dependent transport of D-glucose and D-galactose that is blocked by phlorizin, a specific competitive inhibitor of SGLT1 [Hediger et al., 1987b; 1987c; 1991; Birnir et al., 1990].

The human gene for SGLT1 has recently been mapped to the proximal half of chromosome band 22q13.1 by fluorescence in situ hybridization [Turk et al., 1993]. The sequence analysis of SGLT1 cDNA amplified by polymerase chain reaction (PCR) has revealed a single genetic mutation in family members afflicted with glucose/galactose malabsorption syndrome [Turk et al., 1991; 1994]. To date, this abnormality may involve the substitution of an adenine base for that of a guanine base at

position 92 [Turk et al., 1991], or, the substitution of a guanine base for an adenine base at position 93 [Turk et al., 1994]. These base substitutions modify the final protein product at the level of amino acid position 28, resulting in an amino acid residue change from ASP 28 to Asn 28 [Turk et al., 1991], and ASP 28 to Gly 28 respectively [Turk et al., 1994]. Thus, in these pedigrees, a single missense mutation produces a severe impairment of sodium dependent transport of both glucose and galactose [Turk et al., 1991; 1994].

The Na^+/glucose cotransporter proteins from rabbit and human small intestine are comprised of 662 and 664 amino acids respectively [Hediger and Rhoads, 1994]. There is 84% identity and 94% homology between these polypeptide sequences [Wright, 1992]. Rat kidney SGLT1 is a protein comprised of 665 amino acid residues that shares an 86% and 87% identity with rabbit and human SGLT1 respectively [Lee et al., 1994]. In addition to kidney SGLT1, a low affinity Na^+/glucose cotransporter, SGLT2, an apparent isoform of SGLT1, has been identified in the early proximal tubule of the kidney [Kanai et al., 1994]. Comparative studies have demonstrated highly conserved SGLT1 DNA and protein sequences across broad phylogenetic ranges [Hirayama et al., 1991; Ahearn et al., 1992]. No apparent sequence homology has been observed between SGLT1 and the family of facilitative glucose transporters (the GLUTS) in Escherichia coli [Hediger et al., 1987a]. Mammalian intestinal SGLT1 is thought to belong to a cotransporter superfamily of genes which mediate the sodium-dependent transport of a variety of substrates including neutral amino acids [Kong et al., 1993], nucleosides [Pajor and Wright, 1992], myo-inositol [Kwon et al., 1992], as well as both proline and pantothenic acid from Escherichia coli [Hediger, 1989; Jackowski and Alix, 1990].

SGLT1 Structure-Function

The SGLT1 protein identified in both the proximal convoluted tubule of the kidney and in the absorptive epithelium of the small intestine is a polypeptide with an apparent M_r of approximately 75,000 [Hediger and Rhoads, 1994]. Based on the predicted amino acid sequence and secondary structure analyses SGLT1 (human and rabbit small intestine, rat kidney) is proposed to span the plasma membrane 12 times [Hediger et al., 1989; Lee et al., 1994]. N-linked glycosylation at Asp 248, between membrane spans 5 and 6, suggests this to be a hydrophillic loop located on the extracellular surface of the BBM [Hirayama, 1992]. The evidence supporting N-linked glycosylation would place the NH_2- and COOH- terminals on the cytoplasmic side of the 12M model. Using immunogold fluorescent microscopy with an antibody directed against residues 564-575 of rabbit intestinal SGLT1, Takata et al. (1991) demonstrated that the hydrophillic COOH- terminal of SGLT1 resides on the cytoplasmic side of the MVM in proximal tubule cells. The structural model proposed for SGLT1 is shown in Figure 2.

The core glycosylation of SGLT1 takes place in the endoplasmic reticulum (ER) with additional processing probably occurring while the protein is en route from the ER to the MVM [Hirayama and Wright, 1992]. Posttranslational processing of SGLT1 increases the apparent molecular mass of SGLT1 by approximately 15 kDa [Hirayama et al.,1992]. It would appear that N-linked glycosylation is required for the structural and functional expression of SGLT1 [Birnir et al., 1990; Hirayama and Wright, 1992]. Birnir et al. (1990) reported that tunicamycin, a specific inhibitor of asparagine-linked glycosylation, inhibits SGLT1 expression by approximately 80% in COS-7 cells grown and transfected in the presence of this inhibitor. While N-linked glycosylation may be essential for the proper processing, membrane targeting, insertion, protein

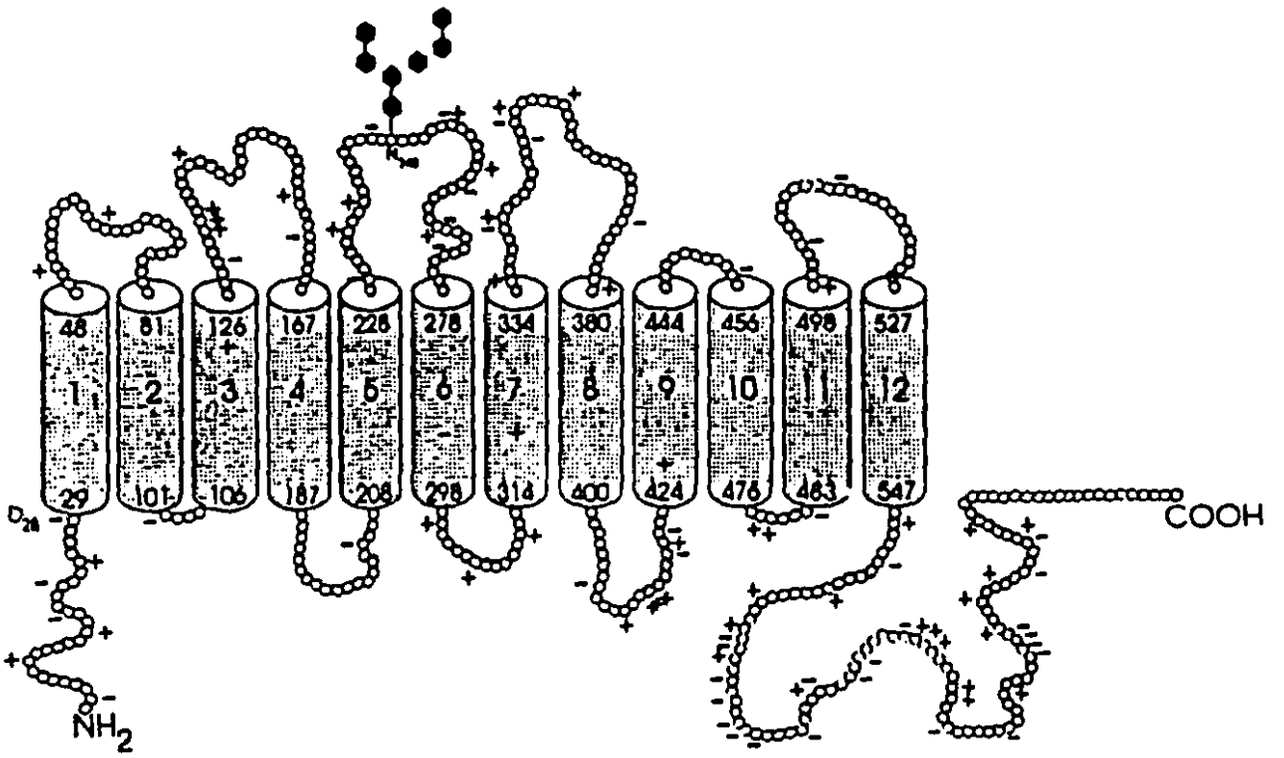


Figure 2:
Structural model of human SGLT1 with 12 membrane-spanning regions. Hydrophobic COOH-terminal is predicted to be on the cytoplasmic surface of the cell plasma membrane.

Adapted from: Hediger, M.A., and Rhoads, D.B. Physiological Reviews, 1994.

stability, and transporter function of SGLT1, the mechanisms whereby glycosylation influences SGLT1 function remains to be elucidated.

SGLT1 is an asymmetric protein. Using MVM vesicles from both renal proximal tubules and small intestine, Na^+-dependent glucose uptake was shown to take place only from the luminal to the intracellular side of the MVM [Hediger, 1987]. Functional studies conducted in a variety of systems have demonstrated that SGLT1 exhibits Michaelis Menten-type kinetics [Smith, 1992; Koepsell, 1993; Hediger and Rhoads, 1994]. While the actual mechanism whereby glucose and Na^+ are cotransported by SGLT1 requires further definition, it is generally accepted that two Na^+ are translocated for each molecule of glucose transported by SGLT1 [Bennett and Kimmich, 1992; Hediger and Rhoads, 1994]. Bennett and Kimmich (1992) have proposed two separate Na^+ binding events occurring at two distinct Na^+ binding sites which influence conformational change of the SGLT1 protein. Briefly, one Na^+ binds in a potential-dependent manner increasing the affinity of SGLT1 for glucose. This event is followed by glucose binding, and the binding of a second Na^+ in a potential-independent manner [Bennett and Kimmich, 1992]. The mechanisms whereby the empty SGLT1 carrier reorients itself within the MVM once these substrates have been released into the cytoplasm requires further study.

A putative membrane-bound regulatory subunit for SGLT1, designated RS1, has recently been cloned [Veyhl et al., 1993]. RS1-homologous mRNA has been detected in the small intestine [Veyhl et al., 1993]. Although SGLT1 alone is able to translocate glucose with sodium, the RS1 subunit has been shown to increase the Vmax of sodium-dependent glucose transport [Weber, 1991; Veyhl et al., 1993; Koepsell and Spangenberg, 1994]. The insertion of the RS1 into the BBM may be responsible for enhanced SGLT1 transport activity occurring in enterocytes lining the upper levels of the crypt-villus

axis. Whether RS1 modulates the transport activity of all components of the SGLT1 superfamily, such as the Na^+/D-glutamine, Na^+/D-alanine, or Na^+/nucleoside transporters remains to be determined [Koepsell, 1993; Veyhl et al., 1993].

Several Na^+/glucose cotransport mechanisms possessing unique kinetic properties have been described in the small intestine. Freeman and Quamme (1986) described a low affinity/high capacity transport system located in the jejunum, and a high affinity/low capacity system found throughout the jejunoileum of young rats. During development, the low affinity system of Na^+/glucose transport develops in the distal small intestine so that both systems are found along the length of the small intestine in the adult rat [Freeman and Quamme, 1986]. Using rabbit SGLT1 expressed in Xenopus laevis oocytes, Hirayama et al. (1994) have demonstrated that H^+ can substitute for Na^+ in driving glucose transport. H^+ has been shown to support a higher maximum transport than Na^+, but appears to reduce transporter affinity for glucose [Hirayama et al., 1994]. Given that both low affinity/high capacity and high affinity/low capacity glucose transport has been demonstrated in the small intestine, the authors suggest that SGLT1 can mediate either type of glucose transport dependent upon the driver cation [Hirayama et al., 1994].

Intestinal-SGLT1 Expression

The distribution of SGLT1 mRNA along the crypt-villus axis has been examined using in situ hybridization. To date, evidence suggests that SGLT1 mRNA appears in enterocytes at the base of the villus and the levels of SGLT1 mRNA increase towards the mid-portion of the villus [Freeman et al., 1993; Hwang et al., 1991; Koepsell, 1993; Weber, 1991]. These results are consistent with SGLT1 gene transcription occurring at the onset of enterocyte differentiation [Hwang et al., 1991; Hediger and Rhoads, 1994].

Immunoprecipitation techniques, Western blotting, and immunocytochemical approaches using anti-SGLT1 antibodies have best clarified the location of SGLT1 protein to the enterocyte MVM. Four monoclonal antibodies directed against the porcine renal Na^+/glucose cotransporter have been shown to interact with the intestinal Na^+/glucose cotransporter, binding to polypeptides of M_r 75,000 and M_r 47,000. Using these antibodies, antigenic sites were demonstrated in intestinal MVM showing densities highest in the jejunum, intermediate in the ileum, and lowest in the duodenum [Haase, 1990]. Immunohistochemical studies using a polyclonal antibody directed against the rabbit intestinal SGLT1 have localized the Na^+/glucose cotransporter in the jejunum of the adult rat [Takata et al., 1992]. Immunoblotting has revealed the onset of SGLT1 expression at the crypt-villus junction and the demonstration of antigenic sites in the adult rat duodenum, jejunum, and ileum [Takata et al., 1992]. In absorptive epithelial cells situated between the crypt-villus axis and the middle of the villi, positive supranuclear staining (Golgi apparatus), in addition to MVM staining for SGLT1 has been observed [Takata et al., 1992]. Coupled with evidence of increased SGLT1 mRNA abundance associated with cell differentiation, these results suggest that SGLT1 is expressed as enterocytes migrate and mature along the crypt-villus axis.

Regulatory Control in the Small Intestine

The regulatory control of SGLT1 expression and function has been investigated in the small intestine in a number of models. In humans, SGLT1 mRNA and aboral gradients of functional activity have been detected in the fetal small intestine as early as 17 weeks gestation [Davidson et al., 1992; Malo and Berteloot, 1987]. Mammals with short gestations, such as the rat, do not demonstrate Na^+/glucose transport activity until approximately 80% of gestation has been completed [Buddington and Diamond, 1989; Buddington,

1994]. Developmental increases in glucose uptake activity in the small intestine have been demonstrated in the rat, with sharp increases in activity observed to coincide with the weaning process [Toloza and Diamond, 1992]. Interestingly, measurements of SGLT1 mRNA abundance in the small intestine of postnatal rats do not appear to change significantly throughout development [Miyamoto et al., 1992]. Similarly, the normal decrease in SGLT1 expression and activity associated with rumen development in weanling lambs does not coincide with levels of SGLT1 mRNA abundance [Freeman et al., 1993; Lescale-Matys et al., 1993]. In young ruminants, the introduction of glucose into the small intestine results in a 60- to 90-fold increase in SGLT1 activity and protein expression that is accompanied by a meagre 2-fold increase in SGLT1 mRNA abundance [Lescale-Matys et al., 1993]. These findings suggest that post-transcriptional events play an important role in regulating the functional expression of SGLT1 during postnatal development [Miyamoto et al., 1992; Lescale-Matys et al., 1993; Shirazi-Beechey et al., 1991].

In contrast, Miyamoto et al. (1993) reported increases in intestinal SGLT1 activity (V_{max}) that were paralleled by an increased SGLT1 mRNA abundance in mature rats after only three days of ingesting a high glucose diet. A number of studies support the observation that high carbohydrate diets are stimulatory to intestinal glucose absorption through increases in the number of SGLT1 transporters occupying the MVM [Ferraris et al., 1992a; Ferraris and Diamond, 1992b; Ferraris et al., 1993]. It has been suggested that increased SGLT1 mRNA abundance in rats consuming a high glucose diet reflect increased rates of SGLT1 mRNA transcription, not post-transcriptional modification [Miyamoto et al., 1993].

An enhanced uptake of glucose has been documented in the diabetic small intestine [Ferraris et al., 1993; Miyamoto et al., 1991]. In rodents, Miyamoto et al. (1991) reported significant increases in SGLT1 transport activity that were

apparent within 5 days following streptozotocin-induced diabetes. Enhanced SGLT1 transport activity was not accompanied by increases in SGLT1 mRNA until 10 days had elapsed following diabetes induction, an observation which favors a post-transcriptional regulatory control of SGLT1 [Miyamoto et al., 1991].

Attempts have been made to identify the regions along the crypt to villus axis that are responsive to signals regulating SGLT1 expression. An upregulation of intestinal SGLT1 activity has been demonstrated following carbohydrate-rich diets [Ferraris et al., 1992a; 1992b; Ferraris et al., 1993; Miyamoto et al., 1993]. Compared to mice fed low-carbohydrate diets, mice consuming high-carbohydrate diets for two weeks demonstrated greater numbers of SGLT1 along the entire crypt-villus axis as assessed by phlorizin binding [Ferraris et al., 1992a]. Given the time course of change in phlorizin binding along the crypt-villus axis, it was concluded that the signal responsible for SGLT1 regulation and/or modulation following dietary carbohydrate manipulation was perceived exclusively by crypt cells [Ferraris et al., 1992b]. These findings suggested that for SGLT1, crypt cells are programmed irreversibly following manipulation of dietary carbohydrate levels, and are not subject to reprogramming during enterocyte migration along the villus [Ferraris and Diamond, 1992b].

Burant et al. (1994) have suggested that the enhanced intestinal glucose uptake observed in diabetic rats results from increased levels of SGLT1 mRNA in mature enterocytes as well as the expression of SGLT1 in less mature enterocytes. It would appear that this altered pattern of gene expression observed in diabetes effectively increases the number of functional SGLT1 transporters along the crypt-villus axis, thereby increasing glucose uptake [Burant et al., 1994; Wild et al., 1994]. Whether SGLT1 expression and activity is regulated and/or modulated predominantly at transcriptional, translational, or post-translational levels during enterocyte

migration along the crypt-villus axis requires further investigation. Taken together, the evidence to date suggests that the regulatory control of SGLT functional expression may vary with species, developmental stage, diet, and disease state.

POSTNATAL REGULATORY MECHANISMS DURING ONTOGENY

In the postnatal small intestine altered patterns of enterocyte proliferation, differentiation, migration rates, membrane composition, and physical characteristics regulate hydrolase and nutrient transport activities [Meddings and Theisen, 1989; Levin, 1994; Buddington, 1994]. In addition, developmental shifts in gene expression and the processing of gene products determine the postnatal expression and functional activity of these proteins [Freund et al., 1990; Levin, 1994]. One of the central problems in the field of developmental biology has been to determine the signals responsible for eliciting the aforementioned developmental events. Despite an extensive investigation of putative internal (ie. circulating hormones, local factors) and external signals (ie. dietary factors) the signals controlling postnatal intestinal maturation and the mechanistic basis of regulatory control have yet to be satisfactorily defined.

Significance of Diet

The exclusive consumption of colostrum and milk during the first two postnatal weeks provides the immature animal with nutrients, immunoglobulins, hormones, and growth factors [Weaver and Walker, 1989]. During the third postnatal week a qualitative dietary change is made as the rat pup gradually weans from a diet that is high fat, high protein, low carbohydrate (mother's milk), to a low fat, low protein, carbohydrate rich diet [Henning, 1981; 1987; Toloza and Diamond, 1992]. At this time, the structural and functional changes occurring in the rat small intestine constitute an

adaptive response which facilitates nutrient absorption during a period of dietary transition [Toloza and Diamond, 1992]. The regulation of these developmental changes then, allows the postnatal animal to absorb nutrients in a manner that closely matches variable growth requirements and changing dietary constituents [Meddings and Theisen, 1989; Karasov, 1992; Buddington, 1992; Diamond, 1992].

While dietary constituents may influence the onset or magnitude of postnatal intestinal development, dietary factors alone probably do not provide the trigger for structural and functional maturation of the small intestine. In rats, prolonged nursing has been shown to inhibit the natural decline of lactase activity, but has no effect on the developmental patterns of maltase or sucrase activities [Henning, 1982]. Rats prevented from normal weaning (dry milk) demonstrate similar growth patterns and age-related nutrient transport capacities for glucose, galactose, and fructose compared to normally weaned rats (chow) suggesting a "hard-wired" regulation of nutrient transporter development [Toloza and Diamond, 1992]. Elevations in glucocorticoid levels secondary to animal stress may confound findings of precocious digestive tract maturation in studies featuring premature and artificial weaning [Buddington, 1992; Yeh et al., 1987]. Investigations using intestinal isografts in preweanling rats suggest that nutrient hydrolase ontogeny (sucrase and lactase) may be cued by a "hard-wired" local trigger localized or synthesized at the level of the intestinal mucosa [Yeh and Holt, 1986; Diamond, 1986; Yeh et al., 1987]. Studies using intestinal explants implanted into the kidney, subcutaneous space, or cultured <u>in vitro</u> have demonstrated normal patterns of functional maturation in the absence of usual luminal constituents [Yeh and Holt, 1986]. In contrast, Castillo et al. (1992) reported that intraluminal nutrients are required for the formation of jejunoileal gradients for DNA, protein, and sucrase, but were less

essential for the developmental decline of ileal lactase. The maturational decline of lactase may result from the accelerated cellular turnover characteristic of the mature small intestine, not because of reduced synthetic rates of enzyme [Tsuboi et al., 1981; 1992]. Alternately, recent work has revealed a two- to fourfold decline in the transcriptional rates of lactase during weaning which paralleled respective regional lactase specific activities [Krasinski et al., 1994].

These studies appear to suggest that while intraluminal nutrients probably do not "trigger" intestinal ontogeny, certain absorptive functions and/or intestinal compartments may be more responsive to modulation by dietary components during intestinal maturation. Moreover, intraluminal nutrients may "fine-tune" preprogrammed intestinal maturational processes.

Significance of Circulating Hormones

Changes in circulating hormones have been extensively studied as putative "trigger signals" effecting intestinal development in the postnatal rat. Both thyroid hormones and glucocorticoids are attractive candidates for the regulation of postnatal intestinal development since surges in the circulating levels of these hormones occur in the second and third postnatal week respectively, that is, just prior to the onset of weaning [Walker, 1988]. Independently, both hormones have elicited the precocious expression of intestinal enzymes [Yeh, 1975; 1978; 1989; Koldovsky, 1974; Tsuboi, 1986]. Evidence suggests, however, that the postnatal decline of lactase is more responsive to alterations in levels of thyroid hormone, while SI expression is regulated mainly by changes in glucocorticoid levels.

In rats, hypothyroidism has been shown to delay postnatal small intestinal maturation, with a full reversal of this effect apparent following the administration of exogenous T_4 [Yeh and Moog, 1974]. In a rodent model of adult

hyperthyroidism, duodenal and jejunal lactase mRNA abundance was shown to be significantly reduced in hyperthyroid animals compared to hypothyroid rats suggesting that the regulatory control of lactase expression can be modulated at the transcriptional level [Hodin et al.,1992]. In contrast, Yeh et al. (1991) suggest that cortisone and thyroxine together may cooperatively modulate the decline of lactase in suckling rats at the posttranscriptional level. In support of the argument favouring the posttranslational developmental regulation of lactase, Liu et al. (1992) reported on thyroxine-induced alterations in processing and increased degradation of lactase during postnatal development in the rat. Other investigators have suggested that the normal maturational decline of lactase in the postnatal rat are attributable to the accelerated cell turnover associated with weaning and associated hormonal fluxes, not diminished rates of lactase synthesis [Yeh et al., 1991; Tsuboi et al., 1992].

Yeh et al. (1989) reported on the cooperative modulation of jejunal sucrase expression during development by both thyroxine and cortisone. The treatment of suckling rats (postnatal day 12) with a single injection of either thyroxine or cortisone was found to elicit dose-dependent increases in jejunal sucrase activity [Yeh et al., 1989]. In addition, sucrase activity and immunodetectable SI were induced in the jejunum of adrenalectomized suckling rats following T_4 treatment [Yeh et al., 1989]. Later work supports the cooperative modulation of sucrase expression by T_4 and cortisone in suckling rats when studied at earlier time points [Yeh et al., 1991].

Despite these findings, glucocorticoids are thought to play the predominant role in the postnatal induction of sucrase. In normal suckling rats, SI mRNA and SI enzyme activity has been detected within 12 hours of a single injection of cortisone at postnatal day 10 [Leeper and Henning, 1990]. These findings have been supported at the level of SI mRNA

responsiveness [Nanthakumar and Henning, 1993]. Work exploring the precocious induction of sucrase-isomaltase in adrenalectomized suckling rats by exogenous glucocorticoids demonstrated that precocious SI enzyme activity is paralleled by the appearance of SI mRNA [Nanthakumar and Henning, 1993]. Alternately, rat pups adrenalectomized on postnatal day 9 were found to demonstrate a latent appearance of SI mRNA and activity when compared to sham-operated controls [Nanthakumar and Henning, 1993].

The small intestine of the postnatal rat appears to lose its responsiveness to glucocorticoid-induced developmental increases in sucrase activity at approximately postnatal day 18 [Henning, 1981]. Recent work suggests that the effects of glucocorticoids on SI gene expression differ markedly at different developmental time points [Nanthakumar and Henning, 1993]. Given the glucocorticoid-induced precocious expression of SI in sucklings following stress, the natural increases in circulating levels of this hormone may provide a survival mechanism for precocious intestinal maturation should pups suffer the precocious loss of their dam [Henning, 1985].

Despite extensive investigations, it would appear that the roles of thyroxine and glucocorticoids in modulating the developmental expression of absorptive functions in the postnatal rat require further clarification.

THE POLYAMINES

Biochemistry and Metabolism

The polyamines are organic polycations of low molecular weight [Heby, 1981; Pegg and McCann, 1982; Pegg, 1986; Scalabrino et al., 1991]. They are ubiquitous molecules present in virtually all prokaryotes, eukaryotic cells (plants and mammals), yeasts, viruses, and bacteriophages [Pegg and McCann 1982; Scalabrino et al., 1991]. They can exist as true polyamines or metabolic products in cells and physiological fluids [Heby, 1981; Scalabrino et al., 1991]. In mammalian

cells, the chief polyamines are putrescine, spermidine, and spermine [Heby and Persson, 1990].

The structural formulae for the three main polyamines are as follows [Scalabrino, 1991]:

Putrescine $H_2N-(CH_2)_4-NH_2$

Spermidine $H_2N-(CH_2)_3-NH-(CH_2)_4-NH_2$

Spermine $H_2N-(CH_2)_3-NH-(CH_2)_4-NH-(CH_2)_3-NH_2$

Polyamines have been recognized as important cellular constituents that are essential to cell growth, division and differentiation [Pegg and McCann, 1982; Pegg, 1986]. When cells are depleted of polyamines using polyamine biosynthetic inhibitors, cellular proliferation is inhibited [Heby and Persson, 1990]. The supplementation of exogenous polyamines to polyamine-depleted cells re-establishes normal growth and differentiation [Heby and Persson, 1990]. Cellular polyamine content, uptake, biosynthesis, metabolism, degradation and concentration appear to be highly regulated by a myriad of possible mechanisms [Pegg and McCann, 1982; Pegg, 1986]. Significant variations in polyamine distribution have been demonstrated between and within species, and appear to be dependent upon growth conditions, cell growth rates, and the physiological age of the animal, organ or cell type [Pegg and McCann, 1982; Pegg, 1986; Scalabrino et al., 1991; Yoshinaga et al., 1993].

Intracellular concentrations of the polyamines are very small with spermidine and spermine present at millimolar concentrations, and putrescine at nanomolar concentrations [Scalabrino et al, 1991]. Polyamines are made available to the cell through the activity of an intracellular biosynthetic and interconversion pathway, or from exogenous sources (ie. food, bacterial production) via a distinct active transport system [Pegg and McCann, 1982; Pegg, 1986; Osborne and Seidel, 1990]. An energy dependent, saturable, ouabain insensitive

uptake of putrescine has been demonstrated in isolated rat enterocytes [Kumagai and Johnson, 1988; Osborne and Seidel, 1990]. Similarly, a saturable, ouabain sensitive, temperature dependent carrier that is distinct from that involved in the transport of putrescine has been described for spermidine and spermine [Kumagai et al., 1989; Kobayashi et al., 1993]. The gut lumen may be a significant source of polyamines for the body as well as for direct use by the small intestinal mucosa given the existence of an enterohepatic circulation of absorbed polyamines [Osborne and Seidel, 1990]. While the fate of absorbed polyamines requires further study, the significance of luminal polyamine transport has been established by the accumulation of radiolabelled polyamines in practically every body organ following the intragastric administration of ^{14}C-labelled putrescine, spermidine and spermine [Bardocz et al., 1990; 1993]. The basolateral uptake of intraperitoneally administered ^{14}C-polyamines has also been demonstrated in rat small intestine following the stimulation of intestinal growth by Phaseolus vulgaris lectin phytohaemagglutinin [Bardocz et al., 1990].

In all mammalian cells and many lower eukaryotes, the enzyme ornithine decarboxylase (ODC) is the initial and rate-limiting step in the polyamine biosynthetic pathway. ODC is a pyridoxal phosphate-dependent enzyme that is present in small amounts in quiescent cells [Pegg and McCann, 1982; Pegg, 1986]. ODC activity is increased several hundred-fold during periods of growth resulting from an increased rate of ODC synthesis that is achieved in part by increases in the amount of ODC mRNA [Holm et al., 1989; Heby and Persson, 1990]. Recent evidence points to multiple, independent regulatory signalling pathways for ODC in cultured intestinal epithelial cells. In the IEC-6 cell line, for example, the exposure of serum-starved cells to exogenous putrescine has been shown to decrease ODC activity while having no significant effect on ODC mRNA content [Ginty et al., 1990]. In vivo, ODC activity

in the intestinal mucosa may be mediated by circadian rhythms [Fujimoto et al., 1992], the presence or absence of luminal nutrients [Tabata and Johnson, 1986a; 1986b] and humoral factors in response to feeding [Tabata and Johnson, 1986b].

Putrescine, produced by the decarboxylation of ornithine by ODC, is the first polyamine in the intracellular biosynthetic pathway [Pegg and McCann, 1982; Pegg, 1986]. Putrescine, spermidine, and spermine have been shown to be metabolically interconvertible [Seiler, 1990]. The intra-cellular interconversion of polyamines has been demonstrated following fasting, exposure to toxic agents, and by exposure to excess spermidine [Pegg and McCann, 1982]. Polyamine interconversion is a function of enzymatic activity. ODC has one of the shortest half-lives known for a mammalian enzyme; 7 to 30 minutes in most mammalian cells [Iwami et al., 1990], approximately 22 minutes in rat small intestinal mucosa [McCormack, 1991]. It is the very short half-life of ODC and the key interconversion enzymes that regulates cellular polyamine content and concentration in response to various stimuli [Pegg and McCann, 1982; Pegg, 1986]. It is generally accepted that the intracellular biosynthesis of polyamines is regulated by negative feedback control mechanisms involving biosynthetic regulation at the levels of transcription, translation, and post-translational modification [Heby and Persson, 1990]. The physiological importance of intracellular polyamine interconversion remains to be determined, however, it follows from the close cellular regulation of these amines that this pathway is a safety feature that prevents the concentration levels of the main polyamines from exceeding beneficial limits. Figure 3 shows the polyamine intracellular interconversion pathway.

The polycationic structure of the polyamines most probably accounts for many of their physiological actions [Heby, 1981; Pegg and McCann, 1982; Scalabrino et al., 1991]. The vast number of biochemical functions attributed to the polyamines

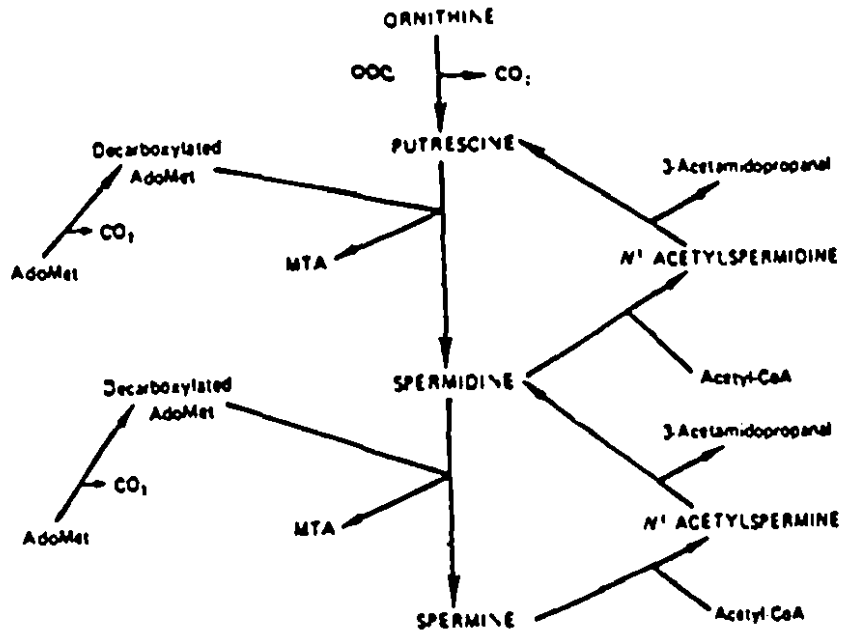


Figure 3:
Pathway for biosynthesis and interconversion of polyamines.

Adapted from: Pegg, A.E
Biochem. J., 1986.

prohibits an indepth review on this subject. Generally speaking, polyamines have been shown to be significantly involved in the regulation and/or enhancement of synaptic activity, gene expression [Heby, 1981; Heby and Persson, 1990; Scalabrino, 1981; Pegg, 1982], RNA synthesis [Heby, 1981; Heby and Persson, 1990; Scalabrino, 1991; Raina, 1975; Tabor, 1984], and protein expression [Heby and Persson, 1990]. Polyamines have also been shown to be important elements in the maintenance of nuclear morphology [Heby and Persson, 1990]. Evidence of this is demonstrated by cells auxotrophic for polyamines which eventually develop severe chromosomal damage and cease to replicate [Pohjanpelto, 1982; Nordling, 1990].

Effects on Cellular Growth, Proliferation and Differentiation

Both prokaryotic and eukaryotic cells have an absolute requirement for polyamines in order for cell growth and duplication to occur [Heby and Persson, 1990]. Some of the most convincing evidence supporting the requirement of polyamines for cell growth and division comes from <u>in vitro</u> and <u>in vivo</u> studies using reversible and irreversible inhibitors of polyamine biosynthesis and metabolism. The in situ inhibition of ODC in HTC cells using α-methylornithine, a reversible competitive inhibitor specific for ODC, resulted in rapid decreases in concentrations of intracellular putrescine and spermidine followed by inhibition of DNA synthesis and cellular proliferation [Mamont, 1976]. Cellular growth and proliferation was restored with the addition of exogenous polyamines to this system. <u>In vitro</u>, the exposure of HTC cells, L1210 mouse leukemia cells, mouse mammary EMT6 sarcoma cells, human small cell lung carcinoma cells, human colon cancer cells, and MA 160 human prostate adenoma cells to α-difluoromethylornithine (DFMO), an irreversible inhibitor of ODC that permanently shuts down polyamine biosynthesis, demonstrate significantly reduced rates of cellular

proliferation. The administration of exogenous polyamines resulted in the reversal of the aforementioned observations [Mamont, 1978; Pegg, 1982]. In vivo, DFMO has been shown to suppress or delay intestinal mucosal proliferation associated with intestinal adaptation following jejunectomy [Luk and Baylin, 1983], cytotoxic injury [Luk and Baylin, 1980], burn injury [Chung et al., 1993], stress damage [Wang and Johnson, 1992], and during lactation [Yang et al., 1984]. DFMO has been shown to prevent increases in ODC activity and associated cellular growth processes during embryogenesis [Fozard, 1980], and when administered to suckling rats, DFMO prevents the natural intestinal maturation processes required for weaning [Luk 1980]. Weanling rats presented with a 2% DFMO drinking solution demonstrate diarrhoea characteristic of malabsorption accompanied by decreases in intestinal mucosal weight, DNA and total protein, leucine amino peptidase activity, sucrase and maltase activities, and enterokinase activity [Alarcon et al., 1987].

While many studies have focused on ODC activity and the effects of polyamines on cell proliferation, differentiation and growth in a variety of models of intestinal adaptation, intracellular polyamines derived from endogenous or exogenous sources may also be important to the process of mucosal restitution following damage to the mucosa. Using a model of stress-induced intestinal mucosal damage, Wang and Johnson (1992) reported significant decreases in microscopic damage to the duodenal mucosa only 4 hours following stress in DFMO-treated rats administered luminal spermidine. Since a 4 hour interval is too short a time-period for cell replacement to have occurred, it has been suggested that luminal polyamines may promote early mucosal restitution whereby viable cells from areas adjacent to, or beneath the injured surface, migrate to replace sloughed off damaged epithelial cells [Wang and Johnson, 1992]. In vitro, however, the re-supply of polyamines to DFMO-treated IEC-6 cells has not been shown to

restore normal cell migration within a time-frame approximating that required for early epithelial restitution [McCormack et al., 1993].

Evidence for the Role of Polyamines in Intestinal Adaptation

Polyamines are thought to play a critical role in the regulation of intestinal mucosal growth [Dembinski et al., 1984; Luk, 1980; 1990]. The mechanisms involving ODC and the polyamines in the regional and compartmental regulation of intestinal adaptation are obscure. In the small intestine, ODC activity and polyamine intracellular content and concentration may vary with cell position along the crypt-villus axis [Sepulveda, 1982; Fitzpatrick et al., 1986; Luk and Yang, 1988; Bamba et al., 1990]. For example, ODC activity appears to be greater in enterocytes at the villus tip than in proliferating crypt cells [Fitzpatrick et al., 1986; Johnson et al., 1989; Bamba et al., 1990], with a gradient in ODC activity observed from villus-tip to crypt [Luk and Yang, 1988]. Bamba et al. (1990) reported no significant differences in either spermidine or spermine concentrations between villus tip and crypt cell fractions isolated from either the jejunum or ileum of adult rats. Dramatic reductions in ODC activity along the entire crypt-villus axis have been shown in jejunum and ileum harvested from adult rats fasted for 24 hours or more [Fitzpatrick et al., 1986; Luk and Yang, 1988; Bamba et al., 1990]. Johnson et al. (1989) reported little, or no immunoreactive ODC in crypt cells isolated from fasted rats. Significant increases in ODC activity have been shown in isolated villus tip and mid-villus cells harvested from fasted adult rats that were refed for 2 hours prior to sacrifice [Fitzpatrick et al., 1986]. Increases in ODC activity have not been demonstrated in isolated crypt cells following refeeding [Fitzpatrick et al., 1986; Luk and Yang, 1988], however, immunoreactive ODC has been detected in crypt cells isolated from rats refed for

2 hours following a 48 hour fast [Johnson et al., 1989]. Increased levels of ODC protein have been demonstrated in rat intestinal crypt cells following exposure to trophic substances such as gastrin and epidermal growth factor (EGF) [Johnson et al., 1989]. It is possible that the uptake of luminal polyamines by crypt cells and not ODC activity may be the trigger for crypt cell proliferation [Fitzpatrick et al., 1986]. Alternatively, crypt cells may require low levels of ODC activity for proliferation while higher ODC activity detected in isolated villus cells may reflect increased cellular requirements for differentiation.

In neonatal rats, increases in both ODC activity and mucosal polyamine content have been observed at weaning (approximately postnatal day 21), a period of maximal mucosal growth [Luk, 1980]. A significantly delayed postnatal intestinal maturation is evident in the ODC-deficient sparse-fur mutant mouse thereby supporting observations that both ODC activity and mucosal polyamine content have important roles in the intestinal adaptation that occurs during weaning [Malo, 1986]. Increases in ODC activity and intracellular polyamine content have been observed in a variety of models featuring intestinal adaptation such as: following fasting/re-feeding [Maudsley, 1976; Tabata and Johnson, 1986a; Luk, 1988], feeding of plant lectins [Bardocz, 1990], chemotoxic injury (cytosine arabinoside) [Luk, 1980], mucosal restitution following burn injury [Chung et al., 1993], mucosal repair after ischemia-reperfusion [Fujimoto et al., 1991], hyperplasia following parasitic infection with Trichinella spiralis [Wang et al., 1991], during adaptive hyperplasia following jejunectomy [Luk, 1983; 1984; Bamba et al., 1990], adaptive hyperplasia following Streptozotocin-induced diabetes [Younoszai et al., 1993], during lactation [Yang et al., 1984], following bowel obstruction [Osborne and Seidel, 1989], extensive bowel resection and pancreatic biliary diversion [Luk, 1987]. Following 60% cutaneous burn injury in rats,

increases in both ODC enzyme activity and polyamine biosynthesis have been observed prior to the mucosal repair of the small intestine [Chung et al., 1993]. In rodents, exogenous polyamines, in particular spermidine and spermine, have been shown to substitute for endogenous polyamines in effecting the repair of stress-induced duodenal mucosal damage [Wang and Johnson, 1992]. Interestingly, the adaptive ability of the gut mucosa, as measured by ODC activity and mucosal polyamine levels, is reported to be markedly attenuated with increasing age [Yoshinaga et al., 1993].

The Significance of Luminal Polyamines

It has been suggested that the direct contact between luminal polyamines and the intestinal mucosa may stimulate growth [Seidel, 1985]. Polyamines derived from both the gut microflora and the diet contribute to the luminal polyamine population. Using a model of intestinal obstruction-induced hyperplasia, Osborne and Seidel (1989) suggested the importance of bacteria-derived polyamines in adaptive hyperplasia by demonstrating a reduced trophic response in the colonic mucosa proximal to the obstructive ligature in response to a course of oral antibiotics. The Thiry-Vella loop model, an <u>in vivo</u> system whereby the proximal jejunal mucosa of adult rats is separated from the remaining gastrointestinal tract, supports the argument for diet-derived polyamines. In this model, a 10-fold increase in ODC activity was observed following the injection of normal intestinal contents, but not lyophilized (polyamine-free) gut contents [Tabata and Johnson, 1986).

Both human and rat milk have been shown to contain the three main polyamines [Pollack et al., 1990; 1992; Romain et al., 1992]. Compared to human milk, rat milk appears to contain higher concentrations of both putrescine and spermidine, with a trend towards an increase in the concentration of these polyamines over the lactation period

[Pollack et al., 1992; Romain et al., 1992]. Increases in the spermidine concentrations of rat milk have been demonstrated between days 16 to 18 of lactation [Romain et al., 1992], an important time-frame during the weaning process. Of interest, rat chow has been shown to be approximately 150 times richer in putrescine and spermine, and 30 times richer in spermidine than rat milk [Romain et al., 1992]. Given that pre-weanling and weaning rats are known to nibble at solid material, including rat chow, in addition to ingesting rat milk, it would appear that high concentrations of diet-derived polyamines, probably in excess of 1000 nmol/day [Buts et al., 1993], are present in the lumen of the rat small intestine during the weaning period.

Human milk has been shown to contain higher concentrations of the main polyamines, especially spermidine and spermine, compared to commercial infant formulas [Romain et al., 1992]. Higher polyamine concentrations have been detected in soy protein-based formulas and high protein formulas devised for premature infants compared to standard infant formulas, however, these levels were either comparable or less than the concentrations found in human milk [Pollack et al., 1992]. It has been suggested that the possible protective effect of human milk against allergies may be due, in part, to high concentrations of polyamines favouring intestinal maturation in breast-fed babies [Romain et al., 1992]. In adult humans, the transport of luminal polyamines may also be of some nutritional consequence. A wide variety of foods subjected to HPLC analysis for polyamines have shown variable contents and concentrations (see Figure 4) of the main polyamines [Bardocz et al., 1993]. To date, no studies have reported on the importance of diet-derived polyamines in human health.

Effects on Small Intestinal Ontogeny in Rodents

While research has traditionally focused on hormones or local mediators as the regulatory mechanisms of intestinal

POLYAMINE COMPOSITION AND CONTENT OF COMMON FOODS

FOOD TYPE	PUTRESCINE nmol/g or ml	SPERMIDINE nmol/g or ml	SPERMINE nmol/g or ml
beef	22-32	39-47	113-165
chicken	32-33	63-65	291-296
cod	300-337	7-11	15-32
wheat bread	6-10	147-169	35-45
rice	11-15	9-11	40-50
pasta	11-12	48-50	52-64
orange	1081-1579	61-67	-
pear	268-275	208-524	40-244
apple	5-19	15-19	-
soya bean	18-74	229-428	147-170
mushroom	1-2	236-279	4-6
potato	229-261	101-109	24-28
tomato	106-1386	11-17	-
milk	1-2	2-4	1-2
cheddar,new	115-227	557-751	118-194
cheddar,aged	7409-7427	1361-1392	115-198

Figure 4:

Adapted from: Bardocz et al.,
J. Nutr. Biochem., 1993.

maturation in suckling rats, the influence of the polyamines on postnatal intestinal maturation has been less thoroughly studied. A number of studies have shown that the administration of exogenous polyamines to suckling rats can elicit a precocious maturation of both intestinal morphology and function. In a study where rats were administered either spermidine (10 μmol) or spermine (6 μmol) on postnatal days 12, 13, and 14, and sacrificed on day 15, Dufour et al. (1988) demonstrated increases in sucrase and maltase specific activities to adult levels. Although lactase activity was significantly reduced in polyamine treated rats as compared to controls, the low levels of lactase activity characteristic of adult rats were not achieved [Dufour et al.,1988]. Analagous results were obtained from an identical model using an earlier postnatal time point; spermine (6 μmol) was administered to rats on postnatal day 7, 8, and 9, with sacrifice on postnatal day 10 [Dufour et al., 1988]. In addition, the administration of exogenous spermidine or spermine has been shown to significantly increase the mucosal concentrations of these polyamines in this model [Dufour et al., 1988].

A dose-dependent effect for spermine has been established during the early suckling period in rats [Dufour et al., 1988; Buts et al., 1993]. A significant premature induction of sucrase, maltase and aminopeptidase activities has been demonstrated in suckling rats with oral doses of spermine equal to 1 μmol/day over a four day period (postnatal day 10 to 14), however doses of 2.5 μmol/day were required to achieve adult levels of activity [Buts et al., 1993]. In contrast, a premature decline in lactase activity could only be elicited with spermine doses of 2.5 μmol/day [Buts et al., 1993].

Wild et al. (1993) demonstrated that rats given oral doses of spermidine (6 μmol) either 1 or 3 days prior to sacrifice on postnatal day 10 showed a precocious appearance of sucrase activity, increases in Na^+K^+ ATPase activity with the establishment of a proximal to distal gradient, reduced

lactase activity in the proximal and distal small intestine, and increased ODC activity, as compared with controls. Adult levels of activity were attained for both sucrase and Na^+K^+ ATPase by animals receiving spermidine for 3 days prior to sacrifice [Wild et al., 1993]. In addition, spermidine-treated neonatal rats exhibited partial to complete disappearance of the endosomal complex and giant supranuclear lysosome characteristic of immature ileal enterocytes [Wild et al., 1993]. Dufour et al. (1988) reported a marked loss of the ileal supranuclear vacuole following the administration of either spermidine or spermine to suckling rats. In contrast to results reported by Dufour et al.(1988), Wild et al.(1993) have shown increases in mucosal ODC activity in their model. In this system, the reduced responsiveness of ODC to feedback inhibition by exogenous polyamines may reflect an augmentation of cellular proliferation, or an induction of ODC activity in response to a glucocorticoid surge [Wild et al., 1993]. Georges et al. (1990) have demonstrated a time-dependant reversal of polyamine-induced precocious maturation with respect to lactase, sucrase, and maltase activities when polyamine exposure (8 μmol spermine) is terminated.

Recent work has suggested a possible relationship between the oral administration of spermine and the secretion of corticosteroids [Buts et al., 1993; Kaouass et al., 1994a]. Buts et al., (1993) reported an endogenous secretion of corticosterone in 60% of suckling rats receiving oral doses of spermine (5 μmol/dose) over a 4 day period as compared to controls. Adrenalectomy of pre-weanling (postnatal day 10) rats has been shown to diminish spermine-induced precocious increases in sucrase and maltase activity [Kaouass et al., 1994a]. In a subsequent study, stimulated secretions of ACTH and corticosterone were observed in pre-weanling rats (postnatal day 11) following oral spermine administrations as compared to controls [Kaouass et al., 1994b]. It has been suggested that this observed stimulation of the hypophysial-

adrenal axis could be activated by gastrointestinal hormones released from the small intestine in response to ingested spermine [Kaouass et al., 1994b]. A polyamine-mediated intestinal maturation by gastrointestinal hormones is supported by findings that the intradistal, but not intraperitoneal introduction of spermine to suckling rats significantly enhances the specific activities of both maltase and sucrase along the entire small intestine [Kaouass et al., 1994a]. Furthermore, spermine does not appear to enhance the disaccharidase activities of treated intestinal explants in organ culture [Kaouass et al., 1994a]. The evidence appears to suggest that the actual mechanism(s) mediating the polyamine-induced precocious maturation of the postnatal small intestine may involve both direct and indirect trophic effects.

While the effects of polyamines on disaccharidase and Na^+K^+ ATPase ontogeny in the postnatal rat have been explored, there is a paucity of information on the significance of the polyamines in the mediation of intestinal maturation with respect to nutrient transporters. Previous work has shown enhanced Na^+K^+ ATPase activity in response to spermidine in the small intestine of suckling rats [Wild et al., 1993]. Given that the Na^+/glucose cotransporter exploits the sodium gradient generated by the Na^+K^+ ATPase for the transport of luminal glucose and galactose into the enterocyte, polyamines may play a role in the postnatal ontogeny of SGLT1 in the rodent small intestine. To our knowledge, the modulation of the intestinal Na^+/glucose cotransporter by exogenous polyamines <u>in vivo</u> remains to be investigated.

STATEMENT OF PURPOSE

In the postnatal rat, the ontogeny of the small intestine is characterized by an orderly set of structural and functional changes at the level of the mucosa which culminate with the expression of the adult phenotype after weaning. This adaptive response facilitates nutrient absorption throughout the transition from a milk-based diet to one of solid foods [Toloza and Diamond, 1992]. A reciprocal regulation of lactase and sucrase-isomaltase gene expression coincides with this dietary transition [see literature review]. In addition, the small intestine demonstrates developmental and regional increases in Na^+K^+ ATPase activity [Sauriol, 1993], which may enhance the sodium-dependent absorption of nutritive substances to accommodate alterations in nutritional status and diet composition.

Although the developmental changes associated with weaning have been the focus of considerable investigation, the factors regulating gene expression during this period remain unclear. Putative modulators of postnatal intestinal ontogeny include glucocorticoids, thyroxine, growth factors, neural factors, cell matrix interactions, luminal nutrients, and mucosal polyamines. Increased concentrations of mucosal polyamines and increases in ornithine decarboxylase activity have been shown in the intestinal mucosa of rats during weaning [Luk et al., 1980]. The oral administration of polyamines has been shown to elicit a precocious maturation of intestinal form and function in suckling rats [Dufour et al., 1988; Wild et al., 1993; 1994; Kaouass et al., 1994a; 1994b], which may be reversed with the termination of exogenous polyamine exposure in a time-dependent fashion [Georges et al., 1990].

The effects of polyamines on the transcriptional and post-transcriptional events mediating the functional expression of ornithine decarboxylase (ODC), the Na^+/glucose cotransporter (SGLT1), sucrase-isomaltase (SI), and the Na^+K^+ ATPase α_1 and

β_1 subunit isoforms, during postnatal small intestinal ontogeny in the rat have not been described. To this end, two experiments were designed to investigate the developmental and regional expression of ODC, SGLT1, SI, and the Na^+K^+ ATPase α_1 and β_1 subunit isoforms.

Experiment 1 - Development Study

The central hypothesis of this study is that the postnatal acquisition of a mature profile of digestive enzymes and transport mechanisms at weaning is associated with age-dependent alterations in transcriptional and/or post-transcriptional processes. The overall goal of this study is to characterize the developmental and regional expression of mRNA and protein for SGLT1, SI, and the Na^+K^+ ATPase α_1 and β_1 subunits isoforms. The postnatal profile of ODC mRNA abundance will be examined since ODC is a marker enzyme of cellular growth and differentiation.

Specific Aims:

1. To measure the postnatal developmental patterns for levels of total RNA, microvillus membrane protein (MVM), and basolateral membrane (BLM) protein in the rat small intestine.
2. To measure the developmental and regional expression of mRNAs encoding ODC, SGLT1, SI, and the Na^+K^+ ATPase α_1 and β_1 subunit isoforms, in the postnatal rat small intestine.
3. To examine the developmental and regional protein expression of SGLT1, SI, and the Na^+K^+ ATPase α_1 and β_1 subunit isoforms in the postnatal rat small intestine.

Experiment II - Precocious Development Study with Washout

The central hypothesis of this study is that exposure to exogenous spermidine prior to the onset of weaning prematurely alters transcriptional and/or post-transcriptional processes thereby resulting in a precocious expression of the adult phenotype of intestinal form and function. Furthermore, these

alterations may be abrogated in a time-dependent fashion following the termination of oral spermidine exposure. The overall goal of this study is to characterize any alterations in the developmental and regional expression of SGLT1, SI, and the Na^+K^+ ATPase subunit isoforms following spermidine exposure. Whether these alterations persist in the absence of exogenous spermidine will also be examined. Given that increases in ODC activity have been demonstrated in the rat small intestine following consecutive spermidine exposures prior to weaning [Wild et al., 1993; 1994], the developmental and regional expression of ODC mRNA following spermidine exposure and the withdrawal of exogenous spermidine, require definition. Since glucocorticoids have been attributed a role in postnatal rat intestinal ontogeny [see literature review], changes in serum cortisol in response to spermidine exposure and following the withdrawal of exogenous spermidine await description.

Specific Aims:

1. To measure the effects of spermidine exposure and the withdrawal of spermidine exposure on body weight and small intestinal weight in suckling rats.
2. To measure the effects of spermidine exposure and terminating exogenous spermidine exposure on total RNA, MVM protein, and BLM protein isolated from the small intestine of suckling rats.
3. To measure the effects of spermidine exposure and the withdrawal of oral spermidine on the developmental and regional expression of mRNAs encoding ODC, SGLT1, SI, and the Na^+K^+ ATPase α_1 and β_1 subunit isoforms, in suckling rats.
4. To examine the effects of spermidine exposure and the termination of oral spermidine on the developmental and regional protein expression of SGLT1, SI, and the Na^+K^+ ATPase

α_1 and β_1 subunit isoforms in the pre-weanling rat small intestine.

5. To measure the levels of serum cortisol in suckling rats exposed to exogenous spermidine, and following the withdrawal of this exposure.

MATERIALS AND METHODS

I. EXPERIMENTAL MODELS

Experiment I - Developmental Study

Study Design

The developmental study was designed to determine whether alterations in transcriptional and/or posttranscriptional processes are associated with the maturation of digestive enzymes and transport mechanisms required for carbohydrate absorption in the postnatal rat. Table 1 (pg. 45) illustrates the study design.

The main objective of Experiment I was to characterize the molecular and cellular events associated with the ontogenic profile of expression of ODC, SGLT1, SI, and the Na^+K^+ ATPase α_1 and β_1 subunit isoforms. At each developmental time point mRNA abundance for ODC, SGLT1, SI, and the Na^+K^+ ATPase α_1 and β_1 subunit isoforms was measured in both the proximal and distal small intestine by Northern blotting. Similarly, the developmental profiles of SGLT1, SI, and the Na^+K^+ ATPase α_1 and β_1 subunit isoform protein expression were measured in proximal and distal small intestine by Western blotting. Age- and regional-dependent differences in mRNA abundance and protein were analyzed quantitatively by transmittance laser densitometry.

Study Protocol

Rats were sacrificed on postnatal days 3, 7, 10, 14, 21, 28, and 35 by carbon dioxide narcosis followed by decapitation. At each time point, the entire small intestine distal to the ligament of Treitz and proximal to the cecum was excised, flushed with ice cold saline, and divided into three equal segments corresponding to proximal, middle, and distal small intestine. Intestinal segments were weighed, snap frozen in liquid nitrogen, and stored at -70° C until time of assay. Only proximal and distal segments were analyzed in this study. The small intestinal mucosa from adult (350-400 g) male Sprague-Dawley rats was used as the reference tissue.

TABLE 1. STUDY DESIGN
Experiment I - Developmental Study

Postnatal Age at Sacrifice	n=# rats
Day 3	15
Day 7	17
Day 10	15
Day 14	17
Day 21	15
Day 28	15
Day 35	15
Adult	12

Table 1: Study design showing postnatal timepoints at sacrifice.

Statistical Analysis

All results for Experiment I were expressed as the arithmetic mean ± SEM. A one-way analysis of variance was used to test H_0 with postnatal age as the main effect. Group means were compared using Duncan's multiple comparison procedure. The Student's T-test (two-tailed, unpaired) was used to test within-group regional differences. Significance was set at $p < 0.05$.

Experiment II - Precocious Development Study with Washout

Study Design

Experiment II was designed to examine any alterations in transcriptional and/or posttranscriptional processes leading to the precocious expression of the adult phenotype of intestinal form and function in suckling rats following exposure to oral spermidine. In addition, this study sought to investigate whether, in the absence of spermidine exposure, any precocious development would persist. The study design is illustrated in Table 2 (pg. 47).

The main objective of Experiment II was to examine the molecular and cellular events associated with the expression of ODC, SGLT1, SI, and the Na^+K^+ ATPase α_1 and β_1 subunit isoforms following spermidine exposure, and to establish whether these alterations persisted following the termination of oral spermidine exposure. Levels of mRNA abundance were measured for ODC, SGLT1, SI, and the Na^+K^+ ATPase α_1 and β_1 subunit isoforms in the proximal and distal small intestine by Northern blotting. Western blotting was performed to measure SGLT1, SI, and Na^+K^+ ATPase α_1 and β_1 subunit isoform protein expression in proximal and distal regions of the small intestine. Transmittance laser densitometry was used to analyze levels of mRNA and proteins. Serum cortisol was measured by radioimmunoassay for littermates randomly chosen within each experimental treatment group.

TABLE 2. STUDY DESIGN

Experiment II - Precocious Development Study

TREATMENT GROUP	SACRIFICE DAY 10	WASHOUT DAY 10, 11, 12	SACRIFICE DAY 13
Spermidine Group	3-4 pups/dam n=31		3-4 pups/dam n=21
Placebo Group	3-4 pups/dam n=31		3-4 pups/dam n=23

Table 2: Study design showing group allocation for the washout protocol.

Study Protocol

The study protocol is illustrated in Figure 5 (pg. 49). On postnatal day 7, littermates were randomly assigned to receive either experimental feedings or placebo for three consecutive days. Suckling rats were orally fed the experimental solution or placebo between 0800 and 1000 hours using a Gilson Pipetman (Mandel Scientific Co.) set to expel 25 μl of solution. The experimental feeding consisted of 8 μmol of spermidine (Sigma Chemical Co.) in 25 μl of saline solution. An equivalent volume of saline solution served as the placebo. Animals did not receive spermidine or placebo during the three day washout period extending from postnatal day 10 to day 12. Pups were killed on postnatal day 10, or after the washout period on day 13.

Rat pups from each group were killed between 0800 and 1000 hours, that is, within the same time frame as the administration of spermidine or placebo. Truncal blood was collected at the time of sacrifice and the recovered serum stored at -20° C until time of assay. Small intestinal segments from rat pups and mucosal scrapings from adult male rats were harvested and stored until time of assay as described in Experiment I.

Statistical Analysis

All results for Experiment II were expressed as the arithmetic mean ± SEM. A two-way analysis of variance was used to test H_o with treatment and age as main effects. Group means were compared using Duncan's multiple comparison procedure. Regional differences were determined by Student's T-test (two-tailed, unpaired). The significance level was set at $p < 0.05$.

FIGURE 5 - STUDY PROTOCOL

Experiment II - Precocious Development Study

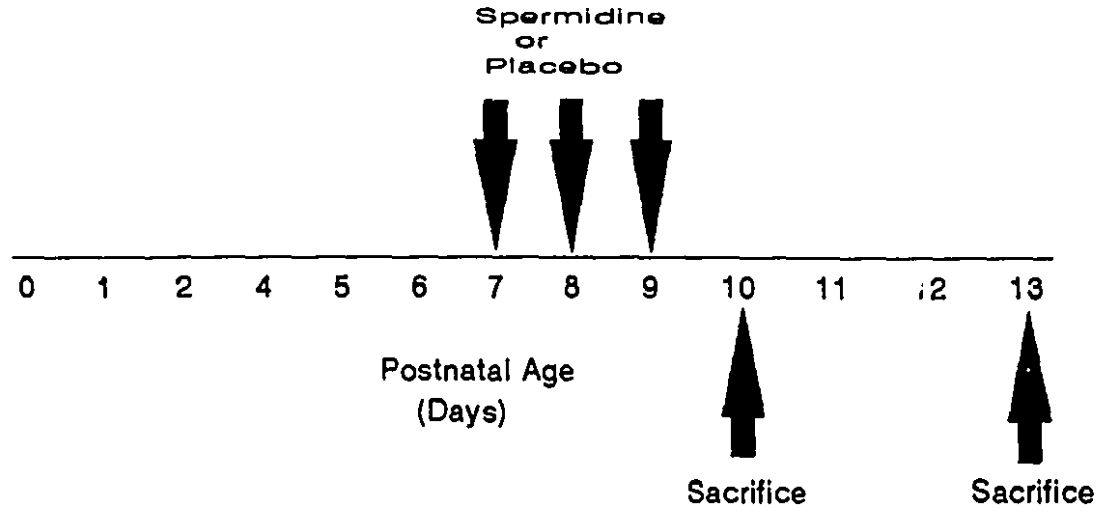


Figure 5: Study protocol showing the administration of spermidine or placebo.

II. MATERIALS

Animal Management

Pregnant Sprague-Dawley rats (day 18 of gestation) were purchased from Charles River Canada Ltd. (Saint Constant, Que.). Animals were acclimatized for 48 hours before parturition at the animal care facilities of the Donner Building (McGill University). The date of birth of the litter was designated as day 0. When necessary, litters were culled to twelve pups on postnatal day 1 to ensure approximately equivalent growth rates between pups. Pups were allowed to suckle until time of sacrifice. Dams were provided with water and conventional rat chow (Purina) ad libitum throughout the study period. All animals were maintained on a twelve hour light/dark cycle (lights on 0700-1900 hrs.) in a climate controlled environment (21° C).

Reagents

Spermidine (N-[3-Aminopropyl]-1,4-butanediamine) was purchased from Sigma Chemical Co. (St-Louis, Mo.).

The protease inhibitors used in the preparation of all protein membrane fractions, that is, aprotinin, leupeptin, and phenylmethylsulfonyl fluoride (PMSF) were purchased from Boehringer Mannheim Biochemica (Montreal, Que). The Biorad reagent used in protein quantitation was obtained from Biorad Canada Ltd. (Mississauga, Ontario). Chemicals and other materials used in the separation of proteins by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western Blot analysis of proteins were obtained from a variety of commercial sources and were of reagent grade.

The RNase inhibitor diethyl pyrocarbonate (DEPC) was obtained from Sigma Chemical Co. (St. Louis, MO). Positively charged nylon membranes, 4-morpholinepropanesulfonic acid (MOPS), anti-digoxigenin-AP, fab fragments and Lumigen PPD were purchased from Boehringer Mannheim Biochemica (Montreal, Que). All chemicals and other materials used in RNA isolation

from snap-frozen tissues, RNA fractionation, and Northern blot analysis of RNA were obtained from a variety of commercial sources and were of molecular biology grade. X-OMAT AR-5 film was purchased from Eastman Kodak CO. (Rochester, N.Y.).

Competent Escherichia coli cells (MAX Efficiency DH5α^{Tm} Competent Cells) were purchased from GIBCO BRL (Gaithersburg, Md.). The QIAEX Gel Extraction Kit was purchased from QIAGEN Inc. (Chatsworth, CA). Hexanucleotide mixture, dNTP labelling mixture, klenow enzyme, nitroblue tetrazolium salt (NBT solution), and 5-bromo-4-chloro-3-indolyl phosphate (X-phosphate) were obtained from Boehringer Mannheim (Montreal, Que.).

The radioimmunoassay kits (DSL-2000) used in the quantitative measurement of serum cortisol were purchased from Diagnostic Systems Laboratories Inc. (Webster, Texas).

III. ANTIBODIES

SGLT1

The polyclonal antibody against the Na^+/glucose transporter protein (SGLT1) was a generous gift from Dr. M. Kasahara of Kyorin University School of Medicine, Tokyo, Japan. The anti-SGLT1 antibody, raised in rabbit, was directed against synthetic peptides corresponding to amino acids 564-575 of the deduced amino acid sequence of rabbit intestinal SGLT1 [Hediger et al., 1987]. This antibody detects an 84 kDa protein in the adult rat small intestine [Takata et al., 1991].

Sucrase-Isomaltase

Dr. Ward Olson of William S. Middleton Memorial Veterans Hospital and Department of Medicine, University of Wisconsin-Madison, kindly provided the polyclonal antibody against rat SI. The SI antibody, raised in rabbit, was prepared from purified SI protein isolated from the small intestine of the adult rat [Olsen and Korsmo, 1977]. When used for immunoblot-

ting on adult rat small intestinal MVM proteins, this antibody detects sucrase at 116-120 kDa, isomaltase at 140 kDa, a high mannose form (completely glycosylated) of SI at 210 kDa, and an incompletely glycosylated form of SI at approximately 194 kDa [Lorenzsonn, Korsmo, and Olsen, 1987].

Na^+K^+ ATPase α_1 and $ß_1$ Isoform Subunits

The polyclonal antibodies directed against rat Na^+K^+ ATPase α_1 (#06-167) and $ß_1$ (#06-170) subunit isoforms (isoform specific) were obtained from Upstate Biotechnology Inc. (UBI, Lake Placid, NY) [Shyjan and Levensson, 1989]. The rat Na^+K^+ ATPase α_1 and $ß_1$ fusion proteins were produced using recombinant techniques by transfecting E.coli with rat cDNA specific for the α_1 and $ß_1$ isoform subunits fused to the E.coli trpE gene. The α_1 subunit fusion protein corresponds to residues 338-518 of the deduced amino acid sequence from rat Na^+K^+ ATPase cDNA. The $ß_1$ subunit fusion protein corresponds to residues 152-340 of the amino acid sequence deduced from rat Na^+K^+ ATPase cDNA. The affinity purified Na^+K^+ ATPase α_1 (#06167) and $ß_1$ (# 06-170) fusion proteins detect BLM proteins of 110-112 kDa and 50-55 kDa respectively [Shyjan and Levensson, 1989].

Anti-Rabbit IgG

Radiolabelled [^{125}I] Anti-Rabbit IgG, whole antibody from goat was purchased from New England Nuclear Research Products, DuPont Canada Inc. (Mississauga, Ont). This radiolabelled goat anti-rabbit antibody was used as a secondary antibody in the detection of each polyclonal primary antibody used in experiments I and II.

IV. cDNA PROBES

SGLT1

Dr. Nicholas Davidson of the University of Chicago generously provided plasmid pGEM-4Z containing the human SGLT1 cDNA

clone isolated from an adult human jejunum cDNA library. The SGLT1 cDNA clone includes nucleotides 201-2343 of the published cDNA sequence [Hediger et al., 1989]. The 2.1 kb SGLT1 insert was cloned into the EcoR1 restriction site of the pGEM-4Z vector. Digoxigenin-labelled SGLT1 cDNA probe recognizes a transcript of 4.8 kb in size [Davidson et al., 1992].

Sucrase-Isomaltase

Dr. Peter Traber of the University of Pennsylvania Medical Center kindly provided plasmid pGEM-4Z containing the rat SI cDNA cloned from rat villus tip SI mRNA. The rat SI clone was amplified from reverse transcribed RNA using oligonucleotide primers designed from both the rabbit and human SI cDNA sequence [Traber, personal communication]. The 0.85 kb SI fragment was cloned into pGEM-4Z at the EcoR1 restriction site. Digoxigenin-labelled SI cDNA probe recognizes a transcript of approximately 6.0 kb in size.

Na^+K^+ ATPase α_1 and β_1 Isoforms

Dr. J. Lingrel of the department of Molecular Genetics, University of Cinncinati, kindly provided the pUC-18 vector containing Na^+K^+ ATPase α1 subunit insert and the pKC expression vector containing Na^+K^+ ATPase β_1 subunit insert. The 0.3 kb α_1 segment was cloned into the pUC-18 vector between the EcoR1 and Pst1 restriction sites. The 0.3 kb β_1 insert was cloned into the pKC vector between the NCoI and STuI restriction sites. Digoxigenin-labelled Na^+K^+ ATPase α_1 and β_1 probes recognize transcripts of 3.7 kb and 2.7 kb, respectively [Orlowski and Lingrel, 1988].

Ornithine Decarboxylase

Dr. Perry J. Blackshear of the Howard Hughes Institute, University of Chicago, generously provided the pUC-19 vector containing ODC cDNA cloned from a rat testicular library. The 1.9 kb ODC insert was cloned into the pUC-19 vector at the

EcoR1 restriction site. Labelled ODC cDNA probe detects two major transcripts of 2.6 kb and 2.2 kb, and a minor transcript at 1.7 kb [Blackshear et al., 1989].

V. BACTERIAL TRANSFORMATION

Plasmid DNA (100 ng) was introduced into competent Escherichia coli (MAX Efficiency DH5α™ Competent Cells) by heat shocking the cells in a 42° C waterbath for 45 seconds without agitation. Heat shocked E. coli containing plasmid DNA were grown in a non-selective growth medium, S.O.C. medium (2% tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSo_4$, 20 mM glucose), at 37° C for 1 hour with agitation to allow the synthesis of plasmid-encoded antibiotic resistant proteins. Upon reaching log phase growth, serial dilutions (1:10, 1:100, 1:1000) of transformation culture were plated out on agar medium containing ampicillin (50 μg/ml) to permit the identification of plasmid-containing colonies. Following an overnight incubation at 37° C, the number of colonies were counted and the efficiency of transformation determined.

Transformation efficiency is defined as the number of colony forming units (CFU) per μg of control DNA. In a control experiment to determine transformation efficiency, pUC19 control DNA (0.05 ng) was introduced into 100 μl of MAX Efficiency DH5α™ Competent Cells, grown and plated out as previously described. The efficiency of transformation was calculated by counting the number of colonies from each control dilution and was determined to be 93-97% efficient.

VI. PLASMID DNA ISOLATION

Colonies were harvested and allowed to grow overnight at 37° C in LB broth (Lennox L Broth Base) containing ampicillin (50 μg/ml). Plasmid DNA was purified from E.coli cells using the alkaline lysis method [Sambrook et al., 1989]. The saturated culture was centrifuged at 4000 x G for 15 minutes,

the LB broth removed by aspiration, and the resultant bacterial pellet resuspended in a solution containing 50 mM glucose, 25 mM Tris-HCl, and 10 mM EDTA. Cells were then lysed in an alkali solution (0.2N NaOH, 1% SDS) followed by dispersion of the bacterial lysate in a 5 M potassium acetate/glacial acetic acid solution. Following a brief centrifugation at 12,000 rpm to pellet the lysate, plasmid DNA was precipitated with isopropanol, washed in 70% alcohol, and once again centrifuged to pellet the purified DNA. Finally, plasmid DNA was purified over a Sephadex column and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Purity and yield were determined using spectrophotometry by measuring sample absorbance at 260 nm and 280 nm and calculating the OD_{260}/OD_{280} ratio respectively.

To cleave cDNA inserts from their respective vectors, plasmid DNA was incubated with the appropriate restriction endonuclease for 90 minutes at 37° C. The restriction cutting reaction was terminated with 0.5M EDTA, and the DNA precipitated with 3M sodium acetate and 100% ethanol for 1 hour at 20° C. DNA was recovered by centrifugation and the pellet washed with 70% ethanol to remove any contaminants. The DNA was then pelleted, resuspended, and size fractionated on a 0.7% (ODC, SGLT1, SI) or 1.5% (Na^+K^+ ATPase α_1 and β_1) agarose gel. Staining of the gel with ethidium bromide permitted the DNA to be visualized under ultraviolet light. The DNA insert was cut from the agarose, extracted, and purified from the agarose gel using the QIAEX Gel Extraction Kit (Qiagen Inc., CA). Using this method DNA fragments were adsorbed to silica gel particles (QIAEX) in the presence of a high salt buffer [Vogelstein and Gillespie, 1979]. Purified DNA was eluted from QIAEX particles with a small volume of TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0) and stored at -20° C until labelling with digoxigenin.

VII. DIGOXIGENIN LABELLING OF cDNA

Digoxigenin, a steroid hapten, was used to label DNA for all molecular probes used in the Northern blot analysis of samples. All DNA probes were labelled with DIG-11-dUTP using the random primed labelling technique according to an optimized standard protocol [Feinberg and Vogelstein, 1983]. The DNA template (10 ug/ml) was heat denatured for 10 minutes at 95° C, snap cooled, and incubated with labelling reagents (10X hexa-nucleotide mixture, dNTP labelling mix, DNA polymerase I Klenow enzyme) for approximately 20 hours at 37° C. The labelling reaction was terminated with 0.5 M EDTA, followed by precipitation with 4 M LiCl and absolute ethanol. The labelled DNA was pelleted and washed with 70% ethanol for further purification. The final labelled cDNA probe was resuspended in 40 ul of TE buffer prior to quantitation.

Digoxigenin-labelled cDNA was quantified using colorimetric detection techniques according to the manufacturer's specifications [Boehringer Mannheim, 1993]. Briefly, serial dilutions of labelled control DNA and labelled experimental DNA were spotted onto a nylon membrane and fixed by cross-linking with ultraviolet light. To prevent the non-specific binding of antibody, membranes were equilibrated in maleate buffer (100mM NaCl, 100mM maleic acid, pH 7.5) and blocked in a 1% Blocking reagent (Boehringer Mannheim) for 30 minutes. Membranes were then incubated with anti-digoxigenin-alkaline phosphatase antibody at a dilution of 1:5000 for 30 minutes. To remove unbound antibody, membranes were briefly washed with maleate buffer containing 0.3% Tween™ 20. Membranes were then incubated with a Tris-buffer solution (100mM Tris-HCl, 100 mM NaCl, pH 9.5) to activate the alkaline phosphatase conjugated to the anti-digoxigenin antibody. Colorimetric detection was carried out by incubating membranes in a dimethylformamide solution containing nitroblue tetrazolium salt (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (X-phosphate). Color development was allowed to proceed in the dark without

agitation for a maximal period of 16 hours. Membranes were washed in distilled deionized water to terminate the color development reaction. The concentrations of labelled DNA were estimated by comparing the spot intensities of the experimental DNA dilutions to the labelled control dilutions.

Following quantitation, all digoxigenin-labelled cDNA probes were diluted in prehybridization solution (7% SDS, 50% deionized formamide, 5x SSC, 2% Blocking Reagent, 50 mM/l sodium phosphate, pH 7.0, and 0.1% N-lauroylsarcosine) and heat denatured in a 95° C waterbath prior to use.

VIII. ANALYTICAL PROCEDURES

A. Preparation of Membrane Fractions

Microvillous Membranes

Using 3 to 5 animals in each experimental group, segments of whole intestine from proximal or distal small intestine were randomly pooled to obtain 0.8 to 1.0 gram of either tissue for sample processing. Mucosal scrapes from proximal and distal small intestine, as well as whole colon, were randomly pooled from 3 adult animals for the preparation of reference membrane fractions. All steps in the preparation of microvillous membranes and crude basolateral membrane fractions were carried out on ice or at 4° C to inhibit protease activity. The protease inhibitors aprotinin (5 μg/ml), leupeptin (2.5 μg/ml) and PMSF (0.5 μM/ml), each diluted 1/1000, were added to homogenization buffers, prior to tissue homogenization. Microvillous membranes (MVM) were isolated according to a modification of Crane's (1973) calcium chloride precipitation method [Kessler et al., 1978] using magnesium chloride. Tissues were homogenized using a Polytron (Brinkman, Burlington, Ont) in a 500 mM mannitol, 10 mM HEPES buffer (pH 7.4) containing the aforementioned protease inhibitor cocktail. The resulting suspension was diluted 1:6 with distilled, deionized water, to which 1 M $MgCl_2$ was added to a final concentration of 10 mM. The dilute homogenate was then

agitated for 20 minutes in an ice bath to maximize the precipitation of cell nuclei, mitochondria, basolateral membrane proteins, and cellular debris. The suspension was centrifuged at 3000 X G for 15 minutes and the resultant supernatant collected and transferred to ultracentrifuge tubes. The supernatant was then centrifuged at 20,000 X G for 30 minutes, and the resulting pellet resuspended in a 100 mM Mannitol, 10 mM HEPES buffer solution (pH 7.4). The re-suspension was subjected to centrifugation at 20,000 X G for 30 minutes. The final pellet containing microvillous membranes was resuspended in 10 mM Tris (pH 7.4).

Crude Basolateral Membrane (BLM) Fractions

Crude BLM fractions were prepared according to the method of Jorgenson (1988). Tissues were homogenized in an ice-cold homogenization buffer (pH 7.5) containing 0.25 M sucrose, 150 mM NaCl, 30 mM Tris using a Polytron homogenizer (Brinkman, Burlington,Ont.). The homogenate was centrifuged at 6,000 x G for 15 minutes. The resulting pellet was re-extracted with the aforementioned homogenization buffer and was again centrifuged at 6,000 x G for 15 minutes. The collected supernatant was then subjected to centrifugation at 45,000 x G for 45 minutes. The final pellet enriched in BLM was resusupended in homogenization buffer (pH 7.5).

Quantitation of Proteins

Microvillous membrane and crude BLM fraction protein quantitation was carried out according to the Bradford method [Bradford, 1976] using a Biorad protein microassay reagent (Biorad, Montreal, Que.). This colorimetric method involves quantitating the binding of Coomassie Brilliant Blue dye to the unknown protein and is designed to quantify from 1 to 10 μg of protein. Sample protein dye binding was compared to the binding of a standard protein at different concentrations. The reference protein used in this assay was bovine serum

albumin (BSA).

In the determination of membrane protein concentration, aliquots from each MVM and BLM fraction sample were measured at A_{595} using a Spectrophotometer (Spectronic 1001 Plus, Milton Roy Co.). All sample aliquots were measured in triplicate and averaged values were compared to a standard curve generated using the protein standard (BSA). Typical protein concentrations ranged from 5.0 to 8.5 μg/ul. Following quantitation, sample aliquots were stored at -70° C until time of assay.

B. Western Immunoblotting

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis [SDS-PAGE]

Microvillous and crude basolateral membrane proteins were resolved by SDS-PAGE under denaturing conditions with constant current as described by Laemmli (1970). Sample MVM and enriched BLM fraction aliquots containing 80 μg of protein were heat denatured at 65° C for 10 minutes and solubilized in sample buffer containing 4% (w/v) SDS and 4M urea (reducing agents), as well as 0.01% (v/v) Bromophenol blue dye for visualization. All samples were analyzed on a 7.5% polyacrylamide gel allowing for an optimal protein resolution range of 30 to 120 kDa. Electrophoresis was carried out at room temperature with a constant current of 30 amps, using a Hoefer minigel apparatus (Fisher Inc., Montreal).

Immunoblotting

Following separation by SDS-PAGE, proteins were immobilized on a solid support by electroblotting to a nitrocellulose membrane [Towbin, 1979]. Electrotransfer was carried out using a Genie Transfer apparatus (Idea Scientific, Montreal). Briefly, gels were vertically oriented between two electrode panels completely submerged in a tank containing transfer buffer (25 mM Tris, 192 mM glycine; pH 8.3). Electrotransfer was carried out at 4° C for 100-120 minutes at maximal voltage and

current settings. All membrane blots were reversibly stained with ponceau S[3-hydoxy-4-(2-sulfo-4-[4-sulfophenylazo]-phenyl-azo)-2, 7-naphthalenedisulfonic acid] to evaluate equivalent protein loading between samples. Blots showing equivalent sample protein concentrations were used for subsequent Western immunoblotting. Blots were rinsed with Tris-buffered saline solution containing 0.5% TWEEN™ 20 (TTBS,pH 7.5) to remove ponceau stain prior to the saturation of unoccupied protein binding sites (blocking).

To saturate non-specific protein binding sites, membranes were incubated (blocked) overnight in BLOTTO (bovine lacto transfer technique optimizer): 5% w/v skim milk Tris-buffered saline solution containing 0.5% Tween 20™ (TTBS, pH 7.5). Blocked membranes were subsequently probed with specific antibodies to identify the antigens of interest. All blots were incubated (with agitation) with one of the following primary antibodies (diluted in 5% BLOTTO) for 2 hours at room temperature: SGLT1 (diluted 1:500), sucrase-isomaltase (diluted 1:3000), Na^+/K^+ ATPase α_1 (diluted 1:200) and $ß_1$ (diluted 1:500) fusion proteins. Following this primary incubation, membranes were briefly washed with TTBS (pH 7.5) to remove residual, unbound primary antibody. Blots were then incubated with radiolabelled secondary antibody [^{125}I] anti-rabbit IgG whole antibody from goat directed against primary antibody for 1 hour at room temperature. The radiolabelled secondary antibody was diluted 1:1500 in 5% BLOTTO (pH 7.5).

Following a series of final washes in TTBS (pH 7.5) to remove residual secondary antibody, membranes were air dried and exposed to X-OMAT AR-5 film using intensifying screens at -70° C for 24 to 72 hours. The relative band densities were determined by transmittance laser densitometry using a SciScan™ 5000 imaging unit (United States Biochemical [USB] Corporation, Cleveland, Ohio).

C. PREPARATION OF TOTAL RNA

Isolation of Total RNA

Using 3 to 5 animals in each experimental group, segments of whole intestine from proximal or distal small intestine were randomly pooled to obtain 0.8 to 1.0 gram of either tissue for sample processing. Mucosal scrapes from proximal and distal small intestine were randomly pooled from 3 adult rats for the isolation of adult reference RNA. All steps in the isolation of RNA, RNA sample preparation, and RNA fractionation were carried out at 4° C to inhibit endogenous RNase activity. In addition, RNA degradation by exogenous RNases was reduced by treating all glassware, solutions, and related materials with the RNase inhibitor Diethyl pyrocarbonate (DEPC), followed by autoclaving or sterile filtering prior to use.

Total RNA was isolated according to the method of Chomczynski and Sacchi (1987). Tissues were homogenized in a denaturing solution containing guanidinium thiocyanate/2-mercaptoethanol (1ml/100mg tissue) using a sterilized Polytron (Brinkman, Burlington, Ont.). To this mixture, 2M sodium acetate (pH 4.0) was added (0.1ml/100mg tissue) and mixed thoroughly by inversion. To further denature nucleoproteins, water saturated phenol (1ml/100mg of tissue) was added followed by 49:1 chloroform/isoamyl alcohol (0.2ml/100mg of tissue), mixed thoroughly by inversion, and incubated at 4° C for 15 minutes. Samples were centrifuged at 10,000 x G for 20 minutes at 4° C to separate RNA from contaminating DNA, proteins, and other cellular constituents. The aqueous phase containing RNA was carefully removed avoiding the cloudy interface separating the aqueous from organic phases and mixed with an equal volume of ice-cold isopropanol to precipitate extracted RNA. RNA precipitation was allowed to proceed for approximately 1 hour at -20° C. Samples were subsequently centrifuged at 10,000 x G for 10 minutes at 4° C to pellet the precipitated RNA, and the supernatant discarded.

A second RNA extraction/purification step was performed by dissolving the pelleted RNA once again in denaturing solution (0.3ml/100 mg of tissue), and re-precipitating the RNA with 3M sodium acetate (pH 4.0)and 2 volumes of ice-cold 100% ethanol. The re-precipitation of RNA was allowed to proceed for approximately 1 hour at -20° C. Samples were again centrifuged at 10,000 x G for 10 minutes at 4° C, and the resulting supernatant discarded.

To remove any residual, contaminating guanidinium thiocyanate and/or salt, RNA was washed by resuspending the pellet in ice-cold 75% ethanol (1ml/100mg of tissue), vortexing the resulting precipitate, and incubating the suspension for 10 minutes on ice. Samples were subsequently centrifuged at 10,000 x G for 5 minutes at 4° C, the supernatant discarded, and the pelleted RNA air dried. The RNA was resuspended in 50 to 100 μl of DEPC-treated water until completely dissolved.

Quantitation of Total RNA

RNA quantitation was achieved using ultraviolet absorption spectrophotometry at an optical density of 260 nm. The purity of the RNA obtained was determined by calculating the ratio of absorbance at 260 nm to the absorbance at 280 nm. Acceptable ratios fell within the range of 1.6 to 2.0. Samples showing 260/280 ratios below the accepted range indicated were subjected to phenol/chloroform re-extraction and ethanol precipitation to remove contaminating protein. RNA integrity was determined by analyzing a small quantity of RNA, approximately 5 μg/sample, on a 1% agarose/formaldehyde gel run at high voltage (80 V) for 1 hour. Intact RNA showed clear ribosomal RNA bands corresponding to the 28s and 18s rRNA subunits when the gel was placed on a UV transilluminator. Only intact RNA was used for Northern blotting. Following quantitation, all samples were stored at -70° C until time of assay.

D. Northern Blotting

Agarose-Formaldehyde Gel Electrophoresis

Total RNA (20-30 μg) was fractionated on a 1.5% agarose gel containing 2.2M formaldehyde as a denaturing agent. Electrophoresis proceeded at 4° C for approximately 3 hours at 55V. RNA integrity was assessed using a UV transilluminator and a photograph documenting intact RNA and equal lane loading was obtained. The fractionated RNA was then transferred to a positively charged nylon membrane (Boehringer Mannheim) by capillary action using 10X SSC (3M NaCl, 0.3 M Na_3 citrate ;pH 7.0) transfer buffer [Sambrook, Fritsch and Maniatis, 1989]. Following transfer, RNA was crosslinked to the membrane by exposure to UV light. Cross-linked membranes were stored in 2X SSC prior to Northern blotting analyses.

Hybridization

Hybridization techniques were carried out according to optimized standard protocols for use with digoxigenin-labelled cDNA probes [Boehringer Mannheim, 1993]. All blots were incubated in a high SDS/formamide northern prehybridization solution (7% SDS, 50 mM sodium phosphate buffer, pH 7.0, 50% formamide, 2% blocking Reagent, 5X SSC, 0.1% lauroylsarcosine) for 4-6 hours at 50° C. In a hybridization bag, membranes were incubated with one of the aforementioned digoxigenin-labelled probes, diluted in northern prehybridization solution (500 ng/10 ml). All probes were subjected to heat denaturation at 95° C for 10 minutes prior to use. Hybridization was allowed to proceed at 50-52° C for approximately 16 hours.

Following the hybridization of probe to RNA, membranes were subjected to a series of post-hybridization washes to remove any unannealed probe from the blots, thereby reducing background. Briefly, blots were washed at room temperature in a 2X wash solution (2X SSC containing 0.1% SDS) followed by high stringency washes at 68° C in 0.1X wash solution (0.1X SSC

containing 0.1% SDS). All subsequent incubations were performed at room temperature with agitation. Blots were briefly equilibrated in maleate buffer (100mM NaCl, 100mM maleic acid, pH 7.5) and blocked in 1% Blocking reagent (Boehringer Mannheim) for 2 hours to prevent any non-specific binding of antibody to the membranes. Membranes were then incubated with anti-digoxigenin-alkaline phosphatase antibody at a dilution of 1:10,000 for 30 minutes. To remove unbound antibody, membranes were washed in maleate buffer containing 0.3% TWEEN™ 20.

Chemiluminescent detection was initiated by briefly equilibrating membranes in a Tris-buffer solution (100 mM Tris-HCl, 100 mM NaCl, pH 9.5) to activate the alkaline phosphatase conjugated to the anti-digoxigenin antibody. Hybridized probe was detected through the reaction of the bound antibody conjugate with the chemiluminescent substrate used, Lumigen™ PPD. The chemiluminescent signal was obtained by exposing blots to X-OMAT AR-5 film for optimal exposure times of 12 to 24 hours. The relative band densities on the autoradiographs were measured by transmittance laser densitometry using a SciScan™ 5000 imaging unit (USB Corporation, Cleveland, Ohio).

E. Quantitation by Transmittance Laser Densitometry

Northern and Western blotting techniques were used to examine the mRNA abundance and protein expression of SGLT1, sucrase-isomaltase, the Na^+K^+ ATPase α_1 and β_1 isoforms, and ornithine decarboxylase in the postnatal rodent small intestine as previously described. The relative abundance of each mRNA and protein was determined from multiple scans of autoradiograms by transmittance laser densitometry using the Sci Scan™ 5000 high resolution scanner (USB Corporation, Cleveland, Ohio). This unit produces an image by measuring the intensity of light transmitted through samples on autoradiograms by a fluorescent lamp with high output in the green

region of the spectrum. Image analysis was performed using Bioanalysis™ software (USB Corporation, Cleveland, Ohio).

For Western blots, all membranes were reversibly stained with Ponceau S to evaluate equivalent protein loading between samples following the electrotransfer of proteins. The relative abundance of each protein was measured from 3 to 4 autoradiograms and the values obtained expressed as percentages of those in the reference adult rat.

The relative abundance of mRNA detected on Northern blots was determined by scanning densitometry of the total transcript area of 3 to 4 autoradiograms. The values obtained for each mRNA were expressed as percentages of those in the reference adult rat. Initially, all membranes were to be additionally probed with a glyceraldehyde 3-phosphate (GAPDH) digoxigenin-labelled cDNA to control for differences in total RNA loaded in each lane. While this transcript is known to be constitutively expressed in the adult small intestine [Burant et al., 1994], GAPDH mRNA appears to be developmentally regulated in the postnatal rat small intestine (Appendix I). Previous studies have suggested that intestinal ß-actin mRNA is developmentally regulated in the postnatal rat and rabbit [Sebastio et al., 1987; Sebastio et al., 1989]. Consequently, mRNA levels were not normalized to GAPDH mRNA or ß-actin in this work. To verify RNA integrity and the uniformity of RNA concentration per lane, the residual ethidium bromide-stained 18S and 28S ribosomal species were visualized by ultra violet illumination following the transfer of RNA to nylon membrane.

F. Radioimmunoassay for Serum Cortisol

Radioimmunoassay for serum cortisol was conducted on serum samples from littermates randomly chosen within the placebo and experimental feeding groups at each time point within the washout protocol (Experiment II). All serum samples stored at -20° C from the time of collection were allowed to reach room temperature, and were gently mixed prior to assay. No grossly

hemolyzed or lipemic samples were used in this analytical procedure. Similarly, all assay reagents were allowed to reach room temperature and inverted to ensure thorough mixing prior to use. All standard and control reagents, as well as unknown samples, were assayed in duplicate.

Cortisol Standards A through G (approximately 0, 0.5, 2.0, 4.0, 10.0, 20.0, and 60.0 μg/dl or, 13.8, 55.2, 138, 276, 552, and 1656 nmol/l respectively), Cortisol Serum Control Levels I and II (corresponding to low (4.0 μg/dl or 110.4 nmol/l) and high (20 μg/dl or 552 nmol/l) levels of human serum cortisol respectively), and 25 μl of each unknown sample were delivered to the bottom of appropriately labelled test tubes using a Gilson Pipetman (Mandel Scientific Co.). Five hundred μl of Cortisol [I^{125}] Reagent was then immediately added to each tube. Following this, all tubes, except the designated total count tubes, received 500 μl of Cortisol Antiserum Complex. All tubes were gently agitated and incubated in a 37° C water bath for 45 minutes. All tubes, except total count tubes, were then subjected to centrifugation at 1500 x G at 4° C for 20 minutes.

Following this, all tubes, with the exception of total count tubes, were decanted and counted in a gamma counter for 1 minute. The average counts per minute (CPM) for each standard, control, and unknown sample were calculated and applied to determine the Percent Bound (% B). A ratio of %B over the mean counts of the zero standard (Bo) was then determined for all standards, controls, and samples.
Scatchard plots were constructed from this data to obtain the cortisol concentration of each unknown sample which were compared to the accepted normal range of values for postnatal day 10-13 rats (approximately 0-0.4 μg/dl or 0-11.04 nmol/l) [Henning, 1985].

RESULTS

EXPERIMENT I: DEVELOPMENTAL STUDY

Experiment I sought to characterize the developmental patterns of mRNA abundance and protein expression for SGLT1, sucrase-isomaltase (SI), and the Na^+K^+ ATPase in the postnatal rat small intestine. Since SGLT1 and SI are located on the enterocyte MVM, and the Na^+K^+ ATPase is restricted to the BLM, the yields of MVM and BLM protein obtained during postnatal development were also examined. In addition, yields of total RNA were measured in this experiment to evaluate whether levels of total RNA correlated with increased mucosal growth during postnatal development. Given that intestinal growth and maturational processes are altered with weaning, the ontogenic profile of ornithine decarboxylase (ODC), a marker enzyme of cellular growth and differentiation, was examined at the level of mRNA.

Developmental Profile of Total RNA Yields

Total RNA was isolated from both the proximal and distal small intestine of postnatal rats at specific developmental time points as previously described. A summary of these results is presented in Table I-1 (pg. 82) and Figure I-1A (pg. 83).

In this experiment, the yields obtained for total RNA showed significant age-dependent ($P<0.0001$) increases in the small intestine during postnatal development (Table I-1). In the proximal small intestine significant increases in yields of total RNA occurred at postnatal day 14 compared to earlier time points, followed by a 2-fold increase in the yield of total RNA from day 14 to day 35. However, yields of total RNA obtained from the proximal small intestine of the adult reference rat were still significantly higher than those at postnatal day 35.

In the distal small intestine significant increases in the yields of total RNA occurred between days 14 and 21. Yields

of total RNA again increased significantly by postnatal day 28. These levels were maintained at day 35 and equalled the values obtained for the reference adult rat. A 7- to 8-fold increase in the yields of total RNA was noted between postnatal day 3 and adult time points in the proximal and distal small intestine, respectively, indicating significant cellular growth during development. Significant differences between the proximal and distal portions of the small intestine occurred at postnatal day 14 and in the reference adult rat (Figure I-1A).

In summary, the largest single increase in the yield of total RNA occurred between days 10 and 14 in the proximal small intestine, whereas this growth in the distal small intestine occurred slightly later between days 14 to 21.

Developmental Profile of MVM Protein Yields

Results for MVM protein fractions for the proximal and distal small intestine are presented in Table I-1 (pg. 82) and Figure I-1B (pg. 83).

Significant age-dependent ($P<0.0001$) increases in the yields of MVM protein were found in the postnatal small intestine (Table I-1). In the proximal small intestine yields of MVM protein were significantly increased at weaning (postnatal day 21). The yields of MVM protein reached adult values at postnatal day 35. Gradual, age-dependent increases in the yields of MVM protein were seen in the distal small intestine beginning at postnatal day 14, with adult values reached by day 28. Yields of MVM protein increased approximately 3- to 4-fold between postnatal day 3 and day 35 in the proximal and distal small intestine, respectively. Significant regional (proximal versus distal) differences were observed at postnatal day 10 ($P<0.01$), at weaning (day 21, $P<0.05$), and in fully weaned rats at days 28 and 35 ($P<0.05$) (Figure I-1B).

In summary, the largest single increase in the yield of MVM protein occurred between days 14 and 21 in the proximal small intestine, whereas the largest single increase in the distal small intestine occurred earlier between days 3 and 7. These regional increases in MVM protein did not parallel those observed for total RNA suggesting that these two fractions do not have similar growth patterns.

Developmental Profile of BLM Protein Yields

BLM protein fractions were isolated from the proximal and distal small intestine as previously described. These results are presented in Table I-1 (pg. 82) and Figure I-1C (pg. 83).

Significant, age-dependent ($P<0.0001$) increases were found for yields of BLM protein obtained from the postnatal small intestine (Table I-1). A 2.5-fold increase in the yields of BLM protein was noted between postnatal day 7 and adult time points in both the proximal and distal small intestine. In the proximal small intestine yields of BLM protein increased significantly between postnatal days 21 and 28. Adult values were reached by postnatal day 35. In the distal small intestine yields for BLM protein increased at postnatal days 14, 28, and 35. The yields of BLM protein obtained at day 35 exceeded reference adult values. Significant regional (proximal versus distal) differences in yields of BLM were found early in postnatal development at days 7 ($P<0.05$), 10 ($P<0.01$), at weaning ($P<0.01$), and in the reference adult rat ($P<0.05$) (Figure I-1C).

In summary, the largest single increase in the yield of BLM protein occurred between postnatal days 21 and 28 in both the proximal and distal small intestine. These increases occurred at a later postnatal age than those reported for total RNA and MVM protein suggesting that growth is not uniform in different cellular compartments during postnatal development.

Developmental Profile of Ornithine Decarboxylase

Ornithine decarboxylase (ODC) is a marker enzyme of cellular growth and differentiation that it known to increase in activity in the rat small intestine at weaning [see literature review]. The present work examines the ontogeny of ODC mRNA abundance in both the proximal and distal portions of the small intestine at specified time points during postnatal development. A summary of these results is presented in Table I-2 (pg. 84) and Figure I-2 (pg. 85). Figures I-2A and I-2B (pg. 86) show representative Northern blots of ODC in the proximal and distal small intestine, respectively.

In the rodent small intestine the digoxigenin-labelled ODC cDNA probe hybridized to two major bands at 2.6 kb and 2.2 kb, respectively. In addition, the ODC probe detected a minor 1.7 kb transcript at postnatal days 21, 28, 35, and in the reference adult rat (Figures I-2A and I-2B).

Significant increases in ODC mRNA abundance were found in both the proximal ($P<0.0011$) and distal ($P<0.0001$) small intestine (Table I-2). In the proximal small intestine, a surge in measured ODC mRNA between postnatal days 14 and 21 significantly increased ODC mRNA abundance from earlier low levels of expression. However, levels of ODC mRNA were comparable at postnatal days 21, 28, 35, and in the reference adult rat. In the distal small intestine ODC mRNA abundance was increased between postnatal days 7 and 10, days 14 and 21, and days 28 and 35. By postnatal day 35, the levels of ODC mRNA were 1.5-fold greater than the values measured for the reference adult. No significant regional differences were found between the proximal and distal portions of the small intestine for ODC (Figure I-2).

In summary, the single greatest increase of ODC mRNA abundance occurred just prior to weaning in both the proximal and distal segments of the small intestine between postnatal days 14 and 21.

Developmental Profile of SGLT1

SGLT1 is a nutrient transporter protein located on the MVM of the mature enterocyte that demonstrates altered gene expression and transport activity in response to dietary change, age, nutritional status, and diabetes [see review of literature]. In Experiment I, the developmental profiles of SGLT1 mRNA abundance and protein expression were examined in the rat small intestine at specific postnatal time points. A summary of these results is presented in Table I-3 (pg. 87) and Figure I-3A, B (pg. 88).

mRNA Abundance

Figures I-3A1 (pg. 89) and I-3A2 (pg. 90) show representative Northern blots of SGLT1 in the proximal and distal small intestine, respectively. The digoxigenin-labelled SGLT1 cDNA probe hybridized to a single 4.8 kb transcript in the small intestine at each postnatal time point analyzed (Figures I-3A1 and I-3A2).

Significant age-dependent increases in SGLT1 mRNA abundance were found in both the proximal ($P<0.0013$) and distal ($P<0.0001$) small intestine (Table I-3). In the proximal small intestine gradual increases in SGLT1 mRNA were observed from postnatal days 3 to 14. SGLT1 mRNA abundance was similar at postnatal days 14, 21, 28, and 35. Measured values of SGLT1 mRNA were only significantly increased in the reference adult compared to these earlier developmental time points.

In the distal small intestine, SGLT1 mRNA was significantly increased at day 10 compared to earlier values. Thereafter, significant increases in SGLT1 mRNA were observed post-weaning, with adult values reached in fully weaned rats at days 28 and 35. Significant regional differences between the proximal and distal portions of the small intestine were found early in postnatal development at day 3 ($P<0.01$), day 7 ($P<0.01$), and day 10 ($P<0.05$) (Figure I-3A). At postnatal day 35 the levels of SGLT1 mRNA were 2-fold greater ($P<0.01$) than those found in the proximal small intestine (Figure I-3A).

In summary, the single greatest increase of mRNA abundance in the distal portion of the small intestine occurred between postnatal days 21 and 28. Although developmental increases in SGLT1 mRNA abundance were reported for both regions of the small intestine, only the distal region demonstrated increased levels of SGLT1 mRNA that were associated with the process of weaning. The proximal small intestine had attained post-weaned values prior to weaning.

Protein Expression

Figures I-3B1 (pg.89) and I-3B2 (pg. 90) show representative Western Blots of SGLT1 in the proximal and distal small intestine, respectively.

SGLT1 protein expression demonstrated significant age-dependent increases in both the proximal ($P<0.0001$) and distal ($P<0.0001$) small intestine (Table I-3). SGLT1 protein was detected at all postnatal time points chosen for this study. Also, it is with interest that we report alterations in the apparent molecular weight of this transporter protein during postnatal development. In both the proximal and distal small intestine of suckling rats (days 3, 7, and 10), the SGLT1 signal was detected at approximately 70-74 kDa. At postnatal day 21 the SGLT1 protein detected on autoradiograms showed a slight upward shift in molecular weight compared to the earlier time points. Similarly, slight increases in the apparent molecular weight of SGLT1 were observed at days 28 and 35. The SGLT1 protein was detected at 84 kDa in the small intestine of the reference adult rat.

In the proximal small intestine significant increases in SGLT1 protein expression occurred just prior to the onset of the weaning between postnatal days 10 and 14 (Table I-3). Adult values were reached at postnatal day 28, while a 1.5-fold decrease from these levels was observed at day 35. These findings may reflect a plateau of SGLT1 protein expression prior to the establishment of adult levels. In the distal small intestine, SGLT1 protein increased 3-fold between

postnatal days 14 and 21. The levels of SGLT1 protein in the distal small intestine were not different between days 21, 28, and in the reference adult. Significant ($P<0.05$) regional (proximal versus distal) differences of SGLT1 protein expression were measured at postnatal day 14 (Figure I-3B).

In summary, the single greatest increase in SGLT1 protein expression occurred in the proximal segment of the small intestine between postnatal days 10 and 14, whereas the greatest increase in the distal small intestine occurred later, between days 14 and 21. These increases do not closely parallel those reported for SGLT1 mRNA abundance in the proximal and distal portions of the small intestine suggesting that SGLT1 mRNA and protein expression may be regulated regionally and independently.

Developmental Profile of Sucrase-Isomaltase

Sucrase-isomaltase (SI) is a marker MVM enzyme of postnatal intestinal development in the rat. In this species, a well documented appearance and surge in SI activity occurs in the third postnatal week [see literature review]. Recent work suggests that SI mRNA abundance parallels SI enzyme activity in the postnatal small intestine [see literature review]. In this study, the ontogeny of SI mRNA abundance and protein expression was examined during postnatal development. A summary of these results is presented in Table I-3 (pg. 87) and Figure I-3C,D (pg. 88).

mRNA Abundance

Figures I-3C1 (pg. 91) and I-3C2 (pg. 92) show representative Northern blots of sucrase-isomaltase (SI) in the proximal and distal small intestine, respectively. In the rodent small intestine the digoxigenin-labelled SI cDNA probe hybridized to a single mRNA transcript at approximately 6 kb (Figures I-3C1 and I-3C2).

Significant age-dependent increases in SI mRNA abundance were found in both the proximal and distal small intestine

($P<0.0001$) (Table I-3). In the proximal small intestine SI mRNA was first detected at postnatal day 21. Levels of SI mRNA were increased almost 2-fold by day 35 compared to days 21 and 28. SI mRNA abundance was still further increased (1.5-fold) in the reference adult compared to postnatal day 35. In the distal small intestine low levels of SI mRNA were first detected at postnatal day 21. SI mRNA abundance increased 3- and 6-fold by postnatal day 35 compared to postnatal days 28 and 21, respectively. SI mRNA abundance was further increased (1.5-fold) between postnatal day 35 and the reference adult.

In summary, in both the proximal and distal segments of the small intestine the single greatest increase in SI mRNA abundance occurred between postnatal days 14 and 21 (Figure I-4C). These findings indicate that SI gene expression is closely associated with weaning. Additional increases in the levels of SI mRNA between postnatal days 21 and 35 indicate that SI gene expression continues to be developmentally upregulated in newly weaned rats.

Protein Expression

Figures I-3D1 (pg. 91) and I-3D2 (pg. 92) show representative Western blots of SI in the proximal and distal small intestine, respectively.

Postnatal age significantly increased SI protein expression in the proximal ($P<0.0001$) and distal ($P<0.0001$) small intestine (Table I-3). In proximal small intestine SI protein (116 kDa) expression was first detected at postnatal day 21 (Figures I-3D1 and I-3D2). SI protein significantly increased (1.6-fold) between days 21 and 28, and remained at these levels in day 35 rats (Table I-3). Measured values of SI protein in weaning (day 21) and fully weaned (days 28 and 35) rats did not reach reference adult levels. In the distal small intestine, only low levels (9-12% of adult levels) of isomaltase protein (140 kDa) were expressed at early developmental time points corresponding to postnatal days 3,

7, 10, and 14. SI protein was first detected at postnatal day 21 with measured levels exceeding those of the reference adult. Thereafter, the levels of SI protein dropped to adult levels by day 28, with further 2-fold decreases found by day 35 compared to adult reference values. Significant regional (proximal versus distal) differences in SI expression were observed at postnatal days 14 ($P<0.05$), 21 ($P<0.05$), and 35 ($P<0.05$) (Figure I-3D).

In the proximal small intestine the isomaltase protein (140 kDa) in the SI complex was detected by postnatal day 21 (Figure I-3D1). By contrast, levels of isomaltase protein were detected in the distal small intestine at each developmental time point assayed (Figure I-3D2). In addition, an incompletely glycosylated form of the SI complex (194 kDa) was detected in both the proximal and distal small intestine at weaning (day 21), in newly weaned (days 28 and 35) rats, and in the adult. A faint signal representative of the high mannose (completely glycosylated) SI complex (210 kDa), was observed in both regions of the rat small intestine at postnatal days 21, 28, 35 and in the reference adult.

In summary, the single greatest increase in SI protein expression occurred in both the proximal and distal portions of the small intestine between postnatal days 14 and 21. These findings for SI protein parallel the results reported for SI mRNA abundance and indicate a close association of SI mRNA and protein expression occurring during weaning.

Developmental Profile of Na^+K^+ ATPase α_1

The α_1 isoform is a catalytic subunit of the Na^+K^+ ATPase that is located on the BLM of the mature enterocyte. This enzyme complex establishes and maintains intracellular sodium gradients that are exploited by nutrient transporters, such as SGLT1 that are restricted to the enterocyte MVM [see literature review]. In this study, the ontogeny of Na^+K^+ ATPase α_1 isoform mRNA abundance and protein expression was

examined in the postnatal rat small intestine. A summary of these results is presented in Table I-4 (pg. 93) and Figure I-4A,B (pg. 94).

mRNA Abundance

Figures I-4A1 (pg. 95) and I-4A2 (pg. 96) show Northern blots representative of the Na^+K^+ ATPase α_1 isoform in the proximal and distal small intestine, respectively. The digoxigenin-labelled Na^+K^+ ATPase α_1 isoform cDNA probe hybridized to a single 3.7 kb mRNA in the rodent small intestine (Figures I-4A1 and I-4A2).

Significant age-dependent increases in Na^+K^+ ATPase α_1 isoform mRNA abundance were found in the proximal ($P<0.0001$) and distal ($P<0.0001$) small intestine (Table I-4). In the proximal small intestine the levels of Na^+K^+ ATPase α_1 subunit mRNA showed gradual increases at postnatal days 7, 21, 28, and 35. Adult levels were reached by postnatal day 35. Na^+K^+ ATPase α_1 subunit mRNA abundance increased almost 4-fold between postnatal day 3 and day 35 time points. In the distal small intestine the levels of Na^+K^+ ATPase α_1 mRNA increased between postnatal days 7 and 10, and these levels were maintained between days 10 and 21. Na^+K^+ ATPase α_1 mRNA abundance increased 2.5-fold by day 28 compared to previous values, and reached adult levels at this time. Na^+K^+ ATPase α_1 mRNA further increased between days 28 and 35 and exceeded adult values. Significant regional (proximal versus distal) differences in Na^+K^+ ATPase α_1 mRNA occurred at postnatal day 7 ($P<0.05$), and in fully weaned rats at days 28 ($P<0.05$) and 35 ($P<0.05$) (Figure I-4A).

In summary, the single greatest increase of Na^+K^+ ATPase α_1 mRNA abundance in the distal portion of the small intestine occurred between postnatal days 21 and 28. Although developmental increases in Na^+K^+ ATPase α_1 mRNA abundance were reported for both regions of the small intestine, only the distal region demonstrated increased levels of α_1 mRNA that were closely associated with the process of weaning.

Protein Expression

Figures I-4B1 (pg. 95) and I-4B2 (pg. 96) show representative Western blots for the Na^+K^+ ATPase α_1 subunit isoform in the proximal and distal small intestine, respectively. Immunodetectable Na^+K^+ ATPase α_1 was identified in the rat small intestine at approximately 112 kDa (Figures I-4B1 and I-4B2).

Na^+K^+ ATPase α_1 protein expression showed significant age-dependent increases in both the proximal ($P<0.0002$) and distal small intestine ($P<0.0001$) (Table I-4). In the proximal small intestine gradual developmental increases in Na^+K^+ ATPase α_1 protein expression were seen by postnatal days 7, 10, 28, and 35. Adult levels of expression were reached by fully weaned rats at days 28 and 35. Although these developmental increases were gradual, immunodetectable Na^+K^+ ATPase α_1 increased almost 2-fold between suckling (day 14) and fully weaned rats (day 35). In the distal small intestine Na^+K^+ ATPase α_1 protein expression increased significantly between postnatal days 10 and 14, days 14 and 21, and again between days 21 and 35. Although adult levels of this isoform were expressed at day 14, these values were exceeded by weanling (day 21) and weaned (days 28 and 35) rats. Significant regional (proximal versus distal) differences in Na^+K^+ ATPase α_1 protein expression occurred in suckling rats at postnatal days 10 ($P<0.05$) and 14 ($P<0.05$), and in weanlings (day 21, $P<0.05$) (Figure I-4B).

In summary, the single greatest increase in Na^+K^+ ATPase α_1 protein expression in the distal small intestine occurred between postnatal days 10 and 14. The values for Na^+K^+ ATPase α_1 mRNA abundance in the distal small intestine between days 10 and 14 do not reflect the reported increase in α_1 protein. This finding suggests that Na^+K^+ ATPase α_1 mRNA and protein expression may not be closely associated immediately preceding the onset of the weaning process. By contrast, the gradual developmental increases in Na^+K^+ ATPase α_1 mRNA abundance in

the proximal small intestine generally paralleled α_1 protein expression in the proximal intestinal segment.

Developmental Profile of Na^+K^+ ATPase β_1

The β_1 isoform is a highly glycosylated subunit of the Na^+K^+ ATPase that appears to facilitate the assembly and insertion of the mature enzyme complex into the enterocyte BLM [see literature review]. Given these observations, the β_1 subunit isoform may regulate Na^+K^+ ATPase activity in the small intestine. In this study, the developmental profile of Na^+K^+ ATPase β_1 isoform mRNA abundance and protein expression was examined in the postnatal rat small intestine. A summary of these results is presented in Table I-4 (pg. 93) and Figure I-4C,D (pg. 94).

mRNA Abundance

Northern blots representative of Na^+K^+ ATPase β_1 subunit isoform mRNA abundance in the proximal and distal small intestine are presented in Figures I-4C1 (pg. 97) and I-4C2 (pg. 98), respectively. The digoxigenin-labelled Na^+K^+ ATPase β_1 subunit isoform cDNA probe hybridized to a single 2.7 kb mRNA in the rat small intestine (Figures I-4C1 and I-4C2).

Significant age-dependent increases in Na^+K^+ ATPase β_1 isoform mRNA abundance were found in both the proximal ($P<0.0001$) and distal ($P<0.0001$) small intestine (Table I-4). In the proximal small intestine the levels of Na^+K^+ ATPase β_1 mRNA were significantly increased between postnatal days 10 and 14, and again between days 14 and 28. The levels of Na^+K^+ ATPase β_1 mRNA were similar between days 28 and 35, but did not reach adult values. β_1 isoform mRNA increased by 1.5-fold between postnatal days 14 and 35. In the distal small intestine Na^+K^+ ATPase β_1 mRNA abundance was significantly increased between postnatal days 10 and 14, and again between days 21 and 35. The levels of β_1 mRNA measured at days 21 and 28 were similar to adult values, however, by day 35 β_1 mRNA abundance had exceeded adult levels. β_1 mRNA abundance

increased 1.5-fold between weanling (day 21) and fully weaned rats at day 35. Significant regional (proximal versus distal) differences in β_1 mRNA were seen at days 7 ($P<0.01$), 21 ($P<0.01$), 28 ($P<0.05$) and 35 ($P<0.01$) (Figure I-4C).

In summary, the greatest similar increases in Na^+K^+ ATPase β_1 mRNA abundance occurred in both the proximal and distal segments of the small intestine between postnatal days 10 and 14, and days 21 and 28. These findings indicate that the levels of Na^+K^+ ATPase β_1 mRNA are developmentally regulated in association with both the onset of weaning and post-weaning adaptations.

Protein Expression

Figures I-4D1 (pg. 97) and I-4D2 (pg. 98) show Western blots representative of β_1 isoform protein expression in the proximal and distal small intestine, respectively. The immunodetectable Na^+K^+ ATPase β_1 subunit isoform was identified in the rat small intestine at approximately 50 kDa (Figures I-4D1 and I-4D2).

Significant age-dependent increases in the levels of β_1 isoform protein were found in both the proximal ($P<0.0001$) and distal ($P<0.0001$) small intestine (Table I-4). In the proximal small intestine Na^+K^+ ATPase β_1 isoform expression increased significantly between postnatal days 14 and 21, days 21 and 28, and again in fully weaned rats between days 28 and 35. The levels of β_1 isoform exceeded adult values by postnatal day 35. β_1 isoform expression increased almost 3-fold between weaning (day 21) and fully weaned (day 35) rats. In the distal small intestine Na^+K^+ ATPase β_1 isoform expression increased significantly between days 14 and 21, with further increases between days 21 and 28. At postnatal day 28 the levels of β_1 isoform protein reached values that were intermediate to those reported for day 35 and adult rats. The levels of β_1 isoform protein significantly exceeded reference adult values by postnatal day 35. Significant regional (proximal versus distal) differences of Na^+K^+ ATPase

β_1 subunit isoform expression were only observed at days 10 and 14 ($P<0.05$) and at weaning (day 21, $P<0.05$) (Figure I-4D).

In summary, the single greatest increase in Na^+K^+ ATPase β_1 subunit isoform expression in the proximal small intestine occurred in newly weaned rats between postnatal days 28 and 35. Interestingly, this increase in protein was preceded by a similar increase in β_1 mRNA abundance reported between days 21 and 28. By contrast, the single largest increase in Na^+K^+ ATPase β_1 protein expression in the distal small intestine occurred between postnatal days 14 and 21. In the distal small intestine increases in β_1 mRNA abundance between days 10 and 14 preceded the increases in β_1 protein. These findings indicate that increases in Na^+K^+ ATPase β_1 mRNA generally precede β_1 isoform protein expression during postnatal development in the rat small intestine. Together these findings suggest that Na^+K^+ ATPase β_1 gene expression is developmentally regulated in the postnatal rat small intestine in association with the process of weaning.

Summary of Main Results for Experiment I

In conclusion, significant age-dependant increases in mRNA and protein were demonstrated for ODC, SGLT1, SI, and the Na^+K^+ ATPase α_1 and β_1 subunit isoforms in Experiment I. Similarly, age-dependant increases in the yields of total RNA, MVM protein, and BLM protein were demonstrated, however no associations between the growth patterns of these cellular compartments were found in this study. Signalling an increase in cellular growth and differentiation, mucosal ODC mRNA abundance was increased with the onset of weaning. Similarly, both SI mRNA and protein also showed the greatest increases in abundance with the onset of weaning. By contrast, mRNA content did not reflect protein expression for both SGLT1 and the Na^+K^+ ATPase α_1 subunit isoform during postnatal development, with the greatest increases occurring in pre-weaned and weaning rats for protein expression, and in post-

weaned rats for mRNA abundance. Furthermore, Na^+K^+ ATPase $ß_1$ isoform protein expression also did not closely parallel $ß_1$ mRNA content during postnatal development, with the greatest increases found prior to weaning and post-weaning (days 21 to 28) for $ß_1$ mRNA, and in weaning and post-weaned (days 28 to 35) rats for $ß_1$ protein. In Experiment I, the developmental profiles of intestinal enzyme and transporter gene expression were generally dissimilar in the proximal and distal regions of the postnatal rat small intestine. Of the variables examined in this experiment only SI gene expression demonstrated similar developmental profiles for mRNA and protein expression in both the proximal and distal small intestine.

Table I-1: Effect of Age on RNA, Microvillus Membrane(MVM) and Basolateral Membrane(BLM) Protein Yields from Rat Small Intestine

Yield (mg/g gut)	Postnatal Age (days)							Adult Reference	One-way ANOVA	
	3	7	10	14	21	28	35		F	p
Total RNA										
Proximal	0.60 ± 0.23 d	0.77 ± 0.20 d	1.02 ± 0.20 d	1.95 ± 0.20 c	2.00 ± 0.20 c	2.51 ± 0.20 bc	2.99 ± 0.20 b	4.21 ± 0.20 a	35.79	0.0001
Distal	0.35 ± 0.24 d	0.55 ± 0.21 cd	0.80 ± 0.21 cd	1.14 ± 0.21 c**	1.85 ± 0.21 b	2.90 ± 0.21 a	3.12 ± 0.21 a	2.97 ± 0.21 a*	30.06	0.0001
MVM Protein										
Proximal	1.10 ± 0.15 e	1.56 ± 0.13 d	1.79 ± 0.13 d	1.89 ± 0.13 d	2.66 ± 0.13 c	2.92 ± 0.13 bc	3.35 ± 0.13 a	3.09 ± 0.13 ab	37.28	0.0001
Distal	0.58 ± 0.18 e	1.30 ± 0.15 d	1.30 ± 0.15 d**	1.63 ± 0.15 cd	1.84 ± 0.15 bc*	2.14 ± 0.18 ab*	2.43 ± 0.18 a*	2.38 ± 0.15 a	14.20	0.0001
BLM Protein										
Proximal	NA	7.78 ± 0.44 d	8.93 ± 0.50 d	9.18 ± 0.44 cd	10.38 ± 0.44 c	15.91 ± 0.44 b	18.17 ± 0.50 a	19.41 ± 0.44 a	114.03	0.0001
Distal	NA	5.71 ± 0.47 d*	6.91 ± 0.47 d**	8.53 ± 0.54 c	8.74 ± 0.47 c**	15.47 ± 0.47 b	17.97 ± 0.54 a	14.80 ± 0.47 b*	98.08	0.0001

NA, not available.
Values are means ± SEM of 3-4 samples.
Within a row, means with the same letter are not significantly different from each other ($p>0.05$, one-way ANOVA).
* $p<0.05$, distal versus proximal (Student's t-test).
** $p<0.01$, distal versus proximal (Student's t-test).

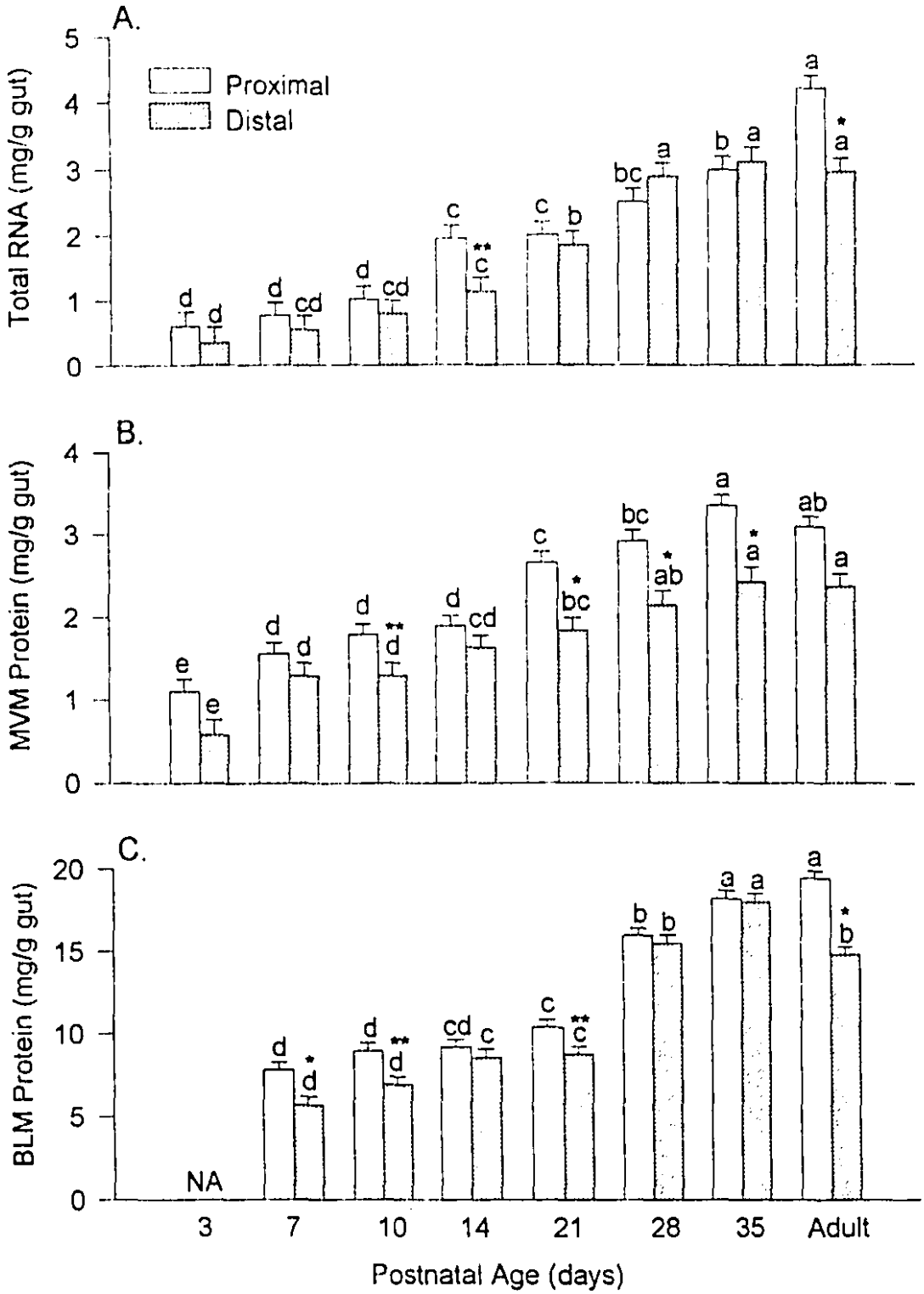


Figure I-1. Effect of age on RNA, microvillus membrane(MVM) and basolateral membrane(BLM) protein yields from rat small intestine. Within each site (proximal or distal), bars with the same letters are not significantly different from each other ($p>0.05$, one-way ANOVA). Distal versus proximal comparisons were done using the Student's t-test where * $p<0.05$ and ** $p<0.01$. NA indicates data not available.

Table I-2: Effect of Age on Ornithine Decarboxylase(ODC) mRNA Abundance in Rat Small Intestine

Autoradiogram Density (% of Adult Reference)	Postnatal Age (days)								One-way ANOVA	
	3	7	10	14	21	28	35	Adult Reference	F	p
ODC mRNA										
Proximal	46.0 ± 24.1 b	52.0 ± 25.2 b	47.3 ± 14.9 b	46.3 ± 11.1 b	90.3 ± 24.5 a	111.4 ± 28.6 a	107.7 ± 19.5 a	100.0 a	6.32	0.0011
Distal	26.0 ± 4.6 d	23.7 ± 8.4 d	58.3 ± 20.5 c	49.7 ± 11.5 c	96.0 ± 10.4 b	107.3 ± 9.0 b	147.0 ± 14.9 a	100.0 b	43.04	0.0001

Values are means ± SEM of 3 samples.
Within a row, means with the same letter are not significantly different from each other ($p>0.05$, one-way ANOVA).
No significant differences were observed between proximal and distal intestine ($p>0.05$, Student's t-test).

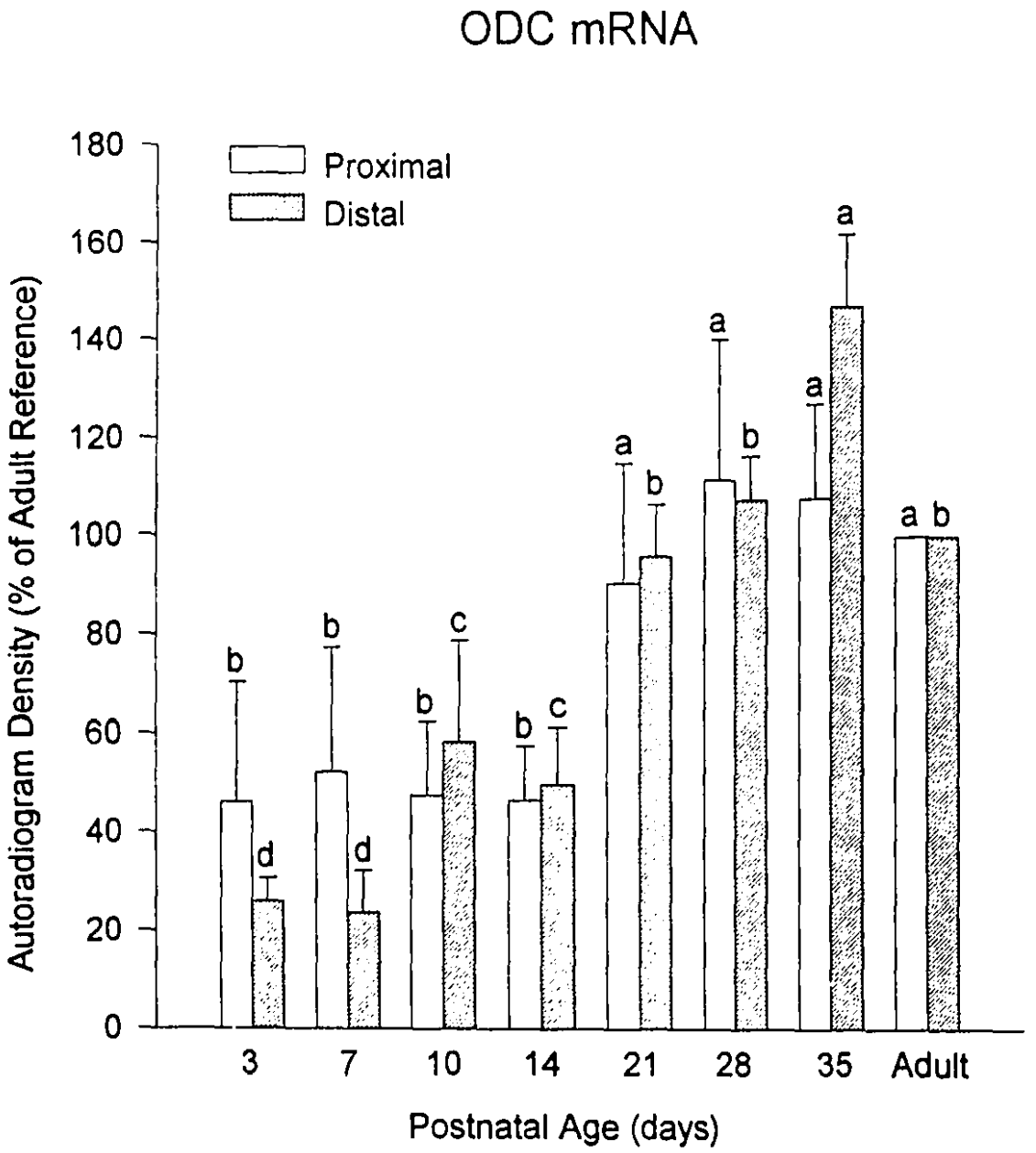


Figure I-2. Effect of age on ornithine decarboxylase(ODC) mRNA abundance in rat small intestine. Within each site (proximal or distal), bars with the same letters are not significantly different from each other ($p>0.05$, one-way ANOVA). No significant differences were observed between proximal distal intestine ($p>0.05$, Student's t-test).

Figure I-2A: Northern Blot of Ornithine Decarboxylase Proximal Small Intestine

Northern blot analysis (representative of n=3) of ornithine decarboxylase (ODC) mRNA abundance in total RNA isolated from rat proximal small intestine during development. 20 μg of total RNA from time points corresponding to postnatal day 3, 7, 10, 14, 21, 28, 35, and adult were fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled ODC cDNA probe for 16 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kilobases (kb) and 5.0 kb respectively. The ODC cDNA probe detects two major transcripts of 2.7 kb and 2.2 kb, and a minor hybridizing species at 1.7 kb.

Figure I-2B: Northern Blot of Ornithine Decarboxylase Distal Small Intestine

Northern blot analysis (representative of n=3) of ODC mRNA abundance in total RNA isolated from rat distal small intestine during development. 20 μg of total RNA from time points corresponding to postnatal day 3, 7, 10, 14, 21, 28, 35, and adult were fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled ODC cDNA probe for 16 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb respectively. The ODC cDNA probe recognizes transcripts of 2.7 kb, 2.2 kb, and 1.7 kb.

Figure I-2A: Northern blot
Ornithine Decarboxylase in the Proximal Small Intestine

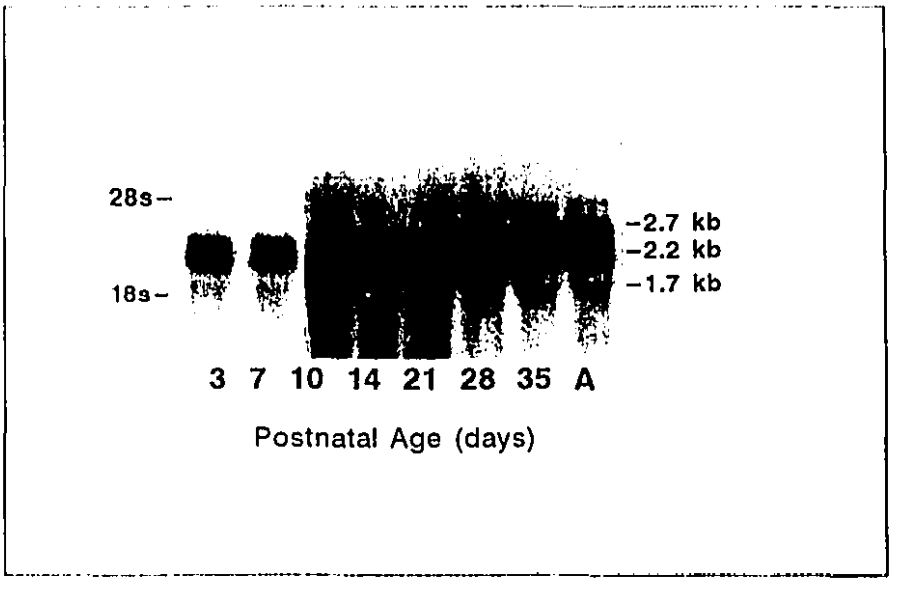


Figure I-2B: Northern Blot
Ornithine Decarboxylase in the Distal Small Intestine

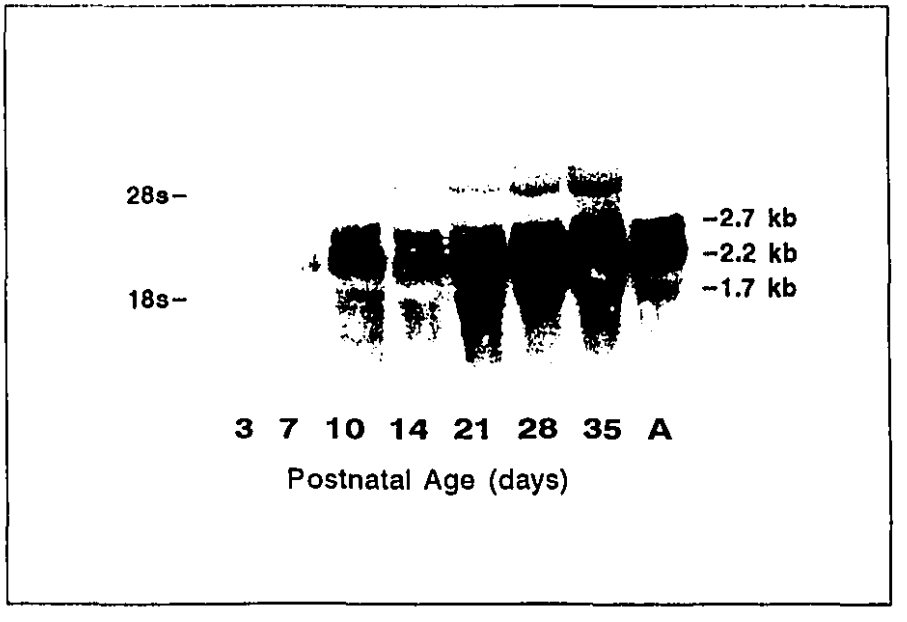


Table I-3: Effect of Age on RNA and Microvillus Membrane(MVM) Protein Abundance of the Sodium-dependant Glucose Transporter(SGLT1) and Sucrase-Isomaltase(SI) in Rat Small Intestine

Autoradiogram Density (% of Adult Reference)	Postnatal Age (days) 3	7	10	14	21	28	35	Adult	One-way ANOVA F	p
SGLT1										
mRNA										
Proximal	49.9 ± 10.4 c	55.6 ± 3.4 bc	60.4 ± 11.1 bc	74.5 ± 19.2 b	78.1 ± 16.3 b	76.9 ± 4.0 b	69.9 ± 3.8 b	100.0 a	6.13	0.0013
Distal	5.0 ± 2.9 d**	13.0 ± 4.5 d**	28.5 ± 12.7 c*	42.0 ± 18.3 c	62.0 ± 6.7 b	96.3 ± 13.2 a	103.1 ± 8.1 a**	100.0 a	58.02	0.0001
MVM Protein										
Proximal	57.0 ± 17.4 cd	36.7 ± 11.8 d	40.7 ± 13.6 d	88.3 ± 15.9 ab	90.3 ± 4.7 ab	100.3 ± 19.3 a	67.0 ± 7.2 c	100.0 a	12.00	0.0001
Distal	38.7 ± 9.2 b	35.0 ± 7.8 b	31.3 ± 5.8 b	33.0 ± 12.5 b*	103.0 ± 23.4 a	95.3 ± 3.1 a	NA	100.0 a	29.55	0.0001
SI										
mRNA										
Proximal	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	25.0 ± 6.2 c	27.7 ± 6.5 c	51.3 ± 15.8 b	100.0 a	91.21	0.0001
Distal	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	12.7 ± 2.0 cd	23.9 ± 6.9 c	74.7 ± 29.0 b	100.0 a	40.86	0.0001
MVM Protein										
Proximal	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	50.3 ± 18.8 c	80.0 ± 13.9 b	70.3 ± 11.1 b	100.0 a	63.93	0.0001
Distal	8.5 ± 4.1 d	10.1 ± 5.2 d	10.6 ± 4.2 d	12.5 ± 4.9 d*	110.0 ± 4.4 a*	97.3 ± 6.5 b	47.0 ± 8.2 c*	100.0 b	236.20	0.0001

NA, not available.
Values are means ± SEM of 3-4 samples.
Within a row, means with the same letter are not significantly different from each other (p>0.05, one-way ANOVA).
* p<0.05, distal versus proximal (Student's t-test).
** p<0.01, distal versus proximal (Student's t-test).

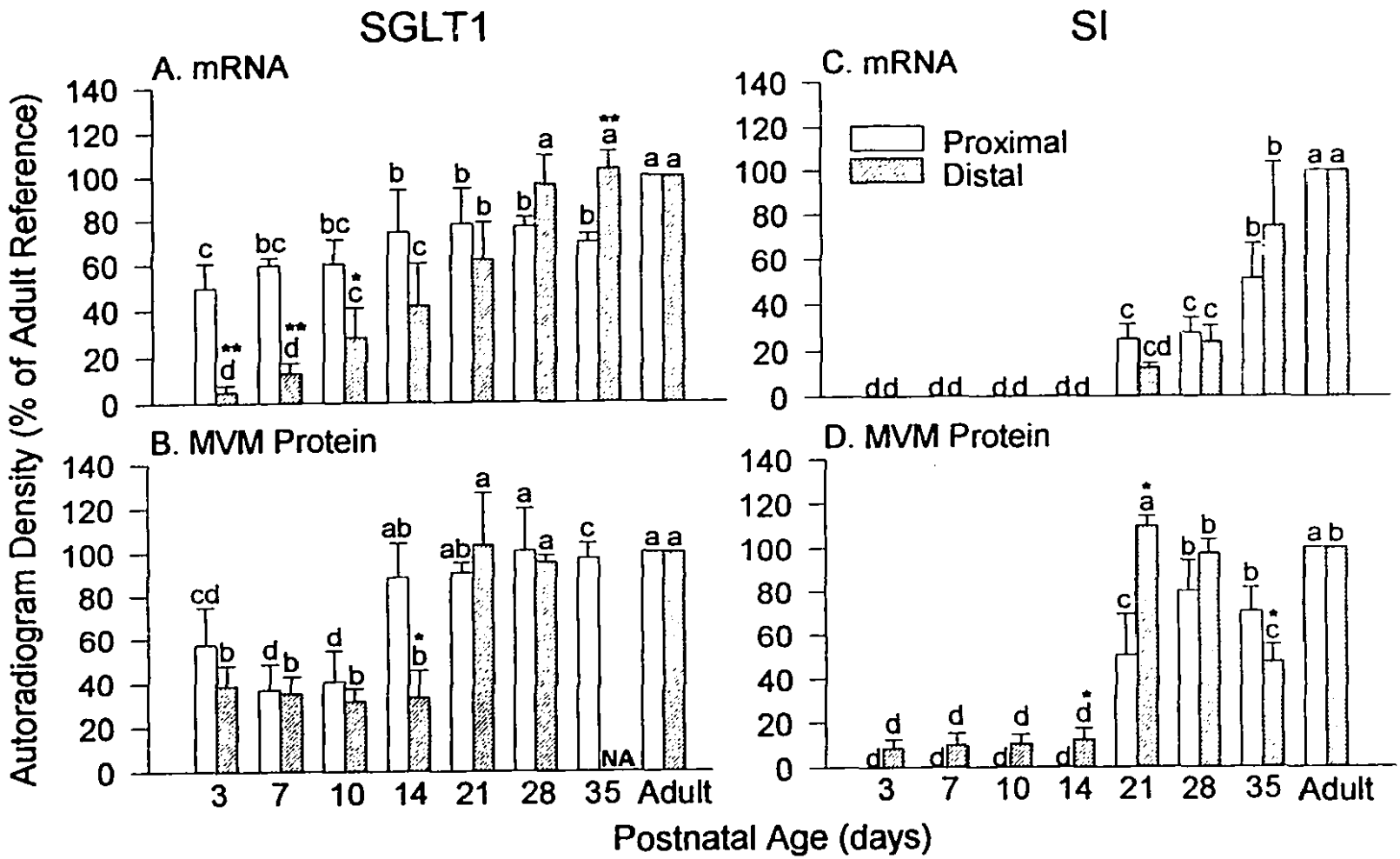


Figure I-3. Effect of age on mRNA and microvillus membrane(MVM) protein abundance of the Na^+-dependent glucose transporter(SGLT1) and sucrase-isomaltase(SI) in rat small intestine. Within each site (proximal or distal), bars with the same letters are not significantly different from each other ($p>0.05$, one-way ANOVA). Distal versus proximal comparisons were done using the Student's t-test where * $p<0.05$ and ** $p<0.01$. NA indicates data not available.

Figure I-3A1: Northern Blot of SGLT1 Proximal Small Intestine

Northern blot analysis (representative of n=3) of SGLT1 abundance in total RNA isolated from rat proximal small intestine during development. 20 μg of total RNA from time points corresponding to postnatal day 3, 7, 10, 14, 21, 28, 35, and adult were fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled SGLT1 cDNA probe for 12 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb respectively. The SGLT1 cDNA probe detects a signal at 4.8 kb.

Figure I-3B1: Western Blot of SGLT1 Proximal Small Intestine

Western blot analysis (representative of n=3) of SGLT1 expression in MVM protein fractions from rat proximal small intestine during development. 80 μg of MVM protein from time points corresponding to day 3, 7, 10, 14, 21, 28, 35, and adult was separated on a 7.5% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE). Adult MVM from colon (80 μg) was run as a negative reference protein. Proteins were electrotransferred to a nitrocellulose membrane for 100 minutes at 4 °C. Western blots were incubated with the polyclonal anti-SGLT1 1° antibody for 2 hours at room temperature with agitation. Immunodetection was carried out following a standard protocol using a [^{125}I]-goat anti-rabbit 2° antibody, and an autoradiograph obtained. The polyclonal anti-SGLT1 antibody detects the mature SGLT1 protein at approximately 84 kilodaltons (kDa).

Figure I-3A1: Northern Blot
SGLT1 in the Proximal Small Intestine

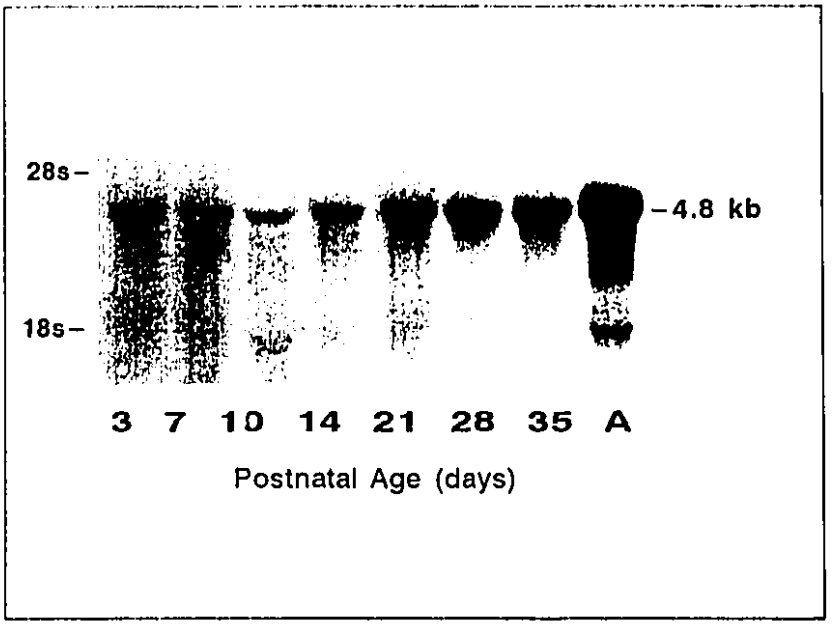


Figure I-3B1: Western Blot
SGLT1 in the Proximal Small Intestine

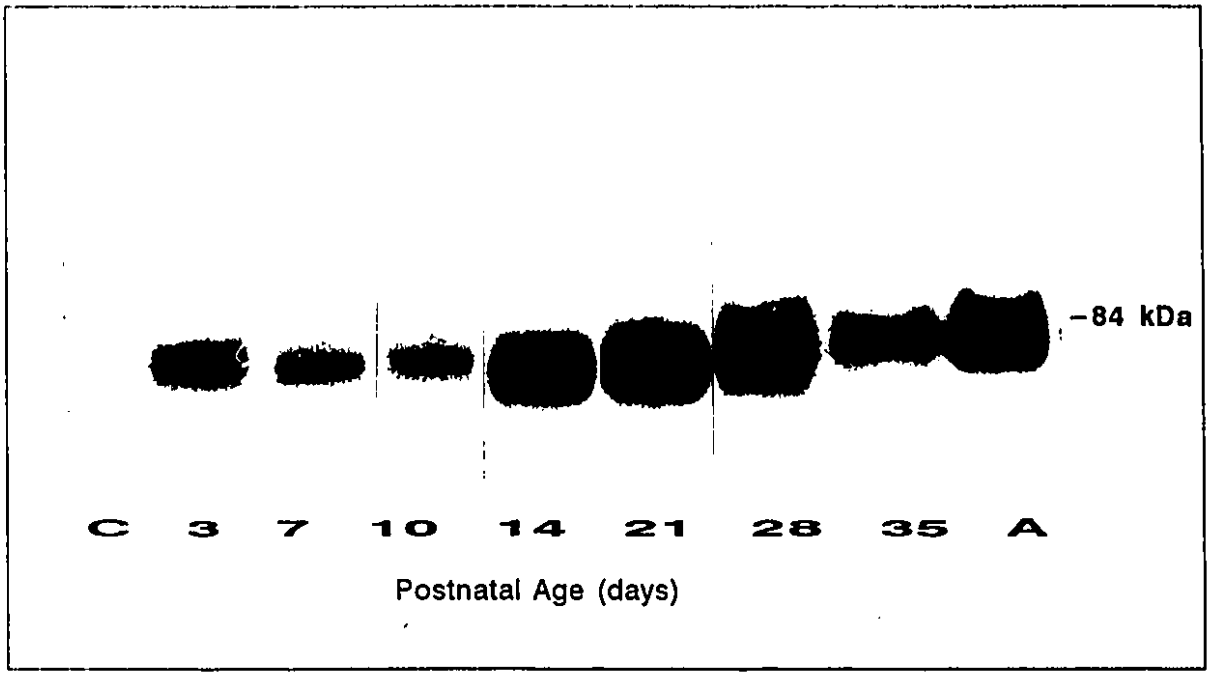


Figure I-3A2: Northern Blot of SGLT1 Distal Small Intestine

Northern blot analysis (representative of n=3) of SGLT1 abundance in total RNA isolated from rat distal small intestine during development. 20 μg of total RNA from time points corresponding to postnatal day 3, 7, 10, 14, 21, 28, 35, and adult were fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled SGLT1 cDNA probe for 12 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb respectively. The SGLT1 cDNA probe detects a signal at 4.8 kb.

Figure I-3B2: Western Blot of SGLT1 Distal Small Intestine

Western blot analysis (representative of n=3) of SGLT1 expression in MVM protein fractions from rat distal small intestine during development. 80 μg of MVM protein from time points corresponding to day 3, 7, 14, 21, 28, and adult was separated on a 7.5% gel by SDS-PAGE. Adult MVM from colon (80 μg) was run as a negative reference protein. Proteins were electrotransferred to a nitrocellulose membrane for 100 minutes at 4 °C. Western blots were incubated with the polyclonal anti-SGLT1 1° antibody for 2 hours at room temperature with agitation. Immunodetection was carried out following a standard protocol using a [^{125}I]-goat anti-rabbit 2° antibody, and an autoradiograph obtained. The polyclonal anti-SGLT1 antibody detects the mature SGLT1 protein at approximately 84 kD.

Figure I-3A2: Northern Blot
SGLT1 in the Distal Small Intestine

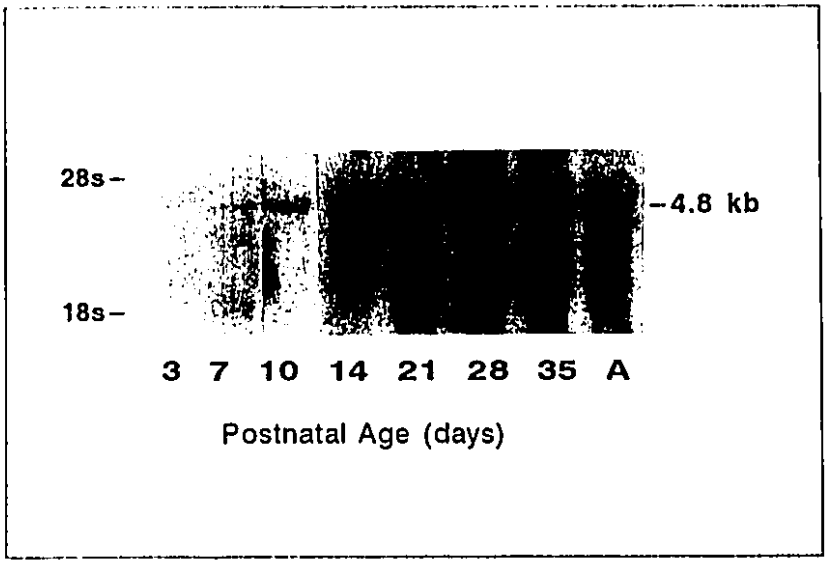


Figure I-3B2: Western Blot
SGLT1 in the Distal Small Intestine

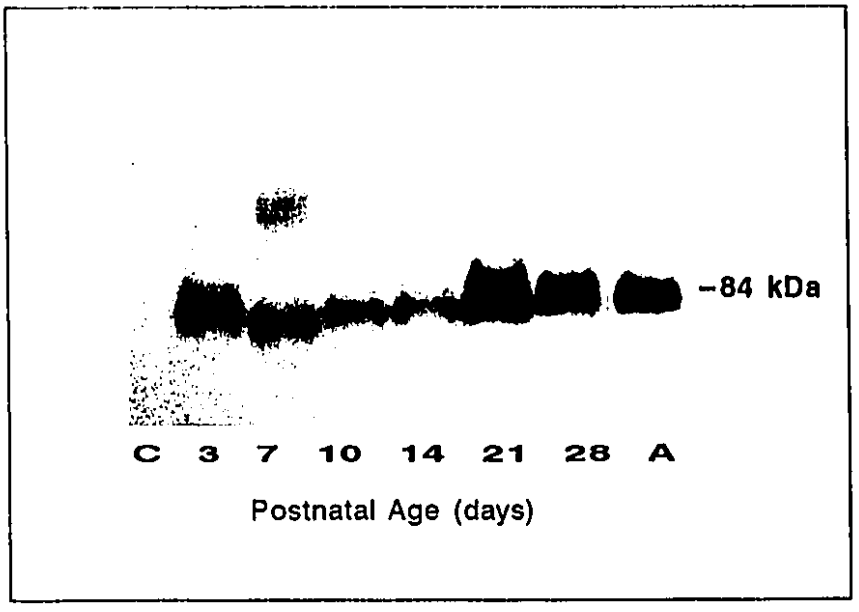


Figure I-3C1: Northern Blot of Sucrase-Isomaltase Proximal Small Intestine

Northern blot analysis (representative of n=3) of SI mRNA abundance in total RNA isolated from rat proximal small intestine during development. 20 μg of total RNA from time points corresponding to postnatal day 3, 7, 10, 14, 21, 28, 35, and adult were fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled SI cDNA probe for 12 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb respectively. The SI cDNA probe detects a signal at approximately 6.0 kb.

Figure I-3D1: Western Blot of Sucrase-Isomaltase Proximal Small Intestine

Western blot analysis (representative of n=3) of SI expression in MVM protein fractions isolated from proximal small intestine during development. 80 μg of MVM protein from time points corresponding to day 3, 7, 10, 14, 21, 28, 35, and adult was separated on a 7.5% gel by SDS-PAGE. Proteins were electrotransferred to a nitrocellulose membrane for 120 minutes at 4 °C. Western blots were then incubated with the polyclonal anti-sucrase-isomaltase 1° antibody for 2 hours at room temperature with agitation. Immunodetection was carried out following a standard protocol using [^{125}I]-goat anti-rabbit 2° antibody, and an autoradiograph obtained. The polyclonal antibody directed against SI detects sucrase at approximately 116-120 kD, isomaltase at 140 kD, an incompletely glycosylated sucrase-isomaltase complex at approximately 194 kD, and a high mannose (completely glycosylated) complex at 210 kD.

Figure I-3C1: Northern Blot
Sucrase-Isomaltase in the Proximal Small Intestine

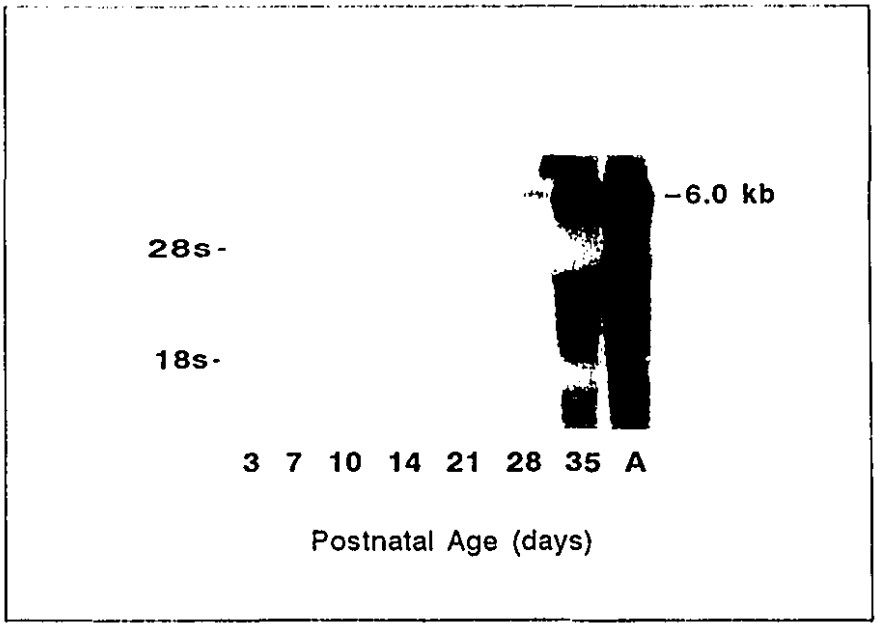


Figure I-3D1: Western Blot
Sucrase-Isomaltase in the Proximal Small Intestine

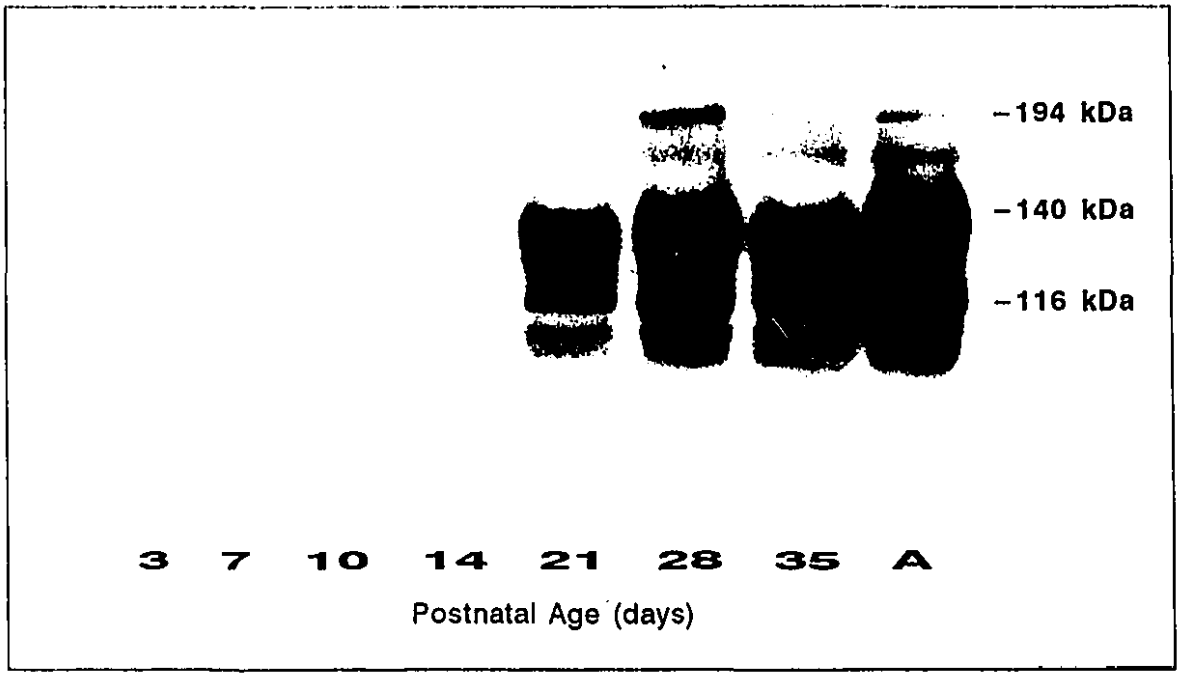


Figure I-3C2: Northern Blot of Sucrase-Isomaltase Distal Small Intestine

Northern blot analysis (representative of n=3) of SI mRNA abundance in total RNA isolated from rat distal small intestine during development. 20 μg of total RNA from time points corresponding to postnatal day 3, 7, 10, 14, 21, 28, 35, and adult were fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled SI cDNA probe for 12 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb respectively. The SI cDNA probe detects a signal at approximately 6.0 kb.

Figure I-3D2: Western Blot of Sucrase-Isomaltase Distal Small Intestine

Western blot analysis (representative of n=3) of SI protein expression in MVM protein fractions isolated from distal small intestine during development. 80 μg of MVM protein from time points corresponding to day 3, 7, 10, 14, 21, 28, 35, and adult was separated on a 7.5% gel by SDS-PAGE. Proteins were electrotransferred to a nitrocellulose membrane for 120 minutes at 4 °C. Western blots were then incubated with the polyclonal anti-SI 1° antibody for 2 hours at room temperature with agitation. Immunodetection was carried out following a standard protocol using [^{125}I]-goat anti-rabbit 2° antibody, and an autoradiograph obtained. The polyclonal antibody directed against SI detects sucrase at approximately 116-120 kD, isomaltase at 140 kD, an incompletely glycosylated sucrase-isomaltase complex at approximately 194 kD, and a high mannose (completely glycosylated) complex at 210 kD.

Figure I-3C2: Northern Blot
Sucrase-Isomaltase in the Distal Small Intestine

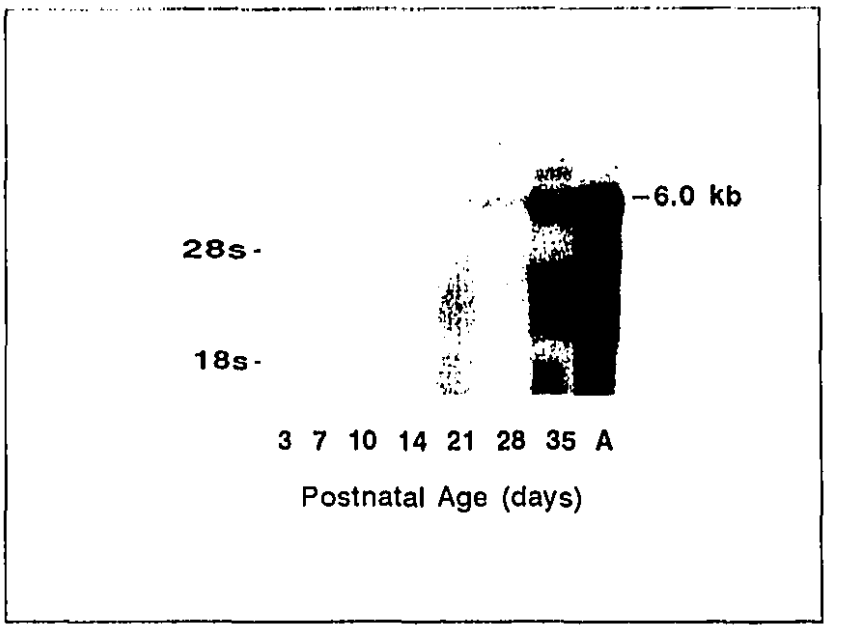


Figure I-3D2: Western Blot
Sucrase-Isomaltase in the Distal Small Intestine

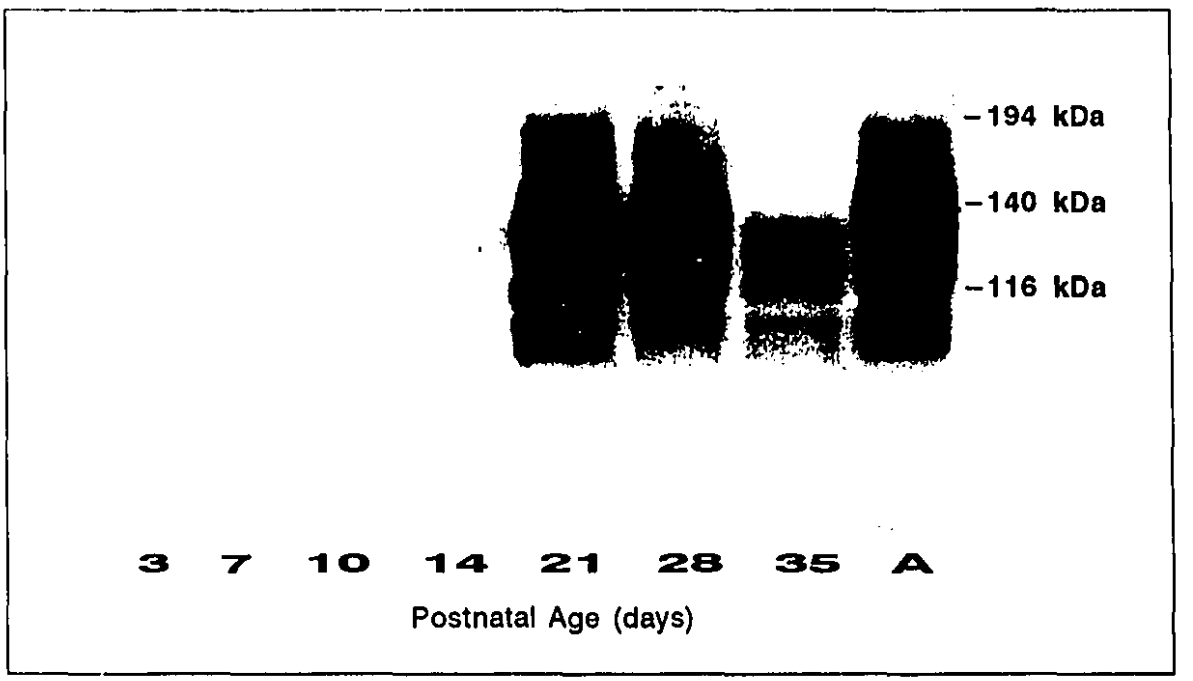


Table I-4: Effect of Age on mRNA and Basolateral Membrane(BLM) Protein Abundance of NaK-ATPase α1 and β1 Subunits in Rat Small Intestine

Autoradiogram Density (% of Adult Reference)	Postnatal Age (days)							Adult Reference		One-way ANOVA	
	3	7	10	14	21	28	35			F	p
NaK-ATPase α1											
mRNA											
Proximal	22.6 ± 3.7 e	41.0 ± 8.9 de	38.3 ± 6.5 de	39.6 ± 11.1 de	48.2 ± 12.5 cd	62.9 ± 16.7 bc	83.0 ± 18.3 ab	100.0	a	14.53	0.0001
Distal	19.0 ± 6.2 d	20.3 ± 3.5 d*	40.8 ± 7.1 c	43.4 ± 6.3 c	44.0 ± 9.7 c	109.3 ± 5.7 b*	128.9 ± 13.3 a*	100.0	b	90.46	0.0001
BLM Protein											
Proximal	44.7 ± 15.3 d	73.6 ± 7.9 c	89.8 ± 6.9 bc	75.7 ± 4.1 bc	101.9 ± 20.0 bc	108.3 ± 19.2 ab	129.7 ± 7.4 a	100.0	ab	8.71	0.0002
Distal	55.4 ± 5.9 d	61.8 ± 7.4 d	55.9 ± 10.4 d*	98.0 ± 9.7 c*	125.9 ± 4.0 b*	132.1 ± 7.8 b	140.1 ± 11.6 a	100.0	c	53.36	0.0001
NaK-ATPase β1											
mRNA											
Proximal	11.8 ± 5.8 e	26.7 ± 11.3 de	31.7 ± 12.9 d	56.5 ± 13.9 c	57.5 ± 9.7 c	82.5 ± 13.9 b	83.6 ± 8.4 b	100.0	a	35.22	0.0001
Distal	30.1 ± 6.3 f**	41.7 ± 11.7 ef	50.6 ± 9.3 e	77.5 ± 13.9 d	90.2 ± 7.1 cd**	115.5 ± 18.1 b*	133.4 ± 14.5 a**	100.0	bc	41.12	0.0001
BLM Protein											
Proximal	12.0 ± 3.1 e	17.1 ± 3.5 e	23.7 ± 4.0 e	23.2 ± 2.9 e	41.0 ± 4.1 d	78.1 ± 10.7 c	121.2 ± 14.9 a	100.0	b	107.59	0.0001
Distal	37.4 ± 11.5 d	21.4 ± 4.7 d	42.9 ± 5.9 d*	42.8 ± 5.2 d*	98.2 ± 22.4 c*	121.7 ± 21.4 ab	136.2 ± 12.7 a	100.0	bc	34.45	0.0001

Values are means ± SEM of 3 samples.
Within a row, means with the same letter are not significantly different from each other ($p>0.05$, one-way ANOVA).
* $p<0.05$, distal versus proximal (Student's t-test).
** $p<0.01$, distal versus proximal (Student's t-test).

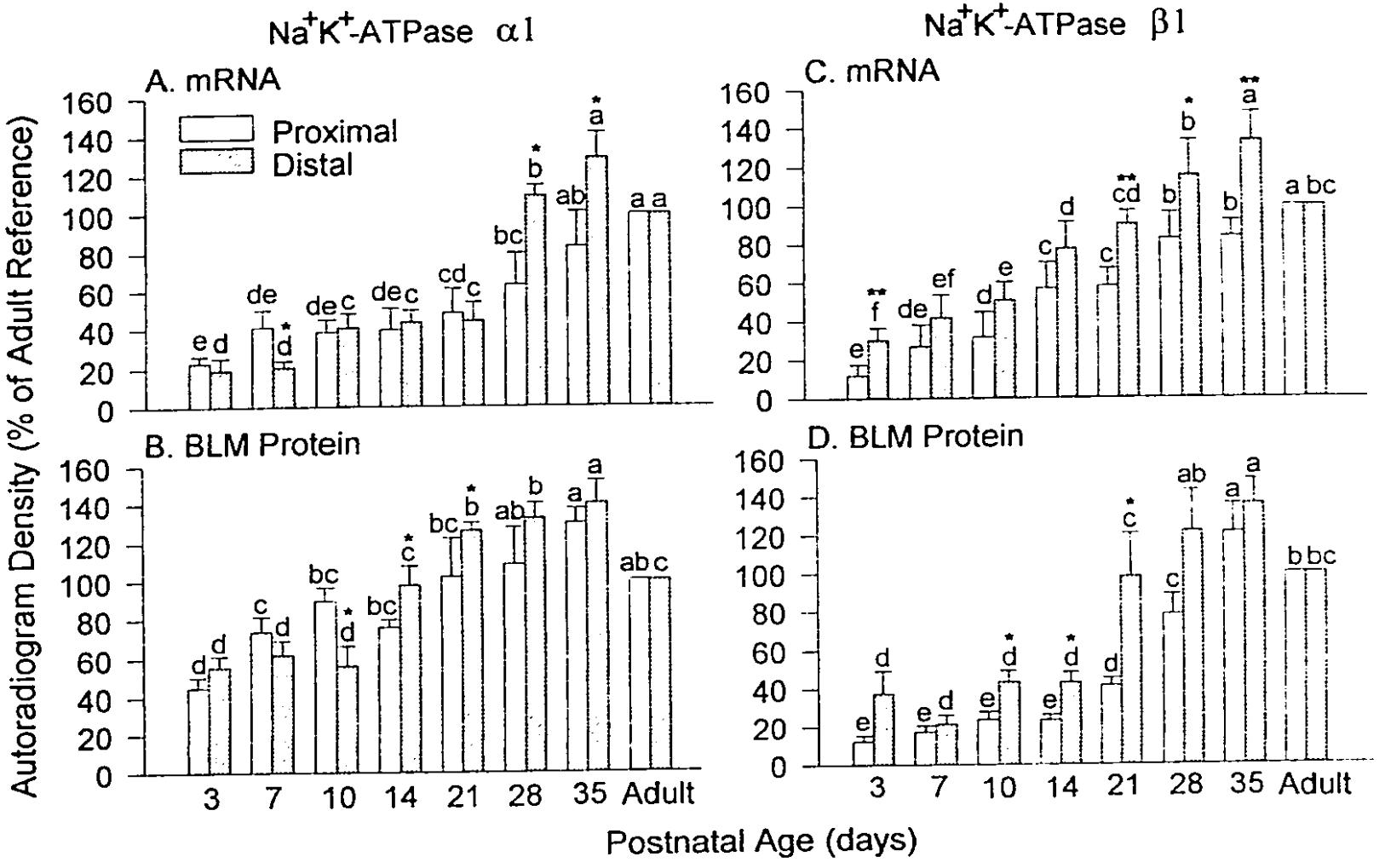


Figure I-4. Effect of age on mRNA and basolateral membrane(BLM) protein abundance of the Na·K·-ATPase α1 and β1 subunits in rat small intestine. Within each site (proximal or distal), bars with the same letters are not significantly different from each other($p>0.05$, one-way ANOVA). Distal versus proximal comparisons were done using the Student's t-test where * $p<0.05$ and ** $p<0.01$.

Figure I-4A1: Northern Blot of the Na^+K^+ ATPase α_1 isoform Proximal Small Intestine

Northern blot analysis (representative of n=3) of Na^+K^+ ATPase α_1 isoform mRNA abundance in total RNA isolated from rat proximal small intestine during development. 20 μg of total RNA from time points corresponding to postnatal day 3, 7, 10, 21, 28, 35, and adult were fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled Na^+K^+ ATPase α_1 isoform cDNA probe for 12 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb respectively. The Na^+K^+ ATPase α_1 isoform cDNA probe detects a signal at 3.7 kb.

Figure I-4B1: Western Blot of the Na^+K^+ ATPase α_1 isoform Proximal Small Intestine

Western blot analysis (representative of n=3) of Na^+K^+ ATPase α_1 isoform expression in BLM protein fractions from rat proximal small intestine during development. 80 μg of BLM protein from time points corresponding to day 3, 7, 10, 14, 21, 28, 35, and adult was separated on a 7.5% gel by SDS-PAGE. Proteins were electrotransferred to a nitrocellulose membrane for 100 minutes at 4 °C. Western blots were incubated with the polyclonal anti-Na^+K^+ ATPase α_1 isoform 1° antibody for 2 hours at room temperature with agitation. Immunodetection was carried out following a standard protocol using a [^{125}I]-goat anti-rabbit 2° antibody, and an autoradiograph obtained. The Na^+K^+ ATPase α_1 fusion protein detects the mature Na^+K^+ ATPase α_1 isoform at approximately 112 kD.

Figure I-4A1: Northern blot
NaK ATPase α1 Isoform in the Proximal Small Intestine

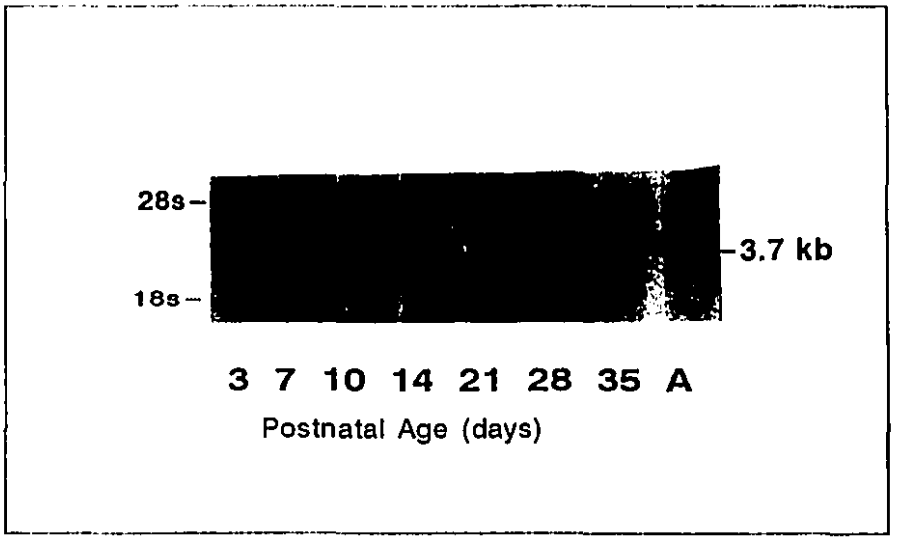


Figure I-4B1: Western blot
NaK ATPase α1 Isoform in the Proximal Small Intestine

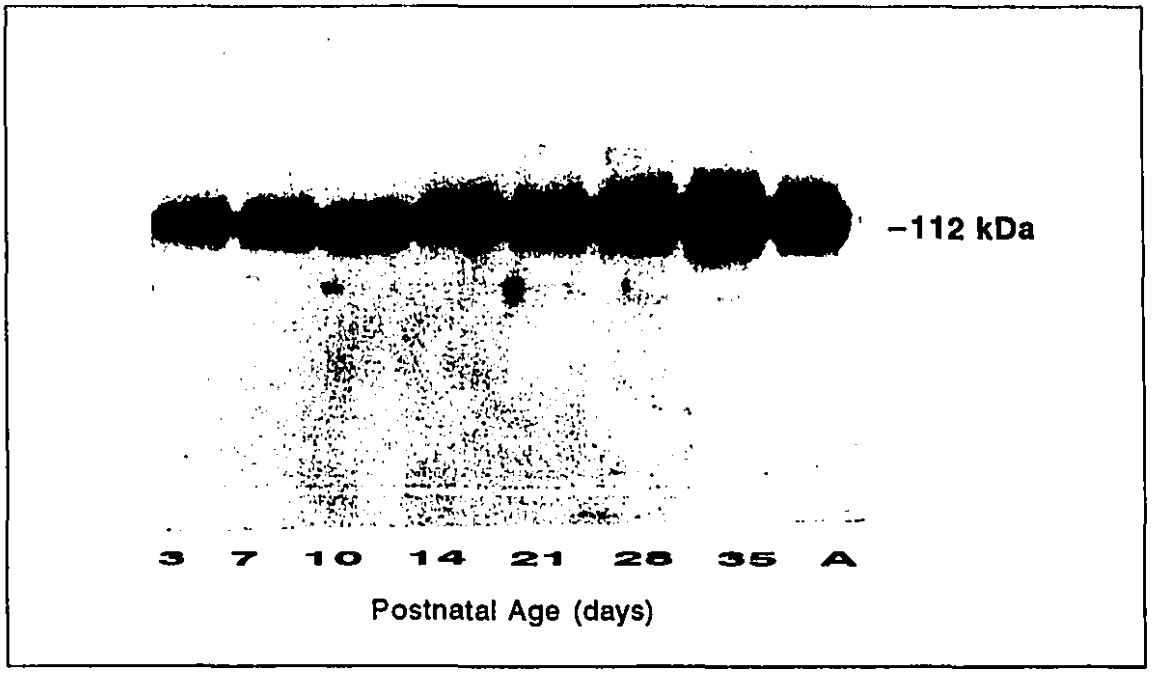


Figure I-4A2: Northern Blot of the Na^+K^+ ATPase α_1 isoform Distal Small Intestine

Northern blot analysis (representative of n=3) of Na^+K^+ ATPase α_1 isoform mRNA abundance in total RNA isolated from rat distal small intestine during development. 20 μg of total RNA from time points corresponding to postnatal day 3, 7, 10, 14, 21, 28, 35, and adult were fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled Na^+K^+ ATPase α_1 isoform cDNA probe for 12 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb respectively. The Na^+K^+ ATPase α_1 isoform cDNA probe detects a signal at 3.7 kb.

Figure I-4B2: Western Blot of the Na^+K^+ ATPase α_1 isoform Distal Small Intestine

Western blot analysis (representative of n=3) of Na^+K^+ ATPase α_1 isoform expression in BLM protein fractions from rat distal small intestine during development. 80 μg of BLM protein from time points corresponding to day 3, 7, 10, 14, 21, 28, 35, and adult was separated on a 7.5% gel by SDS-PAGE. Proteins were electrotransferred to a nitrocellulose membrane for 100 minutes at 4 °C. Western blots were incubated with the polyclonal anti-Na^+K^+ ATPase α_1 isoform 1° antibody for 2 hours at room temperature with agitation. Immunodetection was carried out following a standard protocol using a [^{125}I]-goat anti-rabbit 2° antibody, and an autoradiograph obtained. The Na^+K^+ ATPase α_1 fusion protein detects the mature Na^+K^+ ATPase α_1 isoform at approximately 112 kD.

Figure I-4A2: Northern blot
NaK ATPase œ1 Isoform in the Distal Small Intestine

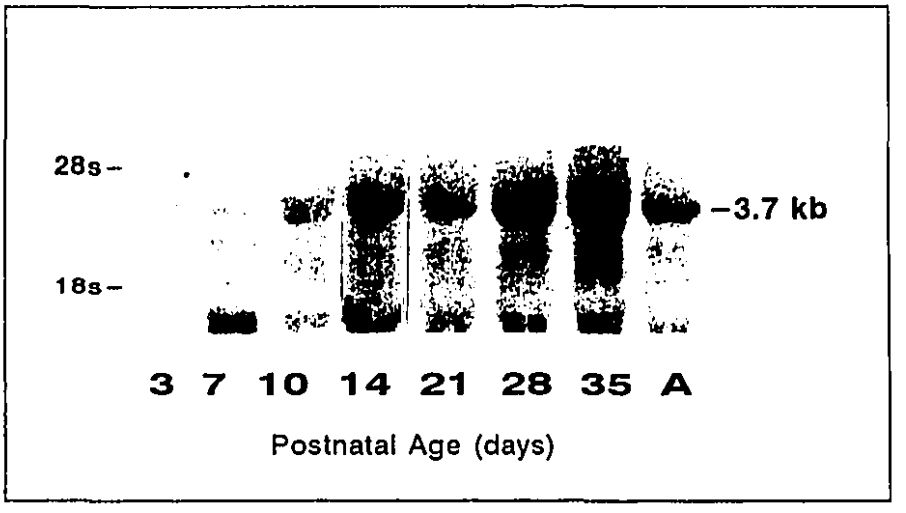


Figure I-4B2: Western blot
NaK ATPase œ1 Isoform in the Distal Small Intestine

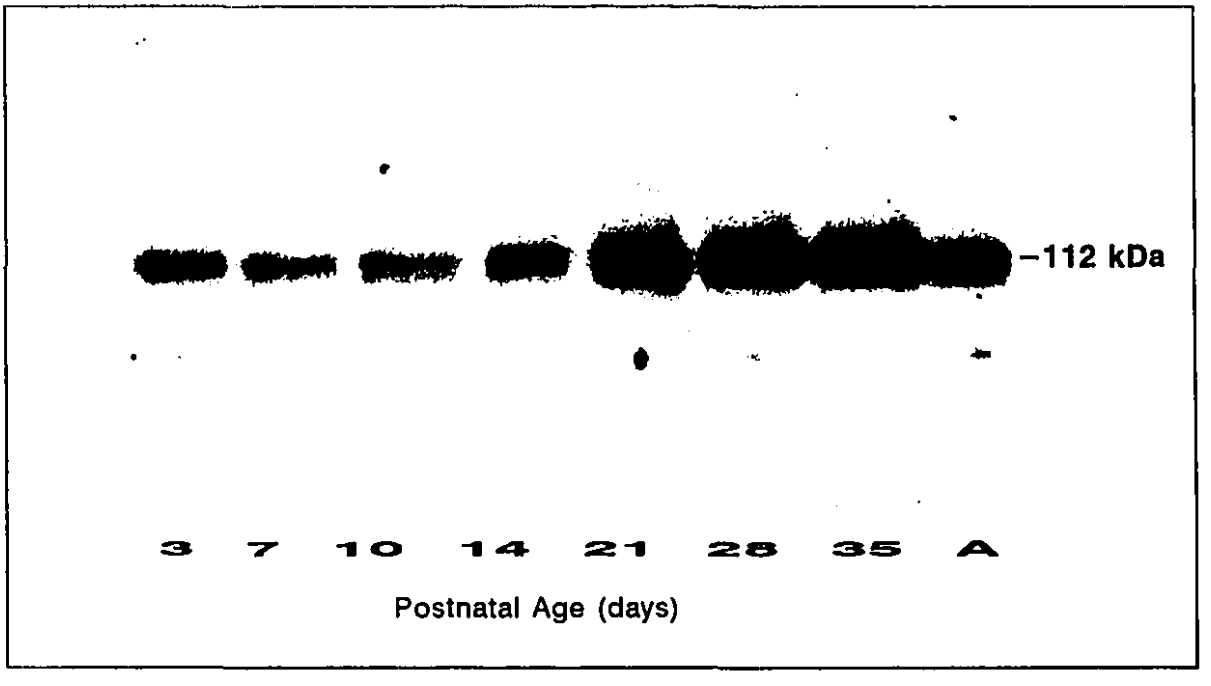


Figure I-4C1: Northern Blot of the Na^+K^+ ATPase $ß_1$ isoform Proximal Small Intestine

Northern blot analysis (representative of n=3) of Na^+K^+ ATPase $ß_1$ isoform mRNA abundance in total RNA isolated from rat proximal small intestine during development. 20 μg of total RNA from time points corresponding to postnatal day 3, 7, 10, 14, 21, 28, 35, and adult were fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled Na^+K^+ ATPase $ß_1$ subunit cDNA probe for 12 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb respectively. The Na^+K^+ ATPase $ß_1$ isoform cDNA probe detects a signal at 2.7 kb in rat small intestine.

Figure I-4D1: Western Blot of the Na^+K^+ ATPase $ß_1$ isoform Proximal Small Intestine

Western blot analysis (representative of n=3) of Na^+K^+ ATPase $ß_1$ subunit isoform expression in BLM protein fractions from rat proximal small intestine during development. 80 μg of BLM protein from time points corresponding to day 3, 7, 10, 14, 21, 28, 35, and adult was separated on a 7.5% gel by SDS-PAGE. Proteins were electrotransferred to a nitrocellulose membrane for 100 minutes at 4 °C. Western blots were incubated with the polyclonal anti-Na^+K^+ ATPase $ß_1$ subunit 1° antibody for 2 hours at room temperature with agitation. Immunodetection was carried out following a standard protocol using a [^{125}I]-goat anti-rabbit 2° antibody, and an autoradiograph obtained. The Na^+K^+ ATPase $ß_1$ fusion protein detects the mature Na^+K^+ ATPase $ß_1$ isoform at approximately 50-55 kD.

Figure I-4C1: Northern Blot
NaK ATPase ß1 Isoform in the Proximal Small Intestine

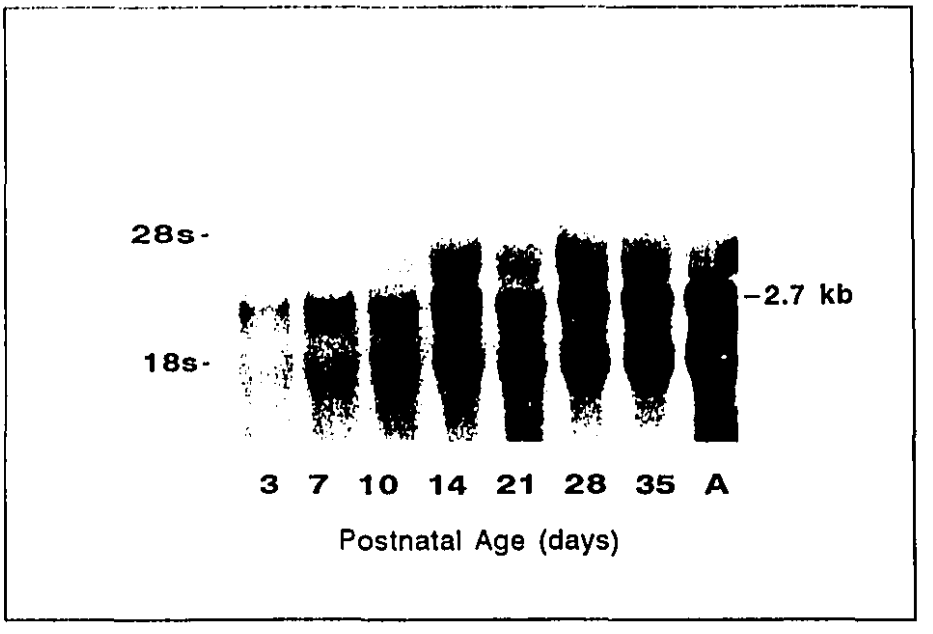


Figure I-4D1: Western Blot
NaK ATPase ß1 Isoform in the Proximal Small Intestine

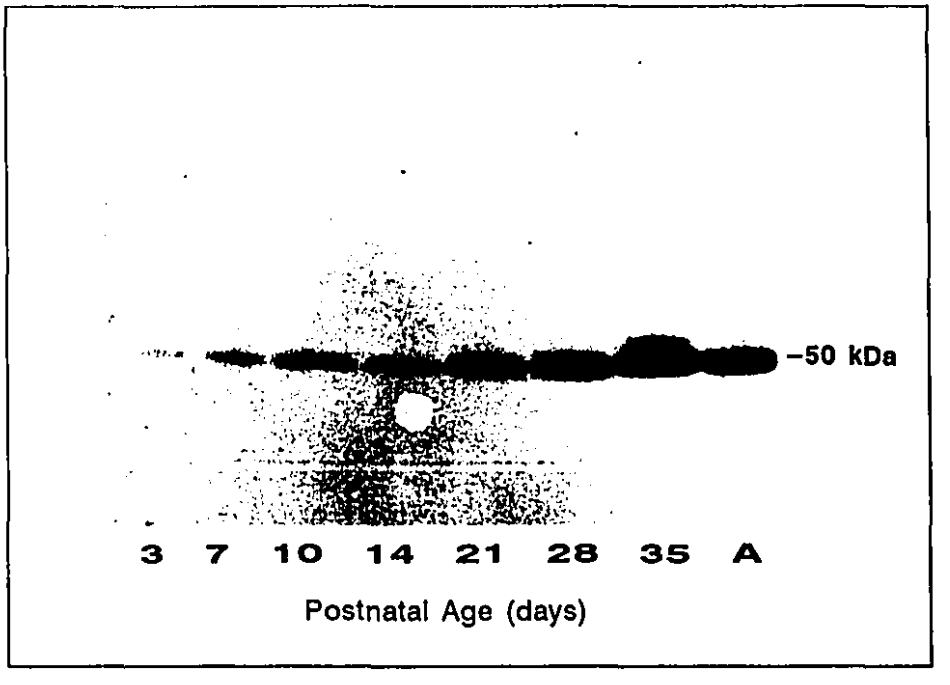


Figure I-4C2: Northern Blot of the Na^+K^+ ATPase $ß_1$ isoform Distal Small Intestine

Northern blot analysis (representative of n=3) of Na^+K^+ ATPase $ß_1$ isoform mRNA abundance in total RNA isolated from rat distal small intestine during development. 20 μg of total RNA from time points corresponding to postnatal day 3, 7, 10, 14, 21, 28, 35, and adult were fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled Na^+K^+ ATPase $ß_1$ subunit cDNA probe for 12 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb respectively. The Na^+K^+ ATPase $ß_1$ isoform cDNA probe detects a signal at 2.7 kb in rat small intestine.

Figure I-4D2: Western Blot of the Na^+K^+ ATPase $ß_1$ isoform Distal Small Intestine

Western blot analysis (representative of n=3) of Na^+K^+ ATPase $ß_1$ subunit isoform expression in BLM protein fractions from rat distal small intestine during development. 80 μg of BLM protein from time points corresponding to day 3, 7, 10, 14, 21, 28, 35, and adult was separated on a 7.5% gel by SDS-PAGE. Proteins were electrotransferred to a nitrocellulose membrane for 100 minutes at 4 °C. Western blots were incubated with the polyclonal anti-Na^+K^+ ATPase $ß_1$ subunit 1^o antibody for 2 hours at room temperature with agitation. Immunodetection was carried out following a standard protocol using a $[^{125}I]$-goat anti-rabbit 2^o antibody, and an autoradiograph obtained. The Na^+K^+ ATPase $ß_1$ fusion protein detects the mature Na^+K^+ ATPase $ß_1$ isoform at approximately 50-55 kD.

Figure I-4C2: Northern Blot
NaK ATPase ß1 Isoform in the Distal Small Intestine

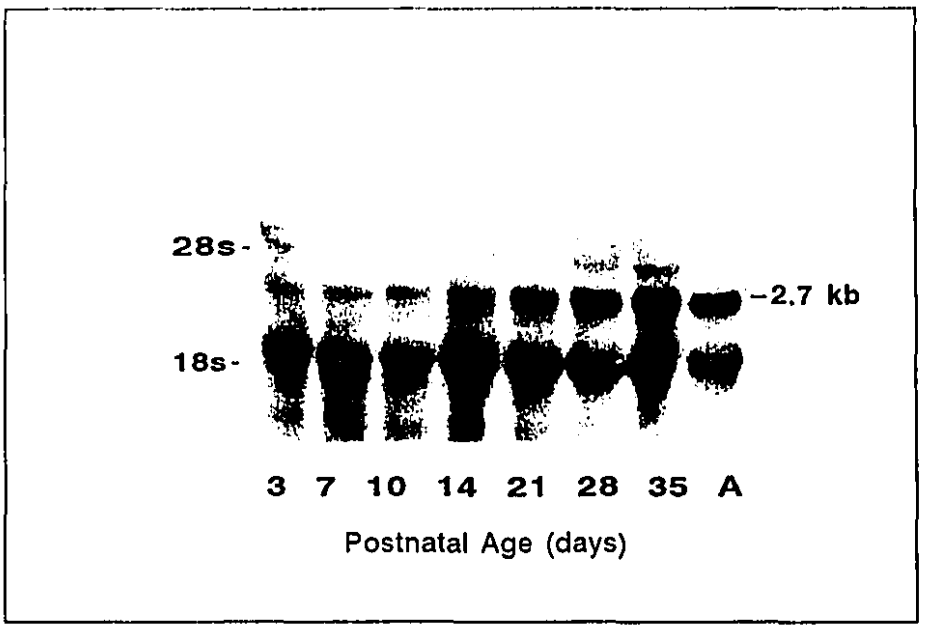
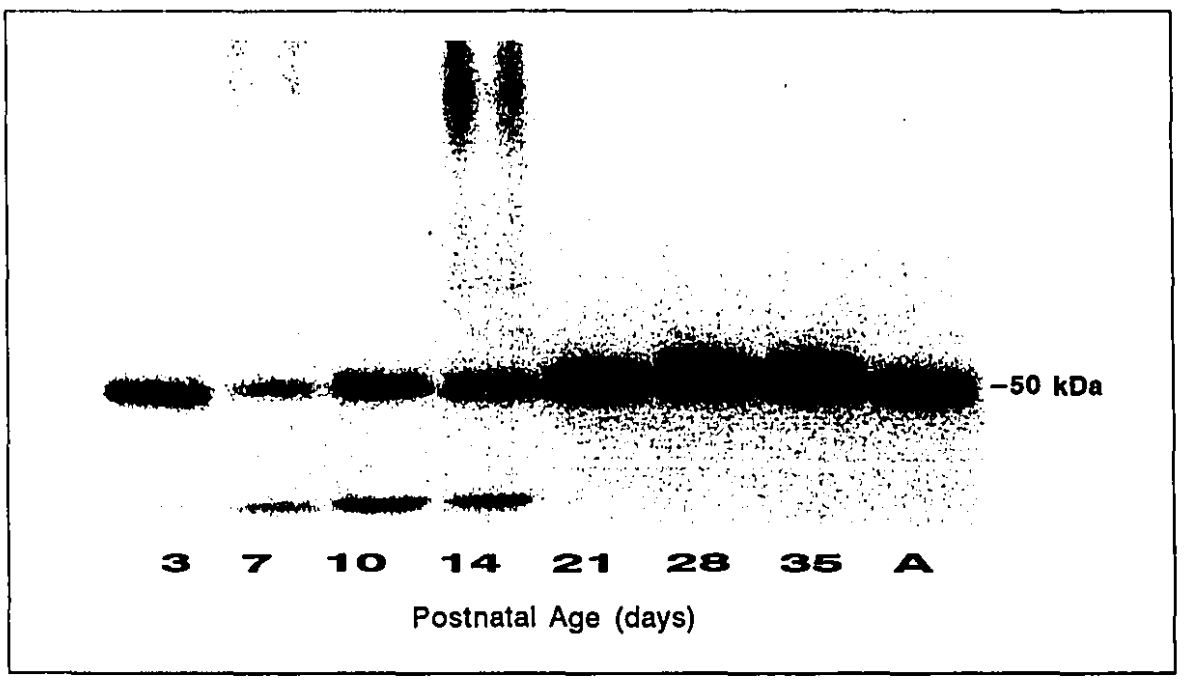


Figure I-4D2: Western Blot
NaK ATPase ß1 Isoform in the Distal Small Intestine



EXPERIMENT II: PRECOCIOUS DEVELOPMENT STUDY WITH WASHOUT

The oral administration of spermidine or spermine has been shown to precociously induce an adult pattern of morphology and enzyme expression in suckling rats [see literature review on the polyamines]. These precocious effects appear to be reversible following the termination of exogenous polyamine exposure [see review on polyamines]. These effects have not been examined at the levels of mRNA abundance or protein expression.

Experiment I reported on the developmental profiles for SGLT1, SI, and Na^+K^+ ATPase α_1 and $ß_1$ isoform mRNA abundance and protein expression, as well as ODC, for which only mRNA was examined, in both the proximal and distal small intestine of the postnatal rat. In view of the findings reported in Experiment I, Experiment II sought to examine whether exposure to spermidine prior to the onset of weaning could precociously induce developmental increases in mRNA abundance and/or protein expression for ODC, SGLT1, SI, and the Na^+K^+ ATPase α_1 and $ß_1$ subunit isoforms. In addition, the effects of exogenous spermidine on body growth, intestinal growth, serum cortisol, yields of total RNA, and yields of membrane protein (MVM and BLM) were examined.

Effect of Spermidine Exposure on Body Growth

The polyamines are essential for cell growth and may promote anabolic events [see review on polyamines]. These beneficial effects are dependant on intracellular polyamine concentrations which are known to be closely regulated according to growth requirements [see review on polyamines]. The exposure to toxic concentrations of polyamines may have negative effects on body growth. In Experiment II both body weight and total intestinal weight were measured to assess the effects of spermidine (8 μmol/day) on body growth. These results are presented in Table II-1 (pg. 115).

In this study all rats remained healthy, however

significant treatment-dependent ($P<0.0132$) effects on animal body weight were observed (Table II-1). At postnatal day 10 there were no significant differences in body weight between rats receiving consecutive exposures to spermidine and those receiving the placebo. With the withdrawal of oral spermidine however, the body weight of day 13 rats was significantly decreased compared to age-matched controls. These results indicated that while consecutive exposures to spermidine (8 μmol/day) did not compromise growth in suckling rats, usual postnatal body growth was not maintained when exogenous spermidine was withdrawn. There were significant age-dependant effects ($P<0.0001$) for body weight since measurements were taken at different postnatal ages (Table II-1). The levels of spermidine provided in this study did not affect total intestinal weight at either postnatal time point, however significant age-dependant differences ($P<0.0001$) were found (Table II-1).

Effect of Spermidine on Serum Cortisol Levels

In the postnatal rat plasma glucocorticoid levels are increased at the onset of weaning and in response to stress [see review on regulatory mechanisms of ontogeny]. These elevations in plasma glucocorticoid concentrations have been associated with the promotion of developmental processes in the small intestine of the postnatal rat [see literature review]. In Experiment II, the effect of spermidine exposure on serum cortisol levels was examined. A summary of these results is presented in Table II-1 (pg. 115).

The serum cortisol levels measured for spermidine-treated rats at both experimental time points ranged from 0.03 to 0.04 μg/dl (0.90 to 1.05 nmol/l) and were within the normal range for postnatal rats between days 10 and 13 (Table II-1). This data suggests that rats were not stressed by the administration of exogenous spermidine at these time points. The levels of serum cortisol measured in rats receiving the

placebo ranged from 0.09 to 0.40 μg/dl (2.59 to 11.04 nmol/l) and were within the normal range limit set for postnatal rats between days 10 and 13. Although within normal limits, the serum cortisol levels measured for the placebo group at day 10 (0.40 μg/dl or 11.04 nmol/l) were higher than those found for the other placebo- and spermidine-treated groups, and may reflect an initial technical error in assay procedures. Owing to the small amounts of serum available from each rat in the placebo group, this assay could not be repeated to re-examine serum cortisol levels. Since the day 10 placebo group showed no precocious induction of the intestinal enzymes or transporter studied when compared to the developmental time frames described in Experiment I, we assumed that these levels were not sufficiently elevated to affect the study comparisons. The significant age-dependant ($P<0.0252$), treatment-dependant ($P<0.0020$), and age * treatment interaction effects ($P<0.0204$) found for serum cortisol in this experiment reflect the significant difference found between day 10 placebo group values and those of the other groups examined (Table II-1).

In summary, these findings indicate that any precocious development following spermidine exposure could not be attributed to elevated levels of serum cortisol.

Effect of Spermidine Exposure on Yields of Total RNA

Total RNA was isolated from both the proximal and distal small intestine of day 10 and day 13 rats in the experimental and placebo groups. A summary of these results is presented in Table II-2 (pg. 116) and Figure II-1A (pg. 117).

In the proximal small intestine spermidine exposure had no significant effect on the yields of total RNA obtained (Table II-2). In the distal small intestine the significant treatment-dependant ($P<0.0458$) and age-dependant ($P<0.0096$) differences found for yields of total RNA reflect the greater yields of total RNA measured for spermidine-treated day 13

rats compared to the other placebo- and spermidine-treated groups (Table II-2).

In summary, these results indicate that consecutive exposures to oral spermidine did not alter the yields of total RNA in either the proximal or distal intestinal segments of day 10 rats. However, in the distal small intestine of day 13 rats the withdrawal of spermidine exposure increased the yields of total RNA to the levels reported for weanling rats in Experiment I.

Effect of Spermidine Exposure on MVM Protein Yields

The results summarizing the effect of spermidine exposure on yields of MVM protein isolated from the proximal and distal small intestine are presented in Table II-2 (pg. 116) and Figure II-1B (pg. 117).

There were no significant differences in MVM protein yields in the proximal or distal small intestine for spermidine or placebo groups at either time point examined (Table II-2 and Figure II-1B). In this experiment, the levels of MVM protein measured in both intestinal segments were similar to values reported for the same developmental time frame examined in Experiment I. Significant regional (proximal versus distal) differences were measured in the placebo group at postnatal days 10 ($P<0.01$) and 13 ($P<0.05$) (Figure II-1B).

In summary, these results indicate that exposure to exogenous spermidine did not influence the yields of MVM protein in the proximal or distal small intestine at the developmental time points examined in this experiment.

Effect of Spermidine Exposure on BLM Protein Yields

BLM protein fractions were isolated from both the proximal and distal small intestine of day 10 and day 13 rats in the spermidine- and placebo-treated groups. These results are summarized in Table II-2 (pg. 116) and Figure II-1C (pg. 117).

Spermidine exposure had no significant effect on the BLM

protein yields obtained from the proximal or distal small intestine (Table II-2 and Figure II-1C). Age-dependant increases in the yields of BLM protein occurred between day 10 and day 13 time points in both proximal ($P<0.0012$) and distal ($P<0.0001$) segments (Table II-2). Significant regional (proximal versus distal) differences ($P<0.01$) were measured at postnatal day 10 in both placebo and spermidine treatment groups (Figure II-1C). The yields of BLM protein measured at days 10 and 13 in this experiment were similar to the regional values reported in Experiment I for this developmental time frame.

In summary, these findings indicate that spermidine exposure did not influence yields of BLM protein obtained from either the proximal or distal small intestine at the time points examined in this experiment.

Effect of Spermidine Exposure on Ornithine Decarboxylase

In Experiment II we examined whether spermidine exposure could precociously induce developmental increases in ODC mRNA at postnatal day 10, and whether these effects would persist in the absence of oral spermidine by day 13. These results are summarized in Table II-3 (pg. 118) and Figure II-2 (pg. 119). Figures II-2A and II-2B (pg. 120) show representative Northern blots of ODC in the proximal and distal small intestine, respectively.

In the rodent small intestine the digoxigenin-labelled ODC cDNA probe hybridized to two major bands at 2.6 kb and 2.2 kb, respectively. In the reference adult the ODC probe detected a minor 1.7 kb transcript (Figures II-2A and II-2B).

In the proximal small intestine ODC mRNA abundance was significantly increased (1.6-fold) at postnatal day 10 following consecutive exposures to spermidine compared to the placebo group (Table II-3 and Figure II-2). These increases were not maintained by postnatal day 13 following the withdrawal of oral spermidine. Similar levels of ODC mRNA

were measured at day 10 in control rats and in spermidine-treated rats at day 13. By day 13 the placebo group demonstrated levels of ODC mRNA content that were intermediate to both the spermidine-induced and placebo levels of mRNA measured at postnatal day 10. Significant age * treatment interaction effects ($P<0.0082$) were found for ODC mRNA measured in the proximal small intestine because the precocious induction of ODC mRNA levels by spermidine at postnatal day 10 did not persist following spermidine withdrawal by day 13 (Table II-3).

In the distal small intestine ODC mRNA abundance increased by 1.4-fold from control levels at postnatal day 10 in response to oral spermidine (Table II-3 and Figure II-2). By postnatal day 13 the levels of ODC mRNA dropped to control values in the absence of exposure to spermidine. ODC mRNA content was similar in the day 10 placebo group and by day 13 in both the placebo- and spermidine-treated groups. Both significant age-dependant ($P<0.0086$) and age * treatment interaction effects ($P<0.0173$) were found for ODC mRNA content in the distal small intestine since ODC mRNA was precociously induced by spermidine at day 10, but did not persist following the withdrawal of spermidine by day 13 (Table II-3).

In summary, these findings indicate that orally fed spermidine can induce precocious increases in ODC mRNA abundance in both proximal and distal segments of the immature small intestine. The exposure to exogenous spermidine must be continued however, for these precocious increases to be maintained at these developmental time points.

Effect of Spermidine Exposure on SGLT1

In Experiment II, we examined the effects of spermidine exposure on SGLT1 mRNA abundance and protein expression in both proximal and distal segments of the small intestine prior to weaning. A summary of these results is presented in Table II-4 (pg. 121) and Figure II-3A,B (pg. 122).

mRNA Abundance

Figures II-3A1 (pg. 123) and II-3A2 (pg. 124) show representative Northern blots of SGLT1 in the proximal and distal small intestine, respectively. The digoxigenin-labelled SGLT1 cDNA probe hybridized to a 4.8 kb mRNA transcript in proximal and distal segments for each experimental group and the reference adult (Figures II-3A1 and II-3A2).

In the proximal small intestine SGLT1 mRNA abundance was increased in spermidine-treated rats at day 10 compared to age-matched controls (Table II-4). By postnatal day 13 the SGLT1 mRNA contents measured for both placebo- and spermidine-treated groups were at levels intermediate to those found for placebo- or spermidine-treated rats at day 10. The significant age * treatment interaction effects ($P<0.0302$) found for SGLT1 mRNA abundance in the proximal small intestine indicate that while spermidine exposure could induce precocious increases in SGLT1 mRNA content at day 10, previous exposure to spermidine did not alter the levels of SGLT1 mRNA content compared to age-matched controls at day 13 (Table II-4).

In the distal small intestine consecutive exposures to spermidine increased SGLT1 mRNA abundance at day 10 compared to age-matched controls (Table II-4). Following spermidine withdrawal the levels of SGLT1 mRNA measured by day 13 did not differ from the spermidine-induced levels found at day 10, however, these levels were significantly lower than those measured for the day 13 placebo group. These findings indicated that while continued spermidine treatments were not required to maintain the spermidine-induced precocious increases in SGLT1 mRNA measured at day 10, previous spermidine exposure prevented SGLT1 mRNA content from reaching the levels measured for the day 13 placebo group. The significant age-dependant effects ($P<0.0029$) found for SGLT1 mRNA content indicated that SGLT1 mRNA abundance was increased

with postnatal age in the distal small intestine. Significant age * treatment interaction effects ($P<0.0188$) were also found for SGLT1 mRNA abundance in the distal small intestine indicating that the precocious increases in SGLT1 mRNA content at day 10 following consecutive spermidine exposures did not persist by day 13 following spermidine withdrawal (Table II-4).

Protein Expression

Figures II-3B1 (pg. 123) and II-3B2 (pg. 124) show representative Western blots of SGLT1 in the proximal and distal segments of the small intestine, respectively. In spermidine-treated day 10 rats the SGLT1 protein detected on autoradiograms showed a slight upward shift in molecular weight in the proximal small intestine compared to the day 10 placebo group. These changes in molecular weight were consistent with age-dependant alterations to immunodetectable SGLT1 observed during postnatal development.

In the proximal small intestine SGLT1 protein expression was significantly increased with spermidine exposure from control levels at postnatal day 10 (Table II-4). The spermidine-induced levels of SGLT1 protein at day 10 were not different from those measured for the placebo group at day 13 indicating that consecutive spermidine exposures could prematurely induce increases in SGLT1 protein expression. By postnatal day 13 however, the levels of SGLT1 protein were significantly reduced from the precociously induced levels following the withdrawal of exogenous spermidine treatments.

SGLT1 protein expression increased significantly in the distal small intestine of spermidine-treated rats at postnatal day 10 compared to age-matched controls (Table II-4). These precocious increases in the levels of immunodetectable SGLT1 were not maintained in the absence of spermidine exposure by postnatal day 13. At postnatal day 13 SGLT1 protein expression was not different in the placebo- and spermidine-treated groups.

In summary, both SGLT1 mRNA abundance and protein expression were precociously increased in both the proximal and distal small intestine by consecutive exposures to spermidine. While SGLT1 mRNA content did not differ between spermidine-treated groups at days 10 and 13, the precocious spermidine-induced increases in SGLT1 protein expression were not maintained when exogenous spermidine treatments were withdrawn. The significant age * treatment interaction effects generally found for SGLT1 mRNA abundance and protein content reflect the finding that consecutive spermidine exposures could induce precocious maturation of SGLT1 at postnatal day 10, whereas spermidine-induced changes did not persist in the absence of spermidine treatments to day 13.

Effect of Spermidine Exposure on Sucrase-Isomaltase

In Experiment II we examined whether spermidine exposure could precociously induce developmental increases in SI mRNA and protein expression at postnatal day 10, and also whether these effects would persist following the withdrawal of spermidine treatments. These results are summarized in Table II-4 (pg. 121) and Figure II-3C, D (pg. 122).

mRNA Abundance

Figures II-3C1 (pg. 125) and II-3C2 (pg. 126) show Northern blots representative of SI mRNA abundance in the proximal and distal small intestine, respectively. In the reference adult small intestine the digoxigenin-labelled SI cDNA probe hybridized to a 6.0 kb mRNA transcript. The 6.0 kb SI mRNA was not detected in either the spermidine-treated or placebo groups at postnatal days 10 or 13 in the proximal or distal small intestine (Figures II-3C1 and II-3C2).

Spermidine exposure had no effect on SI mRNA abundance in either the proximal or distal small intestine at the time points studied in Experiment II (Table II-4 and Figure II-3C). These findings are in accordance with results reported for unmanipulated development in Experiment I.

Protein Expression

Figures II-3D1 (pg. 125) and II-3D2 (pg. 126) show Western blots representative of SI in the proximal and distal small intestine, respectively.

In the proximal small intestine spermidine exposure significantly increased SI protein expression at postnatal day 10 compared to age-matched control values (Table II-4 and Figure II-3D). The low levels of SI protein measured in control and spermidine-treated rats at day 13 were similar to the spermidine-induced values reported at day 10. The levels of SI protein detected at these early ostnatal time points ranged from 1.5% to 7% of the adult reference values.

Spermidine exposure induced a significant 3-fold increase of SI protein expression in the distal small intestine of day 10 rats compared to the placebo group (Table II-4 and Figure II-3D). The levels of SI protein in day 10 spermidine-treated rats were similar to placebo values at postnatal day 13. The withdrawal of exogenous spermidine exposures significantly decreased SI protein expression from spermidine-induced levels by postnatal day 13. In the absence of spermidine treatments SI protein expression at day 13 was similar to that measured for day 10 controls. The levels of SI protein expression in the distal small intestine at these early postnatal time points generally ranged from 7% to 20% of the adult reference values.

The significant age * treatment interaction effects on SI protein expression found for the distal ($P<0.0001$) small intestine (Table II-4) reflect the precocious induction of SI protein by spermidine that occurred at postnatal day 10, but not at day 13. These findings indicate that exogenous spermidine must be continually supplied at these developmental time points to produce a precocious expression of SI protein in the distal segment of the small intestine.

In summary, detectable levels of SI mRNA could not be precociously induced by spermidine exposure at the early

postnatal time points examined prior to weaning. Although undetectable, very low levels of SI mRNA may have been present given that spermidine exposure could induce precocious increases in SI protein expression. The findings reported here suggest that a continued supply of exogenous spermidine is required to maintain precocious developmental increases in SI protein expression. Since low levels of SI protein were detected in the distal small intestine prior to weaning, it was not unexpected that distal values for SI protein were greater than those measured in the proximal small intestine for both the control and spermidine-treated groups.

Effect of Spermidine Exposure on Na^+K^+ ATPase α_1

In Experiment II we examined the effects of spermidine exposure on Na^+K^+ ATPase α_1 mRNA abundance and protein expression in the postnatal rat small intestine prior to the onset of weaning. A summary of these results is presented in Table II-5 (pg. 127) and Figure II-4A, B (pg. 128).

mRNA Abundance

Figures II-4A1 (pg. 129) and II-4A2 (pg. 130) show Northern blots representative of Na^+K^+ ATPase α_1 in the proximal and distal small intestine, respectively. The digoxigenin-labelled Na^+K^+ ATPase α_1 cDNA probe hybridized to a 3.7 kb mRNA in the small intestine for each treatment group and the adult reference (Figures II-4A1 and II-4A2).

In the proximal segment of the small intestine Na^+K^+ ATPase α_1 mRNA abundance was significantly increased by spermidine exposure in day 10 rats compared to age-matched controls (Table II-5 and Figure II-4A). Following the withdrawal of exogenous spermidine treatments the levels of α_1 mRNA by day 13 were 2-fold lower than levels measured in both spermidine-treated rats at day 10 and in day 13 controls. Na^+K^+ ATPase α_1 mRNA abundance in spermidine-treated rats at day 13 was not significantly different from the control levels measured at day 10. The significant age * treatment interaction effects

($P<0.0001$) found for Na^+K^+ ATPase α_1 mRNA content in the proximal small intestine reflect the finding that α_1 mRNA abundance could be precociously induced by spermidine exposures at postnatal day 10, but not day 13 (Table II-5). These results indicate that in the proximal small intestine suckling rats require continued exposures to exogenous spermidine to elicit precocious increases in Na^+K^+ ATPase α_1 mRNA abundance. By contrast, spermidine exposure had no significant effect on Na^+K^+ ATPase α_1 mRNA in the distal segment of the small intestine (Table II-5 and Figure II-4A).

Protein Expression

Figures II-4B1 (pg. 129) and II-4B2 (pg. 130) show Western blots representative of the Na^+K^+ ATPase α_1 subunit isoform in the proximal and distal small intestine, respectively. The immunodetectable Na^+K^+ ATPase α_1 subunit isoform was identified at 112 kDa in proximal and distal regions of the small intestine for all treatment groups and the reference adult rat (Figures II-4B1 and II-4B2).

Spermidine exposure had no significant effect on Na^+K^+ ATPase α_1 isoform protein expression in either the proximal or distal small intestine (Table II-5 and Figure II-4B). While spermidine exposure precociously induced Na^+K^+ ATPase α_1 mRNA abundance in the proximal small intestine at postnatal day 10, α_1 isoform protein expression was not affected by these increases.

In summary, these findings suggest that α_1 isoform expression did not closely parallel changes in Na^+K^+ ATPase α_1 mRNA abundance in the proximal small intestine following spermidine exposure. In the distal small intestine spermidine exposure did not induce the precocious maturation of Na^+K^+ ATPase α_1 mRNA or protein content at the postnatal time points examined in Experiment II.

Effect of Spermidine Exposure on Na^+K^+ ATPase B_1

In Experiment II we also examined whether spermidine exposure could precociously induce developmental alterations in Na^+K^+ ATPase $ß_1$ mRNA abundance and protein expression prior to weaning. A summary of these results is presented in Table II-5 (pg. 127) and Figure II-4C, D (pg. 128).

mRNA Abundance

Figures II-4C1 (pg. 131) and II-4C2 (pg. 132) show Northern blots representative of Na^+K^+ ATPase $ß_1$ in the proximal and distal small intestine, respectively. The digoxigenin-labelled Na^+K^+ ATPase $ß_1$ cDNA probe hybridized to a 2.7 kb mRNA in the proximal and distal small intestine for each experimental group and the adult reference rat (Figures II-4C1 and II-4C2).

In the proximal small intestine Na^+K^+ ATPase $ß_1$ mRNA abundance was significantly increased in spermidine-treated rats at postnatal day 10 compared to the levels measured for age-matched controls (Table II-5 and Figure II-4C). These spermidine-induced levels were similar to those measured for the placebo group at day 13 indicating the precocious maturation of $ß_1$ mRNA in spermidine-treated day 10 rats. Following the withdrawal of exogenous spermidine treatments the levels of $ß_1$ mRNA were reduced 2-fold from day 10 spermidine-induced values by postnatal day 13, and were similar to those measured for the placebo group at postnatal day 10.

By contrast, in the distal small intestine consecutive exposures to spermidine did not alter Na^+K^+ ATPase $ß_1$ mRNA abundance at postnatal day 10 when compared to age-matched controls (Table II-5 and Figure II-4C). By postnatal day 13 the levels of $ß_1$ mRNA measured for placebo- and spermidine-treated groups were significantly greater than day 10 values. Following the withdrawal of exogenous spermidine exposure the levels of $ß_1$ mRNA were still lower at day 13 compared to age-matched controls. The significant age-dependant effects

($P<0.0001$) found for Na^+K^+ ATPase β_1 mRNA abundance in the distal small intestine reflect the increased β_1 mRNA content measured for placebo- and spermidine-treated groups at postnatal day 13 compared to day 10 levels.

Significant age * treatment interaction effects were found for β_1 mRNA abundance in both the proximal ($P<0.0003$) and distal ($P<0.0003$) small intestine (Table II-5). In the proximal small intestine these effects reflect findings that the levels of β_1 mRNA were precociously induced by spermidine at day 10, but not by day 13 following spermidine withdrawal. The effects found in the distal small intestine reflect the finding that spermidine exposure did not precociously alter Na^+K^+ ATPase β_1 mRNA abundance by day 10, however previous spermidine exposure could induce increased levels of β_1 mRNA by day 13.

Together these results suggest that consecutive exposures to exogenous spermidine induced increases in Na^+K^+ ATPase β_1 mRNA abundance in the proximal, but not the distal small intestine at postnatal day 10. When spermidine exposures were terminated the spermidine-induced levels of Na^+K^+ ATPase β_1 mRNA in the proximal small intestine did not persist by day 13. In the distal small intestine previous spermidine exposure seemed to prevent the levels of Na^+K^+ ATPase β_1 mRNA from reaching the values measured for age-matched controls.

Protein Expression

Figures II-4D1 (pg. 131) and II-4D2 (pg. 132) show Western blots representative of the Na^+K^+ ATPase β_1 subunit isoform in the proximal and distal small intestine, respectively. The Na^+K^+ ATPase β_1 subunit isoform protein was detected in the rodent small intestine at approximately 50 kDa (Figures II-4D1 and II-4D2).

Spermidine exposure had no effect on Na^+K^+ ATPase β_1 protein expression in the proximal small intestine (Table II-5 and Figure II-4D). In the distal small intestine consecutive exposures to spermidine had no effect on the levels of Na^+K^+

ATPase β_1 protein expressed at day 10. By day 13 however, the levels of β_1 isoform protein were significantly reduced following the withdrawal of exogenous spermidine when compared to those measured for the day 13 placebo group. In the distal small intestine the significant age * treatment interaction effects found for Na^+K^+ ATPase β_1 protein ($P<0.0083$) reflect the finding that β_1 protein expression was significantly decreased by day 13 following spermidine withdrawal, however consecutive spermidine exposure did not increase β_1 protein content at postnatal day 10 when compared to age-matched controls (Table II-5).

In summary, these results suggest that Na^+K^+ ATPase β_1 protein expression does not parallel alterations in β_1 mRNA abundance following spermidine exposure in the proximal small intestine. These effects may be regional since β_1 protein expression generally followed alterations in mRNA abundance in the distal small intestine. These findings concur with results reported in Experiment I describing a poor association between levels of Na^+K^+ ATPase β_1 mRNA and protein expression in the developing small intestine.

Summary of Main Results for Experiment II

In conclusion, the precocious induction of intestinal development observed following the exposure of suckling rats to exogenous spermidine appears to be dependant on both the variable and the developmental age being examined. Overall, the results of Experiment II indicate that spermidine exposure must be continued for spermidine-induced precocious development to persist in the small intestine of suckling rats. These observations are supported by findings such as those obtained for ODC mRNA, SGLT1 protein, SI protein (distal segment only), Na^+K^+ ATPase α_1 and β_1 mRNA (proximal segment only), as well as β_1 mRNA and protein content (proximal and distal segments, respectively), whereby the spermidine-induced precocious maturational increases measured at postnatal day 10

compared to placebo values did not persist by day 13, or were reduced below age-matched control values, following the withdrawal of exogenous spermidine. Not all the variables examined in Experiment II, such as SI mRNA, could be modulated by spermidine exposure, and moreover, not all spermidine-induced alterations in mRNA content were paralleled by similar changes in protein expression.

In general, the results of Experiment II indicate that when spermidine exposure induces precocious maturation in the small intestine of suckling rats the developmental alterations are not permanent.

Table II-1: Effect of Age and Spermidine on Animal Characteristics

	Day 10		Day 13		Two-way ANOVA					
					age		treatment		age*treatment	
	Placebo	Spermidine	Placebo	Spermidine	F	p	F	p	F	p
Body Weight (g)	22.15 ± 1.94 c (31)	21.07 ± 1.80 c (31)	28.79 ± 5.96 a (23)	25.72 ± 4.48 b (21)	62.94	0.0001	4.51	0.0132	6.37	NS
Total Gut Weight (g)	0.89 ± 0.19 a (31)	0.89 ± 0.29 a (31)	1.30 ± 0.21 b (23)	1.19 ± 0.23 b (21)	63.38	0.0001	2.00	NS	0.84	NS
Serum Cortisol (ug/dl)	0.40 ± 0.13 a	0.03 ± 0.02 b	0.09 ± 0.07 b	0.04 ± 0.03 b	5.26	0.0252	10.42	0.0020	5.66	0.0204
(nmol/l)	11.04 ± 3.46 a (14)	0.90 ± 0.64 b (20)	2.59 ± 1.81 b (16)	1.05 ± 0.87 b (16)						

Values are means ± SEM of number of samples shown in brackets.
Within a row, means with the same letter are not significantly different from each other ($p>0.05$, two-way ANOVA).
NS, not significant ($p>0.05$).

Table II-2: Effect of Age and Spermidine on RNA, Microvillus Membrane(MVM) and Basolateral Membrane(BLM) Protein Yields from Rat Small Intestine

Yield (mg/g gut)	Day 10		Day 13		Two-way ANOVA					
					age		treatment		age*treatment	
	Placebo	Spermidine	Placebo	Spermidine	F	p	F	p	F	p
Total RNA										
Proximal	1.58 ± 0.44	2.06 ± 0.53	1.91 ± 0.21	2.25 ± 0.51	4.31	NS	1.82	NS	0.16	NS
Distal	1.42 ± 0.43 b	1.82 ± 0.38 b	1.64 ± 0.26 b	2.24 ± 0.53 a	9.47	0.0096	4.96	0.0458	1.04	NS
MVM Protein										
Proximal	1.75 ± 0.11	1.61 ± 0.25	1.68 ± 0.36	1.16 ± 0.26	0.06	NS	0.14	NS	0.98	NS
Distal	1.22 ± 0.22 **	1.42 ± 0.17	1.15 ± 0.26 *	1.46 ± 0.15	6.13	NS	0.02	NS	0.22	NS
BLM Protein										
Proximal	8.34 ± 0.55 b	8.25 ± 0.57 b	9.61 ± 0.80 a	9.81 ± 0.65 a	18.61	0.0012	0.01	NS	0.24	NS
Distal	6.95 ± 0.52 b**	6.72 ± 0.24 b**	9.04 ± 0.54 a	9.57 ± 1.07 a	54.39	0.0001	0.19	NS	1.27	NS

Values are means ± SEM of 3-5 samples.
Within a row, means with the same letter are not significantly different from each other ($p>0.05$, two-way ANOVA).
NS, not significant ($p>0.05$).
* $p<0.05$, distal versus proximal (Student's t-test).
** $p<0.01$, distal versus proximal (Student's t-test).

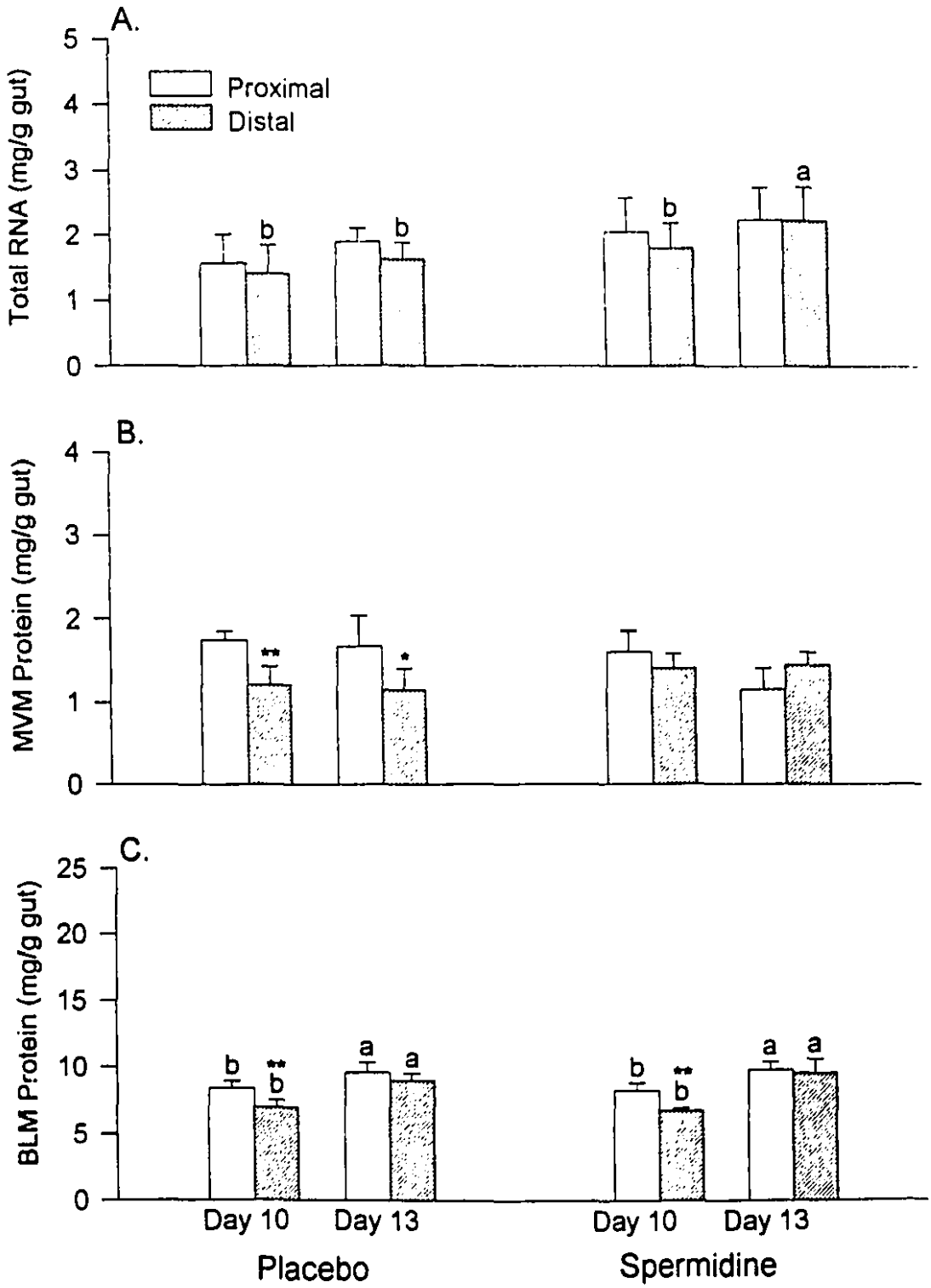


Figure II-1. Effect of age and spermidine on RNA and protein yields from rat small intestine. Within each site (proximal or distal), bars with the same letters are not significantly different from each other ($p>0.05$, two-way ANOVA). Distal versus proximal comparisons were done using the Student's t-test where * $p<0.05$ and ** $p<0.01$.

Table II-3: Effect of Age and Spermidine on Ornithine Decarboxylase (ODC) mRNA Abundance in Rat Small Intestine

Autoradiogram Density (% of Adult Reference)	Day 10		Day 13		Two-way ANOVA					
					age		treatment		age*treatment	
	Placebo	Spermidine	Placebo	Spermidine	F	p	F	p	F	p
ODC mRNA										
Proximal	38.7 ± 10.9 b	63.5 ± 25.8 a	52.2 ± 10.1 ab	28.7 ± 8.9 b	1.95	NS	0.01	NS	10.00	0.0082
Distal	39.5 ± 3.7 b	57.5 ± 11.0 a	37.2 ± 11.0 b	26.0 ± 4.0 b	11.97	0.0086	0.47	NS	8.94	0.0173

Values are means ± SEM of 3-4 samples.
Within a row, means with the same letter are not significantly different from each other ($p>0.05$, two-way ANOVA).
NS, not significant ($p>0.05$).
No significant differences were observed between proximal and distal intestine ($p>0.05$, Student's t-test).

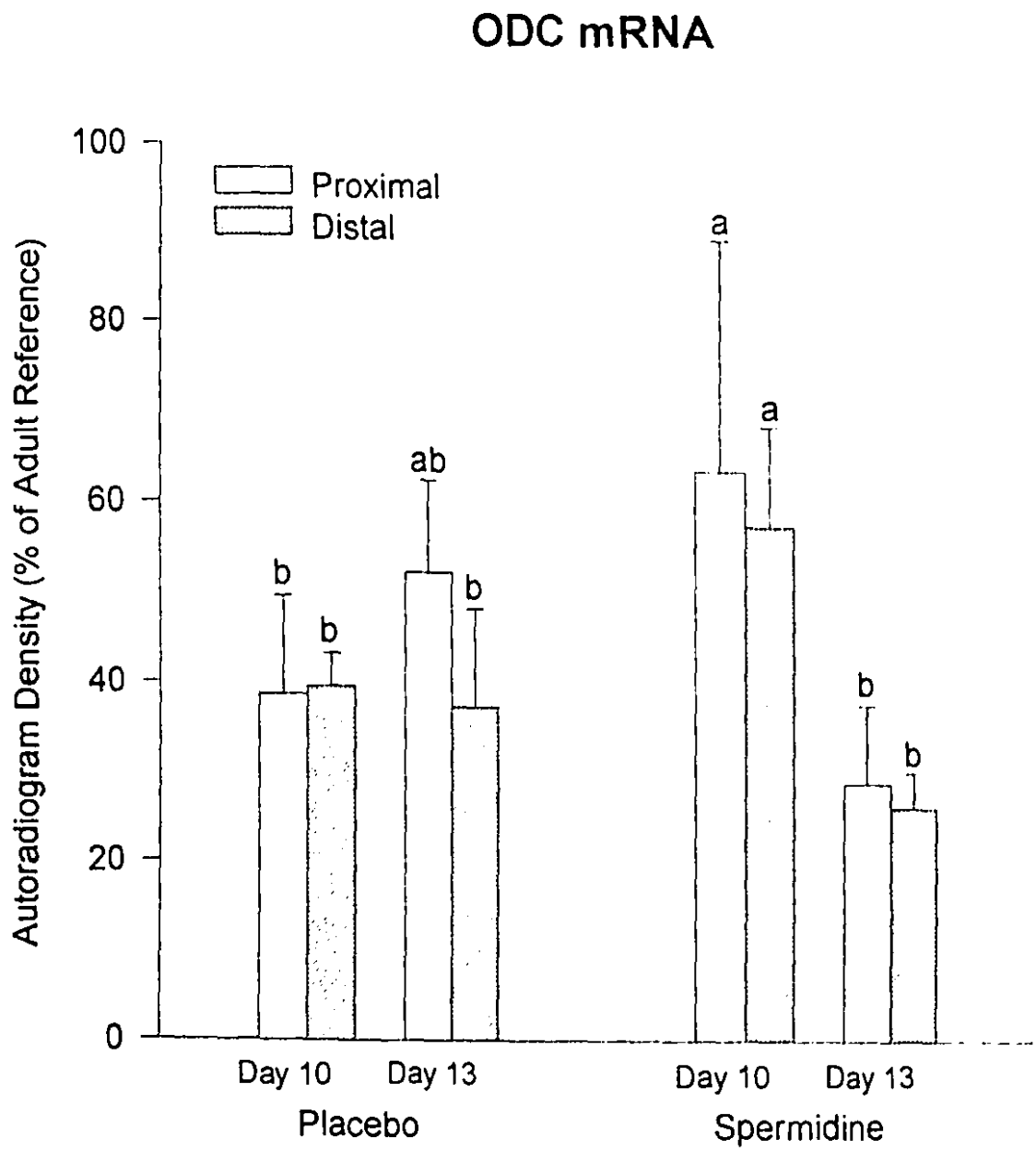


Figure II-2. Effect of age and spermidine on ornithine decarboxylase(ODC) mRNA abundance in rat small intestine. Within each site (proximal or distal), the same letters are not significantly different from each other ($p>0.05$, two-way ANOVA). No significant differences were observed between proximal and distal intestine ($p>0.05$, Student's t-test).

Figure II-2A: Northern Blot of Ornithine Decarboxylase Proximal Small Intestine

Northern blot analysis (representative of n=3) of ornithine decarboxylase (ODC) mRNA abundance in total RNA isolated from rat proximal small intestine following exposure to spermidine (S) or placebo (P) as outlined in Experiment II. 30 μg of total RNA from time points corresponding to postnatal day P10, S10, P13, S13, and adult was fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled ODC cDNA probe for 16 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb respectively. The ODC cDNA probe detects two major transcripts of 2.7 kb and 2.2 kb, and a minor hybridizing species at 1.7 kb.

Figure II-2B: Northern Blot of Ornithine Decarboxylase Distal Small Intestine

Northern blot analysis (representative of n=3) of ODC mRNA abundance in total RNA isolated from rat distal small intestine following exposure to spermidine (S) or placebo (P) as outlined in Experiment II. 30 μg of total RNA from time points corresponding to postnatal day P10, S10, P13, S13, and adult was fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled ODC cDNA probe for 16 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb respectively. The ODC cDNA probe recognizes transcripts of 2.7 kb, 2.2 kb, and 1.7 kb.

Figure II-2A:Northern blot
Ornithine Decarboxylase in the Proximal Small Intestine

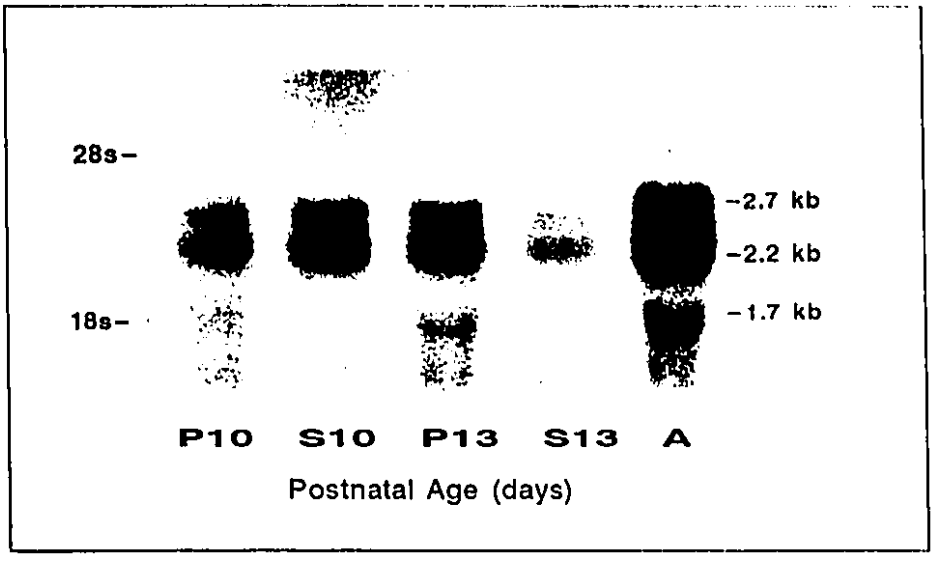


Figure II-2B Northern Blot
Ornithine Decarboxylase in the Distal Small Intestine

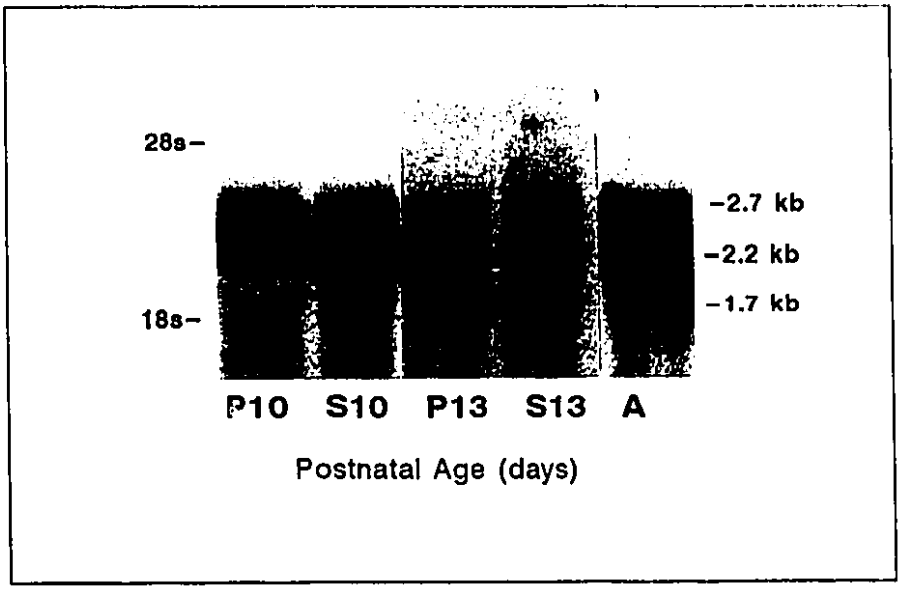


Table II-4: Effect of Age and Spermidine on mRNA and Microvillus Membrane(MVM) Protein Abundance of the Sodium-dependent Glucose Transporter(SGLT1) and Sucrase-Isomaltase(SI) in Rat Small Intestine

Autoradiogram Density (% of Adult Reference)	Day 10		Day 13		Two-way ANOVA					
					age		treatment		age*treatment	
	Placebo	Spermidine	Placebo	Spermidine	F	p	F	p	F	p
SGLT1										
mRNA										
Proximal	21.7 ± 4.0 b	35.4 ± 6.6 a	30.1 ± 11.0 ab	25.0 ± 7.3 ab	0.14	NS	1.06	NS	6.04	0.0302
Distal	18.0 ± 5.6 c	24.9 ± 3.4 b*	35.1 ± 6.0 a	28.0 ± 3.0 b	17.89	0.0029	0.00	NS	8.63	0.0188
MVM Protein										
Proximal	41.3 ± 10.0 b	77.4 ± 13.0 a	77.7 ± 3.0 a	36.3 ± 4.0 b	0.26	NS	0.34	NS	57.49	0.0001
Distal	21.1 ± 4.0 c	67.8 ± 8.0 a	47.8 ± 22.0 ab	34.0 ± 14.0 bc	0.20	NS	4.00	NS	13.93	0.0058
SI										
mRNA										
Proximal	ND	ND	ND	ND	Statistics not applicable					
Distal	ND	ND	ND	ND						
MVM Protein										
Proximal	1.4 ± 0.3 b	6.3 ± 2.1 a	5.9 ± 4.0 a	4.2 ± 2.0 ab	1.03	NS	1.57	NS	7.12	0.0285
Distal	7.0 ± 3.0 b	21.3 ± 4.0 a*	23.0 ± 4.0 a**	10.8 ± 2.0 b*	2.15	NS	0.32	NS	50.45	0.0001

ND (0.0 ± 0.0)
Values are means ± SEM of 3-4 samples.
Within a row, means with the same letter are not significantly different from each other (p>0.05, two-way ANOVA).
NS, not significant (p>0.05).
* p<0.05, distal versus proximal (Student's t-test).
** p<0.01, distal versus proximal (Student's t-test).

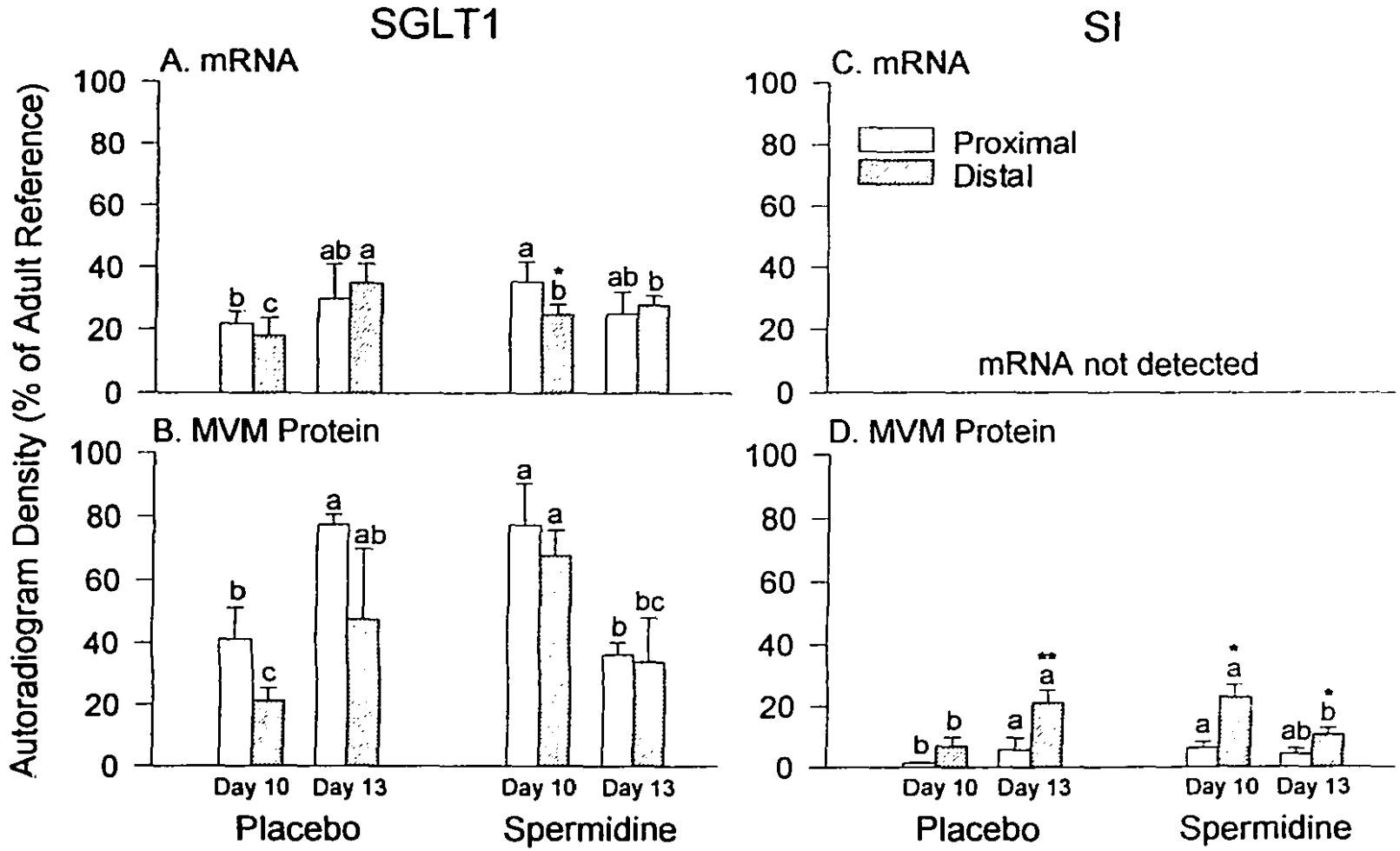


Figure II-3. Effect of age and spermidine on mRNA and microvillus membrane(MVM) protein abundance of the Na^+-dependent glucose transporter(SGLT1) and sucrase-isomaltase(SI) in rat small intestine. Within each site (proximal or distal), bars with the same letters are not significantly different from each other $p>0.05$, two-way ANOVA). Distal versus proximal comparisons were done using the Student's t-test where * $p<0.05$ and ** $p<0.01$.

Figure II-3A1: Northern Blot Of SGLT1 Proximal Small Intestine

Northern blot analysis (representative of n=4) of SGLT1 abundance in total RNA isolated from rat proximal small intestine following exposure to spermidine (S) or placebo (P) as outlined in Experiment II. 30 μg of total RNA from time points corresponding to postnatal day P10, S10, P13, S13, and adult was fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled SGLT1 cDNA probe for 16 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb respectively. The SGLT1 cDNA probe detects a signal at 4.8 kb.

Figure II-3B1: Western Blot of SGLT1 Proximal Small Intestine

Western blot analysis (representative of n=3) of SGLT1 expression in MVM protein fractions from rat proximal small intestine following exposure to spermidine (S) or placebo (P) as outlined in Experiment II. 80 μg of MVM protein from time points corresponding to day P10, S10, P13, S13, and adult was separated on a 7.5% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE). Adult MVM from colon was run as a negative reference protein. Proteins were electrotransferred to a nitrocellulose membrane for 100 minutes at 4 °C. Western blots were incubated with the polyclonal anti-SGLT1 1° antibody for 2 hours at room temperature with agitation. Immunodetection was carried out following a standard protocol using a [^{125}I]-goat anti-rabbit 2° antibody, and an autoradiograph obtained. The polyclonal anti-SGLT1 antibody detects the mature SGLT1 protein at approximately 84 kilodaltons (kDa).

Figure II-3A1:Northern Blot
SGLT1 in the Proximal Small Intestine

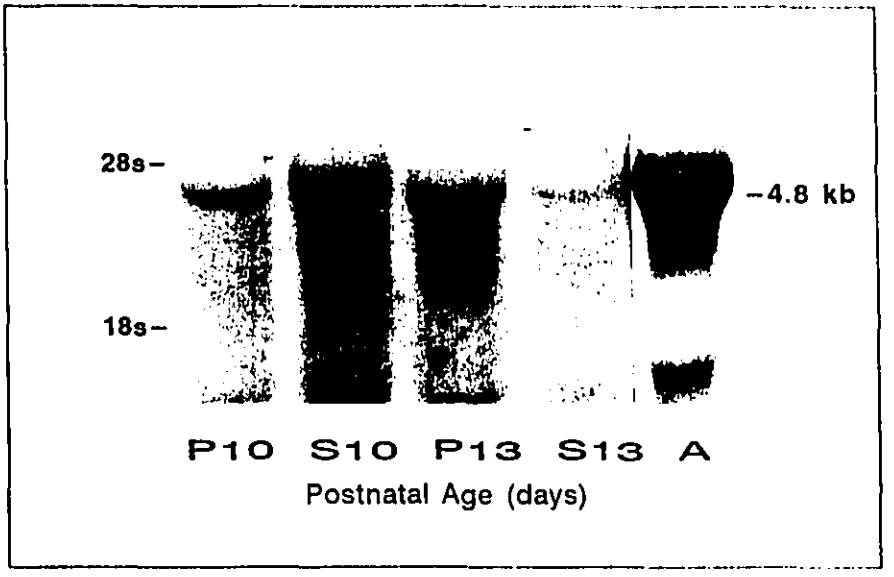


Figure II-3B1:Western Blot
SGLT1 in the Proximal Small Intestine

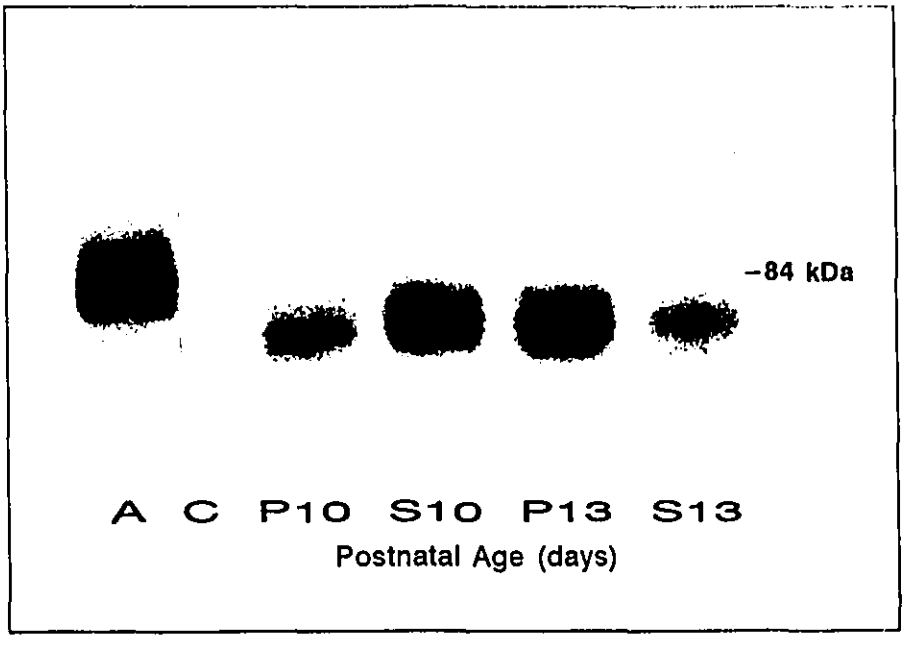


Figure II-3A2: Northern Blot of SGLT1 Distal Small Intestine

Northern blot analysis (representative of n=3) of SGLT1 abundance in total RNA isolated from rat distal small intestine following exposure to spermidine (S) or placebo (P) as outlined in Experiment II. 30 μg of total RNA from time points corresponding to postnatal day P10, S10, P13, S13, and adult was fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled SGLT1 cDNA probe for 16 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb respectively. The SGLT1 cDNA probe detects a signal at 4.8 kb.

Figure II-3B2: Western Blot of SGLT1 Distal Small Intestine

Western blot analysis (representative of n=4) of SGLT1 expression in MVM protein fractions from rat distal small intestine following exposure to spermidine (S) or placebo (P) as outlined in Experiment II. 80 μg of MVM protein from time points corresponding to day P10, S10, P13, S13, and adult was separated on a 7.5% gel by SDS-PAGE. Adult MVM from colon was run as a negative reference protein. Proteins were electrotransferred to a nitrocellulose membrane for 100 minutes at 4 °C. Western blots were incubated with the polyclonal anti-SGLT1 1° antibody for 2 hours at room temperature with agitation. Immunodetection was carried out following a standard protocol using a [^{125}I]-goat anti-rabbit 2° antibody, and an autoradiograph obtained. The polyclonal anti-SGLT1 antibody detects the mature SGLT1 protein at approximately 84 kDa.

Figure II-3A2:Northern Blot
SGLT1 in the Distal Small Intestine

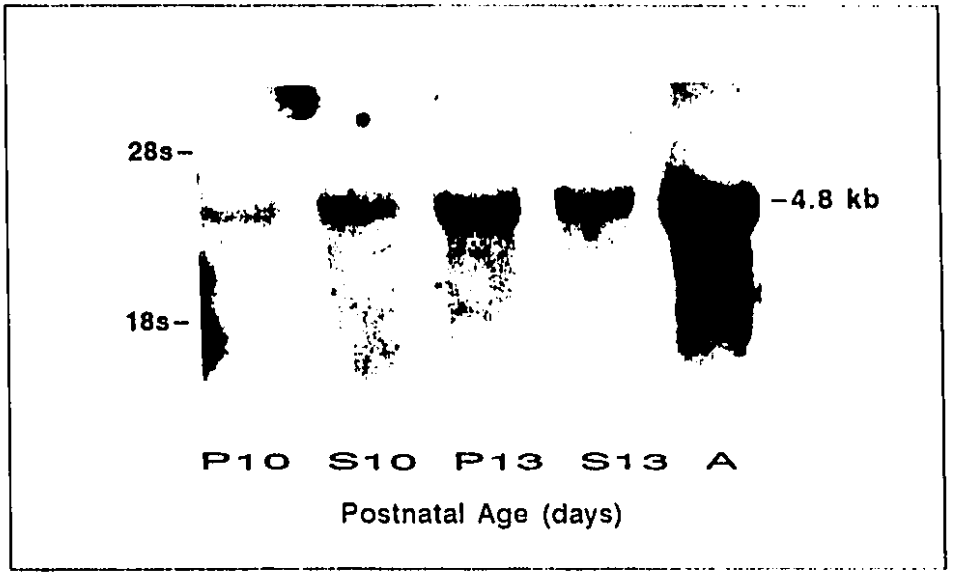


Figure II-3B2:Western Blot
SGLT1 in the Distal Small Intestine

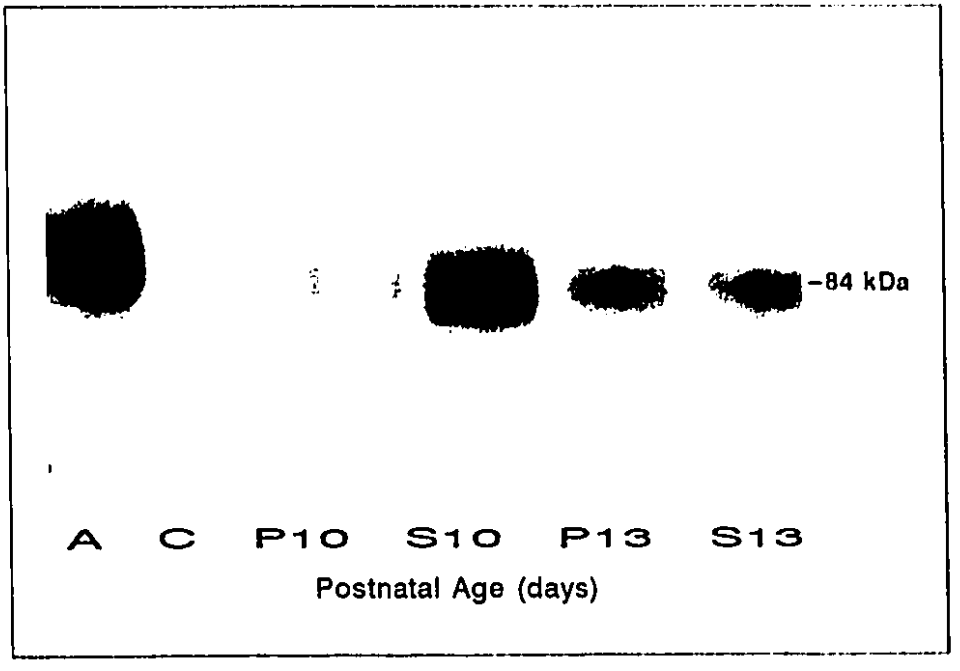


Figure II-3C1: Northern Blot of Sucrase-Isomaltase Proximal Small Intestine

Northern blot analysis (representative of n=3) of SI mRNA abundance in total RNA isolated from rat proximal small intestine following exposure to spermidine (S) or placebo (P) as outlined in Experiment II. 30 μg of total RNA from time points corresponding to postnatal day P10, S10, P13, S13, and adult was fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled SI cDNA probe for 16 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb respectively. The SI cDNA probe detects a signal at approximately 6.0 kb.

Figure II-3D1: Western Blot of Sucrase-Isomaltase Proximal Small Intestine

Western blot analysis (representative of n=4) of SI expression in MVM protein fractions isolated from proximal small intestine following exposure to spermidine (S) or placebo (P) as outlined in Experiment II. 80 μg of MVM protein from time points corresponding to day P10, S10, P13, S13, and adult was separated on a 7.5% gel by SDS-PAGE. Adult MVM from rat colon was run as a negative reference protein. Proteins were electrotransferred to a nitrocellulose membrane for 120 minutes at 4 °C. Western blots were then incubated with the polyclonal anti-sucrase-isomaltase 1° antibody for 2 hours at room temperature with agitation. Immunodetection was carried out following a standard protocol using [^{125}I]-goat anti-rabbit 2° antibody, and an autoradiograph obtained. The polyclonal antibody directed against SI detects sucrase at approximately 116-120 kDa, isomaltase at 140 kDa, an incompletely glycosylated sucrase-isomaltase complex at approximately 194 kDa, and a high mannose (completely glycosylated) complex at 210 kDa.

Figure II-3C1:Northern Blot
Sucrase-Isomaltase in the Proximal Small Intestine

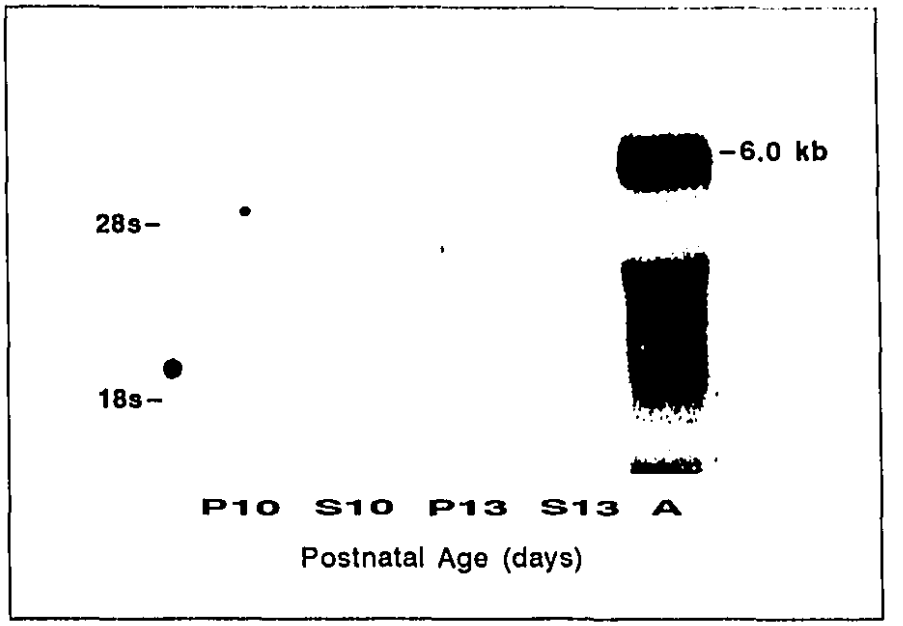


Figure II-3D1:Western Blot
Sucrase-Isomaltase in the Proximal Small Intestine

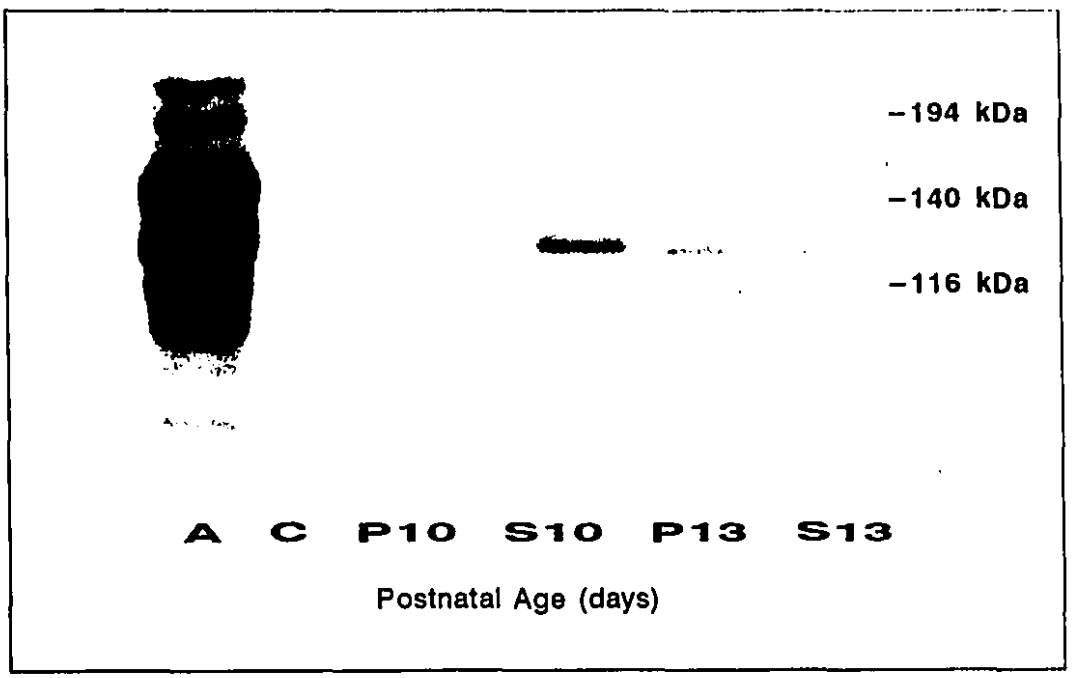


Figure II-3C2: Northern Blot of Sucrase-Isomaltase Distal Small Intestine

Northern blot analysis (representative of n=4) of SI mRNA abundance in total RNA isolated from rat distal small intestine following exposure to spermidine (S) or placebo (P) as outlined in Experiment II. 30 μg of total RNA from time points corresponding to postnatal day P10, S10, P13, S13, and adult was fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled SI cDNA probe for 16 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb respectively. The SI cDNA probe detects a signal at approximately 6.0 kb.

Figure II-3D2: Western Blot of Sucrase-Isomaltase Distal Small Intestine

Western blot analysis (representative of n=4) of SI expression in MVM protein fractions isolated from distal small intestine following exposure to spermidine (S) or placebo (P) as outlined in Experiment II. 80 μg of MVM protein from time points corresponding to day P10, S10, P13, S13, and adult was separated on a 7.5% gel by SDS-PAGE. Adult MVM from rat colon was run as a negative reference protein. Proteins were electrotransferred to a nitrocellulose membrane for 120 minutes at 4 °C. Western blots were then incubated with the polyclonal anti-SI 1° antibody for 2 hours at room temperature with agitation. Immunodetection was carried out following a standard protocol using [^{125}I]-goat anti-rabbit 2° antibody, and an autoradiograph obtained. The polyclonal antibody directed against SI detects sucrase at approximately 116-120 kDa, isomaltase at 140 kDa, an incompletely glycosylated sucrase-isomaltase complex at approximately 194 kDa, and a high mannose (completely glycosylated) complex at 210 kDa.

Figure II-3C2:Northern Blot
Sucrase-Isomaltase in the Distal Small Intestine

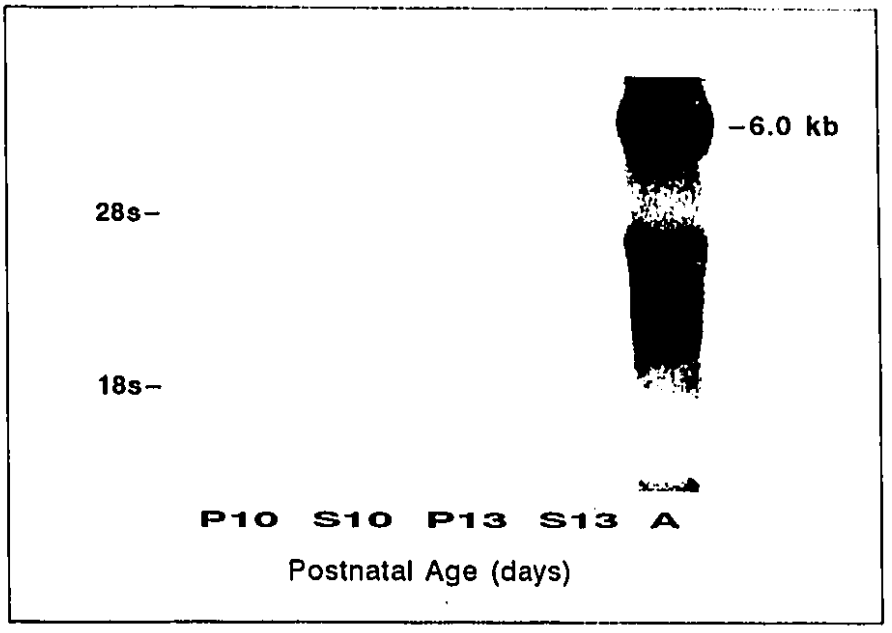
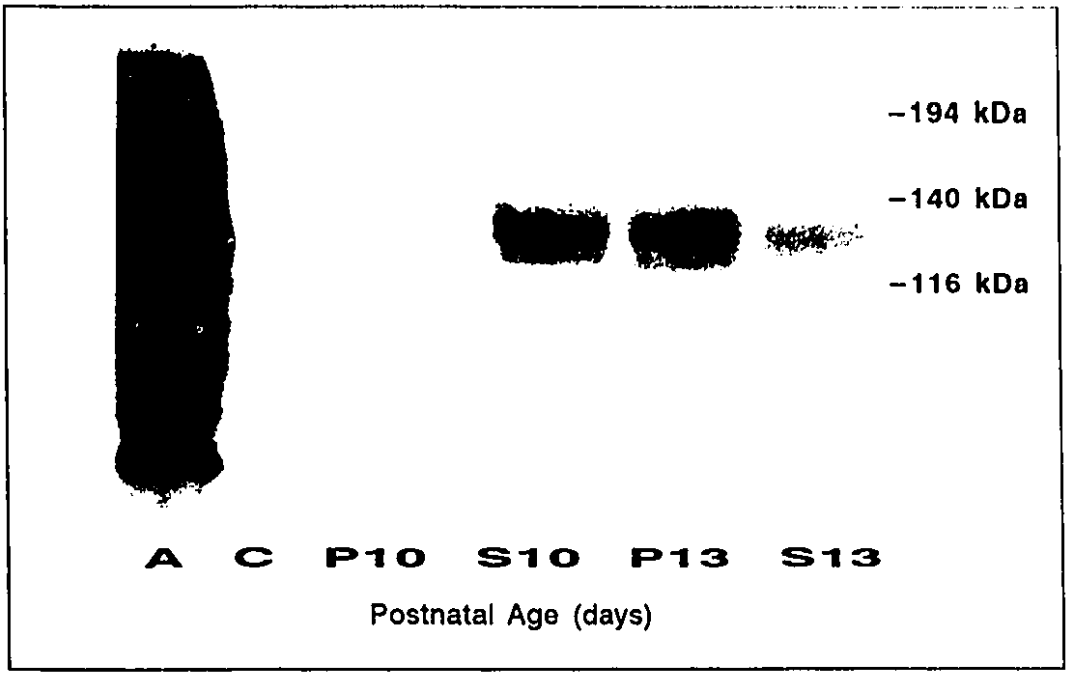


Figure II-3D2:Western Blot
Sucrase-Isomaltase in the Distal Small Intestine



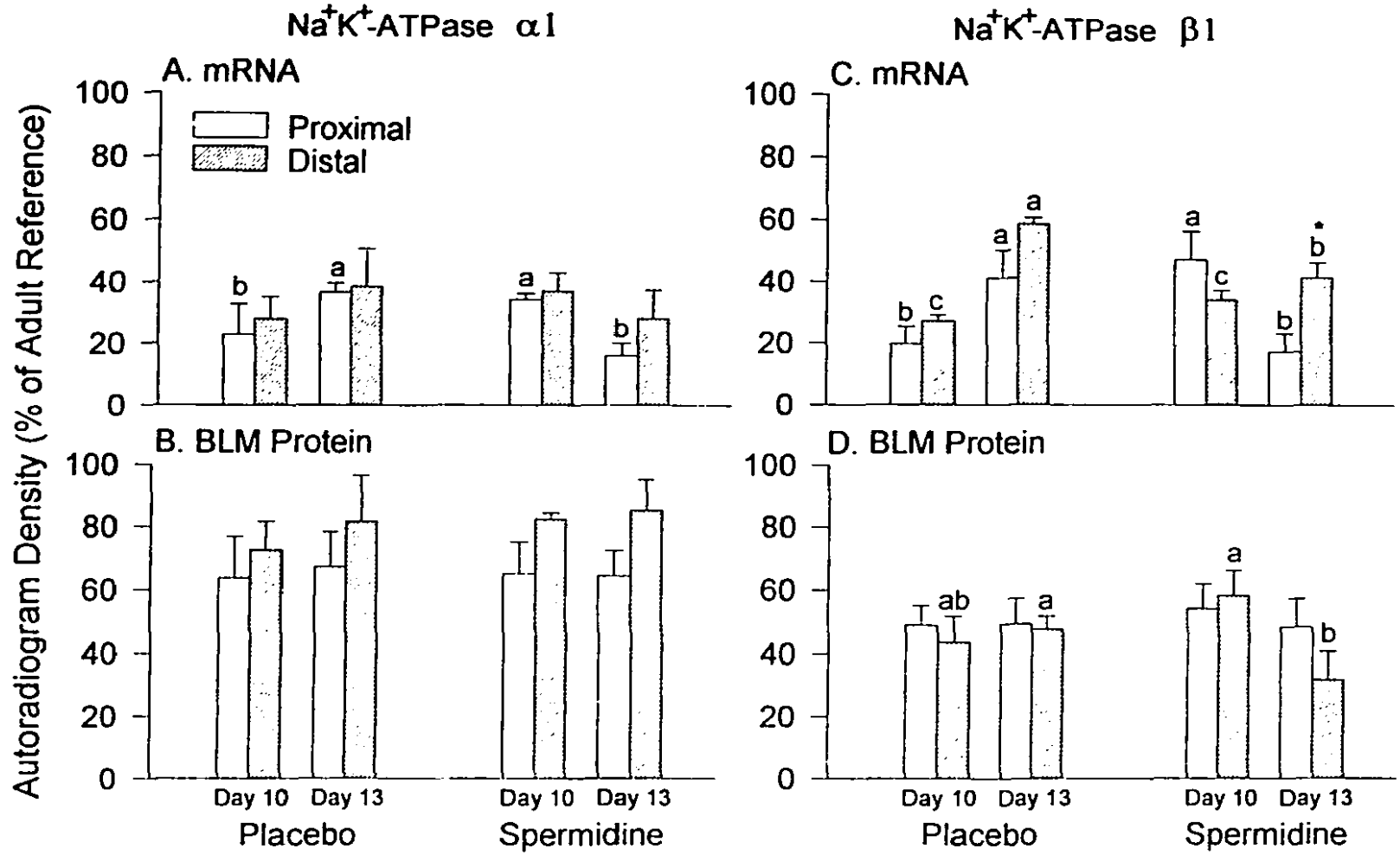


Figure II-4. Effect of age and spermidine on mRNA and basolateral membrane(BLM) protein abundance of the Na+K+-ATPase α1 and β1 subunits in rat small intestine. Within each site (proximal or distal), bars with the same letters are not significantly different from each other(p>0.05, two-way ANOVA). Distal versus proximal comparisons were done using the Student's t-test where * p<0.05 and ** p<0.01.

Figure II-4A1: Northern Blot of the Na^+K^+ ATPase α_1 isoform Proximal Small Intestine

Northern blot analysis (representative of n=3) of Na^+K^+ ATPase α_1 isoform mRNA abundance in total RNA isolated from rat proximal small intestine following exposure to spermidine (S) or placebo (P) as outlined in Experiment II. 30 μg of total RNA from time points corresponding to postnatal day P10, S10, P13, S13, and adult was fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled Na^+K^+ ATPase α_1 isoform cDNA probe for 16 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb, respectively. The Na^+K^+ ATPase α_1 isoform cDNA probe detects a signal at 3.7 kb.

Figure II-4B1: Western Blot of the Na^+K^+ ATPase α_1 isoform Proximal Small Intestine

Western blot analysis (representative of n=3) of Na^+K^+ ATPase α_1 isoform expression in BLM protein fractions from rat proximal small intestine following exposure to spermidine (S) or placebo (P) as outlined in Experiment II. 80 μg of BLM protein from time points corresponding to day P10, S10, P13, S13, and adult was separated on a 7.5% gel by SDS-PAGE. Proteins were electrotransferred to a nitrocellulose membrane for 100 minutes at 4 °C. Western blots were incubated with the polyclonal anti-Na^+K^+ ATPase α_1 isoform 1° antibody for 2 hours at room temperature with agitation. Immunodetection was carried out following a standard protocol using a [^{125}I]-goat anti-rabbit 2° antibody, and an autoradiograph obtained. The Na^+K^+ ATPase α_1 fusion protein detects the mature Na^+K^+ ATPase α_1 isoform at approximately 112 kD.

Figure II-4A1:Northern Blot
NaK ATPase α1 Isoform in the Proximal Small Intestine

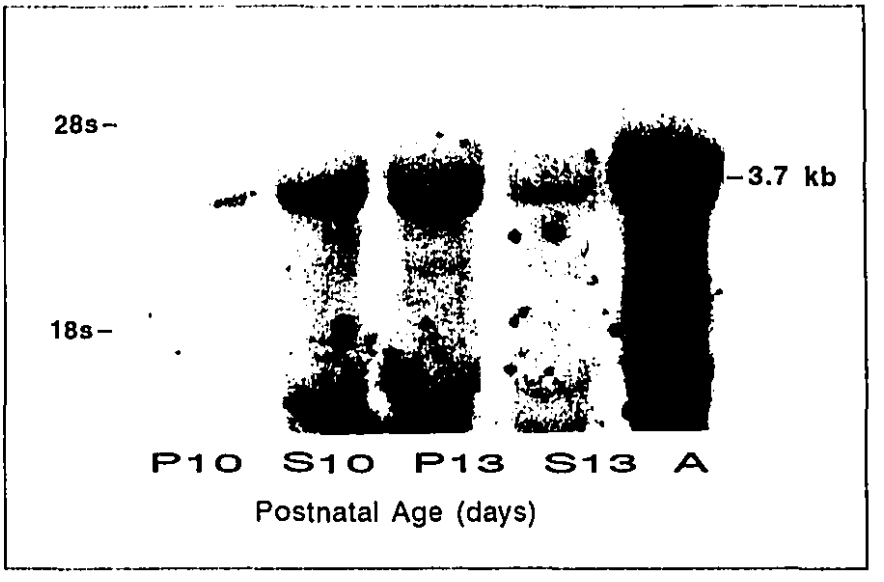


Figure II-4B1:Western Blot
NaK ATPase α1 Isoform in the Proximal Small Intestine

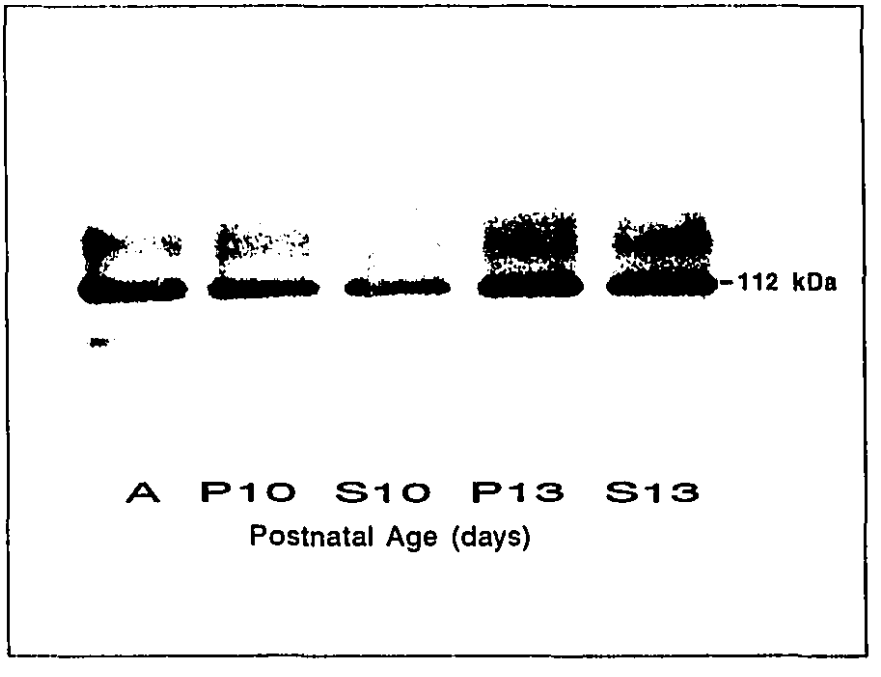


Figure II-4A2: Northern Blot of the Na^+K^+ ATPase α_1 isoform Distal Small Intestine

Northern blot analysis (representative of n=3) of Na^+K^+ ATPase α_1 isoform mRNA abundance in total RNA isolated from rat distal small intestine following exposure to spermidine (S) or placebo (P) as outlined in Experiment II. 30 μg of total RNA from time points corresponding to postnatal day P10, S10, P13, S13, and adult were fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled Na^+K^+ ATPase α_1 isoform cDNA probe for 16 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb, respectively. The Na^+K^+ ATPase α_1 isoform cDNA probe detects a signal at 3.7 kb.

Figure II-4B2: Western Blot of the Na^+K^+ ATPase α_1 isoform Distal Small Intestine

Western blot analysis (representative of n=3) of Na^+K^+ ATPase α_1 isoform expression in BLM protein fractions from rat distal small intestine following spermidine exposure (S) or placebo (P) as outlined in Experiment II. 80 μg of BLM protein from time points corresponding to day P10, S10, P13, S13, and adult was separated on a 7.5% gel by SDS-PAGE. Proteins were electrotransferred to a nitrocellulose membrane for 100 minutes at 4 °C. Western blots were incubated with the polyclonal anti-Na^+K^+ ATPase α_1 isoform 1° antibody for 2 hours at room temperature with agitation. Immunodetection was carried out following a standard protocol using a [^{125}I]-goat anti-rabbit 2° antibody, and an autoradiograph obtained. The Na^+K^+ ATPase α_1 fusion protein detects the mature Na^+K^+ ATPase α_1 isoform at approximately 112 kD.

Figure II-4A2:Northern Blot
NaK ATPase α1 Isoform in the Distal Small Intestine

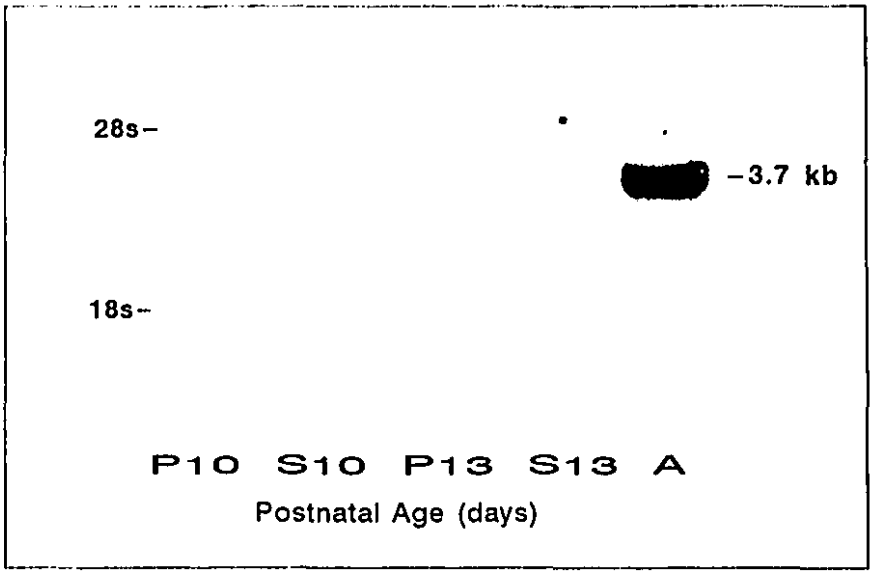


Figure II-4B2:Western Blot
NaK ATPase α1 Isoform in the Distal Small Intestine

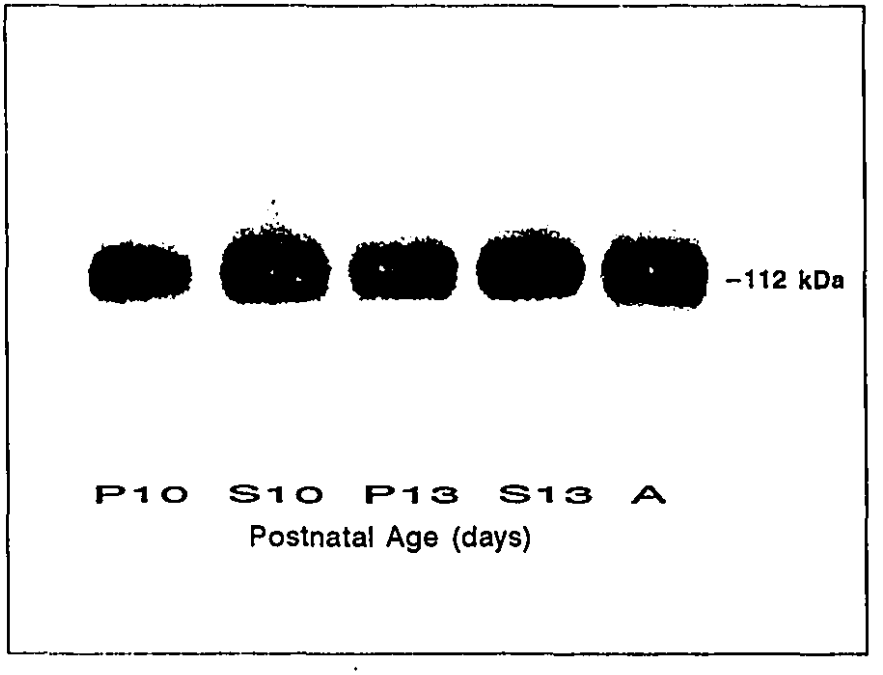


Figure II-4C1: Northern Blot of the Na^+K^+ ATPase β_1 isoform Proximal Small Intestine

Northern blot analysis (representative of n=3) of Na^+K^+ ATPase β_1 isoform mRNA abundance in total RNA isolated from rat proximal small intestine following exposure to spermidine (S) or placebo (P) as outlined in Experiment II. 30 μg of total RNA from time points corresponding to postnatal day P10, S10, P13, S13, and adult was fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled Na^+K^+ ATPase β_1 subunit cDNA probe for 16 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb respectively. The Na^+K^+ ATPase β_1 isoform cDNA probe detects a signal at 2.7 kb in rat small intestine.

Figure II-4D1: Western Blot of the Na^+K^+ ATPase β_1 isoform Proximal Small Intestine

Western blot analysis (representative of n=3) of Na^+K^+ ATPase β_1 subunit isoform expression in BLM protein fractions from rat proximal small intestine following exposure to spermidine (S) or placebo (P) as outlined in Experiment II. 80 μg of BLM protein from time points corresponding to day P10, S10, P13, S13, and adult was separated on a 7.5% gel by SDS-PAGE. Proteins were electrotransferred to a nitrocellulose membrane for 100 minutes at 4 °C. Western blots were incubated with the polyclonal anti-Na^+K^+ ATPase β_1 subunit 1° antibody for 2 hours at room temperature with agitation. Immunodetection was carried out following a standard protocol using a [^{125}I]-goat anti-rabbit 2° antibody, and an autoradiograph obtained. The Na^+K^+ ATPase β_1 fusion protein detects the mature Na^+K^+ ATPase β_1 isoform at approximately 50-55 kDa.

Figure II-4C1:Northern Blot
NaK ATPase ß1 Isoform in the Proximal Small Intestine

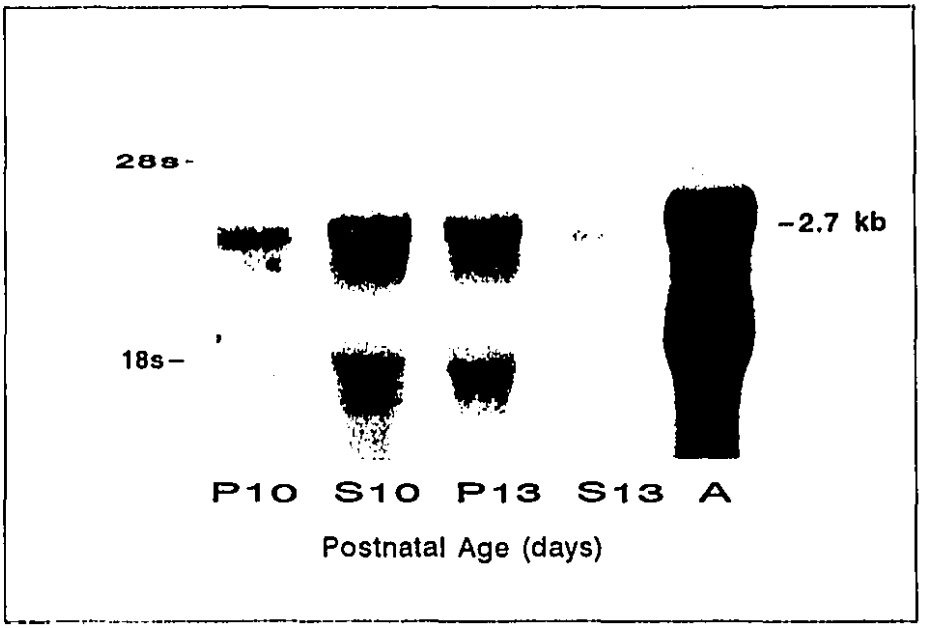


Figure II-4D1:Western Blot
NaK ATPase ß1 Isoform in the Proximal Small Intestine

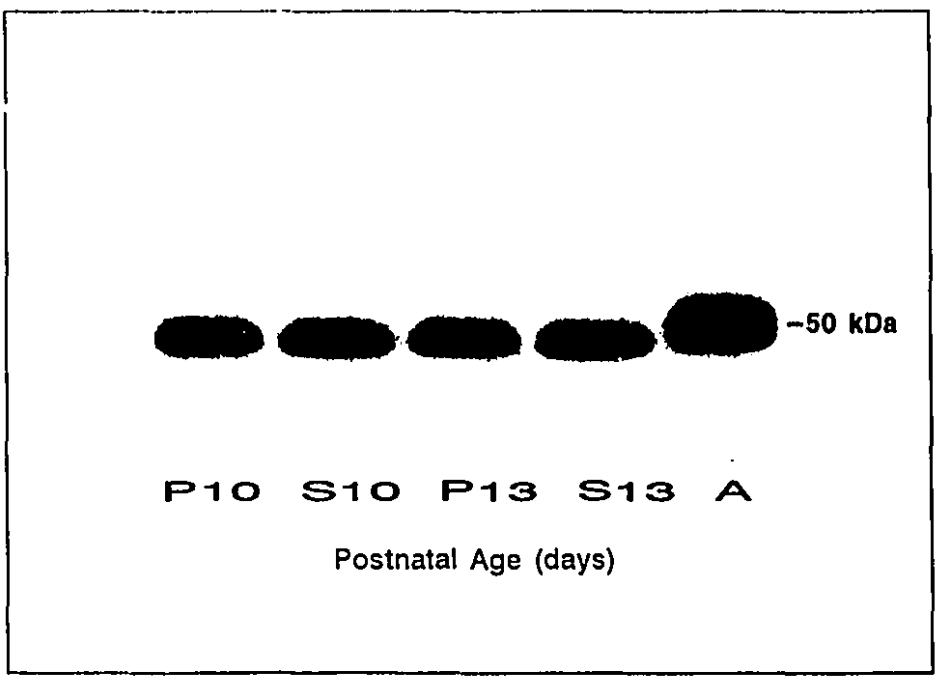


Figure II-4C2: Northern Blot of the Na^+K^+ ATPase β_1 isoform Distal Small Intestine

Northern blot analysis (representative of n=3) of Na^+K^+ ATPase β_1 isoform mRNA abundance in total RNA isolated from rat distal small intestine following exposure to spermidine (S) or placebo (P) as outlined in Experiment II. 30 μg of total RNA from time points corresponding to postnatal day P10, S10, P13, S13, and adult was fractionated on a 1.5% agarose gel, and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled Na^+K^+ ATPase β_1 subunit cDNA probe for 16 hours with agitation. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained. The 18s and 28s rRNA correspond to 2.0 kb and 5.0 kb respectively. The Na^+K^+ ATPase β_1 isoform cDNA probe detects a signal at 2.7 kb in rat small intestine.

Figure II-4D2: Western Blot of the Na^+K^+ ATPase β_1 isoform Distal Small Intestine

Western blot analysis (representative of n=3) of Na^+K^+ ATPase β_1 subunit isoform expression in BLM protein fractions from rat distal small intestine following exposure to spermidine (S) or placebo (P) as outlined in Experiment II. 80 μg of BLM protein from time points corresponding to day P10, S10, P13, S13, and adult was separated on a 7.5% gel by SDS-PAGE. Proteins were electrotransferred to a nitrocellulose membrane for 100 minutes at 4 °C. Western blots were incubated with the polyclonal anti-Na^+K^+ ATPase β_1 subunit 1° antibody for 2 hours at room temperature with agitation. Immunodetection was carried out following a standard protocol using a [^{125}I]-goat anti-rabbit 2° antibody, and an autoradiograph obtained. The Na^+K^+ ATPase β_1 fusion protein detects the mature Na^+K^+ ATPase β_1 isoform at approximately 50-55 kDa.

Figure II-4C2:Northern Blot
NaK ATPase ß1 Isoform in the Distal Small Intestine

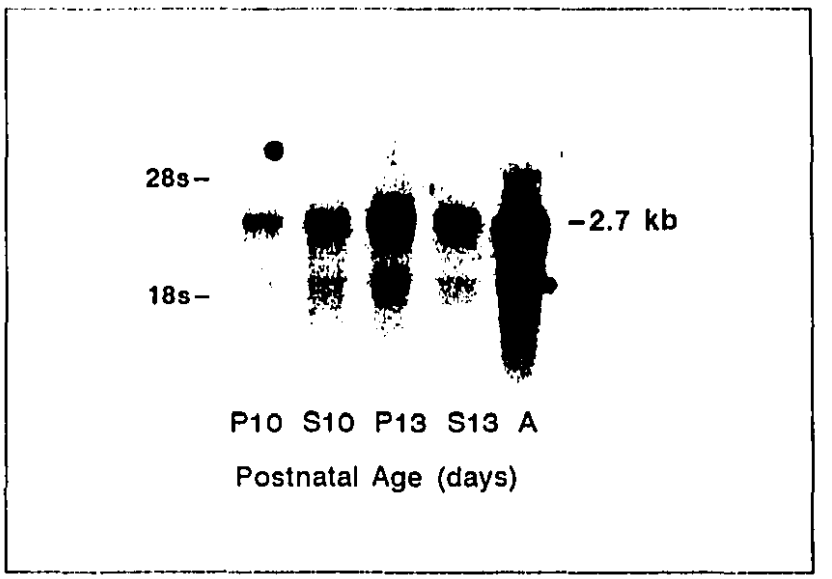
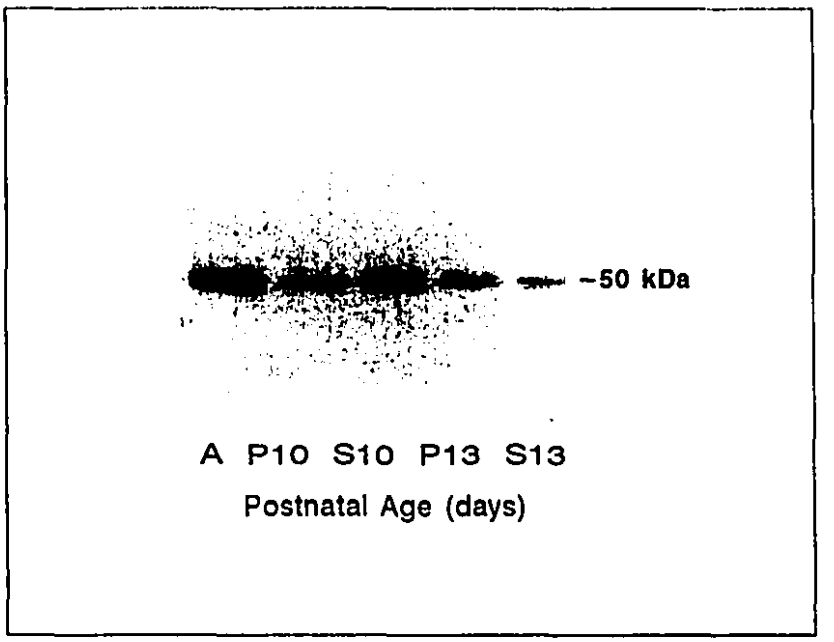


Figure II-4D2:Western Blot
NaK ATPase ß1 Isoform in the Distal Small Intestine



DISCUSSION

During postnatal development the rat small intestine acquires a mature profile of digestive enzymes and transport mechanisms to accommodate the characteristic dietary transition that occurs at weaning. While the developmental profile of sucrase-isomaltase, located on the enterocyte microvillus membrane, has been well characterized in the rat, the ontogeny of SGLT1, also located on the microvillus membrane, and the Na^+K^+ ATPase, restricted to the enterocyte basolateral membrane, have received less attention. To our knowledge, this is the first comprehensive study describing the developmental profiles of both mRNA abundance and protein expression for SGLT1, sucrase-isomaltase, and the Na^+K^+ ATPase α_1 and $ß_1$ subunit isoforms in the postnatal rat small intestine. In addition, we report age-dependant increases in mucosal ODC mRNA abundance during postnatal development in the rat small intestine.

In this study we found the onset of age-dependant increases in mRNA content and protein expression to occur around weaning, and the extent to which these developmental alterations were continued in the post-weaned period, to be dependant upon both the variable and the intestinal region (proximal or distal small intestine) under examination. For SGLT1 we found an early gradual increase in mRNA content in the proximal small intestine with levels unchanged by postnatal day 14 and thereafter. In the distal small intestine however, SGLT1 mRNA showed the greatest developmental increases in post-weaned rats between days 21 and 28. The developmental profile for SGLT1 protein expression did not closely parallel reported alterations in SGLT1 mRNA content in the proximal small intestine and showed regional differences in postnatal regulation with adult levels of protein reached by days 14 and 21 in the proximal and distal small intestine, respectively. We found gradual increases in Na^+K^+ ATPase α_1 subunit mRNA abundance in the

proximal small intestine that reached adult levels post-weaning by day 35. However in a similar fashion to that reported for SGLT1, the main developmental increase for α_1 subunit mRNA in the distal small intestine occurred in post-weaned rats between days 21 and 28. While α_1 isoform protein expression in the proximal small intestine generally paralleled the developmental profile described for α_1 mRNA, the main age-dependant changes in α_1 isozyme expression in the distal small intestine did not closely parallel alterations to distal mRNA content with significant increases in isozyme abundance occurring prior to weaning at day 14, and at weaning. We found the greatest increases in Na^+K^+ ATPase β_1 mRNA to occur prior to weaning (days 10 to 14), and post-weaning (days 21 to 28) in both regions of the small intestine examined, however the main developmental increase in β_1 isozyme protein occurred post-weaning (days 28 to 35) and in weaning rats (days 14 to 21) in the proximal and distal small intestine, respectively. Both Na^+K^+ ATPase α_1 and β_1 gene expression continued to increase in the proximal and distal small intestine of post-weaned rats surpassing adult levels by postnatal day 35. In both proximal and distal regions of the small intestine the greatest developmental increases in ODC mRNA abundance, and sucrase-isomaltase mRNA and protein expression, were found in weaning rats (days 14 to 21).

To our knowledge, the present work is the first to report the effects of exogenous spermidine exposure on both the mRNA and protein expression of SGLT1, sucrase-isomaltase, and the Na^+K^+ ATPase α_1 and β_1 subunit isoforms in the suckling rat small intestine. Previous studies have generally examined the precocious effects of spermine or spermidine on the functional activity of enzymes known to be reciprocally regulated during weaning [Dufour et al., 1988; Georges et al., 1990; Buts et al., 1993; Wild et al., 1993; Kaouass et al., 1994]. In addition, this study presents the first report of the

precocious effects of spermidine exposure on mucosal ornithine decarboxylase (ODC) mRNA abundance in the small intestine of the suckling rat. We report the precocious induction of intestinal development by spermidine exposure to be dependant on the variable and the intestinal region being examined, as well as, the schedule of spermidine administration. In this study, the consecutive exposure of suckling rats to spermidine induced a precocious maturation of ODC mRNA abundance, SGLT1 gene expression, and sucrase-isomaltase protein expression in both the proximal and distal small intestine at postnatal day 10. Spermidine-induced precocious increases in Na^+K^+ ATPase α_1 and β_1 subunit isoform mRNA abundance were found at day 10 in the proximal small intestine only, and were not accompanied by similar alterations in isozyme protein expression. Spermidine exposure had no precocious effect on Na^+K^+ ATPase α_1 and β_1 mRNA and protein expression in the distal small intestine at postnatal day 10. The precocious effects of consecutive spermidine administration on CDC mRNA abundance, Na^+K^+ ATPase α_1 and β_1 mRNA in the proximal small intestine, SGLT1 protein expression, and sucrase-isomaltase protein abundance in the distal small intestine did not persist by day 13 following the withdrawal of spermidine treatments. The levels of SGLT1 mRNA in both segments of the small intestine and SI protein in the proximal small intestine were not altered from spermidine-induced levels by day 13 following the termination of spermidine administration. While consecutive spermidine exposures did not induce precocious developmental increases in Na^+K^+ ATPase β_1 gene expression in the distal small intestine, the previous exposure to spermidine prevented the levels of β_1 mRNA and protein from reaching those measured for the age-matched controls at day 13. Overall, the results of this study show that when exposure to exogenous spermidine promotes precocious alterations in intestinal enzyme and transporter expression, it appears that spermidine must be continuously supplied for these alterations to persist in

suckling rats.

The remainder of this discussion is presented in two parts. The first section discusses the developmental profiles of gene expression reported on in Experiment I, while the second part focuses on the precocious effects of spermidine on intestinal development in the suckling rat.

Developmental Profiles of Gene Expression

In this study we report age-dependant alterations in mRNA abundance and protein expression for each variable examined, with developmental increases in gene expression generally coordinated with the weaning process. However, the postnatal ontogeny of SGLT1 in the rat small intestine presents an interesting variation on this theme in that SGLT1 mRNA abundance only showed early age-dependant alterations that were not associated with weaning in the proximal small intestine, while major developmental increases in SGLT1 mRNA content were found at weaning and thereafter in the distal small intestine. Miyamoto et al. (1992) have reported that the levels of SGLT1 mRNA in the rat jejunum do not change during postnatal intestinal ontogeny from day 5 to adulthood. By contrast, we report a gradual increase in the levels of SGLT1 mRNA in the proximal small intestine from postnatal days 3 to 14, with the levels remaining constant thereafter. A further interpretation of our findings in comparison with those reported by Miyamoto and colleagues (1992) is difficult since the authors did not quantify SGLT1 mRNA abundance earlier than postnatal day 5 and therefore may not have detected the gradual increase in SGLT1 mRNA that we report spanning the postnatal period from days 3 to 14. Moreover, Miyamoto and co-workers (1992) did not report a statistical analysis of their quantitative data and therefore may have overlooked subtle statistical age-dependant differences in jejunal SGLT1 mRNA content in the early postnatal period.

By contrast to findings in the proximal small intestine, SGLT1 mRNA abundance in the distal small intestine increased during weaning (days 14 to 21), and then again in the immediate post-weaning period (days 21 to 28) when adult levels were reached. To our knowledge, the developmental profile of SGLT1 mRNA in the distal small intestine of the postnatal rat has not been previously described. The results of this work indicate that SGLT1 mRNA content increases in association with weaning in the distal segment of the postnatal small intestine. Given these findings we speculate that the distal increases in SGLT1 mRNA may represent an intestinal adaptive response to meet the increased nutrient load of the adult diet. The induction of SGLT1 mRNA abundance and transport activity has been demonstrated in both the adult rat jejunum and the ovine small intestine in response to the infusion of dietary sugars [Miyamoto et al., 1993; Lescale-Matys et al., 1993]. SGLT1 mRNA content may be similarly induced in the rat distal small intestine at weaning when dietary carbohydrate and nutrient volumes are increased from pre-weaning levels. Developmental increases in SGLT1 mRNA content in the proximal small intestine may not occur at weaning as reported for the distal segment, since the postnatal ontogeny of SGLT1 in the proximal small intestine may reflect the induction of SGLT1 mRNA in utero given that the upper gastrointestinal tract is dependant in part on nutrients such as glucose provided from swallowed amniotic fluid for normal development [Buddington, 1993].

The present work reports significant increases in SGLT1 protein expression in pre-weanling (days 10 to 14) and weaning (days 14 to 21) rats in the proximal and distal small intestine, respectively. In the distal small intestine SGLT1 protein expression generally paralleled alterations in SGLT1 mRNA abundance in weaning rats. These results suggest that increases in both SGLT1 mRNA and protein are required in the distal segment of the small intestine during the intestinal

adaptation associated with weaning. SGLT1 mRNA content measured in the proximal segment of the small intestine did not correspond with the 2-fold increase in SGLT1 protein expressed prior to the onset of weaning. A similar discordance for intestinal SGLT1 has been described in ruminants whereby 60- to 90-fold increases in SGLT1 protein and transport activity, accompanied by mere 2-fold increases in SGLT1 mRNA abundance, have been demonstrated following intestinal infusions of sugars [Lescale-Matys et al., 1993]. In the weaning lamb, Shirazi-Beechey and co-workers (1991 and 1993) reported that intestinal SGLT1 mRNA content does not correspond with the significant downregulation of SGLT1 activity and protein expression characteristic of normal postnatal development in this species. Together, these findings suggest that increases in protein expression may "fine-tune" SGLT1 gene expression in the proximal small intestine during postnatal development.

SGLT1 is a glycosylated integral membrane protein [Hirayama and Wright, 1992]. In this study, we report gradual upward shifts in the molecular weight of the SGLT1 protein in both the proximal and distal small intestine during postnatal development. Immunodetectable SGLT1 increased in molecular weight by approximately 10-14 kDa from postnatal day 21 to adulthood. These increases in the apparent molecular weight of SGLT1 are consistent with posttranslational modifications to this protein, such as glycosylation, during intestinal ontogeny. In sheep, an apparent increase in the molecular weight of immunodetectable SGLT1 corresponding to approximately 14 kDa has also been reported in mature enterocytes compared to crypt cells following the intestinal infusion of sugars [Lescale-Matys et al., 1993]. The 14 kDa discrepancy in the molecular weight of SGLT1 reported by Lescale-Matys and co-workers (1993) was attributed to glycosylation of the transport protein. To date, the glycosylation of SGLT1 has not been shown to be important for

transporter function [Hirayama and Wright, 1992; Hediger and Rhoads, 1994]. In ovines, posttranslational modifications, such as protein glycosylation, are suggested to regulate SGLT1 activity during postnatal development and in response to luminal sugar infusions in ruminants [Lescale-Matys et al., 1993]. In the present work, the apparent upward increase in the molecular weight of SGLT1 suggested an increased glycosylation of this transporter protein during intestinal ontogeny. Future work to prove the association between protein glycosylation and the increased molecular weight of SGLT1 with maturation could involve digesting the sugar residues on the SGLT1 protein using specific glycosidases prior to the analysis of immunodetectable SGLT1 by Western blotting. Following the removal of terminal sugar residues, SGLT1 should be detected at the same molecular weight for each developmental time point examined (possibly 70-74 kDa) if protein glycosylation is responsible for the age-dependant upward shift in molecular weight observed in this study. In addition, concomitant measurements of SGLT1 transport activity and the expression of immunodetectable protein would permit further comment on the developmental associations between the glycosylation of SGLT1 and enhanced transport activity that has been suggested by other authors [Shirazi-Beechey et al., 1991; Lescale-Matys et al., 1993].

In view of the function of the Na^+K^+ ATPase in maintaining the electrochemical gradients required for SGLT1 transport activity, we sought to characterize the developmental profiles of Na^+K^+ ATPase α_1 and β_1 subunit isoform mRNA abundance and protein expression in the postnatal rat small intestine. Previous work from this laboratory has demonstrated significant age-dependent increases in Na^+K^+ ATPase activity during postnatal intestinal development and the establishment of proximal to distal gradients of activity in post-weaned rats [Sauriol, 1993]. In the present work we report significant age-dependant increases in Na^+K^+ ATPase α_1 and β_1

subunit isoform gene expression during postnatal ontogeny thereby extending previous work on the Na^+K^+ ATPase enzyme from this laboratory to the mRNA level.

In this study we found significant gradual increases in Na^+K^+ ATPase α_1 mRNA abundance that were generally paralleled by α_1 isoform expression throughout postnatal development in the proximal small intestine. These findings are in accordance with those reported by Zemelman et al. (1992) describing age-dependant, coordinate increases in α_1 mRNA abundance and isoform expression in the proximal rat small intestine between the second postnatal week and adulthood. A further comparison of these results and those of the present work is limited however, since Zemelman and co-workers (1992) did not examine Na^+K^+ ATPase α_1 gene expression at developmental time points earlier than day 14, or in the post-weaned period prior to adulthood. The findings reported in the present work indicate that gradual age-dependant increases in α_1 mRNA and protein content do occur in the proximal small intestine of the suckling rat prior to the onset of weaning.

By contrast, we report the largest single increase in Na^+K^+ ATPase α_1 mRNA abundance in the distal small intestine to be temporally associated with the weaning process. In this study Na^+K^+ ATPase α_1 mRNA continued to rise in the distal small intestine after weaning and exceeded adult levels of abundance in post-weaned rats by postnatal day 35. Immunodetectable α_1 in the distal small intestine also increased in association with weaning and showed similar post-weaning rises, but did not closely parallel alterations in α_1 mRNA content. These recent findings are also in accordance with those previously reported from our laboratory whereby the levels of Na^+K^+ ATPase α_1 isoform reached or exceeded adult levels of expression by postnatal day 30 [Sauriol, 1993].

The findings for Na^+K^+ ATPase α_1 mRNA abundance and protein expression reported in the present work are in accordance with the profile of enzyme activity described by Sauriol (1993) in

as much as adult profiles of gene expression were established in the post-weaned rat. In the present work the developmental profile of immunodetectable α_1 expression reported in both the proximal and distal small intestine however, does not closely parallel the Na^+K^+ ATPase activity described by Sauriol (1993) prior to the post-weaned period. Given that the α_1 subunit features the active site for Na^+-, K^+-, and ATP-binding on the enzyme complex, an association between the developmental profiles of α_1 protein expression and Na^+K^+ ATPase activity would be expected. A possible explanation for this apparent discrepancy could be that mechanisms other than α_1 isoform protein abundance "fine-tune" Na^+K^+ ATPase activity during postnatal development.

In contrast to the catalytic activity of the α subunit, the ß subunit is thought to facilitate the assembly, transport and insertion of the Na^+K^+ ATPase into the BLM [Horisberger et al., 1991]. Given this role in the functional expression of the Na^+K^+ ATPase, the $ß_1$ isoform would be expected to demonstrate a similar developmental profile to that of α_1. Zemelman and co-workers (1992) have described the coordinate expression of $ß_1$ isoform mRNA with that of α_1 isoform mRNA in both the proximal and distal small intestine of two-week old and adult rats. In the present work, the levels of Na^+K^+ ATPase $ß_1$ mRNA were found to be developmentally regulated in association with the onset of weaning and the process of weaning in both the proximal and distal small intestine. The results of this study suggest that the developmental profile of $ß_1$ mRNA is coordinated with that of α_1 in the distal small intestine only, since the levels of α_1 mRNA in the proximal small intestine increase gradually during postnatal ontogeny. Overall, the age-dependant alterations in $ß_1$ mRNA abundance preceded, but did not closely parallel the developmental rises in $ß_1$ protein expressed in both intestinal regions examined in this study. In both the proximal and distal small intestine alterations in the expression of immunodetectable $ß_1$ occurred

at weaning and in the post-weaned period. These findings suggest that the developmental patterns of Na^+K^+ ATPase $ß_1$ gene expression are regulated by increases in both mRNA and protein content.

A novel finding reported in the present work was the detection of an additional band signal for immunodetectable Na^+K^+ ATPase $ß_1$ in the distal small intestine of suckling rats compared to postnatal day 21 time points and thereafter. This "extra" band was identified at a molecular weight that was significantly below that of the 50 kDa $ß_1$ protein. Previous work from our laboratory has shown increased levels of glycosylation in proteins isolated from the BLM of suckling rat small intestine [Sauriol, 1993]. Glycosylation was heaviest in the distal small intestine and showed a spontaneous reduction that was associated with the weaning process [Sauriol, 1993]. Despite these findings, the "extra" protein band associated with immunodetectable $ß_1$ in the suckling small intestine in the present work is not thought to represent an increase in the glycosylation of the principal $ß_1$ protein since it appears as a distinct band at a considerably lower molecular weight than the $ß_1$ isoform. More likely, this band may represent a small regulatory protein. Given its pattern of expression prior to the onset of weaning this "protein" may be involved in maintaining low levels of Na^+K^+ ATPase activity in the immature small intestine. Further experimentation is required to establish the molecular nature and functional significance of this putative low molecular weight protein.

At this time it is unclear what comparisons can be made between the developmental expression profiles of the Na^+K^+ ATPase α_1 and $ß_1$ isoforms in the postnatal rat small intestine. Overall the results of the present work suggest that both these isoforms are developmentally regulated in association with the processes of intestinal adaptation at weaning. Given that the Na^+K^+ ATPase establishes the electrochemical gradient

in the enterocyte that is exploited by many intestinal nutrient transporters located on the MVM, we might predict the coordinate developmental expression of this BLM enzyme with that of SGLT1. While we report SGLT1 gene expression to be developmentally regulated in association with the process of weaning, the results of this work demonstrate similar, but not close parallels between the ontogenesis of the Na^+K^+ ATPase isoforms and SGLT1 in the postnatal rat small intestine.

Sucrase-isomaltase is an accepted apical marker of rat intestinal ontogeny given the abrupt appearance and steep rise in SI activity characteristic of weaning and post-weaning, respectively [Henning, 1981]. Given the close physical and functional association of sucrase-isomaltase (SI) with SGLT1 on the enterocyte apical membrane, this work also sought to characterize the ontogenic profiles of SI mRNA abundance and protein expression in the postnatal rat small intestine. In the present work, SI mRNA was first detected at postnatal day 21 in both the proximal and distal small intestine, and continued to increase from weanling levels in post-weaned rats. These findings concur with those of Krasinski et al. (1994) who reported the initial detection of SI mRNA in the proximal small intestine at postnatal day 21 using ribonuclease protection assays. The results of this study also agree with work by Krasinski and co-workers (1994) describing lower levels of SI mRNA in the distal small intestine at postnatal day 21 compared to levels measured in more proximal segments. Other authors have reported SI mRNA to be first detectable in the jejunum at postnatal day 18 [Leeper and Henning, 1990]. Since SI mRNA content was not measured at other developmental time points between postnatal days 14 and 21 in this study we are unable to comment on the precise day at which SI mRNA appeared during the weaning process.

In this study age-dependant increases in SI protein content were also found in the proximal and distal small intestine,

however the profiles of SI protein expression did not parallel alterations in SI mRNA abundance after weaning. We report the most divergent developmental patterns of SI mRNA abundance and protein expression in the distal small intestine at postnatal day 35, corresponding to decreased protein expression relative to SI mRNA content. These findings concur with those of Leeper and Henning (1990) who have reported the greatest divergence between SI activity and mRNA content to occur in the distal small intestine during the post-weaning period by day 36. Since SI is a complexly glycosylated enzyme complex [Lorenzsonn et al., 1987], it is possible that alterations in the glycosylation of the SI protein could modulate SI activity in post-weaned rats. In the present study, the disparity in SI mRNA abundance and protein expression found in the distal small intestine at postnatal day 35 is probably related to developmental alterations in the glycosylation patterns of SI.

Although both SGLT1 and SI are restricted to the enterocyte MVM, the present work clearly demonstrates the divergent developmental profiles of these two apical membrane proteins. However, the temporal relationship between the age-dependant increases in SGLT1 and SI gene expression would appear to ensure that the digestive capacity of the intestine matches the characteristic dietary transition and substrate load encountered by the rat at weaning. The differences in both the regional and developmental profiles of SGLT1 and SI suggest that different regulatory factors may induce the onset of the postnatal developmental expression of these genes. For SI, the evidence presented to date indicates that surges in the levels of glucocorticoids measured just prior to weaning play a predominant role in the abrupt induction of SI mRNA and enzyme activity [Leeper and Henning, 1990; Nanthakumar and Henning, 1993; Hoffman and Chang, 1993]. It is currently unknown what factors regulate the developmental profile of SGLT1 gene expression in the rat small intestine. Given the different regional developmental profiles of SGLT1 gene

expression found in this study, we can speculate that the qualitative and quantitative dietary changes associated with the weaning process may play a role in the modulation of SGLT1 expression and activity during postnatal development. Given that dietary substrates, such as sucrose, have been shown to induce increases in SI mRNA abundance and enzyme synthesis within a matter of hours [Broyart et al., 1990], qualitative alterations to the diet around weaning may "fine-tune" SI gene expression and activity in post-weaned rats.

In the third postnatal week the rat small intestine undergoes rapid growth processes converting the immature mucosa to the mature phenotype, thus accommodating the characteristic dietary transition that takes place at weaning [Buddington, 1993]. Ornithine decarboxylase activity is known to be highest in tissues undergoing cellular growth and proliferation [Heby and Persson, 1990]. Luk et al. (1980) have reported increased ODC activity in the intestinal mucosa of weaning rats compared to pre-weaning levels, coinciding with the period of maximal mucosal growth in this species. In the present work we demonstrated postnatal increases in ODC mRNA abundance in association with the process of weaning in both the proximal and distal small intestine. In these intestinal regions surges in the levels of ODC mRNA between postnatal days 14 and 21 significantly increased ODC mRNA abundance at weaning (day 21) compared to the earlier postnatal time points studied. These results indicate that increased levels of ODC mRNA abundance at weaning parallel the reported developmental increases in mucosal ODC enzyme activity [Luk et al. 1980]. Together, these findings suggest that developmental alterations in mucosal ODC activity are, at least in part, regulated by increased ODC mRNA abundance. However, ODC may be regulated at multiple cellular levels since enzyme and protein levels can be altered without changes in ODC mRNA content, and conversely, mRNA content can increase without affecting ODC activity [Tabata and Johnson, 1986;

Sertich and Pegg, 1987; Persson et al., 1988; Ginty et al., 1990; Chung et al., 1992]. During periods of mucosal proliferation alterations to both ODC mRNA and protein expression have been described in the regulation of ODC [Chung et al., 1992 and 1993]. Further comment on the levels of developmental regulation for ODC in this study would require concomitant measurements of ODC mRNA abundance, protein expression, and enzyme activity. From the results of the present work we can only speculate that increased levels of ODC mRNA at weaning play a role in the regulation of postnatal ontogeny in the rat small intestine.

Of interest, the levels of ODC mRNA measured in the rat distal small intestine in this study continued to increase after weaning, exceeding adult reference values by postnatal day 35. Other authors have reported profound increases in ileal ODC activity during periods of mucosal growth and adaptation, such as with refeeding, in adult rats [Fujimoto et al., 1992; Kuwayama and Naito, 1993]. In the absence of measurements for ODC activity in this study, it is not clear if the levels of ODC mRNA in the distal segment of the small intestine reflect increased enzyme activity at this postnatal time point. It is with interest that in addition to increased ODC mRNA content, we also reported findings of increased Na^+K^+ ATPase α_1 and β_1 mRNA abundance, exceeding adult values by day 35 in the distal segment of the small intestine only, as well as increased levels of SGLT1, Na^+K^+ ATPase α_1, and β_1 mRNA abundance in the distal small intestine relative to those measured in the proximal segment at this postnatal time point. Since intestinal mucosal growth and adaptation continues in the post-weaned period (days 21 to 40) [Buts and DeMeyer, 1981], the high mRNA content measured for these enzymes and transporter may reflect ongoing growth and maturational processes in the distal small intestine as signalled by ODC.

In the present work we examined developmental alterations in both mRNA abundance and protein expression in defined

segments of the postnatal rat small intestine. While this work has provided a global picture of the levels at which these intestinal enzymes and nutrient transporter may be regulated during postnatal ontogeny, we are unable to comment on the precise molecular and cellular mechanisms regulating their gene expression. Clearly, future work should employ immunocytochemical and <u>in situ</u> hybridization techniques to determine the relative importance of transcriptional and/or posttranscriptional events in the developmental regulation of ODC, SGLT1, SI, and the Na^+K^+ ATPase α_1 and β_1 subunit isoforms along both the crypt-villus axis and in defined intestinal segments. In addition, a better quantitative analysis of the developmental alterations in mRNA abundance could be achieved using ribonuclease protection assays [Roller et al., 1989; Sambrook et al., 1989].

In conclusion, we have shown the developmental regulation of both mRNA abundance and protein expression for SGLT1, sucrase-isomaltase, and the Na^+K^+ ATPase α_1 and β_1 subunit isoforms to be temporally associated with the process of weaning in the postnatal rat. Furthermore, we have shown developmental increases in ODC mRNA abundance to be in accordance with previous accounts of increased ODC activity in the mucosa of weaning rats. From these findings we conclude that while the ontogenesis of the intestinal enzymes and transporter examined in this work are coordinated to facilitate nutrient absorption around weaning, the individual developmental profiles of gene expression are not necessarily coordinated. This point is illustrated by the observation that in this study the developmental profiles of mRNA expression for SGLT1, SI, and the Na^+K^+ ATPase α_1 and β_1 isoforms were generally dissimilar. A comparison of the ontogeny for SGLT1 and SI located on the enterocyte apical membrane further illustrates this point in that adult levels of SGLT1 protein were measured at postnatal days 1[illegible] in the proximal small intestine, while SI protein was only first

detected by day 21 and had not yet reached adult levels of expression. By contrast, the developmental profiles of SGLT1 and SI protein expression were coordinated in the distal small intestine with adult levels of protein abundance reached by postnatal day 21. While the postnatal ontogeny of Na^+K^+ ATPase α_1 and $ß_1$ isoform protein expression did not parallel that observed for SGLT1 protein in the proximal intestinal segment, these variables showed similar developmental patterns of protein expression at weaning (day 21) in the distal small intestine. The developmental patterns of SI and Na^+K^+ ATPase α_1 and $ß_1$ isoform gene expression were generally dissimilar. Overall, in this study the greatest developmental increases in gene expression in the postnatal rat small intestine, with the exception of SGLT1 mRNA levels in the proximal segment of the intestine, occurred with the onset of weaning and thereafter.

Precocious Development Following Spermidine Exposure

Previous work from this laboratory has demonstrated a precocious development of intestinal morphology, enzymatic function, and Na^+/glucose transport activity in the suckling rat following the oral administration of exogenous spermidine [Wild et al., 1993; 1994]. The present work extends these findings by reporting the differential effects of spermidine exposure on the mRNA abundance and protein expression of selected intestinal enzymes and the Na^+/glucose co-transporter.

To our knowledge, the effects of spermidine exposure on SGLT1 mRNA abundance and protein expression have not been previously described in the postnatal rat small intestine. In the present work, increases in both mRNA abundance and protein expression were observed in the proximal and distal small intestine following consecutive exposures to oral spermidine. These spermidine-induced increases in SGLT1 gene expression at postnatal day 10 occurred prior to the usual onset of age-dependant developmental increases previously described in

Experiment I for SGLT1 in the rat. In addition to the reported precocious increases in SGLT1 protein content, a slight upward shift in the molecular weight of immunodetectable SGLT1 was observed at postnatal day 10 in both proximal and distal segments of the small intestine following consecutive spermidine exposures. This spermidine-induced shift in the molecular weight of SGLT1 was similar to the age-dependant increases in molecular weight that were previously described in Experiment I for the SGLT1 protein during postnatal development in the rat small intestine. Since the mature SGLT1 is a glycosylated integral membrane protein [Hirayama and Wright, 1992], these alterations to the molecular weight of this protein suggest that SGLT1 undergoes posttranslational modifications, such as glycosylation, during spermidine-induced precocious maturation. To support this, previous work from this laboratory has revealed spermidine-induced increases in both the fucose and galactose content of the enterocyte MVM prior to the onset of weaning that are consistent with precocious intestinal maturation [Wild et al., 1994]. Together, these findings indicate that consecutive exposures to spermidine can induce a precocious maturation of SGLT1 mRNA abundance and protein expression in the small intestine of suckling rats.

Georges et al. (1990) have described a time-dependent reversibility of spermine-induced intestinal maturation for disaccharidases accompanying the termination of consecutive treatment exposures. It is with interest that we report the precocious effects of consecutive spermidine exposures to be reduced in a time-dependent fashion in the absence of exogenous spermidine treatments. In the present work, SGLT1 protein expression was significantly reduced in the proximal small intestine of the spermidine-treated washout group at postnatal day 13 compared to both spermidine-treated day 10 rats, and the day 13 placebo group. Surprisingly, by day 13 the previous exposure to spermidine had reversed the

precocious maturation found at day 10 to the levels expressed by the day 10 placebo group. These findings contrast with those reported by Georges et al. (1990) whereby spermine-induced precocious increases in disaccharidase activity reverted to levels measured in age-matched controls, not to lower levels of activity, in a time-dependant fashion following the withdrawal of exogenous spermine. Georges and co-workers (1990) unfortunately did not describe SGLT1 activity in their work thereby limiting a comparison to our results. In the present work, the effects of spermidine withdrawal on SGLT1 protein were not as striking in the distal small intestine given that spermidine-induced increases in SGLT1 protein content were not maintained by day 13 in the absence of spermidine, but were similar to those of the day 13 placebo group. In addition, the washout spermidine-treated group showed no residual upward shifts in the apparent molecular weight of immunodetectable SGLT1 in either segment of the small intestine examined in this study. These findings suggest that the putative spermidine-induced posttranslational events assumed to alter the molecular weight of the SGLT1 protein, as well as spermidine-induced increases in SGLT1 protein content, do not persist in the absence of exogenous spermidine. The significance of these results is unknown. Clearly, a further analysis of these findings is required to ascertain whether SGLT1 transport activity was altered with the apparent reversion of this protein to a less mature form.

Previous studies have described the precocious induction of sucrase specific activity in the suckling rat small intestine following the administration of exogenous polyamines [Dufour et al., 1988; Wild et al. 1993; Buts et al., 1993; and Kaouass et al., 1994]. To our knowledge, the present work is the first to describe the effects of spermidine exposure on SI mRNA and protein expression in the suckling rat. In this study, there was no significant induction of SI mRNA following consecutive spermidine exposures. These results suggest that

spermidine exposure had no detectable effect on the transcriptional regulation of SI at these developmental time points. Given these findings for SI mRNA, it is with interest that we report an increased expression of SI protein in both the proximal and distal small intestine following consecutive exposures to spermidine. Previous investigations suggest that levels of SI mRNA closely parallel SI protein levels and activity in the weaning rat [Leeper and Henning, 1990; Nanthakumar and Henning, 1993; Krasinski et al., 1994]. Therefore in the present work low levels of SI mRNA, although undetectable, must have been present for SI protein synthesis to occur. The abrupt appearance of SI mRNA, accompanied by low levels of SI activity, has been demonstrated less than one day following the administration of exogenous glucocorticoids to adrenalectomized rats at postnatal day 10 [Nanthakumar and Henning, 1993]. Plasma corticosteronemia has been reported by Buts et al. (1993) in spermine-treated suckling rats exhibiting precocious SI activity, however the precocious induction of SI mRNA and protein expression were not examined in their study. Kaouass and co-workers (1994) have described a reduced induction of precocious intestinal enzyme activity and diminished levels of SI activity in adrenalectomized spermine-treated suckling rats, but they did not report the effect of these findings on SI mRNA and protein content. Their findings would suggest that surges in glucocorticoid levels in response to exogenously administered polyamines may influence the precocious maturational processes described in the suckling rat intestine. However, in the present work, spermidine exposure did not increase serum cortisol from the levels expected for this developmental time frame. While we may speculate that surges in endogenous glucocorticoids following exposure to exogenous polyamines may have a permissive effect on SI gene expression in the neonate, it is unlikely that spermidine-induced elevations in plasma glucocorticoids are solely required to enhance SI mRNA

detection. More likely, the neonatal levels of SI mRNA were too low to be detected given the methodology employed in this study which is primarily used to detect adult levels of SI mRNA.

Of interest, the present work showed an enhanced expression of SI protein in the distal small intestine following consecutive spermidine exposure relative to the levels measured in the proximal small intestine. Given the technical difficulty in measuring distinct protein bands in this enzyme complex by densitometry, we propose that these increases in SI expression in the distal small intestine also reflect the measurement of an increased maltase protein content. Georges et al. (1990) have reported the specific activity of maltase to be highest in the distal small intestine of suckling rats until the proximal to distal gradients of maltase enzyme expression are achieved at weaning. Furthermore, previous studies have reported increases in maltase activity following the consecutive administration of exogenous polyamines to suckling rats [Dufour et al., 1988; Georges et al., 1990; Wild et al., 1993; Buts et al., 1993; Kaouass et al., 1994]. These studies support our proposal that the measurement of a spermidine-enhanced expression of maltase protein in the distal small intestine contributed to the high spermidine-induced levels of SI protein measured in this intestinal region relative to the proximal intestinal segment.

Previous work from this laboratory has described significant precocious increases in Na^+K^+ ATPase activity following spermidine exposure for 1 or 3 days prior to sacrifice at postnatal day 10 [Wild et al., 1993]. To our knowledge the effects of spermidine exposure on Na^+K^+ ATPase α_1 and β_1 isoform mRNA abundance and protein expression have not been described. Overall, the results of this study do not agree with earlier observations reported by Wild and co-workers (1993) describing spermidine-induced increases in Na^+K^+ ATPase activity. In the present work, Na^+K^+ ATPase α_1 and β_1

isoform mRNA abundance increased from control levels following consecutive spermidine exposures in the proximal but not the distal small intestine. There were no spermidine-induced precocious increases in either α_1 or β_1 isoform protein content measured in the intestinal segments examined. It is unknown whether spermidine-induced alterations in Na^+K^+ ATPase activity occurred in the absence of precocious isoform expression since enzyme activity was not measured in this study. It seems unlikely that increases in Na^+K^+ ATPase activity following spermidine exposure would accompany these findings since enzyme activity has been found to correlate with Na^+K^+ ATPase isozyme gene expression during normal postnatal development [Zemelman et al., 1992]. In addition, Zemelman and co-workers (1992) have described the coordinated precocious maturation of Na^+K^+ ATPase activity with isozyme gene expression in the small intestine of suckling rats following cortisone treatment thereby providing further evidence that induced enzyme activity is preceded by enhanced Na^+K^+ ATPase gene expression. A further analysis of Na^+K^+ ATPase gene expression and enzyme activity following spermidine exposure is warranted to clarify the findings of the present work.

Given the precocious development observed for SGLT1 and SI expression following spermidine exposure in this study, we sought to examine the effects of spermidine exposure on ODC, a known marker of intestinal adaptive growth, at the level of mRNA abundance. The effects of spermidine exposure on ODC mRNA abundance in the suckling rat small intestine have not been previously described. In the present work, consecutive spermidine exposures induced significant increases in ODC mRNA abundance from control levels in both the proximal and distal small intestine. These findings agree with the levels of ODC activity described by Wild and co-workers (1993) whereby significant increases in ODC enzyme activity were measured following consecutive exposure to spermidine for three days prior to sacrifice at postnatal day 10. This observation

suggests that increases in ODC mRNA and activity are induced when consecutive spermidine exposures are first administered to suckling rats at postnatal day 7. Other studies however, have reported disparate findings with regard to ODC activity following the exposure of suckling rats to exogenous polyamines. Initial work by Dufour et al. (1988) described no change in ODC specific activity following a three day consecutive exposure to either spermine or spermidine starting at postnatal day 12. By contrast, a 27-fold depression of ODC activity has been reported in the jejunum of spermine-treated suckling rats at postnatal day 14 compared to controls [Buts et al., 1993]. The discordant findings for ODC activity reported by other investigators may have been influenced by the later postnatal age at which exogenous polyamines were administered. It is possible that the immature small intestine is more responsive to the precocious induction of ODC by polyamines when exposure occurs prior to postnatal day 10, as was the case in the present work and also that of Wild et al. (1993). In any event, given the tight regulation of intracellular polyamine concentrations through the negative feedback inhibition of ODC [Iwami et al., 1990], ODC activity would be expected to decrease following the administration of exogenous polyamines to suckling rats to prevent mucosal polyamine concentrations from exceeding beneficial limits. However, ODC activity is not necessarily associated with changes in ODC mRNA abundance given the multiple regulatory levels that have been described for this enzyme during periods of mucosal growth [Holm et al., 1989; Chung et al., 1992a, b]. It is plausible then for ODC mRNA abundance to be increased following exogenous spermidine treatments without coordinate increases in ODC enzyme activity. Future work in this area should include concomitant measurements of ODC gene expression and activity following exogenous polyamine exposure to verify if the spermidine-induced precocious expression of ODC mRNA reported in the present work is accompanied by similar

alterations in enzyme activity.

It is with interest that we report the unexpected effects of spermidine withdrawal on specific variables examined in this study following previous consecutive spermidine exposure. In this study, consecutive spermidine exposures of 8 μmol/day were not found to affect whole body or total intestinal weight. Previous studies have shown that whole body growth may be promoted by the supplementation of moderate amounts of polyamines to the diet [Smith, 1990; Buts et al., 1993]. In avians however, toxic concentrations of putrescine have been shown to depress food intake and consequently weight gain [Smith, 1990]. In the present work, we report a depressed weight gain in rats whose consecutive treatments of oral spermidine had been terminated for a three day interval prior to sacrifice. While possible, it seems unlikely that the depressed body weight observed in this group reflects spermidine toxicity from earlier consecutive exposures. In the event of spermidine toxicity, intestinal weight gain would most likely be depressed resulting from the increased energetic costs associated with polyamine excretion from intestinal tissues [Smith, 1990]. No such depression in total intestinal weight was observed in these rats. Significant precocious reductions in intestinal lactase activity have been demonstrated in suckling rats following consecutive exposures to spermine [Dufour et al., 1988] and spermidine [Wild et al., 1993]. Since maternal milk is still the primary source of nutrients for rats at postnatal day 13, it is feasible that depressed weight gain in this group is a consequence of reduced lactose digestion resulting from hypolactasia. Georges et al. (1990) reported significant reductions in ileal lactase activity for up to three days following the termination of consecutive exposures to spermine. Whole body weight gain was not examined in the aforementioned study. In the present work, none of the experimental groups demonstrated symptoms characteristic of lactose maldigestion. Clearly, the

evaluation of lactase after the termination of spermidine exposure is warranted to clarify this issue. In future work. it would be of interest to examine the time dependency of spermidine treatment effects on weight gain over a longer washout interval.

In addition, we found that the effects of spermidine-induced precocious development were also not maintained for gene expression when exogenous spermidine treatments were withdrawn. In this study, the previous exposure to exogenous spermidine prevented the levels of SGLT1 mRNA from reaching the levels measured in the placebo group in the distal small intestine. Moreover, the spermidine-induced increases in ODC mRNA abundance, Na^+K^+ ATPase isozyme mRNA abundance, SGLT1 protein, and SI protein (in the distal small intestine only), were not maintained and showed depressed levels compared to the placebo group by postnatal day 13 after spermidine treatments had been withdrawn. This reversion to lower levels of gene expression observed following the withdrawal of spermidine treatments could reflect a diminished mucosal growth and/or a dependence on exogenous polyamines for growth following consecutive exposures. These findings may suggest an "addiction" to exogenous spermidine whereby the mucosa of the suckling rat small intestine becomes unable to produce the levels of polyamines required to sustain normal developmental processes and growth in the absence of an exogenous source of polyamines. The mechanism(s) responsible for these apparent reversions in gene expression and depressed whole body growth following the termination of spermidine exposure is(are) unknown and warrant further investigation over a longer washout period.

In summary, the results of the present work show that exposure to exogenous spermidine at early postnatal time points prior to weaning can promote precocious developmental alterations in the mRNA abundance of ODC, SGLT1 and the Na^+K^+ ATPase α_1 and β_1 subunit isoforms, as well as to the expression

of SGLT1 and SI proteins. It appears however, that continued spermidine exposure must be provided for the observed precocious alterations to persist in suckling rats. Moreover, this work suggests that previous exposure to exogenous spermidine may induce mechanisms which compromise the normal developmental processes expected to resume in the small intestinal mucosa following the withdrawal of spermidine treatments. The significance of these observations to the maturation of intestinal absorptive function is unknown. We conclude that consecutive exposures to exogenous spermidine do not permanently alter the normal developmental patterns of gene expression for ornithine decarboxylase, SGLT1, sucrase-isomaltase, and the Na^+K^+ ATPase α_1 and β_1 subunit isoforms in the small intestinal mucosa of suckling rats.

Conclusion

Other authors have shown that the administration of exogenous polyamines to young rats prior to the onset of weaning can elicit a precocious maturation of both intestinal morphology and enzyme activity [Dufour et al., 1988; Georges et al., 1990; Buts et al., 1993; Wild et al., 1993; Kaouass et al., 1994]. The polyamines are thought to be key intracellular messengers involved in the modulation of postnatal intestinal ontogeny in the rat since mucosal ODC and polyamine content are known to increase substantially with weaning [Luk et al., 1980]. Although the polyamine content of rat milk increases during lactation, and increased levels of the polyamines are available to weaning rats with the ingestion of non-milk substances [Pollack et al., 1992], the question as to whether the polyamines are natural inducers of postnatal intestinal ontogeny remains unanswered.

In the present work, we conclude that exogenous polyamines are trophic substances for the immature small intestinal mucosa, since the administration of oral spermidine to young rats prior to the usual onset of intestinal maturation

associated with weaning could elicit a precocious development of mRNA and/or protein expression for the intestinal enzymes and the nutrient transporter examined in this study. The spermidine-induced precocious alterations to gene expression however, were not maintained once spermidine treatment was terminated, suggesting that a continued supply of exogenous spermidine is required to maintain this precocious development. The observation that gene expression could revert to immature levels following spermidine withdrawal points to a dependency on exogenous sources of spermidine to maintain enhanced maturational change. It would appear that spermidine-induced alterations to the gene expression of variables examined in this work are not permanent in the suckling rat small intestine.

The biological significance of milk-borne polyamines in the regulation of intestinal development is presently unknown. While the main polyamines are known to be present in human milk, the polyamine content and composition of commercial infant formulas does not parallel that of maternal milk throughout the lactation period, and is currently not supplemented with exogenous polyamines [Pollack et al., 1992]. Whether the ingestion of formulas supplemented with polyamines could enhance intestinal development in the premature infant or could promote intestinal closure to the uptake of macromolecules in neonates is an area for future study. Given that spermidine-induced precocious development was not permanent in this study, and a reversion to immature forms was observed for some variables following the termination of spermidine exposure, we caution the future study of polyamine supplementation to humans. Clearly, the mechanisms influencing both spermidine-induced precocious development and the events observed following spermidine withdrawal must be thoroughly resolved before human studies can be contemplated.

BIBLIOGRAPHY

Ahearn,G.A., Behnke,R.D., Zonno,V., and Storelli,C. (1992) Kinetic heterogeneity of Na-D-Glucose cotransport in teleost gastrointestinal tract. Am J Physiol 263 (Regulatory Integrative Comp Physiol 32):R1018-R1023.

Alarcon,P., Lebenthal,E., and Lee,P.C. (1987) Effect of difluoromethyl ornithine (DFMO) on small intestine of adult and weanling rats. Digest Disease Science 32(8):883-888.

Ausubel,F.M., Brent,R., Kingston,R.E., Moore,D.D., Seidman, J.G., Smith,J.A., and Struhl,K. (eds.) (1989) Current Protocols in Molecular Biology, chapter 4.1. Greene Publishing Associates and Wiley-Interscience, New York.

Auvinen,M., Paasinen,A., Andersson,L.C., and Holtta,E. (1992) Ornithine decarboxylase activity is critical for cell transformation. Nature 360:355-358.

Bamba,T., Vaja,S., Murphy,G.M., and Dowling,R.H. (1990) Role of polyamines in the early adaptive response to jejunectomy in the rat: Effect of DFMO on the ileal villus:crypt axis. Digestion 46(Suppl.2):410-423.

Bamba,T., Vaja,S., Murphy,G.M., and Dowling,R.H. (1990) Effect of fasting and feeding on polyamines and related enzymes along the villus:crypt axis. Digestion 46(suppl. 2):424-429.

Bardocz,S., Brown,D.S., Grant,G., and Pusztai,A. (1990) Luminal and basolateral polyamine uptake by rat small intestine stimulated to grow by Phaseolus vulgaris lectin phytohaemagglutinin in vivo. Biochim Biophys Acta 1034:46-52.

Bardocz,S., Grant,G., Brown,D.S., Ewen,S.W.B., Stewart,J.C., and Pusztai,A. (1991) Effect of fasting and refeeding on basolateral polyamine uptake and metabolism by the rat small bowel. Digestion 50:28-35.

Bardocz,S., Grant,G., Brown,D.S., Ralph,A., and Pusztai,A. (1993) Polyamines in food-implications for growth and health. J Nutr Biochem 4:66-71.

Beaulieu,J-F., Nichols,B., and Quaroni,A. (1989) Posttranslational regulation of sucrase-isomaltase expression in intestinal crypt and villus cells. J Biol Chem 264(33): 2000-20011.

Bennett,E., and Kimmich,G.A. (1992) Na^+ binding to the Na^+-glucose cotransporter is potential dependent. Am J Physiol 262 (Cell Physiol. 31):C510-C516.

Birnir,B., Lee,H-S., Hediger,M.A., and Wright,E.M. (1990) Expression and characterization of the intestinal Na^+/ glucose cotransporter in COS-7 cells. Biochem Biophys Acta 1048:100-104.

Blackshear,P.J., Manzella,J.M., Stumpo,D.J., Wen,L., Huang,J-K., Oyen,O., and Young,W.S. (1989) High level, cell-specific expression of ornithine decarboxylase transcripts in rat genitourinary tissues. Molecular Endocrinology 3:68-78.

Broyart,J-P., Hugot,J-P., Perret,C., and Porteu,A. (1990) Molecular cloning and characterization of a rat intestinal sucrase-isomaltase cDNA. Regulation of sucrase-isomaltase gene expression by sucrose feeding. Biochim Biophys Acta 1087:61-67.

Buddington,R.K. (1992) Intestinal nutrient transport during ontogeny of vertebrates. Am J Physiol 263 (Regulatory Integrative Comp. Physiol. 32):R503-R509.

Buddington,R.K. (1994) Nutrition and ontogenetic development of the intestine. Can J Physiol Pharmacol 72:251-259.

Buddington,R.K., and Diamond,J.M. (1989) Ontogenetic development of intestinal nutrient transporters. Annu Rev Physiol 51:601-619.

Burant,C.F., Flink,S., Depaoli,A.M., Chen,J., Lee,W-S., Hediger,A., Buse,J.B., and Chang,E.B. (1994) Small intestine hexose transport in experimental diabetes: increased transporter mRNA and protein expression in enterocytes. J Clin Invest 93:578-585.

Burant,C.F., and Saxena,M. (1994) Rapid reversible substrate regulation of fructose transporter expression in rat small intestine and kidney. Am J Physiol 267(1):G71-G79.

Burant,C.F., Takeda,J., Brot-Laroche,E., Bell,G.I., and Davidson,N.O. (1992) Fructose transporter in human spermatozoa and small intestine is GLUT5. J Biol Chem 267:14523-14526.

Buts,J-P., and De Meyer,R. (1981) Postnatal proximodistal development of the small bowel mucosal mass in growing rats. Biol. Neonate 40:62-69.

Buts,J-P., De Keyser,N., Kolanowski,J., Sokal,E., and Van Hoof,F. (1993) Maturation of villus and crypt cell functions in rat small intestine. Role of dietary polyamines. Digestive Diseases and Sciences 38(6):1091-1098.

Castillo,R.O., Feng,J.J., Stevenson,D.K., and Kwong,L.K. (1992) Maturation of jejunoileal gradients in rat intestine: The role of intraluminal nutrients. Biol Neonate 62:351-362.

Chomczynski,P., and Sacchi,N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162, 156-159.

Chung,D.H., Evers,M., Townsend,C.M., Huang,K.F., Herndon,D.N., and Thompson,J.C. (1993) Role of polyamine biosynthesis during gut mucosal adaptation after burn injury. Am J Surg 165:144-149.

Chung,D.H., Evers,M., Townsend,C.M., Herndon,D.N., Ko,T.C., Uchida,T., and Thompson,J.C. (1992) Cytokine regulation of gut ornithine decarboxylase gene expression and enzyme activity. Surgery 112:364-369.

Davidson,N.O., Hausman,A.M.L., Ifkovits,C.A., Buse,J.B., Gould,G.W., Burant,C.F., and Bell,G.I. (1992) Human intestinal glucose transporter expression and localization of GLUT5. Am J Physiol. 262 (Cell Physiol. 31):C795-C800.

Dembinski and Johnson,L.R. (1984) Stimulation of mucosal growth by a dietary amine. Am J Physiol 247(Gastrointest. Liver Physiol.10):G352-G356.

Desjeux,J.-F. (1989) Congenital selective Na^+/D-glucose cotransport defects leading to renal glycosuria and congenital selective intestinal malabsorption of glucose and galactose. In The Metabolic Basis of Inherited Disease, ed. C. Scriver, A. Beaudet, W. Sly, D. Valle, pp.2463-78. New York: McGraw-Hill.

Diamond, J.M. (1986) Hard-wired local triggering of intestinal enzyme expression. Nature 324:408-409.

Dufour,C., Dandrifosse,G., Forget,P., Vermesse,F., Romain,N., and Lepoint,P. (1988) Spermine and spermidine induce intestinal maturation in the rat. Gastroenterology 95:112-116.

Farman,N., Coutry,N., Logvinenko,N., Blot-Chabaud,M., Bourbouze,R., and Bonvalet,J.P. (1992) Adrenalectomy reduces α_1 and not β_1 Na^+K^+ ATPase mRNA expression in rat distal nephron. Am J Physiol 263 (Cell Physiol. 32):C810-C817.

Ferraris,R.P., Casirola,D.M., and Vinnakota,R.R. (1993) Dietary carbohydrate enhances intestinal sugar transport in diabetic mice. Diabetes 42:1579-1587.

Ferraris,R.P., Villenas,S.A., Hirayama,B.A., and Diamond,J. (1992a) Effect of diet on glucose transporter site density along the intestinal crypt-villus axis. Am J Physiol 262 (Gastrointest. Liver Physiol. 25):G1060-G1068.

Ferraris,R.P., Villenas,S.A., and Diamond,J. (1992b) Crypt-villus site of glucose transporter induction by dietary carbohydrate in mouse intestine. Am J Physiol 262 (Gastrointest. Liver Physiol. 25):1069-G1073.

Fitzpatrick,L.R., Wang,P., Eikenburg,B.E., Haddox,M.K., and Johnson,L.R. (1986) Effect of refeeding on polyamine biosynthesis in isolated enterocytes. Am J Physiol 250 (Gastrointest. Liver Physiol. 13):G709-G713.

Freeman,H.J., and Quamme,G.A. (1986) Age-related changes in sodium-dependent glucose transport in rat small intestine. Am J Physiol 251 (Gastrointest. Liver Physiol. 14):G208-G217.

Freeman,T.C., Wood,I.S., Sirinathsinghji,D.J., Beechey,R.B., Dyer,J., and Shirazi-Beechey,S.P. (1993) The expression of the Na^+/glucose cotransporter (SGLT1) gene in lamb small intestine during postnatal development. Biochim Biophys Acta 1146:203-212.

Freund,J-N., Torp,N., Duluc,I., Foltzer-Jourdainne,C., Danielsen,M., and Raul,F. (1990) Comparative expression of the mRNA for three intestinal hydrolases during postnatal development in the rat. Cell Mol Biol 36:729-736.

Fujimoto,K., Granger,D.N., Johnson,L.R., Price,V.H., Sakata,T., and Tso,P. (1992) Circadian rhythm of ornithine decarboxylase activity in small intestine of fasted rats. PBESM 200:409-413.

Fujimoto,K., Granger,D.N., Price,V.H., and Tso,P. (1991) Ornithine decarboxylase is involved in repair of small intestine after ischemia-reperfusion in rats. Am J Physiol 261 (Gastrointest. Liver Physiol. 24): G523-G529.

Fuller,P.J., and Verity,K. (1990) Mineralocorticoid receptor gene expression in the gastrointestinal tract: distribution and ontogeny. J Steroid Biochem 36(4):263-267.

Georges,P., Dandrifosse,G., Vermesse,F., Forget,P., Deloyer,P., and Romain,N. (1990) Reversibility of spermine-induced intestinal maturation in the rat. Digestive Diseases and Sciences 35(12):1528-1535.

Ghishan,F.K., and Wilson,F.A. (1985) Developmental maturation of D-glucose transport by rat jejunal brush-border membrane vesicles. Am J Physiol 284 (Gastrointest. Liver Physiol. 11):G87-G92.

Ginty,D.D., Marlowe,M., Pekala,P.H., and Seidel,E.R. (1990) Multiple pathways for the regulation of ornithine decarboxylase in intestinal epithelial cells. Am J Physiol 258 (Gastrointest. Liver Physiol. 21): G454-G460.

Haase,W., Heitmann,K., Freise,W., Ollig,D., and Koepsell,H. (1990) Characterization and histochemical localization of the rat intestinal Na^+-D-glucose cotransporter by monoclonal antibodies. Eur J Cell Biol 52:297-309.

Heby,O., and Persson,L. (1990) Molecular genetics of polyamine synthesis in eukaryotic cells. TIBS 15:152-158.

Hediger, M.A., Coady, M.J., Ikeda, T.S., and Wright, E.M. (1987a) Expression cloning and cDNA sequencing of the Na^+/glucose co-transporter. Nature 330:379-381.

Hediger,M.A., Ikeda,T., Coady,M., Gundersen,C.B., and Wright,E.M. (1987b) Expression of size-selected mRNA encoding the intestinal Na/glucose cotransporter in Xenopus laevis oocytes. Proc Natl Acad Sci USA 84:2634-2637.

Hediger,M.A., Turk,E., and Wright,E.M. (1989) Homology of the human intestinal Na^+/glucose and E. coli Na^+/proline cotransporters. Proc Natl Acad Sci USA 86:5748-5752.

Hediger,M.A., Turk,E., and Wright,E.M. (1991) Homology of the human intestinal Na^+/glucose and Escherichia coli Na^+/proline cotransporters. Proc Natl Acad Sci 86:5748-52.

Hediger,M.A., and Rhoads,D.B. (1994) Molecular physiology of sodium-glucose cotransporters. Physiol. Rev. 74(4):993-1026.

Henning,S.J. (1978) Permissive role of thyroxine in the ontogeny of jejunal sucrase. Endocrinology 102:9-15.

Henning,S.J. (1981) Postnatal development: coordination of feeding, digestion, and metabolism. Am J Physiol 241 (Gastrointest. Liver Physiol. 4):G199-G214.

Henning,S.J. (1985) Ontogeny of enzymes in the small intestine. Annu Rev Physiol 47:231-245.

Henning,S.J., Helman,T.A., and Kretchmer,N. (1975). Studies on normal and precocious appearance of jejunal sucrase in suckling rats. Biol Neonate 26:249-262.

Hirayama,B.A., Loo,D.D.F., and Wright,E.M. (1994) Protons drive sugar transport through the Na^+/glucose cotransporter (SGLT1). J Biol Chem 269(34):21407-21410.

Hirayama,B.A., Smith,C.D., and Wright,E.M. (1992) Secondary structure of the Na^+/glucose cotransporter. J Gen Physiol 100:19a-20.

Hirayama,B.A., Wong,H.C., Smith,C.D. Hagenbuch,B.A., Hediger,M.A., and Wright,E.M. (1991) Intestinal and renal Na^+/glucose co-transporters share common structures. Am J Physiol 261 (Cell Physiol. 30):C296-C304.

Hirayama,B.A., and Wright,E.M. (1992) Glycosylation of the rabbit intestinal brush border Na^+/glucose cotransporter. Biochim Biophys Acta 1103:37-44.

Hirano,H. (1992) Immunohistochemical localization of Na^+-dependent glucose transporter in rat jejunum. Cell Tissue Res 267:3-9.

Hodin,R.A., Chamberlain,S.M., and Upton,M.P. (1992) Thyroid hormone differentially regulates rat intestinal brush border enzyme gene expression. Gastroenterology 103:1529-1536.

Hoffman,L.R., and Chang,E.B. (1993) Regional expression and regulation of intestinal sucrase-isomaltase. J Nutr Biochem (4):130-141.

Holm,I., Persson,L., Stjernborg,L., Thorsson,L., and Heby,O. (1989) Feedback control of ornithine decarboxylase expression by polyamines. Biochem. J 258:343-350.

Holt,P.R., and Yeh,K-Y. (1992) Effects of starvation and refeeding on jejunal disaccharidase activity. Digestive Diseases and Sciences 37(6):827-832.

Horisberger,J.D., Lamas,V., Krahenbiuhl,J.P., and Rossier,B.C. (1991) Structure function relationship of Na,K-ATPase. Ann Rev Physiol 53:565-584.

Hunziker,W., Speiss,M., Semenza,G., and Lodish,H.F. (1986) The sucrase-isomaltase complex: Primary structure, membrane orientation, and evolution of a stalked, intrinsic brush border protein. Cell 46:227-234.

Hwang,E-S., Hirayama,B.A., and Wright,E.M. (1991) Distribution of the SGLT1 Na^+/glucose cotransporter and mRNA along the crypt-villus axis of rabbit small intestine. Biochem Biophys Res Comm 181(3):1208-1217.

Ikeda,T.S., Hwang,E.S., Coady,M.J., Hirayama,B.A., Hediger, M.A., and Wright,E.M. (1989) Characterization of a Na^+/glucose cotransporter cloned from rabbit small intestine. J Membrane Biol 110:87-95.

Iwami,K., Wang,J-Y., Jain,R., McCormack,S., and Johnson,L.R. (1990) Intestinal ornithine decarboxylase:half-life and regulation by putrescine. Am J Physiol 258 (Gastrointest. Liver Physiol. 21): G308-G315.

Jackowski,S., and Alix,J.H. (1990) Cloning, sequence, and expression of the pantothenate permease (panF) gene in Escherichia coli. J Bacteriol 172:3842-3848.

Jansson,M., Nilsson,B-O., Rosengren,E., Ekstrom,J., and Lundgren,O. (1993) Effects of luminal stimuli on polyamine metabolism in the small intestine of the rat:the role of enteric nerves. Acta Physiol Scand 149:483-490.

Johnson,L.R., Tseng,C-C., Wang,P., Tipnis,U.R., and Haddox,M.K. (1989) Mucosal ornithine decarboxylase in the small intestine:localization and stimulation. Am J Physiol 256 (Gastrointest. Liver Physiol. 19): G624-G630.

Jumawan,J., and Koldovsky,O. (1978) Comparison of the effect of various doses of thyroxine on jejunal disaccharidases in intact and adrenalectomized rats during the first three weeks of life. Enzyme 23:206-209.

Kanai,Y., Lee,W-S., You,G., Brown,D., and Hediger,M.A. (1994) The human kidney low affinity Na^+/glucose cotransporter SGLT2: delineation of the major renal reabsorptive mechanism for D-glucose. J Clin Invest 93:397-404.

Karasov.,W.H. (1991) Tests of the adaptive modulation hypothesis for dietary control of intestinal nutrient transport. Am J Physiol 263 (Regulatory Integrative Comp. Physiol. 32):R496-R502.

Karasov,W.H., and Diamond,J.M. (1983) Adaptive regulation of sugar and amino acid transport by vertebrate intestine. Am J Physiol 243:G443-G462.

Kaouass,M., Deloyer,P., and Dandrifosse,G. (1994) Intestinal development in suckling rats:direct or indirect spermine action? Digestion 55:160-167.

Kaouass,M., Sulon,J., Deloyer,P., and Dandrifosse,G. (1994) Spermine-induced precocious intestinal maturation in suckling rats: possible involvment of glucocorticoids. J Endocrinology 141:279-283.

Kayano,T., Burant,C.F., Fukumoto,H., Gould,G.W., Fan,Y-S., Eddy,R.L., Byers,M.G., Shows,T.B., Seino,S., and Bell,G.I. (1990) Human facilitative glucose transporters. J Biol Chem 265(22):13276-13282.

Kessler,M., Acuto,O., Storelli,C., Murer,H., Muller,M., and Semenza,G. (1978) A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush border membranes. Their use in investigating some properties of D-glucose and choline transport systems. Biochim Biophys Acta 506:136-154.

Kobayashi,M., Iseki,K., Saitoh,H., and Miyazaki,K. (1992) Uptake characteristics of polyamines into rat intestinal brush-border membrane. Biochem et Biophysica Acta 1105:177-183.

Koepsell,H.K., Korn,A., Raszeja-Specht,S., Bernotat-Danielowski,D. (1988) Characterization and histochemical localization of the rat intestinal Na^+-D-glucose cotransporter by monoclonal antibodies. J Biol Chem 263:18419-18429.

Koepsell,H., and Spangenberg,J. (1994) Topical Review. Function and presumed molecular structure of Na^+-D-Glucose cotransport systems. J Membrane Biol 138:1-11.

Kong,C-T., Yet,S-F., and Lever,J.E. (1993) Cloning and expression of a mammalian Na^+/amino acid cotransporter with sequence similarity to Na^+/glucose cotransporters. J Biol Chem 268:1059-1512.

Krasinski,S.D., Estrada,G., Yeh,K-Y., Yeh,M., Traber,P.G., Rings,E.H.H.M., Buller,H.A., Verhave,M., Montgomery,R.K., and Grand,R.J. (1994) Transcriptional regulation of intestinal hydrolase biosynthesis during postnatal development in rats. Am J Physiol 267 (Gastrointest. Liver Physiol. 30): G584-G594.

Kumagai,J., Jain,R., and Johnson,L.R. (1989) Characteristics of spermidine uptake by isolated rat enterocytes. Am J Physiol 256 (Gastrointest. Liver Physiol. 19):G905-G910.

Kuwayama,H., and Naito,T. (1993) Effects of prostaglandins on ornithine decarboxylase activity in rat small intestine. Digestive Diseases and Sciences 38(6):1087-1090.

Kwon,H.M., Yamauchi,A., Uchida,S., Preston,A.S., Garcia-Perez,A., Burg,M., and Handler,J.S. (1992) Cloning of the cDNA for a Na^+/myo-inositol cotransporter, a hypertonicity stress protein. J Biol Chem 267:6297-6301.

Lebanthal,E. (1989) Concepts in gastrointestinal development. In Human Gastrointestinal Development, ed. E. Lebanthal pp. 3-18, New York, Raven Press.

Lee,W-S., Kanai,Y., Wells,R.G., and Hediger,M.A. (1994) The high affinity Na^+/glucose cotransporter. Re-evaluation of function and distribution of expression. J Biol Chem 269(16):12032-12039.

Leeper,L.L., and Henning,S.J. (1990) Development and tissue distribution of sucrase-isomaltase mRNA in rats. Am J Physiol 258 (Gastrointest. Liver Physiol 21):G52-G58.

Lescale-Matys,L., Dyer,J., Scott,D., Freeman,T.C., Wright,E.M., and Shirazi-Beechey,S.P. (1993) Regulation of the ovine intestinal Na^+/glucose cotransporter (SGLT1) is dissociated from mRNA abundance. Biochem J 291:435-440.

Levin,R.J. (1994) Digestion and absorption of carbohydrates - from molecules and membranes to humans. Am J Clin Nutr 59 (suppl):690s-698s.

Liu,T., Reisenauer,A.M., and Castillo,R.O. (1992) Ontogeny of intestinal lactase: posttranslational regulation by thyroxine. Am J Physiol. 263 (Gastrointest. Liver Physiol. 26):G538-G543.

Lorenzsonn,V., Korsmo,H., and Olsen,W.A. (1987) Localization of sucrase-isomaltase in the rat enterocyte. Gastroenterology 92:98-105.

Luk,G.D., Marton,L.J., Baylin,S.B. (1980) Ornithine decarboxylase is important in intestinal mucosal maturation and recovery from injury in rats. Science 210:195-198.

Luk,G.D., and Yang,P. (1987) Polyamines in intestinal and pancreatic adaptation. Gut 28(S1):95-101.

Luk,G.D., and Yang,P. (1988) Distribution of polyamines and their biosynthetic enzymes in intestinal adaptation. Am J Physiol 254(Gastrointest. Liver Physiol. 17):G194-G200.

Malo,C., Qureshi,I.A., and Letarte,J. (1986) Postnatal maturation of enterocytes in sparse-fur mutant mice. Am J Physiol 250 (Gastrointest. Liver Physiol. 13):G177-G184.

Malo,C., and Berteloot,A. (1987) Proximo-distal gradient of Na^+-dependent D-glucose transport activity in the brush border membrane vesicles from the human fetal small intestine. FEB 220(1):201-205.

Mamont,P.S., Bohlen,S., McCann,P.P., Bey,P., Schuber,F., and Tardif,C. (1976) α-Methylornithine, a potent competitive inhibitor of ornithine decarboxylase blocks proliferation of rat hepatoma cells in culture. Proc Natl Acad Sci USA 73: 1626-1630.

Mamont,P.S., Duchesne,M-C., Grove,J., and Bey,P. (1978) Anti-proliferative properties of DL-α-difluoromethylornithine in cultured cells. A consequence of the irreversible inhibition of ornithine decarboxylase. Biochem Biophys Res Commun 81:58-66.

McCormack,S.A., and Johnson,L.R. (1991) Role of polyamines in gastrointestinal mucosal growth. Am J Physiol 260 (Gastrointest. Liver Physiol.23):G795-G806.

McCormack,S.A., Viar,M.J., and Johnson,L.R. (1993) Polyamines are necessary for cell migration by a small intestinal crypt cell line. Am J Physiol 264 (Gastrointest. Liver Physiol. 27): G367-G374.

Meddings,J.B., DeSouza,D., Goel,M., and Thiesen,S. (1990) Glucose transport and microvillus membrane physical properties along the crypt-villus axis of the rabbit. J Clin Invest 85:1099-1107.

Meddings,J.B., and Theisen,S. (1989) Development of rat jejunum: lipid permeability, physical properties, and chemical composition. Am J Physiol. 256 (Gastrointest. Liver Physiol. 19):G931-G940.

Meddings,J., and Westergaard,H. (1989) Intestinal glucose transport using perfused rat jejunum in vivo: model analysis and derivation of corrected kinetic constants. Clinical Science 76:403-413.

Miyamoto,K-I., Hase,K., Takagi,T., Fujii,T., Taketani,Y., Minami,H., Oka,T., and Nakabou,Y. (1993) Differential responses of intestinal glucose transporter mRNA transcripts to levels of dietary sugars. Biochem J 295:211-215.

Miyamoto,K-I., Hase,K., Taketani,Y., Minami,H., Oka,T., Nakabou,Y., and Hagihira,H. (1991) Diabetes and glucose transporter gene expression in rat small intestine. Biochem Biophys Res Commun 181:1110-1117.

Miyamoto,K-I., Hase,K., Taketani,Y., Minami,H., Oka,T., Nakabou,Y., and Hagihira,H. (1992) Developmental changes in intestinal glucose transporter mRNA levels. Biochem Biophys Res Comm 183(2):626-631.

Murnane,M-J., and Tam,S.W. (1992) Isolation and characterization of RNA from snap-frozen tissues and cultured cells. J Nutr Biochem 3:251-260.

Nanthakumar,N.N., and Henning,S.J. (1993) Ontogeny of sucrase-isomaltase gene expression in rat intestine: responsiveness to glucocorticoids. Am J Physiol 264 (Gastrointest. Liver Physiol. 27):G306-G311.

Neeb,M., Kunz,U., and Koepsell,H. (1987) Identification of a D-glucose binding Na^+-D-glucose cotransporter. J Biol Chem 262:10718-10727.

Orlowski,J., and Lingrel,J.B. (1988) Tissue-specific and developmental regulation of rat Na,K-Atpase catalytic α isoform and ß subunit mRNAs. J Biol Chem 263(21):10436-10442.

Osborne,D.L., and Seidel,E.R. (1990) Gastrointestinal luminal polyamines: cellular accumulation and enterohepatic circulation. Am J Physiol 258 (Gastrointest. Liver Physiol. 21):G576-G584.

Pajor,A.M., and Wright,E.M. (1992) Cloning and functional expression of a mammalian Na^+/nucleoside cotransporter. J Biol Chem 267(6):3557-3560.

Peerce,B.E., Cedilote,M., and Clarke,R.D. (1993) Role of carboxyl and sulfhydryl residues on rabbit small intestinal brush-border membrane Na^+-glucose cotransporter. Am J Physiol 264 (Gastrointest. Liver Physiol. 27):G294-G299.

Peerce,P., and Clarke,R. (1990) Isolation and reconstitution of the intestinal Na^+/glucose cotransporter. J Biol Chem 265:1731-1736.

Peng,H., and Lever,J.E. (1993) Polyamine regulation of Na^+/glucose symporter expression in LLC-PK_1 cells. J Cell Physiol 154:238-247.

Pegg,A.E., and McCann,P.P. (1982) Brief Review. Polyamine metabolism and function. Am J Physiol 243 (Cell Physiol. 12):C212-C221.

Pegg,A.E. (1986) Review Article. Recent advances in the biochemistry of polyamines in eukaryotes. Biochem J 243:249-262.

Pollack,P.F., Koldovsky,O., and Nishioka,K. (1990) Polyamine content of rat and human milk and infant formula. Ped Res 27:289A.

Pollack,P.F., Koldovsky,O., and Nishioka,K. (1992) Polyamines in human and rat milk and in infant formulas. Am J Clin Nutr 56:371-375.

Roller,R.J., Kinloch,R.J., Hiraoka,B.Y., Li,S.-L., and Wasserman,P.M. (1989) Gene expression during mammalian oogenesis and early embryogenesis: quantification of three messenger RNAs abundant in fully grown mouse oocytes. Development 106:251-261.

Romain,N., Dandrifosse,G., Jeusette,F., and Forget,P. (1992) Polyamine concentration in rat milk and food, human milk, and infant formulas. Pediatric Research 32(1):58-63.

Sambrook,J., Fritsch,E.F., and Maniatis,T. (1989) Molecular Cloning: A Laboratory Manual, 2d ed, chapter 7. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Sauriol,N. (1993) Alterations in Na,K-ATPase expression in defined small intestine segments during postnatal development. M.Sc. Thesis dissertation, McGill University, Montreal, Quebec.

Scalabrino,G., Lorenzini,E.C., and Ferioli,M.E. (1991) Polyamines and mammalian hormones. Part I: Biosynthesis, interconversion and hormone effects. Molecular and Cellular Endocrin 77, 1-35.

Scalabrino,G., and Lorenzini,E.C. (1991) Polyamines and mammalian hormones. Part II: Paracrine signals and intracellular regulators. Molecular and Cellular Endocrin 77, 37-56.

Schmidt,U.M., Eddy,B., Fraser,C.M., Venter,J.C., and Semenza,G. (1983) Isolation of (a subunit of) the Na^+/D-glucose cotransporter(s) of rabbit intestinal brush border membranes using monoclonal antibodies. FEBS Lett 161:279-283.

Shirazi-Beechey,S.P., Hirayama,B.A., Wang,Y., Scott,D., Smith,M.W., and Wright,E.M. (1991) Ontogenetic development of lamb intestinal sodium-glucose cotransporter is regulated by diet. J Physiol 437:699-708.

Shirazi-Beechey,S.P., Hirayama,B.A., Wang,Y., Scott,D. et al. (1993) The expression of the Na^+/glucose cotransporter $SGLT_1$ gene in lamb small intestine during post-natal development. Biochim Biophys Acta 1146:203-212.

Shyjan,A.W., and Levenson,R. (1989) Antisera specific for the alpha 1, alpha 2, alpha 3, and beta subunits of the Na,K-ATPase: Differential expression of alpha and beta subunits in rat tissue membranes. Biochemistry 28:4531-4535.

Smith,T.K. (1990) Effect of dietary putrescine on whole body growth and polyamine metabolism. Proc. Exp. Biol. Med. 194(4):332-336.

Smith,C.D., Hirayama,B.A., and Wright,E.M. (1992) Baculovirus-mediated expression of the Na^+/glucose cotransporter in Sf9 cells. Biochim Biophys Acta 1104(1):151-159.

Tabata,K., and Johnson,L.R. (1986a) Ornithine decarboxylase and mucosal growth in response to feeding. Am J Physiol 251 (Gastrointest and Liver Physiol):G270-G274.

Tabata,K., and Johnson,L.R. (1986b) Mechanism of induction of mucosal ornithine decarboxylase by food. Am J Physiol 251 (Gastrointest. Liver Physiol. 14):G370-G374.

Takata,K., Kasahara,T., Kasahara,M., Ezaki,O., and Hirano,H. (1991) Localization of Na^+-dependent active type and erythrocyte/hepG2-type glucose transporters in rat kidney: Immunofluorescence and immunogold study. J Histochem Cytochem 39(3):287-298.

Takata,K., Kasahara,T., Kasahara,M., Ezaki,O., and Hirano,H. (1992) Immunohistochemical localization of Na^+-dependent glucose transporter in rat jejunum. Cell Tissue Res 267:3-9.

Thorens,B. (1993) Facilitated glucose transporters in epithelial cells. Ann Rev Physiol 55:591-608.

Toloza,E.M., and Diamond,J. (1992) Ontogenetic development of nutrient transporters in rat intestine. Am J Physiol 263 (Gastrointest. Liver Physiol. 26):G593-G604.

Traber,P.G., Yu,L., Wu,G.D., and Judge,T.A. (1992) Sucrase-isomaltase gene expression along crypt-villus axis of human small intestine is regulated at level of mRNA abundance. Am J Physiol 262 (Gastrointest. Liver Physiol. 25):G123-G130.

Tsuboi,K.K., Schwartz,S.M., Burrill,P.H., Kwong,L.K., and Sunshine,P. (1979) Sugar hydrolases of the infant rat intestine and their arrangement on the brush border membrane. Biochim Biophys Acta 554:234-248.

Tsuboi,K.K., Kwong,L.K., Neu,J., and Sunshine,P. (1981) A proposed mechanism of normal intestinal lactase decline in the postweaned animal. Biochem Biophys Res Commun 101:642-652.

Tsuboi,K.K., Kwong,L.K., Sunshine,P., and Castillo,R.O. (1992) Mechanism of maturational decline of rat intestinal lactase-phlorizin hydrolase Biochem J 282:107-113.

Turk,E., Klisak,I., Bacallao,R., Sparkes,R.S., and Wright,E.M. (1993) Assignment of the human Na^+/glucose cotransporter gene SGLT1 to chromosome 22q13.1. Genomics 17:752-754.

Turk,E., Martin,M.G., and Wright,E.M. (1994) Structure of the human Na^+/glucose cotransporter gene SGLT1. J Biol Chem 269(21):15204-15209.

Turk,E., Zabel,B., Mundlos,S., Dyer,J., and Wright,E.M. (1991) Glucose/galactose malabsorption caused by a defect in the Na^+/glucose cotransporter. Nature 350:354-356.

Veyhl,M., Puschel,B., Sprangenberg,J., Dekel,C., and Koepsell,H. (1992) Cloning of the ß-subunit of the Na^+-D-glucose symporter. FASEB J 6:A1459.

Veyhl,M., Spangenberg,J., Puschel,B., Poppe,R., Dekel,C., Fritzsch,G., Haase,W., and Koepsell,H. (1993) Cloning of a membrane-associated protein which modifies activity and properties of the Na^+-D-glucose cotransporter. J Biol Chem 268(33):25041-25053.

Vogelstein,B., and Gillespie,D. (1979) Preparative and analytical purification of DNA from agarose. Proc Natl Acad Sci USA 76:615-619.

Wang,J-Y., and Johnson,L.R. (1992) Luminal polyamines substitute for tissue polyamines in duodenal mucosal repair after stress in rats. Gastroenterology 102:1109-1117.

Wang,J-Y., Johnson,L.R., Tsai,Y-H., and Castro,G.A. (1991) Mucosal ornithine decarboxylase, polyamines, and hyperplasia in infected intestine. Am J Physiol. 260 (Gastrointest. Liver Physiol. 23): G450-G51.

Weaver,L.T., and Walker,W.A. (1989) Transport of macromolecules in the neonate. In Human Gastrointestinal Development. E. Lebanthal (ed). New York, Raven Press, pp.731-749.

Weber,W., Puschel,B., Steffgen,J., Koepsell,H., and Scharz,W. (1991) Comparison of a Na^+/D-glucose cotransporter from rat intestine expressed in oocytes of Xenopus laevis with the endogenous cotransporter. Biochim Biophys Acta 1063:73-80.

Wild,G.E., Daly,A.S., Sauriol,N., and Bennett,G. (1993) Effect of exogenously administered polyamine on the structural maturation and enzyme ontogeny of the postnatal rat intestine. Biol Neonate 63:246-257.

Wild,G.E., Searles,L.E., Harding,F., and White,E. (1994) Effects of polyamines on sucrase-isomaltase and Na/glucose transporter gene expression in the small intestine of the postnatal rat. Poster Presentation to the American Gastroenterological Association Meeting, New Orleans.

Wild,G.E., Searles,L.E., Thompson,J.A., Merlin,S., and Thomson,A.B.R. (1994) Alterations in the pattern of glucose and fructose transporter, sucrase-isomaltase and NaK ATPase gene expression in the small intestine of the diabetic rat. Topic Forum presented to the American Gastroenterological Association Meeting, New Orleans.

Wright,E.M. (1993) The intestinal Na^+/glucose co-transporter. Annu Rev Physiol 55:575-589.

Yang,P., Baylin,S.B., and Luk,G.D. (1984) Polyamines and intestinal growth: absolute requirement for ODC activity in adaptation during lactation. Am J Physiol 247 (Gastrointest. Liver Physiol. 10):G553-G557.

Yeh,K-Y., and Holt,P.R. (1986) Ontogenic timing mechanism initiates the expression of rat intestinal sucrase activity. Gastroenterology 90:520-526.

Yeh,K-Y., and Moog,F. (1974) Intestinal lactase activity in the suckling rat: influence of hypophysectomy and thyroidectomy. Science Wash. D.C. 182:77-79.

Yeh,K-Y., and Moog,F. (1975) Development of the small intestine in the hypophysectomized rat. II. Influence of cortisone, thyroxine, growth hormone and prolactin. Dev Biol 47:173-184.

Yeh,K-Y., and Moog,F. (1977) Influence of thyroidectomy, adrenalectomy, and administration of thyroxine or cortisone on the growth of the intestine of the suckling rat, and on the development of intestinal alkaline phosphatase and disaccharide activities. J Exp Zool 200:337-347.

Yeh,K-Y., Yeh,M., and Holt,P.R. (1989) Differential effect of thyroxine and cortisone on jejunal sucrase expression in suckling rats. Am J Physiol 256 (Gastrointest. Liver Physiol. 19):G604-G612.

Yeh,K-Y., Yeh,M., and Holt,P.R. (1987) Hormonal regulation of adaptive intestinal growth in artificially reared rat pups. Am J Physiol. 253 (Gastrointest. Liver Physiol. 16):G802-G808.

Yeh,K-Y., Yeh,M., and Holt,P.R. (1991) Thyroxine and cortisone cooperate to modulate postnatal intestinal enzyme differentiation in the rat. Am J Physiol 260 (Gastrointest.Liver Physiol. 23):G371-G378.

Yeh,K-Y., Yeh,M., and Holt,P.R. (1991) Intestinal lactase expression and epithelial cell transit in hormone-treated suckling rats. Am J Physiol. 260 (Gastrointest. Liver Physiol. 23):G379-G384.

Yeh,K-Y., Yeh,M., and Holt,P.R. (1991) Thyroxine and cortisone cooperate to modulate postnatal enzyme differentiation in the rat. Am J Physiol. 260 (Gastrointest. Liver Physiol. 23):G371-G378.

Yoshinaga,K., Ishizuka,J., Evers,B.M., Townsend,C.M., and Thompson,J.C. (1993) Age-related changes in polyamine biosynthesis after fasting refeeding. Experimental Gerontology 28:565-572.

Younoszai,M.K., Parekh,V.V., and Hoffman,J.L. (1993) Polyamines and intestinal epithelial hyperplasia in streptozotocin-diabetic rats. PSEBM 202:206-211.

Zemelman,B.V., Walker,W.A., and Chu,S.W. (1992) Expression and developmental regulation of Na^+, K^+ adenosine trophosphatase in the rat small intestine. J Clin Invest 90:1016-1022.

APPENDIX

Figure A1i: Ethidium Bromide Stain of Total RNA in the Proximal Small Intestine

Ethidium bromide stain of total RNA isolated from the rat proximal small intestine during development. This photo documents the integrity of samples of 20 μg total RNA from time points corresponding to postnatal day 3, 7, 10, 14, 21, 28, 35, and adult following fractionation on a 1.5% agarose gel.

Figure A1ii: Northern Blot of Glyceraldehyde 3-Phosphate (GAPDH) in the Proximal Small Intestine

Northern blot showing the developmental profile of GAPDH mRNA abundance in the postnatal rat proximal small intestine. 20 μg of total RNA from time points corresponding to postnatal day 3, 7, 10, 14, 21, 28, 35, and adult were fractionated on a 1.5% agarose gel (as depicted in Figure A1i), and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled GAPDH cDNA probe for 16 hours at 42 C^0. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained.

Figure A1i:Ethidium Bromide Stain of Total RNA in the Proximal Small Intestine

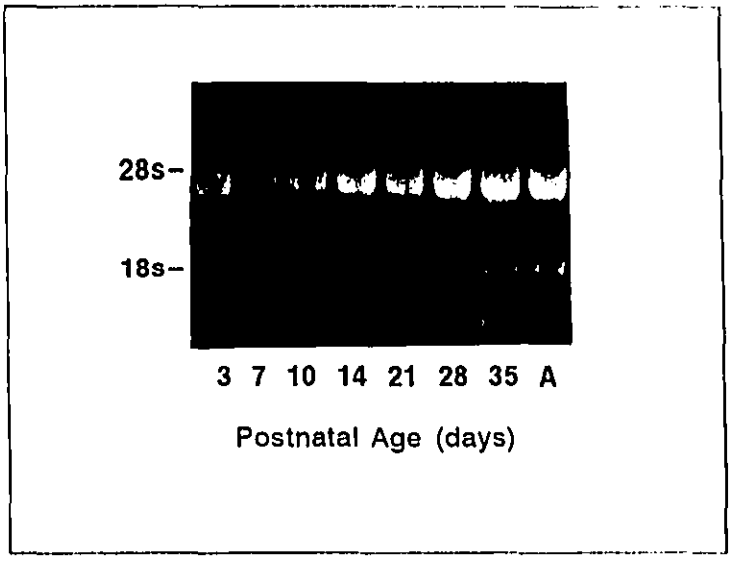


Figure A1ii:Northern Blot
GAPDH in the Proximal Small Intestine

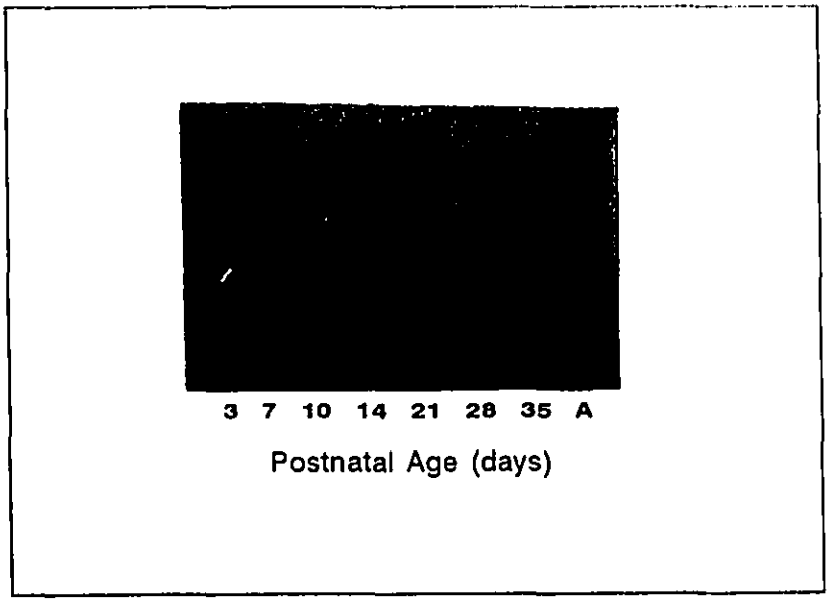


Figure A2i: Ethidium Bromide Stain of Total RNA in the Distal Small Intestine

Ethidium bromide stain of total RNA isolated from the rat distal small intestine during development. This photo documents the integrity of 20 μg total RNA from time points corresponding to postnatal day 3, 7, 14, 21, 28, 35, and adult following fractionation on a 1.5% agarose gel.

Figure A2ii: Northern Blot of Glyceraldehyde 3-Phosphate (GAPDH) in the Distal Small Intestine

Northern blot showing the developmental profile of GAPDH mRNA abundance in the postnatal rat distal small intestine. 20 μg of total RNA from time points corresponding to postnatal day 3, 7, 14, 21, 28, 35, and adult were fractionated on a 1.5% agarose gel (as depicted in Figure A2i), and transferred to a positively charged nylon membrane by capillary action. Northern blots were incubated with the digoxigenin-labelled GAPDH cDNA probe for 16 hours at 42 C^0. Standard post-hybridization Northern blot analysis procedures including chemiluminescent detection were followed, and an autoradiograph obtained.

Figure A2i:Ethidium Bromide Stain of Total RNA in the Distal Small Intestine

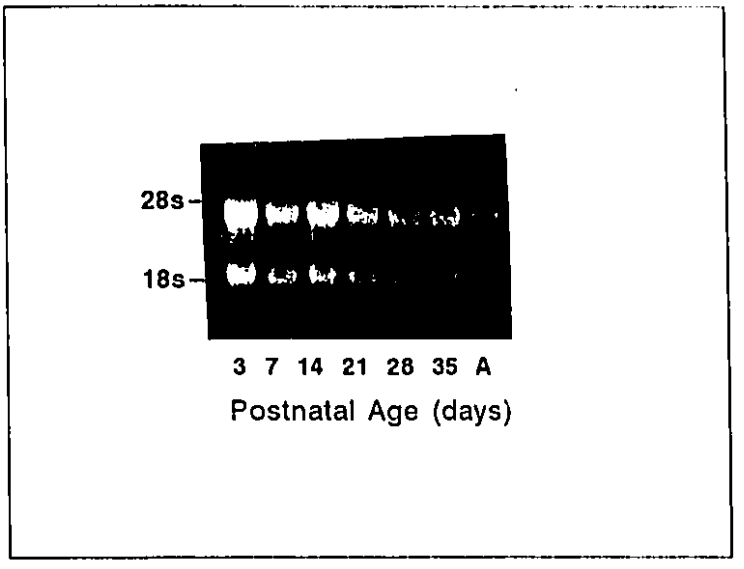


Figure A2ii:Northern Blot GAPDH in the Distal Small Intestine