

BIOBEHAVIOURAL DETERMINANTS
OF
COLITIS-INDUCED ANOREXIA

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BIOBEHAVIOURAL DETERMINANTS OF COLITIS-INDUCED ANOREXIA

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ABSTRACT

Although acute inflammation of the colon (colitis) results in a large suppression of eating, the mechanisms underlying this anorexia remain unknown. Since interleukin-1 (IL-1) receptors are implicated in colitis-induced anorexia and since IL-1 stimulates the release of oxytocin (OT), a neuropeptide implicated in the control of eating, I evaluated the role of OT in the rat model of colitis induced by intrarectal infusion of 2,4,6-trinitrobenzene sulfonic acid (TNB). Antagonism of central OT by a specific OT receptor antagonist did not diminish the magnitude or time course of colitis-induced anorexia. Similarly, no elevations in plasma OT levels correlated with the colitis-induced anorexia, which has been interpreted to mean that malaise was not associated with the anorexia. Subsequently, I examined whether the suppression of food intake could be explained by an exaggerated satiety response to the post-ingestive consequences of food intake that normally signal meal termination. To obtain experimental control over post-ingestive consequences, intragastric preloads were delivered prior to a meal. The various preloads used did not result in a greater suppression of food intake in TNB-treated rats. However, these experiments did reveal significant suppression in the rate of intake for rats with colitis, which suggests a possible shift in response to taste. "Taste Reactivity" measures were taken to assess this possibility. Colitis resulted in a specific change in behaviour and movements that are interpreted to indicate that the food was perceived as less positive.

Together, these studies suggest that neither central OT, malaise, nor exaggerated response to post-ingestive consequences are mediators of the colitis-induced anorexia. The shift in Taste Reactivity measures suggests the anorexia may be mediated by a change in taste hedonic perception.

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DICTIONARY OF ABBREVIATIONS

AD	= active drip	IR	= Intrarectal
ANOVA	= analysis of variance	L	= locomotion
AVP	= arginine-vasopressin	LTP	= lateral tongue protrusions
CCK	= cholecystokinin	MM	= mouth movements
CR	= chin rubs	MPO	= Myeloperoxidase
CRF	= corticotrophin releasing factor	OT	= oxytocin
ETOH	= ethanol	OTA	= oxytocin antagonist
FF	= forelimb flails	PD	= passive drip
G	= gapes	PE	= polyethylene
HS	= head shakes	PL	= paw licks
HW	= head wash	PT	= paw treading
IBD	= Inflammatory Bowel Disease	PVN	= paraventricular nuclei
icv	= intracerebroventricular	R	= rearing
ID	= internal diameter	RF	= real fed
IG	= intragastric	SAL	= saline
IL-1	= Interleukin -1	TNB	=2,4,6-trinitrobenzenesulfonic acid
IO	= intraoral	TP	= tongue protrusions
ip	= intraperitoneal	TR	= taste reactivity

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GENERAL INTRODUCTION

Inflammatory diseases of the gastrointestinal tract, such as Crohn's disease and ulcerative colitis, are often associated with weight loss and reduced food intake (anorexia) (MacPherson & Pfeiffer, 1976; McCallum, Grill, Lange, Planky, Glass & Greenfeld, 1985). This anorexia contributes to the morbidity and mortality connected with Crohn's disease by increasing nutritional deficit, weight loss, and retarding growth and healing (Griffiths, Nguyen, Smith, McMillan & Sherman, 1993; Kirschner, 1988; Motil, Grand, Davis-Kraft, Ferlic & Smith, 1993). Alternatively, improved nutrition has been implicated in the remission of IBD (González-Huix, de León, Fernández-Banares, Esteve, Cabré, Acero, Abad-Lacruz, Figa, Guilera, Planas & Gassull, 1993; Royall, Jeejeebhoy, Baker, Allard, Habal, Cunnane & Greenberg, 1994; Thomas, Taylor & Miller, 1993). Despite the potentially serious harmful effects of reduced food intake, and the potential benefits of improving nutrition, the mechanisms influencing and mediating this suppression of food intake remain unidentified.

Inflammatory Bowel Disease (IBD) is a group of chronic diseases that are primarily diagnosed in children and adolescents (Griffiths *et al*, 1993; Motil *et al*, 1993; Stokes, 1992). IBD is characterized by abdominal pain, diarrhea, and inflammation of the gastrointestinal tract (Anderson, Sutherland & Hassall, 1989). The fact that these symptoms are associated with a chronic disease, that has no known cure, adds to the

importance of trying to better understand IBD for economical reasons alone. The two diseases, ulcerative colitis and Crohn's disease are similar, yet have distinct differences in pathology. In ulcerative colitis, generally the inflammation in the mucosa extends continuously from the rectum toward the large bowel, with ulcers confined to the mucosa (Anderson *et al*, 1989). However, the main differences between Crohn's disease and ulcerative colitis are that in Crohn's disease, the inflammation is not continuous, it can affect any part of the gastrointestinal tract, the ulcers in Crohn's disease can be transmural, and the inflammation is often associated with granulomas (Anderson *et al*, 1989).

The etiology for Crohn's disease and ulcerative colitis has not yet been elucidated, although genetic and environmental factors, such as nutrition, smoking, and infection, have been proposed (Williams, 1991; Kurata, 1994; Persson, Ahlbom & Hellers, 1992). These diseases can be diagnosed using X-ray, endoscopy, histology, patient and doctor report (Hildebrand, Karlberg & Kristiansson, 1994; Harms, Blomer, Bertele-Harms, Shmerling, Konig, Spaeth & The Study Group on Crohn's Disease in Children and Adolescents, 1994). Since a diagnosis can be made, but the cause remains unknown, the main focus of therapy has been control of symptoms.

A common symptom of IBD is weight loss (Anderson *et al*, 1989; Mingrone, Greco, Benedetti, Capristo, Semearo, Zoli & Gasbarrini, 1996; Royall, Greenberg, Allard, Baker & Jeejeebhoy, 1995). Because IBD is frequently diagnosed in children, delayed growth and delayed puberty are common, especially in Crohn's disease (Ferguson & Sedgewick, 1994; Hendricks, Williams, Stoker, Schoenfeld, Walker & Kleinman, 1994;

Hildebrand *et al*, 1994). The delay in growth cannot be accounted for by steroid therapy. Rather, it appears to be the result of malnutrition (Motil *et al*, 1993). In fact the decrease in weight precedes the decrease in height and has been reported as the first symptom of IBD, occurring several years before the appearance of other gastrointestinal symptoms (Hildebrand *et al*, 1994; Motil *et al*, 1993).

Malnutrition is observed in IBD patients, and could result from three primary sources: malabsorption caused by gastrointestinal damage, increased energy expenditure related to inflammation, and a decrease in food intake (Anderson *et al*, 1989; Mingrone *et al*, 1996). Studies with Crohn's disease patients indicate that weight loss is observed in patients who do not demonstrate malabsorption or increased energy expenditure, which suggests that decreased food intake is primarily responsible for the weight decrease. These findings indicate that research should focus on food intake and nutrition (Rigaud, Angel, Cerf, Carduner, Melchior, Sautier, René, Apfelbaum & Mignon, 1992).

The daily caloric intake of patients with IBD can be affected by malaise (feeling of visceral illness), fear of abdominal pain, fear of diarrhea, or a decrease in appetite (Royall *et al*, 1995; Rigaud *et al*, 1992; Stokes, 1992). Crohn's patients with decreased food intake do not report greater nausea, or pain, but they do report a decrease in appetite (Rigaud *et al*, 1992). The importance of decreased appetite as a contributor to disease morbidity is also supported in clinical research with children. Decreased appetite was found to be one of the most important indexes for disease activity in children (Harms *et al*,

1994). While aspects such as depression, anxiety or gastrointestinal malfunction may be involved, it appears that anorexia is a major contributor to malnutrition in IBD.

Research indicating that improved nutrition may be involved in remission of IBD adds to the importance of understanding the mechanisms mediating the anorexia (González-Huix *et al*, 1993; Royall *et al*, 1994; Thomas *et al*, 1993). Retrospective examination of information, from patients who were admitted to hospital with acute exacerbations of IBD, indicated that nutritional support by enteral feeding improved nutritional status and decreased the need for surgical intervention (Gassull, Abad, Cabré, González-Huix, Giné & Dolz, 1986). A randomized, controlled study of children with active Crohn's disease indicated that nutritional treatment with elemental diet resulted in similar improvements to treatment with high-dose steroids in disease activity and remission (Thomas *et al*, 1993). Other studies have supported the role for nutrition in IBD therapy. For example, one study reported that treatment with polymeric enteral nutrition is as safe and effective as steroid treatment in inducing short term remission in active Crohn's disease (Gonzalez-Huix *et al*, 1993). Additionally, nutritional support has been reported to improve body composition for patients with Crohn's disease (Royall *et al*, 1995).

Given the potential harm of anorexia, and the potential benefits from improving nutritional status of IBD patients, further research was required to elucidate the mechanisms that mediate the anorexia associated with colitis. Research into mechanisms is difficult to conduct in humans, thus animal models of colitis were required. Several

animals models for colonic inflammation have been developed, but the best animal model for each research question must be chosen. An ideal animal model for the human disease would be a model with similar disease course (chronic) and symptoms such as diarrhea, abdominal pain, inflammation of the gastrointestinal tract, weight loss and anorexia. The tissue damage should be similar to that reported for the human disease. Further, an animal model should be easily accessible, and affordable, and easy to induce and maintain to allow for reproduction and verification of any research. Most important in a model used for research into the anorexia associated with colitis, the model should result in a robust, reproducible suppression of food intake.

Many animal models have been developed that, while useful models for colitis, are not ideal for research into the mechanisms mediating anorexia. For example, chronic colitis, with relapses, develops spontaneously in cotton top tamarins. The spontaneous model mimics the course of the disease in humans, but cotton top tamarins are an endangered species and, consequently, access to this species is limited (Morris, Beck, Herridge, Depew, Szewczuk & Wallace, 1989). Spontaneous lesions have also been found in the colons of rats, mice and dogs, pigs and monkeys. Unfortunately, the occurrence of tissue damage could not be controlled, making replications of experiments difficult. In addition, many of the symptoms were not similar to IBD (MacPherson & Pfeiffer, 1976).

As an alternative to spontaneous models, colitis can be induced by administration of different substances into the colon. For example, bacteria such as *Escherichia coli*

cause inflammation of the intestinal tract that can be induced when required and replicated easily. However, the inflammation demonstrated no evidence of autoimmune involvement, which suggested that the course of the disease was not similar to the colitis that was being modeled (MacPherson & Pfeiffer, 1976). Alternatively, lesions in the colon can be induced in rats by vasoactive agents, such as acetylcholine or methacholine. These treatments result in a colitis that is reproducible, but the symptoms disappear quickly, making research into mechanisms difficult. Also, the morphology resulting from the lesions is not similar to that found in the tissue of human patients with IBD (MacPherson & Pfeiffer, 1976).

Another option for animal models, where the inflammation is induced rather than spontaneous, includes colitis induced by chemicals. Infusion of chemicals, such as acetic acid or 2,4,6-trinitrobenzene sulfonic acid (TNB), into the colon of rats has been shown to cause an inflammation that is chronic, reproducible and economically feasible (MacPherson & Pfeiffer, 1976; McHugh, Castonguay, Collins & Weingarten, 1993; Morris *et al*, 1989). In particular, TNB treatment in rats results in a rat model that has similar symptomatology to IBD (Allgayer, Deschryver & Stenson, 1989; Grisham, Volkmer, Tso & Yamada, 1991; Morris *et al*, 1989; Yamada, Marshall, Specien & Grisham, 1992). In the model, inflammation is induced in the distal colon (colitis) of male albino Sprague-Dawley rats by one intrarectal (IR) infusion of TNB dissolved in 50% ethanol (ETOH). This treatment causes an inflammation in addition to ulcers, luminal narrowing and smooth muscle hypertrophy (Allgayer *et al*, 1989; Yamada *et al*, 1992).

Also, the inflammation is transmural and granuloma formation occurs following treatment with TNB (Morris *et al*, 1989), which is characteristic in the tissue damage reported for Crohn's disease, indicating that the tissue damage observed in the model has similarities to IBD.

The exact mechanism by which the inflammation is induced in IBD remains to be elucidated; however, one hypothesized etiology for IBD was used for the development of inflammation in the TNB model. It has been suggested that the mucosal barriers of the gastrointestinal tract are compromised first, allowing an antigen to cross the mucosa into the lumen. There, the antigen activates the immune system, resulting in inflammation. In the TNB model, the vehicle, ETOH, acts to break down the mucosal barriers of the colon. TNB itself is then able to cross into the mucosa from the lumen. TNB is a hapten, which can combine with tissue protein to form a highly antigenic molecule that activates the immune system (Morris *et al*, 1989). Additionally, oxygen radicals are released during the metabolism of TNB and can also induce inflammation (Yamada *et al*, 1992). In IBD, oxygen radicals released by neutrophils, epithelial cells and smooth muscle cells have been implicated in causing the tissue damage (Yamada *et al*, 1992; Yue, Sun, Dunn, Yin & Wong, 1996), while oxygen radical scavengers inhibit the inflammation (Yue *et al*, 1996). This suggests that the course of the inflammation and damage in the model have similarities to that of IBD.

Overall, the TNB model fulfils several criteria of a good model: it was developed based on a hypothesis of the etiology of IBD, there are similarities in the tissue damage to

IBD and the model is accessible, affordable and easy to reproduce. In addition, symptoms including diarrhea and weight loss have been observed in the TNB model (McHugh, Weingarten, Keenan, Wallace & Collins, 1993 b). The course of colitis is not a replication of the actual disease, since it is not spontaneous, nor does it last a life time. However, this is not a serious deficiency for research focused on understanding the mechanisms mediating food intake as long as the model results in a reproducible suppression of food intake. Research has shown that, associated with the colitis caused by IR treatment with TNB, there is an immediate, but transient, suppression of food intake. The anorexia is greatest after the first day of treatment (approximately 70% suppression), returning to normal by the fourth day (Hansell, Collins, Verbalis, Stricker & Weingarten, 1993; Kustra, Amarelo, Collins & Weingarten, 1995; McHugh *et al*, 1993 b). This suppression of 24 hour food intake is observed with solid powdered chow, liquid diet and elemental diet, without any significant alteration of water intake, indicating behavioural specificity (McHugh *et al*, 1993 b). The pattern of anorexia has also been replicated in other laboratories (Hogaboam, Jacobsen, Collins & Blennerhassett, 1995), supporting the findings that the TNB model of colitis results in a robust, reproducible anorexia.

Research focusing on food intake within the context of the TNB model has not only shown that the anorexia is a robust, reproducible phenomenon (McHugh *et al*, 1993 a, b) but has helped to characterize the phenomenon. The degree of anorexia is significantly correlated with the degree of tissue inflammation, as indexed by myeloperoxidase activity (MPO), an enzyme released from neutrophils (Weingarten,

1996). While the suppression of food intake returns to baseline levels by the fourth day following treatment with TNB, there is also an associated weight loss that returns to baseline levels only after anorexia is alleviated, approximately 5 days following treatment (McHugh, Collins & Weingarten, 1994). Further, meal pattern analysis has shown that the anorexia is not caused by refusal or inability to eat, in fact anorexic rats initiate meals with the same frequency as control rats. Rather, the suppression of food intake is expressed through a reduction in meal size (McHugh *et al*, 1993 a).

The exact mechanisms mediating meal size reduction are still unknown, although some work has been done to examine this issue. Inflammation in the colon alters eating behaviour (McHugh *et al*, 1993 a), which suggests that communication between the site of inflammation and the feeding centres in the brain must occur. There is preliminary data suggesting that the signal is not likely to be communicated from the gut neurally since neither the elimination of vagal pathways nor the area postrema eliminated the anorexia (Weingarten, Ladenheim, Emond & Collins, 1993). Instead, inflammatory mediators released from the gut may mediate the suppression of food intake. For example, the cytokine interleukin-1 (IL-1) is involved in mediating inflammation and is released into circulation during colitis (Brynskov, Nielson, Ahnfelt-Ronne & Bendtzen 1992; Chance & Fisher, 1991; Plata-Salaman, French-Mullen, 1992) and administration of IL-1 into the periphery results in a suppression of food intake (Daun & McCarthy, 1993; Plata-Salaman, Oomura & Kai, 1988). McHugh and colleagues (1994) demonstrated that peripheral and central administration of a receptor antagonist to IL-1 attenuated the anorexia induced by

TNB treatment. These findings indicate that IL-1 is a possible mediator of the colitis-induced anorexia. Additionally, research with a cyclooxygenase inhibitor indicated that the suppression of feeding is mediated in part by prostaglandins in the colon (McHugh *et al*, 1994; McHugh, Weingarten & Collins, 1992). In some fashion, the mediators of inflammation at the level of the gastrointestinal tract must communicate with the brain in order to alter behaviour. The focus of this research is to understand the mechanism by which inflammation in the colon mediates the behaviour of suppressed food intake. In addition to further characterizing the anorexia expressed in the TNB model of colitis, my thesis examines potential biological and behavioural mediators of anorexia. The first chapter will examine the possibility that the peptide, oxytocin, acts as a mediator of anorexia in the brain. Additionally, the possibility that gastric malaise accounts for the suppression of food intake will be addressed, using peripheral levels of oxytocin as a marker. The second chapter will examine whether an exaggerated response to the consequences of food intake could mediate the anorexia observed in TNB-treated rats. The pattern of anorexia during intraoral infusion of food will be characterized in the third chapter and used in the final chapter to determine whether a change in taste perception might mediate the suppression of food intake.

GENERAL METHODS

SUBJECTS:

Male albino Sprague-Dawley rats (Charles River Inc., Canada) were housed individually in clear plastic cages in a colony room with a 12:12 hour light / dark cycle.

FOOD:

The rats were maintained on Purina powdered rat chow until surgery. Following surgery, they were maintained on Purina powdered chow or nutritionally complete Liquid Diet: 385 ml condensed 2% carnation milk, 85 ml water, 0.3 ml Trivisol (vitamins A, D, C, from MeadJohnson), 47 g sugar and 6.32 ml of mineral cocktail (5.69 μ g cupric acetate, 645.6 μ g ferrous gluconate and 98.6 μ g manganese acetate made up to 200 ml in water). Water was available *ad libitum*.

ICV CANNULA SURGERY:

The rats were anaesthetized with intraperitoneal (ip) injections of Ketamine (90 mg/kg) and Xylazine (10 mg/kg) and given 0.006 mg/rat of the analgesic, buprenorphine (Temgesic). A 22 gauge guide cannula (Plastics One, Roanoke, VA) was positioned in the left lateral ventricle (1.8 mm posterior, 0.2 mm lateral and 3.5 mm below bregma), embedded in dental cement, and closed with a dummy cannula.

ICV Infusions: Infusions were made through a 28 gauge internal cannula (Plastics One, Roanoke, VA) connected to a Hamilton microsyringe by 0.01 internal diameter (ID) tubing (Elkay running foot tubing). Infusion rate was 2 μ l / min by hand using a 0.25 μ l syringe or 1.75 μ l / min by pump (Razel Syringe Pump) with a 10 μ l syringe. The internal cannula remained in the guide cannula for a minimum of 1 min following infusions. All control infusions were equal volumes of the vehicle alone, saline (0.9% isotonic).

ICV Cannula Placement: At the end of the experiment, the rats were given 1 ml of Chloral hydrate. Cannula position was determined by infusing 0.5 ml of 1% Neutral Red Dye into the guide cannulae. The brain was then fixed in 10% formalin for a minimum of 24 hours, and sliced to determine the location of the dye.

JUGULAR CATHETER SURGERY:

The rats were anaesthetized by ip injections of either 6.5 mg/kg of Somnotol or 90 mg /kg of Ketamine with 10 mg / kg of Xylazine. Jugular catheters were surgically implanted to allow blood sampling. The jugular catheters (modified from Nicolaidis, Rowland, Meille, Marfaing-Jallat & Pesez, 1974; Smith & Campfield, 1986) of 0.01 ID Silastic tubing, (Dow Corning, Midland, MI) with reinforced Silastic sheeting (Dow Corning, Midland, MI) were inserted into the jugular vein from an incision in the dorsal neck region. The top of the catheter was passed subcutaneously between the eye and the ear to the top of the

skull, where a blunted 20 gauge needle covered by Elkay tubing (0.03 ID running foot standard) was attached. The catheter was secured in a head cap of dental acrylic (Lang, Wheeling, IL). All surface incisions were closed with 3-0 silk and 0.3 ml of the antibiotic, Derapen, was injected intramuscularly to minimize bacterial infections. The jugular catheters were flushed daily with 0.05 ml of 0.9% heparinized saline.

Blood Sampling: A sample of whole blood (1 ml), was drawn at a rate of 0.025 ml/sec from the jugular catheter into a heparinized syringe. If there were difficulties extracting blood, 0.05 ml of pure heparin (Heparin Leo 1000 I.U. / ml, Leo Laboratories Canada Inc.) was infused into the catheter and immediately withdrawn. The blood sample was immediately placed on ice in a heparinized eppendorf tube, and an equal volume of 0.9% sterile saline was infused back into the catheter. After a maximum of half an hour, the blood samples were centrifuged for 3 min in a refrigerated (5° C) Beckman Microfuge E. The extracted plasma was aliquoted into eppendorf tubes and frozen at -70° C. Plasma oxytocin (OT) and arginine-vasopression (AVP) levels were analyzed by Dr. J Verbalis using radioimmunoassay.

INTRAGASTRIC SURGERY:

The rats were anaesthetized with Ketamine and Xylazine (90 mg / kg and 10 mg / kg respectively), and then given 0.02 ml of Temgesic (Buprenorphine 0.006 mg / rat) by ip injection. Intra gastric (IG) cannulae were prepared 24 hours in advance with a 20 gauge needle blunted at both ends, curved and inserted into a 15 cm length of Silastic tubing (0.03 ID, Dow Corning, USA) and secured using a ball of Silastic glue. A 2 cm x 2 cm square of Marlex mesh (Bard Implants, Mississauga, Ont.) was attached approximately 1 cm from the opposite end of the Silastic tubing. A ball of Silastic glue acted as a hub at the very end of the tubing. The hub was inserted into a hole in the stomach and secured using two purse-string sutures so that the Marlex mesh sat on the outside of the stomach. The muscle wall was sutured with catgut, and the remaining tubing extended from the stomach to the top of the head between the skin and the muscle layer. The metal tubing of the cannula was anchored to the skull using skull screws and acrylic dental cement (Lang, Wheeling, IL). All skin incisions were sutured closed using 3.0 silk. The cannulae were flushed daily with sterile water or saline.

INTRAORAL SURGERY:

The rats were anaesthetized with Ketamine and Xylazine (90 mg / kg and 10 mg / kg respectively), and then given 0.02 ml of Temgesic (Buprenorphine 0.006 mg / rat) by ip injection. The bevelled end of a 15 gauge 3.5 inch steel hypodermic needle with the end bulb removed (regular bevel, reusable; Becton Dickinson, Yale) was placed between the

cheek and the gum just anterior to the upper first molar and advanced between the eye and the ear until it emerged slightly midline between the two ears. Silk thread (0.0) was inserted through the needle, and used to pull the intraoral (IO) cannula into place. The IO cannula was constructed of 6 cm of Silastic tubing (0.03 ID Dow Corning, Midland, MI), with 1 cm of polyethylene (PE-90) tubing (Clay Adams, Becton Dickinson, USA) inserted inside, with the free end heat-flanged. The flanged end anchored the IO cannula inside the mouth, and the cannulae extended under the skin exiting at the top of the head. A 20 gauge needle (blunted ends, sides roughened, with a ball of Silastic glue in the centre) was inserted into the end extending from the head. IO cannula from both sides of the mouth were anchored to the skull using skull screws and acrylic dental cement (Lang, Wheeling, IL). If an IG cannulae was also implanted, all three were secured together in the head cap. The cannulae were flushed daily with sterile water or saline.

Intraoral Infusions: IO-feeding cages were made with Plexiglas walls and bottom, with a mesh screen lid and a mirror mounted at approximately a 45° angle underneath the floor to reflect facial movements and diet rejections. The IO infusions were made using an Ismatec (Cole-Parmer) 4 channel pump connected by a blunted 19 gauge needle to the Elkay tubing (0.03 ID running foot standard; Fischer Scientific) that attached to the rats' IO cannulae. The pump was set at 14% capacity to infuse at a constant rate of 1.0 to 1.1 ml/min similar to the rate used by other researchers (Kaplan, Seeley & Grill, 1994). Meals were initiated by turning on the pump, and meal termination was defined as two

rejections of the liquid diet within a 90 sec period (Grill, Spector, Schwartz, Kaplan & Flynn, 1987). A rejection was considered a drop of liquid leaving the mouth and touching the floor. When the first rejection occurred, the pump was turned off for a 30 sec interval and then restarted. If the rat rejected within 60 sec, then the meal was terminated (total of 90 sec after first rejection). If the rat rejected after the 60 sec, then the drip was treated as a first drip, and the procedure was repeated (Kaplan, Seeley & Grill, 1994). The rats were given a minimum of three days experience with IO infusions before testing was begun. When real feeding (RF) was used, the rats were trained for three days to RF from a graduated cylinder in the same cages as the IO feeding occurred.

TRAINING: Liquid Diet Intake and Intraoral Cage

After a minimum of two days recovery following surgery, the rats were introduced to the novel liquid diet overnight. The rats were habituated to a Plexiglas cage in a novel room during 10 minute sessions on two days. Then, the rats were trained to eat during a 4 hr feeding schedule until they maintained constant weight. During the first hour of the feeding schedule, the rats were placed in the novel room in Plexiglas cages with access to graduated bottles of liquid diet. During the final three hours of the feeding schedule, the rats were returned to the home cages in the colony room and given access to graduated bottles of liquid diet. Food intake was recorded at 1 hour and at 4 hours following the beginning of the feeding period.

POST-OPERATIVE CARE: Duratears lubricating ophthalmic ointment (Alcon, Mississauga, Ont.) was applied to both eyes. Furacin antibacterial ointment (Austin Laboratories Ltd., Quebec) was applied to all incisions. Each rat was administered 0.0006-0.009 mg of Temgesic (buprenorphine analgesic) ip. Fluid replacement consisted of a 3 ml subcutaneous injection of 0.9% sterile saline immediately following surgery. Animals were placed under a heat lamp. When infection with large swellings around the headcap was observed (specifically the IO cannulae) the antibiotic, Tylosin (10 mg / kg) was dissolved in the drinking water following surgery.

INDUCTION OF COLITIS: The rats were anaesthetized by ether in a bell-jar. Then, a PE-60 catheter was inserted through the anus, 8 cm past the rectum. Experimental rats were infused with 0.25 ml of liquid TNB (Biochemica, Caledon Labs, Ontario) in 50% ethanol (ETOH) at 100 mg/ml (25 mg / rat). Control rats were infused with 0.25 ml of the vehicle (ETOH) alone.

INFLAMMATION ASSAY: Myeloperoxidase (MPO), an enzyme released by the intracellular granules of neutrophils, was used as a marker to quantify inflammation. Rats were first given an overdose of 1 ml of Chloral hydrate (350 mg / ml). Approximately 3 cm of colonic tissue was collected from the area just above the maximal inflammation,

frozen in liquid nitrogen and stored at -70° C. MPO activity was assessed by homogenizing 50 mg/ml of tissue in hexadecyltrimethyl-ammonium buffer (Polytron Brinkman Homogenizer), centrifuging the mixture and adding an O-Dianisidine solution. A spectrophotometer was used to measure the colorimetric assay (Bio-Teck, EL312, Microplate Biokinetics Reader) in order to determine the level of MPO activity.

CHAPTER 1

Central and Peripheral Oxytocin

In the first chapter, the peptide oxytocin (OT), will be examined as a potential mediator of the anorexia associated with TNB-induced colitis. OT is released into the central nervous system, specifically the brain, and into the peripheral circulatory system (Arletti, Benelli & Bertolini, 1989; Forsling, 1986; Olson, Drutarosky, Stricker & Verbalis, 1991 a). Involvement of OT from the central nervous system in the TNB-induced anorexia would implicate the peptide as a mediator that translates the signal from the gut into a behavioural change in food intake. Alternatively, peripheral OT can be used as a physiological marker for malaise (Olson *et al*, 1991 a; Verbalis, McCann, McHale & Stricker, 1986 a; Verbalis, McHale, Gardiner & Stricker, 1986 b), which will be discussed to greater extent in the final section of this chapter. A temporal correlation of elevated peripheral OT levels with the anorexia would suggest that anorexia was mediated by malaise.

I: CENTRAL OXYTOCIN

Treatment with IR TNB results in a colitis which is associated with a suppression of food intake. The change in feeding behaviour suggests a communication between the gut, the site of inflammation, and the feeding centres in the brain. Inflammatory mediators released from the gut mediate the suppression of food intake. For example, the cytokine, interleukin-1 (IL-1), is involved in mediating inflammation and is released into circulation during colitis (Brynskov *et al*, 1992; Chance & Fisher, 1991; Plata-Salaman *et al*, 1992). Injections of IL-1 can suppress food intake (Daun & McCarthy, 1993) and peripheral and central administration of a receptor antagonist to IL-1 attenuate the anorexia, indicating that IL-1 is a mediator of the colitis-induced anorexia (McHugh *et al*, 1994). In some fashion, the mediators of the inflammation change brain activity in order to alter behaviour; however, these pathways have not yet been established.

Previous work on feeding behaviour has implicated the peptide, oxytocin (OT), as a mediator in the central nervous system involved in the suppression of food intake (Max, Thystere, Chapleur-Chateau, Burlet, Nicholias & Burlet, 1994; Olson *et al*, 1991 a, Olson, Drutarosky, Stricker & Verbalis, 1991 b). For example, central OT levels correlate with decreased food intake, intracerebroventricular (icv) administration of OT inhibits food intake in a dose dependent fashion, and the OT antagonist, vasotocin, eliminates an

anorexia caused by corticotrophin releasing factor (CRF) (Olson *et al*, 1991 b).

Interestingly, research shows that OT is released centrally in response to CRF, and CRF is released in response to the presence of IL-1 (Glowa, Barrett, Russel & Gold, 1991; Olson *et al*, 1991 b; Uehara, Sekiya, Takasugi, Namiki & Arimura, 1989). Consequently, there is a potential pathway that could mediate the colitis-induced anorexia, involving IL-1 which is known to be released from the site of inflammation and to be associated with the TNB-induced anorexia. IL-1 elevations in the brain could cause a release of CRF, which in turn release central OT to mediate the suppression of food intake during colitis.

One method to assess the role of central OT in colitis-induced anorexia is to antagonize OT activity. If central OT is a physiological mediator of the colitis-induced anorexia, then the central administration of an oxytocin antagonist (OTA) should inhibit the suppression of food intake following TNB treatment. Preliminary studies were conducted as a positive control to determine the amount of OTA required to effectively block a suppression of food intake (Appendix 1). If central oxytocin plays a role in colitis-induced anorexia, then the administration of an OTA during the course of the anorexia should result in a decrease of the anorexia.

I: METHODS

Subjects: Twenty-six male rats (200-300 g) were maintained on Purina powdered rat chow in hanging steel cages.

Infusions: Infusion volumes were determined in a preliminary experiment (see Appendix 1). All infusions were in volumes of 7 μ l infused icv at 1.75 μ l / min (Razel Syringe Pump with 10 μ l syringe), 30 min prior to the two hour feeding period. Experimental infusions were 14 μ g (2 μ g / μ l) of the OTA, L-366,948, (Merck Sharpe & Dohme Research Labs, PA) dissolved in saline (SAL), while the control infusions were 7 μ l of SAL alone (see General Methods).

Procedure: Lateral cerebral ventricle cannulae were implanted. L-366,948 maximally antagonizes OT for 2 hours following icv infusion (Arletti *et al*, 1989). Thus, three days after surgery, rats were trained on a 2 hour feeding schedule of Purina powdered rat chow until body weight and intake stabilized. Food intake was measured at 30, 60 and 120 min following food presentation. Baseline intake was determined following a control SAL infusion 30 min prior to a feeding period. Half of the rats were randomly assigned to be treated with liquid TNB and half with ETOH alone (see General Methods). For the following 5 days, half of the TNB-treated rats received icv infusions of OTA, while half received SAL. The same procedure was followed with the ETOH treated rats, resulting in four experimental groups: TNB-SAL, TNB-OTA, ETOH-SAL, ETOH-OTA. Measurements of food intake were made at 30, 60 and 120 min after meal initiation. After 5 days, the colons were collected for MPO analysis and the cannula placement verified (see General Methods).

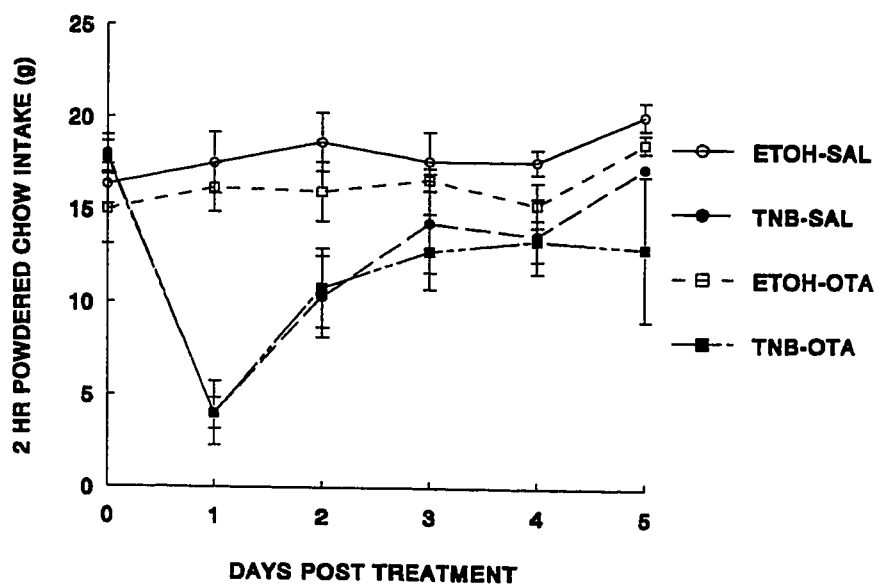
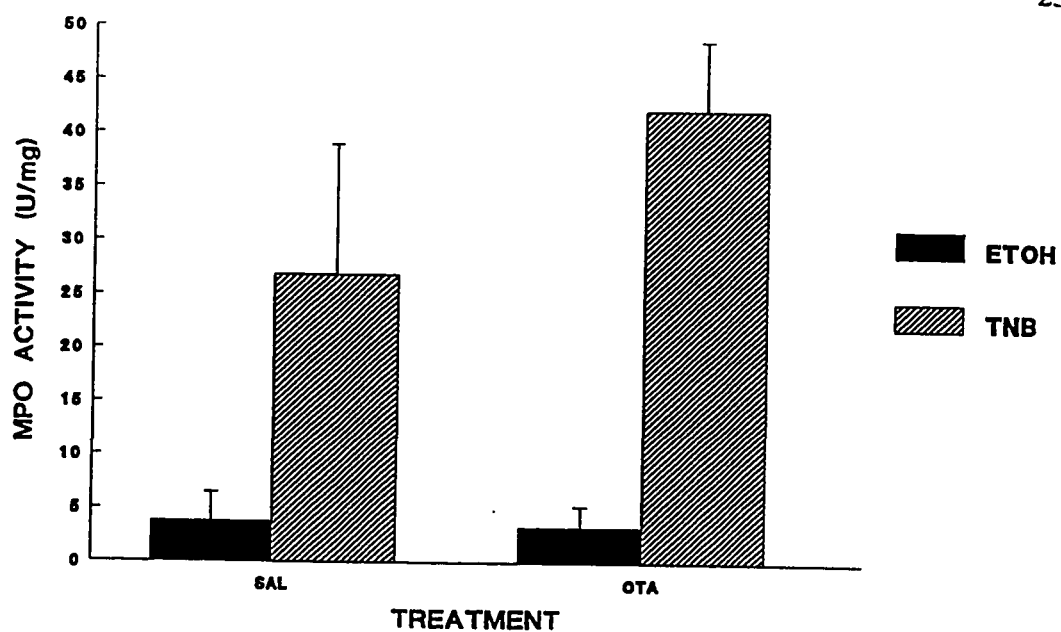
I: RESULTS

Myeloperoxidase Assay: Analysis of the colon samples using analysis of variance (ANOVA) indicated that IR treatment with TNB resulted in significantly more MPO activity than treatment with ETOH ($F(1, 16) = 34.54, p < 0.0001$), but inflammation was not affected by icv infusions (figure 1).

Food intake: Food intake data was collected at 30, 60 and 120 minutes following the initiation of the 2 hour feeding period. The cumulative 2 hour food intake (120 min) (figure 2), was significantly suppressed by treatment with TNB compared to ETOH (3-Way Between-Within ANOVA) ($F(1, 16) = 22.57, p = 0.0002$). The days had a significant effect ($F(4,64) = 15.67, p < 0.0001$), and there was a significant interaction between days and treatment ($F(4,64) = 9.93, p < 0.0001$), indicating that the anorexia followed the expected pattern of an extreme, but transient, suppression of food intake which returns to baseline by Day 5. However, icv infusions of OTA had no effect on the food intake ($F(1,16) = 1.28, p = 0.27$). This pattern of findings was replicated at 30 and 60 minutes following the initiation of a meal, so there was no significant effect of the OTA icv infusions on food intake.

Figure 1: Myeloperoxidase (MPO) activity in the colon for rats receiving saline icv: ETOH-SAL (N= 6), TNB-SAL (N= 3) and rats receiving OTA icv: ETOH-OTA (N= 6), TNB-OTA (N= 5). Data are group means \pm SE.

Figure 2: 2 hr food intake 30 min after icv infusions of 7 μ l of OTA (2 μ g / μ l) or of SAL for baseline and five days following treatment with TNB or ETOH. ETOH-SAL (N= 6), TNB-SAL (N=3) ETOH-OTA (N= 6), TNB-OTA (N= 5). Data are group means \pm SE.



I: DISCUSSION

Treatment with TNB resulted in a significantly greater inflammation than treatment with ETOH, and a significant suppression of food intake. 14 μ g (icv) of the OTA had no significant effect on the anorexia induced by the colitis. However, preliminary experiments (Appendix 1) established that 14 μ g (icv) of the OTA, L-366,948, was capable of eliminating a 50% suppression of food intake induced by CRF release of OT. The positive control enables the conclusion to be drawn that OT is not a central mediator of the colitis-induced anorexia.

Central OT is synthesized primarily in the magnocellular and parvocellular cells of the supraoptic and the paraventricular nuclei (PVN) of the hypothalamus (Ivell, 1986; Leng, Way & Dyball, 1991). Many studies have demonstrated that the PVN is involved in mediating food intake (Monnikes, Schmidt, Raybould & Tacke, 1992; Leibowitz, Hammer & Chang, 1981; Aravich & Sclafani, 1983). However, ablation of the PVN in TNB-treated rats did not attenuate the anorexia (Morrison, 1995). The ablation experiment clearly demonstrated that the integrity of the PVN was not necessary for the expression of the TNB-induced anorexia. Since the PVN is rich in both CRF and OT producing neurons, the experiment also supports the conclusion that central OT is not a mediator of colitis-induced anorexia.

II: PERIPHERAL OXYTOCIN

Elevations in the levels of peripheral OT can be used as a physiological marker for malaise (Olson *et al*, 1991 a; Verbalis *et al*, 1986 a, b). A possible mediator of TNB-induced anorexia is malaise, or the sensation of visceral illness. Malaise and fear of abdominal pain have often been proposed as the reason that patients with IBD decrease their food intake (Anderson *et al*, 1989; Rigaud *et al*, 1992; Royall *et al*, 1995; Stokes, 1992). Malaise has been studied in non-human subjects through behavioural studies such as conditioned taste aversions and changes in behaviour such as grooming and body position (Bernstein, 1991), but malaise is difficult to quantify using these methods. A quantifiable physiological marker of malaise, elevations in peripheral OT, was used to assess the role of malaise in the TNB model of colitis.

Some observations from behavioural studies of the TNB model of experimental colitis suggest that malaise, alone, is not sufficient to account for the suppression of food intake (McHugh *et al*, 1993 a). TNB treated rats were capable of food-appetitive responses such as meal initiation, and were able to maintain feeding in the absence of post-gastric satiety cues (sham feeding) suggesting that malaise was not causing the anorexia (McHugh *et al*, 1993 a).

Systemic OT levels can vary independently from central levels of OT (Arletti *et al*, 1989; Forsling, 1986; Olson *et al*, 1991 a). Peripheral levels of OT are transiently elevated in the blood plasma of rats in a dose related manner after systemic treatment with

agents that reduce food intake in association with gastric malaise (ex. lithium chloride and large doses of cholecystokinin (CCK)) (Burbach, 1986; Flanagan, Verbalis & Stricker, 1989; McCann, Verbalis & Stricker, 1989; Neumann, Landgraf, Takahashi, Pittman & Russell, 1994; Olson *et al*, 1991 a; Renaud, Tang, McCann, Stricker & Verbalis, 1987; Verbalis *et al*, 1986 b). Consequently, the changes in OT levels are sensitive to different amounts of anorexigenics.

Unlike central OT, peripheral OT injections do not reduce food intake (Olson *et al*, 1991 a; Verbalis *et al*, 1986 b), nor do they cause learned taste aversions, suggesting that plasma OT itself does not cause malaise (Arletti *et al*, 1989; Olson *et al*, 1991 a; Verbalis *et al*, 1986 b). Instead, elevations in plasma OT can be used as a quantifiable, physiological marker for malaise (Olson *et al*, 1991 a; Verbalis *et al*, 1986 a, b) and have been used to assess the presence of malaise in several other studies (Chavez, Seeley & Woods, 1995; Pohjanvirta, Unkila & Tuomisto, 1994). To ensure that any changes in plasma OT did not reflect a general activation of the posterior pituitary gland, measurements were also taken of another peptide, Arginine-vasopressin (AVP), which is similar to OT in both form and site of production (Burbach, 1986; Gibbs, 1984; Lang, Heil, Ganten, Hermann, Unger & Rascher, 1983; Verbalis *et al*, 1986 b; Verbalis, Richardson & Stricker, 1987) (see General Methods).

Malaise may mediate the anorexia induced by colitis. Alternatively, it is possible that the rats are experiencing malaise, but that the malaise does not mediate the anorexia. Previous studies support the concept that anorexia can be dissociated from malaise (Ervin,

Birkemo, Johnson, Conger, Mosher & Menius, 1995). This study examined the changes of plasma OT levels (associated with the suppression of food intake following TNB treatment) through samples of blood drawn from a surgically implanted jugular catheter. In order to evaluate the role of malaise in TNB-induced anorexia, the blood samples were examined for elevations of plasma OT which were used as a quantifiable, physiological marker for malaise (Olson *et al*, 1991 a; Verbalis *et al*, 1986 a, b).

IIA: 5 DAYS

An initial experiment was conducted in order to determine whether there were elevations in the plasma levels of OT over 5 days that correlated with anorexia expressed in the rat model of intestinal colitis. Since elevations of plasma OT are used as a physiological marker for malaise (Olson *et al*, 1991 a; Verbalis *et al*, 1986 a, b), a correlation between elevations of plasma OT and suppressed food intake would implicate a role for malaise in mediating the anorexia.

IIA: METHODS

Subjects: Fifteen male rats weighing between 350-450 g at the time of jugular catheter surgery were maintained in steel hanging cages on Purina powdered rat chow.

Procedure: The baseline food intake was established. The rats were anaesthetized with Somnotol (6.5 mg / kg) and jugular catheters were implanted to allow blood sampling (see General Methods). A minimum of two days later, a baseline blood sample of 1 ml was extracted (see General methods). Experimental rats were treated IR with powdered 120 mg / ml (30 mg / rat) of TNB (Kodak Co., Rochester, NY) in 50% ETOH to induce inflammation, while control rats were treated with ETOH alone (see General Methods). On days 1 to 5, blood samples (1 ml) were collected and food intake measured between 10:00 am and 1:00 pm (according to the time baseline data was collected). On day 5 the rats were given an overdose of Somnotol (0.5 ml / rat) and samples of colon were collected and stored for MPO analysis (see General Methods). The OT levels were analyzed, and to ensure that any changes in plasma OT did not reflect a general activation of the posterior pituitary gland, levels of AVP were measured (General Methods).

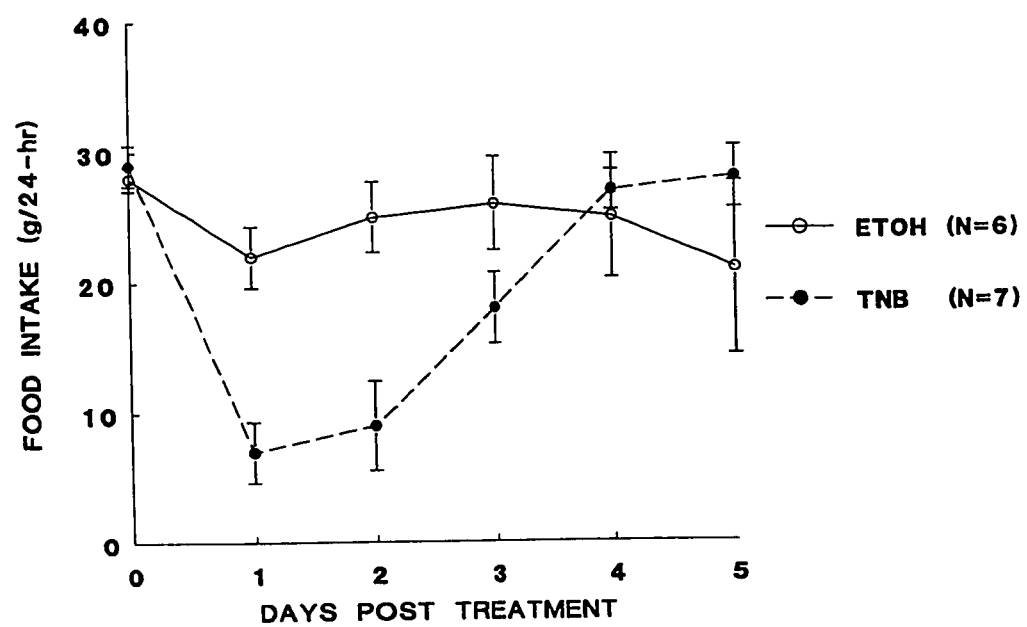
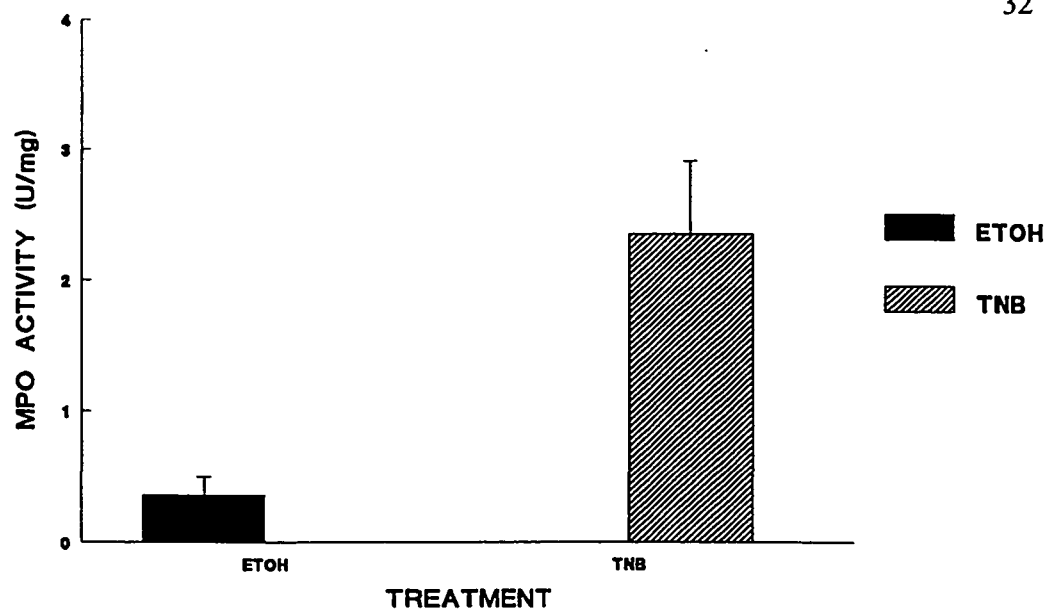
IIA: RESULTS AND DISCUSSION

Myeloperoxidase Assay: Figure 3 shows that IR TNB resulted in greater inflammation of the colon than IR ETOH since the MPO activity was 6.5 times greater than the MPO activity for ETOH ($F(1, 10) = 9.97, p = 0.01$).

Food Intake: The baseline 24 hour food intake (figure 4) of the two groups were the same, and IR administration of ETOH did not change the food intake compared to the

Figure 3: Myeloperoxidase (MPO) activity in the colon after treatment with TNB (N=6) or ETOH (N=6) for rats in Experiment IIA. Data are group means \pm SE.

Figure 4: 24 hr powdered chow intake (g) for baseline and five days after treatment with TNB (N=8) or ETOH (N=7) in Experiment IIA. Data are group means \pm SE.

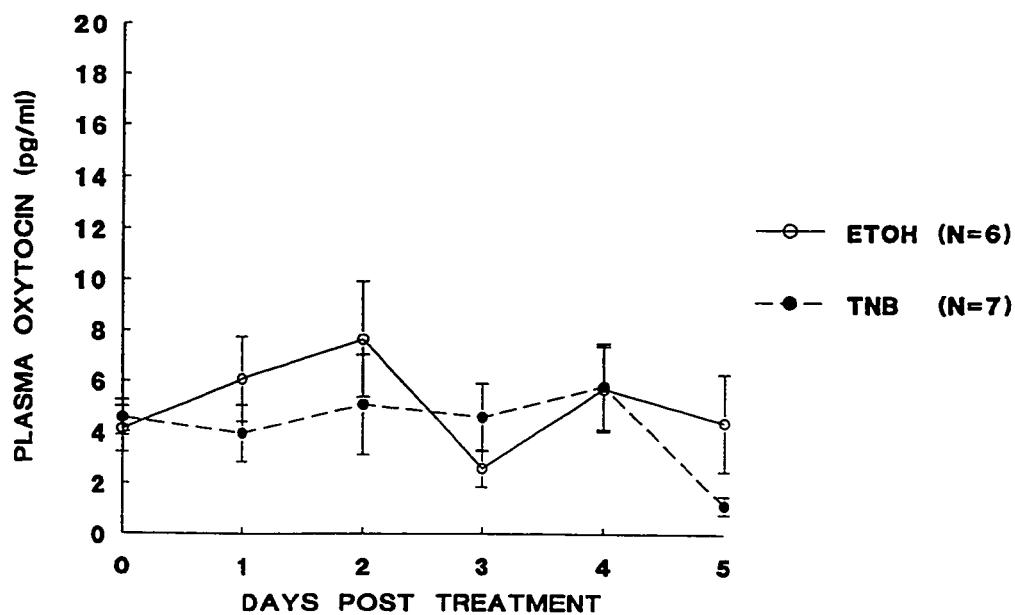
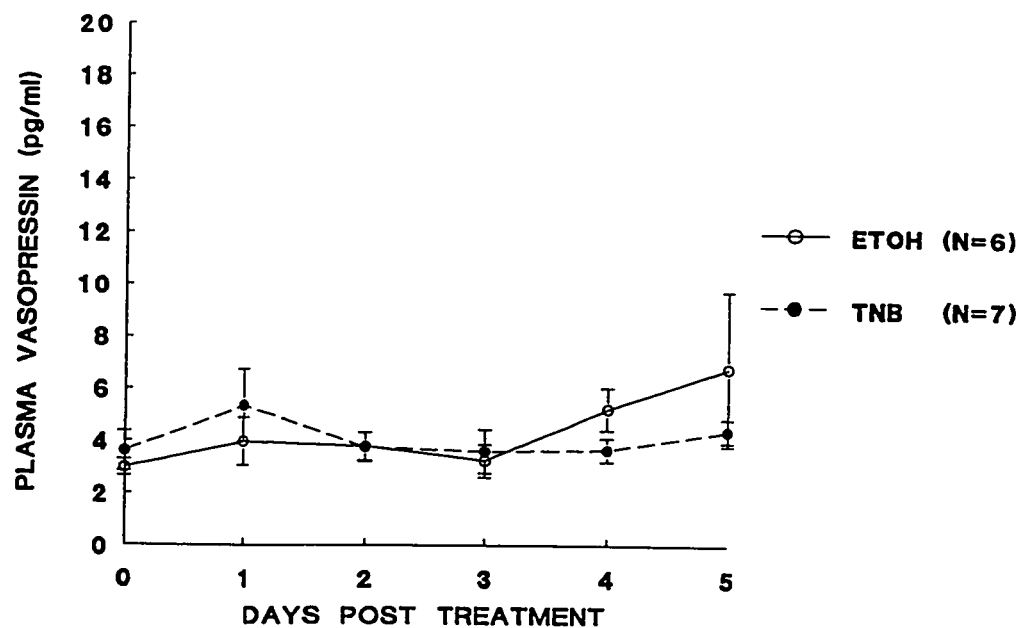


baseline. However, on the first three days, TNB treated rats demonstrated a 67%, 63% and 31% decrease in food intake compared to the control animals. By day 4 the food intake returned to baseline values, following the expected pattern of anorexia, as demonstrated by a significant interaction between Group and Day ($F(4,40) = 10.97$, $p < 0.0000$).

Blood Samples: The plasma levels of AVP (figure 5) were not altered by treatment with TNB or ETOH as determined by a between-within split plot ANOVA ($F(1,6) = 0.38$, $p = 0.38$). This indicated that there was not a general activation of the posterior pituitary gland caused by the experimental treatment. However, neither was there an effect of gastrointestinal inflammation on plasma OT levels (figure 6) when the data were analyzed by a between-within split plot ANOVA ($F(1,7) = 0.35$, $p > .05$). Consequently, when blood samples were taken once a day following induction of colitis, plasma OT levels were not elevated during the anorexia.

Figure 5: Plasma vasopressin (AVP) levels in pg / ml for the baseline and 5 days following treatment with TNB (N=8) or ETOH (N=7) in Experiment IIA. Data are group means \pm SE.

Figure 6: Plasma oxytocin (OT) levels in pg / ml for the baseline and 5 days after treatment with TNB (N=8) or ETOH (N=7) in Experiment IIA. Data are group means \pm SE.



IIB: 24 HOURS

There were no elevations in plasma OT levels detected when blood samples were collected once a day following induction of colitis. However, the changes in plasma oxytocin levels are transient (Giovannelli, Shiromani, Jirikowski & Bloom, 1992; Verbalis *et al*, 1986 a). Consequently, the method of sample collection used in the previous experiment (once in 24 hours) may not have been sufficiently sensitive to detect a transient elevation of OT.

The suppression of food intake is greatest during the first 24 hours following treatment with TNB. Therefore, repeated sampling of the plasma OT levels was conducted during the first day after colitis induction to determine whether there were transient elevations of plasma OT that correlated with the expression of anorexia.

IIB: METHODS

Subjects: Thirteen male rats (300 - 400 g) were maintained in hanging steel cages on Purina powdered rat chow.

Procedure: The rats were anaesthetized for jugular catheter surgery with 90 mg / kg Ketamine and 10 mg / kg Xylazine (see General Methods). After recovery from the surgery, baseline powdered chow intake was established and a baseline blood sample of

1.0 ml was collected between 4:00 - 5:00 pm (General Methods). Immediately after, the experimental animals were treated with powdered TNB and the control animals were treated with ETOH as described in General Methods. Blood samples (1 ml) were collected 3, 5, 8, and 14 hours after treatment, during the dark cycle. Six days following treatment, the rats were given an overdose of Somnotol (0.5 ml) and samples of colon were collected for an MPO assay (see General Methods). The plasma samples were analyzed for AVP and OT levels.

IIB: RESULTS AND DISCUSSION

Food Intake: The suppression of food intake induced by TNB is first significant approximately 8 hours following IR treatment (figure 7). The 24 hour intake of TNB, but not ETOH treated rats, was significantly suppressed compared to the baseline food intake (22.3 g) ($t(1, 12) = 5.87, p < 0.001$) and a between/within split plot ANOVA revealed that the cumulative food intake of TNB-treated animals was significantly less than that of ETOH-treated animals over 24 hours ($F(4, 44) = 9.32, p < 0.0001$).

Blood Samples: The plasma AVP levels (figure 8) were not significantly elevated by TNB treatment, as determined by a between/within split plot ANOVA ($F(1, 11) = 1.44, p = 0.26$), indicating that any elevations of OT were not due to general activation of the pituitary gland.

There was an initial elevation of plasma OT peaking at 5 hours after treatment, indicating that an elevation of OT could be detected. However, the elevation occurred prior to the expression of the anorexia at 8 hours, and thus was not temporally correlated. A between/within split plot ANOVA indicated that over the 24 hours, TNB treatment did not cause a significant elevation in plasma OT levels compared to ETOH treatment ($F(1,11) = 1.97, p = 0.19$), nor did the elevation correlate with the period of anorexia (figure 9). It is possible that the OT may have initiated a series of events that were expressed later through a suppression of food intake. However, since no significant elevations of plasma OT in correlation with the anorexia could be detected by repeated sampling of blood during the greatest period of anorexia, it does not appear that the anorexia is mediated by malaise.

Figure 7: Cumulative powdered chow intake (g) at baseline 3, 5, 8, 14 and 24 hours after treatment with TNB (N=7) or ETOH (N=6) in Experiment IIB. Data are group means \pm SE.

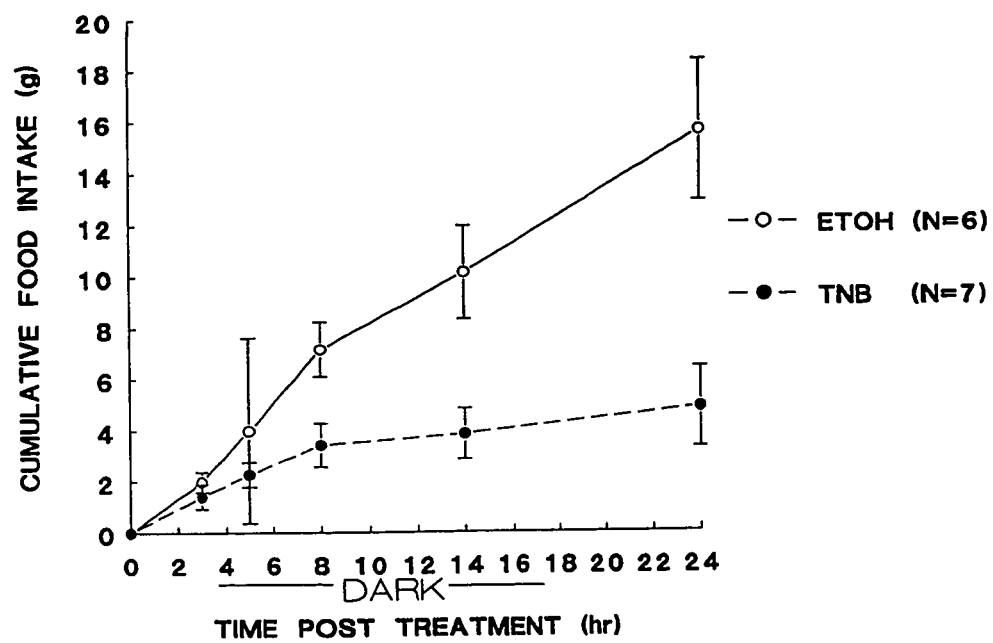
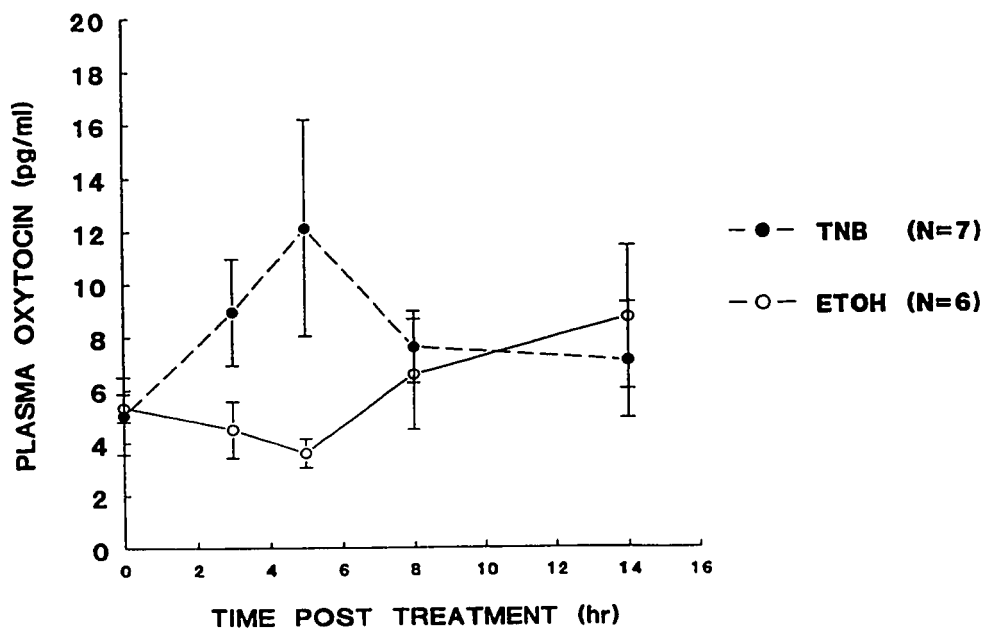
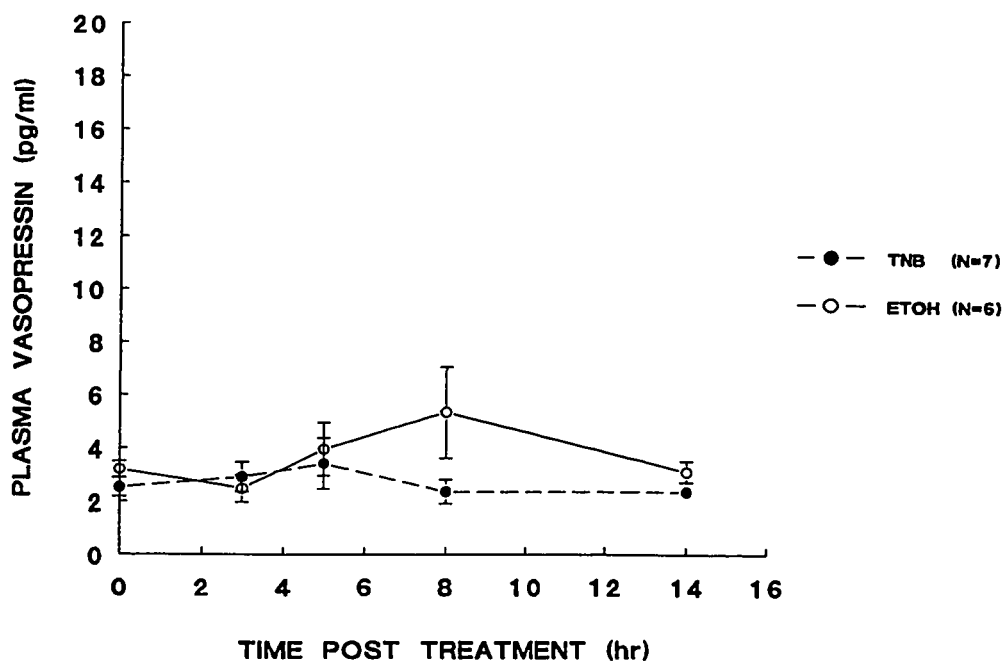


Figure 8: Plasma AVP levels (pg / ml) for baseline 3, 5, 8, 14 and 24 hrs following treatment with TNB (N=7) or ETOH (N=6) in Experiment IIB. Data are group means \pm SE.

Figure 9: Plasma oxytocin (OT) levels (pg / ml) for the baseline 3, 5, 8, 14 and 24 hrs after treatment with TNB (N=7) or ETOH (N=6) in Experiment IIB. Data are group means \pm SE.



IIC: MEALS

No elevations of plasma OT occurred in correlation with anorexia in TNB-treated rats when blood samples were taken once a day following treatment with TNB, or repeatedly in the first 24 hours. However, it is possible that the malaise is only experienced in context of a meal, which neither of the previous experiments examined. Previous research has shown that a gradual rise of plasma OT can be detected during a large meal, although the levels do not reach the same height as that observed with malaise-inducing agents (Verbalis *et al*, 1986 a). Consequently, the final experiment examined whether TNB treated rats had a greater elevation of plasma OT during a meal than controls rats. An elevation of the marker for malaise would indicate that malaise mediated the suppression of food intake within the context of a meal.

IIC: METHODS

Subjects: Twelve male rats (300-450 g) were maintained in hanging steel cages on Purina powdered chow.

Procedure: Jugular catheters were implanted to allow blood sampling (General Methods). After recovery from surgery, the baseline powdered chow intake was established and a

baseline blood sample of 1.0 ml was collected (General Methods). Immediately after, the experimental animals were treated IR with powdered TNB in 50% ETOH and the control animals were treated with ETOH alone (General Methods). On Day 1 following treatment, the rats were familiarized with the testing room. On Day 2, the rats were food deprived for 9 hours between 10:30 am and 7:30 pm. At 7:30 pm, they were placed in the testing room and a baseline blood sample (1.0 ml) was collected before powdered chow was placed in the cage. Blood samples were collected and the food intake was measured 10, 20 and 40 minutes after the initiation of a meal. On day 5, the rats were given an overdose of 1.0 ml of Chloral hydrate, and the colons were collected for MPO analysis (see General Methods).

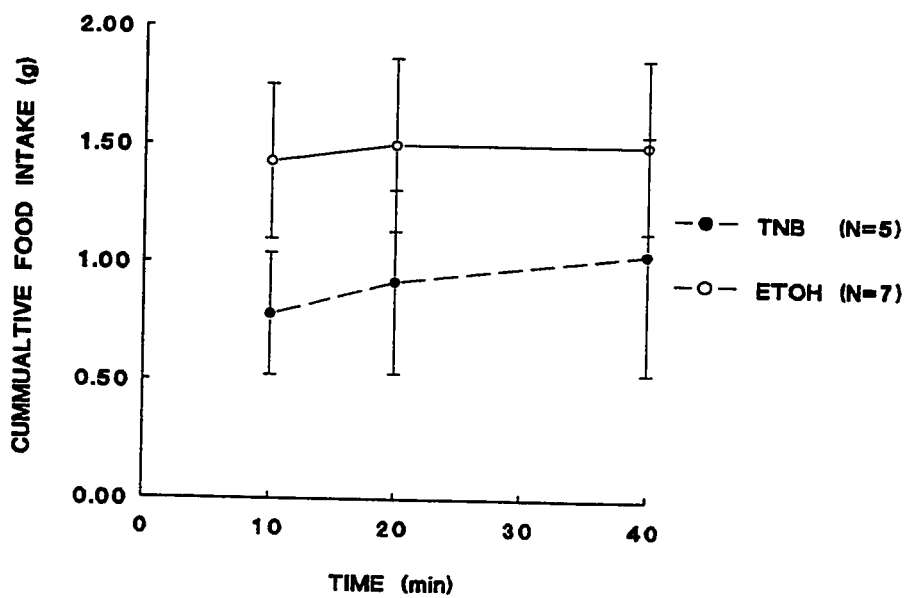
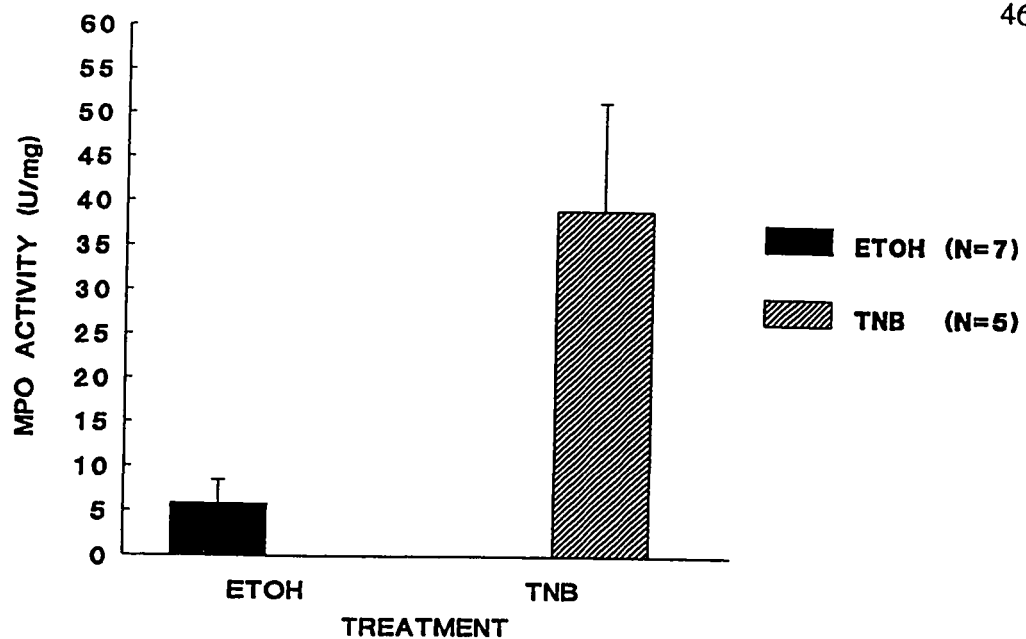
IIC: RESULTS AND DISCUSSION

Myeloperoxidase Assay: The colons of TNB-treated rats were significantly more inflamed than those of ETOH-treated rats ($F(1, 10) = 7.710$, $p < .0189$). The MPO values of TNB treated rats were 6.7 times higher than ETOH treated rats (figure 10).

Food Intake: The food intake during a meal (figure 11) was suppressed in the TNB treated animals. The difference did not reach significance ($F(1, 10) = 1.13$, $p = 0.31$), perhaps because the amount consumed was very small, and the animals were interrupted to measure food intake, resulting in large variation.

Figure 10: Myeloperoxidase (MPO) activity in the colon after treatment with TNB (N=5) or ETOH (N=7) for rats in Experiment IIC. Data are group means \pm SE.

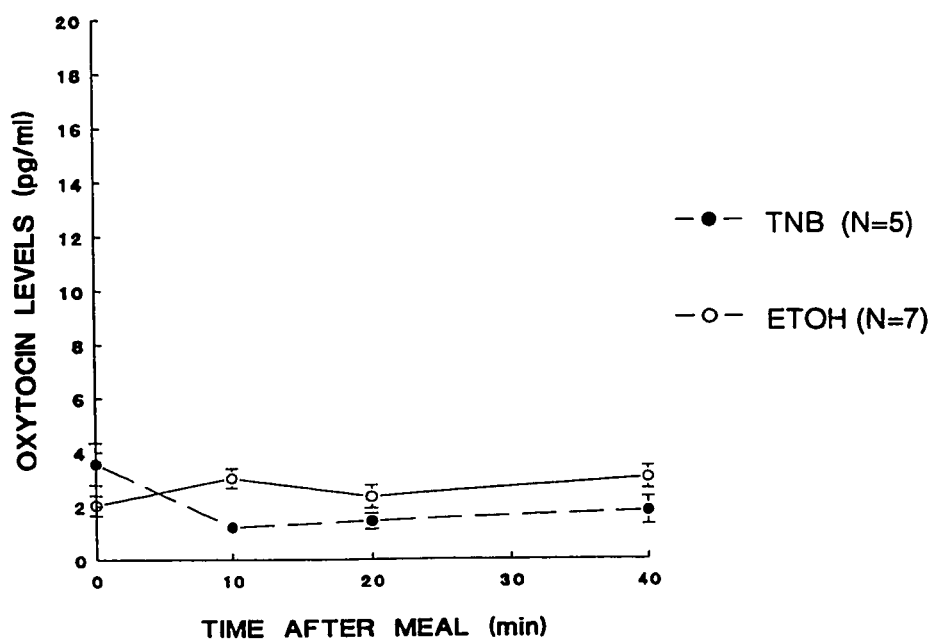
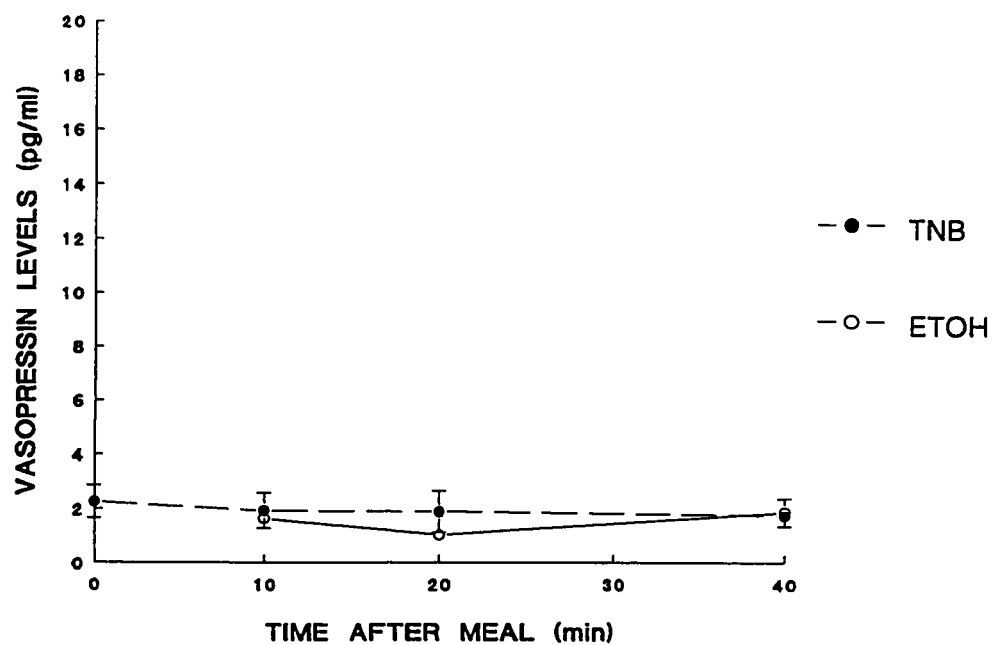
Figure 11: Powdered chow intake (g) at 10, 20 and 40 min after meal initiation in rats treated with TNB (N=5) or ETOH (N=7) in Experiment IIC. Data are group means \pm SE.



Blood Samples: The plasma AVP levels for TNB and ETOH treated animals were not significantly different (figure 12), indicating that any differences in OT levels were not the result of a general activity in the posterior-pituitary gland ($F(1, 9) = 1.83, p = 0.21$). Interestingly, there was a significant elevation in the plasma OT levels (figure 13), but the elevation was for the ETOH control rats, not the TNB-treated rats ($F(1, 10) = 7.155, p < 0.0224$). This indicates that an elevation of OT levels could be detected, but that there was not an elevation of OT plasma levels that correlated with the anorexia induced by colitis.

Figure 12: Plasma vasopressin (AVP) levels (pg / ml) at 10, 20 and 40 min after the initiation of a meal in rats treated with TNB (N=5) or ETOH (N=7) in Experiment IIC. Data are group means \pm SE.

Figure 13: Plasma oxytocin (OT) levels in pg / ml for at 10, 20 and 40 min after the initiation of a meal in rats treated with TNB (N=5) or ETOH (N=7) in Experiment IIC. Data are group means \pm SE.



II: DISCUSSION

An elevation in the plasma level of OT was used as a physiological marker for visceral malaise (Olson *et al*, 1991 a; Verbalis *et al*, 1986 a, b) in the TNB model for IBD. While IR TNB treated rats had significantly greater colonic inflammation (MPO analysis) and a significant suppression of food intake compared to ETOH control rats, no change was detected in either plasma OT or AVP levels when blood samples were collected once a day. However, changes in plasma oxytocin levels tend to be transient (Giovannelli *et al*, 1992; Verbalis *et al*, 1986 a) and thus collection of blood only once a day may not have been sensitive enough to detect a transient elevation of plasma OT.

In order to examine the possible transient elevations of plasma OT levels, blood samples were drawn, repeatedly, within the first 24 hours following treatment, the period of greatest anorexia. The suppression of food intake by a TNB intestinal challenge became apparent during the dark cycle (12 hr light/dark cycle), 8 hours after treatment. Plasma AVP levels were not significantly altered by treatment, indicating that the posterior-pituitary gland was not generally activated by treatment with TNB. While there was a slight increase in plasma OT within the first 6 hours post-treatment, the elevation did not correlate with the anorexia. Therefore, plasma OT elevations during the first 24 hours following treatment with TNB did not correlate temporally with the anorexia.

Verbalis and colleagues (1986 a) observed gradual elevations in the plasma OT of rats during large meals, although not to the same extent induced by malaise-inducing

agents. TNB treated rats may have had a transient rise in plasma OT during a meal, compared to control rats, suggesting malaise acts within the context of a meal. The meal size of TNB-treated rats (Experiment IIC) was smaller than that of controls. However, the trend was not significant, perhaps because food intake was interrupted to collect blood samples and measure food intake, causing a large variation in the small amount eaten within a single meal. Previous work has demonstrated that TNB-treated rats do consume smaller meals than ETOH-treated rats when uninterrupted (McHugh *et al*, 1993 a). While the AVP levels were not different between the two experimental groups, the OT levels demonstrated a significant gradual increase in the ETOH group compared to the TNB group during the meal. These findings support the findings of Verbalis and colleagues (1986 a) of a gradual rise of plasma OT during a meal. They also indicate that an elevation of OT could be detected. While it is possible that malaise sensitized other endogenous factors involved in anorexia; OT levels did not increase in TNB-treated rats, so it is unlikely that malaise, as indexed by OT, plays a role in the anorexia.

The testing procedure was able to detect the elevation of plasma OT, since elevations were detected during the first 6 hours following the use of ether in TNB-treatment, and in the control rats examined during a meal. Despite the fact that elevations of OT could be detected, there were no elevations of plasma OT that correlated with the expression of anorexia in the TNB-model of colitis. This suggests that malaise, as indexed by elevations of plasma OT, does not mediate the suppression of food intake associated with malaise.

CHAPTER 1: GENERAL DISCUSSION

Inflammatory diseases of the gastrointestinal tract in humans, such as Crohn's disease and ulcerative colitis, are often associated with weight loss and reduced food intake (anorexia) (MacPherson & Pfeiffer, 1976; McCallum *et al*, 1985). Anorexia contributes to the morbidity and mortality associated with IBD through increasing nutritional deficit (Kirschner *et al*, 1988), while alleviating malnutrition reduces the need for medication and surgery in IBD patients (Greenberg, Flemming, Jeejeebhoy, Rosenberg, Sales & Tremaine, 1988; Gassull *et al*, 1986). This study has focused on discovering the physiological mediators of colitis-induced anorexia in the rat TNB-model of IBD.

Central OT was assessed as a candidate for a central mediator of colitis-induced anorexia since it has been shown to play a role in food intake (Olson *et al*, 1991 a, b). Antagonism of OT by icv infusion of the OTA, L-366,948, did not alter the pattern of anorexia caused by IR treatment with TNB.

Central OT is primarily synthesized in the magnocellular and parvocellular cells of the supraoptic nucleus and the paraventricular nuclei (PVN) of the hypothalamus (Ivell, 1986; Leng, Dyball & Luckman, 1992; Leng, Way & Dyball, 1991; Van de Kar, Rittenhouse, Li, Qian, Levy & Brownsfield, 1995; Weindl & Sofroniew, 1980). The PVN has been implicated in many studies as a major site for the mediation of food intake

(Aravich & Sclafani, 1983; Leibowitz *et al*, 1981; Monnikes *et al*, 1992). For example, messenger ribonucleic acid for CRF is reduced in the PVN in response to food deprivation (Kiss, Jezova & Aguilera, 1994). The PVN also contains OT synthesizing cells in addition to CRF-like immunoreactive neurons and fibres and CRF receptors (DeSouza, Insel, Perrin, Rivier, Vale & Kuhar, 1985; Griffond, Deray, Mahjaoui-Bouhaddi, Colard, Bugnon & Fellmann, 1994; Jezova, Michajlovskij, Kvetnasky & Makara, 1993; Silverman, Hou-Yun & Chen, 1989). Furthermore, some studies, although not all (Landgraf, Neumann, Holsboer & Pittman, 1995), report the release of OT in the PVN in response to IL-1 (Chang, Ren & Zadina, 1993; Ericsson, Kovacs & Sawchenko, 1994). However, ablation of the PVN did not change the pattern of colitis-induced anorexia either (Morrison, 1995). The results of the ablation study suggest that the PVN is not involved in the receipt of the anorexigenic signal from the intestine, nor the processing of the signal. The combination of the OT antagonist and the PVN ablation studies indicate that OT does not act as a central mediator of colitis-induced anorexia.

A common suggestion for the cause of anorexia in IBD is malaise, the feeling of being unwell (Anderson *et al*, 1989; Rigaud *et al*, 1992; Royall *et al*, 1995; Stokes, 1992). Elevations in the plasma levels of OT can be used as a physiological marker for malaise (Olson *et al*, 1991 a; Verbalis *et al*, 1986 a, b). Plasma levels of OT may be elevated independent of changes in central OT (Arletti *et al*, 1989; Forsling, 1986; Olson *et al*, 1991 a) and do not cause anorexia themselves. The procedures could detect elevations of plasma OT. However, elevations in plasma OT were not correlated with colitis-induced

anorexia when samples were collected over the five days of the anorexia, during the first 24 hours of the greatest anorexia, or during a meal. These observations suggest that malaise, as indexed by elevations of plasma OT, does not contribute to the anorexia induced by TNB treatment.

While the findings of this study do not suggest that there is no malaise involved in colitis, the evidence suggests that malaise cannot account for the anorexia expressed in association with colitis. Other research also indicates that malaise can be dissociated from anorexia in a number of different models of suppressed food intake (Ervin *et al*, 1995). The finding that malaise does not account for the anorexia is in agreement with a visual analogue scale questionnaire given to patients with Crohn's disease, who did not report greater nausea, abdominal pain or fear of diarrhea, although they did report feeling less hunger (Rigaud *et al*, 1992). The agreement between human and rat data adds to the validity of using TNB treatment as a model of colitis. In summary, this study has shown that malaise is not one of the mediators of anorexia in the TNB model of colitis. In addition, OT does not play a role as a central mediator of the colitis, nor is the PVN the brain site that mediates the anorexigenic signal from the periphery.

CHAPTER 2

BEHAVIOURAL: POST-INGESTIVE CONSEQUENCES

The TNB model of colitis has been used to explore the suppression of food intake, anorexia, which is associated with an inflamed bowel. The previous chapter demonstrated that neither central OT nor malaise, as indexed by plasma OT levels, were involved in mediating the colitis-induced anorexia. Meal pattern analysis has previously demonstrated that the TNB-treated rats initiate meals with the same frequency as control rats, and that the anorexia is expressed through smaller meal sizes (McHugh *et al*, 1993 a). These findings suggested that studies of the suppressed food intake should be focused on the termination of meals because the anorexia is mediated by satiety.

Researchers proposed that TNB-treated rats responded in an exaggerated fashion to the physiological changes caused by food entering the stomach and intestine (called the postingestive consequences) (McHugh *et al*, 1993 a). One of the consequences of eating is a feeling of being full, or of satiety, which leads to the end of a meal (Blundell, Lawton & Hill, 1993; Nicolaidis & Even, 1992; Read, French & Cunningham, 1994). Satiety in animals is primarily measured by behaviour, such as the end of a meal, which is mediated by sensory, cognitive, postingestive and post-absorptive processes. The physiological signals released in response to food intake act as satiety cues (Blundell *et al*, 1993; Cooper & Francis, 1993; Smith & Gibbs, 1978; Warwick, Hall, Pappas & Schiffman, 1993;

Weingarten & Gowans, 1991). Consequently, rats with colitis-induced anorexia may initiate meals, but then respond in an exaggerated fashion to the cues released by food intake, eating smaller meals because they terminate the meal more quickly.

Stimulus control over post-ingestive consequences can be achieved through a preparation known as the intragastric (IG) preload. An implanted IG cannula allows food to be delivered directly to the stomach without any oral stimulation, isolating the post-ingestive consequences from any appetitive influences (Ackroff & Sclafani, 1993; Cabanac & LaFrance, 1992; Drucker, Ackroff & Sclafani, 1993; Kaplan, Spector, Grill, 1992; Rolls, 1995; Sclafani, Cardieri, Tucker, Blusk & Ackroff, 1993; Shide Caballero, Reidelberger & Rolls, 1995; Warwick & Weingarten, 1995). A preload is a controlled amount of food that is delivered prior to an established test meal in order to study the response to satiety (Cooper & Francis, 1993; Mook, Atkinson, Johnston & Wagner, 1993 a; Mook, Yoo & Wagner, 1993 b; Rolls, Dimeo & Shide, 1995; Rolls, 1995). The preload in the gastrointestinal tract releases satiety signals that inhibit the amount of food consumed during the test meal, depending on the volume, nutrient density and the time interval of preload administration (Adolf, 1941; Berkun, Kesson & Miller, 1952; Perez, Ackroff & Sclafani, 1994; Smith & Gibbs, 1979). If colitis caused rats to respond in an exaggerated fashion to post-ingestive satiety cues, then an IG preload should cause a greater suppression of food intake during a test meal in TNB-treated rats than in control rats.

Two experiments were conducted in order to determine whether colitis-induced anorexia was mediated by an exaggerated response to post-ingestive consequences. A

preliminary experiment determined the suppression of food intake caused by different IG preload volumes (Appendix 2). This information was then used to determine the volume of preload used in subsequent experiments. In the first experiment, the effect of a 5 ml preload on food intake during a test meal was compared to the effect of a 0 ml preload in animals treated with TNB or ETOH. The rate of intake in the control group (0 ml preload) was also measured during the test meal. In the second experiment, the effect of a larger preload, 10 ml, was assessed with naive rats and compared to a new control group when the rats were treated with TNB or ETOH.

EXPERIMENT 1

PRELOAD- 5 ml

One hypothesis for the suppression of food intake observed during TNB-induced colitis, is that TNB-treated animals have an exaggerated response to post-ingestive satiety cues (McHugh *et al*, 1993 a). An IG preload specifically provides post-ingestive cues that cause a specific suppression of food intake. If TNB treated rats have an exaggerated response to these cues, then a preload that causes little or no suppression in a control rat should cause a significant suppression in TNB treated rats. Since the TNB treatment itself causes a suppression of food intake, the preload volume should be small enough that any exaggerated response to the preload is not masked (ie. if a preload volume is greater than normal intake during anorexia, food intake cannot be suppressed below 0 ml to demonstrate an exaggerated response). A preload of 5 ml was determined to cause little suppression of food intake at 1 or 4 hours in untreated rats (Appendix 2). Consequently, a preload volume of 5 ml was used to determine whether TNB treated rats responded with an exaggerated suppression of food intake compared to controls.

METHODS

Subjects: Twenty-four male Sprague-Dawley albino rats (250 - 350 g)

Procedure: Both IG and IO cannulae were surgically implanted, though the IO cannulae were not used in the experiment. Three days later, the rats were trained on a 4 hour

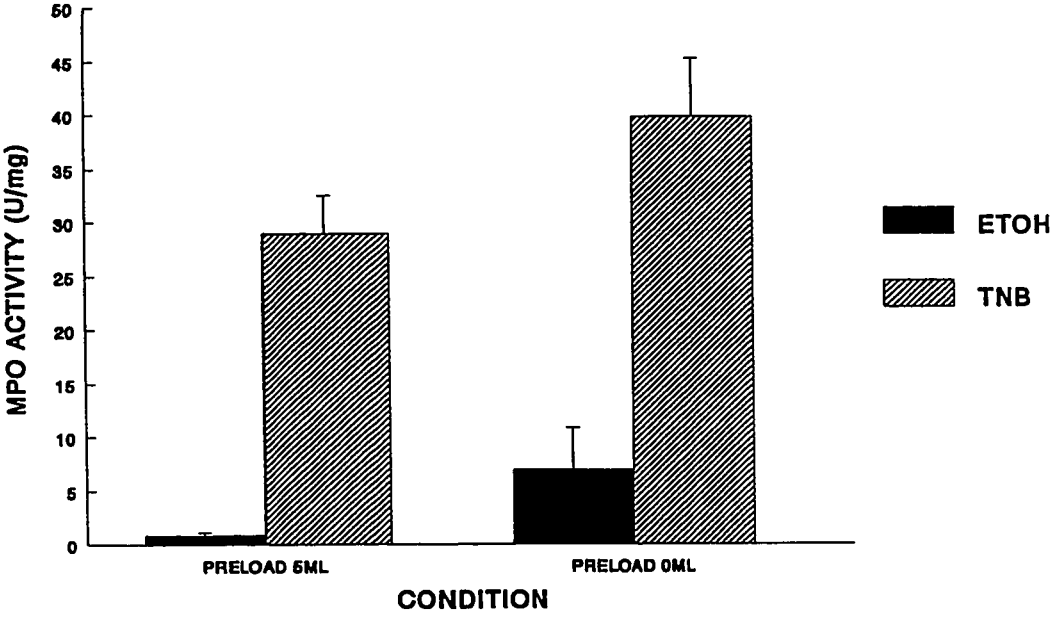
feeding schedule. When a constant weight was maintained on a 4 hour feeding schedule of liquid diet, the rats were treated IR with either TNB or ETOH (see General Methods). On a baseline day, and days 1-5 following IR treatment with either TNB or ETOH, a preload of either 0 ml or 5 ml of liquid diet was delivered through the IG cannula 20 minutes prior to the feeding period. There were four groups (N = 6) determined by IR treatment and preload amount: TNB-0 ml, ETOH-0 ml, TNB-5 ml, ETOH-5 ml. Rats receiving the 0 ml preload were handled in the same fashion as rats receiving the 5 ml preload, but no liquid diet was infused. For the 0 ml preload condition, intakes were measured every 2 minutes during the first hour in order to determine the rate of real feeding intake. Liquid diet intake (real fed from a graduated cylinder) was measured at 1 and 4 hours for all animals on a baseline day, and days 1-5 following IR treatment. After day 6, samples of the colon were collected for MPO analysis (General Methods).

RESULTS

The statistical analysis was run using an ANOVA, with IR treatment and Preload volume as the between-group conditions, and Days post-treatment as the within group condition.

Myeloperoxidase Assay: The rats treated with liquid TNB exhibited significantly more MPO activity in their colons (figure 14) than those treated with the vehicle, ETOH, ($F(1,20) = 63.92, p < 0.001$).

Figure 14: Myeloperoxidase (MPO) activity in the colons of rats treated with TNB (N=6) or ETOH (N=6) after day 5 of the 5 ml-Preload Experiment. Data are group means \pm SE.



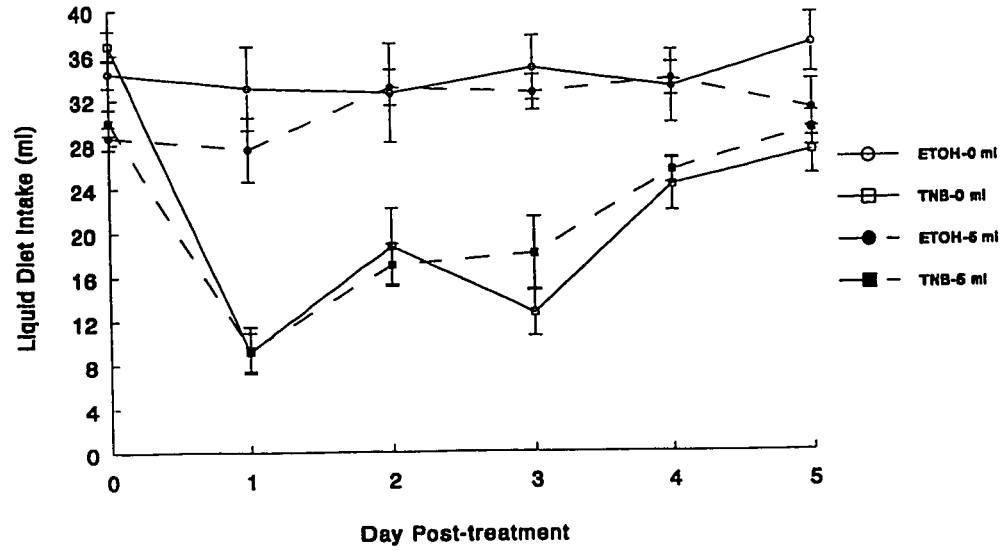
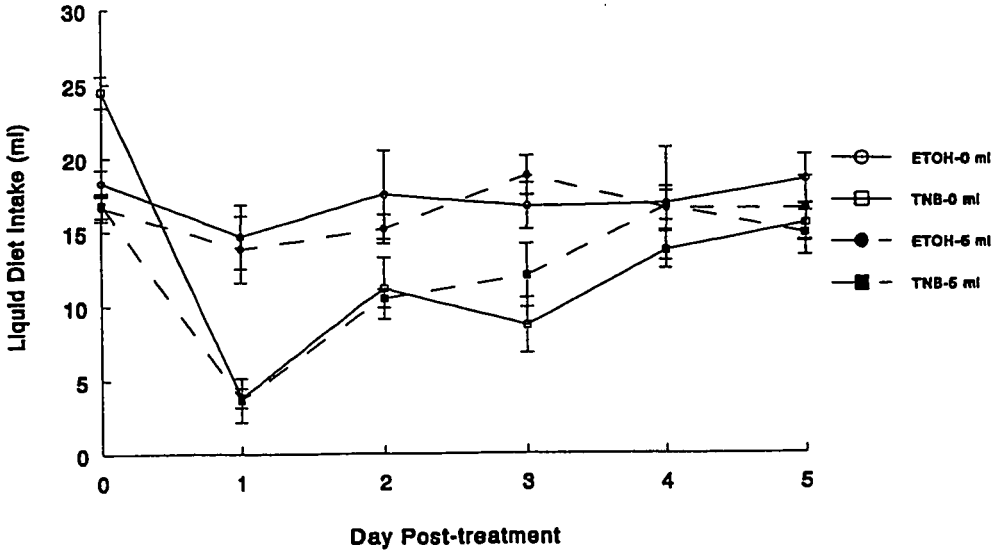
Food Intake:

Test Meal: Analysis of the amount of liquid diet eaten after 1 hour (figure 15) revealed a significant interaction of Treatment by Day ($F(4,80) = 4.88, p = 0.001$). The interaction indicates that the TNB-treated animals decreased their food intake on day 1, but returned to baseline by day 5 in the expected pattern of anorexia (McHugh *et al*, 1993 a). Most importantly though, the 5 ml preload did not have a significant effect on 1 hour food intake for either TNB or ETOH conditions ($F(1,20) = 0.003, p = 0.95$) and there was no interaction between the preload and the treatment ($F(1,20) = 0.30, p = 0.59$). Thus, during the test meal, the preload did not cause a significant suppression of food intake during the 1 hour test meal.

Total Intake: Similar to 1 hour liquid diet intake, the cumulative 4 hour intake (figure 16) was suppressed by treatment with TNB, but returned to baseline by day 5 (Treatment by Day $F(4, 30) = 11.67, p < 0.0001$). In the 4 hour intake, the 5 ml preload did cause a significant decrease in the food intake for both treatment conditions ($F(1, 20) = 6.5, p < 0.05$). However, the suppression of food intake was not greater in TNB-treated animals compared to ETOH-treated animals (Treatment x Preload was not significant $F(1,20) = 0.94, p = 0.34$).

Figure 15: Liquid diet intake (ml) during 1 hour of real feeding on day 0 to day 5 following treatment with TNB or ETOH and delivery of either a 0 ml or a 5 ml preload of liquid diet. N=6 for all four groups. Data are group means \pm SE.

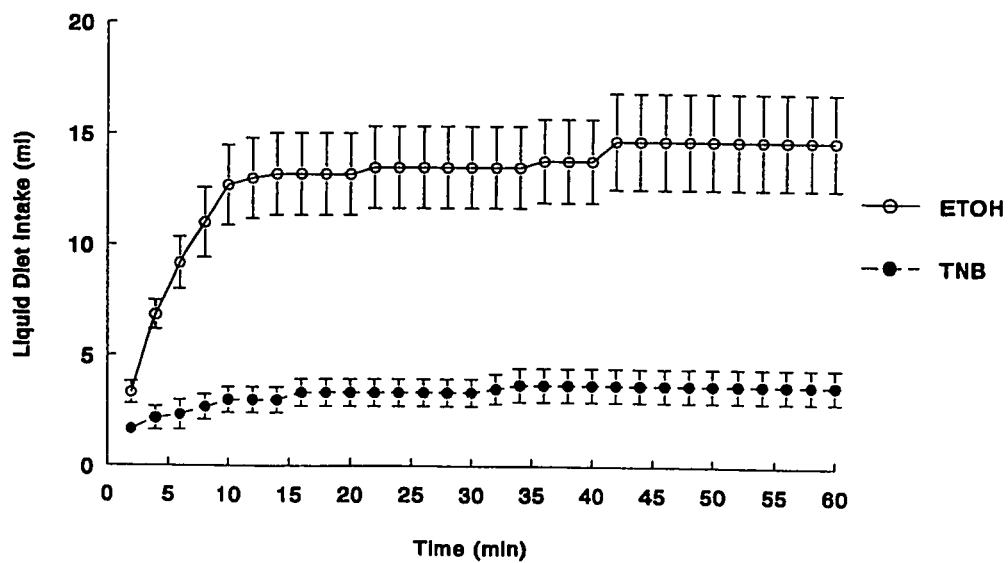
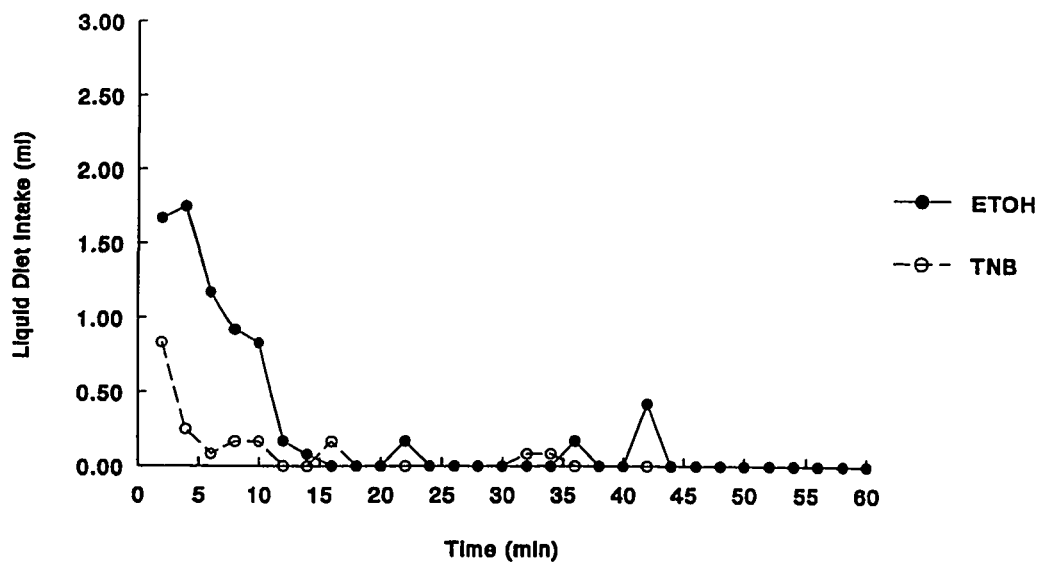
Figure 16: Liquid diet intake (ml) during the cumulative 4 hours of real feeding on day 0 to day 5 following treatment with TNB or ETOH and delivery of either a 0 ml or a 5 ml preload of liquid diet. All four groups had N= 6. Data are group means \pm SE.



Intake Rate: The rate of liquid diet intake was recorded during the first hour for the rats receiving the control 0 ml preload (figure 17, 18). Analysis of the rate of intake was made using an ANOVA with two within-subjects factors (Time and Days) and one between-subjects factor (Treatment). Treatment with TNB significantly decreased the rate of intake compared to treatment with ETOH ($F(1,10) = 11.97, p < 0.01$). There was a significant interaction between treatment group and time following the initiation of a meal ($F(29, 290) = 3.54, p < 0.001$), suggesting that meal duration was altered, and it changed over the days ($F(116,1160) = 1.85, p < 0.001$).

Figure 17: Rate of liquid diet intake (ml / min) when real feeding with 0 ml preload on Day 1 following treatment with TNB (N=6) or ETOH (N=6). Data are group means \pm SE.

Figure 18: Cumulative liquid diet intake (ml) when real feeding with 0 ml preload on Day 1 following treatment with TNB (N = 6) or ETOH (N = 6). Data are group means \pm SE.



DISCUSSION

Rats with TNB-induced colitis did not demonstrate an exaggerated response to the satiety cues induced by an IG preload of 5 ml of liquid diet. Although the colitic rats did initiate meals, supporting the findings of McHugh and colleagues (1993 a), the rate of intake was lower on the first day and remained suppressed for the five days. This suggests that the smaller meals observed in TNB-treated rats were caused by slower intake rates, in addition to shorter meal durations. The suppression of rate occurs immediately at the initiation of a meal, before post-ingestive responses are likely to be involved (Barnfield, Parker, Davies & Miles, 1994).

It could be argued that since the 5 ml preload did not cause a suppression of the 1 hour test meal intake, the pre-load was too small to cause an exaggerated response in the TNB treated rats. The experiment in Appendix 2 indicated that a 10 ml preload caused a 32.2% suppression in 1 hour food intake and a 20.7% suppression of the total 4 hour food intake. Therefore, the effect of a 10 ml preload was examined for its ability to cause an exaggerated suppression of food intake.

EXPERIMENT 2

PRELOAD - 10 ml

A 5 ml preload of liquid diet did not cause an exaggerated suppression of food intake in TNB-treated rats. However, it could be argued that the preload was too small to have any effect on food intake. Therefore, the effect of a larger preload of 10 ml on a test meal was assessed. In this experiment, although a similar procedure was followed, naive rats were used, and the rate of intake was not measured.

Subjects: Twenty-three male Sprague-Dawley albino rats (250-350 g at time of surgery).

Procedure: As in the prior experiment, both IG and IO cannulae were surgically implanted. The IO cannulae were not used in this experiment either, but were included to allow comparison should future experiments be run. The rats were trained on a 4 hour feeding schedule, then treated IR with either TNB or ETOH (see General Methods). On a baseline day, and days 1-5 following IR treatment, a preload of either 0 ml or 10 ml of liquid diet was delivered through the IG cannula 20 minutes prior to the feeding period. There were four groups: TNB-0 ml, ETOH-0 ml, TNB-10 ml, ETOH-10 ml. Rats receiving the 0 ml preload were handled in the same fashion as rats receiving the 10 ml preload, but no liquid diet was infused. Liquid diet intake from a graduated cylinder was measured at 1 and 4 hours for all animals on a baseline day, and days 1-5 following IR

treatment. After day 6, samples of the colon were collected for MPO analysis (General Methods).

RESULTS

The statistical analysis was run using an ANOVA, with the IR Treatment and the Preload volume as the between-group conditions, and Days post-treatment as the within-group condition.

Myeloperoxidase Assay: The MPO analysis was unreliable because the -70°C freezer defrosted prior to the assay. However, TNB-treated rats consistently have greater MPO values, as seen in previous sections (ex. Experiment 1, in Chapter 1, and Experiment 1, in Chapter 2).

Food Intake:

Test Meal: Analysis of the amount of liquid diet eaten during the 1 hour test meal (figure 19) revealed a significant suppression of food intake in response to TNB treatment ($F(1, 19) = 32.56, p < 0.001$). The 10 ml preload of liquid diet also caused a significant suppression of food intake during 1 hour of both TNB and ETOH treated rats ($F(1, 19) = 14.39, p < 0.05$). Most importantly though, there was no interaction between the Preload and the Treatment ($F(1, 19) = 0.13, p = 0.72$), indicating that the 10 ml preload did not

cause an exaggerated suppression of food intake in TNB-treated animals compared to the ETOH control rats. The food intake for all rats increased over the days of the experiment ($F(1, 19) = 13.18, p < 0.0001$).

Home Cage: Analysis of the 3 hour (figure 20) intake indicated that treatment with TNB caused a significant suppression of liquid diet intake compared to ETOH ($F(1, 19) = 41.60, p < 0.001$) that returned to baseline by day 5 ($F(4, 76) = 3.44, p < 0.02$). However, during the 3 hour intake, there was no effect of preload on the food intake ($F(1, 19) = 1.60, p = 0.22$), perhaps the compensation for the preload occurred within the test meal.

Total Intake: The cumulative 4 hour intake (figure 21) was suppressed by TNB ($F(1, 19) = 63.46, p < 0.001$), and there was an interaction between Treatment and Days, indicating that TNB followed the expected pattern of suppression, and returned to baseline by day 5 ($F(4, 76) = 3.09, p < 0.05$). Food intake gradually increased with days for all of the conditions ($F(4, 76) = 6.01, p < 0.001$). The 10 ml liquid diet preload caused a suppression in the 4 hour food consumption of both TNB and ETOH rats ($F(1, 9) = 13.20, p < 0.01$), and had a greater influence on food intake after day 1 ($F(4, 76) = 3.61, p < 0.01$). However, the preload did not cause an exaggerated suppression of food intake for TNB rats compared to ETOH rats, because the interaction between the Preload and the Treatment conditions was not significant ($F(1, 19) = 0.02, p = 0.90$).

Figure 19: Liquid diet intake (ml) during 1 hour of real feeding on day 0 to day 5 following treatment for: TNB-0 ml (N=6), ETOH-0 ml (N=5), TNB-10 ml (N=6), ETOH-10 ml (N=6). Data are group means \pm SE.

Figure 20: Liquid diet intake (ml) during 3 hour of real feeding in the home cage, days 0 to 5 following treatment: TNB-0 ml (N=6), ETOH-0 ml (N=5), TNB-10 ml (N=6), ETOH-10 ml (N=6). Data are group means \pm SE.

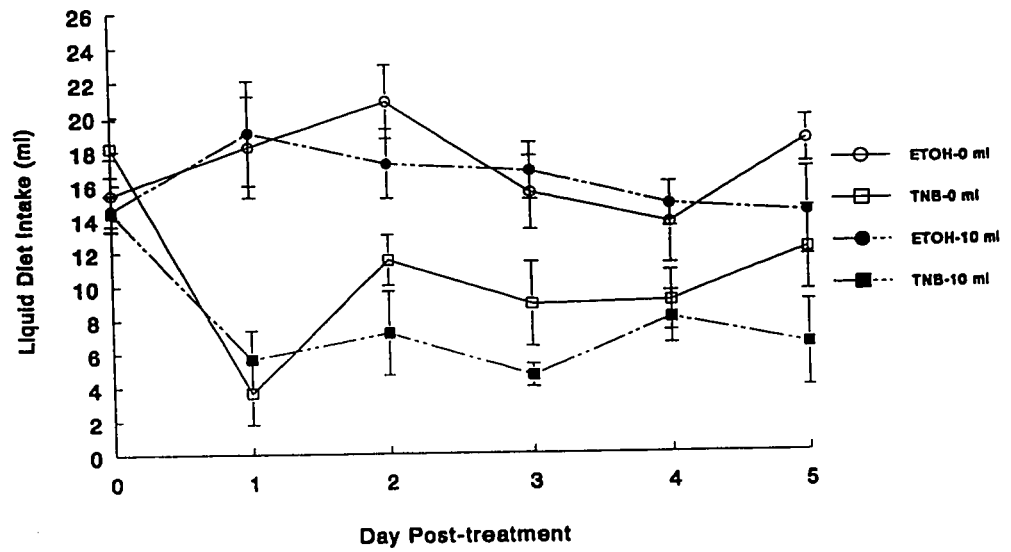
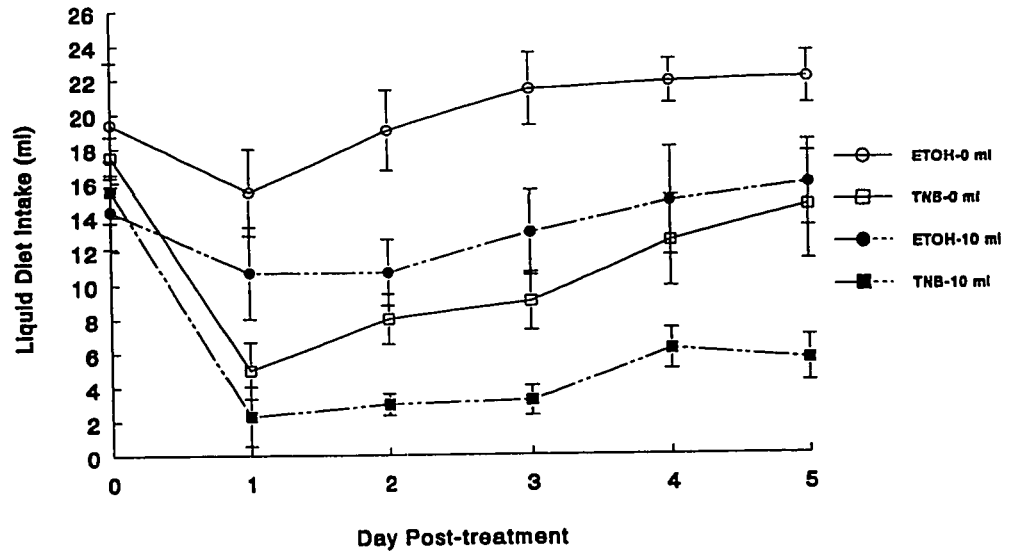
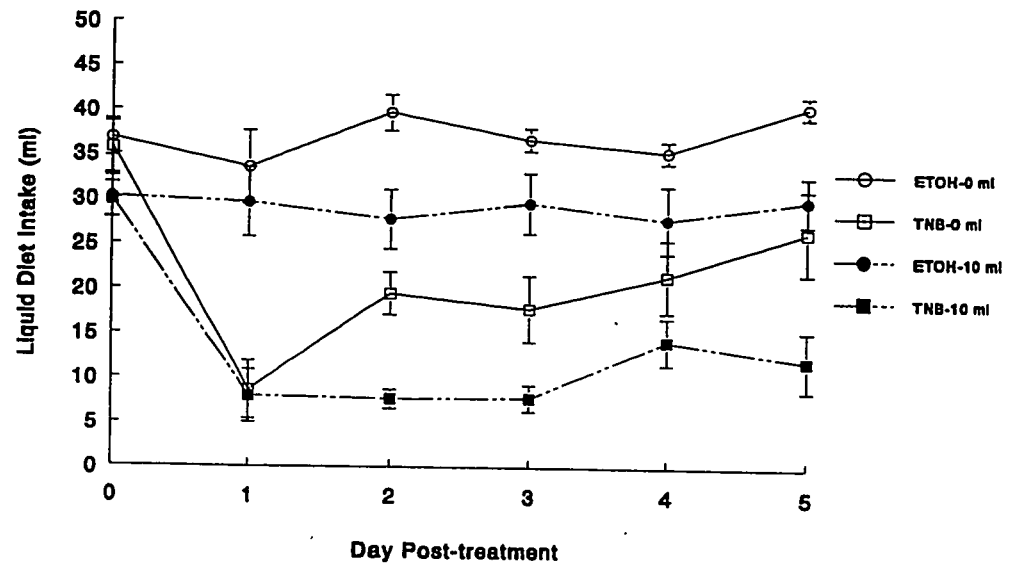


Figure 21: Liquid diet intake (ml) during the cumulative 4 hours of real feeding on day 0 to day 5 following treatment with TNB or ETOH: TNB-0 ml (N=6), ETOH-0 ml (N=5), TNB-10 ml (N=6), ETOH-10 ml (N=6) All four groups had N=6. Data are group means \pm SE.



DISCUSSION

The 10 ml IG preload of liquid diet, unlike 5 ml, was large enough to cause a significant suppression of food intake greater than 20% in the 1 hour and the cumulative (4 hour) intake. Thus, the 10 ml IG preload was large enough to cause post-ingestive consequences. As seen in the previous experiments, the majority of compensation for the preloads occurred during the first hour of intake, since the 3 hour intake in the home cage was unaffected by the preload delivered prior to the 4 hour feeding period. Despite the ability of the 10 ml IG preload to alter food intake, the preload did not cause an exaggerated suppression of food intake in the TNB-treated rats compared to the control rats.

CHAPTER 2: GENERAL DISCUSSION

Previously, researchers have suggested that the anorexia observed in the TNB-model of colitis could be explained by an exaggerated response to satiety cues (McHugh *et al*, 1993 a). There are a number of physiological changes following the entrance of food into the gastrointestinal tract, including gastric distension, the release of peptides into the blood stream (ex. cholecystokinin) and activation of the nervous system (Nicolaidis & Even, 1992; Read *et al*, 1994). The signals released by a meal are then communicated to the brain, where they result in the termination of a meal. It is possible that in some fashion, the consequences of a meal effect an anorexic rat differently from a control rat. For example, the signals produced in the periphery, in response to food, may be different or greater in number. Alternatively, the reception of the signals could be altered, so that the same signal is responded to in an exaggerated fashion. Since previous studies indicated that TNB-treated rats initiated meals with the same frequency as control rats (McHugh *et al*, 1993 a), it seemed reasonable to focus on the termination of meals rather than the initiation.

In order to assess the hypothesis about post-ingestive consequences, different amounts of liquid diet preloads were delivered directly to the stomach. Administration of an IG preload did suppress food intake, supporting previous research that post-ingestive consequences isolated from taste can alter food intake. However, neither an IG preload of

5 ml, nor a larger preload of 10 ml caused a greater suppression of food intake in TNB-treated rats compared to ETOH-treated rats. These results suggests that TNB-treated rats do not react in an exaggerated fashion to post-ingestive satiety cues as previously believed.

Examination of the rate of intake in rats without a preload showed that the rate of intake for TNB-treated rats was significantly slower than the baseline and control rate of intake. The decreased rate of intake was observed as soon as the meal was initiated, and therefore is unlikely to be the result of post-ingestive consequences, and is difficult to reconcile with the hypothesis of early or exaggerated satiety (Barnfield *et al*, 1994). This finding adds credence to the conclusions, drawn above, that an exaggerated response to post-ingestive consequences cannot account for colitis-induced anorexia.

In the study of colitis, attention was previously directed toward the behaviour occurring at the termination of a meal. However, the change in intake rate at the beginning of a meal implies that perhaps attention should be focused on the initiation of a meal. The initial period of food intake, prior to post-ingestive consequences, can be used as an index of the animals appetitive state or the food's palatability (Davis & Smith, 1992). Therefore, it appears possible that the food is perceived as less palatable by rats with colitis-induced anorexia. A technique has been developed to study palatability in rats which is called taste-reactivity (Berridge & Grill, 1983; Grill & Norgren, 1978 a, b).

Overall, the lack of an exaggerated response to a preload of either 5 ml or 10 ml, and the decreased intake rate of liquid diet in TNB-treated rats suggest that the TNB-

induced anorexia cannot be simply explained by an exaggerated response to post-ingestive satiety cues. Instead, attention in the next studies will be focused on alterations in behaviour that occur at the initiation of a meal.

CHAPTER 3

CHARACTERIZATION OF INTRAORAL INTAKE

In the previous chapter, I reported that the rate of food intake is decreased at the beginning of a meal in rats with colitis-induced anorexia. One possible event that could alter the rate of intake at the initiation of a meal is a change in the perceived palatability of food (Barnfield *et al*, 1994). A technique that provides stimulus control over the initiation of a meal is called intraoral (IO) feeding (Kaplan, Seeley, Grill, 1994, a; Kaplan, Siemers & Grill, 1994 b). In this procedure, two cannulae are implanted on both sides of the mouth, between the cheek and the gum of the rat, allowing food to be delivered directly into the mouth. The rat has control over meal termination by allowing the food to passively drip out of the mouth, which signals the end of the IO meal (Grill *et al*, 1987). The IO technique allows an unobstructed view of the facial movements in response to stimuli which can be used as a measure of palatability called taste reactivity (Grill & Norgren, 1978 a). The initiation of IO meals and the rate of delivery are determined by the experimenter which allows greater precision in examining the control of meal sizes (Grill & Bernstein, 1988; Grill & Norgren, 1978 a; Kaplan *et al*, 1994 a, b; Seeley, Kaplan & Grill, 1993).

IO feeding in rats is similar to normal feeding, or real feeding (RF), from a spout. The same oropharyngeal microstructure, with the same rhythmic oromotor behaviour, frequency and volume of swallowing are observed during IO and RF (Kaplan, Spector & Grill, 1990). The mouth movements are organized into bursts which are very similar to licking behaviour (Davis & Smith, 1992; Kaplan, Roitman, Grill, 1995). Although the food is infused directly into the mouth, the cannulae are positioned at the front of the mouth and require mouth movements similar to those required in RF in order to position the diet for swallowing. The behavioural responses of rats that feed IO is also similar to RF. For example, they ingest palatable solutions such as sucrose and reject unpalatable solutions such as quinine (Grill & Norgren, 1978 b) and respond to stimulus concentration (Flynn & Grill, 1988). IO fed rats defend meal size (Seeley, Kaplan & Grill, 1993) and regulate daily caloric intake by increasing intake in response to 48 hour deprivation (Kaplan, Seeley, Grill, 1993; Kaplan *et al*, 1994 b; Seeley, Grill & Kaplan, 1994). Sham feeding is unaltered by IO intake (Grill & Kaplan, 1992), and rats demonstrate a suppression of IO intake in response to preloads, CCK, insulin and dopamine receptor antagonists (Kaplan *et al*, 1994 a; Seeley *et al*, 1994).

Before the IO technique could be explored as a method to examine shifts in perceived palatability, I first needed to establish that the anorexia caused by IR treatment with TNB could be replicated using the IO preparation. In a preliminary study (Appendix 3) IO intake was measured and compared to real fed (RF) intake during a single meal of

liquid diet, with powdered chow available in the home cage, in order to determine if the meal sizes were equivalent. IO and RF methods of food intake were then compared in the TNB model when the rats were under a 4 hour feeding schedule. Finally, the experiment was replicated, with a 4 hour feeding schedule and a 20 hour feeding schedule in order to ensure that the anorexia occurred under different degrees of deprivation.

EXPERIMENT 1

Intraoral Feeding versus Real Feeding in the TNB Model

IO feeding provides stimulus control over the initiation and rate of food intake (Grill *et al*, 1987; Kaplan *et al*, 1994 a), and is a technique used to study Taste Reactivity. However, before the IO technique could be used to study Taste Reactivity it was necessary to determine whether the course of anorexia associated with TNB was also evident in IO-fed meals. Typically, feeding by IO infusion results in larger volumes of food intake than real feeding (RF) (Appendix 3). Consequently, IO intake was compared to RF intake following treatment with either TNB or ETOH to characterize the pattern of anorexia.

METHODS

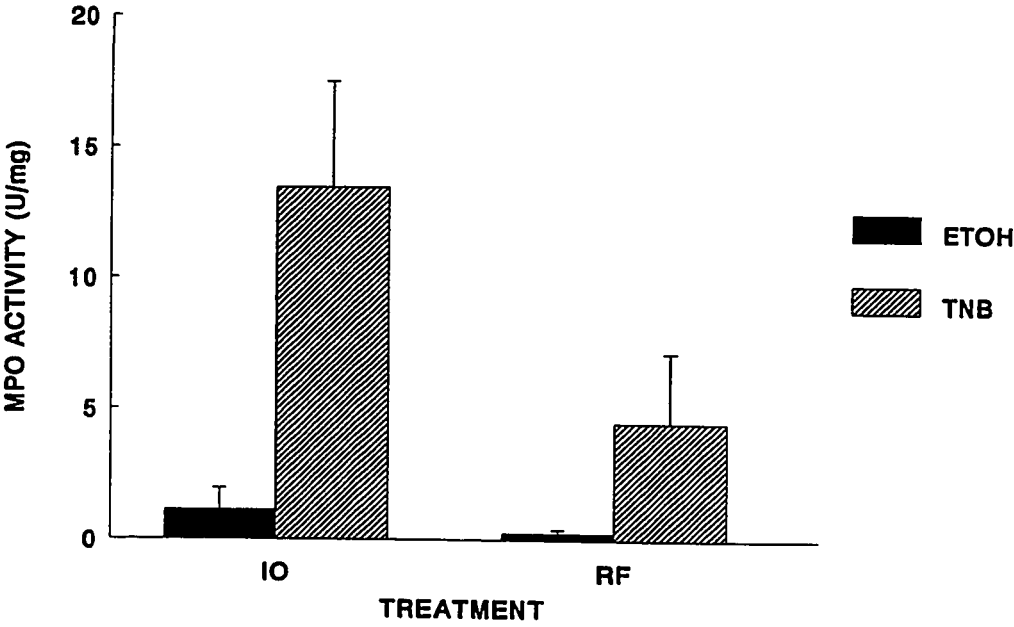
Subjects: Twenty-six male Sprague-Dawley (250 - 350 g at time of surgery) were housed individually in clear plastic cages in a colony room and were maintained on Purina rat pellets until surgery. Following surgery they were maintained on nutritionally complete Liquid Diet (General Methods).

Procedure: IO cannulae were implanted, and a minimum of three days later, the rats were habituated and trained to RF and IO feed nutritionally complete liquid diet in the IO cages (see General Methods). All the rats were trained on a 4 hour feeding schedule. The first hour was spent in the IO cages (either RF or IO), and the final 3 hours were spent in the home cage, RF, with liquid diet available in graduated bottles. Training was completed when each rat had received a total of 3 days of IO training and 3 days of RF training in the IO cages. The rats were then randomly assigned to 4 groups. Half were treated with liquid TNB and half with the vehicle, ETOH (see General Methods). Half of the TNB-treated rats were placed in the IO feeding condition, and half in the RF. The ETOH-treated rats were similarly divided. On days 1 to 5, the liquid diet intakes were measured following the 1 hour in the IO cage and the 3 hours in the home cage. After day 5, samples of the colon were collected (see General Methods).

RESULTS

Myeloperoxidase Assay: The TNB-treated rats had significantly more inflamed colons than the control rats according to the MPO analysis ($F(1, 21) = 10.31, p < 0.005$) (figure 22).

Figure 22: Myeloperoxidase (MPO) activity, after day 5 of treatment, in the colons of rats administered liquid diet, and treated with TNB or ETOH. IO-ETOH (N=7), IO-TNB (N=7), RF-ETOH (N= 6) and RF-TNB (N=5). Data are group means \pm SE.



Food Intake:

Test Meal: The 1 hour food intake (figure 23) in the IO cage was significantly decreased by treatment with TNB ($F(1, 22) = 53.61, p < 0.0001$). The suppression was greater on the first two days, gradually returning towards baseline ($F(5, 110) = 10.34, p < 0.0001$). The intake for all conditions increased over the 5 days ($F(5, 110) = 11.40, p < 0.0001$).

IO intake was significantly higher than the RF intake of liquid diet, a difference of approximately 20 ml throughout the 5 days ($F(1, 22) = 110.29, p < 0.0001$). Most importantly, however, there was not a significant interaction between IR treatment and the type of intake method ($F(1, 22) = 0.29, p = 0.59$), indicating that TNB treatment suppressed the food intake for both IO and RF conditions in the same fashion.

Home Cage: 3 hour real fed intake in the home cage (figure 24), was also suppressed for TNB treated rats compared to ETOH treated rats ($F(1, 22) = 9.73, p = 0.005$). The rats in the RF condition during the test meal ate more than those in IO ($F(1, 22) = 25.86, p < 0.0001$).

Total Intake: The total 4 hour food intake (figure 25), reflected the same pattern as the 1 hour food intake. TNB treatment suppressed food intake compared to the ETOH treatment ($F(1, 22) = 53.85, p < 0.0001$), and IO fed rats consumed more than RF rats ($F(1, 22) = 23.67, p < 0.0001$). Yet, the TNB treatment did not effect IO intake significantly differently from the RF conditions since the interaction between the Treatment and the Intake method was not significant ($F(1, 22) = 2.32, p = 0.14$).

Figure 23: Liquid diet intake (ml) during 1 hour of feeding in the IO cage on day 0 to day 5 following treatment with TNB or ETOH and delivery of food either IO or RF. IO-ETOH (N=7), IO-TNB (N=7), RF-ETOH (N= 6) and RF-TNB (N=6). Data are group means \pm SE.

Figure 24: Liquid diet intake (ml) during 3 hours of real feeding in the home cage on day 0 to day 5 following treatment with TNB or ETOH and delivery of food either IO or RF. IO-ETOH (N=7), IO-TNB (N=7), RF-ETOH (N= 6) and RF-TNB (N=6). Data are group means \pm SE.

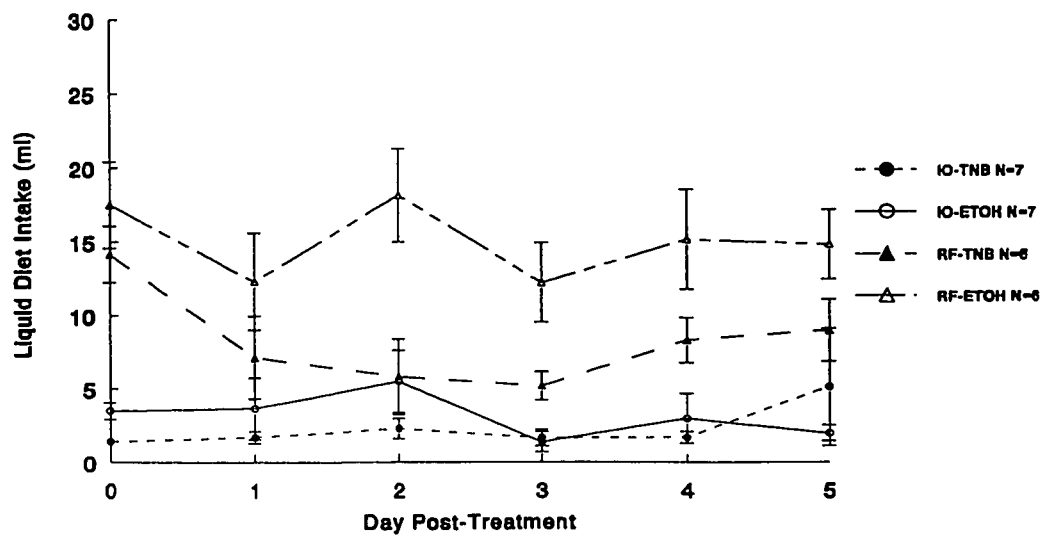
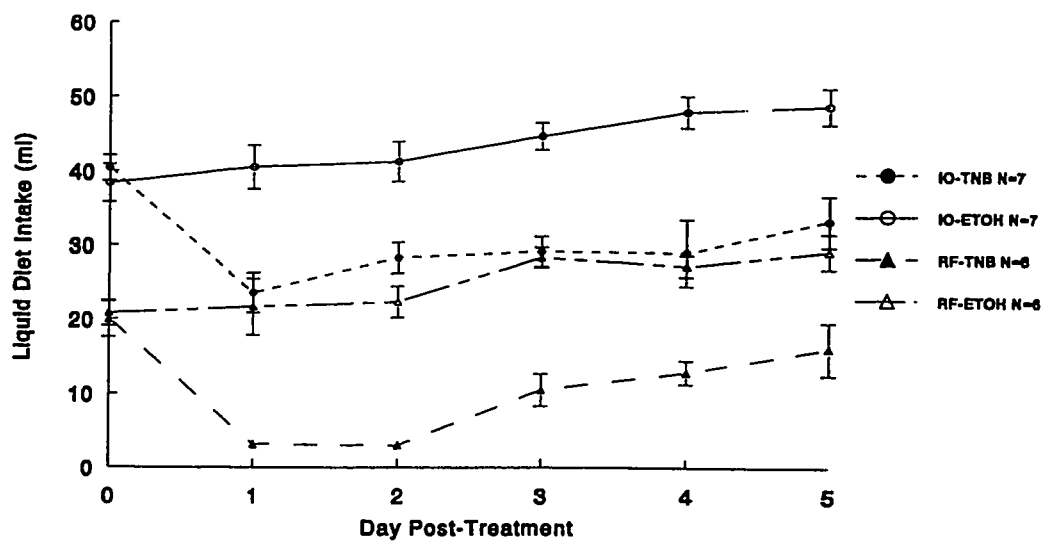
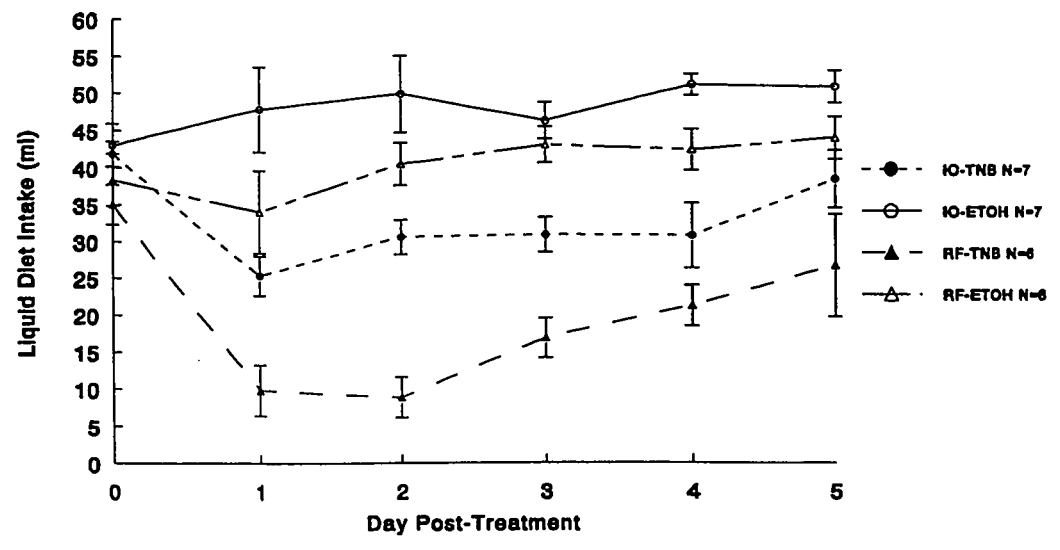


Figure 25: Liquid diet intake (ml) during the cumulative 4 hours of feeding on day 0 to day 5 following treatment with TNB or ETOH and delivery of food either IO or RF. IO-ETOH (N=7), IO-TNB (N=7), RF-ETOH (N= 6) and RF-TNB (N=6). Data are group means \pm SE.



DISCUSSION

Throughout the feeding period, treatment with TNB caused a suppression of food intake compared to the baseline intake, indicating that IO feeding is similar to real feeding. During the 1 hour intake, IO intake was greater than the RF intake (approximately 20 ml), as expected from the preliminary studies (Appendix 3). But, treatment with TNB caused a suppression in IO meals of 41.7%, 30.0% and 27.9% days 1-3, which returned towards baseline. In the RF condition, the suppressions on day 1-3 were 84.2%, 85.0% and 47.0% similar to previous experiments using powdered chow (McHugh *et al*, 1993 a, b). While the suppression for the RF meals was greater than that for the IO meals, statistical analysis indicated that suppression of food intake caused by TNB was not significantly different between the two types of food delivery. Therefore, the pattern of anorexia was replicated in both IO and RF methods of intake.

Some compensation for the large IO meal did occur during the 3 hour RF intake in the home cage, however, this compensation was not sufficient to eliminate the difference in intake when the total intake over 4 hours was examined. The cumulative 4 hour intake for the IO groups were greater than the cumulative 4 hour intake for the RF groups.

Since the treatment with TNB caused a similar anorexia in both groups, it appears that the IO technique could be used to study the TNB model of colitis. While, some compensation for the large IO meal occurred during the 3 hours of real feeding, the compensation was insufficient to defend the 24 hour food intake, and the suppression caused by the TNB over the 4 hours was not as great as that observed in real feeding. It is possible that the 20 hour deprivation (4 hour feeding schedule) used in the experiment might have altered the pattern of intake. In order to ensure that the pattern of anorexia caused by TNB treatment was a robust effect in IO feeding, different degrees of deprivation should be examined.

EXPERIMENT 2

Effect of Deprivation

In Experiment 1, the pattern of anorexia induced by TNB was demonstrated to occur in IO-feeding rats. However, the feeding schedule used resulted in a large amount of deprivation, and may have attenuated the expression of anorexia to some extent. In order to ensure that the pattern of anorexia was a robust event, Experiment 1 was replicated, but the animals were deprived for different periods of time. Following treatment with TNB or ETOH, IO food intake was compared on a 4 hour feeding schedule (20 hour deprivation) and a 20 hour feeding schedule (4 hour deprivation).

METHODS

Subjects: Forty-seven male Sprague-Dawley (200-300 g at time of surgery) were maintained in clear plastic cages on nutritionally complete liquid diet.

Procedure: IO cannulae were implanted, and a minimum of three days later, the rats were habituated and trained to RF and IO feed nutritionally complete liquid diet in the IO cages (General Methods). The rats were randomly assigned to be trained on a 4 or 20 hour feeding schedule. The first hour was spent in the IO cages (either RF or IO), and the final 3 or 19 hours were spent in the home cage, RF, with liquid diet available in graduated

bottles. Training was completed when each rat had received a total of 3 days of IO training and 3 days of RF training in the IO cages and maintained constant body weight. For both 4 hour and 20 hour feeding conditions, half of the rats were treated with liquid TNB and half with the vehicle, ETOH (see General Methods). Half of the TNB-treated rats were placed in the IO intake condition, and half in the RF. The ETOH-treated rats were similarly divided. Consequently, there were a total of 8 groups based on feeding schedule, IR treatment and form of intake. On days 1 to 5, the liquid diet intakes were measured following the 1 hour in the IO cage and the 3 or 19 hours in the home cage. After day 5, colon samples were collected (General Methods).

RESULTS

Myeloperoxidase Assay: The TNB treated rats had significantly greater inflammation in the colon than the ETOH treated rats as assessed by MPO activity ($F(1, 38) = 14.09$, $p < 0.001$), (figure 26). The MPO activity was not influenced by the degree of deprivation ($F(1, 38) = 0.35$, $p = 0.56$).

Food Intake:

Test Meal: The 1 hour intake in the IO cages (figure 27 and 28) was significantly suppressed by treatment with TNB for both feeding schedules ($F(1, 39) = 13.37$, $p < 0.001$), and the suppression was greater for the first 2 days then gradually returned to baseline ($F(4, 156) = 4.23$, $p < 0.01$). The rats with 4 hr feeding schedule (20 hr deprivation) ate significantly more, than the rats on the 20 hr feeding schedule (4 hr

Figure 26: Myeloperoxidase (MPO) activity, after day 5 of treatment, in the colons of rats treated with TNB or ETOH, maintained on different feeding schedules of liquid diet. Rats on a 20 hour feeding schedule: IO-ETOH (N=6), IO-TNB (N=6), RF-ETOH (N=5) and RF-TNB (N=6). Rats on a 4 hour feeding schedule: IO-ETOH (N=5), IO-TNB (N=6), RF-ETOH (N=6) and RF-TNB (N=6). Data are group means \pm SE.

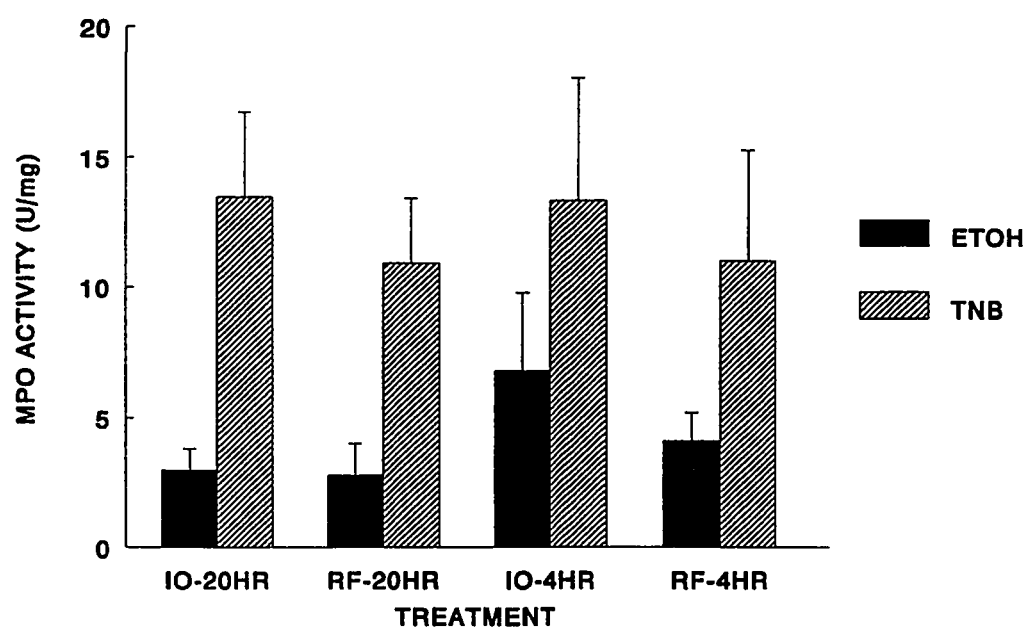
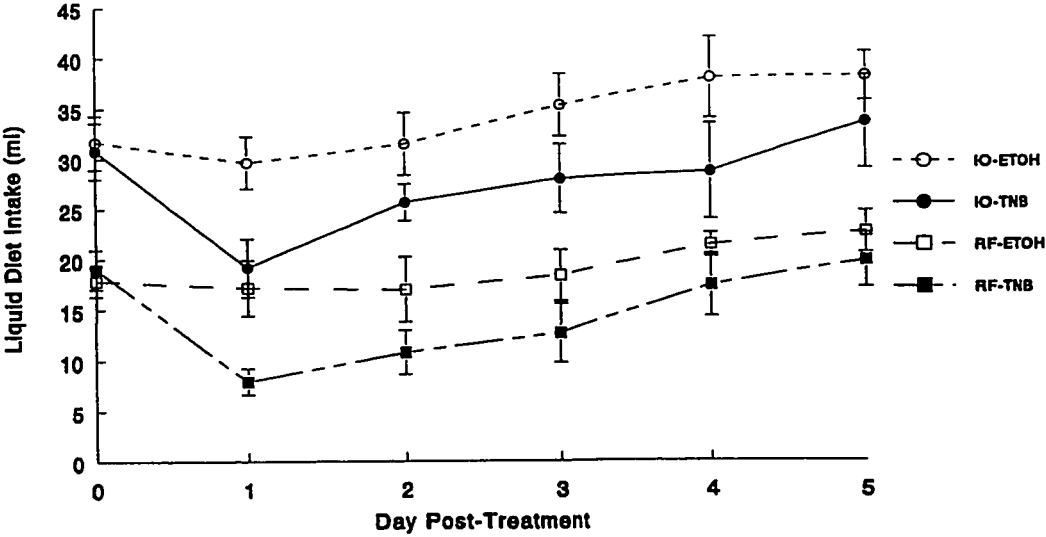


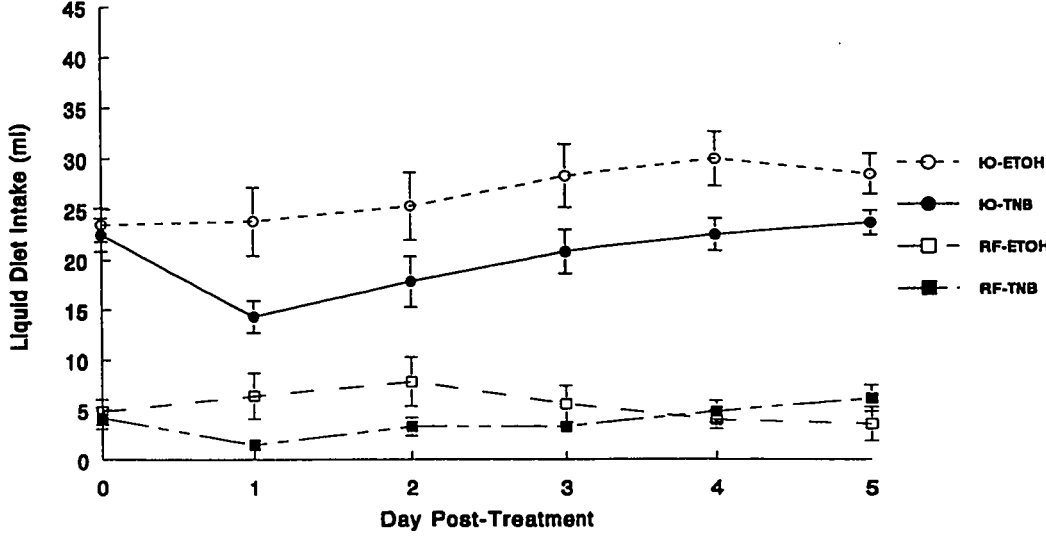
Figure 27: Liquid diet intake (ml) during 1 hour of feeding in the IO cage on day 0 to day 5 for rats on a **4** hour feeding schedule following treatment with TNB or ETOH and delivery of food either IO or RF. IO-ETOH (N=6), IO-TNB (N=6), RF-ETOH (N=6) and RF-TNB (N=6). Data are group means \pm SE.

Figure 28: Liquid diet intake (ml) during 1 hour of feeding in the IO cage on day 0 to day 5 for rats on a **20** hour feeding schedule following treatment with TNB or ETOH and delivery of food either IO or RF. IO-ETOH (N=6), IO-TNB (N=6), RF-ETOH (N=5) and RF-TNB (N=6). Data are group means \pm SE.

4 HR FEEDING GROUP



20 HR FEEDING

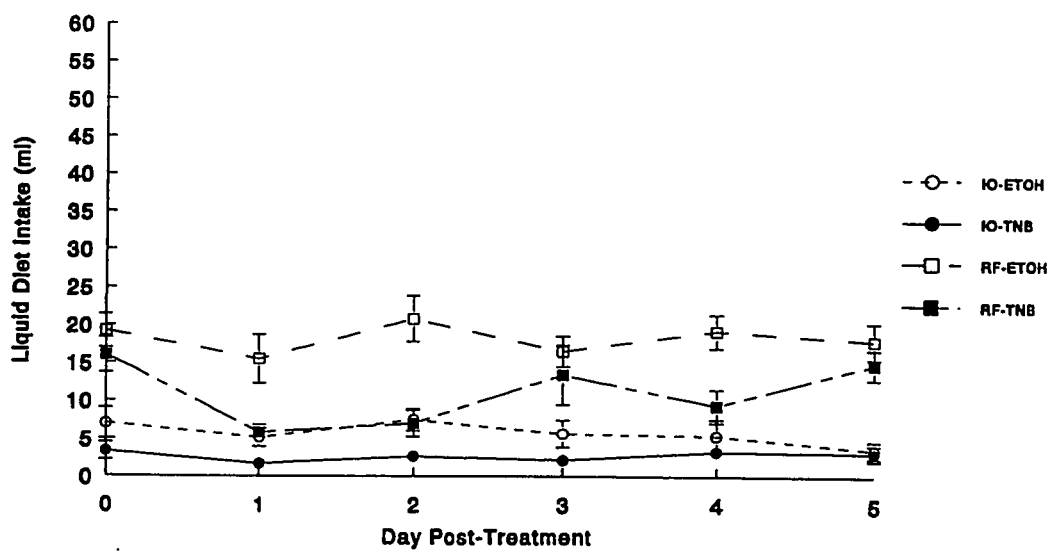
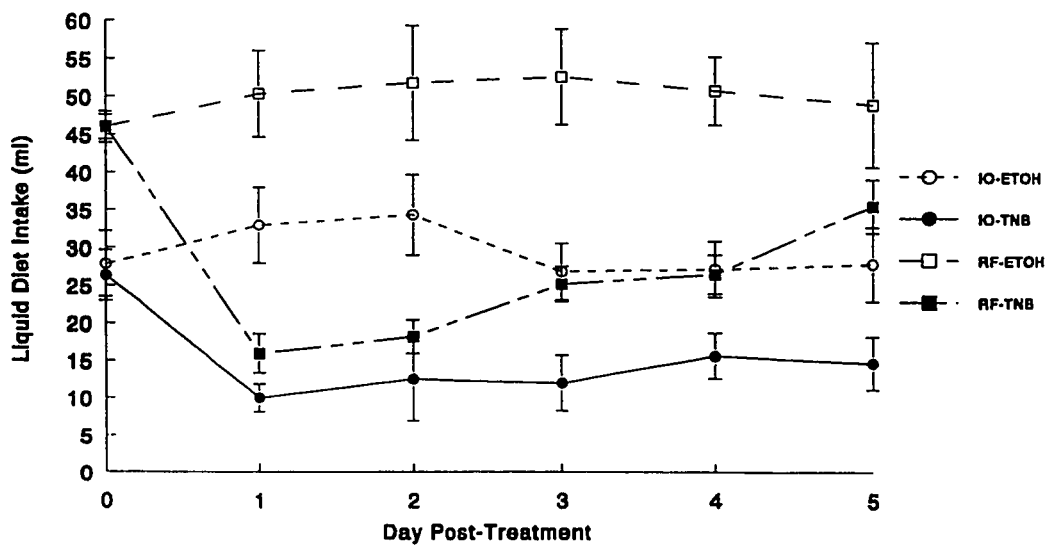


deprivation schedule) ($F(1,39) = 37.82, p < 0.0001$). While all conditions increased intake over the days of the experiment, ($F(4, 156) = 33.48, p < 0.0001$) the rats in the 4 hour feeding schedule condition increased their intake over the days more than the rats on the 20 hour feeding schedule ($F(4, 156) = 5.83, p < 0.001$), and the rats in the IO condition increased their intake more than the rats in the RF condition ($F(4, 156) = 4.00, p < 0.005$). As in Experiment 1, the rats that were fed IO ate significantly more than the rats that were fed RF ($F(1, 39) = 112.49, p < 0.001$). However, there was no interaction between the amount of deprivation due to the food schedule and IR treatment or form of intake (IO or RF), indicating that deprivation does not influence the expression of anorexia ($F(1, 39) = 1.16, p = 0.29$).

Home Cage: The RF intake in the home cage (figure 29, 30), like the 1 hour food intake was suppressed by treatment with TNB compared to ETOH ($F(1, 39) = 46.76, p < 0.0001$), and the suppression followed the same pattern over days ($F(4, 156) = 9.80, p < 0.0001$). Unlike the 1 hour food intake, the rats on the 4 hour schedule of feeding ate significantly less than those on the 20 hour schedule ($F(1, 39) = 106.23, p < 0.0001$) when in the home cage. Similar to the 3 hour intake of Experiment 1, rats that RF during the test meal ate more than the rats that IO fed ($F(1, 39) = 43.69, p < 0.0001$). Intake in the home cage, had an interaction between the feeding schedule and the treatment. TNB-treated rats with food available in the home cage for 19 hours ate more than the TNB rats with food available for only 3 hours, but the largest difference was the greater intake of the ETOH groups in the 19 hour period ($F(1, 39) = 17.04, p < 0.001$).

Figure 29: Liquid diet intake (ml) during 3 hours of feeding in the home cage on day 0 to day 5 for rats on a **4** hour feeding schedule following treatment with TNB or ETOH and delivery of food either IO or RF. IO-ETOH (N=6), IO-TNB (N=6), RF-ETOH (N=6) and RF-TNB (N=6). Data are group means \pm SE.

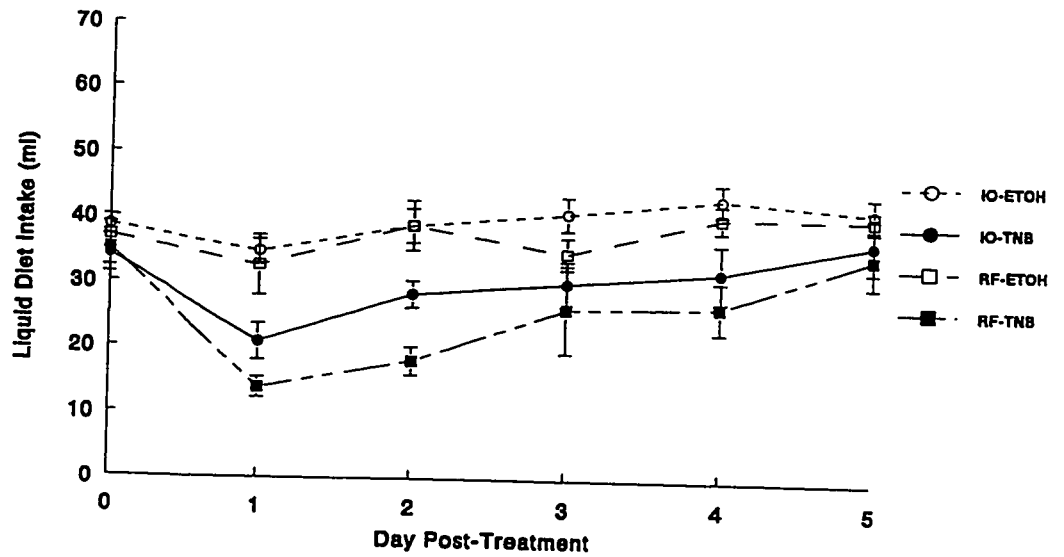
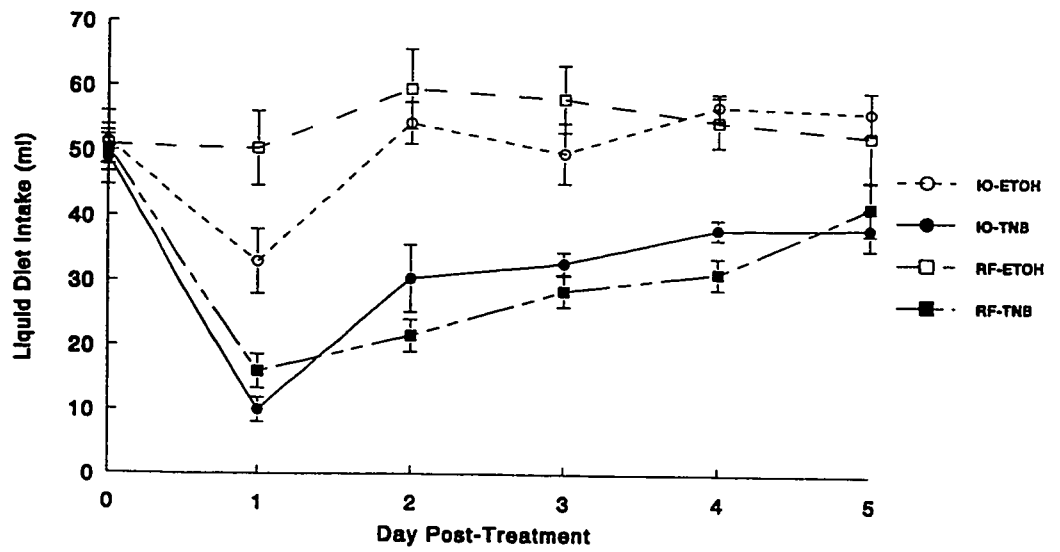
Figure 30: Liquid diet intake (ml) during 3 hours of feeding in the home cage on day 0 to day 5 for rats on a **20** hour feeding schedule following treatment with TNB or ETOH and delivery of food either IO or RF. IO-ETOH (N=6), IO-TNB (N=6), RF-ETOH (N=5) and RF-TNB (N=6). Data are group means \pm SE.

4 HR FEEDING**20 HR FEEDING**

Total Intake: The total intake (figure 31, 32) analysis indicates that treatment with TNB caused a significant suppression of food intake ($F(1,39) = 77.21, p < 0.000$) that returned towards baseline over the days ($F(4, 156) = 10.98, p < 0.000$). The rats increased food intake with days ($F(4, 156) = 59.10, p < 0.001$). Rats on a 4 hour feeding schedule ate less than rats on a 20 hour feeding schedule in total ($F(1, 39) = 15.38, p < 0.001$). However, the difference was primarily due to the ETOH groups (rats with 20 hours to eat, consumed more than rats with 4 hours), while the TNB-treated groups are less affected by the amount of time available to eat ($F(1, 39) = 8.32, p < 0.01$). There was no main effect of the type of intake (IO or RF) on the total amount of liquid diet consumed ($F(1, 39) = 0.44, p = 0.51$). However, there was an interaction between type of intake and the days of the experiment ($F(4, 156) = 3.52, p < 0.01$).

Figure 31: Liquid diet intake (ml) during the cumulative 4 hours of feeding on day 0 to day 5 for rats on a **4** hour feeding schedule following treatment with TNB or ETOH and delivery of food either IO or RF. IO-ETOH (N=6), IO-TNB (N=6), RF-ETOH (N=6) and RF-TNB (N=6). Data are group means \pm SE.

Figure 32: Liquid diet intake (ml) during the cumulative 4 hours of feeding on day 0 to day 5 or rats on a **20** hour feeding schedule following treatment with TNB or ETOH and delivery of food either IO or RF. IO-ETOH (N=6), IO-TNB (N=6), RF-ETOH (N=5) and RF-TNB (N=6). Data are group means \pm SE.

4 HR FEEDING**20 HR FEEDING**

DISCUSSION

Treatment with TNB caused suppression of food intake throughout the experiment. IO intake of liquid diet was greater than RF intake, replicating the findings of the previous experiments in this chapter.

The amount of deprivation induced by the two different feeding schedules did affect food intake. During the first hour, when IO intake was compared to RF intake, the animals that were more deprived ate more. However, during this period, the pattern of anorexia was not significantly changed by the amount of deprivation.

During the RF in the home cage, not surprisingly, the rats with the longer feeding schedule ate more than the rats with the shorter feeding schedule. The difference in feeding schedule had a greater effect on the ETOH control rats than the TNB treated rats, since the ETOH control rat intake increased substantially during the longer feeding period, while the TNB intake did not increase to the same extent. This suggests that the intake following TNB treatment is less influenced by deprivation.

Comparison of the total intake indicates that the amount of deprivation does not significantly affect the pattern of intake of TNB treated rats, but it does influence the level of intake for ETOH treated rats. Thus when calculating the percentage of suppression of TNB compared to ETOH treated rats, it appears that the 4 hour intake has less of a suppression, but the actual volume of intake for TNB treated rats remains consistent. Consequently, the characterization of IO intake indicates that the TNB- induced anorexia is expressed within the parameters of the IO technique.

CHAPTER 3: GENERAL DISCUSSION

Feeding behaviour of the rat appears to be altered when food is delivered directly into the mouth compared to when food is eaten normally, because the intake volume is significantly greater. Several studies by other researchers have also found IO intake to be different from RF. For example, neuropeptide Y, a peptide that normally results in a significant increase in food intake, does not have any effect on the intake of rats that are fed IO (Seeley, Payne & Woods, 1995). Amphetamine and fenfluramine usually cause a suppression of intake, which is not seen at the same doses with the IO technique (Wolgin, Oslan & Thompson; 1988). Similarly, the dopamine antagonists, Raclopride and Schering 233390, cause a suppression of food intake when animals real feed, but not when they are fed IO (Philopena & Smith, 1994).

Dopamine antagonists have previously been reported to attenuate food-anticipatory behaviours without influencing the amount eaten (Weingarten & Martin, 1989). Anticipatory, or preparatory, behaviours are a set of behaviours used to acquire food and can be dissociated from consummatory behaviours which include licking, chewing and swallowing (Seeley *et al*, 1995; Weingarten & Martin, 1989). In the IO technique, the food is delivered directly into the mouth so that anticipatory behaviour is eliminated and the consummatory behaviour is isolated (Swithers-Mulvey & Hall, 1992).

In fact, in each of the cases where the results of IO eating did not agree with that of normal feeding, the authors proposed that the difference was because the preparatory behaviour had been eliminated.

The large meal size resulting from IO feeding could carry a consequence that extends beyond satiety, such as gastrointestinal distress or malaise. However, there has been no evidence of aversion after the voluntary ingestion of mash, sucrose or milk in the large volumes (Berridge, 1991). Alternatively, it could be argued that the large meal was the result of the infusion rate. However, the 1.0 ml rate of infusion was comparable to normal licking rate in rats (see Chapter 2, intake rate), and the duration of meals increased, rather than decreasing or remaining the same.

My findings, that the IO meal size is substantially larger than the RF meal even though the rats control meal termination, imply that the rats did not express the same satiety when fed IO as the when fed from a bottle. This suggests a hypothesis for a model of food intake and meal termination. Perhaps some aspect of the anticipatory behaviour acts as an occasion setter for the post-ingestive consequences of a meal. Between meals, baseline release of signals or firing of neurons occur, that do not carry important information. However, during a meal it is beneficial to respond to all cues that indicate food has been ingested. The anticipatory behaviour might lower the threshold of response to post-ingestive satiety cues, which would result in a normal pattern of satiety and meal termination. However, during the IO meal, normal appetitive behaviours are not present in the rat to lower the response thresholds. Thus, greater cues for satiety (frequency,

duration or intensity) are required to cause the termination of a meal. This hypothesis was explored in the Appendix 4.

Although the food intake was not found to be identical under IO and RF conditions, the experiments demonstrated that a similar pattern of anorexia was expressed in both conditions following treatment with TNB. The finding was replicated in two experiments and was demonstrated to be true under two different conditions of deprivation. This is the essential finding in order to proceed with the research on Taste Reactivity.

In summary, IO intake of liquid diet results in larger meals than RF meals. However, a comparison of RF and IO intake in the TNB model of colitis indicates that the anorexia induced by colitis can be expressed using the IO technique when rats are maintained on liquid diet, under two different conditions of deprivation (4 hours and 20 hours). Consequently, the IO technique can then be used to maintain stimulus control over the initiation of a meal.

CHAPTER 4

TASTE REACTIVITY

Rats treated with IR TNB develop an extreme, transient anorexia in association with the colitis (McHugh *et al*, 1993 a, b). The mediators of the anorexia are still not understood, although it has become clear that the anorexia is not mediated centrally by the peptide oxytocin, nor is it mediated by malaise or an exaggerated response to post-ingestive satiety cues. In examining the intake rate of rats eating from a spout (real feeding, RF) it became apparent that rats treated with TNB ate at a substantially slower rate than control rats treated with the vehicle, ETOH (Chapter 2). The decreased rate of intake occurred at the initiation of the meal, and so was unlikely to be caused by post-ingestive consequences (Barnfield *et al*, 1994; Swithers & Hall, 1994). One possible cause for a decrease in rate of intake is a change in the perception of the food being consumed (Davis & Smith, 1992). The change in perception may be of hedonic value, which will be discussed to greater extent.

Palatability is a hypothetical construct used to define the complex evaluation that, in combination with other factors, determines the response elicited by food. Palatability is an evaluation of food that is not the taste quality nor the intensity, although these are factors which influence the perception of palatability in addition to the internal

physiological state and past learning (Berridge & Grill, 1983; Cabanac, 1971; Ossenkopp, Parker & Spector, 1995).

Palatability has often been considered a single continuous dimension between positive (hedonic) and negative (aversive). Recent research has provided a great deal of information to indicate that, in fact, there are two different systems, one for positive assessment and one for negative assessment (Barnfield *et al*, 1994; Berridge & Grill, 1983; Berridge & Grill, 1984; Berridge & Treit, 1986; Hsiao & Fan, 1993). Although this theory has caused some controversy (Breslin, Spector & Grill, 1992), research has shown that manipulations can alter positive responses to food without altering negative responses (Berridge, 1988; Berridge, 1991; Berridge & Treit, 1986; Doyle, Berridge & Gosnell, 1993; Treit & Berridge, 1990; Treit, Berridge & Schultz, 1987) and alter negative without changing positive (Barnfield *et al*, 1994; Parker, 1994). In addition, the palatability of food can be dissociated from the amount ingested (Berridge & Pecina, 1995; Berridge, Venier & Robinson, 1989; Galaverna, Seeley, Berridge, Grill, Epstein & Schulkin, 1993; Treit & Berridge, 1990), indicating that although ingestion of food is usually linked to palatability, it is possible to eat food that is not palatable, and to avoid food that is judged palatable.

A change in the perception of the palatability of food could result in a change of feeding behaviour (Berridge, Flynn, Schulkin & Grill, 1984, Barnfield *et al*, 1994). The examination of palatability in the TNB model of colitis is supported by the findings of a

clinical questionnaire from patients with Crohn's disease, which indicated that patients with weight loss and decreased hunger also reported a decrease in the sensation of pleasure associated with eating (Rigaud *et al*, 1992). While palatability in rats cannot be examined using questionnaires, a method of examining palatability in rats was developed by Grill and Norgren (1978 a), called taste-elicited consummatory response or taste-reactivity (TR).

Taste Reactivity can be used to examine the role of palatability in the control of ingestive behaviour (Berridge *et al*, 1984; Berridge & Treit, 1986). In the technique of TR, stimuli are infused directly into the mouth through an intraoral (IO) cannula, and the facial and somatic responses are filmed. Rats make highly stereotyped consummatory responses to food called modal fixed action patterns (Berridge & Grill, 1984). The behaviours have typically been classified as ingestive compared to aversive. Tongue protrusions, lateral tongue protrusions and paw licks have been defined as ingestive, while gapes, chin rubs, head shakes, fore-limb flails and head washes have been classified as aversive (Berridge & Grill, 1983) and will be described in greater detail in the methods section. There has been some controversy (Breslin *et al*, 1992; Parker, 1993; Parker & Leeb, 1994) over the classification of mouth movements and passive drips, but they have commonly been defined as neutral (Berridge & Pecina, 1995; Berridge & Fentress, 1985; Berridge *et al*, 1984; Berridge & Treit, 1986; Delamater, Lolordo & Berridge, 1986; Galaverna *et al*, 1993; Treit *et al*, 1987).

Mouth movements are most commonly defined as neutral or weakly ingestive, and have been shown to dissociate from the other movements defined as ingestive (Berridge & Fentress, 1985; Berridge & Treit, 1986). Mouth movements are ingestive, since food is consumed as a result of the motion; however, in this study palatability is being dissociated from simple ingestion. Mouth movements have only weakly been linked to evaluations of palatability and seem to reflect low palatability while lateral tongue protrusions and paw licks reflect high palatability. Consequently, instead of referring to ingestive behaviours, I will refer to hedonic behaviours, and neutral behaviours as expressed by Berridge and Pecina (1995). Hedonic responses have been defined as the unconditioned behavioural response to sweet taste (Berridge & Pecina, 1995).

There is a great deal of support for TR as a measure of palatability. To begin with, it is an actual measure of behaviour rather than a measure of the consequence of behaviour, such as meal size (Ossenkopp *et al*, 1995). In addition, manipulations that alter other measures of taste perception are reflected in TR. For example, TR changes with concentrations of stimuli (Berridge & Grill, 1983), and is altered in response to physiological changes such as salt depletion, satiety and 48 hour deprivation. Consequently, TR has been used to demonstrate that the phenomenon of alliesthesia (the change in affective perception of unchanged stimulus caused by changes in internal state) is also seen in rats (Berridge, 1991; Berridge *et al*, 1984; Berridge & Schulkin, 1989; Cabanac & LaFrance, 1992; Cabanac & Zhao, 1994; Zhao & Cabanac, 1994). In addition, sensory specific satiety, where the hedonic evaluation of a specific food is

decreased after consumption, has also been demonstrated using TR (Berridge, 1991).

Learning can alter perception of food as well. There is evidence that people can learn to like a flavour after continuous exposure, and a similar shift in palatability has been recorded in rats using TR (Zellner, Berridge, Grill & Ternes, 1985). In addition, people can learn to dislike a flavour after it is paired with illness (conditioned taste aversion), and this shift in palatability has been recorded in rats (Breslin *et al*, 1992; Keiffer & Orr, 1992; Ossenkopp & Eckel, 1995; Parker, 1994). Alternatively, drugs, such as morphine and the anxiolytic chlordiazepoxide, increase food intake, and also enhance the positive TR (Berridge, 1988; Berridge & Pecina, 1995; Berridge & Treit, 1986; Doyle *et al*, 1993; Treit & Berridge, 1990; Treit *et al*, 1987). Finally, TR is not altered by events that would not change human palatability ratings, such as shock (Berridge & Treit, 1986).

In Chapter 3, I demonstrated that the anorexia induced by colitis in the TNB model of IBD was also expressed under IO feeding conditions. The IO preparation makes it possible to use the TR technique to study whether palatability influences the amount ingested in the colitis-induced anorexia. The final experiment will use TR to assess whether a change in perceived palatability of food can account for the decrease in the rate of food intake observed in the TNB model.

METHODS

Subjects: Eleven male albino Sprague-Dawley rats (Charles River Inc., Canada) were housed individually in clear plastic cages in a colony room with a 12:12 hour light/dark cycle. The rats were maintained on Purina rat pellets until surgery, then they were maintained on nutritionally complete Liquid Diet (General Methods).

Procedure: IO cannulae were implanted (General methods) and a minimum of two days later, the rats were introduced to the novel IO cages during two 10 min sessions on consecutive days. The rats were trained on a 4 hour feeding schedule of liquid diet (General Methods). The rats were placed in the IO cage for 10 min prior to the initiation of the feeding period. Food intake for the first hour of the feeding schedule was an IO meal in the IO cage (see Chapter 3 for definition of meal termination). The final 3 hours were RF in the home cage. The facial movements during IO meals were recorded from the mirror placed at a 45° angle under the Plexiglas cage using a video camera (Hitachi, CCTV or Newvicon) connected to a videocassette recorder (Zenith VHS HQ or Panasonic Omnivision VHS) and displayed on monitors (Sony or Eaton Viking) as described by Grill and colleagues (1987). The training continued until the rats maintained constant weight, then they were randomly assigned to be treated IR with either TNB or the vehicle, ETOH (General Methods). For days 1 to 5 following treatment, the liquid diet intake was recorded at 1 hour and at 3 hours, and the facial movements were filmed

during the first hour, in the IO cage. After day 5, the rats were given Chloral hydrate (1 ml / rat) and a sample of the colon was collected for MPO analysis (General Methods).

Videotape Analysis:

The first two minutes (as in Parker, 1993) of the facial movement were analyzed following the scoring technique of Berridge and colleagues (Berridge & Grill, 1983; Berridge & Pecina, 1995) by scorers blind to the condition of the rats. Positive hedonic responses included: Tongue Protrusions (TP) = rhythmic protrusions of the tongue along the midline, with the tongue covering the upper incisors; Lateral Tongue Protrusions (LTP) = nonrhythmic extensions of the tongue on either side of the mouth with the tongue lifting the lip laterally as it moves forward; and Paw Licks (PL) = persistent direction of ingestive responses to the paws, with the paws held close to the mouth and lapped for some seconds.

Aversive responses included: Gape (G) = rapid, large amplitude retraction of the corners of the mouth and lower lip to reveal the lower teeth, Chin rubs (CR) = mouth being rubbed along the floor and wall, Head Shake (HS) = rapid side to side movement of head, Forelimb Flail (FF) = rapid shake of both forelimbs along the horizontal plane, Head Wash (HW) = moving the paws across the head rapidly, Paw Treading (PT) = alternate stepping with fore paws in rapid, rhythmic motion, Rearing (R) = raising on hind limbs and Active Drip (AD) = liquid is expelled with force from the mouth, often in combination with HS, FF or CR.

Neutral responses included Mouth Movements (MM) = low amplitude, rhythmic openings of the mandibles, Locomotion (L) = movement around cage and Passive Drip (PD) = fluid accumulates on the mouth and drops off.

Scoring:

When scoring the movements, not all incidences were scored the same way. The units of measure were designed so that equal weight was given to each of the behaviours, independent of frequency (Galaverna *et al*, 1993). The weighting was designed to provide a more detailed and sensitive measure, and to provide a more accurate measure of general palatability (Berridge & Grill, 1984; Berridge & Pecina, 1995). Behaviours such as LTP, G, CR, HW, FF, HS occur in discrete units and were assigned a unit of 1 for each occurrence of the behaviour. Behaviours such as PL, MM, L, R, PT and PD occur as continuous events and were assigned a unit of 1 for each 5 sec of duration. TP occur as a continuous event, but of shorter duration, and so were assigned 1 unit for each 2 sec of duration (Berridge *et al*, 1984; Berridge & Grill, 1984).

RESULTS

Food Intake:

Test Meal: The IO intake during the first hour (figure 33) was significantly suppressed by TNB ($F(1, 9) = 5.98, p < 0.05$). The food intake gradually increased for all the rats over days ($F(4, 36) = 7.74, p < 0.001$).

Home Cage: The 3 hour RF intake from graduated cylinders in the home cage (figure 34) was not significantly effected by IR treatment ($F(1, 9) = 0.26, p = 0.62$). The real fed intake for both groups was small following the IO food intake.

Total Untake: Like the IO intake, the total intake (figure 35) was suppressed by treatment with TNB ($F(1, 9) = 5.95, p < 0.05$), and gradually increased over days ($F(4, 36) = 7.54, p < 0.001$).

Taste Reactivity:

Negative: Examination of the taste-reactivity revealed that there were very few negative movements, and only included FF, HS, HW and rearing (figure 36). When combined together, there was no significant effect of treatment or days ($F(1, 9) = 2.35, p = 0.16$). A post-hoc analysis using Newman-Keuls, indicated that there was increase of negative movements on day 4, however, these did not occur in correlation with the anorexia. It is possible that loosening of IO cannulae in the headcaps could have caused an increase in FF, HS and HW, because these behaviours were recorded more often in the rats with loosened headcaps.

Figure 33: Liquid diet intake (ml) during the IO meal on day 0 to day 5 following treatment with TNB (N=6) or ETOH (N=5). Data are group means \pm SE.

Figure 34: Liquid diet intake (ml) during 3 hours of real feeding in the home cage on day 0 today 5 following treatment with TNB (N=6) or ETOH (N=5). Data are group means \pm SE.

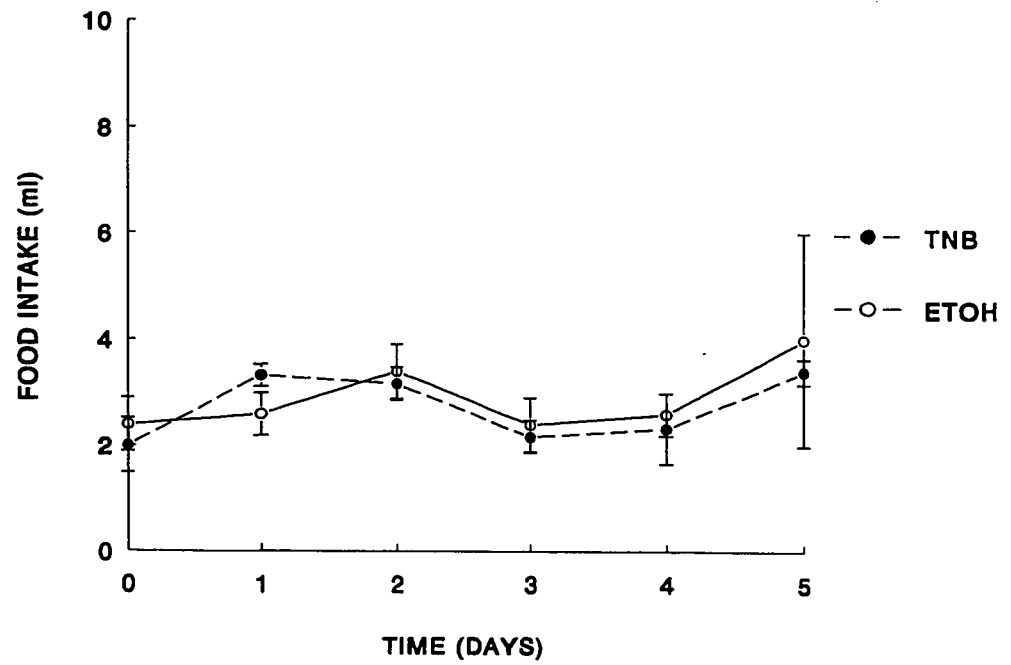
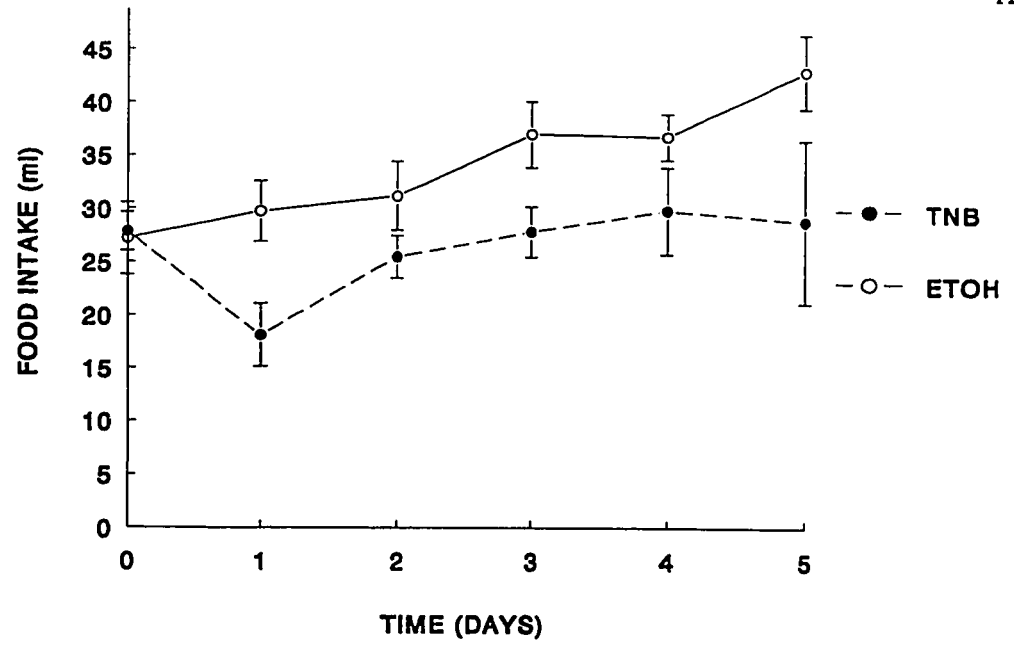


Figure 35: Liquid diet intake (ml) during the cumulative 4 hours of feeding on day 0 to day 5 following treatment with TNB (N=6) or ETOH (N=5). Data are group means \pm SE.

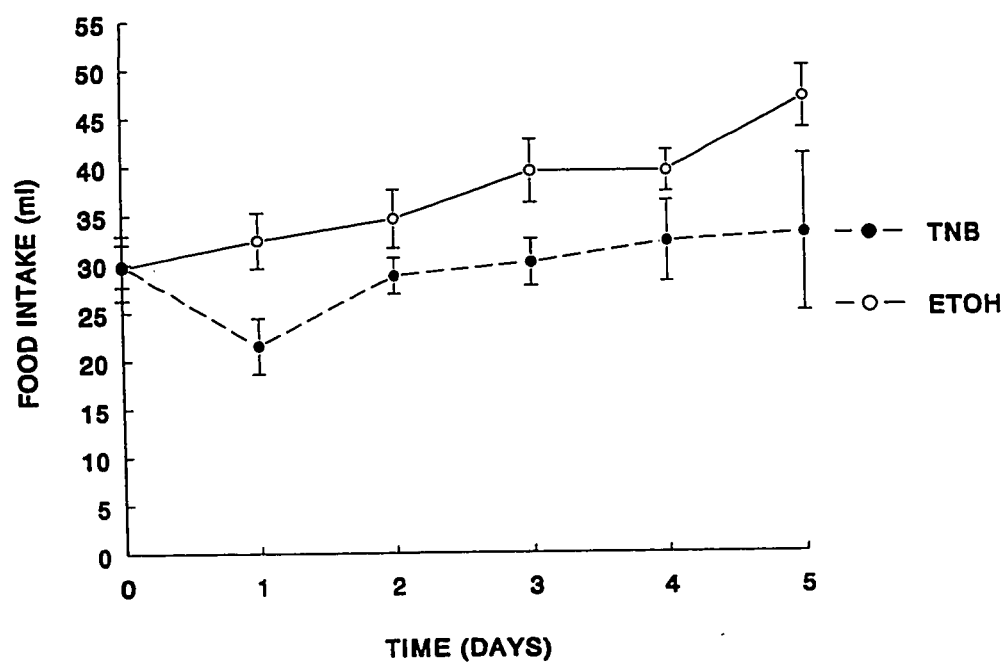
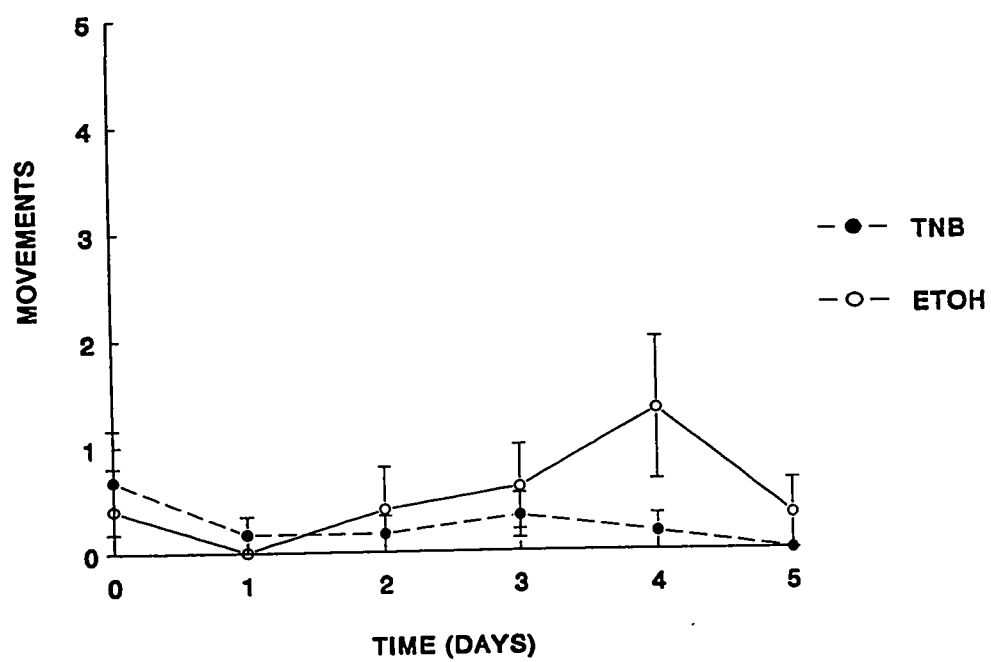


Figure 36: The total Aversive Taste Reactions (forelimb flail, head shake, head wash and rearing) during the first 2 min of an IO meal on days 0 to 5 for rats treated with TNB (N=6) or ETOH (N=5). Data are group means \pm SE.



Positive: The positive movements, when combined together did not show a significant difference between the TNB and ETOH treated rats ($F(1, 9) = 1.32, p = 0.28$). Analysis of the separate measures indicated that LTP (figure 37) were not significantly different for the two conditions ($F(1, 9) = 2.14, p = 0.18$), but the number of LTP's decreased over the days for both conditions ($F(4, 36) = 3.38, p < 0.05$). There was not a significant difference between TNB and ETOH groups for TP either (figure 38) ($F(1, 9) = 3.00, p = 0.12$). However, the number of paw licks (PL) (1 unit = 5 sec) were suppressed as a consequence of treatment with TNB (figure 39) ($F(1, 9) = 6.77, p = 0.03$).

Neutral: Analysis of the neutral movements, revealed that the events were primarily MM (1 unit = 5 sec), with no PD and little locomotion (figure 40). There was not a main effect of treatment ($F(1, 9) = 3.63, p = 0.09$), but there was a significant interaction between treatment and days ($F(4,36) = 3.39, p < 0.05$). TNB treated rats had more neutral movements than the ETOH treated rats, especially on the first day following treatment, the period of greatest anorexia.

Overall, the only significant differences in TR caused by TNB treatment was a significant suppression of the positive movement PL, and a significant elevation of the neutral movement, MM.

Figure 37: The Lateral Tongue Protrusions (LTP) during the first 2 min of an IO meal on days 0 to 5 for rats treated with TNB (N=6) or ETOH (N=5). Data are group means \pm SE.

Figure 38: The Tongue Protrusions (TP) during the first 2 min of an IO meal on days 0 to 5 for rats treated with TNB (N=6) or ETOH (N=5). Data are group means \pm SE.

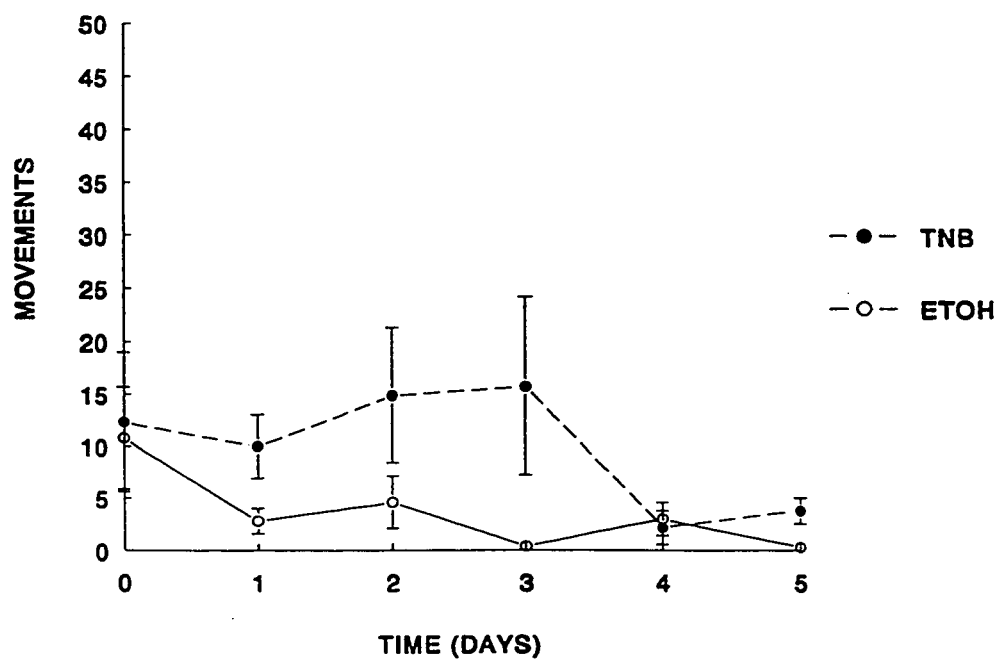
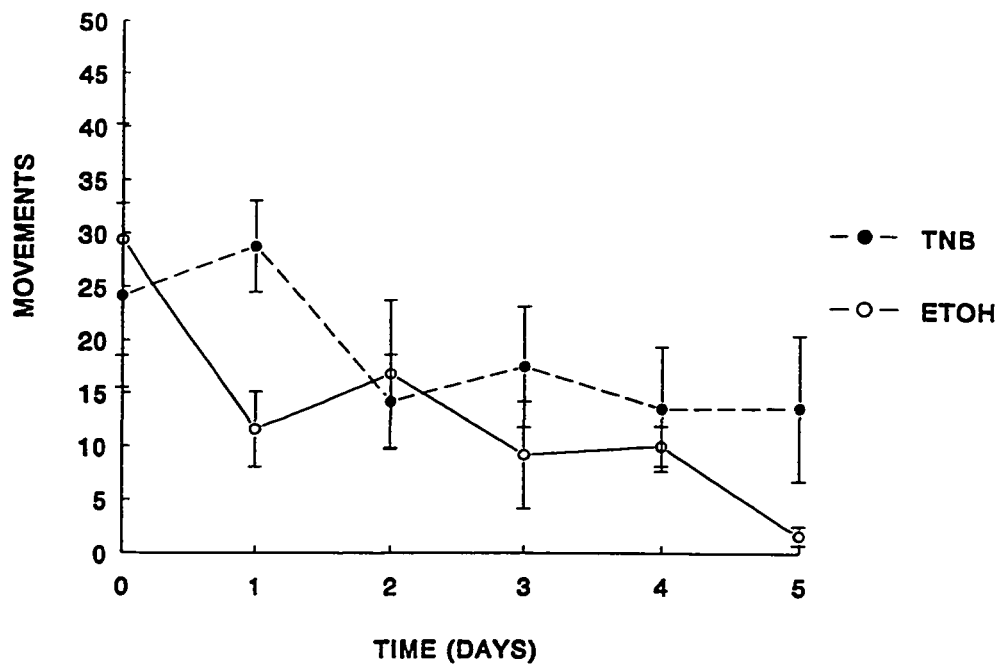
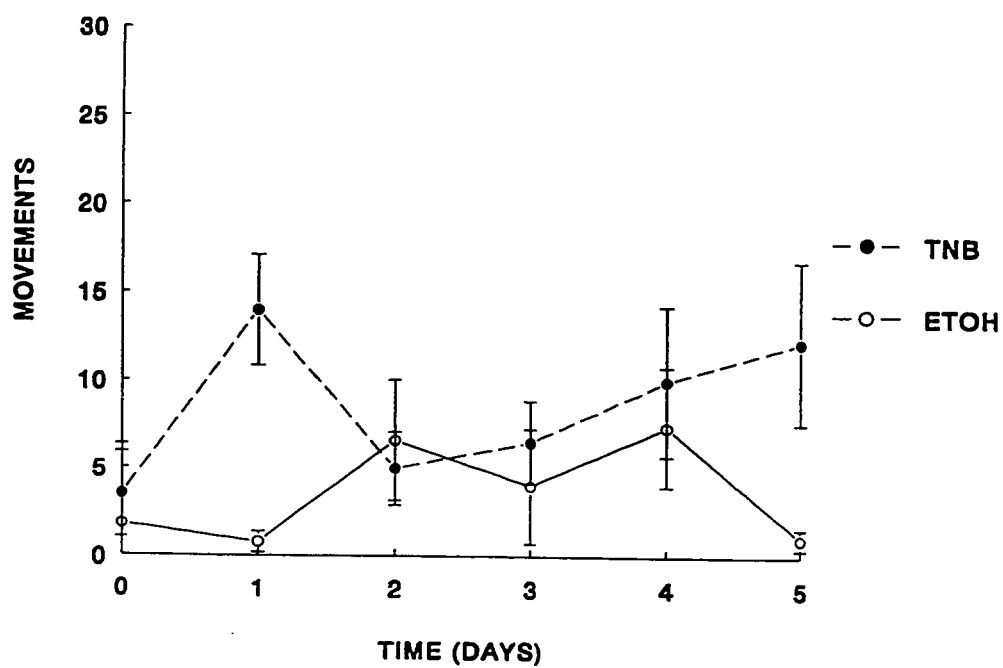
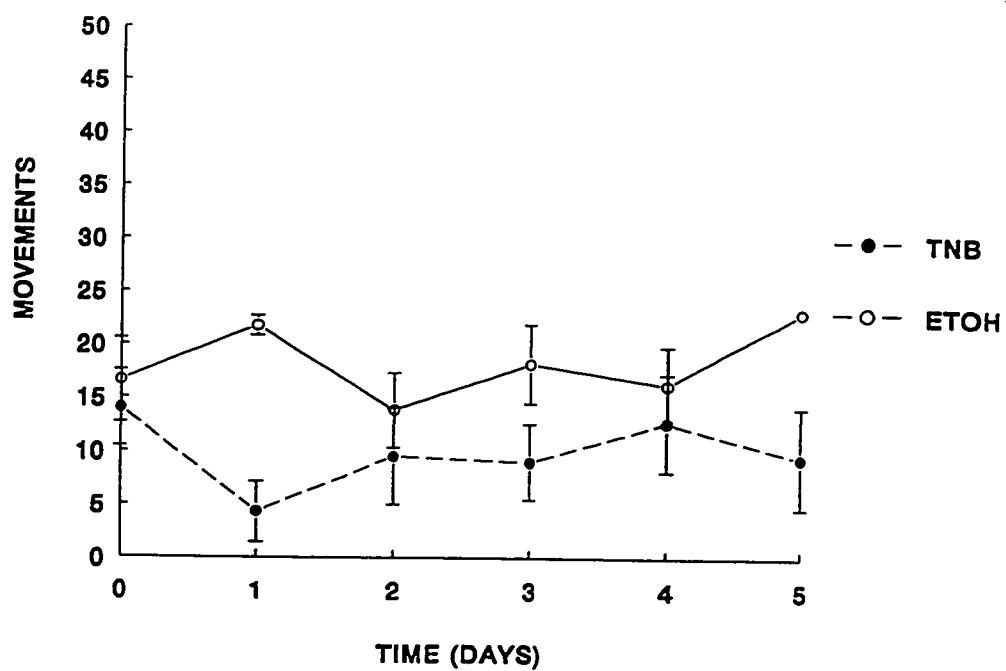


Figure 39: The number of paw licks (PL) during the first 2 min of an IO meal on days 0 to 5 for rats treated with TNB (N=6) or ETOH (N=5). Data are group means \pm SE.

Figure 40: The total Neutral Taste Reactions (mouth movements, locomotion) during the first 2 min of an IO meal on days 0 to 5 for rats treated with TNB (N=6) or ETOH (N=5). Data are group means \pm SE.



DISCUSSION

Examination of the taste-elicited consummatory responses to liquid diet during IO feeding suggest that TR is altered in response to treatment with TNB. TR is a technique used to measure shifts in palatability (Berridge *et al*, 1984; Berridge & Treit, 1986). Since aversive responses were rare during the first two minutes of a liquid diet IO meal, and hedonic responses were common, liquid diet was a palatable diet.

The presence of both aversive and hedonic responses is common in TR tests. Researchers suggest that aversive and hedonic responses are actually two separate systems, and that preference is determined by the proportion of the two responses (Berridge & Grill, 1984). The hedonic response of LTP decreased over the days of the experiment which suggests that the palatability of the diet decreased with experience. A decrease in strongly ingestive responses with repeated experience with a stimuli has been previously recorded (Berridge *et al*, 1984). Interestingly, the amount of liquid diet ingested increased over the duration of TNB experiment, which supports the concept that palatability can be dissociated from ingestion (Berridge & Grill, 1983; Berridge & Pecina, 1995; Berridge *et al*, 1989; Galaverna *et al*, 1993; Treit & Berridge, 1990).

Treatment with TNB did not significantly alter the aversive responses to the liquid diet. However, PL was suppressed in response to treatment with TNB and PL has been identified as one of the highly positive responses, that is difficult to produce with weaker stimuli (Berridge & Pecina, 1995). Also, the number of MM was significantly increased in

TNB treated rats during the greatest period of anorexia. There has been some controversy over the interpretation of MM. Although MM's are part of consummatory behaviour, they do not relate proportionately to positive palatability of taste and may be dissociated from positive hedonic responses (Berridge & Fentress, 1985; Berridge & Grill, 1983; Berridge & Pecina, 1995). MM are the main response elicited by water (Delamater *et al*, 1986), they are present during aversive stimuli such as quinine, (Berridge, Fentress & Parr, 1987; Berridge & Grill, 1983), they are elicited in response to weakly positive stimuli, and disappear with more positive stimuli (Berridge & Pecina, 1995).

In this study, MM increased while PL decreased. In previous experiments where MM increased, independent of the hedonic responses, the MM were interpreted as a decrease in the perception of palatability (Berridge & Fentress, 1985; Berridge & Pecina, 1995). Consequently, it could be argued that, while the food was not perceived as aversive, it was perceived as less hedonically positive, which might account for the observed decrease in rate of consumption (Chapter 2). The observed decrease in hedonic value of the food complements the clinical research in humans, which indicates that the pleasure of eating is decreased (Rigaud *et al*, 1992). Although the shift in palatability may not completely account for anorexia, it may contribute to the decrease in food intake.

Future Research:

If a decrease in the positive hedonic perception of taste contributes to the anorexia seen in association with an inflamed gastrointestinal tract, then future research should establish the physiological mechanisms. The shift in TR in response to the induction of colitis reflects a change in the perception of food in response to a change in physiological state, similar to the phenomenon of alliesthesia (Cabanac, 1979). Cabanac and Zhao (1994) suggested that negative alliesthesia might be mediated by CCK and the vagus nerve. If this is the case in the TNB model, then negative alliesthesia is unlikely to account for the colitis-induced anorexia, since it has previously been shown that CCK and the vagus nerve do not mediate the anorexia (Weingarten *et al*, 1993). However, the duodenal lumen has been indicated as the origin of alimentary negative alliesthesia (Cabanac & Lafrance, 1992), so this may be an area for exploration. Alternatively, research with decerebrate rats indicate that the TR patterns are coded in the caudal brain stem and may be modified within or below the mesencephalon by forebrain controls (Berridge, 1988; Berridge *et al*, 1984). Lesion studies by Kieffer and Orr (1992) suggest that learned palatability shifts are mediated by the gustatory cortex.

Previous research has shown that IL-1 is one of the mediators of the colitis-induced anorexia (McHugh *et al*, 1994). Consequently, an experiment examining the effect of IL-1 on TR could indicate whether this is the mechanism through which IL-1 mediates the feeding behaviour of rats.

GENERAL DISCUSSION

Anorexia adds to the morbidity and mortality associated with Inflammatory Bowel Disease (Griffiths *et al*, 1993; Kirschner, 1988, Motil *et al*, 1993), while improved nutrition can result in remission of the disease (González-Huix *et al*, 1993; Royall *et al*, 1995), yet the mechanisms mediating the suppression of food intake remain unknown. Previous research has established the TNB model as a suitable model to study the feeding suppression associated with colitis. Treatment with TNB results in a robust, highly reproducible pattern of decreased food intake (McHugh *et al*, 1993 a). The focus of this thesis has been to try to determine potential biological and behavioural mediators of the anorexia associated with colitis.

The information from the inflamed tissue in the gut must be communicated to the brain in order to change behaviours such as eating. McHugh and colleagues (1994) have demonstrated that antagonism of central IL-1 receptors, an interleukin which is released from the site of inflammation, attenuates colitis-induced anorexia. IL-1 stimulates the release of CRF in the brain, which suppresses feeding (Uehara *et al*, 1989) and CRF in turn causes the release of central OT, which has been implicated as a central mediator of food intake (Glowa *et al*, 1992; Olson *et al*, 1991 b).

A potential pathway linking the site of inflammation and the change in feeding behaviour might be IL-1 altering CRF levels in the brain which stimulate central OT release.

Administration of an antagonist to OT, L-366, 948, did not alter the course of anorexia, which suggests that central OT is not involved in the pathway that mediates colitis-induced anorexia. Further support for the conclusion that central OT is not a mediator of the anorexia is supplied by an experiment ablating the paraventricular nucleus (PVN) (Morrison, 1995). The PVN has been implicated as a major site for the mediation of food intake, and is also the primary site of OT and CRF production (Ivell, 1986; Van der Kar *et al*, 1994; Weindel & Sofraniew, 1980). Since ablation of the nucleus did not alter the expression of the anorexia, it appears unlikely that either OT or CRF are mediators of colitis induced anorexia.

This thesis has shown that the peptide, OT, does not act centrally as a biological mediator of the anorexia. However, OT is also released peripherally (Arletti *et al*, 1989; Forsling, 1986; Olson *et al*, 1991 a). While peripheral levels of OT do not play a causal role in changing food intake, they have been used as a physiological marker to indicate when food intake is suppressed in response to malaise (Olson *et al*, 1991 a, Verbalis *et al*, 1986 a , b). Malaise, or the feeling of being unwell, has commonly been proposed as the reason that IBD patients decrease their food intake (Anderson *et al*, 1989, Rigaud *et al*, 1992; Royall *et al*, 1995; Stokes, 1992) and should be assessed as a behavioural mediator in the model of colitis. Measurements of the peripheral OT levels of rats were taken in three separate experiments: once each day over the course of the anorexia, during the first

24 hour, and following the initiation of a meal. The elevations of plasma OT did not correlate with the expression of the anorexia. While these findings do not suggest that malaise was not experienced, they suggest that malaise was not the mechanism causing the suppression of food intake.

The conclusion that malaise is not a behavioural mediator of the anorexia is supported by behavioural data which show that TNB-treated rats initiated meals with the same frequency as control rats, maintained pre-treatment water intake and demonstrated the same sham-feed intake as control rats (McHugh *et al*, 1993 a). The IO experiments indicated that rats were not only capable of initiating meals, but of consuming large meals, even when treated with TNB. Therefore, the rats were physically capable of a normal volume of intake, but when real feeding tend to suppress intake (of any medium) in response to colitis. The finding that malaise is not a mediator of anorexia is supported by clinical studies in humans. A questionnaire administered to patients with Crohn's disease indicated that weight loss and decreased food intake were not associated with increased nausea (Rigaud *et al*, 1994). The agreement between research findings with humans and the rat model help to substantiate appropriateness of using the TNB-model to explore the actual mechanisms mediating food intake.

Researchers have hypothesized that inflammation causes an exaggerated response to food which suppresses food intake (McHugh *et al*, 1993 a; Weingarten, 1996). This could mean that inflammation in the gut caused an exaggerated signal to be sent out in response to food in the intestine, or that inflammation modified the brain's reception of a

normal signal. In either case, the response to food is exaggerated, causing early termination of a meal. This hypothesis was supported by the findings that TNB-treated rats initiated meals with the same frequency as control rats. In addition, sham-feeding, which eliminates the post-ingestive consequences of feeding also eliminated the difference in intake between control and anorexic rats (McHugh *et al*, 1993 a).

In order to examine whether an exaggerated response to the post-ingestive consequences of a meal could account for the pattern of anorexia, in Chapter 2 intragastric preloads were administered. Preloads are volumes of food given prior to a test meal. The preloads were administered directly into the stomach in order to isolate the post-ingestive consequences (Ackroff & Sclafani, 1993; Rolls, 1995). However, neither a preload of 5 ml nor a preload of 10 ml caused an exaggerated suppression of food intake during the test meal of TNB-treated rats. This suggests that the anorexia cannot be explained by a simple exaggerated behavioural response to the food following ingestion.

The previous focus of most studies was on the termination of a meal. However, examination of the intake rates in Chapter 2 revealed that, although the anorexic rats initiated meals, the rate of intake was substantially slower than that of control rats. The slow rate of intake was apparent immediately after the initiation of the meal, which suggests that post-ingestive consequences are not likely to be involved in causing the slow rate. This was a particularly surprising finding, because it suggested that instead of focusing on meal termination, that something interesting was happening at the start of the meal. Rate of intake and response to food in the initial period of intake has been

suggested as an index of appetitive behaviour (Davis & Smith, 1992). It is possible that the rate might be slower because the food was perceived as less palatable during the period of anorexia (Barnfield *et al*, 1994; Berridge *et al*, 1984).

Taste-reactivity is a procedure in which the facial and body movements are recorded and scored as positive, negative and neutral in order to assess the palatability of food to rats (Berridge & Grill, 1983; Grill & Norgren, 1978 a). Food is administered through IO cannulae, directly into the mouth so that the view of the face is not obstructed. In an IO meal, the initiation of the meal and the rate of infusion are controlled by the researcher, but the termination of the meal is determined by the rat (Grill *et al*, 1987). In Chapter 3 the pattern of food intake during IO feeding was characterized. A significant anorexia was expressed during an IO meal in response to TNB-induced colitis, although the size of the suppression was less. Interestingly, the IO meal size for all rats was increased by at least two times compared to the intake of a real fed meal.

The larger meal size during IO intake indicates that anorexic rats are capable of eating large meals. It could be argued that the increased meal size was an artifact of the rate of liquid diet infusion. However, the rate of liquid diet infusion is not greater than the rate of liquid diet intake when a rat initiates a meal from a spout (Chapter 2). The rate of intake for rats that are real feeding decreases over the duration of the meal, which is not replicated in the IO preparation. However, in the IO meal, the rats are able to let food drip passively in order to decrease the rate of intake, or to terminate the meal. In fact, meal duration, approximately 15 min. in a real feeding rat, was at least doubled when food

was administered IO. The increase in the duration of the IO meal suggests that the large meal size is unlikely to be an artifact of the infusion rate.

An alternative explanation for the larger meal size could be that some aspect of appetitive behaviour in which the rat prepares to eat a meal is required for the normal expression of satiety. For example, the appetitive behaviour could act as a cue that a meal is about to be initiated. The cue would lower the threshold required for the central response to food, to induce a change in behaviour required for meal termination. During IO meals the appetitive behaviour is eliminated, although all ingestive behaviours remain the same. The post-ingestive signals released in response to an IO meal would have to be greater in magnitude or duration in order to result in the termination of a meal. In Appendix 4, the effect of preloads (real fed or IO) on a real fed test meal were compared to determine whether IO meals resulted in the same termination of a meal as real fed meals. The results were contradictory, so the cause of the large meal intake remains unclear.

Despite the larger meal size, a pattern of anorexia was expressed during the IO meals that was not significantly different from the pattern observed in real fed meals. Thus, the IO technique could be used in the taste-reactivity procedure in order to assess the palatability of food. When the TR test was used, TNB-treated rats demonstrated a significant suppression of the positive movement of paw-licking and a significant increase in the neutral mouth movements compared to control rats. Findings like this have previously been interpreted as a decrease in perceived palatability (Berridge & Fentress,

1985; Berridge & Pecina, 1995). This suggests that perceived palatability of liquid diet may be altered during inflammation of the colon. The change in reactivity to taste may account for the decreased rate of intake, and the consequent suppression of food intake. The findings are similar to clinical reports that people with Crohn's disease with weight loss and decreased hunger also reported a decrease in the sensation of pleasure related to eating (Rigaud *et al*, 1992). The ability of the internal state of an organism to alter the perception of food (alliesthesia), has been documented many times (Cabanac, 1979; Cabanac & Lafrance, 1992; Cabanac & Zhao, 1994; Whitten, 1995). People who are satiated, report that food tastes less palatable than people who are in a state of hunger. The perception of how good food tastes is called its hedonic value (Cabanac, 1979). Therefore, it seems plausible that one of the mediators of the colitis-induced anorexia is a change in the perception of the hedonic value of food.

If the anorexia is mediated by a change in the hedonic value of food, further research will be required to establish the physiological mechanism by which the change occurs, as discussed in Chapter 4. Potential sites to study include the duodenal lumen, the caudal brain stem and the gustatory cortex (Berridge, 1988; Berridge *et al*, 1984; Cabanac & Lafrance, 1992; Kieffer & Orr, 1992). Alternatively, IL-1 is a known mediator of the anorexia associated with TNB treatment (McHugh *et al*, 1993 b). Consequently, an experiment that examined the effect of IL-1 on TR could reveal the mechanism through which IL-1 mediates the feeding behaviour.

In addition, the anorexia should be studied more stringently in the human IBD population. The occurrence and duration of anorexia in humans still remains to be characterized. Expression of anorexia may precede the clinical symptoms of IBD, or even of a flare-up of the disease (Hildebrand *et al*, 1994; Motil *et al*, 1993). The predictive value of the anorexia should be further explored in humans, especially in children because of the potential for growth delay (Ferguson & Sedgewick, 1994; Hildebrand *et al*, 1994). Several questions relating to shifts in perceived hedonic palatability remain to be addressed as well. The shift in palatability remains to be replicated in rats and demonstrated in humans. Also, the parameters affecting the shift, such as types of food and physical states, should be addressed. Finally, the potential for the findings to influence treatment of IBD, perhaps through early identification of the anorexia and guidance of nutritional and steroidal therapy, should be explored.

In conclusion, I have added to the characterization of the TNB-model of colitis, by reporting that the expression of anorexia begins within 8 hours of treatment with TNB. Further, although previous studies have shown that the TNB treated rats initiate meals at the same frequency as control rats (McHugh *et al*, 1993 a), I have demonstrated that the anorexia during real fed (RF) intake is partially mediated by a decrease in the *rate* of food intake. Alternatively, when the rate of intake is controlled, as in IO meals, the anorexia is expressed by shortening the *duration* of meals compared to control animals, although the meal sizes for both are larger than RF meals. In addition, I have demonstrated that the pattern of anorexia caused by TNB is also expressed when rats are fed using the IO

technique. Finally, although the intake of ETOH-treated rats is influenced by the degree of deprivation, the total food intake of TNB-treated rats is not influenced by the amount of deprivation.

In assessing the cause of the anorexia, I have shown that the peptide, central OT, is not a biological mediator in the TNB-model of colitis. Nor can malaise, as indexed by the physiological marker of plasma OT levels, account for the suppression of food intake. Preload studies show that the suppression of food intake cannot be accounted for by an exaggerated behavioural response to post-ingestive satiety cues. However, it is possible that a change in the hedonic perception of food may decrease the amount consumed, and at least partially, account for the anorexia associated with colitis in the rat model of IBD.

APPENDIX I:

CENTRAL OXYTOCIN: OXYTOCIN ANTAGONIST DOSE

Oxytocin (OT) released in the brain plays a role in mediating food intake (Olson *et al*, 1991 a, b), and may be involved in mediating the anorexia observed in the TNB-model. If central OT mediates the suppression of food intake, then infusion of an OT antagonist (OTA) should block the expression of anorexia. However, the amount OTA required to block the anorexic properties of OT was unknown. Preliminary experiments were run in order to determine the *amount* of OTA required to block the anorexia resulting from release of OT, and to act as a *positive control* demonstrating that the OTA could block OT activity.

METHODS

Subjects: Six male rats (300- 450 g) were maintained on Purina powdered rat chow in hanging steel cages.

Infusions: Corticotrophin Releasing Factor (CRF) (human, rat from Bachem, California) dissolved in saline (SAL), was used to release central OT because actual icv infusions of OT resulted in muscle convulsions at levels required to induce anorexia (data not shown). CRF is a potent inhibitor of food intake (Schwartz, Dallman & Woods, 1995). The OTA was L-366,948, (Merck Sharpe & Dohme Research Labs, PA) dissolved in saline (SAL). The control infusions were 7 μ l of SAL alone (see General Methods).

Procedure: Cannulae were implanted in the left ventricle, and after three days, the rats were trained on a 2 hour feeding schedule of powdered chow (see General Methods). Infusions into the icv cannulae were made at approximately 2 μ l / min by hand. Two different infusions were made prior to the feeding period. The first infusion, either OTA or the control (SAL), was made 30 min prior to the feeding schedule. The second infusion, either CRF or SAL, was made 10 min prior to the feeding schedule. The amount of CRF required to cause a 50% suppression of food intake was determined by infusing SAL followed by different amounts of CRF (6 μ g CRF in 6 μ l volume, 4 μ g in 4 μ l or 3 μ g in 6 μ l).

Subsequently, the amount of OTA required to eliminate the 50% anorexia induced by the CRF was determined. Infusions of different amounts of OTA (10 μ g in 5 μ l, 14 μ g in 7 μ l) were followed by infusions of CRF (3 μ g in 6 μ l). To ensure that OTA did not alter food intake when administered alone, control infusions of OTA (14 μ g in 7 μ l) followed by SAL (6 μ l) were made. A final control for the effects of the volume was an infusion of SAL (7 μ l) followed by SAL (6 μ l). The food intake and cannula position were determined according to the procedure in General Methods and Chapter 1, Central Oxytocin: Oxytocin Antagonist.

RESULTS AND DISCUSSION

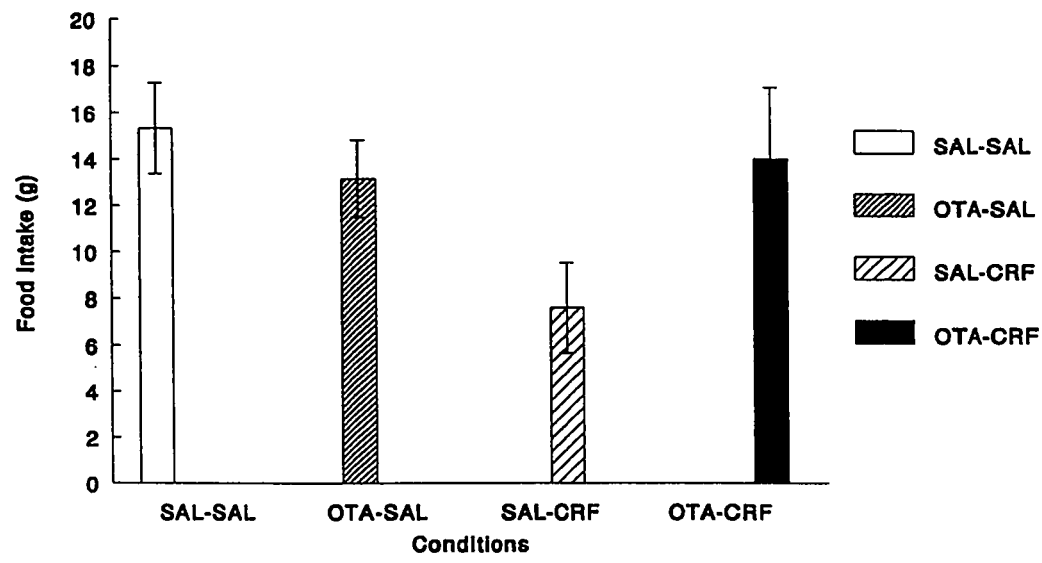
The baseline powdered chow intake with the control, SAL-SAL, injections was 15.33 g (± 1.95 SE). SAL-CRF infusions of 6 μ g in 6 μ l, 4 μ g in 4 μ l or 3 μ g in 6 μ l resulted in suppressions of food intake of 91.5%, 68.0% and 50.4% respectively. Consequently, the amount of CRF required to induce a 50% suppression of food intake was 3 μ g in 6 μ l (7.16 g ± 1.93 SE), and CRF acts to suppress food intake through the release of OT (Schwartz *et al*, 1995).

The amount of the OTA, L-366,948 required to block the 50% suppression of food intake was determined using 3 μ g CRF (in 6 μ l). Infusions of OTA of different amounts (10 μ g in 5 μ l or 14 μ g in 7 μ l) were followed by CRF infusions. 10 μ g of OTA

did not cause a significant change in the intake ($7.16 \text{ g} \pm 2.28 \text{ SE}$) compared to SAL followed by CRF infusions ($7.16 \text{ g} \pm 1.93 \text{ SE}$). However, $14 \mu\text{g}$ of OTA reversed the CRF-induced anorexia and had no significant effect on food intake when administered with control infusions of SAL (OTA-SAL) (see figure 41). Statistical analysis, using a One-Way ANOVA with repeated measures, indicates that the type of infusion had a significant effect on food intake ($F(1, 3) = 4.73, p = 0.016$). Post-hoc analysis by Newman-Keuls Test indicates that the only infusion that reduced the food intake was SAL-CRF ($p < 0.05$).

Consequently, the amount of the OTA, L-366,948, required to eliminate a suppression of food intake caused by the release of OT was determined to be $14 \mu\text{g}$ OTA in $7 \mu\text{l}$ of saline delivered icv. Additionally, the OTA was demonstrated to be capable of eliminating a suppression of food intake induced by the release of central OT - a positive control for the Oxytocin Antagonist Experiment.

Figure 41: 2 hr food intake following icv infusions of 7 μ l followed by 6 μ l (SAL-SAL, OTA-SAL, SAL-CRF, OTA-CRF) in order to establish the amount of L-366,948 OTA that could eliminate a 50% suppression of food intake (N=6). Data are group means \pm SE.



APPENDIX 2:

SUPPRESSION OF FOOD INTAKE IN RESPONSE TO INTRAGASTRIC PRELOADS

A preload is an amount of food delivered prior to the meal period, in order to examine the effect on the test meal. A preliminary experiment was run in order to determine the relationship between a preload of liquid diet delivered directly into the stomach of a rat, and the resulting suppression of food intake. This information was used to determine the preload volumes used in subsequent experiments, examining whether TNB-treated rats responded in an exaggerated fashion to the post-ingestive consequences of the preload (Chapter 2).

METHODS

Subjects: Nine Sprague-Dawley rats (300 - 400 g) were used. For all experiments in this chapter, the rats were housed individually in clear plastic cages in a colony room and maintained on Purina powdered rat chow until surgery. The rats were then trained on a 4 hr feeding schedule of nutritionally complete Liquid Diet (General Methods).

Procedure: IG and IO cannulae were implanted (see General Methods). The IO cannulae were not used in this series of experiments, but were included in the expectation that a later experiment would examine the effect of IG preloads on IO food intake. A minimum of two days after surgery, the rats were trained on a 4 hour feeding schedule until they maintained a constant weight (General Methods). The relationship between the suppression of food intake and the volume of preload was established using preload volumes of 0, 5, 10, 15, 20, 25 or 35 ml of liquid diet. 20 minutes prior to the feeding period, the preload of liquid diet was infused by hand (approximately 2 ml / min) directly into the stomach through the IG cannulae using a 30 cc syringe with tubing (0.03 ID, Elkay, running-foot tubing, Fischer Scientific). Intake of liquid diet from a graduated tube was measured in the testing cage after 1 hour, and then in the home cage after 3 hours, for a total of a 4 hour feeding schedule. The intake following each preload was replicated three times for each rat (randomized order, except for 5 ml and 25 ml which were administered at the end of the experiment in order to extend the range of doses).

RESULTS

Food Intake:

Test Meal: The 1 hour liquid diet intake (figure 42) was significantly suppressed by the preloads ($F(6, 48) = 84.29, p < 0.0001$) when examined by ANOVA with repeated measures. The 10 ml preload caused a 32.2% decrease in food intake compared to the 0 ml preload control, 15 ml caused a 47.7% decrease, while a 35 ml IG preload almost eliminated food intake during the first hour. A Newman-Keuls post-hoc test revealed that the difference between the 0 preload and all other preloads was significant ($p < 0.001$) except for 5 ml ($p = 0.90$), indicating that a 5 ml preload did not cause a significant suppression of the test meal intake.

Home Cage: Liquid diet intake during the 3 hours measured in the home cage (figure 43) was also significantly suppressed by the preloads ($F(6, 48) = 12.86, p < 0.0001$) according to an ANOVA for repeated measures. However, a Newman-Keuls test revealed that the only two preload that were suppressed compared to the 0 preload were 25 and 35 ml ($p = 0.001$).

Total Intake: The cumulative 4 hour intake (figure 44), like the test meal, was significantly suppressed by the IG preloads ($F(6, 48) = 155.20, p < 0.0001$) except for the 5 ml preload as revealed by a post-hoc Newman-Keuls test ($p = 0.16$).

Figure 42: The effect of intragastric (IG) preloads of liquid diet (0, 5, 10, 15, 20, 25 and 35 ml) on subsequent liquid diet (ml) intake during 1 hour of real feeding (N=9). Data are group means \pm SE.

Figure 43: The effect of IG preloads of liquid diet (0, 5, 10, 15, 20, 25 and 35 ml) on subsequent liquid diet (ml) intake during the last 3 hours of real feeding in 4 hour feeding period (N=9). Data are group means \pm SE.

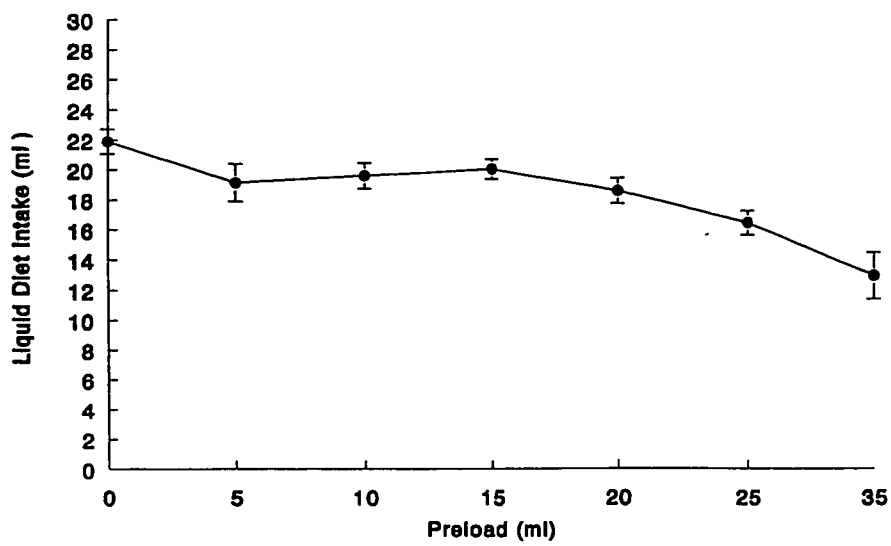
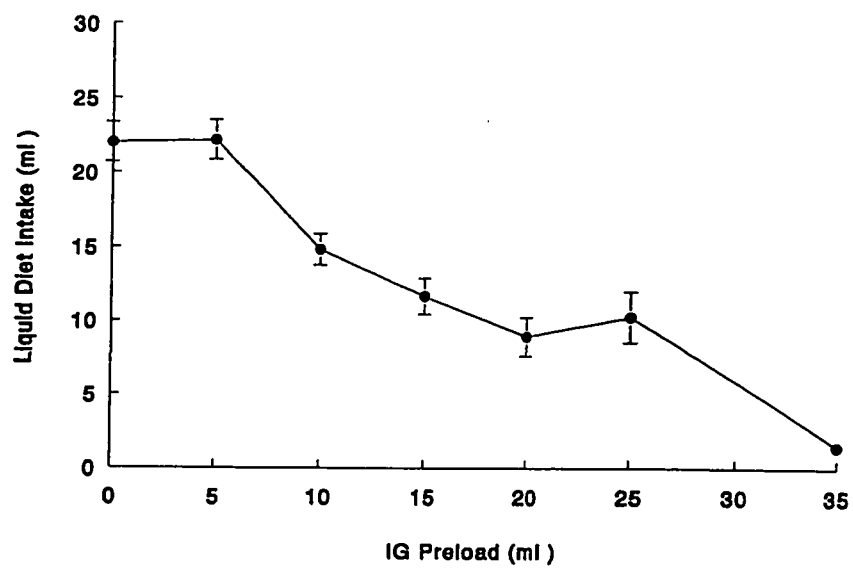
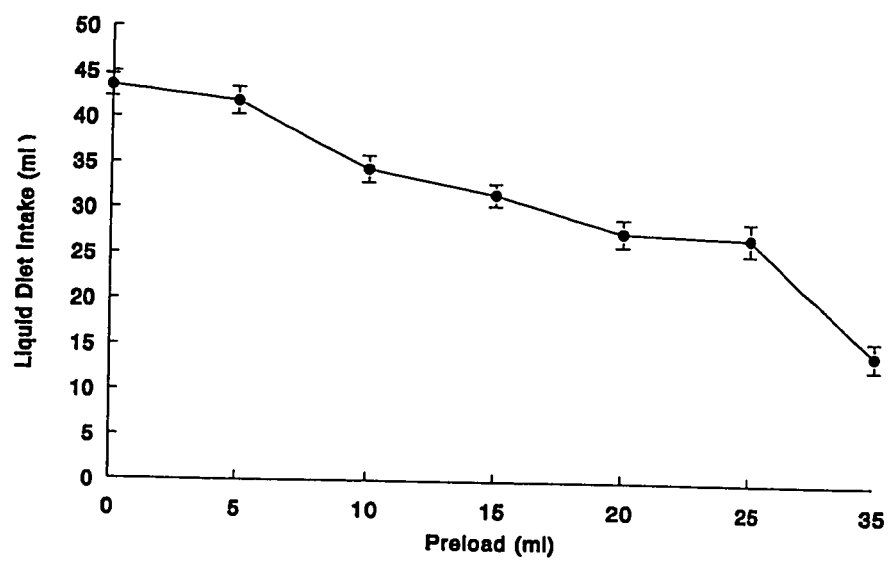


Figure 44: The effect of IG preloads of liquid diet (0, 5, 10, 15, 20, 25 and 35 ml) on the cumulative liquid diet (ml) intake during 4 hours of real feeding (N=9). Data are group means \pm SE.



DISCUSSION

A preload of liquid diet delivered directly into the stomach by an IG cannula resulted in a suppression of food intake in direct proportion to the volume of the preload. The preloads of 5 ml and 25 ml were tested at the end of the experiment when the rats were approximately 100 g heavier, rather than being randomly assigned as the other volumes were. This difference in presentation may have meant that less suppression of food intake was observed compared to other preloads administered at an earlier point in the experiment. Interestingly, the administered preload did not result in an equal volume compensation of food intake during the first hour. This replicates previous findings comparing preloads given IG and RF (real fed) (Berkun *et al*, 1952), and supports the concept that post-ingestive satiety cues alone cannot account for normal satiety (Mook *et al*, 1993 a, b; Nicolaidis & Even, 1992).

A comparison of the 1 hour and 3 hour intakes revealed that any compensation for the preload occurred within the first hour of feeding, except for the largest loads of 25 and 35 ml, which influenced the 3 hour intake. The rats mediated feeding behaviour in a way that defended the daily intake of liquid diet (average 43.4 ± 3.89 ml liquid diet), succeeding for all preloads except the two highest. While the largest preloads, 25 ml and 35 ml, did cause behavioral changes such as chin rubbing which indicate discomfort (Cabanac, 1992), all rats initiated a meal within the feeding period, suggesting that their behaviour and physical motion was not impaired.

The establishment of a preload-suppression curve reveals that the 5 ml preload does not cause a significant suppression of intake during the 4 hour feeding period. One hour liquid diet intake was suppressed 32.2% by a 10 ml preload, and 47.7 % by a 15 ml preload. Four hour intake was suppressed 20.7% by the 10 ml preload, however a 50% suppression of 4 hour intake did not occur until a preload greater than 25 ml. This information was used in the subsequent experiments to determine whether the colitis-induced anorexia in the TNB-treated rats is mediated by an exaggerated response to satiety cues.

APPENDIX 3:

Real versus Intraoral Intake

A series of preliminary studies were run comparing IO to RF intake of different solutions to determine whether a solution resulted in equivalent meal size. In this set of experiments, the surgery technique was altered to improve the method of implanting the IO cannulae and to reduce the loss of cannulae through the development of leaks.

Liquid Diet 10% Sucrose:

Subjects: Seven albino Sprague-Dawley rats (250-300 g at time of surgery) were housed individually in hanging steel cages in the colony room and provided Purina powdered chow ad libitum while in the home cages. Meals in the IO cage were liquid diet (see experiment 1) but 47 g of sucrose were added resulting in a 10% sucrose solution rather than the usual 20%.

Surgery: The initial IO cannula surgery differed from that described in the General Methods, and was more closely based on a technique described by Grill and colleagues (1987) although some modifications were made to reduce the number of cannulae that leaked (adapted from techniques used by Dr. L. Parker and Dr. H. Grill). The bevelled end of a 15 gauge 3.5 inch steel hypodermic needle (regular bevel, reusable; Becton

Dickinson, Yale) was placed between the cheek and the gum just anterior to the upper first molar and advanced between the eye and the ear until it emerged slightly midline between the two ears. A cannulae of PE-90 was threaded through the 15 gauge needle, the needle was removed, a rubber washer (Budlar Flexible Products, Milton) was slipped over the end of the tubing extending from the mouth, which was then heat flanged using a soldering iron. A 20 gauge needle with both ends blunted, the sides roughened and a ball of Silastic glue around the middle was then inserted into the end of PE-90 extending from the top of the head. No head cap was used. 0.3 ml of the antibiotic, Derapin, was injected intramuscularly.

Intraoral Infusions: The IO infusions were made using a Sage Instruments Syringe Pump with a 30 ml glass syringe connected by a blunted 19 gauge needle to the Elkay tubing (0.03 ID running foot standard). The pump infused at a constant rate of 1.1 ml / min.

Induction of Colitis: Colitis was induced as described in the General Methods section with one modification. Experimental rats were infused with 0.25 ml of powdered TNB (Kodak Co., Rochester NY) dissolved in 50% ethanol (120 mg / ml, 30 mg / rat) which induced an equivalent pattern of anorexia to the liquid TNB.

10% Sucrose

Subjects: Four rats (300 - 350 g at time of surgery) were maintained in steel hanging cages with powdered chow. The solution used to compare IO and RF was a 10% sucrose

in distilled water with 2 drops of blue food colouring to make the measurements easier to read.

Surgery: The same surgery used in the Liquid Diet 10% condition was used, except that the IO cannulae were wired together and anchored to the skull with dental cement.

Infusions were as described in Liquid Diet 10%.

10% Glucose

Subjects: Three rats (300 - 350 g at time of surgery) were maintained in steel hanging cages with powdered chow. A solution of 10% glucose in distilled water (2 drops blue food colouring) was used to compare IO and RF intake.

Surgery: The surgery was similar to the General Methods, except that the cannulae were all PE-90 instead of part Silastic tubing.

Intraoral Infusion: As described in the general methods, the multichannel Coleman-Parmer cartridge pump (4 channel) was used in order to run more than one rat at a time. The digital potentiometer was set at 15 (% of rpm) which was a flow rate of 1.1 ml / min.

5% Glucose

Subjects: Eight rats (300-400 g at time of surgery) were maintained in hanging steel cages with powdered chow available in the home cage. A solution of 5% glucose in distilled water, with 2 drops of blue dye, was used to compare IO and RF.

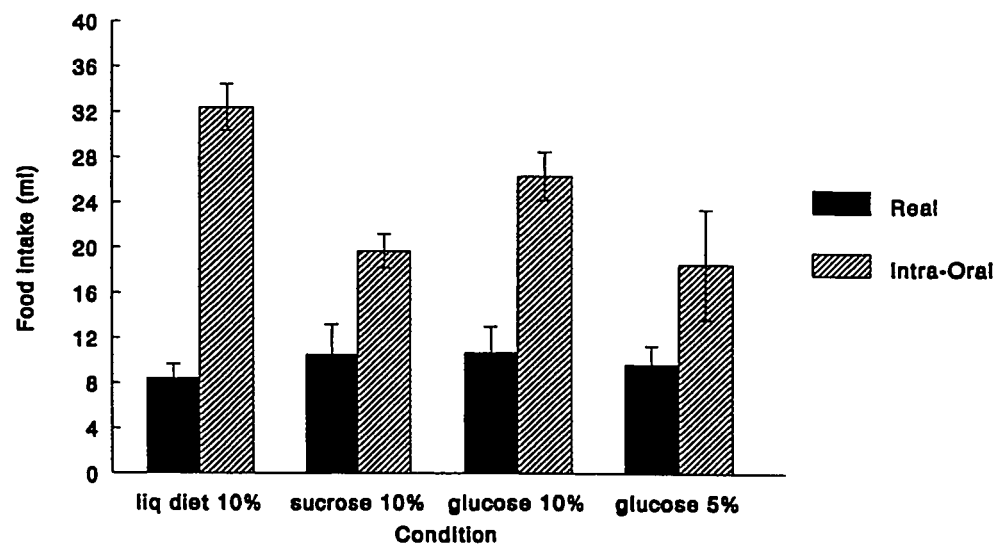
Surgery: See the General Methods for IO surgery and infusion of diet.

Procedure: In all of the conditions, the animals were given a minimum of two days recovery from surgery before training was initiated (General Methods). The rats were trained in the IO cages for three days with the solution delivered IO at 1.1 ml / min, and for three days under the same conditions, but with the solution available to be RF in a graduated bottle. The intakes of each solution infused IO were recorded at the termination of a meal, while RF intake was recorded at 20 min after the initiation of the feeding (based on duration of IO meals, and when the RF had also terminated). At the end of the experiment the rats were given an overdose of chloral hydrate (1 ml).

RESULTS

The IO intake was always greater than the RF intake, even when several solutions were compared: 10% sucrose with liquid diet, 10% sucrose alone, 10% glucose and 5% glucose (figure 45). The IO intake was an average of 2.5 times the RF intake, and even with 5% glucose, the amount eaten IO was 2 times the amount eaten from the bottle.

Figure 45: A comparison of food intake (ml) when real fed (RF) or intraoral fed (IO): liquid diet with 10% sucrose (N=7), 10% Sucrose alone (N=4), 10% Glucose (N=3), 5%Glucose (N=8). Data are group means \pm SE.



DISCUSSION

IO intake resulted in meal sizes at least two times greater during IO than RF. The difference in intake was not isolated to liquid diet, but included pure sucrose and glucose solutions of varied concentrations. The total intake within an IO meal was large, approximately 20-30 ml delivered in 20 min, a value similar to that observed by other researchers (Berridge, 1991; Kaplan *et al*, 1994 a). This large meal was observed even though the rats were not deprived, having Purina powdered chow available in the home cage. Consequently, it does not appear that there is a diet that will have equal amounts eaten when delivered by bottle and IO.

The difference in volume consumed indicates that IO intake is not identical to RF intake, despite the reported similarities (Flynn & Grill, 1988; Grill & Kaplan, 1992; Grill & Norgren, 1978 b; Kaplan, Seeley, Grill, 1993; Kaplan *et al*, 1994 a, b; Seeley *et al*, 1994; Seeley *et al*, 1993). Using the IO technique, the rats have control of the termination of a meal which is similar to RF; however, it appears that they terminate meals after a longer duration, since the rate of IO intake is maintained at a constant 1.1 ml / min. Additionally, since none of the solutions used resulted in equivalent volumes of intake, the nutritionally complete liquid diet will be used.

APPENDIX 4:

INTRAORAL PRELOAD

Examination of food intake during IO infusion of food indicated that the feeding behaviour of the rat appears to be altered when food is delivered directly into the mouth than when eaten normally, because the meal size is much larger. Several studies by other researchers have also found IO intake to be different from RF (Philopena & Smith, 1994; Seeley *et al*, 1995; Wolgin *et al*, 1988), and it has been suggested that the difference is related to changes in preparatory or food-anticipatory behaviours called appetitive behaviours. Anticipatory behaviours are a set of behaviours used to acquire food and can be dissociated from consummatory behaviours which include licking, chewing and swallowing (Seeley *et al*, 1995; Weingarten & Martin, 1989). In the IO technique, the food is delivered directly into the mouth so that anticipatory behaviour is eliminated and the consummatory behaviour is isolated (Swithers-Mulvey & Hall, 1992).

In Chapter 3, I suggested a hypothesis to explain the finding that the IO meal size is substantially larger than the RF meal, even though the rats have control over meal termination. It appears that the rats did not express the same satiety as when fed from a bottle. Perhaps some aspect of the anticipatory behaviour acts as an occasion setter for the postingestive consequences of a meal. Between meals, baseline release of signals may

occur, which are disregarded; however, during a meal it is beneficial to respond to all cues that indicate food has been ingested. Some aspect of the anticipatory behaviour could lower the threshold of response to postingestive satiety cues, which would result in a normal pattern of satiety and meal termination. During the IO meal, normal appetitive behaviours are not present in the rat to lower the response thresholds. Thus, greater cues for satiety (frequency, duration or intensity) are required to cause the termination of a meal.

EXPERIMENT 1

Intraoral Preload vs Real Fed Preload

The intake of rats fed by IO infusion is substantially greater than the intake of rats that real feed (Appendix 3). It seemed possible that some aspect of appetitive behaviour involved in the initiation of a meal, which was eliminated in IO feeding, was required for the normal interpretation of postingestive satiety cues. If this was the case, then a preload delivered IO should cause less suppression of a test meal, than a preload delivered RF. This experiment was designed to determine whether the difference in food intake could be accounted for by differences in satiety.

METHODS

Subjects: Seven Sprague-Dawley rats (250-300 g) were maintained on powdered chow prior to surgery and then maintained on nutritionally complete liquid diet.

Procedure: The IO cannulae were surgically implanted (General Methods) and flushed daily with 50% Listerine (Regular) diluted in water, in order to reduce chance of infection. After a minimum of 3 days, the rats were habituated to the IO cages and trained on a 4 hour feeding schedule of liquid diet (see General Methods for training and IO infusion). During training, the rats were placed in the IO cage for 1 hour, where they received liquid diet either IO or RF (in graduated bottles). The final 3 hours were in the home cage with liquid diet available in graduated bottles. The training was concluded when all rats had received 3 days of IO feeding and 3 days of RF feeding in the IO cages, and the rats maintained constant body weight. On test days, a preload of liquid diet (3, 6, 9 or 12 ml) was delivered in the IO cage, either IO or RF. IO preloads were delivered at 1.0 ml / min, and RF preloads were eaten in under 20 min. 20 min following the end of the preload, a RF test meal was available for 30 min. The rats were then returned to the home cage, where liquid diet was available for 3.5 hours in graduated bottles. The preloads were presented in random order, and each rat received the preload under both IO and RF conditions. After the experiment, the rats were given 1 ml of Chloral hydrate (350 mg / ml) and the colons were collected for MPO assay according to the procedure outlined in the General Methods.

RESULTS

Food Intake:

Test Meal: As expected, the larger the preload administered, the greater the suppression of the RF half hour test meal ($F(3, 18) = 20.58, p < 0.0001$). Most interestingly, the preloads delivered IO caused less suppression of the test meal than the preloads delivered RF ($F(1, 6) = 47.74, p < 0.001$) (figure 46).

Home Cage: There was no significant difference between the RF or IO preload delivery conditions ($F(1, 6) = 0.02, p = 0.90$), nor the preload volumes ($F(3, 18) = 2.68, p = 0.08$) during the 3.5 period of RF in the home cage (figure 47).

Total Intake: However, the 4 hour total intake (figure 48), like the test meal, demonstrated a significant suppression of food intake in response to larger preloads ($F(3, 18) = 9.87, p < 0.001$). Additionally, the RF preloads caused a greater suppression of food intake than the IO preloads ($F(1, 6) = 15.50, p < 0.01$).

DISCUSSION

Preloads of liquid diet decreased the intake of a test meal (0.5 hr) of liquid diet that was available in a graduated bottle. The method of preload delivery did have an effect on the amount that the test meal was decreased. In particular, IO preloads caused less suppression of the test meal than RF preloads. This suggests that the postingestive consequences of IO preloads were less satiating than those of RF preloads.

Figure 46: Liquid diet intake (ml) during a real fed (RF) 0.5 hr test meal
20 min following a preload of liquid diet (3, 6, 9 or 12 ml) delivered
IO or RF (N=7). Data are group means \pm SE.

Figure 47: Liquid diet intake (ml) for 3.5 hours in the home cage following
the test meal and preload of liquid diet (3, 6, 9 or 12 ml) delivered
IO or RF (N=7). Data are group means \pm SE.

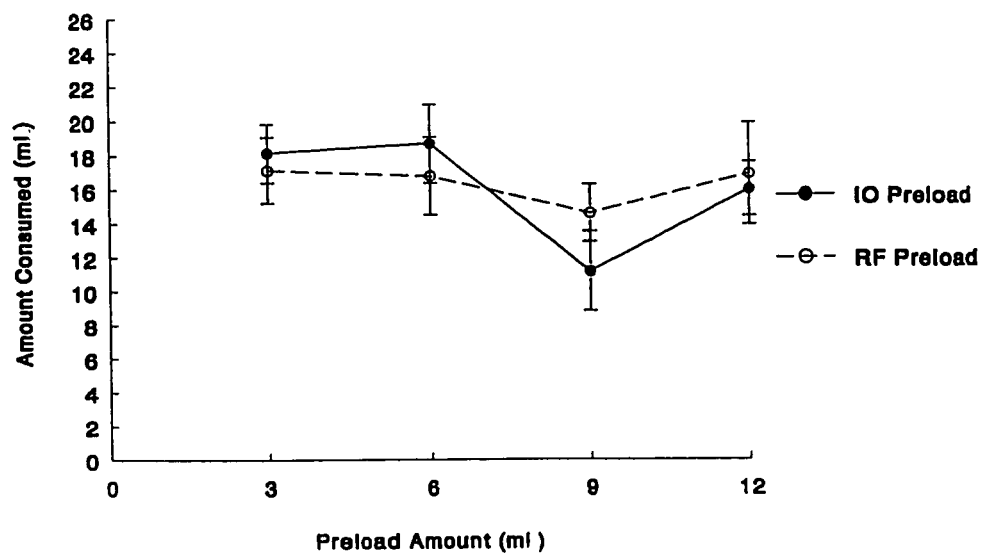
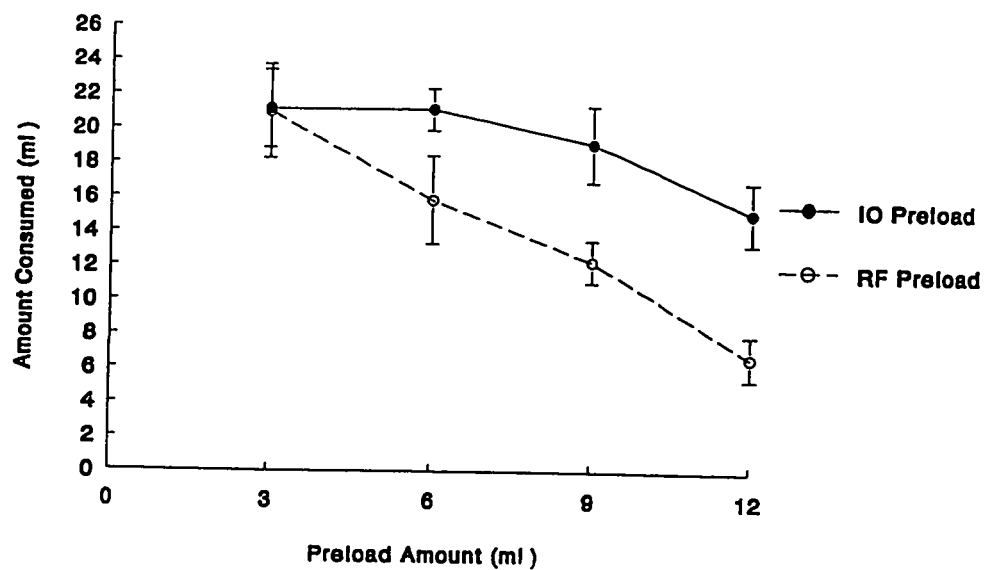
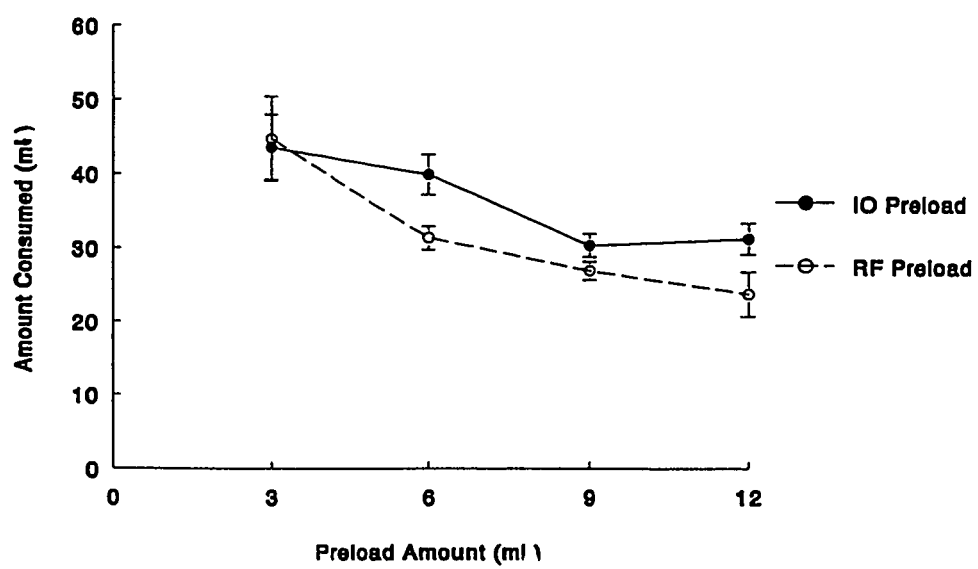


Figure 48: Total liquid diet intake (ml) over the 4 hour feeding period following a preload of liquid diet (3, 6, 9 or 12 ml) delivered IO or RF (N=7). Data are group means \pm SE.



EXPERIMENT 2

PRELOAD INTERVAL

The previous experiment found that an IO preload caused less suppression than a RF preload of a test meal administered RF. This experiment was conducted to see if the results could be replicated, and if the interval between the preload and the test meal effected the suppression of the test meal.

METHODS

Subjects: Nine male Sprague-Dawley (250 - 300 g) were maintained on nutritionally complete liquid diet (General Methods).

Procedure: The IO cannulae were surgically implanted and flushed daily with sterile saline (General Methods). After a minimum of 3 days, the rats were habituated to the IO cages and trained on a 4 hour feeding schedule (see General Methods). During training, the rats were placed in the IO cage for 1 hour, where they received liquid diet either IO or RF (in graduated bottles). The final 3 hours were in the home cage with liquid diet available in graduated bottles. The training was concluded when all rats had received 3 days of IO feeding and 3 days of RF feeding in the IO cages, and the rats maintained

constant body weight. On test days, a preload of liquid diet (12 ml) was delivered in the IO cage, either IO or RF, (which was eaten within 20 min). A RF test meal was available for 30 min following an interval of either 1, 10, 20 or 40 min. The rats were then returned to the home cage, where liquid diet was available for 3.5 hours in graduated bottles, for a total of 3.5 hours food intake. The intervals were presented twice in random order, and each rat received the 12 ml preload alternately under IO or RF conditions. After the experiment, the rats were given 1 ml of Chloral hydrate (350 mg / ml) and the colons were collected for MPO analysis (see General Methods).

RESULTS AND DISCUSSION

Food Intake:

Test Meal: The findings of the Experiment 1 were not replicated in this experiment.

While, the IO preload caused slightly less of a suppression of food intake, the difference was not significant for the test meal (figure 49) ($F(1, 8) = 0.36$, $p = 0.56$). Neither was there an effect of the interval ($F(3, 24) = 1.49$, $p = 0.24$).

Home Cage: The 3.5 hour food intake (figure 50) was not suppressed more by the RF preload than the IO preload ($F(1, 8) = 1.61$, $p = 0.24$), nor did the interval influence the food intake ($F(3, 24) = 2.65$, $p = 0.07$).

Total Intake: The type of preload did not affect total intake (figure 51) ($F(1, 8) = 4.83$, $p = 0.06$). The interval between the preload and the test meal did not have a significant effect on the food intake either ($F(3, 24) = 0.39$, $p = 0.76$).

Figure 49: Liquid diet intake (ml) during a real fed (RF) 0.5 hr test meal at intervals (1, 10, 20 or 40 min) following a 12 ml preload of liquid diet delivered IO or RF (N=9). Data are group means \pm SE.

Figure 50: Liquid diet intake (ml) for 3.5 hours in the home cage following the test meal. The 12 ml preload was administered at 1, 10, 20 or 40 min intervals prior to the test meal either IO or RF (N=9). Data are group means \pm SE.

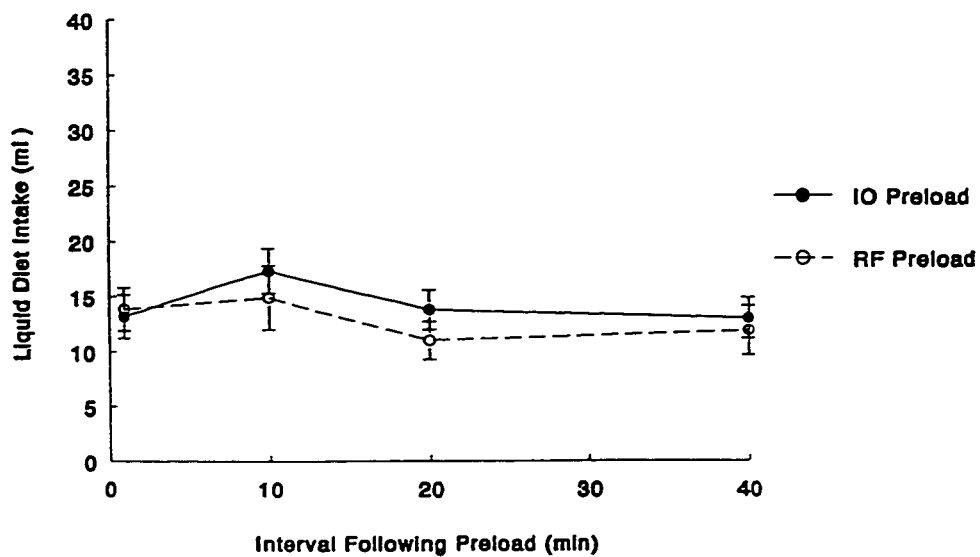
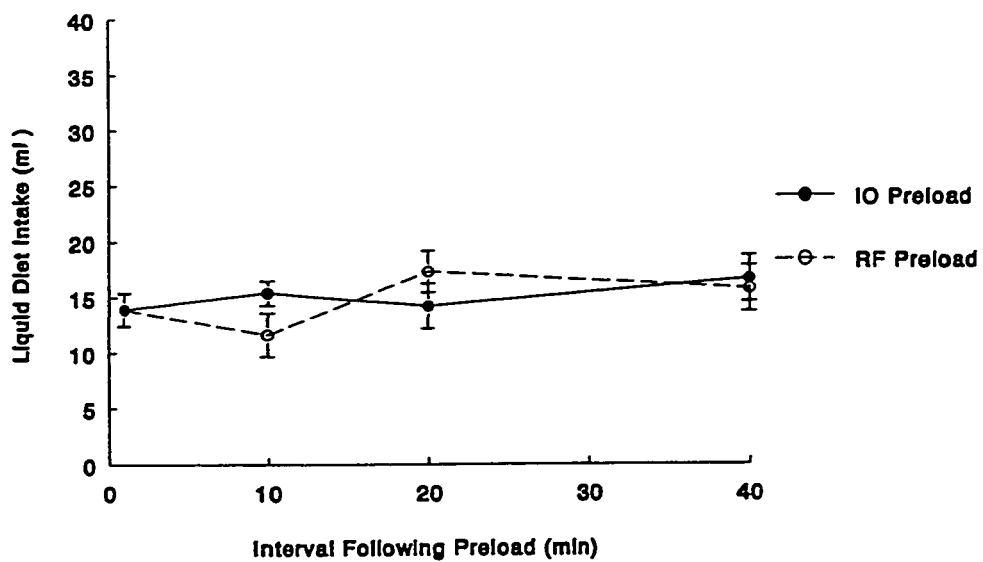
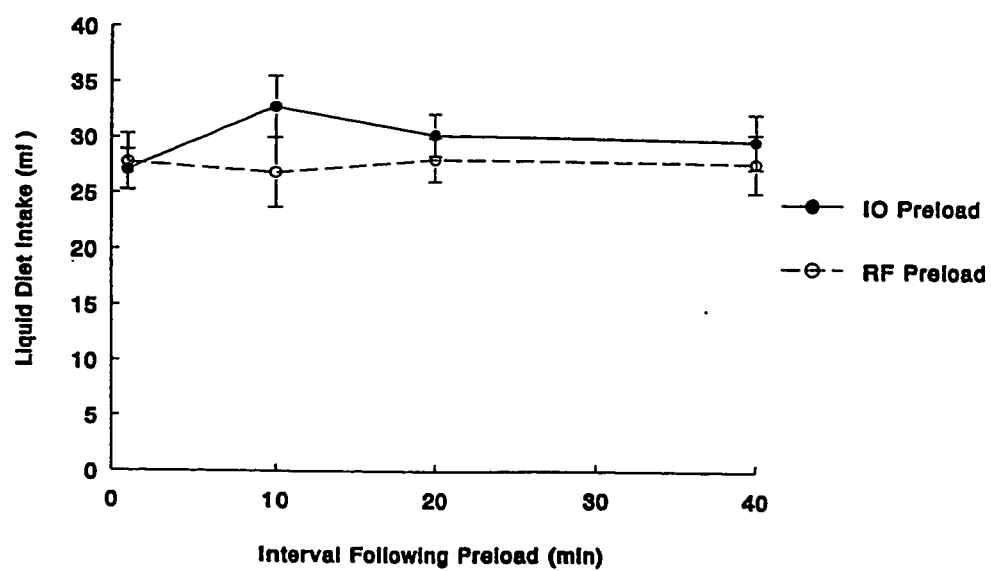


Figure 51: Total liquid diet intake (ml) over the 4 hour feeding period following a 12 ml preload of liquid diet administered at 1, 10, 20 or 40 min intervals prior to the test meal either IO or RF (N=9). Data are group means \pm SE.



APPENDIX 4: GENERAL DISCUSSION

IO meals are substantially larger than RF meals of liquid diet, sucrose and glucose. It could be argued that the larger meal size is a function of rate of infusion. However, the rate of infusion was only 1.0 ml / min, which is a rate used by many other researchers (Grill *et al*, 1987; Kaplan *et al*, 1994 a), and is within the initial rate of intake of real feeding rats (Chapter 2). It could still further be argued that the rate of intake decreases over the meal of a RF rat, but is maintained constant in IO feeding rats. But this maintained rate of intake alone cannot account for the large meals because the durations of IO meals are increased compared to RF rats. When the rate of intake was examined in RF rats, the meal duration was approximately 15 min (see Chapter 2), but when the food was delivered IO, the meals lasted between 20 and 50 min.

The increased meal size and meal duration led to the hypothesis that satiety (the response to the ingestion of food that results in the termination of a meal) was altered by the IO preparation. Since the main factor changed by IO infusion of food is the elimination of appetitive behaviour, it was hypothesized that appetitive behaviour was required for the normal expression of satiety to terminate meals. Studies were conducted comparing the influence of preloads delivered IO or RF on a subsequent test meal. The findings were contradictory, in one case IO resulted in significantly less suppression of food intake than RF, and in one case there was a trend in that direction, but it was not significant. These findings suggest that satiety may be altered by IO infusion of food, however, the conclusions are unclear.

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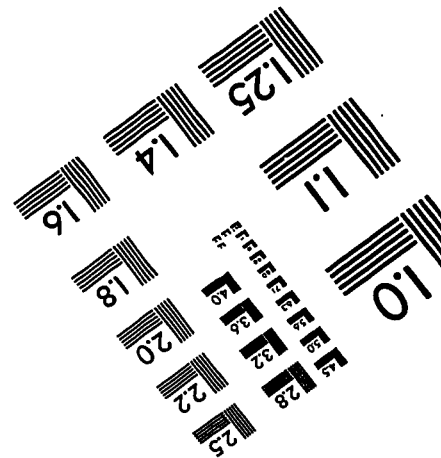
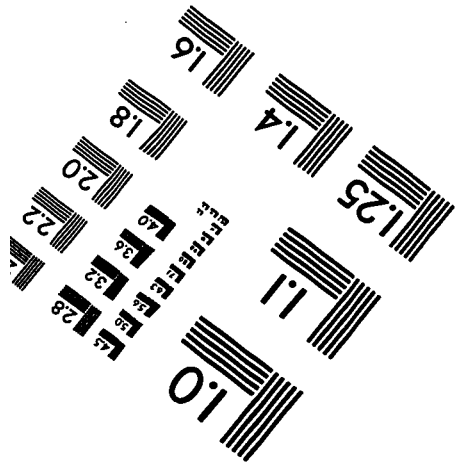
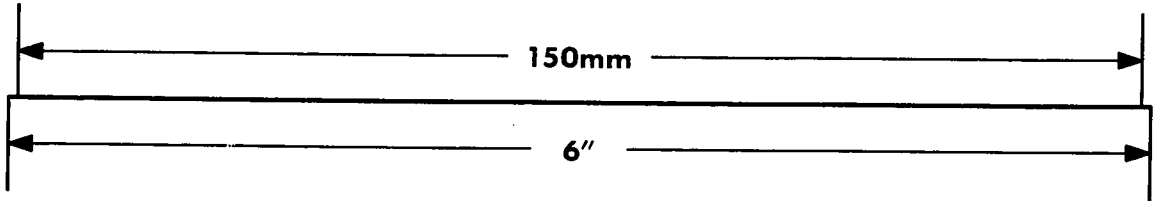
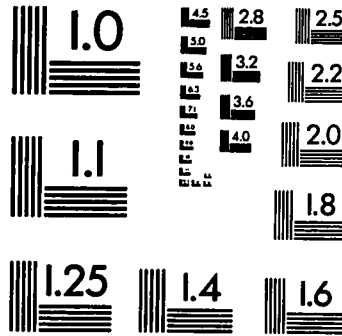
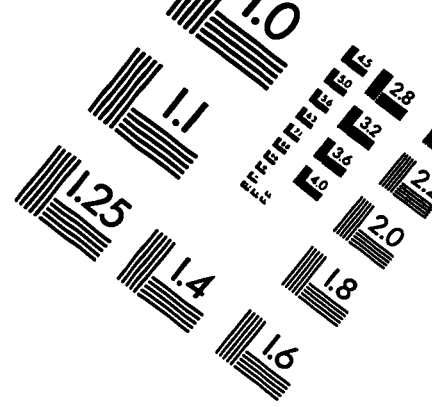
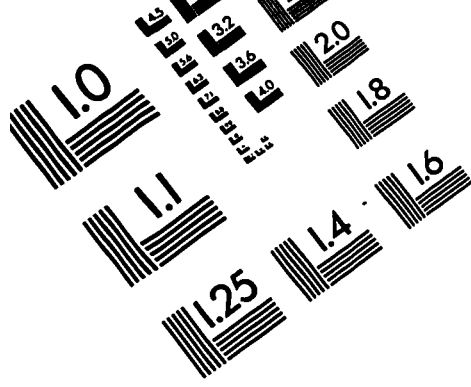
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