

INHIBITION OF GASTRIC EMPTYING IS NEITHER NECESSARY  
NOR SUFFICIENT FOR PEPTIDE-INDUCED SATIETY  
IN THE RAT.

BY

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## ABSTRACT

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This research examines the hypothesis that the satiety effect of cholecystokinin octapeptide (CCK-8) is mediated by changes in gastric emptying. A method for collection of gastric emptying data, the double sampling procedure, is developed and validated for use in the rat. The double sampling technique permits repeated measurements of liquid gastric volume and thus describes the time course of emptying within a single experimental session. Further, the method allows determination of the amount of gastric secretion, volume emptied into the intestines, and amount of gastric load remaining in the stomach. Experiments are presented which: i) demonstrate the utility of the technique; ii) validate its accuracy in determining gastric volume; iii) indicate the stability of measurements obtained with this procedure; and iv) provide a procedure for quantitative evaluation of data obtained with this technique.

Using the double sampling procedure, the ability of CCK-8 to delay gastric emptying and to influence feeding are then compared under similar experimental conditions. The effect of CCK-8 on gastric emptying is assessed in 6 hr deprived rats receiving 10 ml intragastric test loads of either .15M saline or 15% sucrose.

Intraperitoneal (ip) injections of CCK-8 in doses of 1.4-22.4 ug/kg produce a dose-dependent retardation of gastric emptying of both saline and nutrient. Lower doses of CCK-8, .0.01 and 0.1 ug/kg, have no effect on gastric emptying.

The effect of CCK-8 on feeding is assessed in rats tested under the same experimental conditions used in the gastric emptying studies. Doses of CCK-8 capable of retarding gastric emptying also suppress eating in a dose-dependent manner. These findings provide necessary correlational support for the hypothesis that satiety produced by CCK-8 is mediated by inhibition of gastric emptying. However, a further quantitative analysis of the correspondence of the gastric emptying and feeding effects of CCK-8 suggest that retardation of emptying may not account for the entire satiety effect of the peptide.

The next set of studies provide direct tests of whether changes in gastric emptying mediate CCK-induced satiety. If gastric emptying plays a significant role in the satiety produced by CCK-8 then: i) the effects of CCK-8 on emptying and feeding should share similar kinetics, and ii) peptides that inhibit emptying should also inhibit feeding. I show that CCK-8 (5.6 ug/kg) injected coincident with introduction of an intragastric load or presentation of a test meal produces a rapid inhibition of both emptying and feeding.

In contrast, the identical dose of CCK-8 administered 15 min

before testing causes no inhibition of emptying, even though the peptide retains its ability to produce satiety. I also test the abilities of the peptides pentagastrin (100 ug/kg), bombesin (8 & 16 ug/kg) and secretin (2.86, 14.3 & 28.6 ug/kg) to reduce food intake and inhibit gastric emptying. Pentagastrin does not affect food intake or gastric emptying. Bombesin causes a small transient delay in emptying but a large and sustained suppression of eating. High dose secretin (14.3 ug/kg) causes no significant reduction of food intake, even though this dose of secretin inhibits emptying to the same degree as 1.4 ug/kg CCK-8, which does reduce intake. Thus, although CCK-8 does influence the rate of gastric emptying, the present results indicate that the inhibition of emptying by CCK is neither necessary nor sufficient to explain its satiety effect.

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## CHAPTER 1.

### INTRODUCTION

#### 1.1 CHOLECYSTOKININ AND SATIETY

Research regarding the hormonal control of meal size began with the observation that a crude extract of canine intestine decreased food intake when injected into rabbits (Maclagan 1937). However, further study of control of food intake by gut peptides was then delayed until gut peptides were chemically and functionally well characterized. By 1972, there were three such peptides: cholecystokinin (CCK), gastrin and secretin; of these only CCK suppressed food consumption in the rat when administered peripherally (Gibbs, Young & Smith 1973). Since the initial discovery of CCK's effect on feeding, researchers have focused on three questions: i) does CCK suppress feeding via a true process of satiety?, ii) does CCK released from the gut affect feeding?, and iii) how does CCK reduce meal size? The answers to these questions are necessarily related. For example, if CCK is a true satiety signal, then physiological levels must be adequate to reduce meal size. However, these questions are sufficiently

independent to allow their separate pursuit, in that the demonstration that CCK indeed produces satiety need not reveal the physiological basis for the peptide's action. The aim of this thesis is to explore the third question, ie: the mechanism by which CCK affects the amount of food consumed by rats in a single meal. The literature relevant to this question, and the others, is reviewed in this Introduction.

#### What is CCK?

CCK is a peptide of the gastrointestinal tract that occurs in a variety of forms in vivo, which vary in size (Eysselein, Deveney, Sankaran, Reeve, Walsh 1983; Eysselein, Reeve, Shively, Hawk & Walsh 1982). In vitro structure-function analysis has determined that different forms of CCK differ in the efficacy and potency of the biological response they induce (Christophe, Svoboda, Calderone-Attas 1980; Thompson 1973). In comparison to other fragments, the sulfated C-terminal octapeptide (CCK-8) is the smallest form of CCK that yields maximal in vitro responses, and the sulphate moiety is important to the peptide's biological action (Christophe, et al. 1980; Thompson 1973). CCK-8 has become the form employed in feeding experiments, because CCK-8: i) also occurs in vivo (Brand & Morgan 1981), ii) is effective in many preparations and species, and iii) can be synthesized in pure form (Ondetti, Rubin, Engel, Pluscec & Sheehan 1970).

### What is Satiety?

The meal is a functional unit of feeding behavior for humans, rats and other mammals. A meal is characterized by a bout of food consumption with a discrete beginning and an end (Smith 1982). For the purpose of this thesis, satiety is the process whereby responses arising from food in the gut are interpreted by the brain to cause meal termination.

### I.2 DOES CCK SUPPRESS FEEDING VIA A NORMAL PROCESS OF SATIETY?

Following the initial demonstration that CCK reduces meal size (Gibbs, Young & Smith 1973), further investigation sought to establish: i) that CCK yields a robust feeding suppression, and ii) the suppression results from a true process of satiety.

### CCK Inhibits Feeding in Many Species

The inhibitory effect of exogenous CCK-8 on feeding has been repeatedly confirmed for the rat (Collins, Walker, Forsyth & Belbeck 1983; Antin, Gibbs, Holt, Young & Smith 1975), and demonstrated in mice (McLaughlin & Baile 1981; Strohmayer & Smith 1981; Parrot & Batt 1980), hamsters (Micelli & Malsbury 1983), rabbits (Haupt, Anika & Wolf 1978), fowl (Savory & Gentle 1980), pigs (Anika, Haupt & Haupt 1981), sheep (Grofum 1981), monkeys

(Gibbs, Falasco & McHugh 1976) and humans (Pi-Sunyer, Kissileff, Thorton & Smith 1982; Stacher, Steinringer, Schmierer & Winklehener 1982). This mass of findings has firmly established that CCK-8 reduces meal size. These results have been taken to suggest that endogenous CCK may be a postprandial satiety signal.

CCK Inhibits Sham Feeding

When the flow of nutrient from the stomach to the intestines is diverted by allowing the chyme to drain through a gastric cannula (sham feeding), animals continue to feed continuously for much longer than is seen in a normal meal in which chyme enters the intestine (Young, Gibbs, Antin, Holt & Smith 1974). The sham feeding preparation prolongs consumption by preventing the occurrence of satiety signals that arise from the flow of nutrient into the stomach or intestines (Young et al. 1974). These observations support the view that a signal generated by nutrient in the intestines is critical to normal postprandial satiety.

Administration of exogenous CCK-8 is sufficient to suppress sham feeding in rats (Gibbs, Young & Smith 1973), and monkeys (Falasco, Smith & Gibbs 1979). The implication from these studies is that CCK released in vivo (endogenous CCK) may be a physiological satiety signal (Gibbs et al. 1973).

### Intestinal Satiety Hypothesis

The physiological and anatomical data regarding CCK make it a reasonable candidate for the intestinal satiety signal. During the course of a meal, endogenous CCK is released into the bloodstream from secretory cells in the intestinal wall when these cells are stimulated by nutrient (Makhlouf 1974). The sites of endogenous peripheral CCK release are endocrine mucosal cells in the duodenum and proximal jejunum of the gastrointestinal tract in both the rat (Soveny, Mercuri & Hansky 1984; Larsson & Rehfeld 1979; Schneeman & Lyman 1977), and man (Buffa, Solcia, & Go 1976; Polak, Bloom, Rayford, Pearse, Buchan & Thompson 1975). The antrum is a secondary site of CCK release in rats (Soveny, et al. 1984).

The nutrient constituents that cause CCK secretion (secretagogues) are numerous, and vary between species. CCK secretagogues in dogs and humans include: calcium ions (Reeder, Becker & Rayford 1974; Holtermuller, Go & Sizemore 1973), acid (Chen, Chey, Chang & Lee 1985; Himeno et al. 1983; Grossman & Konturek 1974; Meyer, Way & Grossman 1970), L-isomers of the amino acids tryptophan, phenylalanine, valine and methionine (Meyer, Spignola & Jones 1973; Meyer & Grossman 1972; Go, Hofmann & Summerskill 1970), mixed amino acids (Stubbs & Stabile 1985; Himeno, et al. 1983), fatty acids with more than 10 carbons, (Stubbs & Stabile 1985; Hopman, Jansen & Lamers 1984; Himeno, et

al. 1983; Fried et al. 1983; Walsh et al. 1982; Burhol, Rayford, Jorde, Waldum, Schulz & Thompson 1980; Malagelada, DiMagno, Summerskill & Go 1976) and meals comprised of carbohydrates, proteins and fats (Liddle, Morita, Conrad & Williams 1986; Wolf & McGuigan 1984; Himeno, et al. 1983; Meyer 1975).

In rats known secretagogues for CCK include: i) protein (Liddle, Green, Conrad & Williams 1986); ii) trypsin inhibitor; and iii) ethanol (Liddle et al. 1984). CCK secretion in the rat is not stimulated by amino acids, carbohydrates or fats when administered individually, but CCK is released by mixed macronutrient meals, (meals containing carbohydrates, proteins and fats), (Liddle et al. 1986). The facts that exogenous CCK suppresses feeding, and endogenous CCK is released in the course of a meal, raise the possibility that intestinal hormonal signals may contribute to normal satiety.

#### Is the Satiety Effect of Exogenous CCK Due to Malaise?

As Smith (1984) has noted, we cannot conclude from evidence that feeding is suppressed that CCK is a satiety signal, because other internal states may also inhibit intake. If, for example, following CCK administration, animals were sedated or felt ill, then we would also expect them to eat less. Thus, if CCK is a natural satiety signal, then doses of CCK that suppress feeding must not generally suppress behavior or induce malaise. In fact,



exogenous CCK in doses that suppress feeding does not produce gross indications of sickness such as heightened body temperature, or diarrhea (Gibbs, Young & Smith 1973). However, it is possible that CCK suppresses feeding by causing a milder state of malaise.

#### Initial Evidence for Satiety

Sensitive assays for malaise involve comparisons of the effects of CCK on feeding with its effect on other behaviors. CCK-8 does not inhibit drinking of water in normal (Gibbs, Young & Smith 1973; Mueller & Hsiao 1977), or sham feeding (Kraly, Carty, Resnick & Smith 1978), showing that general motor behavior is not suppressed by CCK-8. CCK-8 also induces behaviors inconsistent with malaise. When administered to normal feeding (Antin et al. 1975) or sham feeding (Lorenz, Kreielsheimer & Smith 1979) rats. CCK-8 produces a natural sequence of postprandial grooming and resting behavior that is indicative of satiety. Moreover, following CCK-8 administration animals initiate feeding normally, but finish a meal sooner (Smith, Gibbs & Kulkosky 1982), a finding that is congruent with Booth's (1972) criteria for a satiety signal.

Another test for toxicity is the conditioned taste aversion (CTA) paradigm. Rats learn to avoid a novel-tasting test meal, if after eating that food they become ill or experience malaise (Garcia, Ervin & Koelling 1967). Thus, the malaise-inducing

properties of new compounds is often assessed by testing their ability to support establishment of a taste aversion (see Goudie 1979 for review). In a one-bottle taste-preference test, CCK does not produce a conditioned taste aversion (Holt, Antin, Gibbs, Young & Smith 1974; Gibbs et al. 1973), implying that CCK did not make the rats ill.

#### Evidence for Malaise

From the beginning, Deutsch has been the foremost critic of the CCK-satiety hypothesis (Deutsch & Hardy 1977). Deutsch suggested that that CCK can be shown to produce CTAs if a more sensitive test is used (Deutsch & Hardy 1977). To provide physiological support for the malaise argument, Deutsch showed that exogenous CCK produces changes in intestinal motility, and asserted that such "abnormal" motility patterns indicate sickness (Deutsch, Thiel & Greenberg 1978). Ironically, Deutsch and Gonzalez (1978) then cast doubt on the ability of taste aversion studies to distinguish satiety from malaise by finding that doses of LiCl sufficient to reduce meal size were insufficient to condition taste aversions. Thus if CCK does cause sickness, the degree of malaise is less than that needed for CTA.

In a related approach, the effect of CCK on place conditioning was examined (Swerdlow, van der Kooy, Koob & Wiegner 1983). Swerdlow et al. (1983) argued that if CCK is a satiety

signal then administration of the exogenous peptide should be reinforcing and animals would learn to prefer places associated with CCK. Alternately, if CCK is aversive then rats should avoid locations paired with CCK administration. They demonstrated that CCK administration yielded place-aversions, and so concluded that CCK inhibits feeding by causing illness.

#### Refutation of the Malaise Hypothesis

In answer to Deutsch's contention that CCK supports CTA learning, Gibbs and Smith (1977) identified doses of CCK and LiCl (the substance used typically to induce malaise in CTA) doses that were equally effective in reducing food intake. At these doses only LiCl produced CTA.

CCK and LiCl affect feeding differently. CCK-8 does not affect appetitive behavior, as measured by either runway performance (Cox, Brown, Toney & Wiebe 1984; Cox 1986), or latency to feed in response to food cues (Weingarten 1984).

Another approach to the question of toxicity is to compare the facial oral reactions elicited by flavors previously paired with food, CCK and LiCl (Grill 1985). When the taste reactions were compared, CCK produced taste reactions indicative of natural satiety, and reactions dissimilar to those following LiCl (Grill 1985). These studies suggest that CCK affects satiety in a manner different than that resulting from a toxin.

In humans, peripheral CCK reduces meal size without engendering verbal reports of illness (Kissileff, Pi-Sunyer, Thornton & Smith 1981; Pi-Sunyer et al. 1982; Stacher et al. 1980). Taken in conjunction, the evidence has convinced many researchers that exogenous CCK-8 does not cause reduction of feeding by inducing a state of malaise.

### I.3 DOES ENDOGENOUS CCK CAUSE SATIETY?

Demonstration that the feeding-effect of exogenous CCK-8 is not due to malaise is a necessary step toward establishing that CCK induces satiety. However, the issue of whether feeding is under hormonal control by CCK remains unsettled, because demonstration that exogenous peptide causes a biological effect is not sufficient to claim that endogenous peptide controls that effect (Grossman 1977). To assess whether a peptide performs physiologically one can attempt: i) pharmacological blockade of the effect by a specific antagonist, and ii) correlation of the peptide plasma levels produced by endogenous release with the amounts of exogenous peptide sufficient to yield the effect.

#### Can the Satiety Effect of CCK be Blocked Pharmacologically?

If CCK is a physiological satiety signal, then agents that block the action of CCK should increase meal size. In fact, an

increase in meal size following a CCK antagonist would provide very encouraging evidence that CCK controls feeding because greater food consumption is not likely the result of malaise. The CCK antagonist proglumide (Kaplita & Roebuck 1984; Hahne, Jensen, Lemp & Gardner 1981), has been shown to completely block suppression of feeding by exogenous CCK (Collins et al. 1983), indicating that proglumide would be useful for investigating the role of endogenous CCK in satiety.

Unfortunately, the first attempt to enlarge meal size with proglumide was unsuccessful. Proglumide does not increase intake when given 15 minutes before presentation of a liquid sucrose test meal (Collins et al. 1983). However, this failure may have resulted from use of sucrose as the test meal; because sucrose is not a CCK secretagogue it was unlikely that endogenous CCK was released and, thus, there may have been no peptide for the antagonist to block. To furnish an optimal test situation Shillabeer and Davidson (1984, 1985) gave rats a mixed nutrient preload, chosen to assure endogenous release of CCK, 20 minutes before proglumide administration and test meal presentation. Using this protocol they demonstrated that proglumide increased meal size. However, this finding is not robust. Schneider, Gibbs, and Smith (1986) were unable to replicate Shillabeer and Davidson (1984, 1985) despite using a very similar protocol.

The reason for these inconsistent results with proglumide is

unclear. In the absence of a definitive outcome we are left with two hypotheses: either endogenous CCK is not involved in satiety, or proglumide is a poor antagonist in the feeding situation.

Stronger support for the contention that endogenous CCK signals satiety is provided by investigations of the effect of intraduodenal nutrient on sham feeding.

Infusion of nutrient into the intestines causes rats to stop sham feeding (Leibling, Eisner, Gibbs & Smith 1975), suggesting that intestinal responses to food can signal satiety. In a very similar experiment, proglumide was found to partially block sham feeding suppression by intraduodenal nutrient (Collins, Conover, Forsyth & Weingarten 1985), thus implying that endogenous CCK contributes to intestinal satiety. However, because the blockage achieved was partial, and the intestinal load was larger than what is physiologically normal, Collins et al. (1985) concluded that endogenous CCK alone was not a powerful satiety agent. In a subsequent study, Yox, Stokesberry and Ritter (1987) demonstrated that a more potent antagonist, CR1409, could completely block the suppression of sham feeding produced by intraduodenal the fatty acid: sodium oleate. The ability of CR1409 to antagonize CCK under some conditions implies that endogenous CCK suppresses feeding. However, this implication is seriously weakened by both failure to demonstrate antagonism of exogenous CCK by CR1409 in their test situation, and by the uncertainty that sodium oleate

causes the release of CCK in the rat (Liddle et al. 1986). Without this knowledge it is impossible to conclude that endogenous CCK was involved.

#### Does CCK Cause Satiety in an Endocrine Manner?

The issue of whether endogenous amounts of CCK released during a meal are sufficient to cause satiety through an endocrine mechanism can be addressed by investigating CCK plasma levels. The optimal procedure to establish a physiological effect, is to determine whether exogenous administration of peptide in doses which mimic determined physiological levels also produce the desired response (Grossman 1977). This criterion depends upon the ability to measure plasma levels of the hormone. Initially however, the satiety effect of CCK was not tested in this fashion because specific bioassays and radioimmunoassays have developed only recently.

The question of whether a new biological action of a peptide is physiological can also be approached by comparing the dose of peptide required to yield the new response with the amount needed to elicit an established physiological response (Grossman 1977). Thus, if the peptide yields an effect on the new response at a dose sufficient for an established physiological action, then the new response may be under hormonal control by the peptide. If, on the other hand, the amount of peptide required for the new

response is substantially greater than that for a physiological response, then it is unlikely that the new response is a physiological action of the peptide.

Reidelberger and Solomon (1986), compared doses of CCK-8 sufficient to trigger exocrine pancreatic secretion, with doses of CCK-8 that inhibit feeding. They found that the dose of CCK-8 needed to reduce meal size was 5 times higher than that necessary to cause maximal pancreatic secretion. Because much more CCK was needed to suppress feeding than was necessary to induce pancreatic secretion, they concluded that circulating levels of CCK normally present after feeding are not sufficient to produce satiety.

In a similar study with dogs, the satiety dose of CCK-8 was shown to be larger than the dose of CCK-8 that causes maximal responses in the pancreas and gallbladder (Pappas, Melendez, Strah & Debas 1985). Moreover, loads of intraduodenal nutrient sufficient to stimulate normal pancreatic and biliary responses were insufficient to induce satiety (Pappas et al. 1985).

Indeed, lately, when plasma CCK became measurable, it was found that the concentration of exogenous CCK that effectively inhibited feeding in dogs was 10 to 14 times greater than the physiological levels that normally follow eating (Reidelberger, Kalogeris, Turkelson & Solomon 1987). Thus physiological plasma concentrations of CCK are not likely to signal satiety in rats or dogs, and it is improbable that the feeding-effect is an endocrine



function. However, it may be that large increases in circulating CCK are not necessary for endogenous peptide to reduce feeding. Instead, endogenous CCK may achieve high local concentrations which are not reflected by plasma levels of the peptide, and receptors very proximal to the site of CCK release mediate the effect on feeding by a paracrine or neurocrine mechanism (Collins & Weingarten, 1989). Thus, failure to meet the criterion, that endogenous levels of CCK-8 must inhibit feeding (ie. the peptide must act in a classic endocrine manner), does not dissuade some enthusiasts from pursuing the endogenous CCK satiety hypothesis.

#### I.4 PHYSIOLOGICAL FUNCTIONS OF CCK

The controversy over whether endogenous CCK causes satiety need not discourage investigation of the mechanism whereby CCK administration exerts its satiety-effect. Many would be satisfied if research of CCK's satiety-effect simply lead to pharmacological methods for controlling food intake and obesity. Further understanding of how exogenous CCK reduces meal size could reveal the physiological substrate that controls feeding, even though endogenous CCK is not significantly involved. Consideration of the functions that CCK performs in the gut, in addition to providing heuristic support for the CCK satiety hypothesis, may also provide insights regarding how CCK causes

satiety.

#### Gallbladder: Bile Release

The first gut effect of CCK was described by Ivy and Oldberg (1928), when they found that extracts from duodenal mucosa caused contraction of the gallbladder and bile release in dogs. Others have since demonstrated that CCK is the agent responsible, and verified that CCK-8 causes the gallbladder to empty (Johnson, Marshall & Wilson 1982; Byrnes, Borody, Daskalopoulos, Boyle & Benn 1981; Behar & Biancani 1980; Everson, Braverman, Johnson & Kern 1980; Lin 1975).

It is becoming apparent that gallbladder function is under the hormonal control of CCK. CCK-8 receptors have been found on gallbladder muscle cells (Speigerwalt, Goldfine & Williams 1984; Yau, Makhlof, Edwards & Farrar 1973), implying that CCK is involved in gallbladder motility. Moreover, gallbladder contraction has been found to be positively correlated with endogenous levels of CCK (Fried et al. 1983; Wiener, Inoue, Fagan, Liija, Watson & Thompson 1981). Finally, physiological levels of exogenous CCK-8 have been demonstrated to cause gallbladder contraction, providing strong evidence that endogenous CCK controls bile release (Liddle et al. 1985).

### Pancreas: Digestive Enzyme Secretion

Harper and Raper (1943) discovered an extract of the feline small intestine that caused secretion of enzymes from the pancreas, and called it pancreozymin. Elucidation of the amino acid sequences for pancreozymin and CCK revealed that they were identical peptides (Mutt & Jorpes 1968). Because Ivey and Oldberg (1928) discovered the effect on gallbladder first, the peptide was called cholecystokinin. The targets for the secretory action of CCK are the acinar cells on the pancreas (Steigerwalt & Williams 1981; Sankaran, Goldfine, Deveny, Wong & Williams 1980).

The hypothesis that CCK controls pancreatic secretion is supported by demonstrations that exogenous & endogenous CCK stimulate the release of digestive enzymes (Fried, Ogden, Swierczek, Greely, Rayford & Thompson 1983; Konturek, Tasler & Obtulowicz 1972; Konturek, Radecki, Biernat & Thor 1972; Stening & Grossman 1969). Evidence that a CCK antagonist blocks pancreatic secretion strongly suggests that endogenous CCK has a physiological role in pancreatic enzyme secretion (Stubbs & Stabile 1985).

### Stomach and Pylorus: Control of Gastric Emptying

The first clue that a hormone affects gastric motor function was provided by Farrell & Ivy (1926) in a demonstration that fats instilled into the stomach proper altered the motility of a

transplanted stomach pouch. Later knowledge that CCK is released by fats (Stubbs & Stabile 1985; Hopman, Jansen & Lamers 1984; Himeno, et al. 1983; Fried et al. 1983; Walsh et al. 1982; Burhol et al. 1980; Malagelada et al. 1976) led to the speculation that CCK affects gastric motility.

Studies of isolated stomach smooth muscle cells from the antrum (Bitar, Saffouri & Makhoulf 1982; Morgan, Schmalz, Go & Szurszewski 1978), and fundus (Bitar & Makhoulf 1982; Collins & Gardner 1982), and from fundic muscle strips (Grider & Makhoulf 1986) have shown that CCK receptors exist at these sites and that CCK triggers cellular contraction.

CCK-8 also yields contraction of isolated stomach preparations (Scheurer, Varga, Drack, Burki & Halter 1983a & 1983b; Gerner 1979, Gerner, Haffner & Norstein 1979). However, in the intact animal CCK-8 causes reduction of intragastric pressure (Valenzeula 1976). Extracts from the duodenal mucosa containing cholecystokinin inhibited gastric motility (Dinosa, Chey, Hendricks & Lorber 1969; Johnson, Brown & Magee 1966; Johnson & Magee 1965; Kosaka & Lim 1930), which may result in slower emptying.

Autoradiographic studies have found CCK receptors on the pylorus (Robinson, Moran, Goldrich & McHugh 1987; Smith, Moran, Coyle, Kuhar, O'Donahue & McHugh 1984). CCK causes pyloric muscle to contract (Murphy, Smith & Gibbs 1987, Scheurer et al. 1983a &

1983b; Phaosawasdi & Fisher 1982; Fisher, Lipshutz & Cohen 1973). Furthermore, exogenous CCK-8 also inhibits gastric emptying (Liddle et al. 1986; Mangel & Koegel 1984; Anika 1982; Bertaccini & Scarpignato 1980; Valenzuela & DeFilippi 1981; Yamagishi & Debas 1978; Debas, Farooq & Grossman 1975; Chey, Hitanant, Hendricks & Lorber 1970). These findings support the hypothesis that CCK may be involved in control of gastric emptying.

However, the issue of whether endogenous CCK controls gastric emptying has been controversial. Debas et al. (1975) demonstrated that doses of CCK-8 that yielded half maximal gallbladder and pancreatic responses also inhibited gastric emptying in dogs, suggesting that physiological levels of endogenous CCK may slow emptying. Moreover, gastric emptying is retarded by the CCK secretagogue tryptophan, when delivered in amounts sufficient to cause gallbladder contraction and pancreatic secretion; thus implying the involvement of endogenous CCK (Debas et al. 1975). Nevertheless, the physiological role of CCK in gastric emptying was ambiguous, because a supraphysiological dose of CCK-8 was reported to be necessary to inhibit gastric emptying in man (Valenzuela & DeFillipi 1981). However, Valenzuela & DeFillipi (1981) were unable to determine the plasma concentration of CCK, and thus their study was inconclusive. This controversy was resolved by the finding that in man, gastric emptying is inhibited by a dose of exogenous CCK-8 that produces plasma levels

similar to levels for endogenous CCK released following a meal (Liddle et al. 1986).

#### I.5 WHAT MECHANISM MEDIATES THE SATIETY EFFECT OF CCK?

Knowledge of the digestive functions that CCK performs is good preparation for investigation of how CCK performs the additional role of controlling meal size. The desired outcome from this investigation is identification of the sequence of biological events that CCK initiates which result in satiety. In physical terms, we wish to discover the receptor sites where peripheral CCK acts that are relevant to feeding, and if required, the physiological substrate which conveys the satiety signal to the brain.

##### Mediation by the Brain

It is axiomatic that the brain is an important component to the substrate for CCK's effect on feeding because we know that the central nervous system is involved in all behavior. Therefore, an important first step toward uncovering the satiety mechanism for CCK was to determine whether CCK acts at a peripheral site or directly on receptors in the brain. It has recently been shown that centrally administered CCK-8 suppresses feeding in the rat

(Schick, Stevens, Yaksh & Go 1988; Schick, Yaksh & Go 1986).

Intraventricular CCK also inhibits feeding in sheep (Della-Fera & Baile 1979). Thus, there are receptors within the brain upon which central CCK may act to cause satiety. These brain sites could be responsible for the satiety effect of peripheral CCK. However this possibility is tentative because CCK does not cross the blood-brain barrier (Oldendorf 1981; Passaro, Debas, Oldendorf & Yamada 1982), and therefore central CCK receptors are inaccessible to CCK.

The blood-brain barrier does not totally bar direct contact of circulating hormones from the central nervous system, because there are regions, such as the Area Postrema, where the blood-brain barrier is absent (Weindel 1983). Receptors for CCK do exist on the Area Postrema (Moran, Robinson, Goldrich & McHugh 1986; Zarbin, Innis, Wamsley, Snyder & Kuhar 1983), and thus it is possible that peripheral CCK act directly on the brain to reduce feeding. However, destruction of the Area Postrema does not block the satiety-effect of circulating CCK (Edwards, Ladenheim & Ritter 1986) and therefore it is probable that the feeding-relevant receptors for peripheral CCK lie outside the brain (Smith & Gibbs 1985).

#### Mediation by the Vagus Nerve

Further evidence of the peripheral basis of CCK induced

satiety are the demonstrations that an intact gastric vagus is critical for the peptide's feeding effect. Transection of the vagus nerve below the diaphragm entirely blocks the suppression of normal feeding by CCK-8 (Smith, Jerome, Cushin, Eterno & Simansky 1981; Lorenz & Goldman 1982; Morley, Levine, Kniep & Grace 1982) in rats, and the effect of moderate doses of CCK in hamsters (Miceli 1985). Additional study has shown that the satiety signal depends on the afferent portion of gastric vagal branch (Smith, Jerome & Norgren 1985). Indeed, the discovery of CCK receptors on the rat vagus (Zarbin, Wamsley, Innis & Kuhar 1981), has been taken to suggest that the vagus monitors CCK levels directly (Smith & Gibbs 1984).

#### Mediation by Smooth Muscle

An alternative to the vagal monitoring hypothesis is the proposal that CCK reduces food intake by affecting the muscle tone of the stomach (Smith & Gibbs 1984). This proposal is based upon observations that CCK-8 causes isolated gastric smooth muscle cells to contract (Collins & Gardner 1982). Signals arising from muscular contraction are presumably transmitted to the brain by vagal mechanoreceptors (Paintal 1973).

#### Mediation by Gastric Emptying

Currently, the most clearly articulated hypothesis is that



CCK's satiety effect is mediated by changes in gastric emptying (Moran and McHugh [1982, 1986a,b]; Smith, Moran, Coyle, Kuhar, O'Donahue & McHugh 1984). The argument for the gastric mediation hypothesis is as follows. During the course of a meal, food is swallowed and enters the stomach. A portion of the ingested nutrient then empties from the stomach into the intestines. However, because the rate of ingestion is faster than the rate of emptying, the stomach fills and becomes distended. When gastric distension reaches a critical amount, impulses from muscular stretch receptors (Paintal 1973) become sufficient to signal satiety. Moran and McHugh propose that CCK-8 reduces meal size by causing the retention of food in the stomach, which reduces the amount of food needed to stimulate gastric satiety signals. This hypothesis is attractive because it relates CCK-induced satiety to theories suggesting that gastric volume (Deutsch 1985; Wirth & McHugh 1983) contributes to the control of meal size.

There is much evidence that food in the stomach can influence feeding. In the dog, distension of the stomach by either intragastric nutrient (Janowitz & Grossman 1949) or a balloon (Share, Martyniuk & Grossman 1952) suppresses sham feeding. In the rat, direct intragastric infusions of nutrient support the acquisition of taste preferences for non-nutritive test meals despite prevention of emptying by a pyloric clamp (Deutsch & Wang 1977). The contention that gastric factors can

control meal size independently of intestinal factors was further supported by the demonstration that meal size remained the same regardless of whether the intestines received nutrient (Kraly & Smith 1978). Moreover, rats accurately compensate within a meal when ingested nutrient is removed via a gastric cannula by consuming more food (Deutsch, Young & Kalogeris 1978). In contrast to the canine data, Deutsch and colleagues determined that in addition to gastric distension, the amount of calories in the stomach is an important factor for gastric satiety in rats (Deutsch & Gonzalez 1980, 1981; Deutsch, Gonzalez & Young 1980). Indeed, Deutsch (1985) argues that caloric load estimates derived from sensory receptors for meal volume and caloric concentration are used to control meal size by rats.

The strongest support for the gastric emptying hypothesis is the demonstration in monkeys that gastric infusion of a saline preload can be necessary for CCK to reduce meal size (Moran and McHugh 1982). Moran and McHugh claim that by causing retention of saline in the stomach, CCK-8 lessened the amount of food that need be consumed to release gastric distension satiety signals. Furthermore, they have proposed that the pylorus is the specific site of action for the satiety effect of CCK (McHugh & Moran 1986; Moran & McHugh 1982; Robinson et al. 1987). This view is supported by studies establishing that CCK-8 induces contraction of pyloric muscle both in vitro (Scheurer et al. 1983a & b; Murphy

et al. 1987), and in vivo (Kumar et al. 1987; Fisher et al. 1973; Munk et al. 1977) and that CCK increases pyloric resistance (Fisher et al. 1973; Phaosawasdi & Fisher 1982; Yamagishi & Debas 1978). Moreover, Moran and McHugh have demonstrated that a dense band of CCK receptors exist on the smooth muscle circling the pylorus (Robinson et al. 1987; Smith & Moran et al. 1984) of the rat. This evidence makes the pylorus a viable candidate for the mediation of CCK-8-induced satiety. Moreover, Moran and McHugh's hypothesis is consistent with the suggestion that modulation of stomach emptying rate is a physiological function of CCK (Liddle et al. 1986; Debas et al. 1978).

#### I.6 THESIS QUESTION AND OUTLINE OF EXPERIMENTATION.

The focus of this thesis is the following question: do changes in the rate of gastric emptying mediate the satiating effect of CCK-8 in the rat? Exposition of my thesis research is in three sections. In the first section (Chapter 2), I present experimental validation of a new method for the investigation of gastric emptying in rats. Development of a new method for examining gastric emptying in rats was necessary because established techniques were inadequate to study the course of gastric emptying over time. With the new methodology established, I address three experimental questions.

In Chapter 3, the gastric mediation hypothesis is examined by asking whether doses of CCK that inhibit emptying also suppress feeding. If emptying inhibition is responsible for the satiety effect of CCK, then it is required that the effective dose range for emptying must overlap with that for feeding suppression.

Chapter 4 addresses the issue of whether inhibition of gastric emptying by CCK causes the peptide to induce satiety. Two strategies were taken. First, I study the pharmacokinetics with which CCK influences emptying and feeding. Second, I investigate whether peptides that slow gastric emptying also suppress feeding. If inhibition of gastric emptying per se is critical to the satiating effect of CCK-8, then peptides other than CCK that retard emptying should also reduce feeding.

CHAPTER 2.  
VALIDATION OF NEW PROCEDURE FOR WITHIN-TRIAL  
REPEATED MEASUREMENT OF GASTRIC EMPTYING  
IN THE RAT.

Since food in the gut normally provides stimuli adequate for satiety, physiological psychology has focussed on food-stimulated, gastrointestinal responses as signals for meal termination. Specific interest in the role of gastric emptying in feeding has been fueled by the hypothesis that inhibition of gastric emptying represents the physiological mechanism mediating the satiety effects of cholecystokinin (Moran & McHugh 1986). However, this hypothesis and other possible roles of gastric emptying in satiety, are difficult to assess because of certain limitations of current techniques for monitoring gastric emptying in the rat. These technical constraints are particularly troublesome, because the rat is the animal with which a great deal of the feeding data have been obtained. This chapter describes a new procedure for monitoring gastric emptying in the freely-behaving rat. The method is a modification of a technique employed with humans (Dubois, van Eerdeghe & Gardner 1977). Its major advantage over

current techniques for the rat is the capacity to permit repeated, serial sampling of gastric emptying in a single experimental session without the requirement of periodic evacuation of stomach contents.

The most primitive procedure for measuring both gastric volume and gastric emptying is removal and weighing of contents in various gastrointestinal sites after sacrificing the rat (e.g. Curi, Hell, Bazotte & Timo-Iaria 1984; Davies, Rossi, Panksepp, Bean & Zolovick 1983; Friedman, Ramirez, Wade, Siegle & Granneman 1982; Wiepkema, Prins & Steffens 1972). The disadvantages of this technique are obvious -- it is laborious and no repeated sampling can be performed.

Other studies examining the relationship between stomach emptying and feeding in the rat have employed a serial-sampling procedure (Granneman & Striker 1984; Setler & Smith 1969; Snowden 1970) in which gastric volume is measured by aspirating the entire gastric contents through an indwelling gastric cannula. As first suggested by Hunt and Spurrell (1951), volume of food in the stomach is distinguished from fluid secreted by the stomach by mixing an inert non-absorbable dye, such as phenol red (Ivey & Schedl 1970; Penner & Hollander 1940), with the liquid diet. If the time course of emptying is required, the serial sampling procedure is applied at different times on separate test days (e.g. Granneman & Striker 1984; Hunt & Spurrell 1951; Setler &

Smith 1969; Snowdon 1970).

A major disadvantage of Hunt and Spurrel's (1951) procedure is that only one measurement is permitted per trial because the entire contents of the stomach must be removed. Moran & McHugh (1982) modified the serial sampling procedure by re-filling the stomach after it was evacuated. Although this permitted more than one measurement in each session, the consequences of repeated evacuations and refillings on gastric emptying and physiology are unclear. This modification of serial sampling is certainly problematic for studies relevant to feeding behavior since evacuation of the stomach may confound satiety signals and, in fact, has been used as a procedure for sham feeding (Davis & Campbell 1972).

The most efficient procedure for monitoring emptying is one allowing repeated determinations of gastric volume during a single experimental session without disturbing the normal course of emptying. Such a technique would permit time course studies and, across experimental days, allow a determination of individual variability. A procedure with these qualities, developed by George (1968) and termed the double sampling method, is used with humans (George 1968; Dubois, van Eerdewegh & Gardner 1977). This paper describes and validates this technique for use in rats.

The double sampling method reveals the time course of emptying by the repeated measurement of stomach volume, volume of

gastric secretion and amount of nutrient emptied into the duodenum. The basis of the technique is the principle of dye dilution and the mathematical relationships permitting evaluation of gastric volume and emptying are detailed in the Appendix and elsewhere (George 1968; Dubois, van Eerdewegh & Gardner 1977).

Double-sampling is applied repeatedly in a series of basic steps after infusion of the gastric load (test volume) into the stomach. At the beginning of the test session, a test volume of known volume and dye concentration is infused intragastrically (IG). Gastric dye concentration is determined at time  $t$  by withdrawing a small volume of stomach contents. Then, at  $t'$  a dye solution (probe volume) of known volume and concentration is injected intragastrically and mixed with stomach contents. Another sample is then drawn to determine the gastric dye concentration that results from addition of the probe. The gastric volume is then calculated from the dye concentrations of the probe volume, and the gastric volume before and after sampling. The actual volumes of sample withdrawn or infused are small relative to the volume of gastric contents and, when used with a rat equipped with a chronic gastric cannula, the sampling sequence can be repeated in the freely-behaving animal to obtain the time course of emptying.



## METHODS

### Subjects.

Subjects were male, Long-Evans hooded rats weighing between 350-450 gms at testing. Each rat was implanted with a chronically indwelling gastric cannula according to procedures described elsewhere (Weingarten & Powley 1980) and permitted a minimum of two weeks to recover from surgery. Rats were housed individually in a room maintained at 20° C on a 14:10 light:dark cycle. They had continuous access to water and food (Purina Laboratory Rodent chow) except on test days when they were fasted for 6 hours prior to the emptying test.

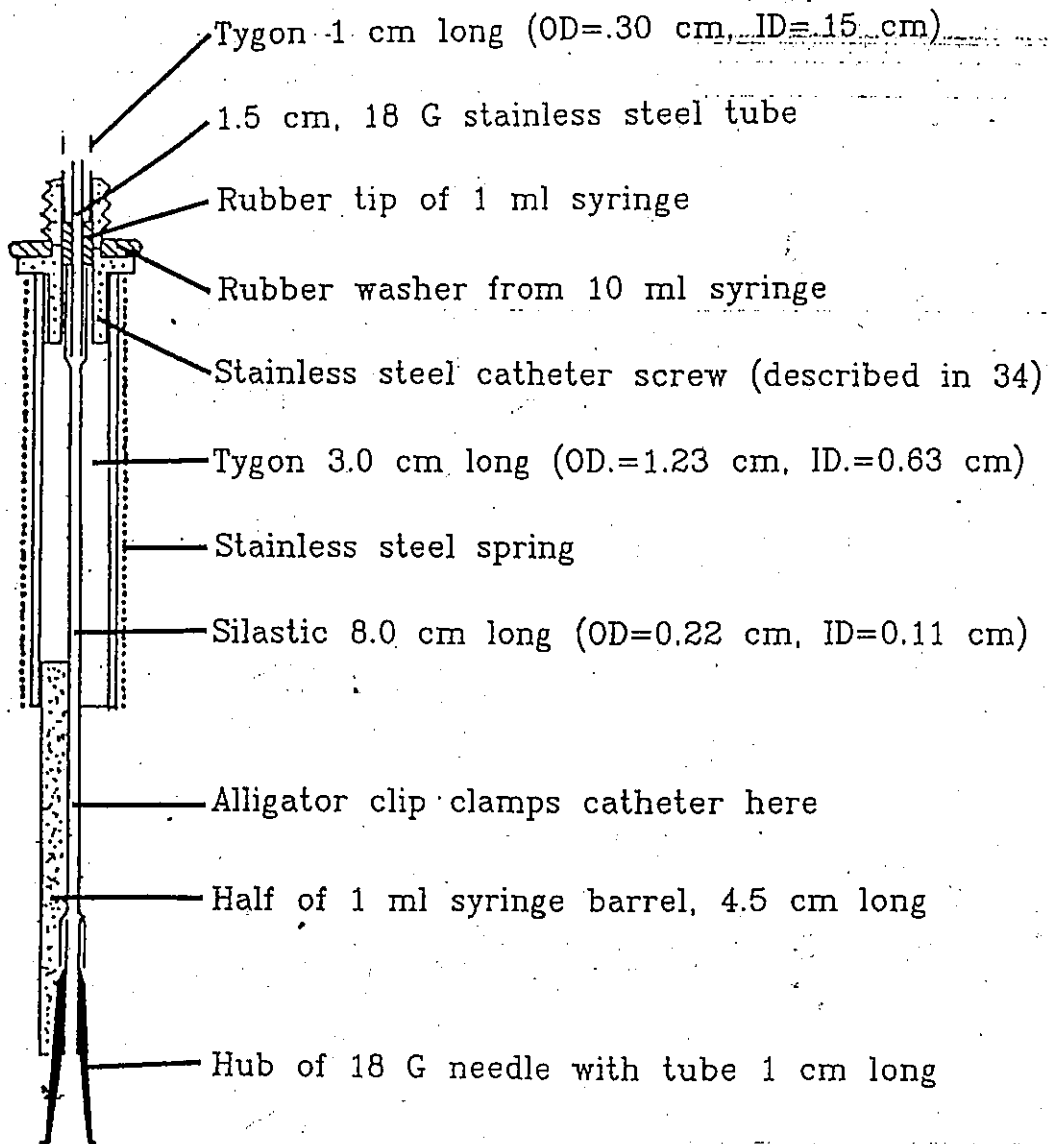
### Materials.

The sampling catheter (Figure 1) is a modification of the collection tube described for use with the gastric cannulated rat (Weingarten & Rowley 1980). A 1.5 cm length of 18 ga stainless steel tubing was pushed through the rubber tip of a 1 ml disposable syringe and fit into the lumen of the collection tube. The exposed tip of the 18 ga tube was protected by a sleeve made from a 1 cm length of Tygon tubing (OD=.30 cm, ID=.15 cm). Four holes 1 mm in diameter were melted through the walls of this sleeve with a hot needle, and its end was left open, to allow mixing and unobstructed collection of gastric contents. The joint between the collection tube and gastric cannula was sealed by a

rubber washer made from the rubber tip of a 10 ml disposable syringe plunger. A 8.0 cm Silastic tube (OD=0.22 cm, ID=0.11 cm) was force fit over the lower end of the 18 ga tube. The other end of this tube was connected to the hub of an 18 ga needle. The entire catheter was protected from damage by the rat by a 3.0 cm long Tygon tube (OD=1.23 cm, ID=0.63 cm) wrapped with a stainless steel spring. The split half of a 1 ml disposable syringe barrel 4.5 cm long was inserted into the protective tubing and projected 4 cm beyond its end. This prevented the rat from pulling the collection tube up into the test cage. The sampling catheter was clamped shut with a small alligator clip.

To prepare for sampling, each animal was removed from its home cage and its stomach was cleared by saline lavage applied through the open cannula. The sampling catheter was then screwed into the gastric cannula and the animal was placed in the experimental cage. The dye used in these experiments was .02% phenol red (Fisher Scientific). The gastric load (test solution), containing 2.5% (v/v) of .02% phenol red, was infused intragastrically at time 0. At time t, 0.5 ml of gastric contents were removed to determine the initial dye concentration. At time t', 1 ml of a phenol red solution (probe; 0.1% v/v) was injected into the stomach and mixed thoroughly with gastric contents by 30 reciprocations of the syringe plunger. Then, 0.5 ml of gastric contents were removed to determine the resulting dye

Figure 1. Cross-sectional diagram of catheter which threads into the indwelling gastric cannula.



concentration.

Dye concentrations were measured by absorption spectrophotometry at a wavelength of 560 nm. Samples were prepared for spectrophotometry by diluting 0.25 ml from each aliquot in 2.75 ml of 0.01M NaOH. These samples were then passed through 0.2  $\mu$ m cellulose filters (13 mm diameter, Sartorius) to remove particulate matter.

#### II.1 EXPERIMENT 1a: COMPARISON OF ESTIMATED AND ACTUAL GASTRIC VOLUMES.

This experiment validates the double sampling procedure by comparing estimates of gastric volume generated by this technique to the actual volume of stomach contents measured directly by complete aspiration of stomach contents.

##### Protocol

Four rats were 6 hrs food-deprived at testing and were prepared for the emptying procedure in the manner described above. Time 0 is the start of the experimental session, and at that time 10 mls of test solution were infused directly intragastrically over a 1 min period. Three test solutions were used: .15M saline, 10% (w/v) sucrose, and 30% sucrose. Since these three substances should empty at different rates (Kalogeris, Reidelberger & Mendel

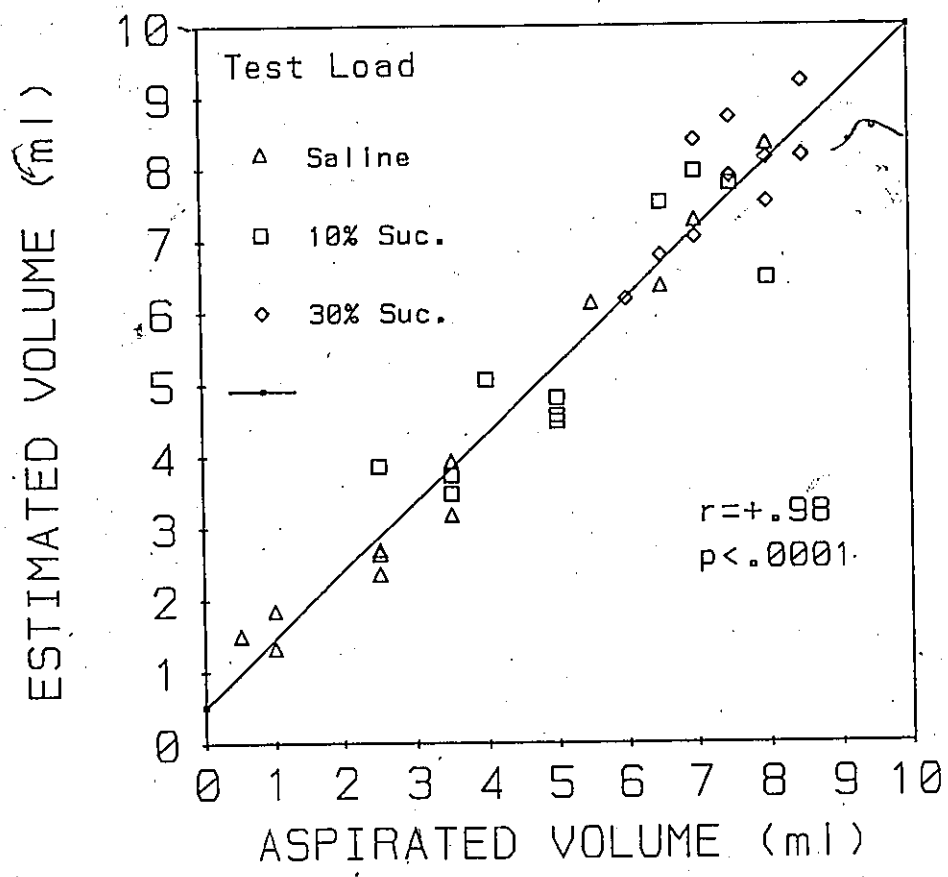
1970; McHugh, Moran & Barton 1975), selection of these infusates assured a reasonable gastric volume even at late sampling times. The double sampling procedure was applied on different days, at 2 min, 20 min, or 40 min, postinfusion. Immediately after the samples used to calculate gastric volume had been drawn, the gastric contents were evacuated through the gastric cannula by aspiration. The volume of gastric contents was measured and compared to volumes calculated on the basis of the double sampling technique. All rats were tested with each test solution at each of the 3 sampling intervals.

#### Results & Discussion

Figure 2 presents the scatterplot correlating gastric volumes estimated by double sampling to those measured directly by stomach aspiration. One sample was lost due to contamination by blood. The Pearson Product-Moment correlation was highly significant,  $r(35) = +.98$ ,  $p < .0001$ , indicating that the double sampling method provided an accurate measurement of gastric volume.

Moreover, rapid withdrawal and reinstatement of the gastric contents while mixing did not cause a visible disturbance of the animals.

Figure 2. Scattergram illustrating the correlation between gastric volumes estimated using the double sampling method and gastric volumes measured directly by aspirating stomach contents.





## II.2 EXPERIMENT 1b: VALIDITY CHECKS WITH REPEATED SAMPLING.

A major advantage of the double sampling procedure is that it permits repeated assessment of gastric state within the same experimental session. The previous experiment demonstrated the validity of the procedure when only one sample was obtained in a test session. However, aspiration of the gastric contents prevented assessment of whether the method was accurate over a series of samples. The validity over repeated sampling is appraised in this experiment, by comparing the mean gastric volumes as determined by aspiration at different times with those measured by repeated double sampling. As well, this experiment demonstrates the capacity of the double sampling procedure to monitor other parameters of gastric function which may be relevant in the control of feeding behavior, such as volume secreted into the stomach and volume emptied.

### Protocol

The four animals used in the previous study were subjects for this experiment. After a 6 hour fast animals were prepared for gastric sampling in the manner described above. Ten mls of test solution; .15M saline, 10% sucrose, or 30% sucrose, were infused at time 0. Only one test solution was used on a particular test day. On all days, the double sampling procedure

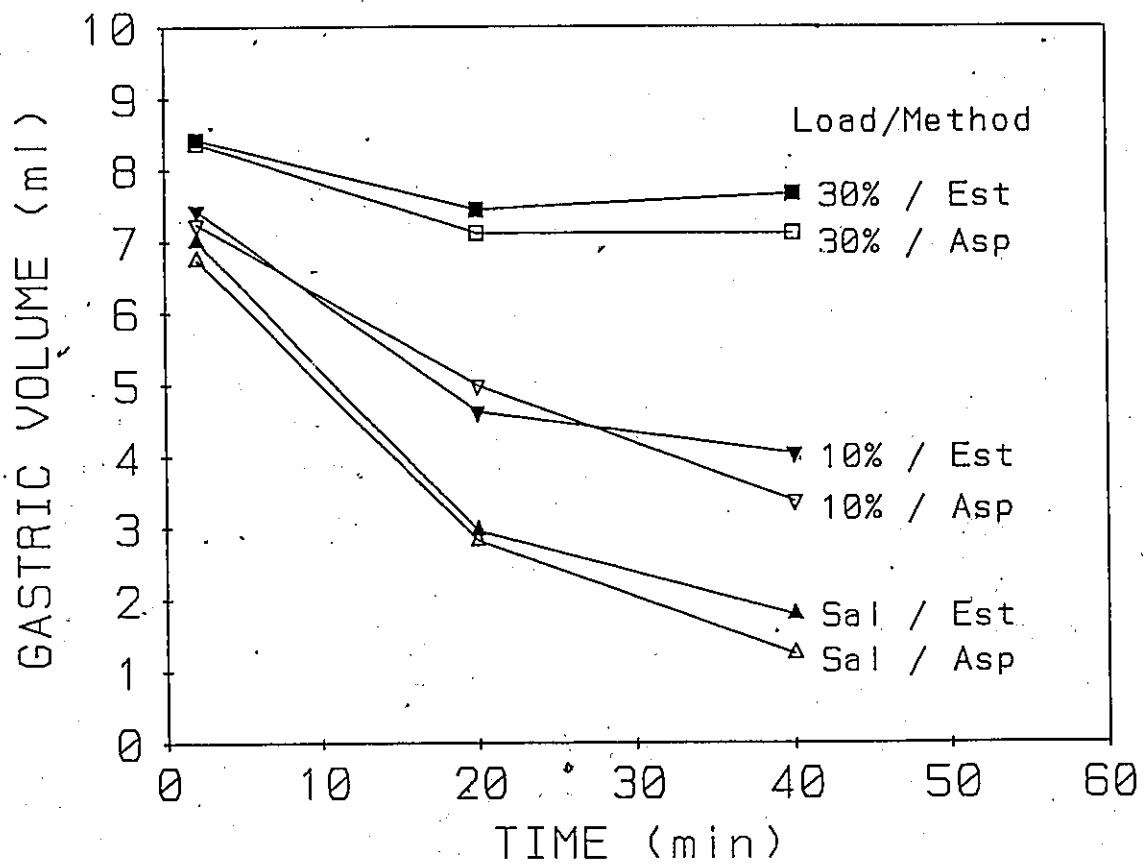
was applied at 2 min, 20 min, and 40 min, postinfusion.

### Results & Discussion

Figure 3 shows gastric volumes remaining estimated by the double sampling technique when the procedure is repeated in the same test session. These estimated values are compared to the actual values measured by stomach aspiration presented in Experiment 1. The repeated sampling procedure was validated: for all infusions,  $\bar{x}$  (34) = +.915,  $p > .001$ . Therefore, repeated application of the double sampling procedure did not alter the normal profile of gastric emptying.

Gastric volume is a function of both the amount of infusate remaining in the stomach and the amount of gastric secretion. A major advantage of the double sampling procedure is that it permits independent measurement of each of these parameters. For example, from the data obtained in this study, the mean rate of secretion for each of the three test conditions was calculated. The average rates of secretion (SEM) were 0.024 (.009) mls/min for saline, 0.021 (0.006) for 10% sucrose and 0.037 (.006) for 30% sucrose. An analysis of variance on the mean secretion rates found no significant differences,  $F(2,6) = 1.37$ ,  $p > .05$ .

Figure 3. Comparison of gastric volumes estimated on basis of double sampling (closed symbols) when the procedure was repeated at 3 time points in the same session. Estimated values are compared to actual aspirated volumes obtained in Experiment 1 (open symbols). Infusates used were .15M saline, 10% and 30% sucrose.



### II.3 EXPERIMENT 2: DEMONSTRATION OF BASELINE STABILITY.

Experiment 2 was performed to demonstrate the ability of double sampling to present a more detailed picture of gastric emptying and to assess the stability of baseline emptying data over a series of trials. As well, a procedure for quantitative analysis of emptying data obtained by double-sampling is presented.

#### Protocol

Five male rats were implanted with a gastric cannula (Weingarten & Powley 1980) and were maintained ad libitum except for 6 hr deprivation prior to testing. Emptying of a 10 ml gastric infusion of .15M saline was assessed on 4 separate test days performed on 4 consecutive days. Gastric volumes were determined at 2, 6, 11, 16, 21, 26, 31, 36, and 41 min, with the time of infusion of the test solution defined as time 0.

#### Data Analysis

To assess the rate of emptying, individual half emptying times ( $t_{1/2}$ ) were calculated from the time course data. The  $t_{1/2}$  values were derived by fitting the power exponential function to the individual test load time-course data (Elashoff et al., 1982) and was defined as the time needed for half of the test load to

leave the stomach. The gastric load remaining at each sampling time was calculated by subtracting the volume secreted at that time from the gastric volume generated by the double sampling procedure.

In addition to  $t_{1/2}$ , the power exponential has the parameter,  $\beta$ , which describes the shape of the emptying curve relative to a simple exponential with the same  $t_{1/2}$ . When  $\beta = 1$ , the emptying curve is perfectly exponential. A  $\beta < 1$  indicates a faster than exponential initial phase of emptying (i.e., approximately the first 10 minutes) followed by a slower-than exponential phase of later emptying. This type of emptying is characteristic of subjects with impaired pyloric function, as in the case of vagotomy and pyloroplasty (Elashoff et al., 1982). A  $\beta > 1$  indicates initial emptying slower than exponential followed by a later phase of emptying more rapid than exponential. In this study, a  $\beta > 1$  is best interpreted as a lag or suppression of emptying in the early phase of gastric emptying. The goodness of fit of the power exponential function to the time courses was measured by the coefficient of determination ( $R^2$ ); the median  $R^2$  is reported for each treatment. As  $R^2$  approaches 1, the fit of the curve to the data becomes perfect.

### Results and Discussion

The time courses for the emptying of 10 ml of saline from

the stomach are presented in Figure 4. On all trials, the infusion volume emptied in an approximately exponential manner, suggesting that liquid gastric emptying is not abnormal in the presence of the gastric cannula. Furthermore, the stability of data obtained with this procedure are indicated by the stability of emptying parameter measurements across trials. Analyses of variance indicated that the mean  $t_{1/2}$ 's and  $\beta$ 's over the 4 trials were not significantly different, ( $F(3,12) = 1.207$  and  $F(3,12) = 0.977$  respectively,  $p$ 's  $> .05$ ). In all cases the median  $R^2$  values approached one, indicating that the power exponential curve fit the time course data well.

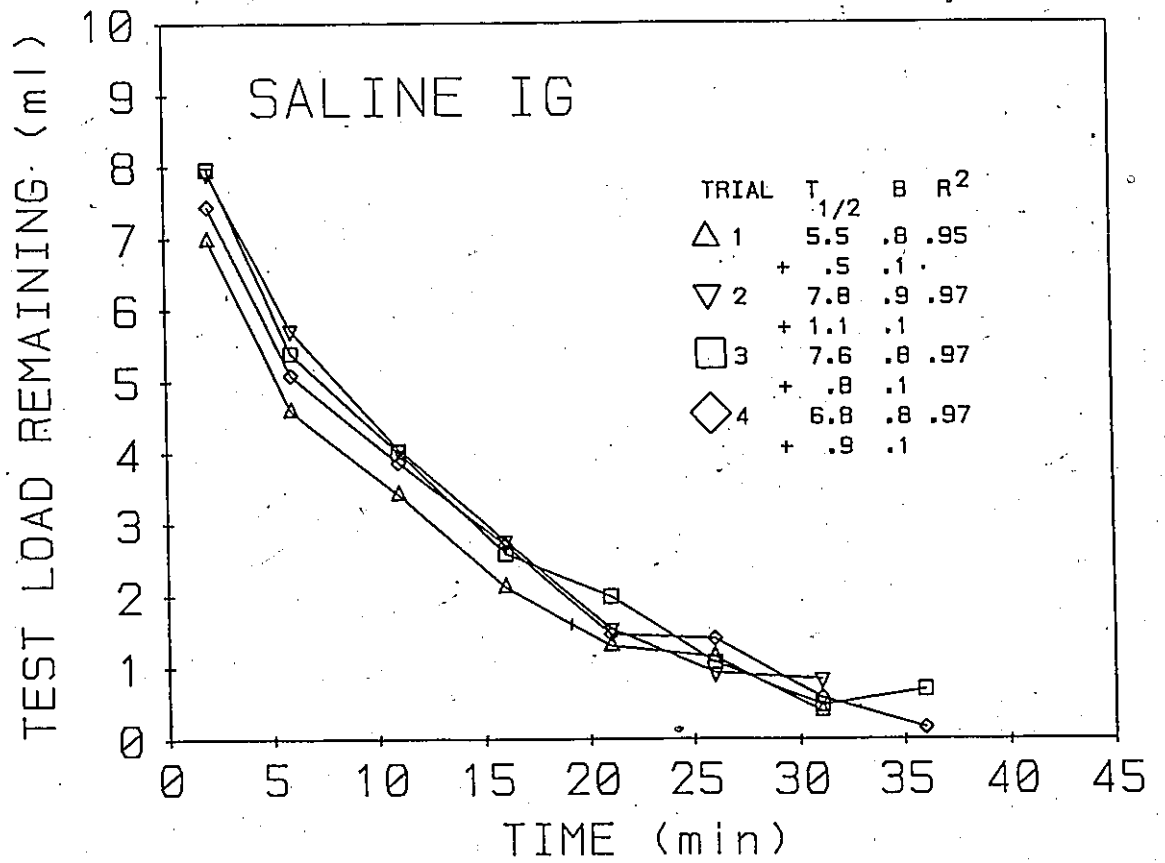
#### SUMMARY AND DISCUSSION

The overall conclusion to be drawn from the results is that the double sampling method provides valid information about gastric volume and permits assessment of gastric secretion in the rat. Because these gastric factors may be measured many times within a trial, the time course for the volume remaining in the stomach can be determined and the results summarized in terms of half emptying time. In comparison with Hunt's (1951) serial sampling method, the double sampling procedure provides the same information, but with much less effort. Furthermore, unlike serial sampling which requires obtaining time course data over many trials, double sampling, which requires only a single

9

Figure 4. Time course of emptying of 10 ml .15M saline on four trials in 6 rats. Included in each panel is the mean half emptying time ( $T_{1/2}$ ),  $\beta$ ,  $R^2$  and corresponding standard errors. See Chapter 2 for explanation of these measures.





session, is not confounded by the possibility that the response of emptying to a treatment changes with repeated trials.

A number of assumptions underly the double sampling method. These assumptions are that: i) the gastric volume does not change while the measurement is being taken (i.e., from time  $t$  to  $t'$ ); ii) no dye is lost from the stomach other than by gastric emptying, and that; iii) the probe infusions and gastric volumes are completely mixed. The fact that the volumes estimated by double sampling correlated highly with the true gastric volumes as determined by aspiration, and the short time interval ( $< 1$  min) required to apply double-sampling, suggests that the first assumption is not a problem. The double sampling procedure did yield volume measurements that were approximately 0.5 ml larger than the aspirated volumes. This small difference, however, likely arises because aspiration does not completely recover the gastric contents, and therefore aspiration slightly underestimates the true fluid volume in the stomach.

The assumption that no changes in the amount of dye in the stomach arise other than by gastric emptying (i.e. none is absorbed) has been demonstrated to be valid in a number of studies (Ivey & Schedl 1970; McHugh & Moran 1979; Penner & Hollander 1940).

The third assumption, that test dye mixes thoroughly in the stomach, is supported by the validation findings in the first

experiment since incomplete mixing would reduce the correlation between estimated and aspirated volume.

A potential limitation of double sampling is that the required gastric cannula might compromise normal emptying by impairing muscular contraction of the forestomach. Gastric emptying of liquids does rely partly on intragastric pressure maintained by muscle tone of the proximal stomach (Keith 1980; Miller, Kauffman, Elashoff, Ohashi, Carter & Meyer 1981; Strunz & Grossman 1978; Yagamishi & Debas 1978). However, the confound introduced by a gastric cannula appears minimal. The fact that the emptying of nutrient was related to intragastric caloric loads, i.e., that nutrient emptied more slowly than saline, (Experiments 1a and 1b) and that saline emptied in the normally-expected exponential manner (Experiment 2) supports the contention that properties of gastric emptying in the cannulated rat resembles those of intact animals. Furthermore, in other preparations, such as those using nutrient loads (Kalogeris, Reidelberger & Mendel 1983), anticholinergics (Brodie 1966; Setler & Smith 1969), or cholecystokinin (Bertaccini & Scarpignato 1981), the presence of a gastric cannula does not change the response of the system compared to the intact animal.

In sum, the double sampling procedure has been validated as providing an accurate measure of gastric volume and emptying in the freely-behaving rat. Its property of allowing multiple

determinations of gastric volume in a single test session makes it valuable for investigations where a time course or detailed picture of gastric emptying is required.

CHAPTER 3.  
COMPARISON OF THE EFFECT OF CCK-8 ON  
EMPTYING AND FEEDING

The gastric mediation hypothesis purporting to explain CCK-induced satiety is supported by observations that CCK-8 in doses capable of inducing satiety also slows gastric emptying in the monkey (Moran & McHugh 1986). The absence of conclusive data in the rat is problematic however, because it is in this species that the great majority of peptide-induced satiety data have been obtained. Furthermore, the validity of the gastric emptying hypothesis is limited since doses of CCK-8 that suppress feeding have not been demonstrated to retard emptying under similar experimental conditions. Since the actions of CCK-8 on emptying or feeding may be substantially influenced by the nature of the test substance used to monitor these functions, the volume and composition of gastric contents, and the manner in which the peptide is administered, it is mandatory that these factors be incorporated into any study that attempts to evaluate the role of gastric emptying in CCK-induced satiety. In this study, we have used the technique of Chapter 2 to compare formally the dose-response relationships and kinetics of the actions of CCK-8 on

gastric emptying and food intake in the rat.

## METHOD

### Subjects

The subjects were male Long Evans hooded rats bred in the McMaster Psychology Department colony from stock originating from Blue Spruce Farms (Altmont, NY). The rats weighed between 375 and 500 grams at the time of testing. The animals were housed in individual hanging cages in a room maintained at 26 degrees Celsius on a 14:10 light:dark cycle. They had Purina rat chow and water ad lib except as indicated in the protocol described below.

### Injections

All injections were made up to a volume of 1 ml with .15M saline and were injected ip. Control injections were equivolumes (1 ml) of .15M saline.

### Preparation of animals and test cages

The animals were 6 hr food deprived before each trial. Experiments were conducted between 15:00 and 17:00 hours. In preparation for every trial, each subject's stomach was flushed with saline lavage applied through the open cannula.

Subjects were tested for both emptying and feeding studies in a Plexiglas cage (10 cm high x 15 cm wide x 25 cm long), with a

slot in the floor through which a tube attached to the gastric cannula could hang. The gastric infusions and dye injections were introduced into the stomach via the gastric catheter, as described in Chapter 2.

### III.1 EXPERIMENTS 3A & 3B: EFFECT OF CCK-8 ON GASTRIC EMPTYING

#### INTRODUCTION

These experiments were performed to assess the dose-response relationship between CCK-8 and the gastric emptying of saline and sucrose in the rat.

#### Protocol

After the stomach was cleaned, the gastric catheter was installed, and saline remaining from the flushing procedure was aspirated from the stomach. In Experiment 3a, the gastric test load was 10 ml of .15M saline. In Experiment 3b, it was 10 ml of 15% (w/v) sucrose. The test loads were infused directly intragastrically through the sampling catheter over a 1 min period. The beginning of the test load infusion was set as  $t=0$  min, and measurements of the volume of the test load remaining in the stomach were performed at 2, 5, 10, 15, 20, 25, 30, 35, and 40 minutes in Experiment 3a, and at 2, 10, 20, 30, 40, 50, and 60 minutes in Experiment 3b.

There were six subjects in each of Experiments 3a and 3b; each rat served as its own control. The doses of CCK-8 employed in Experiment 3 were: 0.01, 0.1, 1.4, 5.6, 11.2 and 22.4 ug/kg. The order of dose administration was randomized across subjects.

#### Data Analysis

To assess the degree of emptying inhibition (Experiment 3), individual  $t_{1/2}$ s and  $\beta$  values were calculated from the time course data. The use of ANOVA trend analysis to study the dose-response relationship between CCK-8 and  $t_{1/2}$  was inappropriate because the treatment levels (peptide dose) did not increase in equal increments. Therefore, Mann's nonparametric test for monotone trend was employed because it does not demand that treatments are equally spaced (Mann 1945). Mann's method is particularly appropriate in these studies because, unlike a trend analysis by ANOVA, it assumes only that the dose-response curve increases monotonically. For similar reasons, the effect of CCK-8 on feeding was also assessed with Mann's method.

To determine which doses of, and at what times, CCK-8 significantly inhibited emptying, one way repeated measure ANOVA's were performed on the test load remaining data. Separate analyses were conducted for times 2, 5 and 10 minutes in the case of saline, and 2, 10 and 20 minute for sucrose. These three initial times were chosen for comparison because CCK-8 was expected to



yield the greatest inhibition at the beginning of each trial. Where warranted, multiple comparisons used Dunnett's one tailed test (Winer 1971) for comparing experimental means with the mean control value.

The emptying of saline was compared to that of sucrose by a two-way ANOVA comparing the time courses following ip saline (test load: Between; time: Within). The significance of the difference between the mean volumes of test load remaining at 2 minutes following ip saline was assessed using a two-tailed t-test for independent samples.

### RESULTS

The average volume of saline test load remaining over time following different doses of CCK-8 is presented in Figure 5. CCK-8 inhibited emptying of saline in a dose-dependent manner. This progressively increasing suppression of emptying with increased doses of peptide was reflected in several parameters of gastric emptying. First, CCK-8 produced a progressive shift of the emptying curve to the right (see Table 1), reflected as a significant increase in mean  $t_{1/2}$  with dose (Mann's test:  $Z = 4.47$ ,  $p < 0.0001$ ). Second, as the dose of CCK-8 increased,  $\beta$  values also increased significantly ( $Z = 4.86$ ,  $p < 0.0001$ ), suggesting that emptying within the first 10 minutes became slower with larger doses of the peptide. Third, analyses of variance

conducted to compare volumes of saline load remaining in the stomach at various time points (see Table 2) revealed that doses CCK-8 of 1.4 ug/kg and above significantly inhibited emptying within 2 min of the IG load.

The time courses for gastric emptying of 15% sucrose (Experiment 3b) are presented in Figure 6. Overall, 15% sucrose emptied more slowly after an initial large release into the duodenum. For the first 10 minutes, time courses for sucrose emptying were similar to saline. The slowed emptying rate with nutrient relative to saline is supported by results of an ANOVA comparing the emptying of these two loads under control conditions. This analysis resulted in a significant interaction of time by load ( $F(4,40) = 7.11, p < 0.001$ ) and inspection of Figures 5 and 6 indicates that this interaction stemmed from a slower emptying of sucrose than saline when saline was injected ip. The different pattern of emptying with nutrient is reflected best in differences in  $\beta$ . In the early phase of gastric emptying (i.e., the first 10 minutes) there is little difference in the emptying of saline and nutrient. For example, a comparison of volumes remaining in the stomach following ip saline when either saline or nutrient were infused IG revealed no differences at 2 min ( $t(10) = .437, NS$ ) or 10 min ( $t(10) = 1.858, NS$ ) postinfusion. However, saline and nutrient did show different patterns of emptying in the later phases. With IG saline, later emptying was

Figure 5. Time courses for the emptying of 10 ml saline test loads following various doses of CCK-8. Test volume remaining is the volume in the stomach corrected for gastric secretion. (N=6).

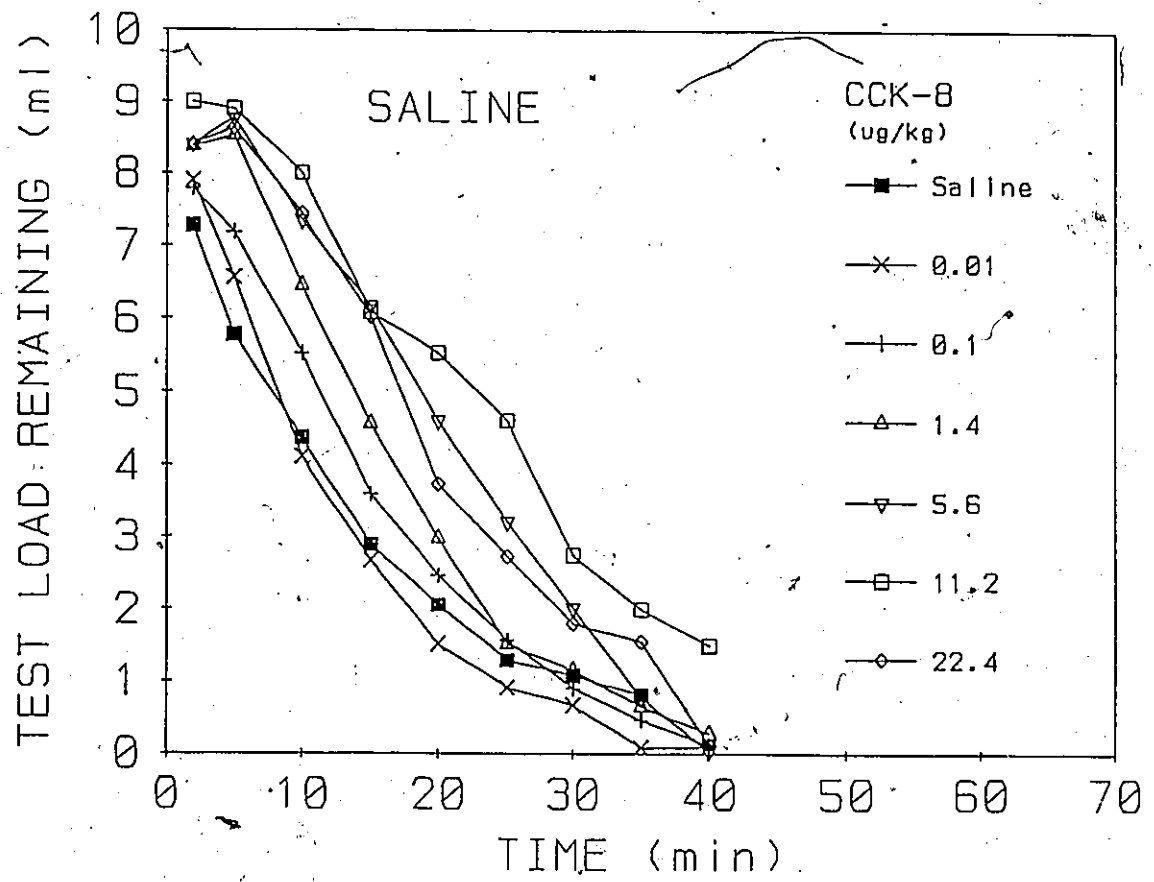


TABLE 1

Effect of CCK-8 on the Gastric Emptying of  
Saline and 15% Sucrose Solutions

Test Load <sup>1</sup>	Dose CCK-8 <sup>2</sup>						
	Saline	.01	0.1	1.4	5.6	11.2	22.4
<u>Saline</u>							
$t_{1/2}$ <sup>3</sup>	7.4 (1.6)	8.0 (1.5)	10.9 (3.0)	13.4 (1.6)	18.6 (3.3)	20.7 (2.3)	18.0 (2.5)
$\beta$ <sup>4</sup>	.81 (.05)	1.06 (.11)	1.16 (.18)	1.14 (.13)	1.95 (.28)	1.74 (.29)	1.65 (.13)
$R^{25}$	.96	.97	.96	.94	.95	.95	.94
<u>Sucrose</u>							
$t_{1/2}$	11.4 (2.6)	10.5 (2.5)	12.4 (3.4)	15.9 (2.2)	19.0 (3.6)	23.2 (2.9)	30.2 (2.2)
$\beta$	.58 (.03)	.55 (.04)	.54 (.05)	.69 (.07)	.82 (.05)	1.03 (.15)	1.15 (.16)
$R^2$	.95	.97	.96	.94	.93	.89	.91

<sup>1</sup>The gastric test load was 10 ml of either .15M saline, or 15% sucrose infused intragastrically at 10 ml/min.

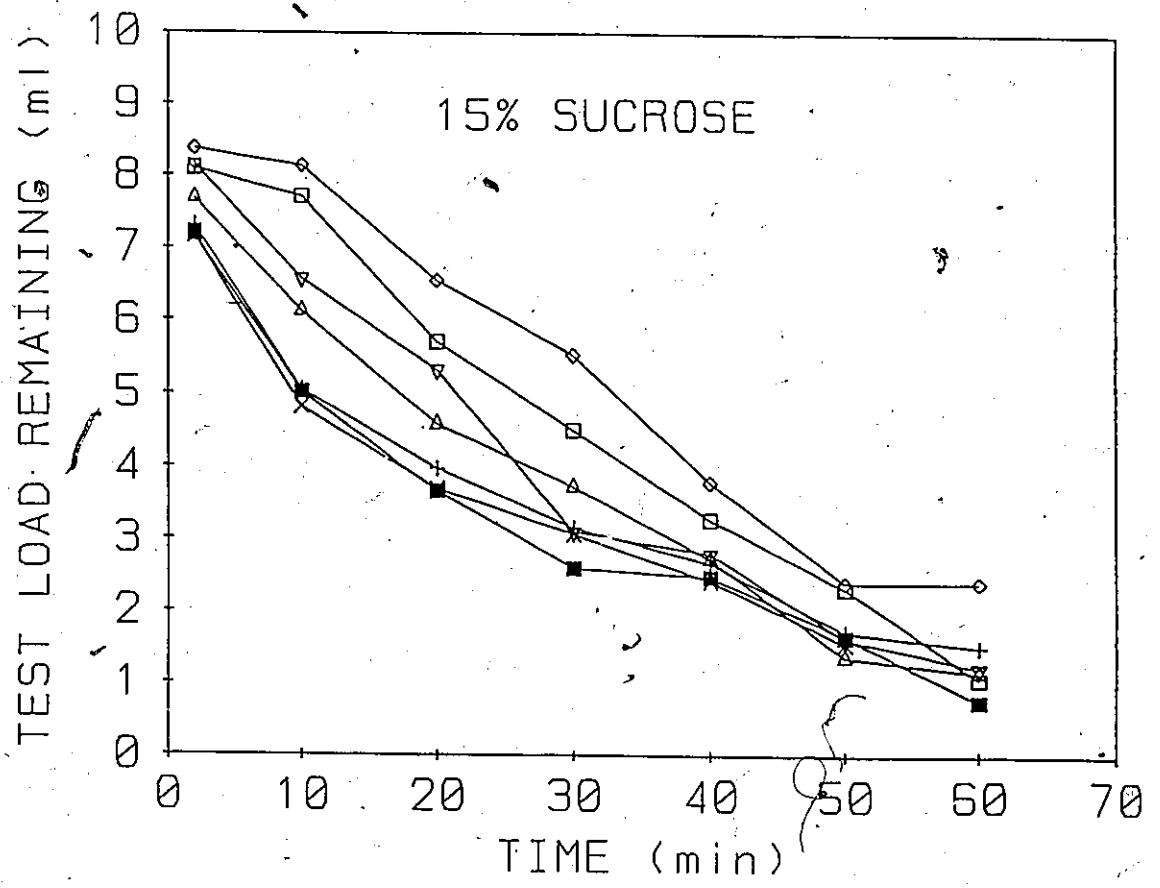
<sup>2</sup>Dose of CCK-8 in ug/kg. All injections were delivered ip in a total volume of 1 ml.

<sup>3</sup>The  $t_{1/2}$  is the average half emptying time (N=6). Numbers in brackets below mean indicate 1 standard error of the mean (SEM).

<sup>4</sup>The  $\beta$  parameter describes the shape of the emptying curve relative to an exponential curve with the same  $t_{1/2}$ . See Chapter 2 for further description.

<sup>5</sup>The median  $R^2$  is the median coefficient of determination of the power exponential curves fitted to the individual time courses for that treatment and indicates the goodness-of-fit.

Figure 6. Time courses for the emptying of 10 ml 15% sucrose test loads following various doses of CCK-8. The curve symbols are labeled as in Figure 1. Test volume remaining is the volume in the stomach corrected for gastric secretion. (N=6).



rapid and followed an exponential curve. In contrast, later emptying of nutrient was slower and linear. This difference was reflected as significant saline versus nutrient differences in  $\beta$ ,  $F(1,10) = 34.78$   $p < 0.0003$ , with  $\beta$ 's following nutrient being smaller than those with IG saline. This difference in  $\beta$  results from the fact that nutrient emptied more slowly than saline in the later phases of gastric emptying.

In spite of the different profile of emptying with sucrose compared to saline, the effects of CCK-8 on sucrose emptying paralleled the data obtained with IG saline. Specifically, with IG sucrose, CCK-8 produced a dose-dependent increase of  $t_{1/2}$  ( $Z = 4.80$ ,  $p < .0001$ ) and  $\beta$  ( $Z = 4.73$ ,  $p < 0.0001$ ). The rapidity of CCK-8's action on emptying was supported most strongly by the fact that the volume of the test load remaining in the stomach even at 2 minutes was greater with increasing dose of CCK-8 ( $Z = 3.95$ ,  $p < 0.0002$ ). CCK-8 only in doses of 5.6 ug/kg and above significantly showed emptying compared to saline (see Table 2). Volumes remaining at doses of .01 and .1 ug/kg CCK-8 were similar to saline in the first 10 min. Following 1.4 ug/kg CCK-8, the volumes remaining appeared to be elevated. However, comparisons using the sensitive one-tailed Dunnet test (Table 2) revealed that this increase was not statistically significant.



TABLE 2

Effect of CCK-8 on Mean Test Load Remaining  
Soon after Peptide Injection

Test Load <sup>3</sup>	Time (min)	Dose CCK-8 <sup>1</sup>							F <sub>2</sub>	
		Sal	0.01	0.1	1.4	5.6	11.2	22.4	F(6,30)	p
<u>Saline</u>										
	2	7.3 <sup>4</sup> (0.3)	7.9 (0.6)	7.8 (0.6)	8.4* (0.2)	8.4* (0.2)	9.0 <sup>+</sup> (0.3)	8.4* (0.01)	2.88	.024
	5	5.8 (0.6)	6.6 (0.9)	7.2 (1.0)	8.5 <sup>+</sup> (0.6)	8.8 <sup>+</sup> (0.3)	8.9 <sup>+</sup> (0.3)	8.9 <sup>+</sup> (0.3)	4.44	.002
	10	4.4 (0.6)	4.1 (0.7)	5.5 (1.2)	6.5 (0.7)	7.4* (0.9)	8.0* (0.7)	7.4* (0.4)	4.49	.002
<u>Sucrose</u>										
	2	7.2 (0.3)	7.2 (0.3)	7.3 (0.3)	7.7 (0.2)	8.2 (0.3)	8.1 (0.6)	8.4* (0.2)	2.26	.064
	10	5.0 (0.6)	4.8 (0.5)	5.1 (0.3)	6.1 (0.3)	6.6* (0.6)	7.7 <sup>+</sup> (0.5)	8.1 <sup>+</sup> (0.4)	10.77	.001
	20	3.7 (0.7)	3.7 (0.5)	4.0 (0.5)	4.6 (0.4)	5.3 (0.8)	5.7* (0.6)	6.6 <sup>+</sup> (0.2)	4.52	.002

<sup>1</sup>Dose of CCK-8 in ug/kg.

<sup>2</sup>The F was derived using a one-way repeated measures ANOVA of the test load data. A separate analysis was conducted for each of the times indicated. Post hoc comparisons were made using Dunnet's one-tailed test. Significantly greater than saline, \* p < 0.05, + p < 0.01.

<sup>3</sup>Test loads were 10 ml of either .15M saline or 15% sucrose infused IG at time 0 min at a rate of 10 ml/min.

<sup>4</sup>Mean volumes (ml) of test load remaining in the stomach (N = 6). Numbers in brackets below mean indicate 1 standard error (SEM).

### III.2 EXPERIMENT 4: EFFECT OF CCK-8 ON FEEDING.

#### INTRODUCTION

To allow clear comparison of the action of CCK-8 on emptying and feeding, the satiety effect of CCK-8 was assessed under similar conditions of food deprivation and gastric manipulation as those in Experiment 3.

#### Protocol

Fifteen subjects were food deprived for 6 hr prior to testing, and, in preparation for each trial, the gastric cannula was opened and the stomach cleaned by saline lavage. The cannula was then reclosed. At time 0, the rats were injected ip with CCK-8 or saline and allowed to consume a 15% (w/v) sucrose solution. (Rats were trained initially to drink the sucrose solution reliably by repeated control trials until their consumption became stable.) Sucrose consumption was measured to the nearest 1 ml, at 5, 10, 15, 20, 25, and 30 minutes after the injection. CCK-8 trials were preceded and followed by control trials in which 1 ml of .15M saline was injected ip. Consumption on saline trials was averaged for each subject to establish the baseline sucrose consumption.

## RESULTS

The mean cumulative consumptions of 15% sucrose over time are shown in Figure 7. Analysis of the meal sizes at 5 min (Table 3) indicated that CCK-8 produced a dose-dependent reduction of intake 5 min after injection ( $F(3,42) = 46.84, p < 0.001$ ). CCK-8, in doses ranging from 1.4-5.6 ug/kg suppressed food intake when assessed at 5, 10 or 30 mins after feeding. Multiple comparisons demonstrated that all the mean consumptions were significantly different from each other (all  $p$ 's  $< 0.01$ ). The dose-dependent suppression of feeding was also apparent at 10 min,  $F(3,42) = 64.62, p < 0.001$ , and 30 min,  $F(3,42) = 25.79, p < 0.001$ .

## Discussion

This study has several advantages over previous work on gastric emptying in the rat, in that it provides simultaneous data on the: i) time course; ii) onset; and iii) degree of emptying inhibition obtained under very similar experimental conditions. Collection of emptying and satiety data in the context of one study is essential to a complete understanding of the relationships between gastric filling, emptying, and satiety.

Doses of CCK-8 in the range of 1.4 to 22.4 ug/kg retard the emptying of saline from the stomach in a dose-dependent manner. This finding is consistent with previous reports of an inhibition of emptying by CCK-8 in rats (Anika 1982; Bertaccini & Scarpignato

Figure 7. Time courses for the cumulative consumption of 15% sucrose meals following various doses of CCR<sub>8</sub>. (N=15).

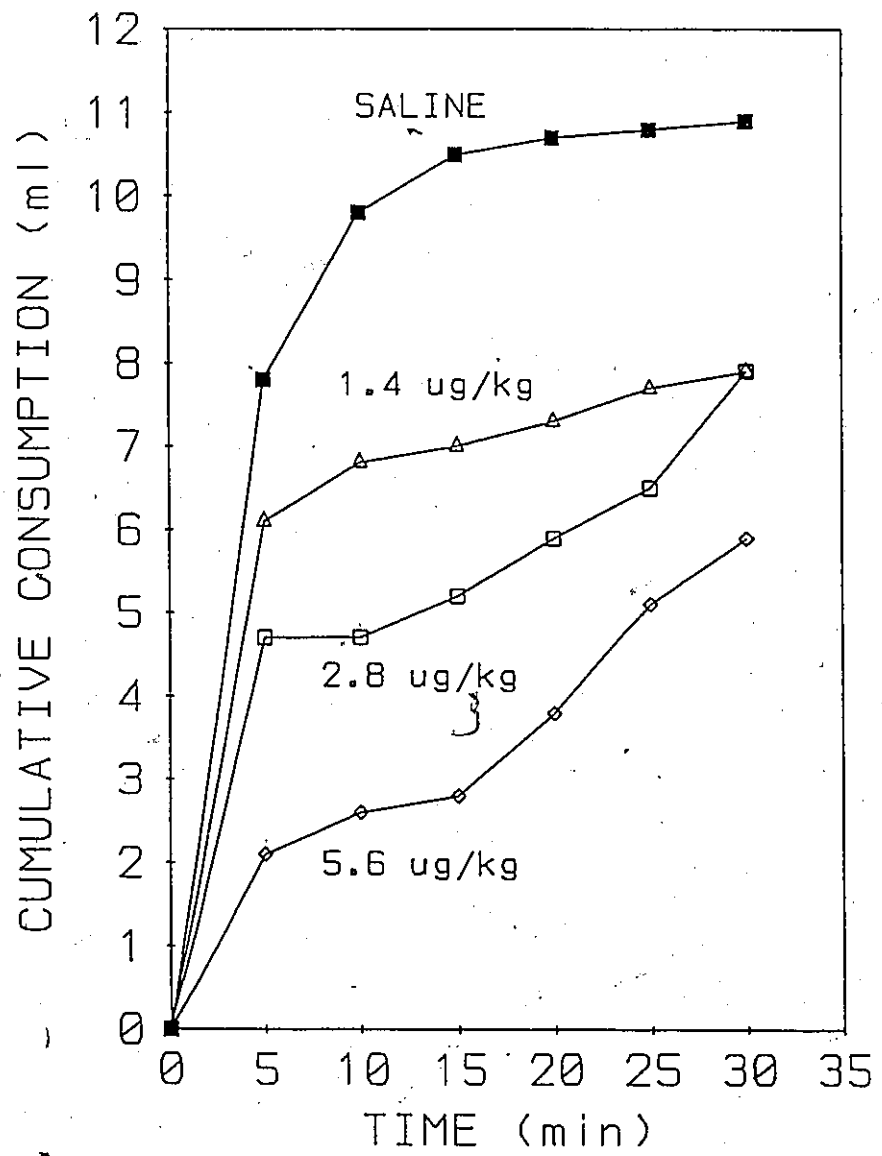


TABLE 3

Effect of CCK-8 on the Consumption of  
15% Sucrose Solution

Consumption	Dose CCK-8 <sup>2</sup>			
	Saline	1.4	2.8	5.6
5 min	7.9 <sup>1</sup> (0.5)	6.1* (0.5)	4.7* (0.7)	2.1* (0.4)
10 min	9.9 (0.6)	6.8* (0.5)	4.7* (0.7)	2.6* (0.4)
30 min	11.2 (0.5)	7.9 <sup>1</sup> (0.6)	7.9 <sup>1</sup> (0.5)	5.9 <sup>+</sup> (0.6)

<sup>1</sup>Mean cumulative consumption (mls) of 15% sucrose solution (N=15).  
Numbers in brackets below mean indicate 1 standard error (SEM).

<sup>2</sup>Doses of CCK-8 are in ug/kg; injections were administered ip at  
time 0.

\*indicates all means significantly different from each other, ( $p < 0.01$ ).

<sup>1</sup>indicates significantly different from saline, ( $p < 0.01$ ).

<sup>+</sup>indicates significantly different from all other means, ( $p < 0.01$ ).

& Schally 1980; Mangel & Koegel 1984), dogs (Yamagishi & Debas 1978; Debas, Farooq & Grossman 1975), hamsters (Miceli 1985), mice (Lotti, Cerino, Kling & Chang 1986,) and humans (Chey, Hitanant, Hendricks et al 1970; Liddle, Morita, Conrad & Williams 1986; Valenzuela & Defilippi 1981). Furthermore, although emptying is generally slower for nutrient solutions, CCK-8 also slowed the emptying of 15% sucrose test loads in a dose-dependent manner. However, the sensitivity of saline and nutrient emptying as assays for the effects of CCK-8 varies slightly. The more rapid emptying characterized by saline IG loads reveals a significant retardation of emptying by doses of CCK-8 as low as 1.4 ug/kg. In contrast, with the overall slowing of emptying produced by nutrient IG, a significant inhibition of emptying by the peptide is first revealed at 5.6 ug/kg CCK-8.

This study was conducted to assess certain predictions instrumental to evaluate the validity of the hypothesis that changes in gastric emptying mediate the satiety effect of CCK-8 (McHugh & Moran 1982). If the gastric emptying hypothesis is correct: 1) CCK-8 must be shown to inhibit emptying especially of the foods used typically to demonstrate the satiety effect of the peptide, and ii) the emptying and satiety effects of CCK-8 must be manifest at similar doses. Both of these predictions were verified.

Although these demonstrations leave open the possibility

that changes in gastric emptying as contribute to CCK's satiety action, they do not demonstrate conclusively that this is the sole mechanism mediating the behavioral effect. In fact, because of the detailed quantitative analyses permitted by the double sampling technique, it seems that some mechanism other than altered emptying rate must participate in CCK-induced satiety. For example, although 1.4 ug/kg CCK-8 does not produce a significant inhibition of emptying within 10 minutes of its administration (see Table 2), it does produce a significant and substantial reduction in eating (approximately 32% suppression) within the same time period. Furthermore, the gastric emptying hypothesis assumes that spontaneous satiety occurs when the stomach has filled to a certain volume; CCK accelerates the onset of satiety by preventing emptying and, thereby forwarding the time at which the stomach reaches that volume. If this hypothesis is correct, the stomach volume at satiety should be the same regardless of whether satiety occurred spontaneously or was accelerated by CCK-8 administration.

Experiment 4 reveals that, meals of 15% sucrose are terminated spontaneously after 10 minutes of eating. The data from Experiment 3b indicate that 5 ml of 15% sucrose are in the stomach at the time of spontaneous satiety. [I also assume that 5 ml is an underestimate of actual stomach volume because loads infused directly IG probably empty faster than orally consumed



food (Cannon 1911, Hunt 1956)]. Therefore, if CCK-8 induces satiety by preventing gastric emptying, the size of meals eaten after CCK administration should, as a minimum, not be less than 5 ml. However, Experiment 3b (see Table 3) reveals that 5.6 ug/kg CCK-8 reduced meal size to 2.6 ml, a value considerably less than the minimum meal size predicted exclusively on the basis of the gastric emptying hypothesis.

Thus, while dose-response and kinetic similarities between the effects of CCK on gastric emptying and feeding in the rat have been demonstrated in this study, the data remain equivocal with respect to validation of the gastric emptying hypothesis.

#### CHAPTER 4.

### CHANGES IN GASTRIC EMPTYING ARE NEITHER NECESSARY NOR SUFFICIENT FOR PEPTIDE INDUCED SATIETY IN THE RAT.

This study uses two approaches to determine the degree to which CCK-induced changes in gastric emptying mediate its satiety action. In Experiment 5, the kinetics of CCK-8's suppression of emptying and feeding are compared. In spite of the short half-life of CCK in plasma, approximately 17 min in rats (Koulischer, Moroder & Deschodt-Lanckman 1982), exogenous CCK-8, reduces feeding even when administered 15 minutes before initiation of a test meal (Collins, Walker, Forsyth & Belbeck 1983; Forsyth, Weingarten & Collins 1985). If suppression of emptying is critical to the satiety effect of CCK, then the ability of CCK-8 to inhibit emptying must also survive a 15 minute delay.

In Experiment 6, the ability of other gut peptides to affect emptying and satiety was examined. If CCK produces satiety because it slows emptying, then any peptide which inhibits emptying to a degree similar to CCK should produce reduction of food intake of similar magnitude.

#### IV.1 EXPERIMENT 5: KINETICS OF FEEDING AND EMPTYING INHIBITION.

##### INTRODUCTION

Cholecystokinin inhibits feeding even when injected 15 minutes before the initiation of eating (Collins, Walker, Forsyth & Belbeck 1983; Forsyth, Weingarten & Collins 1985). This experiment tested whether the effect of CCK-8 on emptying possessed similar kinetics.

##### METHOD

###### Subjects.

Subjects were male, Long-Evans hooded rats weighing between 350-450 gms at testing. Each rat was implanted with a chronically indwelling gastric cannula according to procedures described elsewhere (Weingarten & Powley 1980) and permitted a minimum of two weeks to recover from surgery. Rats were housed individually in a room maintained at 20° C on a 14:10 light:dark cycle. They had continuous access to water and food (Purina Laboratory Rodent chow) except on test days when they were fasted for 6 hours prior to the emptying test.

###### Injections and Protocol

All injections were made up to a volume of 1 ml with .15M

saline and were injected intraperitoneally (ip). Control injections consisted of 1 ml .15M saline. Rats were injected with CCK-8 (5.6 or 11.2 ug/kg) either immediately, or 15 minutes before, an IG test load of .15M saline.

#### Data Analysis

Half emptying times ( $t_{1/2}$ ) and  $\beta$  values were defined as in Experiment 2 (Chapter 2). To test the effects of dose CCK-8 and injection time on emptying inhibition (Experiment 5), two-way repeated measures ANOVAs (Dose CCK-8 by Injection Time) were performed on the  $t_{1/2}$  and  $\beta$  estimates, and upon the test load remaining values for 2, 5, and 10 minutes. Multiple comparisons of the  $t_{1/2}$  and  $\beta$  estimates were performed using the Newman-Keuls procedure. To maximize statistical sensitivity to emptying inhibition, the mean remaining test volumes were compared using Dunnett's test (one-tailed) for experimental treatment vs the control of IP Saline.

The satiety effect of CCK-8 under these experimental conditions was assessed by comparing the intakes of 15% sucrose under saline control trials to intakes following injections of 5.6 ug/kg CCK-8 either immediately, or 15 minutes before, meal presentation.

### RESULTS

Examination of Figure 8 and statistical analysis of corresponding  $t_{1/2}$ s (Table 4) revealed that CCK-8 significantly inhibited emptying,  $F(2,10) = 42.66$ ,  $p < 0.001$  when injected at time 0. Multiple comparisons determined that when injected at time 0, both 5.6 ug/kg ( $q_3 = 5.81$ ,  $p < .01$ ) and 11.2 ug/kg ( $q_5 = 6.18$ ,  $p < .01$ ) CCK-8 significantly raised mean  $t_{1/2}$  over that for saline. An inhibition of the early phase of emptying was also apparent, indicated by a significant increase of  $b$  values with increasing dose ( $F(2,10) = 14.55$ ,  $p < 0.001$ ; see Table 4). Post hoc comparisons revealed that  $\beta$ s following both 5.6 ( $q_4 = 5.81$ ,  $p < .01$ ) and 11.2 ug/kg were significantly higher than control values (respectively:  $q_4 = 5.81$ ,  $q_5 = 6.81$ ,  $p$ 's  $< 0.01$ ).

Injecting the peptide 15 minutes before the test load significantly reduced the ability of the peptide to inhibit gastric emptying. The mean  $t_{1/2}$  following 5.6 ug/kg CCK-8 administered at -15 min was not different than the saline mean at -15 min ( $q_3 = 3.90$ , n.s.). Although 11.2 ug/kg CCK-8 injected at -15 min significantly increased half-emptying time compared to saline ( $q_3 = 5.30$ ,  $p < 0.01$ ), the degree of inhibition was significantly less than when the identical dose was injected at 0 min ( $q_3 = 5.30$ ,  $p < 0.01$ ). The reduced ability of CCK-8 to retard emptying when injected 15 min prior to the IG load was also evident in the analysis of  $\beta$  which revealed that the  $\beta$ 's for CCK-8

Figure 8. Time courses for the emptying of 10 ml saline test loads following CCK-8 (5.6 or 11.2 ug/kg) administered coincident with (0 min) or 15 min before (-15 min) infusion of the test load. Test load remaining is the volume remaining in the stomach corrected for gastric secretion. (N=6).

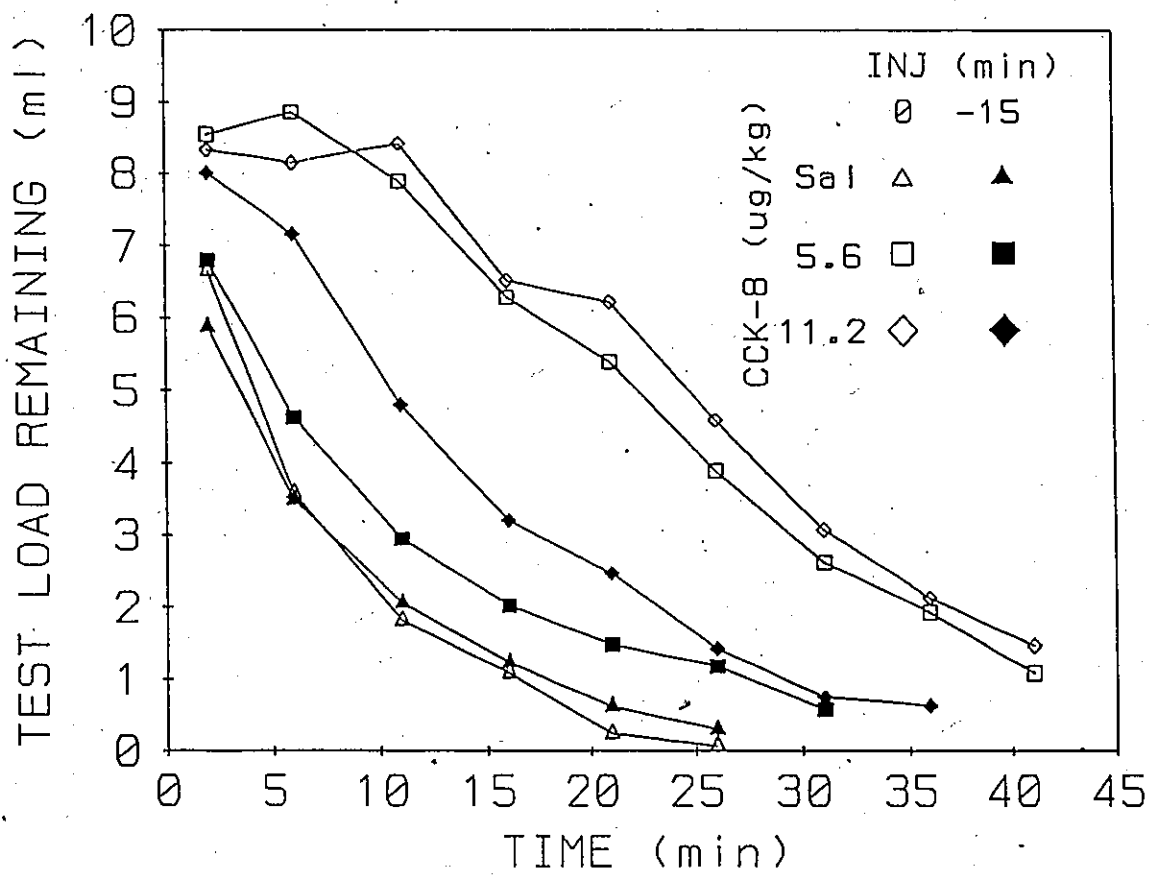


TABLE 4

Effect of Injection Time on the  
Inhibition of Gastric Emptying by CCK-8

Injection Time <sup>1</sup>		Dose CCK-8(ug/kg)		
		Saline	5.6	11.2
0	$t_{1/2}$ <sup>2</sup>	3.8 (0.4)	20.8** (2.3)	23.5** (2.4)
	$\beta$ <sup>3</sup>	0.9 (0.06)	1.7** (0.22)	1.8** (0.22)
	Median R <sup>2</sup>	.99	.92	.88
	-----			
-15	$t_{1/2}$	3.1 (0.4)	5.3 (1.2)	10.4* (1.4)
	$\beta$	0.7 (0.06)	0.7 (0.08)	1.1 (0.06)
	Median R <sup>2</sup>	.98	.99	.98

<sup>1</sup>Represents time (in min) at which CCK-8 was injected relative to infusion of the gastric test load.

<sup>2</sup>The mean  $t_{1/2}$  is the time (min) at which half the test load had emptied. Numbers in brackets below mean indicate 1 standard error of mean (SEM).

<sup>3</sup>See text for interpretation of mean  $\beta$  values.

\*  $p < .01$  when compared to saline.

+  $p < .01$  when compared to corresponding dose of CCK-8 administered at -15 min.



at both 5.6 and 11.2 ug/kg were not significantly greater than that for saline. To be confident that 5.6 ug/kg CCK-8 injected at -15 min did not inhibit emptying, the volumes of test load remaining at times 2, 5, and 10 min after peptide administration were compared, using Dunnet's one tailed test, to those following saline (See Table 5). Stomach volumes following CCK-8 5.6 ug/kg at -15 min were similar to control values at 2, 5 and 10 minutes, and so reinforced the conclusion that 5.6 ug/kg CCK-8 did not inhibit emptying when administered 15 min before the IG load.

Analysis of sucrose consumption at 5 min (see Figure 9 and Table 6) revealed that CCK-8 significantly reduced meal size when injected at 0 min ( $q_4 = 1.20$ ,  $p < 0.01$ ), indicating that the peptide acts rapidly. CCK-8 injected coincident with meal initiation also reduced intake at 15 ( $q_4 = 2.54$ ,  $p < .01$ ) and 30 ( $q_4 = 2.11$ ,  $p < .01$ ) min.

Although the magnitude of eating suppression following CCK-8 at -15 min was significantly reduced compared to feeding after CCK-8 at 0 min ( $q_2$ 's = 0.95 & 2.01 at 5 and 15 min respectively,  $p$ 's < 0.01), CCK-8 administered at -15 min (Table 6) did result in significant reductions of intake at 5, 15 and 30 min, ( $q_3$ 's = 1.10, 2.34 & 1.84 respectively,  $p$ 's < 0.01), indicating that the satiety effect persisted over a 15 min interval.

The results of Experiment 5 indicate different kinetics of the CCK-induced emptying and feeding effects. The time frame over

TABLE 5

Effect of CCK-8 on Test Load Remaining in Stomach  
at Times Indicated after IG Infusion

Time <sup>4</sup> (min)	CCK-8 Injection Time <sup>1</sup>						F(2,10) <sup>3</sup>	p
	0 min			-15 min				
	Dose <sup>2</sup>			Dose				
	Saline	5.6	11.2	Saline	5.6	11.2		
2	6.7 <sup>5</sup> (0.3)	8.6 <sup>+</sup> (0.3)	8.3 <sup>+</sup> (0.2)	5.9 (0.5)	6.8 (0.4)	8.0* (0.3)	3.22	0.082
5	3.6 (0.2)	8.9 <sup>+</sup> (0.5)	8.2 <sup>+</sup> (0.2)	3.5 (0.2)	4.6 (0.7)	7.2 <sup>+</sup> (0.6)	24.62	0.001
10	1.8 (0.3)	7.9 <sup>+</sup> (0.8)	8.4 <sup>+</sup> (0.5)	2.0 (0.2)	2.9 (0.5)	4.8 <sup>+</sup> (0.5)	25.80	0.001

<sup>1</sup>Time (min) at which CCK-8 was injected relative to infusion of the test load.

<sup>2</sup>In ug/kg.

<sup>3</sup>F ratios for Injection by Time of Dose interaction for mean test volumes remaining at 2, 5 & 10 min.

<sup>4</sup>The time at which the test volumes remaining were measured relative to the onset of infusion.

<sup>5</sup>The mean test load remaining. Numbers in brackets below mean indicate 1 standard error (SEM).

\*significantly greater than Saline at 0 min,  $p < 0.05$

+significantly greater than Saline at 0 min,  $p < 0.01$

Figure 9. Cumulative consumption of 15% sucrose following 5.6 ug/kg CCK-8, administered coincident with (0 min) or 15 min before (-15 min) meal presentation. (N=15).

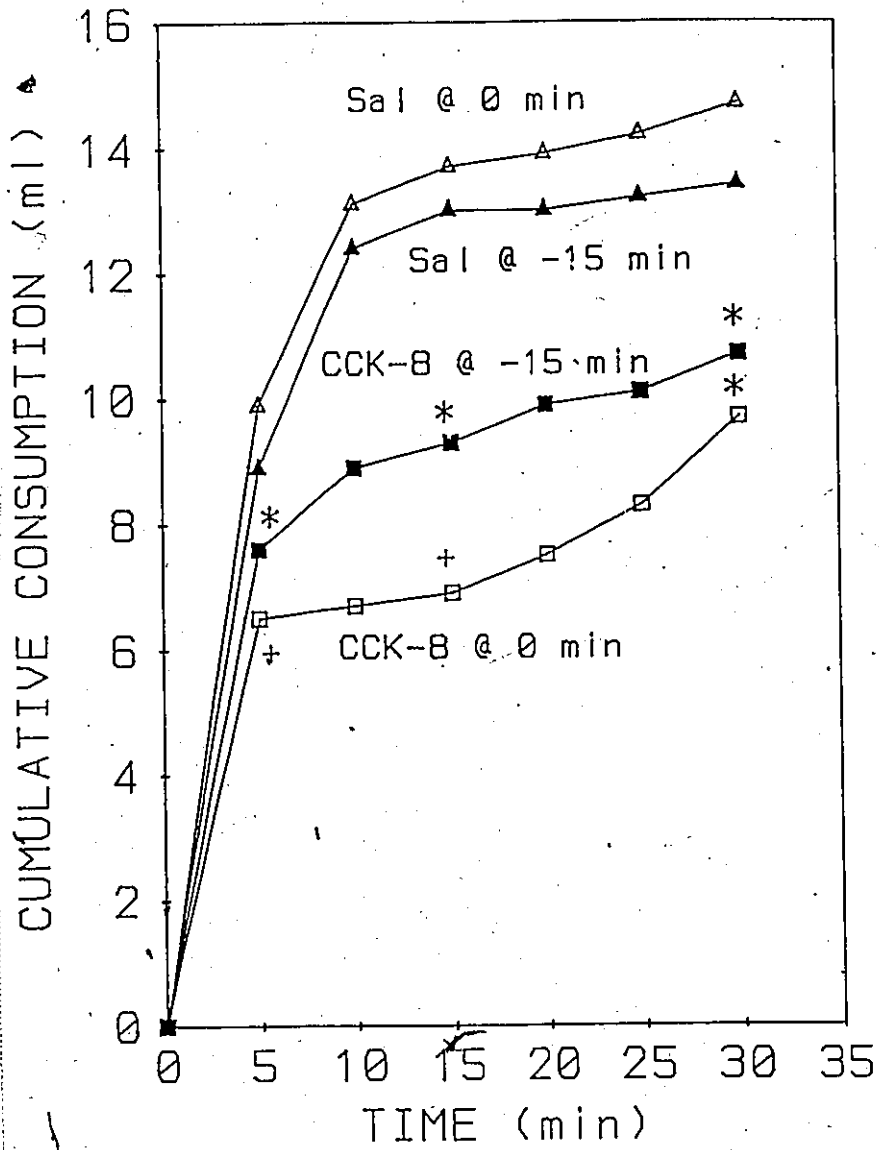


TABLE 6

Effect of Injection time on CCK-8-Induced Satiety

Injection Time <sup>2</sup>	Dose CCK-8 <sup>1</sup>	Consumption <sup>3</sup>		
		5 min	15 min	30 min
0	Saline	10.1 (0.5)	13.7 (1.0)	14.7 (0.8)
	5.6	6.5*+ (0.5)	6.9*+ (0.6)	9.7* (0.8)
-15	Saline	9.2 (0.5)	13.2 (0.8)	13.6 (0.9)
	5.6	7.6* (0.4)	9.3* (0.7)	10.6* (0.8)

<sup>1</sup>In ug/kg.

<sup>2</sup>Time (min) at which CCK-8 was injected relative to presentation of the test meal.

<sup>3</sup>The mean consumptions (in mls) of 15% sucrose at 5, 15 and 30 minutes. Numbers in brackets below mean indicate 1 standard error (SEM).

\* $p < .01$  when compared to corresponding saline condition.

+ $p < .01$  when compared to corresponding dose of CCK-8 when administered at -15 min.

which CCK influences emptying is brief and consistent with the demonstrated short half-life of the peptide (Koulisher, Moroder & Deschodt-Lanckman 1982). In comparison, the ability of CCK-8 to suppress feeding survives longer intervals. The finding that CCK-8 can suppress feeding after losing the ability to retard emptying argues against the hypothesis that emptying inhibition mediates the satiety effect of CCK-8.

#### IV.2 EXPERIMENT 6: EFFECT OF OTHER GUT PEPTIDES ON EMPTYING AND FEEDING.

##### INTRODUCTION

If inhibition of gastric emptying is the mechanism whereby CCK produces satiety, then other peptides which yield an emptying inhibition comparable to CCK-8 should produce a similar suppression of meal size.

##### METHOD

###### Subjects.

Subjects were male, Long-Evans hooded rats weighing between 350-450 gms at testing. Each rat was implanted with a chronically indwelling gastric cannula according to procedures described elsewhere (Weingarten & Powley 1980) and permitted a minimum of

two weeks to recover from surgery. Rats were housed individually in a room maintained at 20° C. on a 14:10 light:dark cycle. They had continuous access to water and food (Purina Laboratory Rodent chow) except on test days when they were fasted for 6 hours prior to the emptying test.

### Injections

Pentagastrin (Peptavlon) was purchased from Ayerst Laboratories, Montreal, Canada. Cimetidine (Tagamet) was purchased from Smith Kline & French Canada Ltd. Bombesin (B 5508) and secretin (S 5014) were purchased from Sigma Co (St. Louis, MO). All injections were made up to a volume of 1 ml with .15M saline and were injected intraperitoneally (IP). Control injections consisted of 1 ml .15M saline.

### Peptide Administration Protocol.

To examine the effect of pentagastrin on emptying, subjects received two injections at time 0 consisting of: i) saline and saline; ii) saline and pentagastrin; iii) cimetidine and saline; or iv) cimetidine and pentagastrin. Doses used were 100 ug/kg pentagastrin and 5 mg/kg cimetidine. Feeding studies used the identical dose of pentagastrin. To examine the ability of bombesin to inhibit gastric emptying, rats were injected with either saline or that peptide at doses of 8 and 16 ug/kg. Feeding

studies with bombesin used the 8 ug/kg dose only. The effect of secretin on emptying used 2.86, 14.3, and 28.6 ug/kg corresponding to doses of 10, 50, and 100 clinical units/kg. Feeding studies only used secretin doses of 14.3 and 28.6 ug/kg. For feeding studies, all injections were administered immediately before the IG load or meal respectively.

#### Data Analysis

The effects of pentagastrin and cimetidine on  $t_{1/2}$ ,  $\beta$  and gastric secretion were assessed using two-way repeated measure ANOVAs. Dunnett's two-tailed was used for post hoc comparisons of the treatment means. One-way repeated measures ANOVAs were used to analyze the effect of bombesin and secretin on  $t_{1/2}$  and  $\beta$ , and multiple comparisons were performed by the Newman-Keuls test.

Feeding data were analyzed with one-way repeated measure ANOVAs on the cumulative sucrose consumptions at 5, 15 and 30 minutes. Multiple comparisons used the Newman-Keuls method.

#### RESULTS

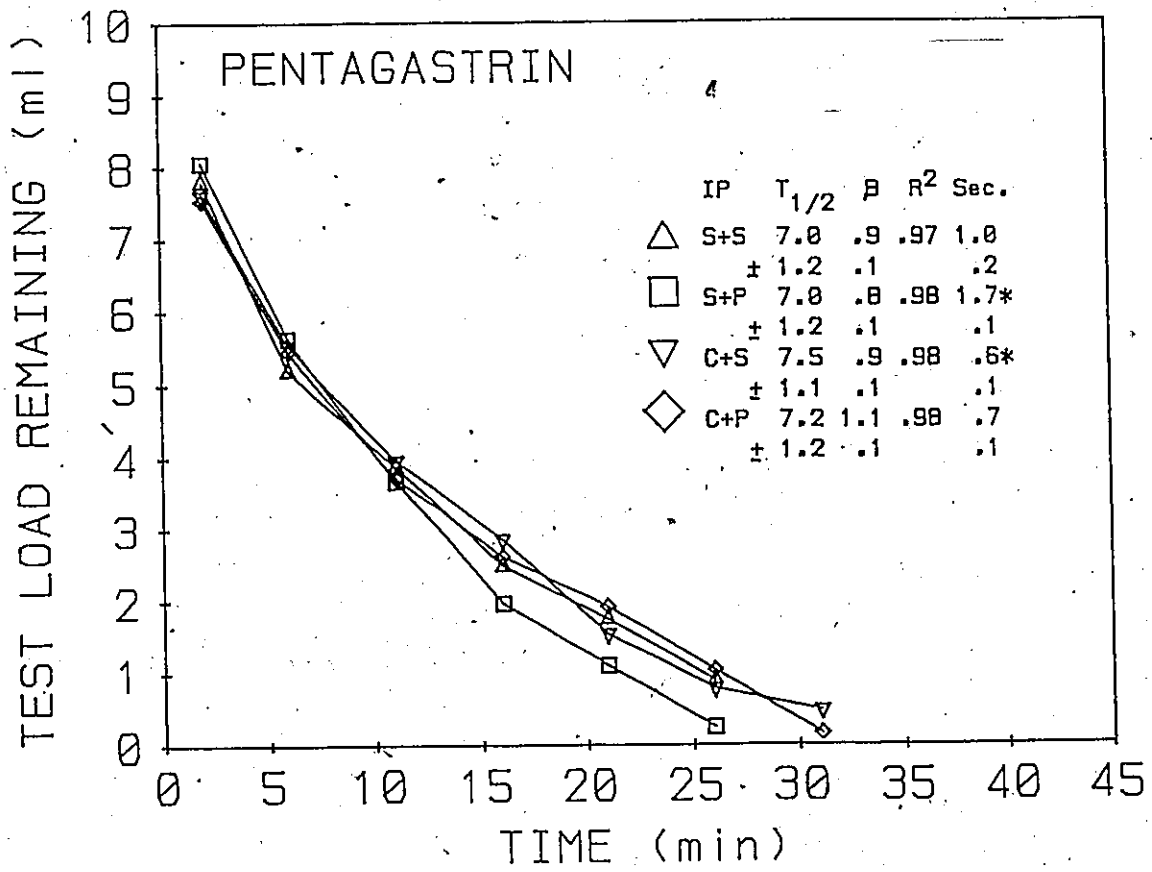
Figure 10 and statistical analysis indicated that 100 ug/kg pentagastrin significantly increased gastric secretion during the first 20 min  $F(1,5) = 9.67$ ,  $p < 0.03$ , an effect which was blocked by cimetidine,  $F(1,5) = 27.98$ ,  $p < 0.005$ . Nevertheless, 100 ug/kg pentagastrin had no effect on the time course of gastric emptying,



Figure 10. Time courses for the emptying of a 10 ml saline IG test load in the following test conditions: (S+S) saline + saline; (S+P) saline + pentagastrin; (C+S) cimetidine + saline; or (C+P) cimetidine + pentagastrin. See text for doses and Chapter 2 for explanation of  $t_{1/2}$ ,  $\beta$ , and  $R^2$  measures. Gastric secretion was measured in ml over the first 20 min.

\*  $p < .05$  Dunnet's two-tailed test. (N=6).

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verified by the absence of significant changes in  $t_{1/2}$ ,  $F(1,5) = 0.02$ , NS, or  $\beta$ ,  $F(1,5) = 0.27$ , NS compared to saline trials. The amount eaten following 100 ug/kg pentagastrin was also not significantly different from control (Table 7).

Bombesin (8 & 16 ug/kg) had only a minor impact on the time course of gastric emptying (Figure 11). Although  $t_{1/2}$  values following administration of bombesin were not significantly different from those following saline  $F(2,10) = 1.19$ ,  $p < 0.345$ , there was a small, but significant, increase in  $\beta$  at both the 8 ug/kg ( $q_2 = 0.17$ ,  $p < .05$ ), and 16 ug/kg, ( $q_3 = 0.21$ ,  $p < 0.05$ ) doses. This increase in  $\beta$ , approximately 20%, is small compared to the 100% increase produced by CCK-8 in Experiment 5, suggesting that bombesin produces only a small and transient retardation of emptying. In spite of the small emptying effects, even the lower dose of bombesin, 8 ug/kg, profoundly suppressed eating (Table 7) measured at 5, 15 and 30 minutes after meal initiation ( $F(1,14)$ 's = 19.10, 48.58 & 46.79 respectively, all  $p$ 's  $< 0.001$ ). The percent suppression produced by bombesin, 49% at 15 min, is similar to the level of inhibition produced by CCK-8.

Secretin significantly inhibited gastric emptying in a dose-related manner, indicated by an increase in  $t_{1/2}$ 's with dose,  $F(3,15) = 31.94$ ,  $p < 0.001$  (Figure 12). Secretin, 14.3 and 28.6 ug/kg, produced elevated, but equivalent, increases in  $t_{1/2}$  over saline ( $q_3 = 4.84$  &  $q_4 = 5.25$  respectively,  $p$ 's  $< 0.01$ ).

TABLE 7

Effect of Pentagastrin, Bombesin and Secretin on  
Consumption of 15% Sucrose

Peptide <sup>1</sup>	Dose Peptide <sup>2</sup>	Consumption <sup>3</sup>		
		5 min	15 min	30 min
Pentagastrin	Saline	8.0 (0.7)	10.3 (0.7)	10.6 (0.7)
	100	7.3 (0.7)	10.1 (0.7)	10.7 (0.7)
Bombesin	Saline	7.7 (0.6)	10.6 (0.8)	10.9 (0.8)
	8	5.1* (0.6)	5.4* (0.7)	5.7* (0.7)
Secretin	Saline	8.1 (0.6)	11.0 (0.7)	11.4 (0.6)
	14.3	7.1 (0.6)	10.5 (0.8)	11.7 (0.5)
	28.6	5.3+ (0.7)	8.1+ (0.6)	9.3+ (0.6)

<sup>1</sup>Peptides were injected immediately before presentation of the test meal.

<sup>2</sup>In ug/kg.

<sup>3</sup>Mean consumptions (in mls) of 15% sucrose at 5, 15 and 30 min. Numbers in brackets below mean indicate 1 SEM.

\*  $p < .01$  compared to corresponding mean for saline.

+  $p < .01$  compared to corresponding means for saline and smaller dose of secretin.

Figure 11. Time courses for the emptying of a 10 ml saline test load after ip injection of saline or bombesin (8 & 16 ug/kg). (N=6).

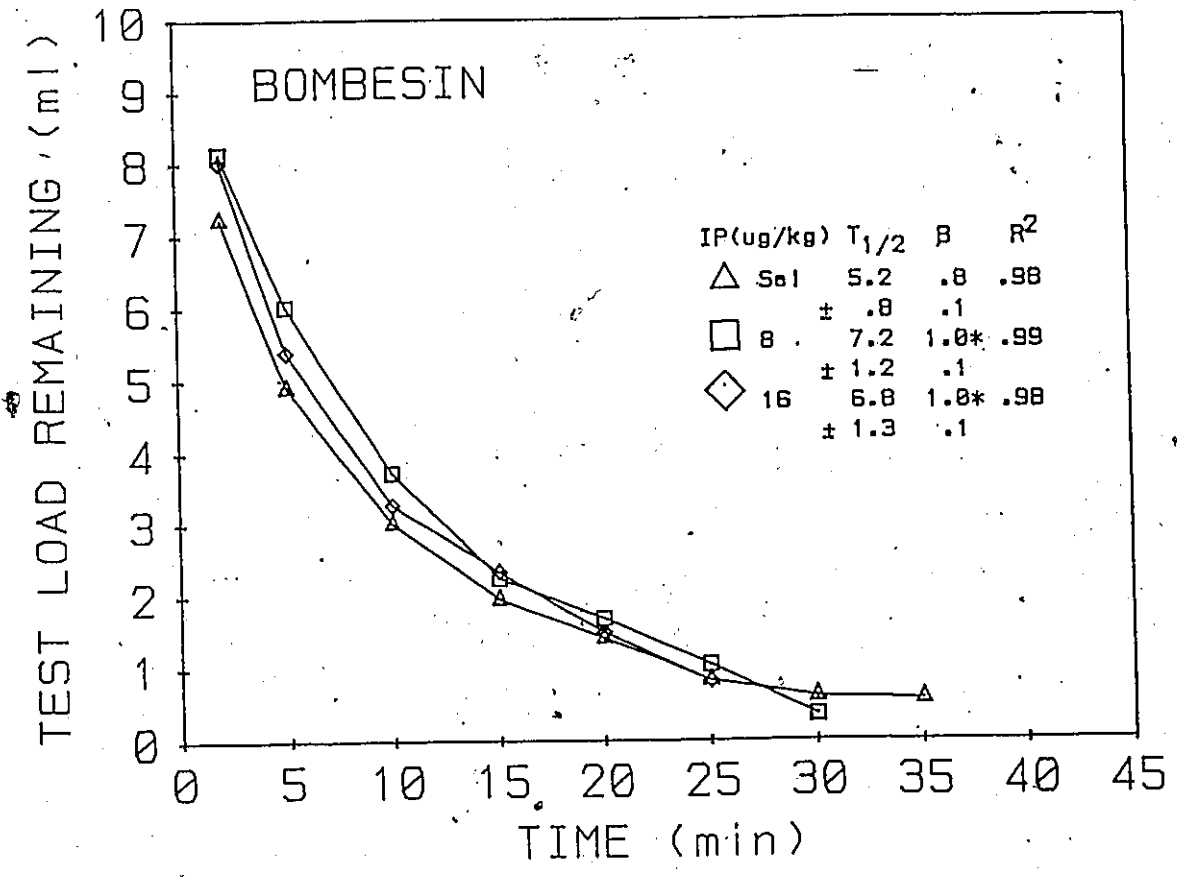
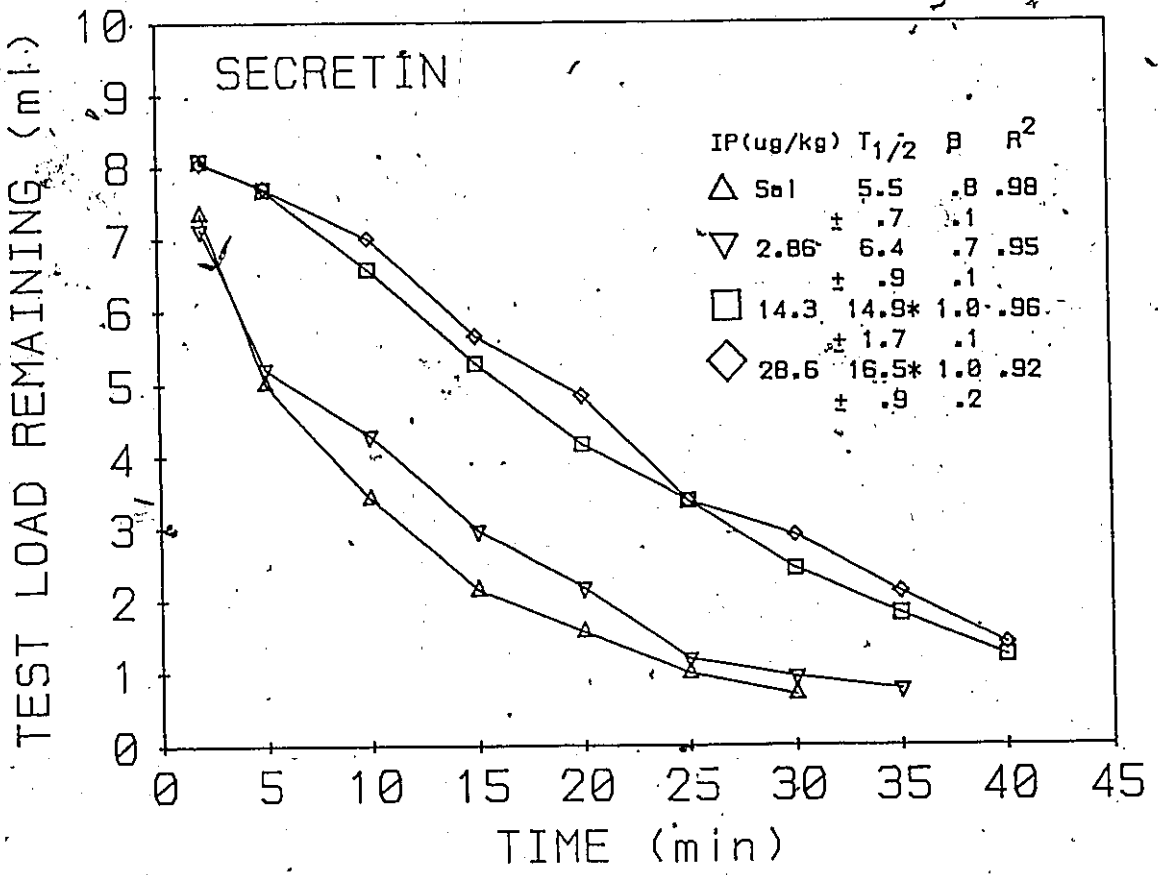


Figure 12. . . Time courses for the emptying of a 10 ml saline test load after ip injection of saline or secretin (2.86, 14.3 & 28.6 ug/kg). (N=6).





The lowest dose of secretin used, 2.86 ug/kg, did not alter the time course of emptying.

Multiple comparisons revealed that only secretin at 28.6 ug/kg significantly reduced sucrose consumption (Table 7).

Secretin 14.3 ug/kg, had no significant effect on sucrose intake, even though this dose retards emptying to the same degree as the highest dose of secretin and 1.4 ug/kg CCK-8 (see Experiment 3).

#### DISCUSSION

The purpose of this study was to examine the importance of gastric emptying in the mediation of peptide-induced satiety. The motivation for these examinations is the necessity of evaluating the most viable hypothesis regarding CCK's mechanism of behavioral action, i.e., that its satiety is mediated by its ability to retard the rate of gastric emptying (McHugh & Moran 1986; Moran & McHugh 1982; Robinson, Moran, Goldrich & McHugh 1987). The results of the current and previous studies (Aylon, Falasco, Smith & Gibbs 1986; Falasco, Joyner, Aylon, Gibbs & Smith 1986; Chapter 3) demonstrating that CCK-8 suppresses gastric emptying in a dose dependent manner are consistent with this hypothesis.

However, the manipulations employed in this study, which investigate CCK's response kinetics and the ability of other peptides to modulate emptying and eating, demonstrate several important dissociations between the emptying response and satiety.

These results are summarized in Table 8 and are discussed in more detail below.

CCK-8 injected coincident with meal initiation produces a dose dependent inhibition of gastric emptying and meal size. These data are consistent with previous reports of an inhibition of gastric emptying with this peptide (Anika 1982; Aylon, Falasco, Smith & Gibbs 1986; Falasco, Joyner, Aylon, Gibbs & Smith 1986; Moran & McHugh 1986; and Chapter 2). This correlation between CCK's effects on emptying and satiety are congenial with the gastric emptying hypothesis. Similarly, the correlation reported in this study between the inability of the structurally-related peptide, pentagastrin, to suppress both emptying and eating is consistent with the predictions of the gastric emptying hypothesis.

The kinetics of the CCK-induced emptying response, however, do not coincide with the kinetics of CCK-induced satiety. Specifically, CCK-8 injected 15 min prior to the emptying test does not affect the rate of gastric emptying. This observation is consistent with the demonstrated short half-life of the peptide in plasma (Koulisher, Moroder & Deschodt-Lanckman 1982). In contrast, administering CCK-8 15 min prior to meal preserved a significant and large suppression of food intake. The suppression of feeding behavior when there is no corresponding change in the rate of gastric emptying suggests that changes in gastric emptying

TABLE 8

Comparison of the Effects of Gastrointestinal Peptides  
on Satiety and Gastric Emptying.

<u>Treatment</u>	<u>Induces Satiety</u>	<u>Inhibits Emptying</u>
CCK-8 (5.6 ug/kg)@ 0 min	Y	Y
Pentagastrin (100 ug/kg)	N	N
CCK-8 (5.6 ug/kg)@ -15 min	Y	N
Bombesin (8 ug/kg)	Y	N
Secretin (14.3 ug/kg)	N	Y

are not necessary for CCK-induced satiety. One possible interpretation of these data is that there exists a subpopulation of peripheral CCK receptors responsible for satiety which are independent of a receptor population mediating CCK-induced emptying changes. Alternatively, the same population of receptors may mediate both responses. However, the time course of the induced emptying changes are comparatively brief, whereas the effect of that peptide on food intake is considerably longer.

The data comparing the effects of bombesin on emptying and satiety further reveal the dissociation between changes in gastric emptying and food intake produced by peptides. Bombesin in a dose of 8 ug/kg produced a profound and reliable suppression of feeding. This finding is consistent with numerous similar demonstrations (Collins, Walker, Forsyth & Belbeck 1983; Kulkosky, Gibbs & Smith 1982; Taylor & Garcia 1985; Wager-Srdar, Morley & Levine 1986). In contrast, this dose of bombesin produced only a trivial effect on the rate of gastric emptying. The effects of bombesin on gastric emptying reported in the literature have been equivocal. Some (Falasco, Joyner, Avilon, Gibbs & Smith 1986; Scarpignato, Micali, Vitualo, Zimbaro & Bertaccini 1982) have found a decrease in gastric emptying with ip. bombesin; others (Porreca & Burks 1983) have failed to find any effect. It is not clear why different investigators have found different results, although considerations such as dose, species, time of peptide

injection relative to test load, and sensitivity of the preparation used to measure gastric emptying, are all candidates. The current study was designed deliberately so that the protocols used to assess the effects of the peptide on emptying and satiety would be identical. Thus, the currently-demonstrated dissociation between the effects of bombesin on emptying and satiety demonstrate that changes in stomach emptying are not necessary for peptide-induced satiety. This conclusion with respect to bombesin is not novel, as investigators have already speculated that the mechanism underlying bombesin-induced satiety differs from that of CCK-induced satiety (Gibbs & Smith 1984).

The secretin data demonstrate that changes in gastric emptying are not sufficient for the induction of satiety. Using our preparation, secretin inhibited gastric emptying in a dose dependent manner, a finding which is consistent with several other reports in the literature relating secretin to gastric emptying (Chey, Hitanant, Hendricks & Lorber 1969; Falasco, Joyner, Avilon, Gibbs & Smith 1986; Valenzeula & Defilippi 1981). In our hands, a dose of 14.3 ug/kg secretin produced an inhibition of gastric emptying identical to that produced by a dose of 1.4 ug/kg CCK-8. However, the critical observation is that the same dose of CCK-8 resulted in a significant inhibition of food intake whereas 14.3 ug/kg secretin, a dose that retarded gastric emptying to a rate equivalent to that of 1.4 mg/kg CCK-8, produced no satiety

whatsoever. These results are consistent with previous reports that secretin does not affect meal size (Falasco, Joyner, Aylon, Gibbs & Smith 1986; Schally, Redding & Lucien 1967).

In summary, these data demonstrate that changes in gastric emptying are neither necessary nor sufficient for peptide-induced satiety. The degree to which changes in gastric emptying contribute to the satiety action of CCK is still somewhat open. The observation that gastric emptying is neither necessary nor sufficient for peptide induced satiety does not suggest that in the case of CCK-8 there is no contribution of inhibition of gastric emptying to the satiety effect of the peptide. The current results suggest, however, that the changes in gastric emptying cannot represent the sole mechanism whereby CCK induces satiety in the rat.

CHAPTER 5  
GENERAL DISCUSSION

The purpose of this thesis was to test the hypothesis that the satiety-effect of peripheral CCK is mediated by inhibition of gastric emptying. This work has provided necessary support for the hypothesis by employing a specially developed procedure (Chapter 2) to demonstrate that doses of CCK-8 sufficient to suppress feeding also retard emptying (Chapter 3). Assessment of the kinetics of emptying and feeding in response to CCK-8 revealed that the correlation was not high (Chapter 4). Moreover, comparison of the emptying and feeding effects of other peptides demonstrated that emptying inhibition is neither necessary or sufficient for satiety (Chapter 4). These dissociations suggest that emptying inhibition and satiety are at best weakly linked.

In addition to testing the gastric mediation hypothesis, this thesis provides new data that address the issue of how CCK-8 induces satiety. The finding that doses of secretin and CCK-8 matched for the inhibition of emptying have differing effects on feeding (Experiment 6), suggests that comparison of the gastrointestinal responses to these peptides may provide a clue to the basis of CCK's satiety effect. Thus the proposal that

contraction of the pyloric sphincter is critical (Robinson et al. 1987) seems unlikely because both peptides induce constriction of the pylorus (Fisher, Lipshutz & Cohen 1973, Phaosawasdi & Fisher 1982). Moreover, the fact that these hormones produce similar decreases in intragastric pressure (Dinoso, Chey, Hendricks & Lorber 1969; Sugawara, Isaza, Curt & Woodward 1969; Valenzuela 1976) indicates that relaxation of the proximal stomach may not account for the behavioral difference.

The hormones do vary in their affect on motility of isolated antral muscle, in that CCK-8 causes contraction (Morgan, Schmalz, Go & Szurszewski 1978; Cameron, Sidney, Phillips, William & Summerskill 1970; Gerner & Haffner 1978) while secretin does not (Cameron et al. 1970; Chey, Kosay, Hendricks, Braverman & Lorber 1969; Schmalz & Beeler 1977). However, antral contractions are not sufficient for the satiety effect of CCK, because gastrin also increases antral motility (Cameron et al. 1970; Gerner & Haffner 1978; Schmalz & Beeler 1977), but has no effect on feeding (Experiment 6; Lorenz et al. 1979). Similarly, the fact that CCK and secretin have opposite effects on intestinal intraluminal pressure (Ramirez & Farrar 1970; Gutierrez, Chey & Dinoso 1974) cannot account for the CCK-induced satiety, because gastrin produces an pressure increase comparable to CCK (Stewart & Burks 1980), but has no effect on feeding. While subject to the hazards of comparing the results across many species and preparations,



this analysis does not point to emptying changes as being central to feeding control by CCK.

The issue of where CCK acts in the periphery to reduce meal size remains open. There are various strategies by which to discover the peripheral CCK "satiety-receptors".

One approach is to seek CCK receptor subpopulations that are sensitive to different CCK antagonists. Once the antagonists are developed, then the satiety receptors may be revealed by determining which antagonist blocks the effect of CCK on feeding. Some progress along this path has already been achieved. CCK antagonists that discriminate receptors in the Area Postrema from pancreatic and intestinal receptors have been developed (Moran, Shnayder, Jensen, Sawyer & McHugh 1987). Application of these antagonists may confirm that CCK receptors in the Area Postrema do not contribute to CCK-induced satiety (Edwards, Ladenheim & Ritter 1986).

The question of where CCK acts in the gut to suppress feeding may be illuminated by recently developed antagonists that are specific to muscular or neural CCK receptors (Takahashi, Yamamura, Kusunoki, Kantoh, Ishikawa & Utsunomiya 1987). These antagonists may tell us whether CCK acts on smooth muscle to inhibit feeding, or if the CCK satiety receptors are directly on nerve afferents.

Another way to localize the satiety-relevant CCK receptors

is to administer CCK into different sites in the circulatory system. Receptor sites close to the entry portal should be indicated by increased efficacy of the CCK infusion. Preliminary work along these lines has been performed in the pig (Houpt 1983). This research revealed that the major site of action for the satiety-effect of CCK lies in the bed of the cranial mesenteric or coeliac arteries. Infusion into the gastric branch of the splenic artery increased feeding. This increase is interesting in light of the knowledge that CCK causes the proximal stomach to relax (Dinosa et al. 1969; Sugawara et al. 1969; Valenzuela 1976), and supports the notion that gastric volume or pressure influences meal size. Portal vein and caudal aortic artery infusions had no effect on feeding, indicating that hepatic CCK receptors are not involved in CCK-induced satiety. In a more recent study direct infusion of CCK-8 into the hepatic-portal vein in rats had no effect on feeding (Greenburg, Smith & Gibbs 1987). Because intraperitoneal administration of CCK-8 produced suppression of feeding, it was inferred that the liver was sequestering or degrading the CCK-8. These results were taken to suggest that if endogenous CCK has a satiating action, then the receptors must be paracrine.

The vagotomy data indicate that at least part of the circuit mediating the peptide's behavioral effects must involve a peripheral (presumably gastrointestinal) component. Although

brain CCK receptors are identified, and they may have some satiety effect, their contribution to satiety induced by peripheral ip. injections of CCK is unclear.

The results of this thesis do not identify the peripheral receptors or the mechanism mediating CCK-induced satiety. However, I believe this thesis does provide a model for how these sites and mechanisms can be identified. First, any putative method must be integrated into the known physiological effects of CCK-8. Then, sensitive procedures for monitoring that function or response must be developed. Finally, rigorous and quantitative experiments must be conducted to evaluate the adequacy of the hypothesis. The gastric mediation hypothesis was attractive because it was the first theory linking CCK's satiety effect to known physiological effects of the peptide. The present data do not support the viability of this hypothesis. As reviewed, there are other peripheral and central sites upon which CCK acts. At present, the receptors mediating pancreatic secretion, smooth muscle motility, and neural receptors, and other unidentified groups, are equally likely candidates for CCK-induced satiety.

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## APPENDIX

### Calculation of Volumes

#### Gastric Volume

The basis for George's (1968) technique is the principle of dye dilution. If one mixes a solution of known volume and dye concentration with an unknown volume, the resulting dye concentration can be used to estimate the unknown volume. Assuming that the unknown volume does not change during its measurement, no dye is lost, and that the probe and unknown volumes are completely mixed we have the following mathematical relationship:

Where:

$V_g$  - Unknown gastric volume

$C_g$  - Initial concentration of dye in  $V_g$

$V_p$  - Volume of probe solution to be mixed with  $V_g$

$C_p$  - Concentration of dye in  $V_p$

$C'_g$  - Concentration of dye in mixture of  $V_g$  and  $V_p$

The mass of dye in  $V_g$  is given by:  $D_g = V_g C_g$

The mass of dye in  $V_p$  is given by:  $D_p = V_p C_p$

The mass of dye in  $V_g + V_p$  is given by:  $D'_g = (V_g + V_p) C'_g$

Now, assuming that no dye is lost during the sampling procedure:

$$\text{i.e.: } D_g + D_p = D'_g$$

$$\text{Therefore: } V_g C_g + V_p C_p = (V_g + V_p) C'_g$$

$$\text{Rearranging: } V_g = V_p (C_p - C'_g) / (C'_g - C_g)$$

The double sampling method is applied in the following manner. First, the contents of the stomach are thoroughly mixed and a small volume,  $V_a$  is removed at time  $t$  to determine the initial dye concentration,  $C_g$ . Immediately after taking  $V_a$ , the probe volume of dye,  $V_p$ , of known concentration,  $C_p$ , is injected and mixed with the volume in the stomach,  $(V_g + V_p)$ . Another sample,  $V_a'$  is then taken at time  $t'$ , to determine the resulting concentration,  $C'_g$ . Thus, the volume of the stomach at time  $t_1$  after  $V_a$  is removed,  $V_g$ , is given by the equation:

$$V_g = V_p(C_p - C'_g) / (C'_g - C_g)$$

The assumption that gastric volume does not change during the measurement may be approximated by making the duration of procedure as short as possible. Dubois et al. (1977) allowed one minute to elapse between the sampling of  $V_a$  and  $V_a'$ . The volume of the first aliquot,  $V_a$  must be added to  $V_g$  to give the true volume of the stomach before sampling,  $V_g$ , therefore:

$$V_g = V_p(C_p - C'_g) / (C'_g - C_g) + V_a$$

The entire procedure can be repeated at time  $t_1$  and at time  $t_2$ , excetera, thus giving a record of the gastric volume over the time following a single meal.

#### Volume Emptied

The estimation of the volume that leaves the stomach is confounded by the fact that the stomach can secrete fluids into the gastric contents. The secretions dilute the concentration of

the dye and hence the volume entering the duodenum cannot be exactly calculated from the amount of dye emptied. However, we can put limits on the volume emptied,  $V_e$ , because we know the dye concentrations,  $C'_{g1}$  and  $C_{g2}$  at times  $t'_1$  and  $t_2$ , respectively.

$$\text{i.e.: } C'_{g1} > C_e > C_{g2}$$

$$\text{Therefore: } D_e/C_{g2} < V_e < D_e/C'_{g1}$$

If one wishes an estimate of  $V_e$ , and stomach samples are taken often enough, say every five or ten minutes, we can take the average of  $C'_{g1}$  and  $C_{g2}$  as being equal to  $C_e$  (Hunt & Spurrell 1951; Snowdon 1970).

$$\text{Therefore: } V_e = (D_e \times 2)/(C'_{g1} + C_{g2})$$

#### Volume Secreted

Once we have estimated the volume emptied it is possible to calculate the volume that has been secreted into the stomach,  $V_s$ . Assuming that the stomach does not absorb water, then the change in gastric volume,  $dV_g$ , occurring between times  $t'_1$  and  $t_2$ , equals the volume secreted minus the volume emptied.

$$\text{i.e.: } dV_g = V_s - V_e$$

Moreover, the change in gastric volume between time  $t'_1$  and  $t_2$  is also equal to the difference between the respective gastric volumes.

$$\text{i.e.: } dV_g = V_{g2} - V_{g1}$$

$$\text{Therefore: } V_{g2} - V_{g1} = V_s - V_e$$

$$\text{Rearranging: } V_s = V_{g2} - V_{g1} + V_e$$

### Volume of Test Load Remaining in Stomach

The gastric volume is not equal to the volume of the test load in the stomach because of secretion. Therefore, to estimate  $V_r$ , the test load remaining at time  $t$ , the volume secreted by time  $t$  is subtracted from the gastric volume at time  $t$ .

$$\text{i.e.: } V_r = V_t - V_{s_t}$$

where:  $V_{s_t}$  = the sum of the volume secreted estimates up until time  $t$ .

### Calculation of Half Emptying Times

Half emptying times were determined according to Elashoff et al. (1982) by solving for the parameter values of a power exponential function that best fit the infusion volume remaining data for each trial.