THE UNIVERSITY OF CALGARY

Bombesin: Effect on Ontogeny of the Gut

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Bombesin: Effect on Ontogeny of the Gut" submitted by Eman M. Karkashan in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

The effect of bombesin (BBN) on ontogeny of the gastrointestinal tract was examined in New Zealand White rabbits. BBN was administered at various concentrations (1.25, 12.5, 30 μg/kg body weight) to suckling rabbits for 13 days starting on day 4 of life intraperitoneally. Control rabbits received saline by the same route. Animals were killed at day 17. While there was no significant effect observed using BBN at concentrations of 1.25 or 12.5 μg/kg on any of the parameters measured in any regions of the gut studied, except the pancreas, BBN administered at concentration 30 μg/kg induced a wide spread trophic effect on the gastrointestinal tract. This trophic effect was characterized by a significant increase in wet weight of liver, stomach, whole small intestine, 10-cm segment of proximal, mid, and distal small intestine as well as their mucosal weight, and colonic mucosal weight in BBN-treated group compared to controls. BBN significantly increased protein and DNA content in the liver, the fundus of stomach, the small intestine, and the distal colon. Maximal stimulation was seen in DNA content suggesting that BBN has a primarily hyperplastic effect. BBN had no effect in inducing precocious maturation of intestinal brush border dissacharidase activites or liver glucokinase activity.

BBN stimulated pancreatic growth as characterized by significant dose-dependent increases in pancreatic weight, protein, and DNA content compared to controls. The

maximum effect was observed using BBN at 30 μg/kg. Pancreatic amylase activity was also significantly increased by all concentrations of BBN. This effect was greatest at 1.25 μg/kg.

The specific BBN receptor antagonist [Leu^{13}-ψ(CH_2NH)Leu^{14}]-BBN, given intraperitoneally for 9 days at 30 μg/kg starting on day 20 of life, significantly reduced the development of pancreatic amylase and lipase activities compared to control, but had no effect on protein or DNA content.

In conclusion, our findings suggest that BBN has a trophic effect on the developing gastrointestinal tract and liver but has no effect on inducing precocious maturation of intestinal brush border dissacharidase activities or liver glucokinase activity. Moreover, it provides evidence of a physiological role of BBN on maturation of the neonatal pancreatic exocrine function.

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1. INTRODUCTION

The gastrointestinal tract undergoes continuing maturation of digestive and absorptive function during the postnatal period. The investigation of these developmental changes has received considerable attention. However, the mechanisms that control this development are not well understood. Hormonal, nutritional, neuronal, genetic, and growth factors have all been implicated.

1.1 POSTNATAL DEVELOPMENT OF THE GASTROINTESTINAL TRACT

In rats and humans, the stomach of the neonate has a low level of mucosal pepsinogen (37), low acid secretion (2,56), and low antral gastrin levels. Serum gastrin level is high, but it has no effect on either gastric acid secretion or DNA synthesis (142) because of a lack of gastrin receptors at this stage of development (142). Secretion of acid and pepsin in response to a variety of other stimuli occurs in suckling rats, but increases following weaning (1,56). Gastric lipase activity is high during the suckling period and decreases considerably with age (81). This high lipase activity is necessary to overcome the physiological pancreatic deficiencies during the suckling period (81).

In the rat, pancreatic amylase and lipase have been found in very small amounts

both in the lumen of small intestine and in the pancreas during the suckling period, and their levels start to increase markedly by the time of weaning (120). While the pancreatic content of trypsinogen and chymotrypsinogen is high during the first two postnatal weeks compared with adult levels, only very small amounts of trypsin and chymotrypsin have been detected in lumen of small intestine (120). This could be explained by immaturity of the process of their secretion (32,153). There are conflicting reports on the ability of the neonatal pancreas to respond to pancreozymin and secretin. Lebenthal et al. (75) reported that a full response to these secretogogues is demonstrable in children at age of 2 years, while Zoppi et al. (165) reported that pancreozymin and secretin are able to stimulate the pancreatic secretion of fluid, protein, and electrolytes in full term newborn.

In most mammalian species lactase activity is high at birth and declines as nursing is completed (70). On the other hand, maltase, isomaltase, trehalase, and sucrase activities are low or absent at birth and increase towards the end of the suckling period (119,125). These developmental changes in enzyme activities reflect the change in principal dietary constituents of carbohydrates from lactose during the suckling period (57) to maltose, isomaltose, and sucrose after weaning (50). In the neonatal period, the small intestinal epithelium is more permeable to water, electrolytes, amino acids, peptides, and lipids (5,126,161), has a high total capacity for glucose uptake (100,160), has elevated absorptive capacity for some metals such as copper (97), and iron (40) and has limited capacity for active Na^+transport (130). This is associated with diminished villus cell Na^+K^+ -ATPase activity (the enzyme

system associated with active Na^+ transport in the basolateral membrane). Electrolyte absorption occurs primarily by diffusion during the suckling period (161). The lack of an adaptive mechanism responsive to differences in osmotic pressure gradients between lumen and serum can explained the greater permeability in the rat intestine during the suckling period (161). In suckling animals, villus enterocytes are rich in thymidine kinase, an enyzme of DNA replication normally present in immature cells (150).

During the postnatal period, the hepatic ketogenic enzymes which are responsible for both fatty acid oxidation and ketone body production are high and decline to the adult level towards the end of the suckling period (6), whereas the activities of glucokinase, fructokinase, and lipogenic enzymes such as ATP citratelyase are low at birth and start to increase by the time of weaning (50). These developmental changes in enzyme activities reflect changing the nature of the diet from high-fat, low-carbohydrate at the suckling period to high-carbohydrate diet after weaning. The liver excretory function is not fully developed during the postnatal period. The active ileal transport of bile acids is deficient (84), and the total bile pool size and bile salt synthesis are reduced compared to the adult (84,137).

1.2 REGULATION OF THE POSTNATAL DEVELOPMENT OF THE GASTROINTESINAL TRACT

1.2.1 HORMONAL FACTORS

The hypothalamic-hypophysial-thyroid-adrenal axis seems to play an important role in the ontogeny of the gastrointestinal tract. However, growth hormone and prolactin are found to have no effect on small intestinal growth in hypophysectomized rats (158).

The involvement of glucocorticoids in the development of the rat intestine has been extensively studied. In the rat, the plasma level of free corticosterone is low throughout the first 12 days of life and then increases dramatically about 48 hr before the start of developmental enzymatic changes in the gut (49,142). In neonatal rats and mice, administration of glucocorticoids during the first two weeks of life induces precocious maturation of the stomach (56,71,113), liver (45,137), pancreas (127), intestinal disaccharidase activities (38), jejunal and ileal pinocytosis (26,61), and many lysosomal hydrolases (103). Adrenalectomy or hypophysectomy delayed but did not prevent the biological clock of development of the gut (89,159). It is believed that in the gastrointestinal tract the time for the initiation of enzymatic development is genetically programmed and that glucocorticoid hormones control only the rate of expression of the intestinal program (52,67). This concept is discussed in more detail below.

The circulating concentration of thyroxine (T4) increases markedly during the second week of life in rats (50) providing circumstantial evidence for a possible role of T4 in intestinal maturation. Administration of T4 to suckling rats causes precocious maturation of the liver (45), intestinal disaccharidases (62,112), gastric pepsinogen (71), and ileal lysosomal hydrolases (66). Hypothyroidism delayed but

did not prevent the biological clock of development of the gut (72,73,157,159). The effect of T4 on sucrase and maltase seems to be secondary to the accompanying changes of serum corticosterone, as it was found that administration of T4 causes an increase in plasma corticosterone (23) and the effect of hypothyrodism on these enzymes can be reversed by glucocorticoid administration (89). Moreover, it was found that T4 had no effect on sucrase and maltase in jejunal explants from suckling mice or rats (3,132). Thus it appears that T4 may play a permissive role in certain aspects of intestinal development (e.g., sucrase, and maltase) (3,89,132). However, it may play a primary role for others (e.g., lactase) as the effect of hypophysectomy on lactase activity is fully restored by administration of T4, but only partially restored by cortisone (157).

1.2.2 NUTRITIONAL FACTORS

Although the major developmental changes in the rat gastrointestinal tract occur concurrently with the onset of weaning, when the diet changes from a high-fat, low-carbohydrate diet to a high-carbohydrate, low-fat diet, it appears that weaning is not the primary cause of digestive and metabolic development in the gut. Most of the developmental changes in the rat gut begin in the third postnatal week in animals prevented from weaning (50). Moreover, it is found that sucrase activity developed normally in isografts of small intestine implanted in kidney capsule (36). However, the dietary change of weaning may modulate the plateau levels of gastrointestinal enzymes (51). Studies done on suckling rabbits showed that increasing nutrient

intake, and not the chronological age per se, induced precocious maturation of the gastrointestinal tract (39). Studies done on the effect of protein-calorie malnutrition on the developing intestine show reduced growth and maturation of the gut compared to controls (15,47). The effect of food intake on development of the gut can be explained by an increase in the rate at which cells are sloughed off the villi (60), direct effects on the gut mucosa from increased exposure to dietary nutrients and stimulation of release of gastrointestinal hormones or increased exposure to growth factors present in mother's milk.

1.2.3 BIOLOGICAL CLOCK

The biological clock is defined as the genetically controlled and predetermined temporal sequence of developmental events in the ontogeny of the gastrointestinal tract. It is preprogrammed in the nucleotide sequence in the DNA molecule and is species specific (76). As mentioned above, the expression of the genetic potential at various times in gastrointestinal development is modulated through other regulatory mechanisms. An example of the biological clock is the developmental clock for the appearance of various enzymes in the digestive system.

1.2.4 NEURAL FACTORS

In the adult rat intestinal epithelium, α-adrenergic stimulation by noradrenaline was found to shorten both cell cycle and mitotic times, whereas, β-adrenergic stimulation by adrenaline or isoprenaline was found to prolong cell cycle and mitotic

times (146). Sympathectomy produced by administration of guanethidine sulfate to the rats during the first 2 weeks of life lowers the mitotic and labeling indices in the ileum and delays the disapperance of the immature vacuolated cells from ileal villi (94). These findings indicate a possible role for autonomic nervous system in the regulation of intestinal cell proliferation during the postnatal period.

1.2.5 GUT HORMONES AND GROWTH FACTORS

Gastrin, cholecystokinin (CCK), and glucagon appear to have no effect on the development of the gut during the postnatal period (14,163). Insulin may play a role in the development of the gut as insulin administration to suckling mice causes precocious maturation of the intestine (88,95). However, no experiments were conducted to study the effect of removing endogenous insulin on intestinal development. There are conflicting reports on the role of epidermal growth factor (EGF), a polypeptide present in high concentration in breast milk in various species (9,104,143), in postnatal development of the gastrointestinal tract. EGF was reported to induce precocious maturation of the intestinal tract, pancreas, and liver in suckling rabbits (109,110), as well as to induce precocious maturation of the intestinal tract in suckling mice (87). Administration of EGF did not, however, induce precocious maturation of the stomach (29) or the intestine (108) in suckling rats. As pointed out by Malo and Menard, endogenous EGF level is low in the neonatal mouse, and the developmental increase in EGF production occurred after the enzymatic changes in the small intestine (87). Therefore, if EGF has an effect on the development of the

gastrointestinal tract, it may be supplied by mother's milk.

Bombesin (BBN) is an attractive candidate for the regulation of the growth and postnatal development of the gastrointestinal tract as it too is found in high concentration in breast milk of many mammalian species (33,74,152). BBN is a tetradecapeptide originally isolated from a methanol extract of the skin of the frog Bombina bombina in 1970 by Erspamer and his coworkers (34). It was identified based on its ability to stimulate contraction of the small intestinal, uterine, and urinary tract smooth muscle after intravenous infusion. Structurally related peptides named alytesin, litorin, and ranatensin where isolated from methanol extracts of the skin derived from other amphibian species (35), whereas, gastrin releasing peptide (GRP), neuromedin B, and neuromedin C were isolated from mammalian species (92,98,99). These peptides vary in length but have a highly conserved carboxy-terminal heptapeptide sequence which is required for immunogenicity as well as for high affinity receptor binding and biological activity (102) (Table 1).

DISTRIBUTION OF BBN

Using anti-BBN anti-sera, BBN has been identified in brain, lung, genitourinary tract and gastrointestinal tract of several mammalian species (138). BBN is widely distributed in intrinsic neurons throughout the gut. In the human gastrointestinal tract, the highest concentrations are found in the fundus, antrum, pylorus, and pancreas with lower concentrations in the duodenum, jejunum, terminal ileum, and colon (42,117). In the rat, high concentrations are present in the fundus, and

Table 1

SEQUENCE OF BBN-LIKE PEPTIDES

BBN	Pyr-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met
GRP	Ala-Pro-Val-Ser-Val-Gly-Gly-Gly-Thr-Val-Leu-Ala-Lys-Met
	Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met
NEUROMEDIN B	Gly-Asn-Leu-Trp-Ala-Thr-Gly-His-Phe-met
NEUROMEDIN C	Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met
ALYTESIN	Pyr-Gly-Arg-Leu-Gly-Thr-Gln-Trp-Ala-Val-Gly-His-phe-Met
RANATENSIN	Pyr-Val-Pro-Gln-Trp-Ala-Val-Gly-His-Phe-Met
LITORIN	Pyr-Gln-Trp-Ala-Val-Gly-His-phe-Met

Note: Sequence homologies relative to BBN are underlined.

colon,whereas moderate concentrations are present in the antrum, duodenum, ileum, and jejunum (148). BBN-immunoreactive nerves are present mainly in the sphincter of Oddi, whereas the muscle layer of the gall bladder, cystic duct, and hepatic duct were only sparsely innervated by BBN (16). BBN-like peptides have been localized in endocrine cells of avian proventriculus (144) and dogfish pyloric stomach (19).

BBN is produced by enteric neurons (30). BBN-immunoreactive nerve fibres are located in the myenteric plexuses of the stomach, small intestine, and colon. Nerve fibres project anally and terminate around other cell bodies in the myenteric plexus throughout the gut. Fibres also project to the circular muscle, submucous ganglia and externally to the coeliac ganglia. In the stomach, nerve fibres extend into the mucosa. Cutting of all ascending and descending pathways in the myenteric plexus resulted in disappearance of the reactive terminals in the myenteric plexus, circular muscle, and the submucous plexus on the anal side. After the mesentric nerves are cut, no change is observed in the intestinal wall but the reactive fibres in coeliac ganglia disappear (20,24). Application of colchicine to the stomach wall caused an accumulation of BBN in gastric myenteric neurons (46). These results strongly suggest that BBN-immunoreactive fibres in coeliac and superior mesenteric ganglia originate in myenteric neurons located in the stomach and small intestine.

BBN RECEPTORS

BBN receptors which recognize the conserved C-terminus of BBN are present in the central nervous system (CNS) (164), gastrointestinal tract (105,147,152), pancreas

(128), dorsal horn of the spinal cord (107), genitourinary tract (138), small cell lung carcinoma (SCLC) cell lines (101), and 3T3 fibroblast cell lines (162). BBN and platelet-derived growth factor (PDGF) are the only known agents that can stimulate the growth of Swiss 3T3 fibroblast in the absence of a second growth-promoting agent (122). BBN-receptor interaction has been demonstrated to induce expression of c-fos and c-myc proto-oncogens (80). Following binding to the receptor, BBN stimulates the breakdown of phosphatidyl inositol 4,5-biphosphate to diacylglycerol and inositol 1,4,5-triphospate (IP3). Diacylglycerol activates protein kinase C and IP3 triggers the release of Ca^{2+} from intracellular stores (123,135). There is an increase in tyrosine kinase activity (123), and a rapid influx of Na^+ into Swiss 3T3 cells via an amiloride-sensitive Na^+/H^+ antiport. This results in an increase in concentration of the intracellular Na^+ and causes cytoplasmic alkalinization (135). Moreover, there is a secondray stimulation of Na^+/K^+ pump activity which increase the level of K^+ and restores the electrochemical gradient for Na^+ (135).

ONTOGENY

BBN-like immunoreactivity has been found at birth in the stomach and colon of rats but not in small intestine and brain during the first 2 days after birth. BBN-like activity starts to increase in the intestine during the first 2 weeks of life and peaks by 16 days (148). BBN-like activity begins to appear in the brain at the end of the first week and continue to rise through the first 4 weeks of life. The concentration of BBN-like peptides is higher in the rat fetal lung relative to adult lung. Spindel et

al. (134) have shown a period of active synthesis of GRP, revealed by a marked elevation in GRP mRNA activity, in the human lung during the period of gestation from 12 to 20 weeks, when bronchial development is most active.

BIOLOGICAL ACTIVITIES OF BBN IN THE GASTROINTESTINAL TRACT

Intravenous infusion of BBN stimulates gastric acid secretion in dogs, cats, and humans (11,27) but not in rats (10). This stimulation follows an increase of gastrin release suggesting that BBN stimulates gastric acid secretion via release of gastrin. A direct action of BBN on the antral G cell was reported (44). Dose-response studies with BBN and GRP demonstrated no significant difference in potency between the amphibian and the mammalian peptide as a stimulant of release of canine gastrin (93). Intracerebral injection of BBN inhibits gastric acid secretion in rats and dogs, an effect which is blocked by transection of the cervical spinal cord but not by adrenalectomy or truncal vagotomy (102,140). Another central effect of BBN is the adrenergic-dependent stimulation of gastric mucous secretion (139).

Intravenous infusion of BBN caused stimulation of enzyme-rich, bicarbonate-poor pancreatic juice in humans, rats, and dogs as well as the release of CCK (8,106). BBN has been found to have a direct effect on pancreatic acinar cells to stimulate amylase secretion (65). Thus BBN may exert its effect on the pancreas directly or indirectly through the release of CCK. In isolated perfused porcine pancreas with intact autonomic nerve supply, electrical stimulation of the vagus nerve causes increased output of GRP (65). Electrical stimulation of the vagus nerve elicits

pancreatic secretory responses in many mammalian species. Therefore, it is quite possible that release of BBN from intrinsic nerves in the pancreas contributes significantly to neural regulation of pancreatic secretion in the pig. Cerebroventricular injection of BBN inhibits protein, volume, and bicarbonate pancreatic secretion in rats (32).

Intravenous infusion of BBN has also a profound effect on gastrointestinal motility. In humans, intravenous infusion of BBN causes an increase of lower esophageal sphincter pressure, delay of solid meal gastric emptying, inhibition of basal mechanical activity of duodenum and jejunum, stimulation of gall bladder contraction and emptying, contraction of antral and pyloric stomach and contraction of ileocecal valve (28). BBN has a direct effect on rat stomach strips in numerous species (43). Cerebroventricular and intrathecal injection of BBN decreased intestinal motility and delayed gastric emptying in rats (116).

In humans, intravenous infusion of BBN produced significant increases in plasma concentrations of gastrin, insulin, pancreatic glucagon, pancreatic polypeptide, CCK, motilin, neurotensin, enteroglucagon, and vasoactive intestinal peptide (41,93). Intracerbroventricular injection of BBN leads to hyperglycemia associated with increased plasma glucagon and decreased plasma insulin in rats (13).

BBN stimulates the proliferation of Swiss 3T3 fibroblasts (121), normal human bronchial epithelial cells (155), some small cell lung carcinomas cell lines (22), antral gastrin cells (77), the pancreas causing acinar hypertrophy and hyperplasia (83) and epithelial-like cells from human normal embryonic intestine (131). In suckling rats,

BBN administration significantly stimulates gastric, colonic, and exocrine as well as endocrine pancreatic cell growth (78,79,111,118).

In vitro, BBN transiently increase electrogenic anion secretion in chicken ileum (18). The short-circuit current respose is inhibited by loperamide, an antidiarrheal drug which binds to opiate receptors present in the intestine (18). Intravenous infusion of BBN reduced net H_2O, Na^+, and CL^- absorption in the canine jejunum in vivo (7).

BBN stimulates fasting secretion of bile acids independant bile flow (69). BBN significantly increases the secretion of biliary bicarbonate produced by duodenal acidification or by secretin in dogs (63).

BBN ANTAGONISTS

There has been considerable interest in the design of BBN receptor antagonists not only to study the physiological role of BBN, but also because BBN appears to act as potent autocrine growth factor in human small cell lung carcinoma (22). The first BBN receptor antagonist available was a substance P analog which was described to prevent BBN-stimulated pancreatic amylase release (58). Since then, various analogs of BBN have been synthesized. The first structural analog was (D-Phe^{12})-BBN in which D-phenylalanine replacement for histidine resulted in a competitive antagonist which has high specificity for BBN receptor on pancreatic acini (48), but which also binds to substance P receptors on brain membranes (96). The affinity of this analog for BBN receptors was only slightly higher than that observed with substance P

analogs. The second analog was [Leu^{13}-ψ(CH_2NH)Leu^{14}]-BBN in which the amino acid linkage (CO-NH) between Leu^{13} and Leu^{14} is replaced by a reduced peptide bond (CH_2NH). This analog has been shown to specifically antagonize the action of BBN on pancreatic acini (21), Swiss 3T3 fibroblasts (21,156), gastric acid secretion in rats (124), growth of certain strains of human small cell lung carcinoma (86,145), and on BBN-induced contractile response on isolated smooth muscle cells from guinea pig (129). This competitive antagonist exhibits a 100-fold improvment in the binding affinity compared to previously reported BBN receptor antagonists and show no affinity for substance P receptors. Recently, an alkylamide BBN analog has been synthesized. This analog has been reported to be 30-fold more potent than any previously described BBN receptor antagonist (149).

1.3 OBJECTIVES

Some studies have examined the effect of BBN on the rat gastrointestinal tract and pancreas during the suckling period (78,79,111,118), but there has been no through study done on the effect of BBN on intestinal enzymatic changes, maturation of the liver, and on the physiological role of BBN on ontogey of the gut. The present study was undertaken to determine:

1. The effect of BBN on growth and maturation of the liver, pancreas, and gastrointastinal tract of suckling rabbits.

2. The physiological role of BBN on growth and maturation of the neonatal pancreas using the specific BBN antagonist [Leu^{13}-$\psi(CH_2NH)Leu^{14}$]-BBN.

2. METHODS

2.1 EFFECT OF BBN ON GROWTH AND POSTNATAL DEVELOPMENT OF THE RABBIT GASTROINTESTINAL TRACT

Pregnant New Zealand Rabbits (Riemens Fur Ranches Ltd., St. Agatha, Ont.) were received in our vivarium on approximately the fifteenth to the twentieth day of gestation. Room environment was maintained at 22°C and 12-hour photoperiods. The day of birth is referred to as day 0. Starting on day 4 of life, experimental animals received BBN (Sigma Chemical, St. Louis, MO) at various concentrations [1.25 (n=6), 12.5 (n=6), 30 (n=10) μg/kg body weight] daily by intraperitoneal (i.p) injection and littermate controls (n=10) received 0.9% saline in equivalent volume by the same route. Animals were weighed daily and killed on day 17 by an intracardial injection of overdose of sodium pentobarbital (2 ml/4.5 kg). The protocol was approved by the University of Calgary Animal Care Service and all procedures were carried out in accordance with the guidelines established by the Canadian Council on Animal Care. The following portions of the gut were removed:

1. STOMACH

Wet weight of the stomach were determined. Portions were taken from fundus, body, and antrum regions of the stomach, homogenized in 2.5 mM disodium ethylene diamine tetraacetate (EDTA) (100 mg tissue/1 ml EDTA) using a polytron (Brinkmann Instruments (Canada) Limited) at position 6 for about 10s, frozen in microcentrifuge tubes using dry ice and acetone, and stored in a -70°C freezer for

later determination of protein and DNA. Protein was measured by the method of Lowry et al. (85), and DNA according to Hinegardner (53).

2. LIVER

Wet weight of the liver was determined. Portions were taken from the liver and homogenized in 2.5 mM EDTA (100 mg/ml), frozen, and stored for later determination of protein and DNA or homogenized in 50 mM triethanolamine, 0.3 M sucrose, 1 mM EDTA buffer, then the homogenate was centrifuged at 100,000 g for 1h at 4°C, and the supernatant was assayed for glucokinase activity as described by Stanley et al. (136).

3. PANCREAS

The pancreas was trimmed free of fat, weighed, homogenized in 0.9% saline (100 mg/ml), frozen, and stored for later determination of protein, DNA, and amylase. Amylase activity was measured by the method of Ceska et al. (17) using Phadebas reagent tablets.

4. SMALL INTESTINE

Wet weight of the whole small intestine (from the ligament of Treitz to the most proximal attachment of the mesoappendix), as well as 10-cm segments of proximal (10-cm distal to the ligament of Treitz), mid (by folding the whole small intestine), and distal (10-cm proximal to the ileocecal junction) were determined. Mucosa was scraped from the intestinal segments with a microscope slide, weighed, homogenized in 2.5 EDTA (100 mg/ml), frozen, and stored for later determination of protein, DNA, as well as sucrase, maltase, and lactase activities. Their activities were

measured by the method of Dahlquist (25).

5. COLON

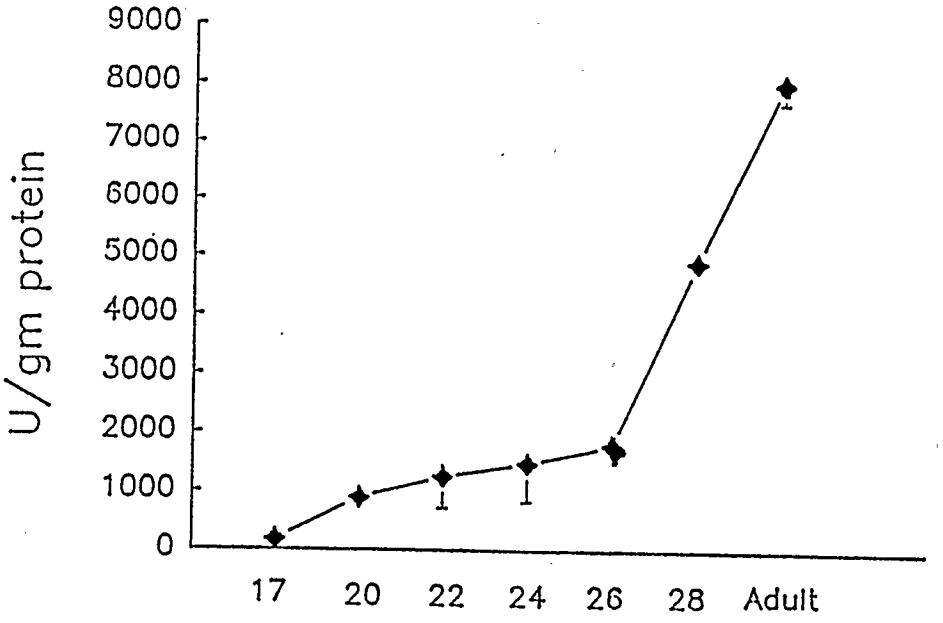
The wet weight of 10-cm segment of the distal colon were determined. Mucosa was scraped, weighed, homogenized in 2.5 mM EDTA (100 mg/ml), frozen, and stored for later determination of protein and DNA.

2.2 THE PHYSIOLOGICAL ROLE OF BBN ON ONTOGENY OF THE RABBIT PANCREAS

A preliminary experiment was carried out to define the normal pattern of postnatal development of pancreatic amylase activity in the rabbit. Animals (n=4-5) were killed at day 17, 20, 22, 24, 26, and 28 as well as at adult stage. A portion of the pancreas was removed, homogenized for 10s in 0.9% saline (100 mg/ml) using a polytron, and frozen for later assay of amylase activity. The normal pattern of postnatal development of pancreatic amylase activity in the rabbit is shown in Fig. 1. Amylase activity is low at day 17 till day 28 when there is a dramatic increase in the amylase activity. Therefore an experiment examining the effect of the BBN antagonist [Leu^{13}-$\psi(CH_2NH)Leu^{14}$]-BBN on the postnatal development of exocrine pancreatic function was carried out from day 20 till day 29 of life.

Starting on day 20 of life, New Zealand White rabbits (Riemens) animals (n=4) received BBN antagonist [Leu^{13}-$\psi(CH_2NH)Leu^{14}$]-BBN (Sigma) at 30 μg/kg body weight, daily i.p for 9 days and littermate controls (n=4) received 0.9% saline in equivalent volume by the same route. Animals were weighed daily. At the end of

Figure 1. Normal postnatal development of pancreatic amylase activity. Points are means ± S.E of 4-5 animals.


U/gm protein
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
17
20
22
24
26
28
Adult

the treatment period, the animals were killed and a portion of the pancreas removed. At this age it was impossible to remove the pancreas in its totality because it was diffuse and islands of pancreatic tissues were surrounded with fat. A portion of the pancreas was homogenized for 10s in 0.9% saline (100 mg/ml) using a polytron. Aliquots were frozen and stored at -70°C for later assay of protein, DNA, as well as amylase and lipase activities. Lipase activity was measured by the method of Mauck et al. (90).

2.3 STATISTICAL ANALYSIS

All results were expressed as means ± standard error of the mean. Statistical comparison between experimental and control groups were made using Student's t test for unparied data. Statistical comparison between experimental groups using different concentrations of BBN and controls was made using one way analysis of variance (ANOVA) followed by a multiple comparison test (Student-Newman-Keules test). Level of significance was set as $P<0.05$.

3. RESULTS

3.1 ROLE OF BBN ON GROWTH AND POSTNATAL DEVELOPMENT OF THE RABBIT GASTROINTESTINAL TRACT

The BBN-treated animals did not exhibit significantly different weight gain compared to controls. There was no significant effect observed using BBN at concentrations 1.25 or 12.5 μg/kg body weight on any of the parameters measured in any region of the gut studied, except the pancreas. Therefore, only the results using BBN at concentration 30 μg/kg body weight are presented except for the results of total intestinal weight, wet weight of intestinal segments , and mucosal weight. However, because of the efficacy of all concentrations of BBN on pancreatic development, the results of all concentrations of BBN on the pancreas are presented.

3.1.1 Body and organ weight

Weight gain and final body weight of BBN-treated animals were not significantly different from control animals (Fig. 2). Wet weight of the stomach was significantly increased by BBN (4.09 ± 0.09 g, $P<0.001$) compared with controls (3.54 ± 0.11 g) (Fig. 3A). Wet weight of the liver was also significantly increased by BBN (9.86 ± 0.65 g, $P<0.05$) compared with controls (8.28 ± o.41) (Fig. 4A). Pancreatic weight was increased after BBN treatment in a dose-dependent manner: 1.25 μg/kg (1.44-fold increase over control, $P<0.01$); 12.5 μg/kg (1.67-fold increase over control, $P<0.01$); and 30 μg/kg (1.94-fold increase over control, $P<0.01$) (Fig. 5A). Total

Figure 2. Effect of BBN at 30 μg/kg body weight on the total body weight. Values are means ± SE. There was no significant difference between control and BBN groups. Cont, control.

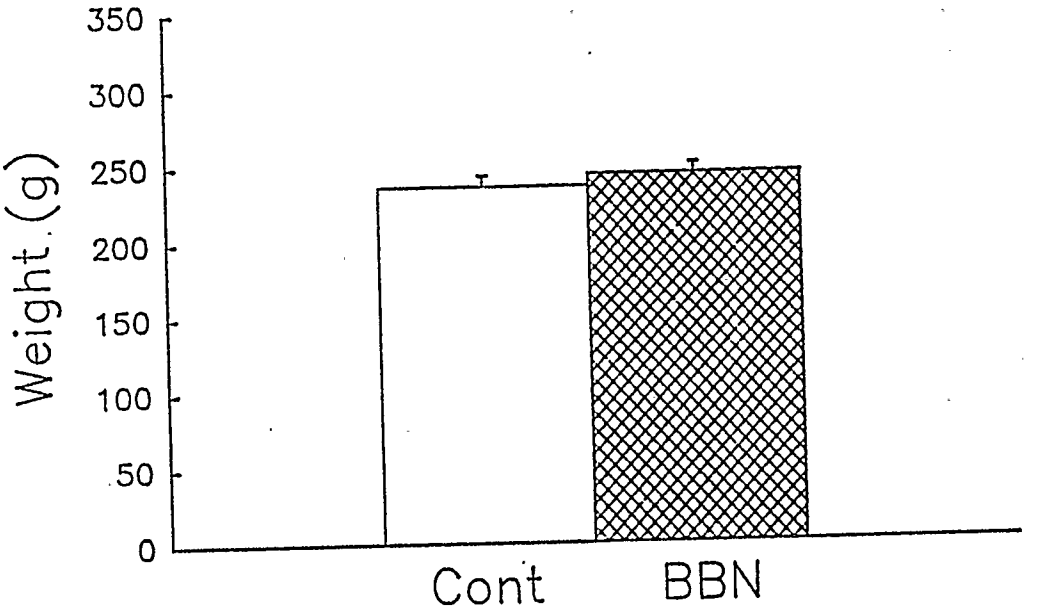

350
300
250
200
150
100
50
0
Weight (g)
Cont
BBN

Figure 3. Effect of BBN at 30 μg/kg body weight on (A) stomach weight, (B) protein, (C) DNA, and (D) protein/DNA (P/DNA) ratio of fundus, body, and antrum regions of stomach. Values are means ± SE. *$P < 0.05$, **$P < 0.005$ compared with controls.

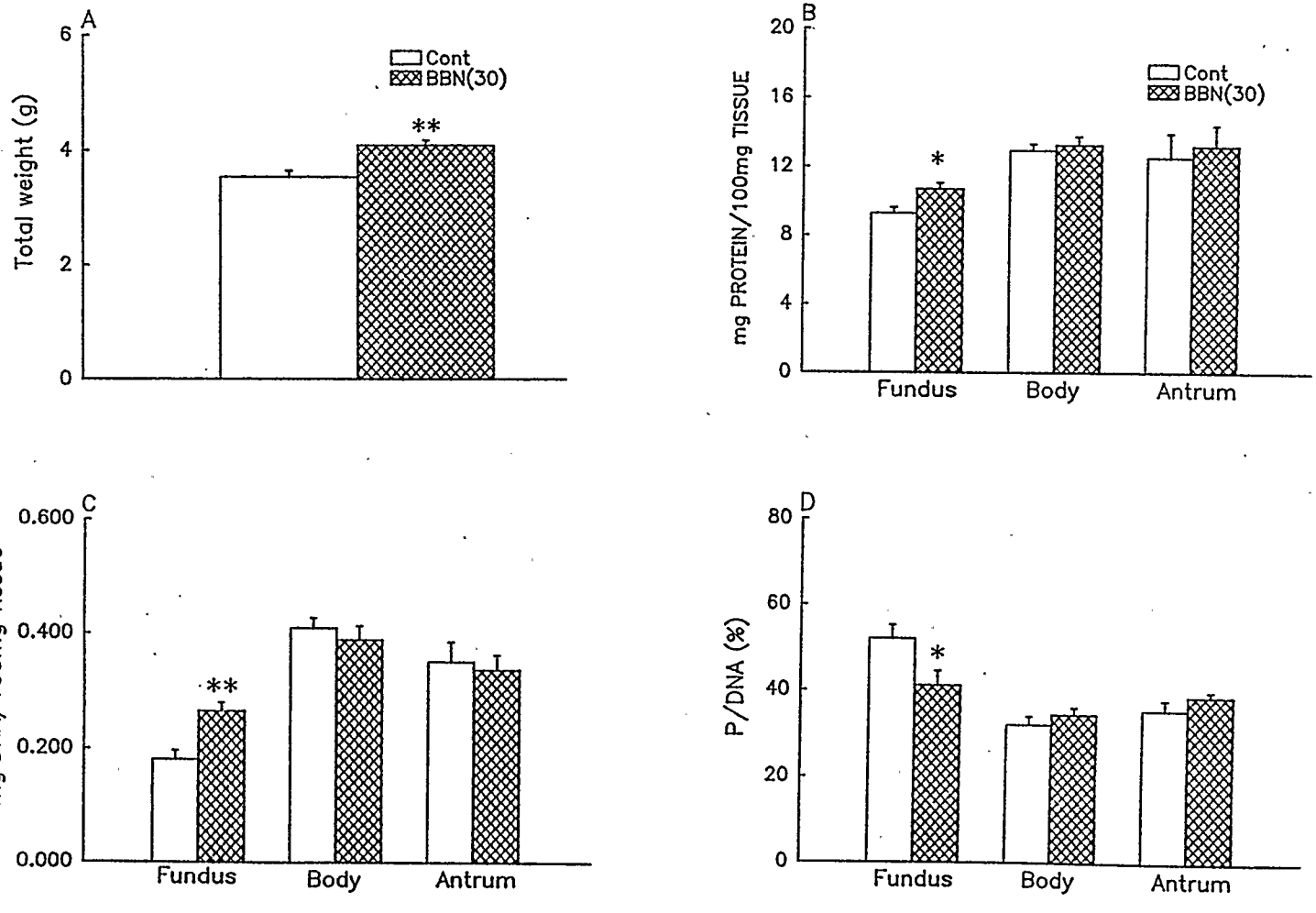
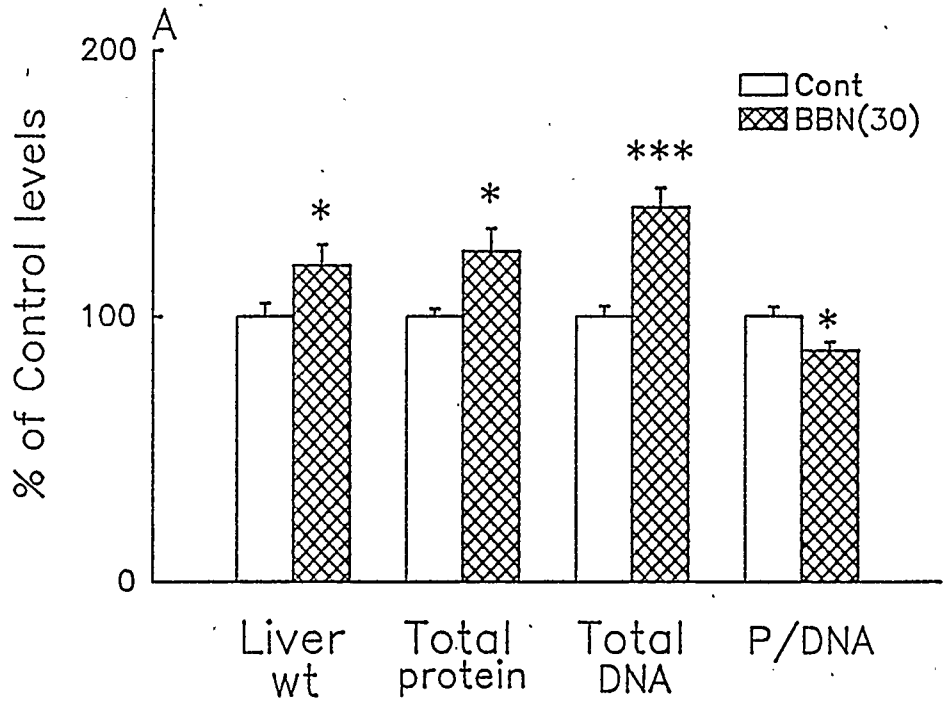

A
Total weight (g)
6
4
2
0
Cont
BBN(30)
**
B
mg PROTEIN/100mg TISSUE
20
16
12
8
4
0
Cont
BBN(30)
*
Fundus
Body
Antrum
C
mg DNA/100mg Tissue
0.600
0.400
0.200
0.000
**
Fundus
Body
Antrum
D
P/DNA (%)
80
60
40
20
0
*
Fundus
Body
Antrum

Figure 4. (A) Effect of BBN at 30 μg/kg body weight on liver weight, total protein, total DNA, and protein/DNA (P/DNA) ratio. Values are percent of control levels. *$P<0.05$ and **$P<0.005$ compared with controls.

(B) Effect of BBN at 30 μg/kg/body weight on liver glucokinase activity. Values are means ± SE. There was no significant differance between control and BBN groups. Cont, control.


A
200
100
0
% of Control levels
Cont
BBN(30)
*
*
***
*
Liver wt
Total protein
Total DNA
P/DNA

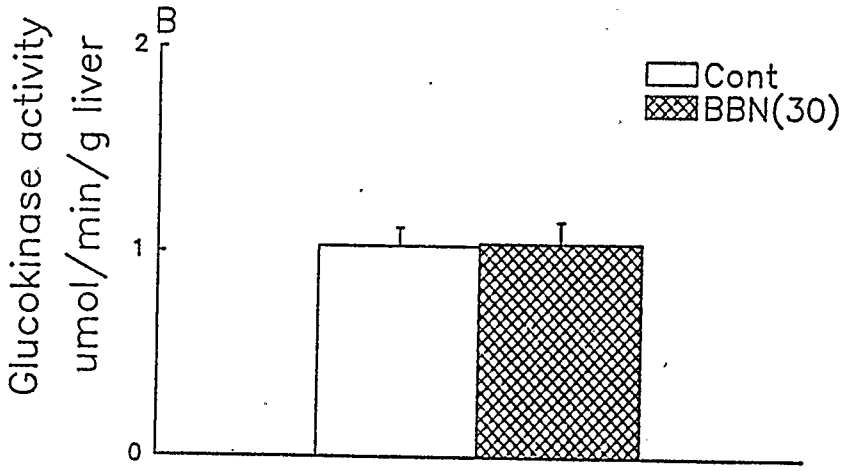
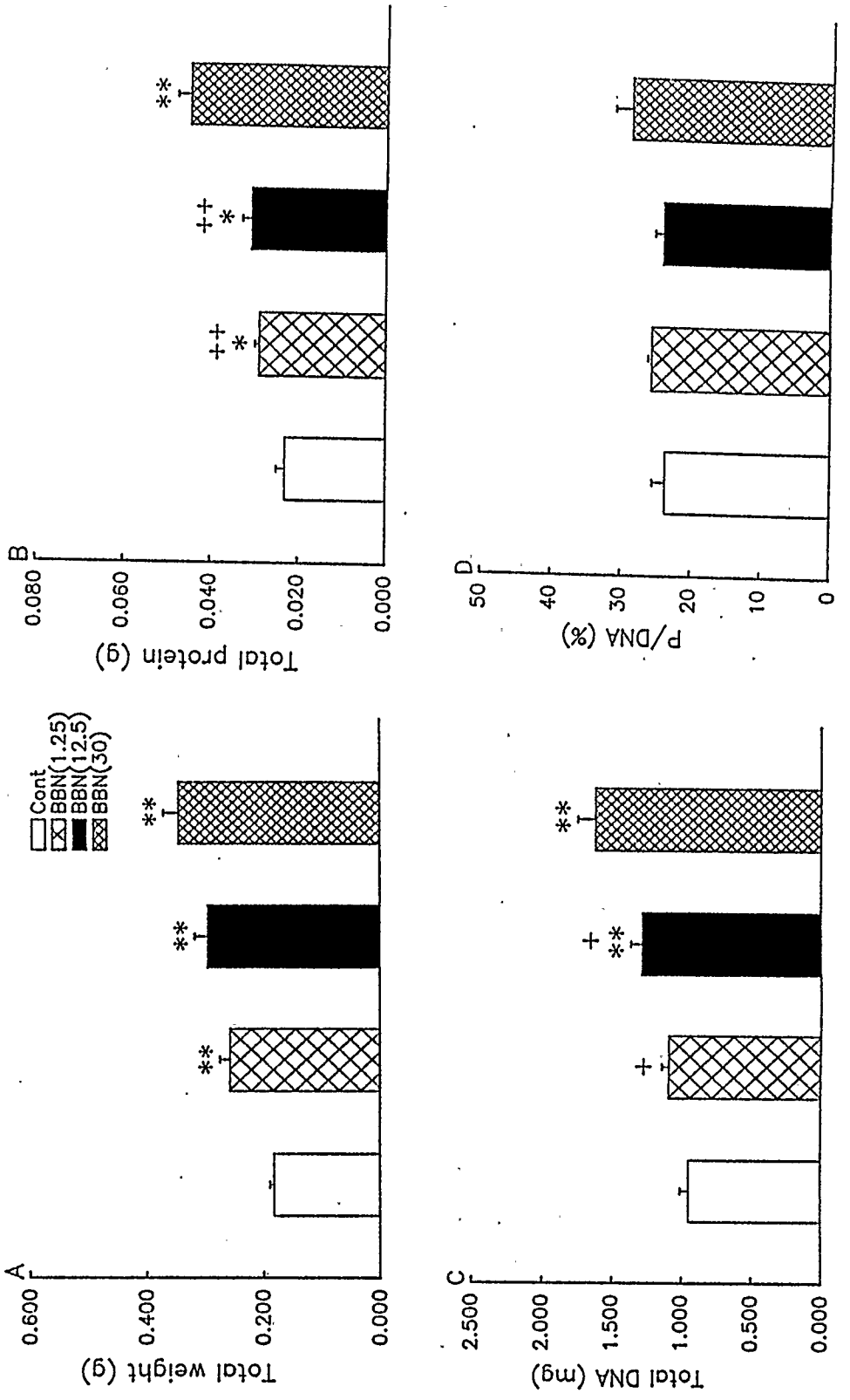

B
2
1
0
Glucokinase activity
umol/min/g liver
Cont
BBN(30)

Figure 5. Effect of BBN at concentrations 1.25, 12.5, and 30 μg/kg body weight on (A) total pancreatic weight,(B) total protein content, (C) total DNA content, and (D) protein/DNA (P/DNA) ratio. Values are means ± SE. *$P<0.05$ and **$P<0.01$ compared with controls. +$P<0.05$ and ++$P<0.01$ compared with 30 μg BBN group.


A
Total weight (g)
0.600
0.400
0.200
0.000
Cont
BBN(1.25)
BBN(12.5)
BBN(30)
B
Total protein (g)
0.080
0.060
0.040
0.020
0.000
C
Total DNA (mg)
2.500
2.000
1.500
1.000
0.500
0.000
D
P/DNA (%)
50
40
30
20
10
0

small intestinal weight, wet weight of all intestinal segments, as well as mucosal wet weight were significantly increased by BBN at a concentration of 30 μg/kg body weight (Fig. 6-8). While wet weight of 10-cm segment of the distal colon of BBN group was not significantly differ from control value, mucosal wet weight was significantly increased by BBN (0.25 ± 0.01 g, $P < 0.003$) compared with controls (0.19 ± 0.01) (Fig. 9).

3.1.2 Stomach protein and DNA

BBN administration significantly increased the protein ($P < 0.01$) (Fig. 2B) and DNA ($P < 0.002$) (Fig. 2C) content in the fundic region with the increase in DNA being proportionally larger as the protein/DNA (P/DNA) ratio was significantly ($P < 0.03$) lower in BBN group compared to controls (Fig. 2D). However, the protein and DNA content , as well as P\DNA ratio in the body and antrum regions of the stomach were not altered by BBN administration (Fig. 2B-D).

3.1.3 Liver protein, DNA, and glucokinase

The increased liver weight in BBN-treated group was reflected by a significant increase in total protein ($P < 0.01$) and total DNA content ($P < 0.0005$) compared with controls (Fig. 3A). The increased in liver weight in BBN-treated group appeared to be mainly due to increased in liver cell number as P\DNA ratio was significantly lower ($P < 0.01$) in BBN group compared to controls (Fig. 3A). BBN appears to have no effect on glucokinase activity (Fig. 3B).

3.1.4 Pancreatic protein, DNA, and amylase activity

There was no significant difference between BBN groups and controls in protein

Figure 6. Effect of BBN at concentrations 1.25, 12.5, and 30 μg/kg body weight on total small intestinal weight. Values are means ± SE. *P<0.05 compared with controls. +P<0.05 compared with 30 μg BBN group.

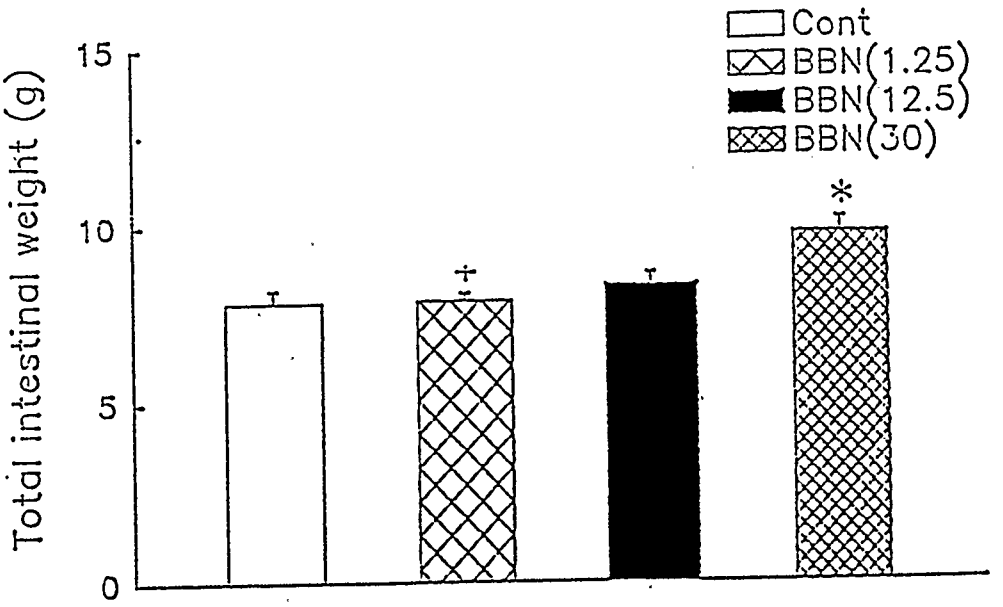

Cont
BBN(1.25)
BBN(12.5)
BBN(30)
Total intestinal weight (g)
15
10
5
0
+
*

Figure 7. Effect of BBN at concentrations 1.25, 12.5, and 30 μg/kg body weight on the total wet weight of 10-cm segments of proximal (P), mid (M), and distal (D) small intestine. Values are means ± SE. *$P<0.05$ and **$P<0.01$ compared with controls. +$P<0.05$ compared with 30 μg BBN group.

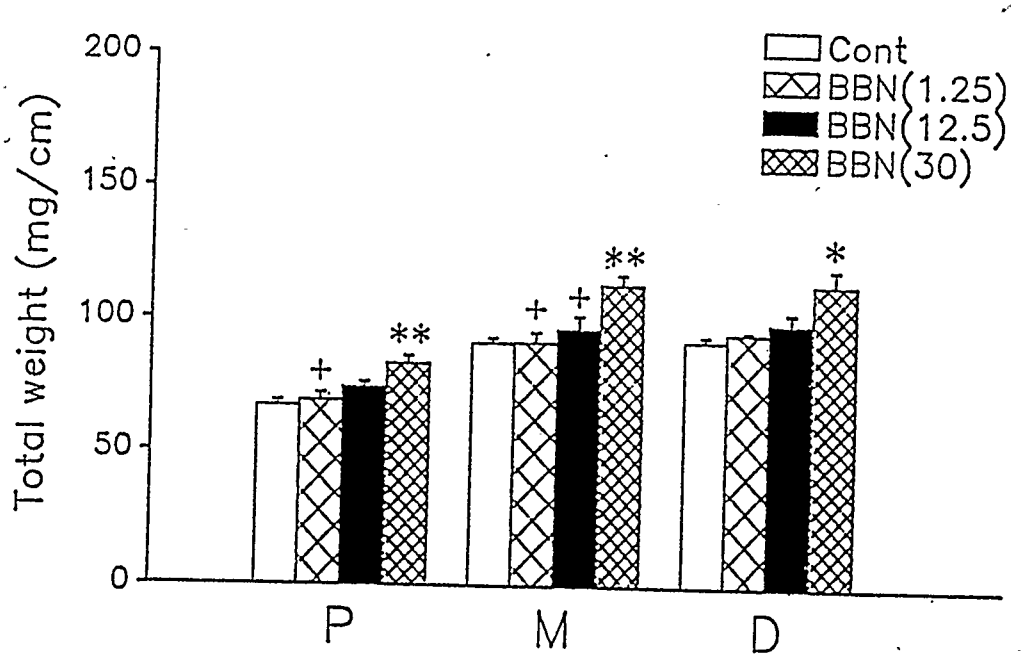

Total weight (mg/cm)
200
150
100
50
0
Cont
BBN(1.25)
BBN(12.5)
BBN(30)
+
**
+
+
**
*
P
M
D

Figure 8. Effect of BBN at concentrations 1.25, 12.5, and 30 μg/kg body weight on the mucosal weight of 10-cm segment of proximal (P), mid (M), and distal (D) small intestine. Values are means ± SE. *$P<0.05$ and *$P<0.01$ compared with controls. +$P<0.05$ compared with 30 μg BBN group.

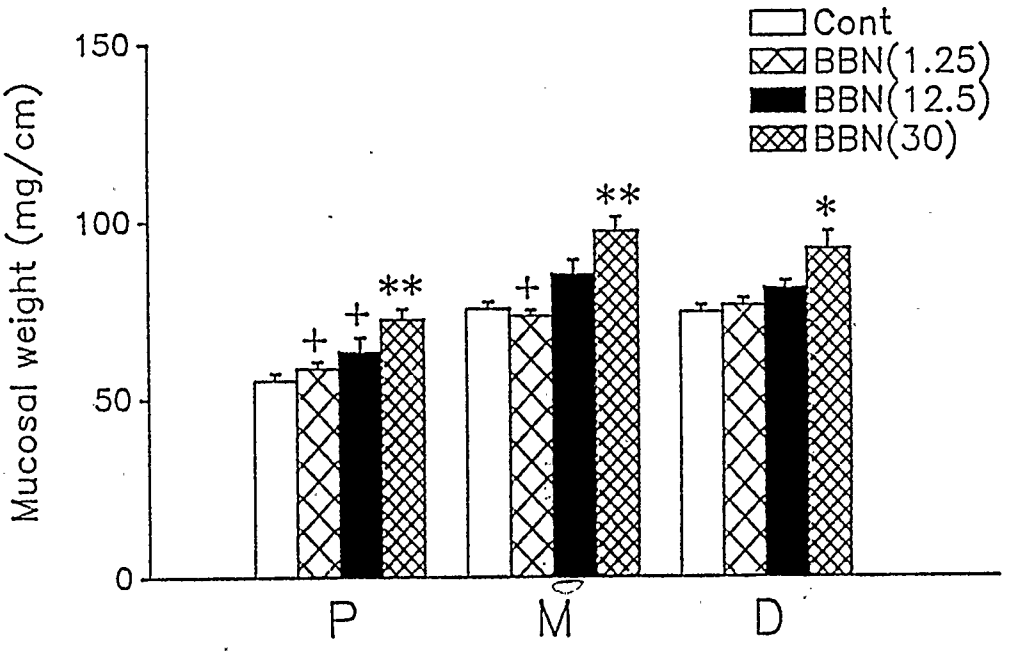
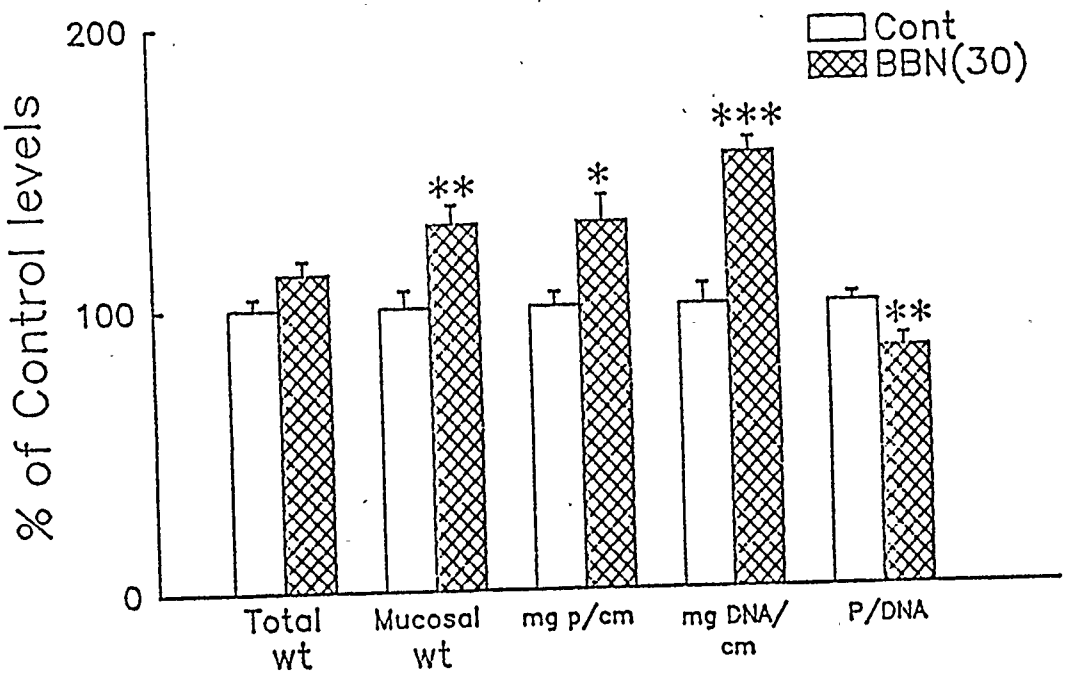

Cont
BBN(1.25)
BBN(12.5)
BBN(30)
Mucosal weight (mg/cm)
150
100
50
0
+
+
**
+
**
*
P
M
D

Figure 9. Effect of BBN at 30 μg/kg body weight on total weight and mucosal weight of 10-cm segment of the distal colon, as well as on the mucosal protein, DNA, and protein/DNA (P/DNA) ratio. Values are percent of control levels. *$P<0.05$, **$P<0.005$, and ***$P<0.0005$ compared with controls.


Cont
BBN(30)
% of Control levels
200
100
0
**
*
***
**
Total wt
Mucosal wt
mg p/cm
mg DNA/ cm
P/DNA

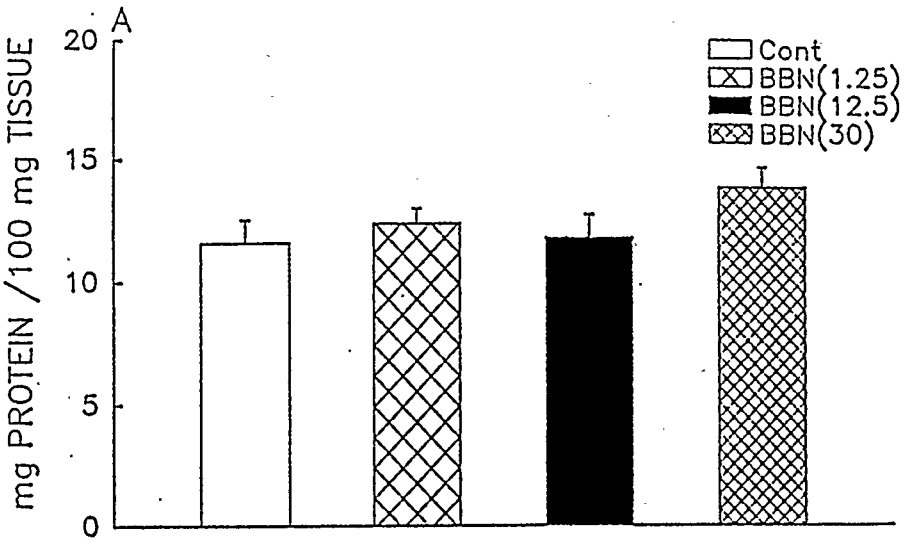
and DNA content when expressed as mg/100 mg tissue (Fig. 10A-B). However, as seen with total pancreatic weight there was a dose-dependent increase in total pancreatic protein, and DNA in all BBN-treated groups. The maximum effect was obsereved with BBN at a dose of 30 μg/kg. BBN 1.25 μg/kg increased protein content 1.3-fold ($P<0.05$), 12.5 μg/kg 1.35-fold ($P<0.05$), and 30 μg/kg 1.96-fold ($P<0.01$). The increase in protein induced by BBN 30 μg/kg was significantly ($P<0.01$) greater than that seen with 1.25 or 12.5 μg/kg (Fig. 5B). DNA content was increased by 1.2-fold after BBN 1.25 μg/kg but it did not reach statistical significance, 1.4-fold by BBN 12.5 μg/kg ($P<0.01$), and 1.7-fold by BBN 30 μg/kg ($P<0.01$) (Fig. 5C). The P/DNA ratio did not differ significantly between the various groups (Fig. 5D).

Amylase activity was significantly increased after BBN treatment in a dose-dependent manner whether expressed as units/gm protein, units/mg DNA, or units/total pancreas (Fig. 11). The maximal effect was at a dose of 1.25 μg/kg. BBN 1.25 μg/kg increased amylase activity expressed as units/gm protein 5.9-fold compared to an increase of 4.8-fold with 12.5 μg/kg and 3.4-fold with 30 μg/kg which was significantly ($P<0.01$) less than that seen with BBN 1.25 μg/kg (Fig. 11A). A similar pattern was seen when amylase activity was expressed as units/mg DNA (Fig. 11B) or units/total pancreas (Fig. 11C).

3.1.5 small intestinal mucosal protein, DNA, and brush border disaccharidase activities

Mucosal protein and DNA of all intestinal segments were significantly increased

Figure 10. Effect of BBN at concentrations 1.25, 12.5, and 30 μg/kg body weight on pancreatic (A) protein and (B) DNA expressed as mg/100 mg tissue. There was no significant differance between control and BBN groups.


A
mg PROTEIN /100 mg TISSUE
20
15
10
5
0
Cont
BBN(1.25)
BBN(12.5)
BBN(30)

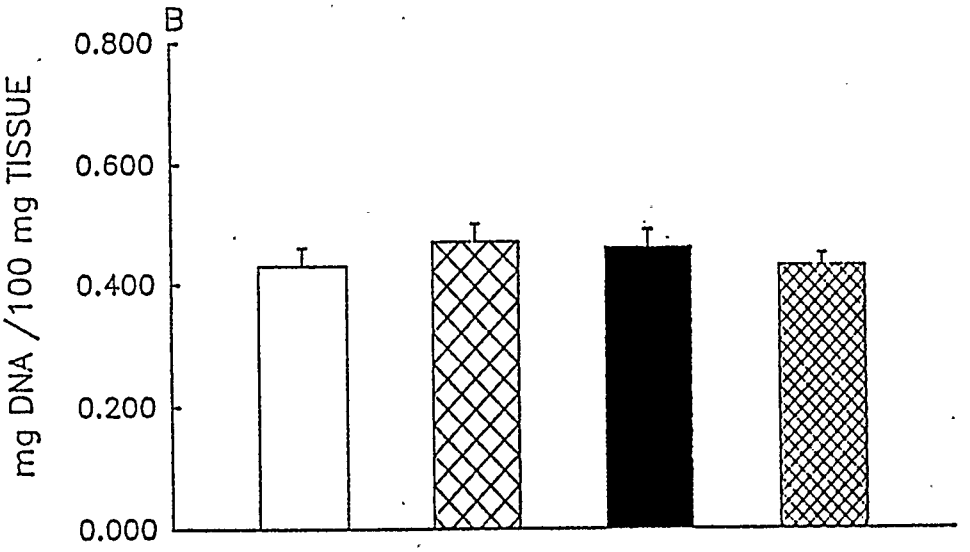
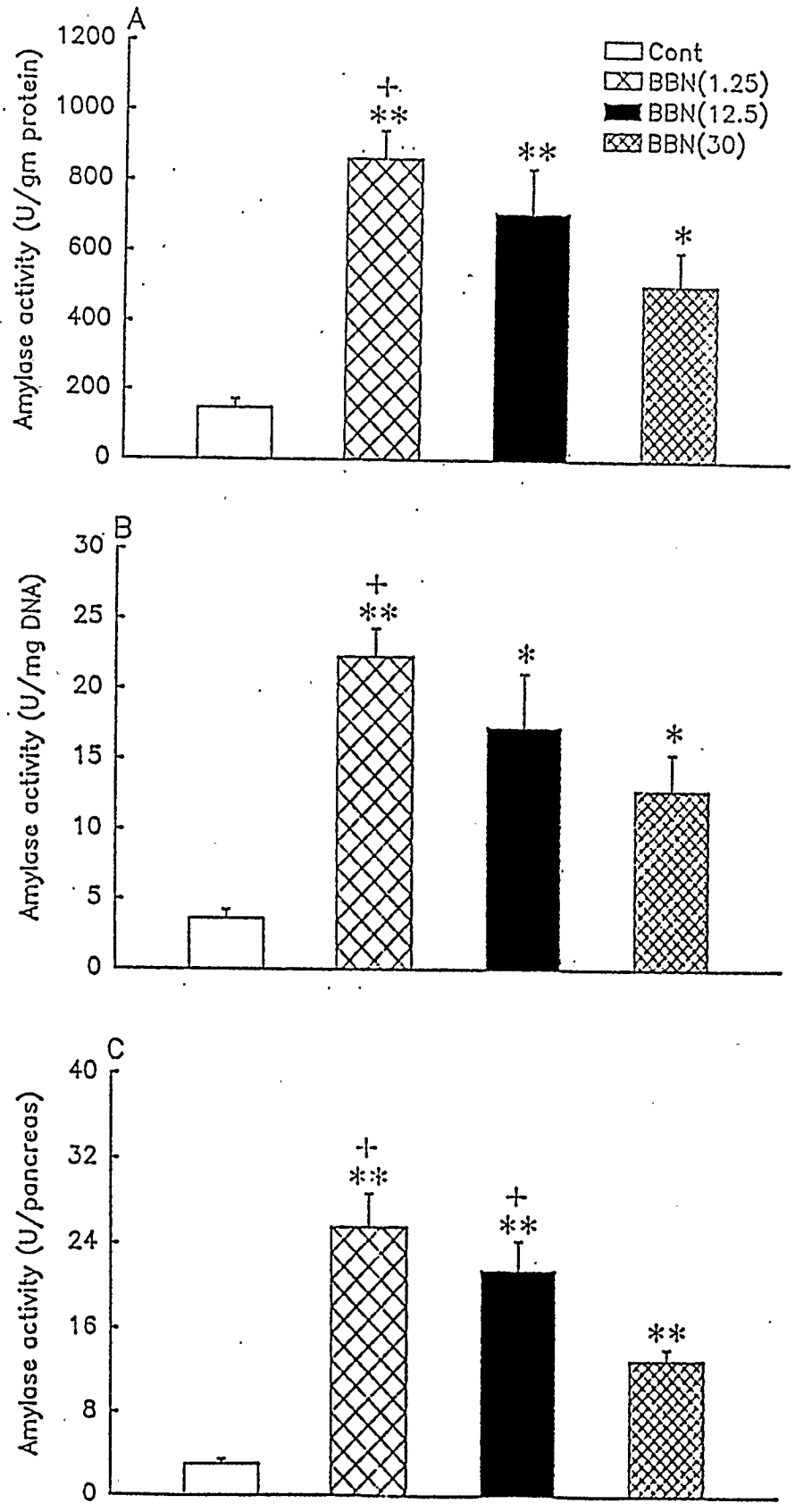

B
mg DNA /100 mg TISSUE
0.800
0.600
0.400
0.200
0.000

Figure 11. Effect of BBN at concentrations 1.25, 12.5, and 30 μg/kg body weight on pancreatic amylase activity expressed as (A) units/gm protein, (B) units/mg DNA or (C) units/pancreas. Values are means ± SE. *P<0.05 and **P<0.01 compared with controls. +P<0.05 compared with 30 μg BBN group.


A
Amylase activity (U/gm protein)
1200
1000
800
600
400
200
0
Cont
BBN(1.25)
BBN(12.5)
BBN(30)
+
**
**
*
B
Amylase activity (U/mg DNA)
30
25
20
15
10
5
0
+
**
*
*
C
Amylase activity (U/pancreas)
40
32
24
16
8
0
+
**
+
**
**

by BBN 30 μg/kg compared to control values. The maximal increase was in DNA content as P/DNA ratio was significantly lower in the proximal ($P<0.008$) and mid ($P<0.005$) intestinal segments of BBN-treated animals (Fig. 12).

BBN administration significantly increased sucrase and maltase activities in proximal and mid segments when expressed as units/gm protein (Fig. 13A and 14A). However, there were no significant differences when enzyme activities were expressed as units/mg DNA (Fig. 13B and 14B). BBN had no effect on lactase activity in any of the intestinal segments studied (Fig. 15).

3.1.6 Colonic mucosal protein and DNA

BBN significantly increased mucosal protein content ($P<0.008$), as well as DNA content ($P<0.0005$) of the distal colon. Again, the maximal increase was in DNA content as P/DNA ratio was significantly ($P<0.005$) lower in BBN-treated animals compared to control values (Fig. 9).

Figure 12. Effect of BBN at 30 μg/kg body weight on mucosal content of (A) protein, (B) DNA, and (C) protein/DNA (P/DNA) ratio of proximal (P), Mid (M), and distal (D) small intestinal segments. Values are means ± SE. *$P<0.05$, **$P<0.005$, and ***$P<0.0005$ compared to controls.

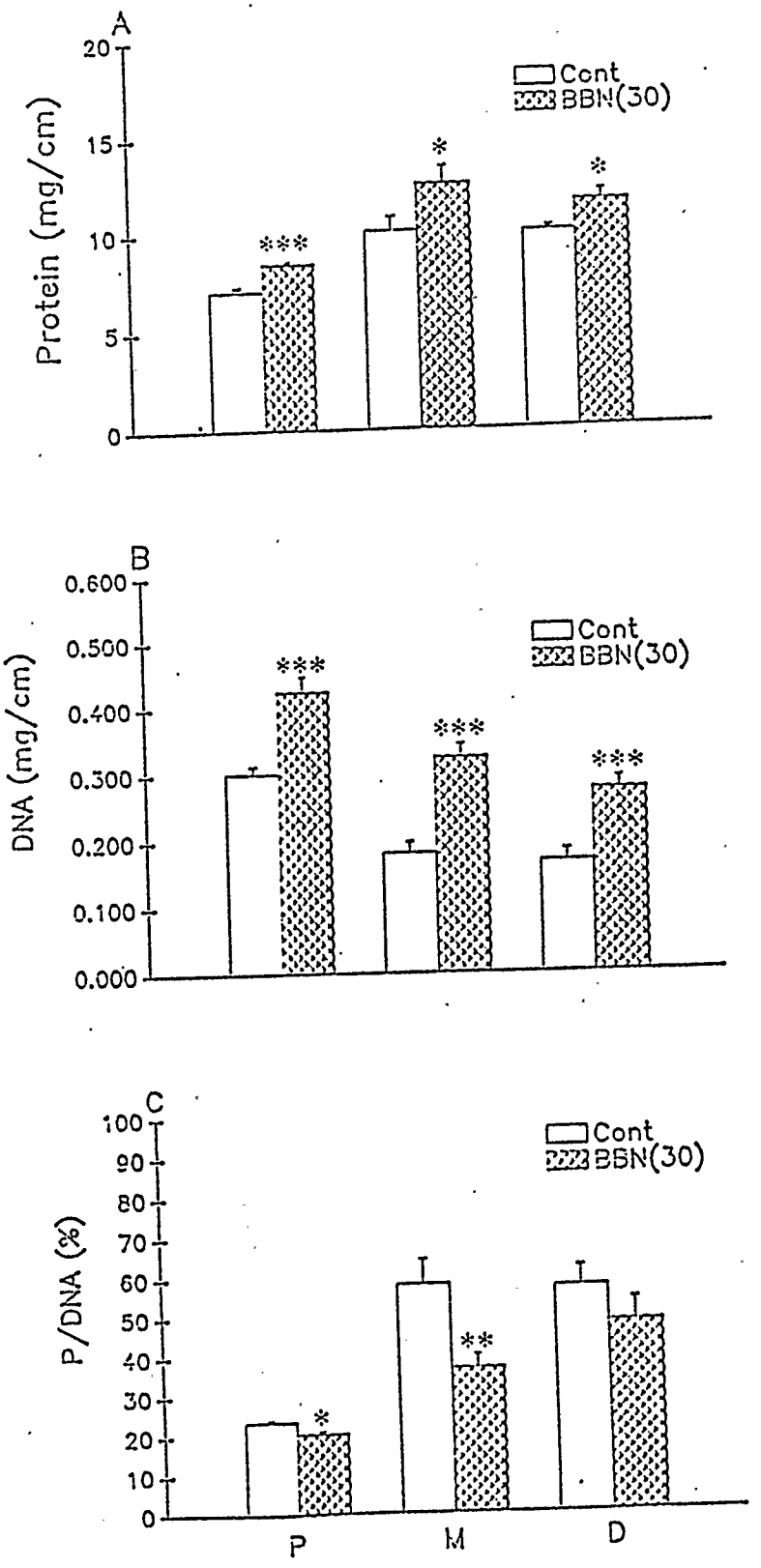

A
Protein (mg/cm)
20
15
10
5
0
Cont
BBN(30)
***
*
*
B
DNA (mg/cm)
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Cont
BBN(30)
***
***
***
C
P/DNA (%)
100
90
80
70
60
50
40
30
20
10
0
Cont
BBN(30)
*
**
P
M
D

Figure 13. Effect of BBN at 30 μg/kg body weight on sucrase activity expressed as (A) units/gm protein and (B) units/mg DNA in proximal (P), mid (M), and distal (D) small intestinal segments. Values are means ± SE. *$P < 0.05$ and **$P < 0.005$ compared with controls.

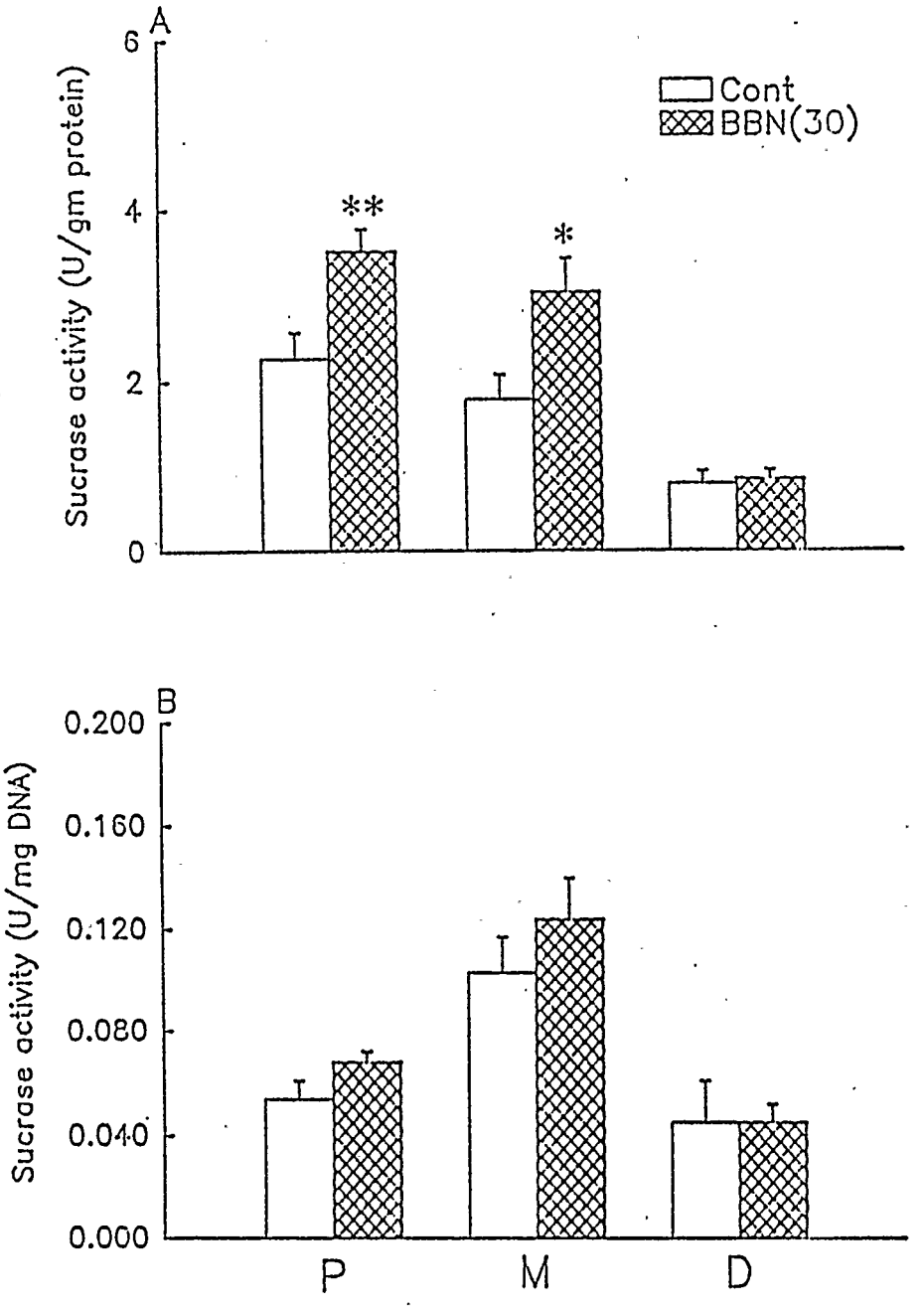

A
Sucrase activity (U/gm protein)
6
4
2
0
Cont
BBN(30)
**
*
B
Sucrase activity (U/mg DNA)
0.200
0.160
0.120
0.080
0.040
0.000
P
M
D

Figure 14. Effect of BBN at 30 μg/kg body weight on maltase activity expressed as units/gm protein (A) and units/mg DNA (B) in proximal (P), mid (M), and distal (D) small intestinal segments. Values are means ± SE. *$P < 0.05$ compared with controls.

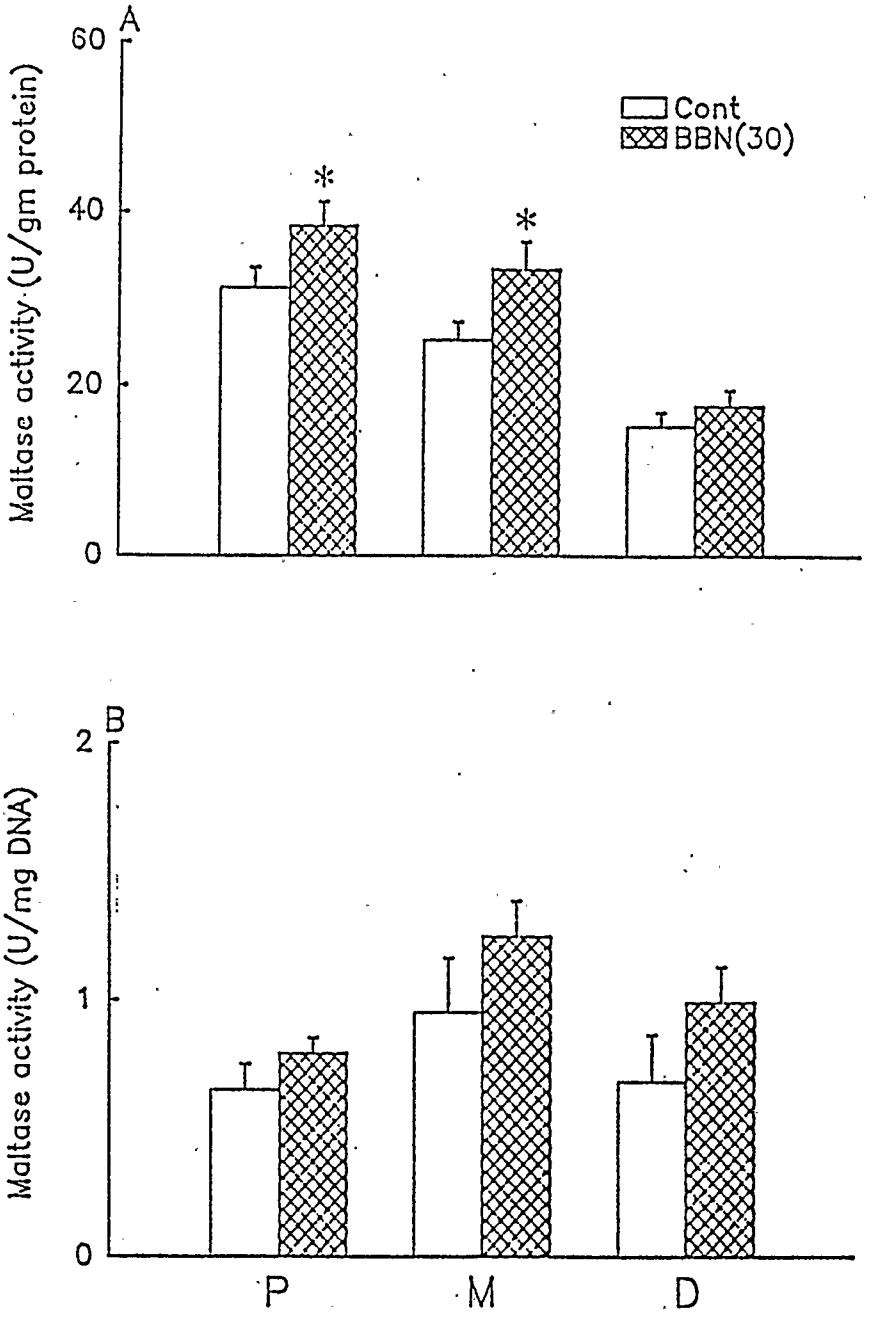
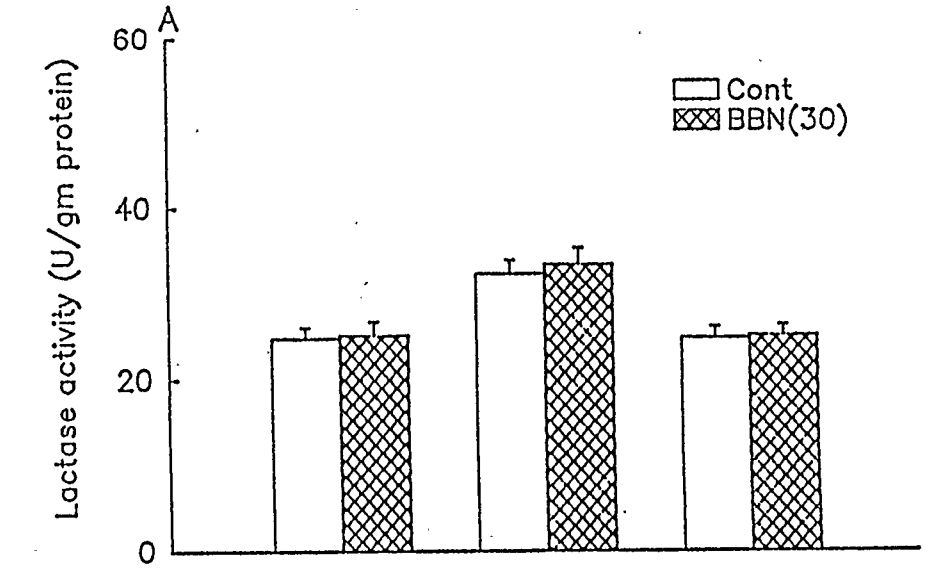
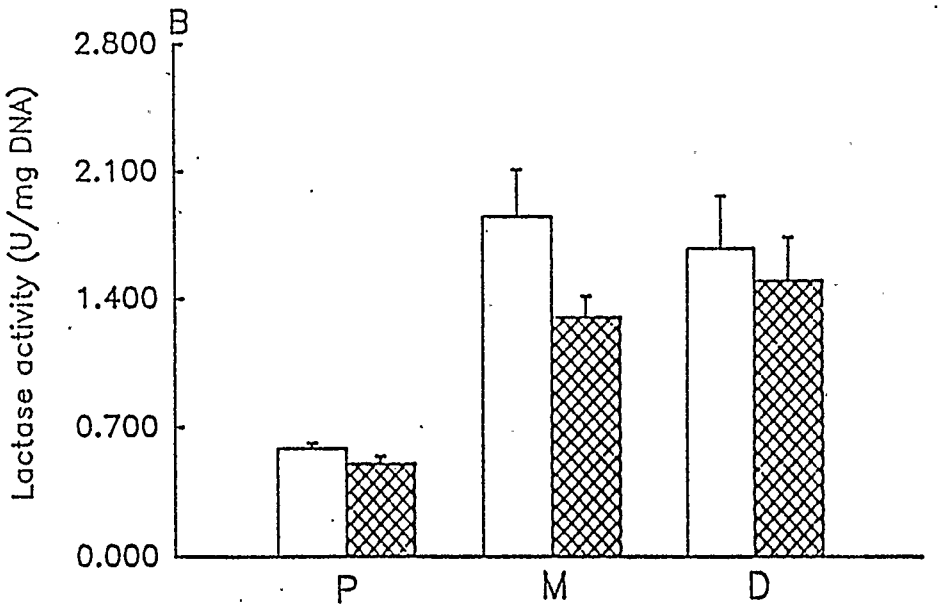

A
60
40
20
0
Maltase activity (U/gm protein)
Cont
BBN(30)
*
*
B
2
1
0
Maltase activity (U/mg DNA)
P
M
D

Figure 15. Effect of BBN at 30 μg/kg body weight on lactase activity expressed as (A) units/gm protein and (B) units/mg DNA in proximal (P), mid (M), and distal (D) small intestinal segments. Values are means ± SE.


A
60
40
20
0
Lactase activity (U/gm protein)
Cont
BBN(30)


B
2.800
2.100
1.400
0.700
0.000
Lactase activity (U/mg DNA)
P
M
D

3.2 THE PHYSIOLOGICAL ROLE OF BBN ON ONTOGENY OF THE RABBIT PANCREAS

3.2.1 Body weight

Weight gain of BBN antagonist-treated animals was not significantly different from control animals (Fig. 16).

3.2.2 Pancreatic protein, DNA, and P/DNA ratio

The BBN antagonist had no significant effect on pancreatic protein and DNA expressed as mg/100 mg tissue or P/DNA ratio in expermental group compared to controls (Fig. 17).

3.2.3 Pancreatic amylase and lipase activities

Amylase and lipase activities were significantly reduced in BBN antagonist-treated animals when it was expressed as units/gm protein ($P<0.05$) or units/gm tissue ($P<0.05$) compared to controls (Fig. 18A,C and 19A,C). However, their activities did not differ significantly between the two groups when expressed as units/mg DNA, although a tendency to decrease was noted in the BBN antagonist-treated group (Fig. 18B and 19B).

Figure 16. Weight gain in control and expermental animals treated with BBN antagonist [Leu^{13}-$\psi(CH_2NH)Leu^{14}$]-BBN at 30μg/kg body weight for 9 days. Values are means ± SE. There was no signicant differance between control and expermental animals.

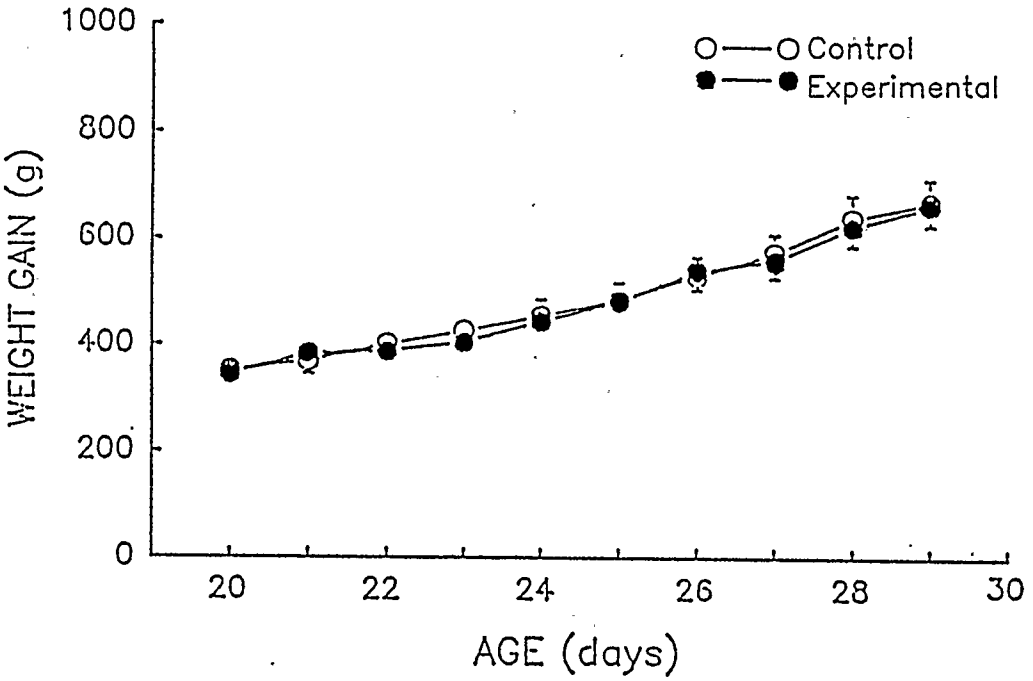

1000
800
600
400
200
0
WEIGHT GAIN (g)
20
22
24
26
28
30
AGE (days)
O—O Control
●—● Experimental

Figure 17. Effect of BBN antagonist [Leu^{13}-ψ(CH_2NH)Leu^{14}]-BBN at 30 μg/kg body weight on pancreatic (A) protein, (B) DNA, and (C) protein/DNA (P/DNA) ratio. Values are means ± SE. There was no significant differance between control and expermental animals.

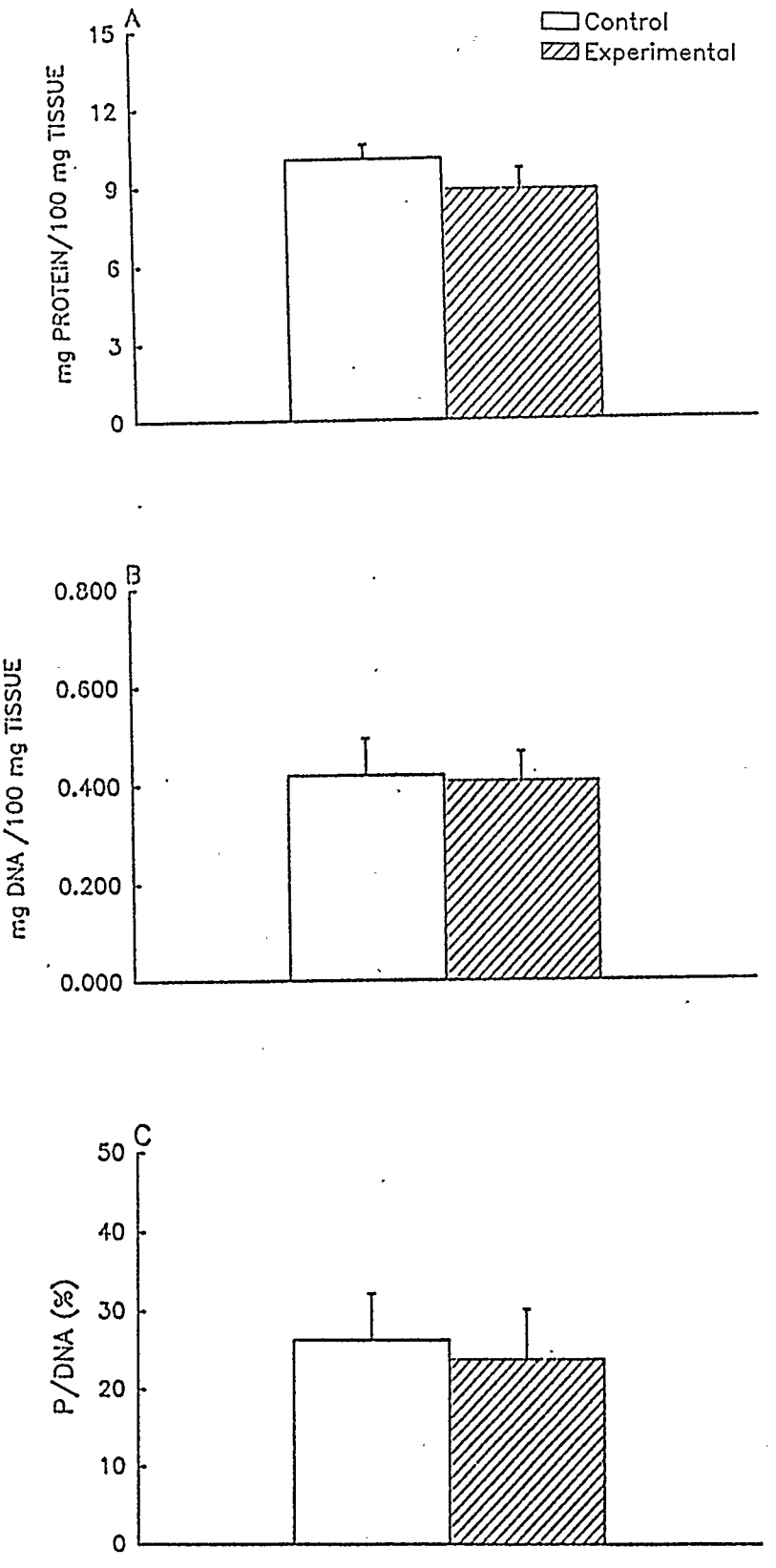

A
Control
Experimental
mg PROTEIN/100 mg TISSUE
15
12
9
6
3
0
B
mg DNA /100 mg TISSUE
0.800
0.600
0.400
0.200
0.000
C
P/DNA (%)
50
40
30
20
10
0

Figure 18. Effect of BBN antagonist [Leu^{13}-$\psi(CH_2NH)Leu^{14}$]-BBN at 30 μg/kg body weight on pancreatic amylase activity expressed as (A) units/gm protein, (B) units/mg DNA, and (C) units/gm tissue. Values are means ± SE. *$P < 0.05$ compared to controls.

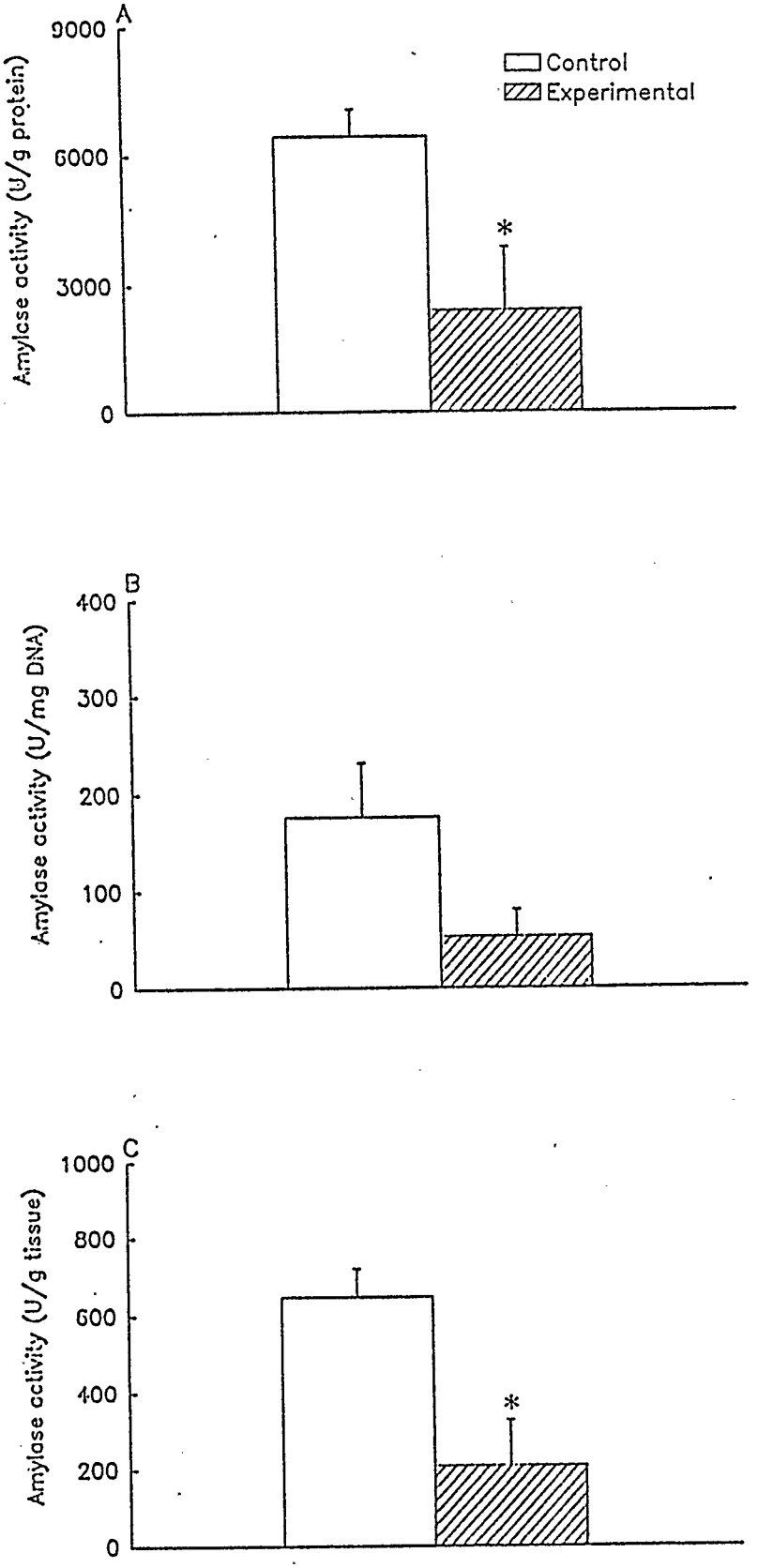
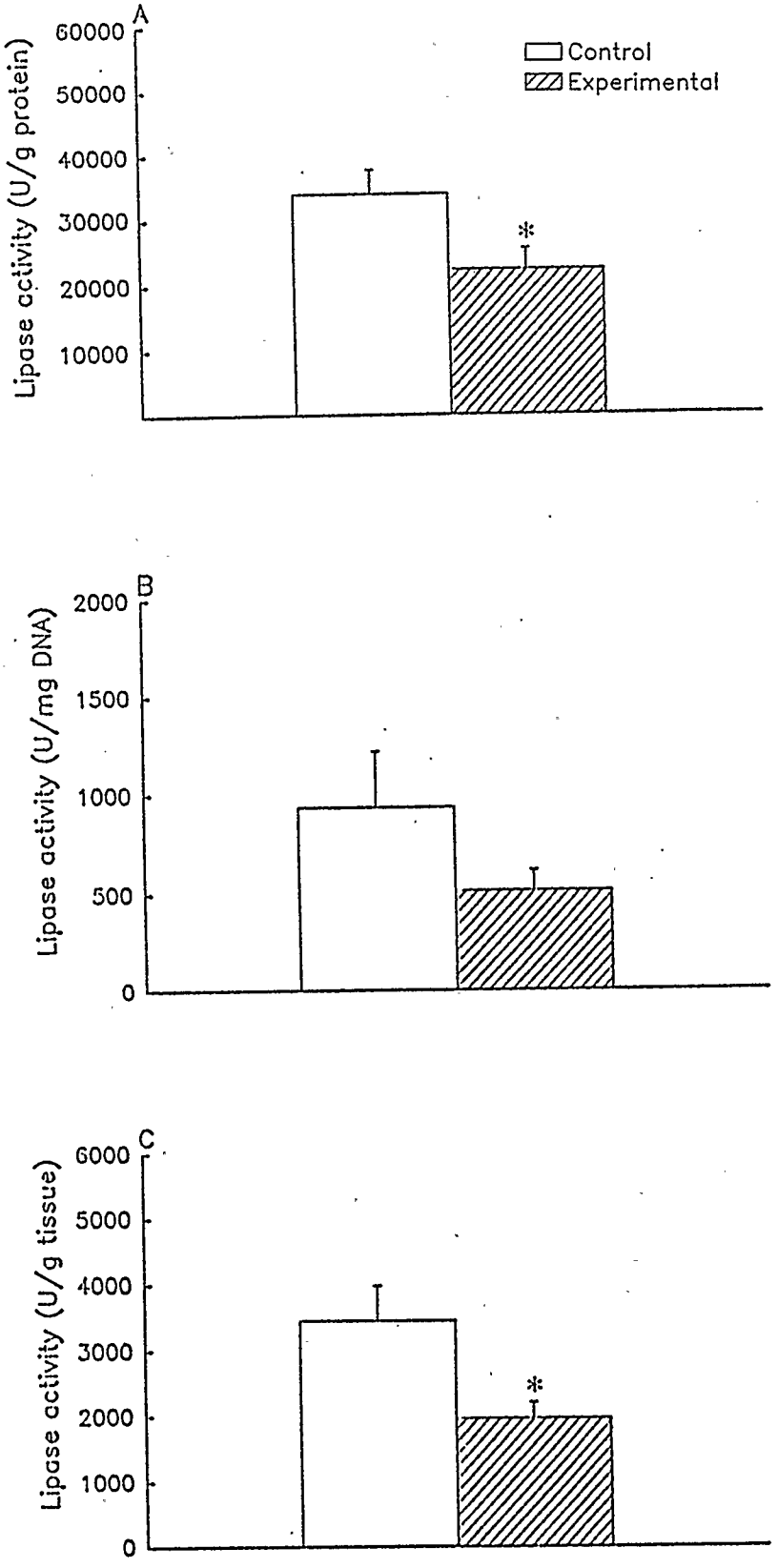

A
9000
6000
3000
0
Amylase activity (U/g protein)
Control
Experimental
*
B
400
300
200
100
0
Amylase activity (U/mg DNA)
C
1000
800
600
400
200
0
Amylase activity (U/g tissue)
*

Figure 19. Effect of BBN antagonist [Leu^{13}-ψ(CH_2NH)Leu^{14}]-BBN at 30 μg/kg body weight on pancreatic lipase activity expressed as (A) units/gm protein, (B) units/mg DNA, and (C) units/gm tissue. Values are means ± SE. *$P < 0.05$ compared to contols.


A
Lipase activity (U/g protein)
60000
50000
40000
30000
20000
10000
Control
Experimental
*
B
Lipase activity (U/mg DNA)
2000
1500
1000
500
0
C
Lipase activity (U/g tissue)
6000
5000
4000
3000
2000
1000
0
*

4. DISCUSSION

The principle aim of this study was to investigate the hypothesis that BBN might have a role in the ontogeny of the rabbit gastrointestinal tract. BBN-like immunoreactant has been discovered in human, bovine, and porcine milk (33,74,152). Similarly we have detected BBN-like activity in lactating rabbit milk (2.3 ng/ml). Some hormones and peptides present in maternal milk such as glucocorticoids, thyroxine, and epidermal growth factor have been reported to promote the maturation of the gastrointestinal tract during the postnatal period (38,87,112,109). Additionally, BBN-like immunoreactivity has been found in the rat gastrointestinal tract during the postnatal period (148). BBN is a potent mitogen which stimulates cellular proliferation in many tissues both in vitro and in vivo and stimulates the growth of Swiss 3T3 fibroblast in the absence of a second growth-promoting agent (22,121,122,155). Chronic BBN administration to the adult rat significantly increases DNA content of oxyntic and colonic mucosa (59), stimulates antral gastrin cell proliferation (77), and causes exocrine pancreatic hypertrophy and hyperplasia (83). BBN also promotes gastric, colonic, and exocrine as well as endocrine pancreatic cell growth in the suckling rat (78,79,111,118). These findings raise the possibility of a role for BBN in intestinal development.

A previous study by Lehy et al. (78) revealed that subcutaneous administration of BBN at dose of 40 μg/kg for 6 days in rats during the second week of life significantly increased the weight of stomach, pancreas, and intestine. There was a

significant increase in fundic and antral mucosal heights, as well as in the density of parietal cells in the fundic mucosa. Biochemical and electron microscopic morphometry studies showed that the increase in pancreatic weight is due to increases in both the acinar cell number and size. Orally administered BBN at dose of 60 μg/kg/day to suckling rats for 5 days during either the first, second, third, or fourth postnatal week of life significantly enhanced the labeling and mitotic indices in the oxyntic, antral, and colonic mucosa as well as in the exocrine and endocrine pancreas. The maximal stimulation of these parameters occurred in the second week of life and this effect of oral BBN disappeared in all tissues after weaning (79). Orogastric BBN administration significantly increased the labeling and mitotic indices in the endocrine pancreatic islets at the end of the first and second weeks of life but did not modify these parameters at the third and fourth weeks of life (118). Our results indicate that BBN is capable of exerting a trophic effect on stomach, liver, pancreas, small intestine, and colon in suckling rabbits. However, it has no effect in inducing precocious maturation of either brush border disaccharidase activities or liver glucokinase activity. These results confirm previous data on the growth-promoting effect of BBN on gastrointestinal tract of the suckling rats, and provide new preliminary data on the effect of BBN on growth and maturation of the liver and on the effect of BBN on intestinal brush border disaccharidase activities during the postnatal period. Moreover, our results provide evidence for a physiological role of endogenous BBN in the development of pancreatic exocrine function.

In the present study, the wet weight of stomach, liver, pancreas, whole small

intestine, full thickness wet weight of proximal, mid, and distal small intestinal segments as well as their mucosal weight, and colonic mucosal weight were significantly increased after BBN administration. This wide spread trophic effect of BBN is in keeping with the wide distribution of BBN receptors in the gastrointestinal tract. BBN receptors are localized in esophagus, gastric fundus and antrum, duodenum, jejunum, ileum, colon, and appendix of the human gastrointestinal tract (151) as well as in gastric fundus and antrum, duodenum, and ileum of the rat gastrointestinal tract (105). BBN receptors have been identified in the pancreas of many species (128).

BBN has been demonstated to increase both protein and DNA content in liver, fundus of the stomach, pancreas, all intestinal segments and in the distal colon. The increase was proportionally greater for DNA content as P/DNA ratio was significantly lower in BBN-treated group compared to controls. Our findings in the colon and pancreas are in agreement with those of Lehy et al. (78). Observations concerning the action of BBN on the stomach present some discrepancies. Lehy et al. (79) reported a trophic effect of BBN on both fundic and antral regions of the rat stomach, but in our study a trophic effect of BBN was observed only in the fundus region of the rabbit stomach. This discrepancy could be explained by species specificity of the response.

BBN significantly increased sucrase and maltase activities in proximal and mid segments when expressed as units/gm protein. Howerver, BBN did not increase their activities to a level of statistical significance when expressed as units/mg DNA. This

indicates that BBN had no effect on inducing precocious maturation of these enzymes, and that the significant increase in their activities when expressed as units/gm protein is due to increased tissue mass. BBN had no effect on lactase activity. Similiar observations have been demonstrated with insulin (95). Insulin has been shown to induce precocious maturation of sucrase and maltase activities expressed as units/gm protein but does not bring about a premature depression of lactase activity, but instead causes an increase (95). BBN had no effect on inducing precocious maturation of liver glucokinase activity.

Our observation on the effect of BBN on the pancreatic amylase is in agreement with those of Papp et al. (111) who administered BBN at dose of 30 μg/kg/day for 10 days from the day of parturition subcutaneously and observed a significant increase in pancreatic weight, total pancreatic protein and DNA contents, as well as total trypsin and amylase activities, but contradicts the results of Lehy et al. (78), who observed a decrease in amylase activity in BBN-treated animals. In another study, suckling rats which were treated subcutaneously every 12 hours for 6 days with different concentrations of BBN (5,10,20,40 μg/kg), starting on day 3 of life showed a dose-dependent increase in total pancreatic protein, but no effect on pancreatic DNA or amylase activity (114). Pollack et al. (115) reported an acute increase in intestinal trypsin activity following enteral administration of BBN in suckling rats. In our study we observed a dose-dependent increase in amylase activity. Interestingly, this effect was greatest at 1.25 μg/kg body weight. The explanation for this observation is not clear, but it could be that increasing the dose of BBN might

stimulate another inhibitory pathway as a cellular regulatory mechanism. The most potent known action of BBN is stimulation of gastrin release and there is a good correlation between plasma gastrin response to BBN and gastric acid response. However, higher doses of BBN caused further elevation of gastrin but did not cause higher rates of acid secretion. This observation was explained by the suggesion that other inhibitory peptides were released, most likely somatostatin, which is also released by BBN (54). A second possibility is that the higher concentrations led to down regulation of BBN-receptor numbers.

The mechanism (s) by which BBN stimulates growth is still to be determined. It could be mediated via the release of other factors as BBN is known to release numerous trophic hormones such as gastrin, CCK, neurotensin, pancreatic polypeptide, pancreatic glucagon, and insulin (41,93). However, gastrin, CCK, and pancreatic glucagon appear to have no effect on development of the gut during the postnatal period (14,163). The trophic effect of pancreatic polypeptide and neurotensin has not been studied during the postnatal period. Insulin released by BBN is known to induce precocious maturation of the intestine during the suckling period (88,95). Alternatively, BBN could stimulate growth of the gastrointestinal tract through a direct effect as BBN receptors have been described throughout the gastrointestinal tract of several mammalian species (105,128,147,151). Additionly, BBN has been demonstrated to have a direct effect on pancreatic acinar cells (65), epithelial-like cells from human normal embryonic intestine (131), and antral gastrin cells (44). However, It could be that both direct and indirect mechanisms exist

simultaneously.

Taking into account (1) the concentration of BBN-like activity in lactating rabbit milk, 2.3 ng/ml, (2) the quantity of milk ingested by the neonatal rabbit daily during the first 2 weeks of life, 10-25 ml/day, and (3) the increasing weight of the neonatal rabbit from ~100g at day 4 to ~240g at day 16, BBN at concentration 1.25 μg/kg would correspond to ~5times the amount of BBN ingested from the milk daily. Since there was no significant effect observed using BBN at concentration 1.25 μg/kg body weight on any of the parameters measured in any region of the gut studied except the pancreas, higher doses were used (12.5 and 30 μg/kg body weight).

Our findings do not conclusively prove that BBN has a physiological influence on growth and postnatal development of the gastrointestinal tract and the liver as the dose of BBN (30 μg/kg) which induced a significant trophic effect was of a pharmacological concentration order as it probably exceeds that which could be obtained from maternal milk. The concentration of BBN-like activity found in lactating rabbit milk is 2.3 ng/ml. The concentration in milk from other mammalian species is 2000, 1200, 235 pg/ml in porcine, bovine, and human milk, respectively (33,74,152). However, the dose used in our study comparable with that used by others in suckling rats [40 μg/kg/day for 7 days (78) or 60 μg/kg/day for 5 days (118)].

There are several ways to demonstrate that BBN might play a physiological role in regulating ontogeny of the gastrointestinal tract. One approach would be to study the effect of removing the source of BBN to determine if this delayed normal

ontogeny of the gastrointestinal tract and then define if the effect could be reversed by replacing it exogenously. This approach is nearly impossible because BBN is derived from many sources including endogenous and exogenous sources in the postnatal period. A second approach is to study the effect of attenuating or abolishing the effect of BBN by a specific antiserum or a selective antagonist. In the present study we used the BBN antagonist [Leu^{13}-$\psi(CH_2NH)Leu^{14}$]-BBN to study the physiological role of BBN in the neonatal pancreas. As mentioned earlier, this potent competitive antagonist has high specificity for BBN receptors and exhibits a 100-fold improvement in the binding affinity compared to previously reported BBN receptor antagonists. This analog has been shown to specifically antagonize the action of BBN on pancreatic acini (21), Swiss 3T3 fibroblasts (21,156), gastric acid secretion in rats (124), growth of certain strains of human small cell lung carcinoma (86,145), and on BBN-induced contractile response on isolated smooth muscle cells from guinea pig (129). These studies suggest that this new BBN antagonist should be a useful agent for studying the role of endogenous BBN on the postnatal development of the gastrointestinal tract and on other BBN-dependant systems. Considering (1) [Leu^{13}-$\psi(CH_2NH)Leu^{14}$]-BBN binds to dispersed pancreatic acinar cell BBN receptor sites with a K_D of 59.6 nM, as compared with 4.4 nM for BBN, to give an antagonist to agonist potency ratio of 1:14 (21) and (2) the amount of BBN ingested in the milk daily during the first 2 weeks of life is ~0.23 μg/kg and assuming that the same amount is ingested during the third and fourth weeks of life, [Leu^{13}-$\psi(CH_2NH)Leu^{14}$]-BBN at concentration 30 μg/kg would correspond to ~130times the daily intake of

BBN from the milk. Taking into accont that the affinity of BBN for BBN receptor on pancreatic acinar cells is 14 times higher than the affinity reported for [Leu^{13}-$\psi(CH_2NH)Leu^{14}$]-BBN, this dose would correspond to ~9times the daily intake of BBN from the milk. BBN antagonist-treated animals did not exhibit significantly different weight gain comparable to controls. There was no significant differenace in pancreatic protein and DNA content expressed as mg/100 mg tissue between the two groups. However, there was a significant reduction in amylase and lipase activities in BBN antagonist group compared to controls. These results suggest that BBN has a physiological role in the development of exocrine pancreatic function during the postnatal period and it may provide a basis for developing new therapeutic strategem for diseases associated with pancreatic defeciency.

In conclusion, our findings suggest that BBN has a trophic effect on the developing gastrointestinal tract and liver but has no effect on inducing precocious maturation of intestinal brush border dissaccharidase activities or liver glucokinase activity. Moreover, it provides evidence for a physiological role of BBN in the maturation of neonatal exocrine pancreatic function.

Future directions of research

Future studies might include an investigation of the role of BBN on other aspects of intestinal functional development such as its effect on intestinal transport of glucose and electrolytes during the postnatal period. Histological studies might be helpful to confirm the biochemical findings.

An other way to prove a physiological role for BBN in the ontogeny of the gastrointestinal tract is the measurement of endogenous BBN in intestinal tissues during the critical period of the postnatal development by radioimmunoassay (12). A rise in BBN level before the start of developmental changes would provide circumstantial evidence for a possible role of BBN in intestinal maturation.

Studies utilizing tissue in culture rather than the whole animal might be helpful to demonstrate a direct effect of BBN on maturation of the gastrointestinal tract. However, studies with tissue in culture must be treated with caution as it is not known to what extent cell changes during culture might affect the cell response to BBN, or how much the artificial cell culture media resemble the cell media in vivo.

Orally administered BBN to suckling rats has been reported to induce a proliferative response in the gastrointestinal tract (118). The mechanism by which BBN reaches its targets is still unknown. Thus it might be interesting to investigate whether BBN activiates luminal receptors without being absorped or whether BBN is absorped and acts locally or systemically on its targets as has been suggested previously for EGF (68,143).

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