GASTRIC FUNCTION
IN THE CONTROL OF
FOOD INTAKE

Studies in patients with eating
disorders and in normal
subjects and laboratory
studies in animals.

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ABSTRACT

In this thesis the relationships between gastric function, satiety and eating disorders are investigated by means of studies in animals and in patients with eating disorders.

Previous work is reviewed and hypotheses presented.

The effects of oral dl-fenfluramine on eating behaviour during a laboratory meal in 15 patients with bulimia nervosa are described. Eating and bulimic symptoms were significantly reduced by the drug.

dl-Fenfluramine also significantly reduced glucose consumption and gastric emptying in Rhesus Monkeys and the two effects were interrelated.

The putative satiety peptide cholecystokinin (CCK) was then studied in immature rats, believed to lack brain CCK receptors. Extensive receptors were found using autoradiography in the rat stomach, present before birth. CCK also inhibited independent milk ingestion and gastric emptying in neonatal rats. The ingestive and gastric inhibitory effects of CCK may be functionally related.

Patients with eating disorders were studied using gamma-camera imaging of radio-labelled meals. Acutely starving (but not low weight refed) patients with anorexia nervosa had delayed gastric emptying suggesting that starvation produces delayed emptying. Patients of normal weight with anorexia nervosa or bulimia nervosa had normal gastric emptying.

Rats were fed on a restricted time schedule, and developed profoundly delayed gastric emptying which improved with refeeding.

Lastly, in a study of hunger and satiety, patients with anorexia nervosa had elevated satiety levels, reported hunger abnormally, and frequently showed correlations between gastric contents (measured on the gamma scan) and affective state and symptoms of eating disorder. This phenomenon, not observed in controls, was termed paraceptivity. Patients with bulimia nervosa reported hunger and satiety normally, but demonstrated paraceptivity.

It is postulated that delayed gastric emptying mediates the reduced feeding observed after dl-fenfluramine and CCK administration and contributes to the maintenance of anorexia nervosa by enhancing satiety and provoking interoceptive distortion.
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CHAPTER 1

INTRODUCTION

An outline of the thesis
This book brings together experimental work performed in several different realms of enquiry. Observations of eating behaviour in animal and human subjects are described alongside studies of gastric physiology and peptide receptor biology. Experimental subjects vary between patients with anorexia and bulimia nervosa, normal human controls, and laboratory animals, some mature, others newborn or even at foetal stages of development. The various hypotheses generated before and over the course of these studies are listed on the following pages. The area of enquiry that is shared by these diverse studies is the role of delayed gastric emptying in the modulation of food intake. The literature relevant to this area, and its application to the specific experimental problems addressed in the thesis, are discussed in chapter 2.

The first research question posed in this work concerns the effect and mechanism of action of dl-fenfluramine, a well known appetite suppressant drug. A standard dose was given, under closely controlled experimental conditions, to patients suffering from bulimia nervosa, a serious eating disorder described in 1979 (Russell, 1979).
Hypothesis 1  Fenfluramine will reduce the amount eaten during a test meal in patients with bulimia nervosa.
Hypothesis 2  Fenfluramine will reduce the incidence of bulimic symptoms in patients with bulimia nervosa.
Hypothesis 3  Fenfluramine will delay gastric emptying of glucose and saline solutions in rhesus monkeys
Hypothesis 4  Fenfluramine will reduce sucrose intake in rhesus monkeys
Hypothesis 5  The delay in gastric emptying due to fenfluramine will correlate highly with the reduction in sucrose intake observed in rhesus monkeys

Hypotheses relating fenfluramine, feeding and gastric emptying
Hypothesis 6 Cholecystokinin receptors will be located in the new-born rat pylorus
Hypothesis 7 If hypothesis 6 is true, new-born rats will be found to reduce their rate of gastric emptying in response to CCK
Hypothesis 8 If hypotheses 6 and 7 are true, new born rats will reduce their food intake in response to CCK.
Hypothesis 9 Desulphated CCK will not affect food intake in new-born rats.
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Psychological state, in patients and in matched controls, was monitored, and, two hours after giving the drug, subjects were given a large amount of food and their food intake and bulimic symptoms were recorded. Both food intake and episodes of bulimia were reduced by dl-fenfluramine, confirming hypotheses 1 and 2. This study gives rise to a number of research questions. dl-fenfluramine might be useful as a therapeutic agent in bulimia nervosa, and this possibility is currently being explored using the d-isomer of fenfluramine. Secondly, the mechanism of action of fenfluramine in bulimia nervosa required investigation. In the present studies, this question was pursued using experiments in the rhesus monkey. The aim was to determine the proportion of the feeding inhibitory effect of dl-fenfluramine that could be attributed to its gastric inhibitory properties, previously described in the rat. First, gastric emptying rates of saline and glucose loads were determined using serial test meals administered by an intragastric cannula. Emptying of both solutions was found to be delayed by dl-fenfluramine, thereby confirming both parts of hypothesis 3. Feeding of a sucrose solution and gastric emptying of the same solution were then studied simultaneously in rhesus monkeys using the same gastric cannulae after dl-fenfluramine. Feeding and gastric emptying were inhibited by the drug, and the effects on the two functions were highly correlated, suggesting that inhibition of gastric emptying is an important mediator of feeding inhibition in rhesus.
monkeys under the conditions of the experiment, confirming hypotheses 4 and 5. From the experiments described in Chapter 3, it can be concluded that dl-fenfluramine inhibits food intake and bulimic symptoms in bulimia nervosa, and that, in the rhesus monkey, a proportion of the feeding inhibitory effect of dl-fenfluramine is attributable to the drug's effect on gastric emptying.

The aims of the studies using dl-fenfluramine were to establish a finding in patients with a defined clinical condition and to design experiments using experimental animals which would help elucidate the important mechanisms. The remaining studies in this book arose from the established finding, in anorexia nervosa, of delayed gastric emptying. Gastric function was studied, in patients with eating disorders and in rats on a restricted diet (Chapters 5 and 6) in order to establish the determinants of delayed gastric emptying in anorexia nervosa, and a specific peptide, cholecystokinin, was studied in rats, to improve understanding of its role in modulation of feeding and gastric emptying. It was believed that cholecystokinin might be of importance in the mediation of delayed gastric emptying in anorexia nervosa. It had been shown to inhibit both food intake and gastric emptying in a dose dependent fashion and, of the compounds suggested as potential mediators of satiety and gastric emptying, cholecystokinin is the peptide about which most information is available regarding its possible role in the modulation of these two functions in animals.
and man. In the present thesis, the experimental work on cholecystokinin is presented in Chapter 4. The studies may be divided into two sections: receptor autoradiographic studies and studies on food intake. It was decided to restrict the work on cholecystokinin to immature and foetal rats. Previous work had suggested that in the adult rat, cholecystokinin receptors were present in high densities in both pyloric sphincter and brain. Studies in the adult would be hampered by difficulties in determining whether a response obtained was mediated centrally or peripherally. In the new born rat, however, cholecystokinin receptors are poorly developed in the brain, so that a feeding response elicited by administered cholecystokinin would be unlikely to be mediated centrally. The aims of these studies were, first, to establish whether, in the new born rat, cholecystokinin receptors could be identified in the pyloric sphincter, and then to study the effects of cholecystokinin on feeding in the neonate. Cholecystokinin receptors were labelled using $^{125}$I-Cholecystokinin in upper gastrointestinal tracts from rat foetuses aged 17 and 20 days, and from rats aged from 1 day to adulthood. Cholecystokinin receptors were, indeed, identified in the neonate, confirming hypothesis 6, as well as in the 17 day foetal stomach, and their distribution proved more extensive in the immature stomach than in the adult. Behavioural experiments to establish the effects on feeding of cholecystokinin in the neonate had to be designed according to the ingestive behaviour of
these animals. Suckling has been shown not to be influenced by cholecystokinin in the newborn and, in the present studies, a test of ingestion was used which bears some similarities to adult feeding tests, namely independent ingestion of milk from the floor of an incubator. Using this methodology it was possible to show that rats as young as one day of age showed inhibition of milk intake after low doses of cholecystokinin, and the demonstration that cholecystokinin inhibited gastric emptying of a saline load in 1 and 3 day old rats suggested that, in the absence of central receptors for cholecystokinin in the neonatal rat, inhibition of milk intake might be mediated by inhibition of gastric emptying, and that hypotheses 7 and 8 appeared to be confirmed. A number of observations of the rat pups were made during the ingestion experiments which confirmed the intake observations: ingestive but not non-ingestive behaviours were found to decrease after cholecystokinin. The specificity of the response was studied in two additional ways. The desulphated form of cholecystokinin was used and found to be ineffective in reducing milk intake (hypothesis 9 confirmed) and 3 day rats subjected to hypertonic dehydration ("thirst" stimulus) were found not to decrease their milk intake after cholecystokinin, in contrast to animals subjected to a "hunger" stimulus (deprivation) which drank less after cholecystokinin (hypothesis 10 confirmed).

Studies of cholecystokinin function therefore supported the
general hypothesis that, in the neonatal rat, an animal which is thought to lack a central cholecystokinin receptor system, reduction of food intake is mediated by inhibition of gastric emptying, itself a function of the interaction of the peptide with gastric cholecystokinin receptors. In the remaining studies, delayed gastric emptying in anorexia nervosa was investigated, and an animal model developed in which the potential role for cholecystokinin in producing delayed emptying could be pursued.

In the research studies described in Chapter 5, patients with anorexia nervosa, bulimia nervosa and normal controls were given radiolabelled meals and gastric emptying rate measured using a gamma camera. It was confirmed that, for solid and for glucose (but not normal saline) meals, gastric emptying was delayed in one group of patients: those who were acutely starving as a result of severe food deprivation, or of intractable self induced vomiting. In those patients who, although underweight, were being treated by intensive refeeding on an inpatient ward, gastric emptying was normal. The major determinant of delayed emptying was therefore recent dietary intake, not weight. These findings confirmed hypotheses 11, 12, and 13 and informed the next series of experiments, in which an animal model of the delayed emptying was produced in rats by subjecting them to a restricted diet schedule. After 4 months, gastric emptying in restricted diet rats was very much slower than in controls, (hypothesis 14 confirmed) and this animal model is currently being investigated to
determine the physiological changes that contribute to delayed emptying and, therefore, treatments that such changes suggest which might help reverse it and, perhaps, contribute to management of anorexia nervosa.

Chapter 6 contains the results of psychological measures that were made during the gastric emptying studies, the physiological results of which are given in the previous chapter. It was reasoned that ratings made on a simple visual analogue scale could be compared with radioactive counts remaining within the stomach after the solid meal so that a number of hypotheses suggested by previous authors could be tested. It was possible to relate prolonged satiety after meals to gastric contents and to show that patients with anorexia nervosa have higher post-prandial satiety ratings which decline abnormally slowly (hypotheses 15 and 16 confirmed) but that these two findings apply equally to patients with slow and normal gastric emptying (hypothesis 17 refuted). It was also found that ratings of hunger in anorexia nervosa were often quite unrelated to the amount of food (represented as radioactive counts) remaining in the stomach, although satiety ratings showed normal correlations with gastric contents (hypothesis 18 partially confirmed). Moreover, in both anorexia and bulimia nervosa, gastric contents were frequently related to analogue scales reflecting symptoms (eg. depression, fatness, urge to vomit), an unexpected finding which has been labelled paraceptivity.

In the final two chapters, the findings of all the studies
are reviewed in turn and their implications discussed. The possible place of dl-fenfluramine in the management of bulimia nervosa is considered as are the mechanisms, peripheral and central, that might contribute to dl-fenfluramine-induced inhibition of feeding and it is suggested that delayed gastric emptying, in the rhesus monkey, is a major contributor to those mechanisms. Cholecystokinin is discussed as a potential physiological mediator of food intake and gastric emptying, and as an example of a peptide with a receptor system that undergoes major changes during development. Lastly, the pathogenesis and significance of delayed gastric emptying and abnormal gastric perceptivity in anorexia nervosa are reviewed and implications for future research discussed.

**General construction of the thesis**

This thesis is arranged in such a way that the middle four chapters (3 to 6) each represent research conducted in one area of study. Thus, chapter 3 is concerned with studies on the clinical and experimental aspect of the drug dl-fenfluramine, chapter 4 with developmental and behavioural aspects of the peptide cholecystokinin, chapter 5 with gastric emptying in eating disorders, and in rats, and chapter 6 with psychological responses of the eating disorder patients. In this introduction, it has been made clear that these diverse areas of study are linked by their emphasis on gastric mechanisms in the modulation of food.
intake. The separation of this work has been necessary because of the wide range of methodologies used and because, except in the case of chapters 5 and 6, experimental groups of subjects were quite distinct. The overall approach was to identify a clinical problem and to pursue it using clinical populations and experimental animals. Some of the research questions can be formulated as follows:

**dl-fenfluramine:**

a. Does dl-fenfluramine influence eating behaviour and bulimic symptoms in an acute experiment?
b. If so, is this a clinically useful effect?
c. What is the contribution of gastric mechanisms to the effect?

**Delayed gastric emptying in anorexia nervosa:**

a. What are the determinants of the phenomenon?
b. What are the physiological mechanisms? eg: is cholecystokinin involved?
c. How can the abnormal gastric function be reversed?
d. What are the therapeutic consequences of reversing the disorder?

**Cholecystokinin**

a. Could disturbed cholecystokinin function underlie delayed gastric emptying in anorexia nervosa?
b. Does cholecystokinin have a role in modulation of gastric function and feeding that could be disturbed in

1these questions were not addressed in the present work.
anorexia nervosa?

c. As the neonatal rat appears to lack central cholecystokinin receptors, are they identifiable peripherally?

d. Does cholecystokinin affect feeding and gastric emptying in the neonate?

e. Is cholecystokinin function disturbed in a rat model of delayed gastric emptying?
CHAPTER 2

THE ROLE OF THE STOMACH IN THE CONTROL OF FOOD INTAKE:

A review of the literature

The stomach has long been thought to exert important regulatory controls on feeding. Cannon and Washburn (1912) reported that the experience of hunger pangs could be traced to gastric contractions, which occurred whenever the subject, (who was the second author), described feelings of hunger localised to the epigastrium. Although subsequent work has cast doubt on the significance of gastric contractions for the experience of hunger, (Penick, et al, 1967, Stunkard and Fox, 1971) changes in stomach function have been shown to exert considerable influence on food intake. Indeed, it has been suggested that modulation of gastric function is a major contributor to the regulation of caloric intake and body weight (McHugh et al, 1975, McHugh and Moran, 1979). In this chapter, the evidence for the role of the stomach in control of feeding will be reviewed, to place in context the clinical and experimental studies that led to the research findings and inferences that form the rest of this thesis.
1. Gastric distension and inhibition of feeding

Distension of the stomach by means of a gastric balloon inhibits food intake in rats (Geliebter, et al, 1986) and in man (Geliebter et al, 1987), and inflation of an intragastric balloon has been used as an adjunct to the treatment of obesity (Brown, et al, 1988). In the rhesus monkey, the role of gastric distension has been well demonstrated by Wirth et al (1983) who showed that, during feeding of a glucose solution, withdrawal of gastric contents via a chronic indwelling cannula results in a subsequent increase in glucose intake exactly equivalent to the volume withdrawn. In the 6 day old rat, Phifer et al (1986) have suggested, using evidence adduced from gastric preloads to influence intake and pyloric nooses to block gastric emptying, that gastric distension is the major signal that these immature animals use to control their intake of food. The rat pups consumed milk via oral infusions until stomach contents were about 6.5% of body weight, regardless of preloads given or whether gastric emptying could proceed or not.

There is evidence that the rate of gastric emptying of many caloric solutions is tightly controlled, at least in man and the rhesus monkey, and that this physiological system may be important in regulation of caloric intake. For example, in the monkey, d-glucose empties from the stomach at a constant (ie linear) rate of about 0.4 kcal/min. This rate of caloric emptying stays constant if glucose concentration is varied over the range 0.2-1.0
Moreover, glucose introduced directly into the duodenum delays the emptying of a saline load placed in the stomach by 2.5 min for every kcal infused (McHugh et al., 1982). As 2.5 min/kcal is an exact reciprocal of 0.4 kcal/min (the constant caloric rate of emptying of glucose in the rhesus monkey) this work provides evidence of a duodeno-gastric feedback loop which controls the rate of egress of calories from the stomach. A solution of lower caloric concentration will empty more quickly than one of high caloric concentration, and gastric distension will be more prolonged after a calorically dense meal. If caloric concentration is doubled, gastric emptying is halved and consumption of nutrient is proportionately reduced. Constancy of caloric emptying also applies to fat and protein solutions, (McHugh and Moran, 1979) although some caloric meals, fructose, for example, do not empty at a constant rate, following an exponential rather than a linear pattern.

Several lines of evidence therefore converge to associate gastric distension with the control of short term food intake. In the next section, inhibition of gastric emptying is seen as an important contributory mechanism in the action of drugs with inhibitory effects on feeding
2. Gastric emptying and drug-induced inhibition of feeding
(a) Cholecystokinin (CCK)

CCK is the name given to a class of peptides sharing a number of chemical and physiological properties. CCK was originally discovered as a factor in blood that led to gall bladder contraction, (Ivy and Oldberg, 1928) and pancreatic exocrine stimulation (Harper and Raper, 1943), actions that were originally thought to be caused by distinct compounds. The name cholecystokinin reflects the stimulatory action of the peptides on gall bladder smooth muscle, whereas the alternative name, pancreozymin, now discarded, reflects their pancreatic stimulatory properties. Although many forms of CCK have been described in mammalian blood, all the information for coding the biological actions of CCK on pancreas and gall bladder resides in the terminal tetrapeptide, (Sankaran et al, 1981) a sequence that is shared by gastrin. CCK is generally found in body tissues as the octapeptide (CCK-8) or the triacontatriapeptide (CCK-33) and biological activity is found to fall by several orders of magnitude if peptides smaller than the C-terminal octapeptide are examined. Moreover, sulphation of the C-7 tyrosine leads to an increase in biological potency by a factor of 100-300x (Sankaran et al, 1980, Jensen et al, 1982). In the work described here, the forms of CCK used were either sulphated CCK-8 or sulphated CCK-33, with desulphated CCK being employed in order to demonstrate the contrast introduced by the loss of the sulphate radical. The
molecular formulae of the sulphated form of CCK and that of gastrin for comparison are illustrated diagrammatically in Figure 1.
FIGURE 1: Molecular formulae of porcine cholecystokinin (C-terminal octapeptide, CCK 8), and of porcine and human gastrin. Note the sulphated tyrosine residue on each peptide.
Inhibition of feeding by CCK

The interest of CCK for students of ingestive physiology was stimulated by the finding, in 1973, that CCK reduced food intake in rats in a dose dependent manner, with no effect on the intake of water (Gibbs et al, 1973). Moreover, CCK also reduced the intake of food during sham feeding, when rats consumed food which was not allowed to reach the duodenum, but drained from a metal fistula leading from the stomach cavity to the anterior abdominal wall (Gibbs et al, 1973a). In the sham feeding rat, desulphated CCK was found to have no effect on intake, suggesting that this action is specifically associated with the more selective Type A CCK receptor (Moran et al, 1986).

It was then discovered that a total abdominal vagotomy effectively blocked the effect of CCK on feeding in the rat and that the essential branch of the vagus is that branch which supplies the stomach (Smith et al, 1981). Indeed, while section of the sensory nerves in the vagus blocked the effect of CCK on feeding, section of the motor nerves did not (Smith et al, 1985). Thus, the sensory information leading to satiety induced by CCK appeared to be transmitted in the sensory vagus. The precise nature of that information has been the subject of much research and controversy. The vagus has been found to contain specific CCK receptors which travel distally in the nerve (Zarbin et al, 1981) and might be distributed on sensory terminals where they could be stimulated directly by CCK either injected intraperitoneally or released from the small
intestine. The other major theory of the peripheral action of CCK is that the peptide inhibits gastric emptying and, by facilitating gastric distension during a meal, reduces food intake. Support for this idea comes from the work in rhesus monkeys of Moran and McHugh (1982). They found that the lowest dose of CCK, given by intravenous infusion, that inhibited gastric emptying was lower than the dose that reduced intake of food. It was thus possible to choose a dose of CCK between these two thresholds that inhibited gastric emptying but not food intake. It was found that when this dose of CCK was administered after introducing a distending volume of saline into the stomach via a gastric cannula, food intake was inhibited. Saline without CCK did not affect feeding: inhibition of food intake was the result of the combined effects of CCK and the gastric saline load. A likely interpretation of these important results is that CCK inhibited gastric emptying of saline (which, in the absence of CCK, empties rapidly from the stomach) and that the resulting gastric distension stimulus led to enhanced satiety and reduced feeding. A similar result has been obtained using a long-acting CCK analogue, the feeding inhibitory effect of which in the rat was enhanced by a gastric preload (Moran et al., 1992). In man, CCK has been found to inhibit the emptying of a saline meal (Robinson et al., 1988) while its administration during meals has produced varying results. Kissileff et al. (1982) in normal weight men and Pi-Sunyer et al. (1982) in obese men demonstrated a reduction in food intake during
infusions of CCK given after a preload snack. Subjects ate at the same rate during the CCK infusion as during placebo, but they stopped eating earlier, precisely the result that would be expected if the major mechanism of action of CCK is inhibition of gastric emptying and premature satiety. The same group demonstrated that a larger preload gave more pronounced inhibition of subsequent food intake (Muurahainen, et al, 1991). Other studies in man have been less convincing. Stacher et al (1979) found that CCK reduced the hunger and activation resulting in experimental subjects while the researchers prepared a tasty meal, while Sturdevant and Goetz, (1976) found contrary effects of an intestinal extract containing 20% pure CCK. When delivered in an intravenous bolus, this preparation inhibited feeding in man, and when given as a slow intravenous infusion feeding was stimulated. Most recently, in patients with the condition bulimia nervosa, intravenous CCK was found not to influence the binge eating characteristic of that disorder (Mitchell, et al, 1986).

The feeding inhibitory action of CCK is therefore not fully established in man. The physiological relevance of CCK to control of feeding in any species has also been the subject of debate. Plasma levels of CCK that can be measured after a meal are of the order of 15-20 nanogram per litre, (Izzo et al, 1984), while reduction of food intake can only be reliably obtained by doses of 1 microgram per kg, or more. Because of this discrepancy, it is argued that the feeding effects of CCK are
pharmacological, in contrast to the pancreatic actions of CCK which can be elicited within the range of concentrations observed post-prandially (Williams et al, 1981). This is a strong argument, and has been challenged by the finding that the CCK receptor blockers, proglumide (Shillabeer and Davison, 1984) and the more potent and selective antagonist L-364,718, (now MK-329, or devazepide) (Hewson et al, 1988, Reidelberger and O'Rourke, 1989, Garlicki, et al, 1990) increase food intake in experimental animals. Moreover, Soybean Trypsin Inhibitor (STI), which releases endogenous CCK, does not inhibit food intake in adult rats, (Smith et al, 1989) even though plasma CCK levels stimulated by STI are well above those achieved by exogenous doses of CCK that strongly inhibit food intake.

On balance, the evidence suggests a role for endogenous CCK in the control of food intake, with a difference between the effect of CCK of endogenous and exogenous origin that is currently unexplained.

Site or sites of action of CCK inhibition of food intake
It is important to know what the concentrations of CCK are at the locus or loci of action after exogenous administration and endogenous release. There are several candidates for the locus of action of CCK. One is at the terminals of the vagus nerve, as already mentioned. A second possible site of action is on the smooth muscle of the pyloric sphincter. Specific receptor sites for radio-iodinated CCK were described by Smith et al (1984) in the
distal part of the pyloric sphincter of the rat, where they could induce pyloric contraction and delay gastric emptying. Analogous receptors have been found in the human pyloric sphincter and an intravenous infusion of CCK has been shown, in humans, to lead to a contraction of the pylorus and antrum with a rapid onset and recovery after the beginning and end of the infusion (Robinson et al, 1988a).

CCK is abundant in the central nervous system, and a central action of the peptide in reducing food intake has also been proposed. Intracerebroventricular (ICV) injections of CCK in sheep reduced feed intake, and ICV administration of an antibody specifically directed against CCK increases feeding in the sheep (Della-Fera et al, 1981). In the rat, ICV infusions have also been shown to reduce food intake, by mechanisms thought to be independent of gastric inhibition (Della-Fera et al, 1990).

The physiological activity of CCK is much reduced by desulphation of the C-7 tyrosine. This has been shown in studies of the pancreatic stimulation, (Brugge and Praissman, 1984, Sankaran et al, 1980) the gall bladder stimulation (Behar and Biancani, 1987) the depolarisation of rat hippocampal neurones (Dodd and Kelly, 1981) and the inhibition of sham feeding (Gibbs et al, 1973a) produced by CCK. The physiological studies suggest one type of receptor responsive to sulphated CCK. However, using radiolabelled CCK binding to homogenized membranes and autoradiographic
demonstration of binding sites have revealed at least two
classes of CCK receptor (Innis and Snyder, 1980). The
first type, found mainly in association with alimentary
structures, and therefore designated Type A (Moran et al,
1986) shows binding characteristics similar to the
physiological properties of CCK. That is, in binding
assays, radiolabelled ligand can be displaced from these
sites using much lower concentrations of unlabelled
sulphated CCK-8, than desulphated CCK-8. These receptors
are found in pancreas, gall bladder and in the pyloric
sphincter. CCK receptors found in the brain have different
properties, with a much lower differential ability between
sCCK and dCCK to displace labelled CCK, and have been
designated Type B (Moran et al, 1986). At pancreatic (Type
A) binding sites, desulphation of SCCK reduces its ability
to displace labelled CCK by a factor of about 300 (Innis
and Snyder, 1980) while in the brain, desulphation either
has no effect on this action of CCK, (Hays et al, 1980) or
reduces it by a factor of between 4 and 50 (Moran et al,
of action for CCK satiety would, therefore, appear to be in
the periphery, where receptor characteristics match
physiological specificity. Other findings point, however,
to the brain as a possible locus of action for CCK.
Physiological bath experiments have strongly suggested the
presence of Type A receptors in the rat CNS (Dodd and
Kelly, 1981) and the inhibition of feeding produced by ICV
CCK has not been obtained using DCCK (Della-Fera and Baile,
1981). In an autoradiographic search for Type A receptors in the rat brain, they were found in highly specific locations: the area postrema, the interpeduncular nuclei and the nucleus of the tractus solitarius (Moran et al, 1986). In the first of these locations, the area postrema, the blood brain barrier is deficient, (Wislocki and Putnam, 1920) and this structure could represent a central site of action for peripherally administered or released CCK. Support for this idea comes from the finding that, in rats with lesions of the area postrema, an effect of CCK on feeding cannot be demonstrated (Van der Kooy, 1984). Against this is the suggestion (Edwards et al, 1986) that area postrema lesions do not, alone, affect CCK satiety, but must be combined with damage to underlying vagal nuclei and their afferent projections. As afferent vagotomy blocks CCK satiety (Smith et al, 1985) it is important to be certain of the extent to which the lesion extends beyond the area postrema, as extensive damage would produce a central vagotomy, rather than a specific lesion of the area postrema. The region of the nucleus of the tractus solitarius has been implicated by the finding that localised injections of CCK into this site, as well as others in the hypothalamus and medial pontine area, were particularly effective in reducing food intake in rats (Schick et al, 1990).
Developmental biology of CCK

The work on CCK receptors suggests that CCK might be acting at central or peripheral sites, or at both, in reducing food intake. Studies in neonatal rats have shown that the appearance of brain CCK receptors is delayed until the second week of life, with only low levels at birth (Hays et al, 1981). Changes in CCK receptors in the developing rat brain fairly closely follow the changes in cerebral CCK itself, measured by radio-immunoassay. Brain CCK levels are very low at birth and rise to adult levels by day 30 (Noyer et al, 1980). In contrast, gut CCK develops more rapidly, with detectable levels in the small intestine of the second trimester foetus in man (Buchan et al, 1981) and an early appearance of gut CCK in the development of the rat (Brand, 1982). There is, therefore, a possibility that CCK receptors would be found in the gut in the immature animal. If gut receptors could be identified at birth, a feeding response to CCK in the neonate would suggest that the site of action of CCK was in the periphery, rather than in the brain, where there are few receptor sites. Feeding behaviour in the neonate is, however, very different from that in the adult, and before investigating the neonatal rat as a potential model for the peripheral actions of CCK, a number of developmental issues must be explored.

The sole source of nutrient intake in the neonatal mammal is maternal milk. In the rat, intake is primarily controlled, not by satiation, but by the amount of milk
provided for the pup in the form of intermittent 'let-downs' (Lincoln et al, 1973). Pups allowed to suckle indefinitely will take in milk until their stomachs are grossly distended (Hall and Rosenblatt, 1977). With such a powerful stimulus to ingestion, it is not surprising, therefore, that CCK does not inhibit intake during suckling in the rat (Blass et al, 1979) until 15 days of age, at which time weaning is beginning. CCK was also observed (Bernstein et al, 1976) to inhibit intake of solid and liquid diet in weanling rat pups. The effect of CCK on gastric emptying in immature rats has been investigated in one study (Houpt & Houpt, 1979). Although no effect was demonstrated this study can be criticised because of the long time (50-81 minutes) between administration of CCK and testing, and between administration of the gastric loads and determination of gastric emptying rate (60 minutes). These intervals are almost certainly many times the plasma half life of CCK, which has been estimated in human plasma at 1.30 minutes (Kanayoma et al, 1985) and the likelihood of demonstrating an effect would be small.

Suckling is therefore not significantly altered by CCK in the neonate, and the question of CCK's effect on gastric emptying remains open. The stimulus to suckling is very powerful, and any inhibitory effect of CCK might be overwhelmed by its intensely rewarding nature. Hall and Bryan (1980) described a method of demonstrating nutrient intake in rat pups ingesting milk independently of the dam. The pups are placed in a warm, humid incubator, on the
floor of which is a paper towel soaked in cows' milk. Over a thirty minute test, rats three days old will ingest the milk, and the increase in weight of the pup over the period of the test accurately reflects intake (Houpt & Epstein, 1973) as long as defaecation and micturition are induced prior to testing by gentle stroking of the perineum (Hall and Bryan, 1980). Observation of pups during the test reveals a variety of behaviours, some reflecting oral activity such as mouthing and other behaviours such as locomotion, reflecting general motor activity. Behaviour under these conditions bears some important resemblances to adult feeding. Intake increases with increasing length of deprivation and, during the test, the occurrence of oral and other behaviours decreases with time, so that the pups demonstrate the reduction in activity during the latter part of the meal characteristic of satiety in adult rats (Antin et al, 1975). This experimental model of feeding, although artificial, appeared to be a promising system in which to test the effects of CCK on ingestion, behaviour and gastric emptying in the neonatal rat.

(b) Gastric emptying and inhibition of feeding by dl-fenfluramine

Inhibition of feeding by dl-fenfluramine

dl-Fenfluramine is a racemic compound that has been in clinical and experimental use for many years as an inhibitor of feeding behaviour (Stunkard et al, 1973, Pinder et al, 1975, Blundell et al, 1979, Silverstone et
al, 1975, Kyriakides and Silverstone, 1979, 1979a). It is chemically closely related to amphetamine, differing in that dl-fenfluramine is n-alkylated and has a trifluoromethyl group on the phenyl ring (Figure 2). These distinctions are associated with a number of differences in the pharmacological properties of the two drugs. While amphetamine is a stimulant, and therefore widely abused, dl-fenfluramine is a CNS depressant, and is rarely abused (Connell, 1975, 1979). Amphetamine is a potent facilitator of dopaminergic transmission, and can produce a schizophrenia-like psychosis (Connell, 1958) and dl-fenfluramine (particularly the l-isomer) shows dopamine receptor antagonist properties (Bettini et al, 1987). In the development of strategies in the clinical management of hyperphagic conditions, dl-fenfluramine might, therefore, be considered for evaluation when other, more dangerous appetite suppressants, such as the amphetamines, would be precluded. Inhibition of food intake by dl-fenfluramine has been observed in human subjects (Kyriakides and Silverstone, 1979, 1979a) in rats (Blundell et al, 1979) and in sub-human primates (Foltin and Schuster, 1983).
FIGURE 2a: Molecular structure of fenfluramine.
In normal human subjects, it suppresses ratings of hunger (Kyriakides and Silverstone, 1979a) and an oral dose of 60mg reduces intake during a test meal by around 60% (Kyriakides and Silverstone, 1979). It is also effective in reducing food intake in obese subjects, and in long term use in obesity, results in a reliable loss of weight which is maintained as long as the drug is taken (Douglas et al, 1983). However, weight returns to premorbid levels after dl-fenfluramine is discontinued, and this apparent need for long term administration has prevented the general acceptance of dl-fenfluramine in the treatment of obesity. In addition, there is some evidence that the use of the drug in association with behavioural approaches to the treatment of obesity reduces the long term efficacy of the latter (Craighead et al, 1981).

Bulimia nervosa: Introduction.

Bulimia nervosa (Russell, 1979, Fairburn and Cooper, 1984) is an eating disorder, allied to anorexia nervosa, in which patients, who are generally young adult females, engage in episodes of overeating (bulimia) during which they may consume very large amounts of food rich in carbohydrate and fat. These episodes of bulimia are generally followed by the induction of vomiting by the patient, in order to rid herself of the ingested food. Other methods used to reduce calorie absorption or increase its metabolism are the taking of large doses of laxatives, excessive and prolonged exercising and abuse of thyroid hormones. Some patients also take diuretic preparations, sometimes prescribed, and
abuse of amphetamines in order to control appetite is also observed. These patients describe an intense fear of putting on weight, and are usually attempting, through dieting, to reduce weight. The dieting may be very severe, and is thought (Russell, 1979, Robinson, 1986) to contribute significantly to the preoccupations with food, and to the episodes of bulimia which characterize their symptomatology. Thus, a reduction of food intake over the previous few hours or days may lead to episodes of bulimia. This form of malnutrition may be called short-term starvation and the response of patients with bulimia nervosa to this pattern of feeding and starving can be compared to the behaviour of rats fed on a restricted time schedule. Over the first few days, they rapidly increase the amount of food eaten during the time allowed (which may be two hours per day) until they are consuming, during that time, enough, at least partially, to compensate for the reduction in access time to food (Curi et al, 1984). The second influence that may contribute to the occurrence of episodes of bulimia is weight loss. Patients with bulimia nervosa have generally lost weight from a previous level, which may have represented a degree of obesity. In a recent series of male patients with bulimia nervosa, all had lost substantial amounts of weight, some from a frankly obese weight to within the normal range, some from the normal weight range to a low weight at which they fulfilled criteria for anorexia nervosa, and one originally obese patient who lost enough weight to satisfy criteria for
anorexia nervosa (Robinson & Holden, 1986). This form of malnutrition may be called long-term starvation and may be compared to the response of rats, underfed for long enough to result in substantial weight loss. When given free access to food, they overeat until their previous weight curve is re-established (Hoebel and Teitelbaum, 1966). Both short-term and long-term starvation may therefore contribute to the over-eating observed in patients with bulimia nervosa. In some patients who have lost large amounts of weight, severe starvation with amenorrhoea may indicate the presence of anorexia nervosa, and a proportion of patients with this disorder are found to engage in regular episodes of bulimia (Casper et al, 1980 Garfinkel et al, 1980). There is, therefore, a significant overlap between anorexia nervosa and bulimia nervosa, and patients may, by alterations in weight, satisfy criteria for each condition at different times.

Bulimia nervosa: Approaches to treatment.

Attempts at treatment of this serious disorder have met with varying degrees of success. Admission to hospital under careful nursing supervision may prevent bulimic episodes, but relapse usually occurs on discharge. Various psychological treatments including cognitive-behavioural and psychodynamic therapy based on individual or group approaches (Fairburn, 1981, Fairburn et al, 1986, Freeman et al, 1985, Huon and Brown, 1985, Lacey, 1983) have been evaluated. In general, intensive therapeutic approaches have been found to be of substantial efficacy in the short
term, and there is also evidence of long-term benefit using these methods.

The role of drug treatment is uncertain. The anticonvulsant drug phenytoin was reported to reduce the incidence of bulimic episodes in eight out of nineteen patients with the 'binge-eating syndrome' in a controlled trial (Wermuth et al, 1977) and in 9 out of 10 'compulsive eaters' with a variety of other diagnoses in an uncontrolled study (Green & Rau, 1974). The other pharmacological approach has been to use antidepressant medication. In an uncontrolled study of monoamine oxidase inhibitors (Walsh et al, 1982), improvement was reported in all six bulimic patients given the drugs, and this result has been corroborated by a subsequent double-blind controlled trial demonstrating the superiority of phenelzine over placebo (Walsh et al, 1985). Two well controlled studies of antidepressant drugs each suggested different conclusions. Imipramine (Pope et al, 1983) was reported to improve eight out of nine patients with bulimia, compared to one out of ten given placebo, a result confirmed by a later study (Agras et al, 1987). However, mianserin (Sabine et al, 1983), although given at a rather low dose, provided no clear benefit in a controlled double blind trial, and amitriptyline was also found to have no effect on the eating disturbance in bulimia nervosa (Mitchell and Groat, 1984), although depressive symptoms were improved. Two other antidepressants, Desipramine (Hughes et al, 1986) and fluoxetine (Freeman, et al, 1988,
have also, in controlled trials been shown to reduce the incidence of bulimic symptoms. Thus, antidepressants with diverse pharmacological actions appear to improve bulimic symptoms, and it is, at present, unclear whether their mode of action is primarily antidepressant, antianxiety or even appetite suppressant in the production of this therapeutic effect.

Appetite suppressant drugs in bulimia nervosa.

It has been shown (Ong et al, 1983) that intravenous methylamphetamine, in a double-blind placebo controlled experiment, significantly reduced the quantity eaten by eight patients with bulimia nervosa. Amphetamines have a number of different actions, including euphoria, suppression of appetite and increased alertness, and it is not clear which of these was responsible for the suppression of eating noted in the patients. Should a similar effect be produced by dl-fenfluramine, which has appetite suppressant but no euphoriant or alerting action (Stunkard et al, 1973), then it might be deduced that suppression of appetite is the mechanism whereby both drugs inhibit eating in bulimia nervosa.

The finding that drug-induced suppression of hunger may modify the symptoms of bulimia nervosa is not all that readily predictable. Patients with bulimia nervosa often deny hunger, sometimes quite vehemently. They express their symptoms more in terms equivalent to a failure of
satiety (Russell, 1979). However, the methylamphetamine study suggested that the use of drugs modifying appetite might prove beneficial in the treatment of bulimia nervosa. For this reason, a preliminary study of the effects of dl-fenfluramine on feeding behaviour in bulimia nervosa was performed, and is reported in chapter 3.

Fenfluramine: Mechanism of action

The mode of action of dl-fenfluramine in the inhibition of feeding is complex. dl-Fenfluramine is a potent inhibitor of the reuptake of serotonin into neurones (Fuxe et al, 1975). Destruction of central serotonin pathways in the rat through lesions of the septal nuclei (Samanin et al, 1972) results in an attenuation of the feeding inhibitory effect of dl-fenfluramine in 18 hour deprived animals during a test meal. There is also a body of opinion suggesting that, by enhancing central serotonin transmission, fenfluramine causes a specific reduction in carbohydrate intake (Wurtman and Wurtman, 1984). While a central site of action for the feeding inhibition of dl-fenfluramine, probably mediated by serotonin pathways, appears established, peripheral sites of action have also been posited. dl-Fenfluramine is thought to enhance cellular uptake of insulin and thereby increase the metabolic rate of adipose cells (Hamet et al, 1986) although the degree to which this contributes to the weight loss observed in obesity is uncertain. Secondly, it has
been suggested that dl-fenfluramine might act, in part, by inhibiting gastric emptying and so enhance satiety in a similar way to that suggested for the inhibition of feeding and gastric emptying produced by cholecystokinin, described above. Davies et al (1983) demonstrated that dl-fenfluramine inhibited gastric emptying in rats. This finding has been replicated in the rat (Baker et al, 1988), the hamster (Rowland and Carlton, 1986a) and in man (Horowitz et al, 1985). As dl-fenfluramine was thought to be ineffective in reducing food intake in rats with lesions of the septal nuclei (Samanin et al, 1972), the relevance of these studies appeared in doubt. However, Davies et al (1983) also showed that, in freely feeding rats with septal lesions, dl-fenfluramine was effective in reducing food intake. This finding suggests that a mechanism similar to that observed for CCK, in which gastric distension appeared to facilitate the feeding inhibitory action of the peptide (Moran and McHugh, 1982), might be relevant to dl-fenfluramine. Accordingly, a series of experiments, designed to investigate the links between gastric emptying, feeding and fenfluramine were conducted in rhesus monkeys.

3. Delayed gastric emptying in anorexia and bulimia nervosa (a) Gastric function in anorexia and bulimia nervosa: studies to date.

The studies already considered suggest that slowing of gastric emptying contributes significantly to the
regulation of caloric intake and to the inhibition of feeding produced by drugs. We now consider the role of altered gastric emptying in the disturbances of eating observed in anorexia and bulimia nervosa. Eating behaviour in anorexia nervosa is diverse. Most patients reduce weight by means of severe dieting, sometimes amounting to starvation (Russell, 1983). Others eat meals, often under pressure to do so from their families, only to dispose of them afterwards by inducing vomiting (Casper et al, 1980, Garfinkel et al, 1980). Yet others engage in episodes of bulimia alternating with a combination self-induced vomiting, laxative abuse, prolonged starvation and other measures, already described, designed to minimize the effect of ingested calories upon weight (Casper et al, 1980, Garfinkel et al, 1980). The aetiology of the condition is complex and contemporary authorities recognize biological factors both inherited and acquired, and the contributions of psychological, family, social and cultural influences in anorexia nervosa (Garfinkel and Garner, 1982). The approach used in the following studies was to concentrate on possible gastric contributions to the disturbances of eating in anorexia and bulimia nervosa. Early studies of gastric function in anorexia nervosa showed normal gastric pressures, using an intragastric catheter (Silverstone & Russell, 1967) or a pressure telemetry "pill", which, when swallowed, sends pressure signals by radio (Crisp, 1967). However, In 1979, Dubois et al reported that the gastric emptying of water and the
gastric output of acid, measured using a gastric tube sampling method, were both abnormally reduced in a series of patients with anorexia nervosa. The same workers later reported that both emptying rate and acid output could be improved using bethanechol, a cholinomimetic drug (Dubois et al, 1980), while Saleh and Lebwohl (1979) found that metoclopramide improved gastric emptying in patients with anorexia nervosa. In 1981, Holt et al, using a nuclear medicine technique, reported slow solid and liquid emptying in 10 patients with anorexia nervosa who were being refed on an inpatient unit. This study can be criticised in two ways. First, the marker for the solid phase of the mixed meal consisted of pieces of paper impregnated with isotope. It is not clear how far these labelled items emptied from the stomach together with solid food. Secondly, the control subjects were not matched for age or sex and, as male subjects have more rapid gastric emptying of solid and liquid meals than females (Notivol et al, 1984) the study has to be interpreted with caution. Russell et al, (1983) described a patient with anorexia nervosa who complained of severe post-prandial epigastric discomfort. Gastric emptying of a radio-labelled egg meal was found to be delayed, with a half emptying time of 119 minutes (normal 50-90 minutes) and an improvement in both gastric emptying to 75 minutes and in analogue ratings of gastric fullness was produced using domperidone, a peripheral dopamine antagonist with gastro-kinetic properties. McCallum et al (1985) found the emptying of solid food to be delayed, but
that of water was normal in anorexia nervosa, and, lastly, Stacher et al (1986) found a significant delay in the emptying of a porridge meal, with improvement in emptying rate after domperidone. Enough evidence has therefore been published to indicate that a disorder of gastric emptying occurs in anorexia nervosa. However, the studies cited suffer from a number of drawbacks. Most importantly, clinically relevant information about the patients is rarely given. It is conceivable that the presence of bulimia, vomiting, laxative abuse, and other symptoms of anorexia nervosa, could influence gastric emptying. Although the patients' weight is generally reported their recent dietary intake is not and a consideration of the relative contributions of the symptoms of anorexia nervosa, as well as the presence or absence of short term starvation, might provide insight into the factors contributing to delayed gastric emptying in anorexia nervosa. Solid emptying has usually been found to be delayed, although the results of liquid emptying studies have been varied. Solid emptying is under different physiological controls from the emptying of liquids, (Kelly, 1980) while caloric and acaloric liquids empty according to different laws (McHugh and Moran, 1979). It is necessary to compare, not only solid with liquid, but liquids containing calories with acaloric liquid meals.
(b) Methodologies applied to the measurement of gastric emptying

Gastric emptying has been measured in a number of ways: 1. The serial test meal method (Hunt and Spurrell, 1951) using a gastric tube. 2. The use of radio-opaque meals (such as Barium Sulphate) or radio-opaque markers and serial abdominal radiographs (Keys et al, 1950, Horton et al, 1965). 3. The use of a radio-pharmaceutical bound to the solid or liquid phase of a mixed meal and a gamma camera with appropriate computer support to allow analysis and visualization of studies (Minami and McCallum 1984). The serial test meal method is firmly established and validated and was used in most of the animal experiments reported here. Radioisotopic methods of measuring gastric emptying are relatively recent developments. Their advantages are the lack of a need for oro-gastric intubation, their use of physiological meals, and the ability to study both solid and liquid emptying simultaneously.

Certain pitfalls in isotopic methods need to be avoided. In the preparation of a solid test meal, the isotope requires to be firmly attached to a constituent of the meal. Secondly, if a single, anterior camera is used, anomalous results may be obtained as food moves in a postero-anterior direction during the initial phase of postprandial gastric activity, when no gastric emptying is

\(^{2}\)Either technetium\textsuperscript{99m} bound to sulphur or tin colloid, to prevent adsorption onto gastro-intestinal mucosa, or Indium\textsuperscript{111} bound to DTPA (Christian et al, 1980).

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occurring, but when the counts obtained anteriorly may actually increase (Christian et al, 1980). This is overcome by using both anterior and posterior cameras and taking the geometric mean of the gastric counts for the analysis. Thirdly, great care must be taken in the interpretation of dual isotope methods. In these, one isotope, say TC⁹⁹m is used to label the solid phase and another, say In¹¹¹ is used for the liquid phase. Because the radiation emitted by these two isotopes appears in different energy bands, they have been used to measure solid and liquid emptying simultaneously. However, the two energy bands have significant overlap (termed "cross-talk") of 30-60% (Dubois, 1983) and for that reason, changes attributed to solid emptying might be arising in the liquid phase and vice versa.

In the gastric emptying studies reported here, attempts were made to overcome the clinical and technical problems that have been incompletely addressed in previous reports.

4. Gastric perceptivity in anorexia and bulimia nervosa

The series of experimental and clinical studies described in this thesis are intended to test the hypothesis that slow gastric emptying contributes to the termination of a meal by provoking gastric distension. The control of feeding behaviour by signals arising in the stomach may be mediated in a number of ways, and the
information may or may not need to reach conscious awareness to influence food intake. In a number of studies, attempts have been made to determine whether, in normal subjects, perception of the state of the stomach, gastric perceptivity (Coddington and Bruch, 1970), is sufficiently precise to contribute significantly to control of food intake. Subjects with eating disorders have also been studied, in order to detect abnormal responses to tests of awareness of gastric function. Two attributes of gastric function have been investigated; gastric motility and gastric contents. Cannon and Washburn (1912) found that hunger pangs were always experienced at times of gastric contractions, measured using a manometer attached to a gastric balloon. Stunkard (1959) and Stunkard and Koch (1964) confirmed these findings and postulated that obese individuals did not, in contrast to normals, associate gastric contractions with hunger. However, their methodology has been criticised (Penick et al, 1967) and, in a subsequent study, in which telemetry of gastric motility was performed using a gastric tube, Stunkard and Fox (1971) found that the relationship between gastric motility, hunger and food intake was "weak and inconsistent" and that defects in the perception of gastric motility did not account for disturbances in hunger or in control of food intake observed in the obese. Moreover, studies of the concurrence of reports of hunger and
measured gastric contractions have been estimated at 58%, not much better than the 50% expected by chance (Whitehead and Drescher, 1980). Silverstone and Russell (1967) studied patients with anorexia nervosa using a gastric tube and measuring intragastric pressure. While the pressure recordings were normal, the authors noted that patients usually denied feeling anything during gastric contractions while normal subjects generally reported feeling empty or hungry. Bruch (1966) has proposed that a cardinal disturbance in anorexia nervosa is in the "accuracy of perception or cognitive interpretation of stimuli arising within the body". She and Coddington (1970) gave a standard volume of liquid food via a gastric tube and found that, while some normal subjects were able to determine with fair accuracy whether or not the nutrient had been introduced (blindly) into their stomachs, 15 subjects with obesity and three patients with anorexia nervosa were significantly less accurate. Garfinkel (1974) gave patients with anorexia nervosa a standard meal and monitored hunger and satiety using questionnaires. He found that patients associated satiety not with gastric fullness but with either unpleasant feelings of bloating, or with no gastric sensation. Much more work is required in this field. On currently available evidence it appears that perception of gastric contractions does not contribute significantly to the experience of hunger and to the control of food intake, although normal subjects may be able to detect gastric contents. There is some evidence
that patients with eating disorders have abnormal perceptions of satiety and are less accurate than normals in detecting gastric contents. No study has, thus far, attempted to relate gastric contents measured during gastric emptying with perceptions of gastric fullness and hunger.
CHAPTER 3

FENFLURAMINE, BULIMIA NERVOSA AND GASTRIC FUNCTION

1. Suppression of eating by fenfluramine in patients with bulimia nervosa

Summary
Fifteen patients with bulimia nervosa received fenfluramine (60 mg po) or placebo under double-blind, randomly ordered conditions. Two hours later food was presented. Significantly less food was eaten after fenfluramine and the quantity eaten was inversely correlated with serum fenfluramine levels. Significantly fewer patients reported bulimic symptoms during the test after fenfluramine, but no significant effect was demonstrated after leaving the ward. Fenfluramine caused drowsiness but did not reduce hunger ratings. Similarly, eating failed to reduce hunger ratings normally in the patients. These findings suggest that in patients with bulimia nervosa, hunger is reported abnormally and eating is suppressed by fenfluramine. Bulimic symptoms were probably reduced by fenfluramine, which may prove to be a useful treatment for bulimia nervosa.

Introduction
Bulimia nervosa is a serious condition in which the patient succumbs to intractable urges to overeat. During
the resulting bulimic episodes, large amounts of easily ingested food, often of high calorific value, are rapidly consumed. These episodes are accompanied by mounting distress and, because of an underlying morbid fear of weight gain, they may be followed by self-induced vomiting, laxative abuse or other means of reducing the 'fattening' effects of the ingested food. Severely restrictive dieting frequently ensues and may be interrupted by another episode of bulimia. The episodes may occur several times in a day and lead to serious physical complications including dental erosion, hypokalaemia, and renal failure (Robinson, 1989).

The term bulimia nervosa (Russell, 1979) has been used to identify a group of patients who suffer bulimic episodes, attempt to mitigate the effects of overeating by self-induced vomiting, laxative abuse or by other means, and who show a morbid concern with weight and body size. This concern is shared by patients with anorexia nervosa, and close links may be seen between the two conditions. Patients suffering from bulimia nervosa frequently give a history of previous true or 'cryptic' anorexia nervosa (Russell, 1979) while, conversely, bulimic episodes may complicate anorexia nervosa (Casper et al, 1980, Garfinkel et al, 1980). Episodes may also occur in patients with no history of anorexia nervosa (Stunkard, 1959, Fairburn, 1982) although dieting is prominent in these patients. There is, therefore, considerable overlap between patients currently suffering from anorexia nervosa, those with a past history of the disorder and those with no such
history, in that they may all have bulimic episodes. The term 'bulimia nervosa' has been applied to bulimic patients with a past history of anorexia nervosa (Russell, 1979) and to patients with bulimic episodes irrespective of their past history (Fairburn, 1982). The term 'bulimia' has also been applied to the latter group of patients, (American Psychiatric Association, 1980) and has been extended to include patients currently suffering from anorexia nervosa, if they have bulimic episodes (Andersen, 1983), reflecting differing usage between various workers. The term bulimia nervosa will here be used to describe patients who suffer episodes of bulimia associated with one of the major compensatory behaviours and who manifest a morbid overconcern with body weight and shape. These patients are not currently severely underweight but generally give a history of anorexia nervosa or of having lost a substantial amount of weight without fulfilling rigorous criteria for anorexia nervosa.

The aetiology of bulimia nervosa remains obscure. Because of the shared psychopathology with anorexia nervosa and the occurrence of the latter in the histories of patients with bulimia nervosa, it may be viewed as a chronic complication of anorexia nervosa. Many patients, although of average weight, are attempting to maintain body weight at a level that is lower than the premorbid weight. Accordingly, it has been suggested that overeating in bulimia nervosa may represent, in part, a physiological response to the patient's attempts to maintain body weight.
at what is, for her, a sub-optimal level (Russell, 1979). This theoretical standpoint implies that patients with bulimia nervosa should be encouraged to regain weight up to the premorbid level, even if premorbid weight was in the obese range. Against this view is the empirical finding that, during apparently successful therapy for bulimia nervosa, patients in whom weight loss is indicated for medical or social reasons can lose weight and still, at least in the short term, show improvement in their bulimic symptoms.

The use of drugs including anticonvulsants, tricyclic antidepressants and monoamine oxidase inhibitors in the management of bulimia nervosa has provided conflicting results about their efficacy, as noted in Chapter 2. Ong et al.'s (1983) study showed that methylamphetamine reduced the incidence of bulimia during a test meal and suggested the possibility of using appetite suppressant drugs in the treatment of bulimia nervosa. Amphetamines have serious unwanted effects such as dependence and psychosis (Connell, 1958) and could not be used in the treatment of these patients. Fenfluramine, however, has little or no potential for abuse (Connell, 1975, 1979) and if it could be shown to prevent episodes of bulimia in an acute experiment, a chronic trial of the drug might be warranted. Fenfluramine has been shown to decrease food intake significantly over the two hours after oral administration of 60 mg although hunger ratings were not reliably suppressed at that dose (Kyriakides & Silverstone, 1979).
In the present investigation the effects of fenfluramine on the consumption of food under controlled conditions, on self-rating scales of hunger, fullness and mood and on the incidence of subsequent bulimic episodes were studied.

Patients

Subjects were drawn from an out-patient clinic treating patients with eating disorders. All were female and satisfied criteria for both bulimia nervosa (Russell, 1979) and bulimia (American Psychiatric Association, 1979). From Table 1, in which clinical data are summarised, it will be noted that 5 patients were maintaining a weight at least 10% below their premorbid weight, while four patients gave a history of having been more than 10% above standard weight. Eight of the patients had in the past fulfilled diagnostic criteria for anorexia nervosa (Russell, 1983) while the rest had a history of weight loss with or without menstrual symptoms, but had not satisfied full diagnostic criteria for anorexia nervosa.

Most of the patients showed fairly severe psychological disturbances in addition to the bulimic symptoms. This is reflected in scores on the Hamilton Depression Rating Scale (Hamilton, 1960) and the Eating Attitudes Test (Garner and Garfinkel, 1979) which are given in Table 2.
<table>
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<tr>
<th>PATIENT</th>
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<th>DURATION</th>
<th>CURRENT WEIGHT</th>
<th>PREVIOUS A.N.³</th>
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<td>KG</td>
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<td>% MPMW²</td>
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<td>15</td>
<td>29</td>
<td>5</td>
<td>66</td>
<td>98</td>
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</table>

MEANS 25.0 4.7 55.5 95.7 94.3 8/15

1: PMW - PREMORBID WEIGHT
2: MPMW: MEAN POPULATION MATCHED WEIGHT (DOCUMENTA GEIGY, 1959)
3. FULFILLED CRITERIA FOR ANOREXIA NERVOSA
*PAST HISTORY OF BEING >10% OVER MPMW

TABLE 1: DEMOGRAPHIC AND CLINICAL INFORMATION
<table>
<thead>
<tr>
<th>PATIENT</th>
<th>BULIMIA &amp; VOMITING (FREQUENCY)</th>
<th>LAXATIVE ABUSE</th>
<th>CURRENT AMENORRHOEA</th>
<th>HDRS1</th>
<th>EAT2</th>
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**MEANS**

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<th>HDRS1</th>
<th>EAT2</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>55.9</td>
</tr>
</tbody>
</table>

1: HDRS - HAMILTON DEPRESSION RATING SCALE  
2: EAT - EATING ATTITUDES TEST - 26 ITEM VERSION (CUT-OFF FOR ANOREXIA NERVOSA 15)  
*: NO VOMITING

**TABLE 2: EATING DISORDER SYMPTOMS AND RESULTS OF RATING SCALES.**
Two typical case histories are presented.

Patient 2 is a 22 year old music student whose weight has been 45 Kg since puberty. At the age of 17 she lost 3 Kg after a febrile illness, began to feel fat, and experienced guilt after eating. Her attempts to diet were interrupted by bulimic episodes during which she would consume over 10,000 Calories in bread, cakes and sweets, induce vomiting, and take 20-30 laxative tablets. On a number of occasions she was not satisfied that all the food had been disposed of, and she would swallow a large number of aspirin tablets, present herself at a casualty department and obtain a stomach washout. In the past, she has stolen compulsively and she is amenorrhoeic. A sister has had anorexia nervosa in the past and her mother is obese. When first seen she was having 4 or 5 bulimic episodes daily and was unable to pursue her music studies.

Patient 11 is a 21 year old au pair girl, who developed anorexia nervosa at the age of 16. She lost weight from 54 Kg to 42 Kg and her periods ceased. At the time, important examinations were imminent and her mother was divorcing her father who suffered from alcoholism and manic depressive psychosis. Because of reading difficulties, the patient had always felt inferior to her successful sister. She has been and remains, over-dependent on her mother. After one year of low weight, she began to have bulimic episodes interspersed with periods of complete starvation lasting up to three days. Her weight has fluctuated between 45 and 73 Kg. She has been severely depressed and has made two suicide attempts.
Methods

Testing was begun in a single room on a research ward at 9.30 am after an overnight fast. At 10.00 am, fenfluramine hydrochloride 60 mg (Servier Laboratories Ltd) or placebo was administered orally under double blind and randomly ordered conditions. The two preparations were administered in two tests, separated by an interval of about one week, enabling a comparison between placebo and fenfluramine to be made. The patient was informed that she was to receive one of two preparations which may or may not influence her eating. Two hours after taking the tablets, when the anorectic action of fenfluramine would be expected to be reaching a peak (Silverstone et al, 1975), a blood sample was taken for serum fenfluramine estimation and the patient was left alone for thirty minutes with a liberal supply of food chosen by her in advance. She had been asked to choose the specific food that she was most likely to eat during a bulimic episode. On presentation of the food, the patient was asked to eat some of what was given and to continue to eat as much as she wished with the 30 minutes. The food was weighed before and after the test and the calorific value of the food consumed was calculated by reference to tables (Documenta Geigy, 1959). During this part of the test, the quantity of food eaten was determined with fair accuracy. However, laboratory conditions differ markedly from the settings in which bulimic episodes in these patients usually occur. Accordingly, the patient was allowed to leave the ward at 12.30 pm and asked to record,
on a standard sheet, for the next five days, bulimic episodes and other food consumed as well as any adverse effects possibly attributable to the medication. In this way, a record of the occurrence of bulimic episodes under natural conditions was obtained.

For purposes of the study, a bulimic episode was said to have occurred when the patient reported consumption of a large amount of food, rapidly, within a discrete period of time. In addition, the patient's subjective assessment of whether the episode constituted a 'binge' or not was used. For different patients, a variety of elements appeared to be important in making this assessment including 'a feeling of loss of control', depressive and self-deprecating thoughts, and the later occurrence of self-induced vomiting, purging or fasting. Although unavoidable, reliance on this subjective evaluation clearly introduces a possible source of error. When the diary sheet was returned, it was discussed in detail with the patient, and it was ascertained that the bulimic episodes recorded fulfilled the above criteria. The eating that had occurred during the test on the ward was also discussed and characterised. For the purposes of designating individual bouts of eating, patients were asked to indicate whether the episode was 1. a bulimic episode, or "binge" 2. a "near binge", being an episode having some characteristics of a "binge" but not reaching the subject's own criteria, 3. a meal containing at least two slices of bread or the equivalent in other sources of carbohydrate, 4. a low
carbohydrate meal, containing less than two slices of bread or equivalent.

From the time the patient took the tablets until leaving the ward, 100mm visual analogue scales (Silverstone et al, 1978) were completed every 30 minutes. These were labelled as follows:-
Alert  I---------------------------------------I  Drowsy

Not at all hungry    Extremely hungry
I---------------------------------------I

Extremely sad    Not at all sad
I---------------------------------------I

Not at all Full    Extremely Full
I---------------------------------------I

Extremely tense    Not at all tense
I---------------------------------------I

No urge to eat    Extreme urge to eat
I---------------------------------------I

Extremely fat    Not at all fat
I---------------------------------------I

In order to allow a comparison to be made between the patients' analogue scale results and those that would be expected from a normal group, a limited experiment was performed using controls. Subjects drawn from the hospital staff, who denied any history of eating disorder were matched with the patients for sex and for age (± 20%) and weight (± 12%) and were tested after an overnight fast.
under the same conditions as the patients. They were given placebo, but were told that they were receiving tablets that may or may not affect their eating. They completed the analogue scales every 30 minutes and after two hours, a sample of blood was taken and the subjects were presented with the same amount of the same food that had been consumed after placebo by the patients with whom they had been paired. They were asked to consume some of the food and to eat as much as they wished within 30 minutes. Written, witnessed, informed consent and the approval of the ethical committee of the Maudsley Hospital were obtained.

Statistical comparisons were made as follows: Calories consumed at 2 hours were compared, using analyses of variance, to determine the effects of drug treatment and of order of presentation of the tablets and, to compare patients with controls. Correlations were derived by calculation of Pearson's r.

The number of patients who characterised the meal two hours after fenfluramine as a bulimic episode was compared with the number after placebo, using McNemar's Chi-squared test for non-independent samples (Hays, 1973).

The McNemar test was also used to compare the incidence of bulimia, vomiting and laxative abuse in the first 20 hours after the tablets, this period representing the half-life of oral fenfluramine (Campbell, 1971) and in the third 20 hours, when little or no pharmacological action would be expected.
The visual analogue scale results were compared, using an analysis of variance with repeated measures. The groups were drug, placebo (patient) and placebo (control), and the repeated measure was timed at 0, 30, 60, 90, 120 and 150 minutes, the last time being post-prandial.

In all analyses, differences were regarded as significant if P<0.05.

Results (Table 3)

**Eating at two hours:** The number of calories consumed by patients having received fenfluramine was significantly smaller than their consumption after placebo: (F(1,26) = 8.46, P<0.01). In the same two-way analysis of variance there was no significant effect of order of presentation of drug or placebo. (F(1,26) = 1.73, P>0.1).

In a one-way analysis of variance including the control group there was a significant treatment effect (F(2,42) = 5.69, P<0.01).

Planned comparison t-tests of differences in mean calories consumed suggest that not only did patients eat more after placebo (mean intake 492 Kcal) than after fenfluramine (234 Kcal), but patients having had placebo ate significantly more than controls who received placebo and were presented with identical quantities of food (312 Kcal) (t>2.29, P<0.05).

After fenfluramine, there was a negative correlation which did not reach statistical significance between plasma drug level at 2 hours and calories consumed (Pearson's r =
Four of the patients (patients 1, 2, 3 and 11) characterised their eating after placebo as a 'definite binge' and one of these (patient 3) induced vomiting on the ward. Two additional patients (14 and 15) reported that the meal had some qualities of a bulimic episode in that it was, for them, excessive consumption of 'forbidden' food, but both felt that the quantity eaten did not satisfy their criteria for a 'binge'. They did classify them as 'near binges' and have been designated as such in Table 2. One of these two patients, who ate 6 single portion chocolate rolls commented "I wanted to eat all 18, but knowing you would be back I was too much of a lady". One patient (4) induced vomiting immediately after leaving the ward while three more (6, 8 and 9) suffered bulimic episodes within four and a half hours of leaving the ward. In contrast, after fenfluramine, no patient complained that eating on the ward constituted a 'binge' or a 'near binge' and none admitted to inducing vomiting after the test. One patient (7) had a bulimic episode immediately after leaving the ward, having received fenfluramine. In summary, there was a significant decrease in the number of patients showing bulimic symptoms ('binges', 'near binges' and vomiting) on the ward after fenfluramine (McNemar Chi-squared = 4.17, df = 1, p<0.05). The incidence of bulimic symptoms arising shortly after leaving the ward and experienced by the patient as closely linked to the meal taken during the test was also lower after fenfluramine, although the difference
was not significant when this period is considered in isolation.

**Eating in the four days following the test:**

**Bulimic episodes:** Seven patients reported bulimic episodes or episodes of vomiting or laxative abuse in the 20-hour period after placebo compared to two after fenfluramine. This difference is not significant (p>.05). Episodes occurring during the ward test were not counted in this comparison.

**Other meals:** Ten of the fifteen diaries gave sufficiently detailed information to allow some analysis of eating patterns, apart from bulimic episodes. There was no significant difference in the number of meals taken in the 24 hours after leaving the ward between drug and placebo. In view of the tendency of patients with bulimia nervosa to restrict carbohydrate intake, and the possibility that fenfluramine might result in an increase of this tendency, meals were classified according to their carbohydrate content (more or less than two slices of bread or equivalent). No significant difference between drug and placebo in the incidence of either type of meal emerged.

**Side-effects**

Ten patients complained of side-effects, either before or after leaving the ward, compared to two after placebo. (McNemar Chi-squared = 4.08, df = 1, P<0.05). The most common effects reported after active drug were drowsiness,
headache, and unsteadiness, while two patients reported euphoria. The effects were mild to moderate in severity, lasted a mean of 21.8 hours, and were not related to the plasma drug level. After placebo, one patient described headache and drowsiness and the other reported euphoria, and two control subjects described moderate loss of appetite for 48 hours.

**Visual Analogue Scale Results (Figures 3a, b)**

Analyses of variance with repeated measures provided tests of significance of effects of treatment (drug, placebo, control), time (0, 30, 60, 90, 120 and 150) and interaction between the two.
Figure 3a

- Controls
- Fenfluramine 60 mg p.o.
- Placebo

**MEAN RATINGS (mm ± sem)**

**HUNGRY**

**URGE TO EAT**

**Time (min)**

67
Figure 3b

- Controls
- Fenfluramine 60 mg p.o.
- Placebo

Mean Ratings (mm ± sem)

Time (min)

FULL

FAT

Meal
Figure legends

Figures 3a, b: Results of visual analogue scale ratings of hunger, urge to eat (Figure 3a), fullness, and fatness (Figure 3b), during test. Fenfluramine (circles) or placebo (triangles for patients, squares for controls) given at time 0. Food available between times 120 and 150. Patients with bulimia nervosa showed no mean change in hunger, and an increase in urge to eat after eating. These abnormal responses were partly corrected by fenfluramine.
Patients rated themselves as increasingly drowsy throughout the test after fenfluramine compared to placebo, maximal at 120 minutes, reflected in a significant effect of treatment \(F(1,28) = 8, P<0.01\) and time \(F(4,112) = 22.2, P<0.01\). Hunger ratings showed no significant effect of treatment but a significant effect of time \(F(5,210) = 5.38, P<0.01\) and a significant interaction \(F(10,210) = 2.20, P<0.05\). Inspection of cell means shows that the effect of time is derived from a reduction in hunger ratings which occurred after eating. However, the interaction effect reflects a difference in the reduction between groups. Mean hunger ratings in controls fell 32.2mm (Planned comparison t-test: \(t = 5.32, P<0.002\)), patients receiving placebo showed a mean fall of 1.7mm after food \(P>.25\) while after fenfluramine a mean fall of 11.7mm occurred \((t = 1.93, 0.05<P<0.1)\). These results suggest first that the patients were rating themselves on a hunger scale differently from controls, and secondly that fenfluramine tended to restore the normal fall in hunger ratings after food. The scale 'urge to eat' showed a pattern in controls very similar to the 'hungry' scale, with a mean fall of 31.6mm after eating. (Planned comparison \(t = 4.91, P<0.001\)). Patients, having received placebo increased their ratings of 'urge to eat' by a mean of 11.8mm \((t = 1.83, 0.05<P<0.1)\) and after fenfluramine ratings fell by a mean of 7.1mm \((P>0.25)\). These varied responses are reflected in a significant interaction effect in the analysis of variance \(F(10,210) = 3.54, P<0.01\).
Post-prandial ratings after placebo were compared with ratings after fenfluramine, using paired t-tests. 'Urge to eat' was significantly lower after the active preparation (t = 2.56, P<0.05) and hunger ratings were lower after fenfluramine, compared to placebo (t = 1.73, 0.05<P<0.1). In contrast, ratings of fullness showed a uniform increase after the meal under all three conditions, reflected in a significant effect of time (F(5,210) = 24.45, P<0.01) but no difference between groups and no interaction.

In order to test whether the differences observed between the groups could be attributed to the different quantities of food eaten under the three conditions, correlation coefficients were determined between calories eaten and ratings of hunger, urge to eat, and fullness. The only correlation approaching significance was between the controls' post-prandial (150m) hunger ratings and calories consumed (r = -0.45, P<0.1), indicating that the proportion of the variance in the ratings contributed by the differences in caloric intake was small.

Results of the other scales reflect additional aspects of the patients' mental state. The scale 'fat' showed a significant treatment effect (F(2,42) = 6.18, P<0.01) and a significant effect of time (F(5,210) = 5.14, P<0.01). The patients rated themselves twice as 'fat' as controls, and subjects under all conditions increased their ratings after eating (Figure 3b).

Patients rated themselves higher than controls on scales 'sad' (mean ratings: patients 37mm, controls 12.6mm, F(2,42
= 9.73, P<0.01) and 'tense' (means 31.6mm, 15.2mm, \( F(2,42) = 4.9, P<0.05 \)) and there was no effect of time and no interaction.
Discussion

In fifteen patients with bulimia nervosa, fenfluramine reduced the quantity of food consumed under laboratory conditions. This reduction in eating is consistent with previous reports of the drug's action in normal volunteers (Kyriakides & Silverstone, 1979) and suggests that, in terms of calories consumed, bulimic patients respond to fenfluramine in a manner comparable to normal subjects. The negative correlation between calories consumed and plasma fenfluramine, although not reaching statistical significance, supports a direct pharmacological effect upon eating behaviour.

Secondly, there appeared to be a significant reduction in abnormal eating during the test, reflected in a difference in the number of patients who described the experience as a 'binge' or a 'near binge'. It is clear that the definition of abnormal eating relies substantially on the subjective report of the patient, for which no standardised assessment is currently available. Characterization of eating episodes was performed, using clinical assessment, taking into account the patient's description of the eating and accompanying affective changes. However, this measure is clearly subject to error.

Fenfluramine failed to produce a significant suppression of bulimic symptoms after leaving the hospital. Five patients suffered episodes of bulimia or vomiting shortly after leaving the ward and in all but one instance, these
patients had received placebo. However, taking the immediate post-test period or the first 20 hours after the drug, no significant effect has been demonstrated. At least two factors may contribute to this. Firstly, the additional support of a hospital environment may be required for the drug to be effective and, secondly, plasma fenfluramine levels may, after several hours, have fallen below a therapeutic range.

The mechanism of action of the drug in suppressing eating in bulimic patients has not been clarified. In the previous study of Ong et al (1983), methylamphetamine resulted in enhanced alertness and euphoria and suppression of hunger. However, the predominant effect of fenfluramine was drowsiness, euphoria was noted in only two patients, and while hunger ratings were reduced post-prandially, this effect failed to reach statistical significance. The effect that the two drugs share, and therefore the most likely mechanism whereby they suppressed eating, is a specific reduction in eating behaviour. The failure of fenfluramine, at the dose used, to suppress ratings of hunger significantly has been noted previously in normal subjects (Kyriakides & Silverstone, 1979), while other findings presented here, namely reduction of food intake and a variety of side-effects, are consistent with the known properties of the drug (Kyriakides & Silverstone, 1979, 1979a, Silverstone et al, 1975). Moreover, the mean duration of side-effects in our patients, 21.8 hours, is consistent with the estimated plasma half-life of
fenfluramine after a single administration, which is 20.3 hours (Campbell, 1971), suggesting that side effects are most likely to occur when plasma levels are greater than 50% of peak levels.

Drowsiness ratings were significantly greater after fenfluramine. However, the suppression of eating seems unlikely to be due to this effect. It is conceivable that the presence of side-effects allowed patients to realise that they had received the active preparation, and thereby compromised the blind nature of the experiment.

The apparent suppression of bulimic episodes seen after fenfluramine can be understood in a number of ways. Patients with bulimia nervosa frequently report that a bulimic episode will invariably follow the consumption of a certain threshold quantity of a high-calorie food (Abraham and Beumont, 1982). Fenfluramine may therefore act by suppressing food intake so that the threshold is not exceeded, and thereby prevent the triggering of a bulimic episode. Secondly, the action of the drug in suppressing bulimia may suggest that there exists, in patients with bulimia nervosa, a disorder of the control of eating behaviour which can be modified by drugs. Thirdly, the abnormal increase in 'urge to eat', seen after the meal in the bulimic patients, was partially reversed by the drug which may therefore act to facilitate mechanisms underlying satiety and thereby suppress bulimia. Current views about the mechanism of action of fenfluramine suggests that the suppression of eating observed after the drug probably
reflects a central effect mediated by serotonergic mechanisms, and a peripheral action involving inhibition of gastric emptying (Davies et al, 1983), demonstrated in rhesus monkeys in the following studies.

The failure of hunger ratings to be suppressed by eating in patients with bulimia nervosa is a phenomenon that has not previously been described. Garfinkel (1974) found that patients with anorexia nervosa were less likely to report gastric fullness after a standard meal, compared to controls, while Robinson et al, (1983) found patients with bulimia to have lower hunger and higher fullness ratings both before and after a standard meal, compared to controls. However, inspection of the data presented in the latter study suggests that their control subjects reported significantly reduced hunger and increased fullness following the meal, while the bulimics showed no changes in mean hunger ratings, but did significantly increase ratings of fullness. These are similar to the present findings and suggest an abnormality in the way patients with bulimia experience or report hunger which merits further study.

Apart from contributing to our understanding of the symptoms of bulimia nervosa, the study of the actions of appetite suppressant drugs may also lead to a useful treatment for this condition, which often resists therapeutic efforts. The present study provides evidence suggesting that fenfluramine influences bulimic symptoms, at least in a ward environment, and points to the need for a controlled trial of the drug given to out-patients over
a period of several weeks. Such a study should be designed
to monitor not only therapeutic effects of the drug in
suppressing bulimic episodes, but also the possible adverse
effects on dietary intake and mood.
### TABLE 3: FOOD INTAKE AND SUBSEQUENT EATING BEHAVIOUR AFTER FENFLURAMINE, 60MG PO, AND PLACEBO IN 15 OUTPATIENTS WITH BULIMIA NERVOSA, AND AFTER PLACEBO IN 15 MATCHED CONTROLS.

* Documenta Geigy. ** DB; definite binge, V; vomiting, NB, near binge. 3: Superscript=hours after leaving hospital. 4: PLA: placebo, and FEN: fenfluramine, 60mg, po, in patients with bulimia nervosa. CTL: Control subjects (placebo). Means with different superscripts* are significantly different.

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* Documenta Geigy. ** DB; definite binge, V; vomiting, NB, near binge. 3: Superscript=hours after leaving hospital. 4: PLA: placebo, and FEN: fenfluramine, 60mg, po, in patients with bulimia nervosa. CTL: Control subjects (placebo). Means with different superscripts* are significantly different.
2. Inhibition of gastric emptying and feeding by fenfluramine in the rhesus monkey

Summary
To assess the mechanism by which the anorectic agent fenfluramine influences feeding, we examined, in Macaca Mulatta the effect of intragastric fenfluramine (2mg/kg) on both feeding and the gastric emptying of saline and glucose test meals. Gastric emptying was measured by the dye-dilution technique, using chronic indwelling intragastric cannulas. The emptying of normal saline was inhibited by fenfluramine as was the initial bolus phase of the emptying of glucose. In contrast, the subsequent controlled phase of glucose emptying was unaffected in two out of four animals, slightly inhibited in one and strongly inhibited in only one monkey. Rhesus monkeys trained to drink a glucose solution to satiety drank, on average, 21% less after fenfluramine. The emptying of the consumed glucose from the stomach was reduced by 39%, whereas gastric volume at satiety was reduced by only 10%. The reduction of intake was strongly related to the degree of inhibition of gastric emptying. These results support an important role for the inhibition of gastric emptying in fenfluramine-induced suppression of feeding.

Introduction
The control of meal size in primates depends in part on gastric mechanisms. It has been shown, for example, that rhesus monkeys equipped with chronic gastric cannulas and drinking a solution of glucose will, when aliquots of gastric contents are removed via the cannula, consume a further amount equivalent to the volume removed (Wirth and McHugh, 1983), suggesting that gastric distension acts as a satiety signal under these conditions. Certain peptides have been implicated as physiological mediators of satiety, and it has been demonstrated that exogenous administration
of one such agent, cholecystokinin, exerts part of its inhibitory effect on feeding by reducing gastric emptying (Moran and McHugh, 1982), thereby facilitating gastric distension.

Fenfluramine is a halogenated derivative of amphetamine and has been shown to reduce food intake in rats (Blundell et al, 1979), monkeys (Foltin and Schuster, 1983), normal humans (Kyriakides and Silverstone, 1979), and, as described above, in patients with bulimia nervosa (Robinson et al, 1985a). The demonstration that fenfluramine reduces hunger ratings (Silverstone et al, 1978) and delays the onset of feeding (Kyriakides and Silverstone, 1979) in humans suggests that the drug may inhibit the initiation of eating. However, fenfluramine reduces meal size without affecting intermeal interval in the rat Blundell et al, 1979), suggesting that it also provokes the termination of a bout of ingestion.

Evidence has been presented showing that fenfluramine inhibits gastric emptying in the rat (Davies et al, 1983, Rowland and Carlton, 1984) and in man (Horowitz et al, 1985). If the gastric inhibitory action of fenfluramine can be confirmed, it, and the gastric distension it facilitates, could be suggested as a peripheral physiological means for the drug's suppression of food intake. The present series of experiments was undertaken to examine the influence of fenfluramine on gastric
emptying in the rhesus monkey and to investigate the role of any observed effect on gastric function in mediating an inhibition on consumption of a glucose solution.

**General methods**

The subjects were five male rhesus monkeys (*Macaca mulatta*) caged individually and weighing between 5 and 14 Kg. Four monkeys were used in each experiment. Each animal was fitted with a chronic indwelling Silastic intragastric cannula that allowed continuous access to the stomach for both the administration of intragastric loads and the withdrawal of gastric contents. The monkeys wore soft leather vests to protect the cannula where it emerged from the skin, and the cannula travelled through a multiflexible steel sheath attached to the back of the cage. The animals were able to move around the cages freely with this sheath attached to the vest. The preparation has been described in more detail elsewhere (McHugh and Moran, 1978). The animals were trained to eat their daily chow intake between 1200 and 1600 h each day. Both the gastric emptying experiments and glucose consumption experiments were carried out between 0900 and 1200 h after 17 h food deprivation. Drinking water was available at all times except during experiments.
Experiment 1.
Effect of Penfluramine on gastric emptying

Methods

Gastric emptying was examined by means of the serial test meal method of Hunt and Spurrell (1951). In preparation for each experiment the monkey's stomach was rinsed with 50 ml of physiological saline to remove any remaining food particles and to check that the stomach was empty. Then a volume of 150 ml of either physiological saline (9g/l NaCl) or D-glucose (125g/l, 0.5 kcal/ml), warmed to 37 degrees centigrade and containing phenol red (31.25 mg/l), was infused through the gastric cannula and left in the stomach for a duration of time, the emptying period. This emptying period was varied from occasion to occasion. Two determinations were obtained for the 5-, 10-, and 20-min emptying periods for saline and the 10-, 20-, 60-, and 90-min emptying periods for glucose.

At the end of each emptying period the contents of the stomach were withdrawn through the cannula, and the stomach was rinsed to gather any remnant volume. The second rinse represents a modification (McHugh et al, 1982) of the original method of Hunt and Spurrell. The volume of the original load still remaining in the stomach and the volume of secretions that had accumulated during the emptying period were then estimated by correcting the total volume
of liquid removed from the stomach by the dilution of the phenol red marker measured by spectrophotometry. Gastric emptying curves were constructed by displaying the volume of the original load remaining in the stomach at the end of each emptying period. It has previously been demonstrated (McHugh and Moran, 1979) that, in the rhesus monkey, physiological saline empties from the stomach in an exponential uniphasic fashion. The regression curves for emptying were therefore constructed with log-transformed volumes of saline remaining in the stomach and the half emptying times estimated. It has also been demonstrated that glucose emptying follows a biphasic function with at first a rapid phase of emptying followed by a slow and linear phase in which glucose calories (estimated from 4 kcal/g of glucose) are emptied from the stomach at a constant rate of approximately 0.4 kcal/min (McHugh et al, 1982). Regression curves for glucose emptying were therefore constructed using the untransformed volumes of glucose remaining in the stomach and the least-squares regression line drawn. This permitted measurement of the rate of glucose emptying during the linear phase (the slope of this regression line) and measurement of the volume of the first rapid phase of glucose emptying from the difference between the Y-intercept of this curve and the 150 ml that actually was infused into the stomach at time 0.

The characteristics of gastric emptying of saline and
glucose with and without fenfluramine were thereby compared. The drug was given by intragastric injection of dl-fenfluramine HCL (2mg/kg; A.H. Robins, Richmond VA, USA) dissolved in 20 ml saline (9g NaCl/l). This dose is in the range that has been shown to suppress food intake reliably in both rhesus monkeys (Foltin and Schuster, 1983) and humans (Kyriakides and Silverstone, 1979, 1979a). The cannulas were washed through with an additional 20 ml of saline. Gastric emptying studies under the drug condition began from 1 to 3 h after the injection of fenfluramine. The differences in volume remaining at given emptying times between drug and control conditions were determined and tested for significance using trend analysis and orthogonal polynomials. For saline emptying, half emptying time and, for glucose emptying, the slope and Y-intercept were also determined and compared using paired t-tests. On some experimental days two or three emptying experiments were completed during the same session. Before proceeding with such additional experiments after a glucose load, the emptying of saline was determined to be sure that the original conditions at the beginning of the day had been re-established. Three to seven days elapsed between trial days, and at the beginning of each day gastric emptying of saline was determined to ensure that it fell within the range that had previously been established for each animal in the drug-free state.
Results

The volume of saline emptied after fenfluramine was significantly less than that obtained under control conditions $[F(1,3) = 48.7, P<0.01]$, as can be seen in Figures 4a-d. The control emptying of saline was rapid and exponential in all four monkeys, with a mean half emptying time of 5.6 min. After fenfluramine the emptying rate was always slower, and the half emptying time was increased to a mean of 21.7 min ($t = 3.31, P<0.05$).

The effect of fenfluramine on gastric emptying of glucose was more complex, as can be seen in figures 5a-d. Under control conditions, emptying was biphasic with an initial bolus represented by the difference between the infused volume of 150 ml and Y-intercept and a second phase of controlled linear emptying at a rate described by the gradient of the regression line. Although the volume of glucose emptied over 90 min was significantly less with fenfluramine $[F(1, 3) = 15.5, P<0.05]$, the overall pattern of response to the drug of the four monkeys differed. Two of the monkeys (Q29, Fig 5a, and P43, Fig 5b) showed no change in the slope of the regression line with the drug, but the intercept on the Y-axis was shifted up, thus indicating a reduction in the amount of glucose passed in the initial bolus. One animal (R37, Fig 5c) showed a similar shift upward of the Y-intercept and a slight
slowing of the rate of emptying, whereas in the fourth animal (B22, Fig 5d) the slope was clearly slowed and the Y-intercept only slightly increased. The mean rate of glucose emptying in the linear phase was 0.28 kcal/min after the fenfluramine, which is not significantly different from the mean emptying rate of glucose under control conditions, 0.38 kcal/min ($t = 1.47, \text{df} = 3, P>0.2$). The mean Y-intercept after fenfluramine (136.0 ml) was, however, significantly greater and thus closer to the original 150-ml volumes than the control mean Y-intercept, 124.0 ml ($t = 3.34, \text{df} = 3, P<0.05$).

To estimate the time course of drug action the volume remaining after each fenfluramine experiment was expressed as a percentage of the control volume remaining, and this proportion was, in a linear regression, compared with the delay between drug administration and testing time (which varied between 60 and 180 min). No significant trend emerged, suggesting that the drug was as active 3 h after administration as at 1 h. Using similar analyses, no effect of repeated administration was observed on the gastric inhibitory action of fenfluramine. Volume of secretions showed no consistent change after drug during either saline or glucose experiments [$F(1, 3) = 3.6, P>0.1$].
Comments

These experiments demonstrate that fenfluramine inhibits gastric emptying. The effect on saline emptying was observed throughout the 20-min period of observation. In contrast, glucose emptying was significantly reduced only in the initial phase of emptying, with subsequent emptying being unaffected in two animals, slightly affected in one, and markedly inhibited in the last. Gastric emptying of solutions such as glucose proceeds in at least two distinct phases (McHugh et al, 1975, 1982). These are, first, the initial bolus that passes through the pylorus immediately after filling of the stomach and, second, the phase of slow and controlled emptying. Both the emptying of saline and the size of the initial bolus of glucose emptying were consistently reduced by fenfluramine. Similar physiological mechanisms may, therefore, underlie saline emptying and the emptying of the initial bolus of glucose.

Experiment 2

Fenfluramine, Gastric emptying and Sucrose consumption

Methods

Before the start of this experiment four monkeys were
trained to drink a sucrose solution (125 g/l, 0.5 kcal/ml) to satiety within 15 min. On experimental days, after a saline gastric rinse, dl-fenfluramine HCL (2 mg/kg) dissolved in saline or an equivalent volume of normal saline vehicle was given as an intragastric injection. The gastric cannula was then cleared with an additional 20 ml normal saline. Each animal was tested on eight occasions with fenfluramine and eight occasions with the saline vehicle. The experimental order was randomized and the experimenter blind to drug treatment. From experiment I it had been established that after 3 drug-free days no residual drug effect was noted on gastric emptying, and tolerance was not observed with repeated testing at this interval. Accordingly, at least 4 days were allowed between a fenfluramine test day and a subsequent test. Drinking water was available until 30 min after drug administration to ensure adequate hydration. Sixty minutes after the intragastric infusion of drug or vehicle solution, subjects were given a drinking bottle containing a quantity of sucrose at least 20% greater than their maximum consumption during training. The solution was labelled with phenol red to facilitate determination of gastric emptying rate, volume of secretions, and spillage. Subjects were allowed to drink for 15 min. During this period the animal's drinking activity (time spent with the mouth in contact with the drinking spout) and locomotor activity (time spent moving) were recorded by the
The experimenter who was blind to the drug treatment. The observer rated the animal's behaviour in 10-s intervals over the entire 15-min observation period using a pair of microswitches linked to a chart recorder. If a behaviour was recorded during a 10-s period, one point was scored, giving a maximum possible of 90 for each activity. Meal termination was said to have occurred at the end of an episode of ingestive behaviour that was followed by at least 2 min free of drinking.

After 15 min the bottle was removed, and the sucrose solution remaining in the bottle was measured. At the same time the stomach contents were rapidly aspirated through the indwelling cannula, two gastric washes of 50 ml of saline were performed to assure the full collection of gastric contents, and any spillage from the drinking bottle was collected from the cage floor.

Gastric contents were analyzed by the dye-dilution technique. This method allowed the estimation of the following volumes:
1) volume of sucrose consumed, 2) volume of sucrose meal remaining in the stomach 15 min after the beginning of sucrose consumption, 3) volume of oropharyngeal and gastric secretions, 4) end gastric volume at 15 min, and 5) volume of sucrose emptied from the stomach in 15 min.

For each animal, analysis of covariance was performed to
yield the mean difference between drug and vehicle for each of the various measures, adjusted for trial number, to allow for changes in behaviour occurring with increasing experience of the experimental set-up. For measures of intake, gastric emptying, end gastric volume and secretions, the means were further adjusted for body weight, with which they generally covaried significantly, by use of linear regression. The differences between resulting adjusted means were tested for significance using t-tests.

Results

Fenfluramine significantly decreased sucrose consumption (mean reduction 99.1 ml; t = 5.29, df = 3, P< 0.02). Fenfluramine also inhibited gastric emptying under these conditions, expressed as a percentage of volume consumed (mean reduction 8.3%; t = 4.39, df = 3, P<0.05). Because the effect of fenfluramine on feeding and gastric emptying was not equally potent in all animals, individual data are presented in Tables 4 and 5.

The inhibitory effect of fenfluramine on feeding was greatest in monkey R37 and was progressively less marked in the other animals (R37>B55>B22>P43). P43 was insensitive to the effects of fenfluramine. The inhibitory effect of fenfluramine on gastric emptying showed the same sequential
pattern across the four monkeys.

Each experiment using fenfluramine was paired with the next experiment in the same animal in which the vehicle preparation was used. Volume consumed and gastric emptying after fenfluramine were expressed as the percentage of consumption and gastric emptying on the day that vehicle was administered to provide measures of the inhibition by the drug of volume consumed and emptied. Correlation coefficients (Pearson's $r$) were determined for each animal between the two measures.

As can be seen in Table 4, the sequence of correlations from highest to lowest is identical to that obtained when the effects of the drug on feeding and on gastric emptying are used to generate similar sequences; i.e., $R37 > B55 > B22 > P43$. In two of the subjects very high correlations between the effect of fenfluramine on ingestion and its effect on gastric emptying were observed. In one animal a lower but still significant correlation was seen, whereas $P43$, the animal that showed little or no effect of fenfluramine on feeding and gastric emptying, showed a correlation that was not significant.

The volume of gastric contents remaining in the stomach at the end of the test was also found to be significantly reduced by fenfluramine ($t = 10.4$, $df = 3$, $P<0.01$). The ratios between the mean end gastric volumes after
fenfluramine and the corresponding mean after vehicle are given in Table 5. The overall mean value was 89.4%. Thus the animals were reaching satiety at a slightly, although significantly, reduced gastric volume after fenfluramine compared with vehicle. As in experiment I, there was no indication that fenfluramine altered the rate of production of upper gastrointestinal secretions (Table 5) and, using linear regression, no evidence of tolerance.

Behavioural observations summarized in Figures 6a-c revealed a significant effect of fenfluramine on ingestive activity (P<0.05) and a trend toward a reduction in locomotor activity (P<0.1). In addition, three of the animals showed earlier termination of the meal after fenfluramine compared with vehicle (P<0.1).

Comments

This experiment shows that the inhibition of gastric emptying observed during experiment I can be demonstrated during feeding. Moreover, there was a clear relationship between the ability of fenfluramine to inhibit gastric emptying and its potency in reducing feeding. The squares of the correlation coefficients in Table 4 indicate the proportion of the variance in the suppression of feeding
that could be explained by inhibition of gastric emptying. This proportion was 92% for R37, 83% for B55, and 50% for B22, and in P43, which did not respond to fenfluramine, the figure is 6%. The feeding inhibitory action of fenfluramine was reflected in a significant reduction in observed ingestive behaviour, and the reported sedative effect of the drug in man (Robinson et al, 1985a) was manifested in the monkey by a reduction in locomotor activity in all subjects.

Discussion

This work demonstrates that the anorectic drug dl-fenfluramine HCL exerts marked inhibitory effects on gastric emptying of saline and glucose meals in the rhesus monkey and that it suppresses consumption of a sucrose solution in the same animal. The simple hypothesis that these two actions of fenfluramine are related seems partially confirmed. The more fenfluramine influenced gastric emptying, the more feeding was inhibited. Feeding tended to persist under the fenfluramine condition until the stomach reached approximately 90% of its volume under the control condition. The inhibitory effects of fenfluramine on feeding were correlated and rank ordered
with its effects on gastric emptying.

Fenfluramine exerted a differential action on the different phases of gastric emptying. Saline emptying was inhibited throughout, whereas glucose emptying was inhibited largely by an action on the initial bolus phase. This suggests that the underlying physiology of the initial bolus phase of glucose emptying is similar to that of saline emptying. At least 3 days were allowed between experiments, and no evidence of tolerance was observed.

The mechanisms by which fenfluramine exerts this inhibitory effect on gastric activity remain to be elucidated. The drug could relax the stomach wall or contract the pyloric sphincter or duodenal musculature. The pyloro-duodenal system is thought to mediate controlled emptying of nutrients such as glucose, the linear portion of the glucose emptying curve. In the present work, the effect of fenfluramine on saline emptying and the initial bolus of glucose emptying were similar, and as both of these functions are thought to be regulated by gastric wall, rather than pyloroduodenal function, it is by reducing gastric tone that fenfluramine is most likely to be exerting its gastric inhibitory effect.

The results suggest that fenfluramine suppresses feeding, in a part, through an inhibition of gastric emptying. It
is also likely that some feeding suppression depends on other actions of fenfluramine because the end gastric volume was significantly less than the control. Davies et al (1983) have noted in rats that midbrain raphe lesions interrupt fenfluramine inhibition of feeding and suggested that central serotonergic pathways may mediate some of the anorexia induced by fenfluramine in 18-h deprived but not in freely feeding animals. In the present study the effects of fenfluramine on the consumption and gastric emptying of liquid meals were investigated. Analogous inhibition of gastric emptying of solid food by fenfluramine has been noted in rats (Rowland and Carlton, 1984) and in man (Horowitz et al, 1985) and in rhesus monkeys it has been observed that gastric preloads shift to the left the dose-response curve for fenfluramine inhibition of solid food intake (Foltin and Schuster, 1983), suggesting that the drug enhances physiological controls of food intake, such as modulation of gastric emptying rate.

Finally, it is possible that the gastric and feeding inhibition produced by fenfluramine are separate unrelated phenomena. Fenfluramine does affect several neurotransmitter systems (Garattini et al, 1975, Prove and Ehrlein, 1983, Sipes et al, 1971, Waton and Kasim, 1975), and its feeding inhibitory effect could derive from one or
several of these systems. However, the correlations and the rank ordering of the effects suggest their integration.
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TABLE 4: Showing mean (sem) volume of sucrose ingested and emptied over a 15 minute test during eight trials in each of four monkeys after dl-fenfluramine, 2mg/kg, ig, or vehicle. The within subject correlation coefficients (rho) between the effect of fenfluramine on volume ingested and on volume emptied are also shown. Fenfluramine reduced sucrose intake and gastric emptying, and the effects were significantly correlated in 3 out of 4 animals. t-tests for corrected individual means: * p<0.05.
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<th>END GASTRIC VOLUME (EGV) ml ± sem</th>
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TABLE 5: Showing mean (sem) volume of upper G-I secretions over a 15 minute test during eight trials in each of four monkeys after dl-fenfluramine, 2mg/kg, ig, or vehicle. The volume of gastric contents at the end of the 15 minute meal (end gastric volume) is also shown after drug and vehicle as well as the ratio between the two measures (EGVfen/EGVveh). Fenfluramine reduced end gastric volume. Animals interrupted intake after fenfluramine when their stomach contents were about 90% of control volume. t-tests for individual means: ** p<0.01.
Figure 4a: Saline emptying in Macaca Mulatta

Effect of dl-fenfluramine, 2mg/kg

- Vehicle
- dl-fenfluramine

Volume remaining, ml

Time, min

0 5 10 15 20 25

Q29
Figure 4b: Saline emptying in Macaca Mulatta

Effect of dl-fenfluramine, 2mg/kg

Volume remaining, ml

Time, min

Vehicle dl-fenfluramine

Vehicle dl-fenfluramine
Figure 4c: Saline emptying in *Macaca Mulatta*

*Effect of dl-fenfluramine, 2mg/kg*

![Graph showing saline emptying with effect of dl-fenfluramine.](image)
Figure 4d: Saline emptying in Macaca Mulatta

Effect of dl-fenfluramine, 2mg/kg

Volume remaining, ml

Time, min

Vehicle  dl-fenfluramine

B22
FIGURE 5a: GLUCOSE EMPTYING IN MACACA MULATTA

Effect of dl-fenfluramine, 2mg/kg

Volume remaining, ml.

Time, min.

Vehicle  dl-fenfluramine

Q29
FIGURE 5b: GLUCOSE EMPTYING IN MACACA MULATTA

Effect of dl-fenfluramine, 2mg/kg

Volume remaining, ml.

Time, min

Vehicle  dl-fenfluramine

P43
FIGURE 5c: GLUCOSE EMPTYING IN MACACA MULATTA

Effect of dl-fenfluramine, 2mg/kg

Vol. remaining, ml.

Time, min

Vehicle  dl-fenfluramine

R37
FIGURE 5d: GLUCOSE EMPTYING IN MACACA MULATTA

Effect of dl-fenfluramine, 2mg/kg

Volume remaining, ml.

Time, min

Vehicle

dl-fenfluramine

B22
Figure 6a: Duration of sucrose meal
Effect of dl-fenfluramine in Macaca Mulatta

Meal termination, Min.

Animal

P43  B22  R37  B55

Vehicle  dl-fenfluramine

p < 0.1
Figure 6b: Ingestive activity during feeding

Effect of dl-fenfluramine in Macaca Mulatta

- **P43**
- **B22**
- **R37**
- **B55**

Vehicle  dl-fenfluramine

*p < .05*
Figure 6c: Motor activity during feeding

Effect of dl-fenfluramine in Macaca Mulatta

Motor activity

Animal

P43  B22  R37  B55

Vehicle  dl-fenfluramine

p<.01
Figure legends

Figure 4a-d: Experiment 1. Gastric emptying of saline (9g/l, 150ml, ig,) in 4 monkeys under control conditions (squares) and after dl-fenfluramine Hcl, 2mg/kg ig (triangles). Results from one animal are given in each figure. At each point a mean of two estimations is given. Fenfluramine significantly inhibited the emptying of saline meals.

Figures 5a-d: Experiment 1. Gastric emptying of glucose solution (0.5 kcal/ml, 150ml, ig,) in 4 monkeys under control conditions (squares) and after dl-fenfluramine Hcl, 2mg/kg ig (triangles). Data from 8 emptying experiments under each condition were used to generate regression lines for individual animals (one figure per animal). Fenfluramine significantly inhibited the initial bolus phase of glucose emptying (Y-intercept) but not the rate of emptying (slope).

Figure 6a-c: Experiment 2. Behavioural observations showing mean time of meal termination (Figure 6a) and mean total scores on ratings of ingestive (Figure 6b) and of locomotor activity (Figure 6c) after vehicle (solid columns) dl-fenfluramine HCl 2mg/kg ig (hatched columns). Means are of eight trials on each preparation.
The role of gastric inhibition in fenfluramine-induced suppression of feeding: Summary and implications for future research

The studies described in this chapter have shown first, that dl-fenfluramine, in an acute experiment, influences feeding in bulimia nervosa. Secondly, in monkeys equipped with chronic gastric cannulae dl-fenfluramine both inhibited gastric emptying and feeding, and the two effects were closely linked, suggesting a causal relationship. The efficacy of fenfluramine in bulimia nervosa suggests that a double-blind controlled therapeutic trial of long-term administration of the drug might suggest that it produces useful improvement in bulimic symptoms and ultimately fenfluramine may have a place, alongside psychotherapeutic approaches, in the management of this eating disorder.

The form of fenfluramine used in both the clinical and the animal studies was the racemic mixture. The feeding inhibitory action of the drug is manifested more potently by the d- form (Thurlby et al, 1985). This suggests a further study in which the effects of d- and l-fenfluramine on gastric emptying are compared. As l-fenfluramine has not been approved for human use, such a study would have to be performed in animals. If the gastric inhibitory action of fenfluramine were similarly confined largely to the d- form, this would add to the
evidence in favour of a peripheral site of action of fenfluramine in the suppression of food intake. The action of d-fenfluramine in suppressing food intake has been antagonized using a specific cholecystokinin (CCK-A) receptor antagonist, MK-329 (Cooper et al, 1990). This suggests an interaction between the function of the peptide, CCK, and the drug, fenfluramine, perhaps at 5HT receptors.
CHAPTER 4

CHOLECYSTOKININ, GASTRIC EMPTYING AND THE ONTOGENY OF FEEDING BEHAVIOUR

1. DEVELOPMENT OF CHOLECYSTOKININ BINDING SITES IN RAT UPPER GASTRO-INTESTINAL TRACT

Summary
Autoradiography using $^{125}$I-labelled Bolton-Hunter-CCK-33 was used to study the distribution of cholecystokinin binding sites at different stages of development in the rat upper gastrointestinal tract. Cholecystokinin (CCK) binding was present in the distal stomach, oesophagus and gastro-duodenal junction in the rat foetus of gestational age of 17 days. In the 20 day foetus, specific binding was found in the gastric mucosa, antral circular muscle and pyloric sphincter. Mucosal binding declined during post-natal development and had disappeared by day 15. Antral binding declined sharply between day 10 and day 15 and disappeared by day 50. Pyloric muscle binding was present in foetal stomach and persisted in the adult. Pancreatic CCK binding was not observed before day 10. These results suggest that CCK may have a role in the control of gastric emptying and ingestive behaviour in the neonatal rat.

Introduction
Several functions have been demonstrated or proposed for the gut-brain peptide cholecystokinin (CCK). Recent interest has centred around a possible role for CCK in the mediation of satiety. Exogenous CCK inhibits food intake
(Gibbs et al, 1973, Moran and McHugh, 1982) but the mechanism by which CCK exerts this action remains to be determined. In some species, centrally administered CCK inhibits feeding (Della-Fera and Baile, 1979, 1981), but CCK is unlikely to cross the blood-brain barrier, and as selective gastric vagotomy blocks the satiety effect of CCK (Smith et al, 1981), a peripheral site of action appears most likely for CCK released or administered peripherally. It has been demonstrated that CCK inhibits gastric emptying (Debas et al, 1975, Moran and McHugh, 1982), and the latter authors have suggested that it is through this action that CCK affects feeding. In support of this hypothesis, Moran and McHugh, with colleagues, have demonstrated the existence of gastric CCK binding sites localized to the circular muscle layer of the pyloric sphincter of the rat (Smith et al, 1984). Contraction of this muscle could reduce the outflow from the stomach and produce inhibition of gastric emptying. CCK systems in the gut and brain appear at different ontogenetic points. The development of brain CCK binding sites (Hays et al, 1981) and the appearance of CCK in rat brain (Beinfeld et al, 1983, Brand, 1982, Noyer et al, 1980) are primarily postnatal, while CCK appears early in the development of the gut in the rat (Brand, 1982). In the human foetus, CCK can be detected in the duodenum and jejunum as early as the second trimester (Buchan et al, 1981). It seems likely, therefore, that as CCK production appears early in the gut, CCK receptors would also prove to be demonstrable early in
ontogeny. If a satiety effect of CCK could be demonstrated in an animal with gut but little brain CCK binding capacity, a good case could be made for the stomach as a major site of action for CCK satiety in that animal. In the adult rat, pyloric CCK receptors are highly localised to a compact band in the distal part of the circular muscle of the pyloric sphincter, where, it is suggested, they may mediate the effect of CCK on gastric emptying. The hypothesis for the present experimental series was that a similar pyloric band of CCK receptors might be located in the neonatal stomach. It was not predicted, however, that a complex ontogenetic pattern of CCK receptor development would be discovered.

Methods

Animals

Sprague-Dawley white rats that were housed individually in hanging cages under constant conditions of temperature and humidity were used. Animals were fed Purina rat chow, and water was available ad libitum.

For foetal studies, timed, pregnant rats were dissected after anaesthetizing with ether at 17 or 20 days of gestation. The small size of the 17-day foetal stomach (diameter 1.5 mm) made study of earlier gestational ages impractical in this species. For studies of postnatal development, pregnant rats were inspected daily and the day
on which a litter was found was designated day 0. The distribution of gastric CCK binding sites was determined at 1, 3, 6, 10, 15, 20, 30, 40, and 50 days of age. Adult animals of over 120 days were also studied. At each age two or four animals were used. Rats were fasted for 12 h before death to ensure that the stomach was empty. Up to the age of 30 days the young were caged with the dam whose diet they shared after weaning. After that age young rats were housed in separate cages in groups of four from the same litter.

Preparation of tissue

Animals were killed by decapitation and the oesophagus, stomach, and duodenum immediately removed and frozen in isopentane at minus 70 degrees centigrade. For study of rats less than 50 days old, the entire stomach with the lower oesophagus and duodenum was then embedded in water-soluble mounting medium (Tissue Tek) and sectioned longitudinally at 25 μm and minus 15 degrees centigrade. For studies of older animals, the pyloric sphincter, with adjacent portions of antrum and duodenum, were removed, embedded in Tissue Tek, and longitudinal 25 μm sections obtained at minus 15 degrees centigrade. The sections were thaw-mounted onto cold gelatin-coated ("subbed") slides and dried in a desiccator jar under partial vacuum at minus 20 degrees centigrade for 24 hours before binding.
CCK Binding

In previous studies (Smith et al, 1984) the optimal conditions for CCK binding in adult rat stomach and brain were defined. With use of these conditions, specific binding in rat brain above 80% was regularly obtained. In the present study identical conditions proved suitable for demonstrating CCK binding sites in the immature stomach. The slide-mounted tissue sections were preincubated in 50mM tris(hydroxymethyl)aminomethane (Tris)HCl (pH 7.4) containing 0.5% bovine serum albumin (BSA) for 20 minutes at 24°C. Slides were then incubated in 50mM Tris-HCl (pH 6.5) containing 0.5% BSA, 0.025% bacitracin, 4 mcg/ml leupeptin, 2 mcg/ml chymostatin, 130 Mm NaCl, 4.7M KCl, 5M MgCl₂, 1 Mm ethyleneglycol-bis(beta-amino ethyl ether)-N,N'-tetra acetic acid (EGTA) and 400 Pm [¹²⁵I]CCK-33 (cholecystokinin triacontatriapeptide iodinated with ¹²⁵I Bolton Hunter reagent to a specific activity of approximately 500 Ci/mmol) for 2 hours at 24°C. Alternate slides (blanks) were incubated in the presence of an excess (1 mcM) of CCK octapeptide (CCK-8) to determine the extent of non-specific binding. After the incubation, slides underwent 9 sequential 10 minute washes in 50mM Tris-HCl (Ph 7.4) containing 0.5% BSA at 4°C. After washing, slides were dried in a stream of warm air and placed in a desiccator jar under partial vacuum for 24 hours to ensure total drying. Dried slides were placed in an X-ray cassette apposed to a sheet of LKB Ultrofilm for 6 to 20 days. In order to estimate the optimal exposure time for
a particular binding run, a section of adult brain that had been subjected to the procedure for labelling binding sites was wiped from the slide onto filter paper and the latter was placed in a gamma counter for one minute to provide an estimate of total binding. A similar section of brain that had been incubated in the presence of excess unlabelled CCK-8 was also wiped and counted to give an estimate of non-specific binding. The counts recorded from the "blank" section, subtracted from the counts from the "total" section, gave an estimate of specific binding. The higher this number, the shorter the exposure time given. During early studies a rough correlation was established between counts per brain section and optimal exposure time and used in subsequent experiments. Nonspecific binding was estimated by determination of the number of counts obtained from a "blank" brain section incubated in the presence of excess unlabelled CCK-8. Nonspecific binding under the conditions employed varied from 16 to 20% of total binding.

Autoradiographic images from the developed film demonstrating total or nonspecific binding were compared with the corresponding tissue sections that had been stained with toluidine blue. By superimposing a slide on its corresponding autoradiograph in a projecting microscope, it was possible accurately to localize areas of specific binding to histological structures in the sections.

Quantitative analysis of autoradiographs was performed using computerized microdensitometry (Loats), allowing
comparison of the optical densities of tissues showing different intensities of binding. The densities were expressed as a percentage of the density of an adult brain section processed during the same binding run and exposed on the same piece of film.
FIGURE 9

FIGURE 10
125-I-CCK33 Binding density at different ages in rats.
FIGURE 10
125-I-CCK33 Binding density at different ages in rats.
**Figure legends**

Key for Figures 7, 8 and 9:

Scale = 1mm

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**Figure 7:**
A. Foetal stomach, **day 17** of gestation; left, toluidine blue stained, 25μm section; middle, autoradiograph showing total binding. ¹²⁵I-CCK-33 binding sites are observed in oesophagus (es), gastric mucosa and gastroduodenal junction; right, non specific autoradiograph, incubated in the presence of unlabelled CCK-8, 1μM.

B. Foetal stomach, **day 20** of gestation left, toluidine blue stained, 25μm section; middle, autoradiograph showing total binding. Specific binding is observed in gastric mucosa (gm) antral circular muscle (ac) and pyloric sphincter (ps); right, non specific autoradiograph.
Figure 8:
A. Day 1 rat upper gastrointestinal tract; left, toluidine blue stained 25μm section; middle, "total" autoradiograph. Specific binding in gastric mucosa (gm), antral circular muscle (ac) and pyloric sphincter (ps). No binding sites are observed in pancreas (pa); right, non-specific autoradiograph. Non-specific binding is seen in duodenum.
B. Day 3 rat upper gastrointestinal tract.; left, toluidine blue stained, 25μm section; middle, "total" autoradiograph. Distribution of specific binding as for Day 1. Pancreas is still negative; right, non-specific autoradiograph.

Figure 9:
A. Day 10 rat upper gastrointestinal tract; left, toluidine blue stained 25μm section; middle, "total" autoradiograph. Specific binding is largely confined to antral circular muscle (ac) and pyloric sphincter (ps). Area of apparent binding within antral lumen is artefactual. Specific CCK binding sites are observed in pancreas (pa); right, non-specific autoradiograph.

B. Day 15 rat upper gastrointestinal tract; left, toluidine blue stained, 25μm section; middle, "total" autoradiograph. Specific binding in pyloric sphincter (ps) and, faintly, in antral circular muscle (ac); right, non-specific autoradiograph. Non-specific binding is seen in duodenal and gastric mucosa.
C. Adult rat gastroduodenal junction; toluidine blue stained, 25μm section; middle, "total" autoradiograph. Specific binding is confined to pyloric sphincter (ps); right, non-specific autoradiograph.

Figure 10. Developmental changes in binding density of $^{125}\text{I}-\text{CCK-8}$ in three gastric tissues: pylorus, antral circular muscle and gastric mucosa. Computerized microdensitometry (Loats) was used to determine optical density of the autoradiographic images (Figs 7-9). For each tissue, optical density (mean of three measurements) was expressed as a percentage of the average density of an autoradiographic image obtained from a 25μm adult brain section processed during the same binding run and exposed on the same piece of LKB Ulstrofilm.

**Results**

**Foetal stomach** (Figure 7). At 17 days of gestation it was possible to discern specific binding in the distal half of the foetal stomach extending into the oesophageal root and the duodenum. By 20 days of gestation (the day before birth) binding could be localized to three distinct areas (Figure 7b). There was heavy binding in the gastric mucosa; a second area of binding was localized to the muscular wall of the distal part of the stomach; and the third was confined to the muscle of the gastroduodenal junction.

**Postnatal development.** At postnatal day 1 (Figure 8a),
binding was found in the gastric mucosa at a higher relative density than that observed in the foetus. High densities of specific binding were found in the smooth muscle of the antrum and in the circular muscle layer at the pyloric sphincter. The distribution of CCK binding sites changed little between day 1 and day 3 (Figure 8b). Between day 3 (Figure 8b) and day 10 (Figure 9a), mucosal binding sites almost disappeared. They remained at very low levels up to day 15 (figure 9b) then declined to zero in the adult animal (Figure 9c). The distribution of antral muscle binding remained extensive up to day 10, then declined sharply between day 10 and day 15 (Figure 9). The relative density of antral binding rose to a peak at 10 days, then fell progressively from day 10 to the adult, at which stage no antral binding was detectable. In the day 40 animal, a small amount of binding was observed just proximal to the pyloric sphincter, and this had disappeared by the next age studied, day 50.

Pyloric CCK binding was visible at all stages of development. Relative binding density increased to a peak at day 10 and then fell to reach adult levels by day 20 (Figure 10), at which stage pyloric specific binding was 75% of adult brain specific binding density. In the adult rat, specific CCK binding, completely inhibited by incubation in the presence of a large excess of unlabelled CCK-8 was found only at the gastroduodenal junction in the circular muscle of the pyloric sphincter.
Samples of pancreas were included in the tissues studied at days 1, 3, 10 and 15 (Figures 8, 9). Specific binding was only observed at the latter two ages, suggesting that CCK binding sites observable by this technique appear in neonatal development between days 3 and 10.

The amount and distribution of CCK binding in the stomach, therefore, undergo a variety of developmental transitions from the day 17 foetus to the adult. In Figure 10, binding density in various gastric tissues, expressed as a percentage of the average density of an adult brain section, is shown for the different ages studied.

**Discussion**

Specific binding sites for cholecystokinin have been located in the gastro-duodenal junction of the neonatal rat where they could mediate CCK-induced contraction of the pyloric sphincter and therefore control of gastric emptying. Developmental changes in the distribution of cholecystokinin binding sites, demonstrated autoradiographically, have not hitherto been reported. The previously recognised locus of binding sites for CCK-33 in the circular muscle of the rat pyloric sphincter has now been shown to be evident at 17 days of gestation and to persist as a dense area of binding into adulthood. For technical reasons it was not possible to define the gestational age at which CCK binding first appears in the rat foetus. CCK binding sites are also found in other
areas of the stomach in the developing rat. The gastric mucosa has extensive binding in the foetus and this persists until day 10 to day 15 of postnatal life. The antral circular muscle is also densely packed with CCK binding sites in foetal and early postnatal life. The extent of this binding also declines with increasing age but more slowly than the mucosal binding. Preliminary evidence is provided that pancreatic CCK binding sites may not appear until several days after birth. The presence of CCK binding sites in foetal stomach suggests that CCK might have a role in gastric morphogenesis.

The ontogeny of both CCK and its binding sites in the gastrointestinal tract differs markedly from its development in the brain. Studies using immunocytochemistry (Larsson, 1977, Larsson and Morch-Jorgensen, 1978) radioimmunoassay (Noyer et al, 1980), and bioassay (Brand, 1982) have suggested that the neonatal rat small intestine contains CCK that can be localized to mucosal cells. Levels increased to a peak between postnatal days 8 and 15 and then gradually falls to adult levels over the next few weeks. In contrast, brain CCK levels in the rat rise from low or undetectable levels at birth to a peak at 20-28 days (Beinfeld et al, 1983, Brand, 1982, McDonald et al, 1982, Noyer et al, 1980), and CCK binding sites in rat forebrain rise from low levels at birth to a peak at day 12, subsequently falling to adult levels by day 26 (Hays et al, 1981). The neonatal rat brain appears, therefore, to bear relatively few CCK
binding sites. The demonstration of an extensive system of CCK binding sites in the gut at day 1, at which stage of development CCK has been shown to inhibit ingestive behaviour and gastric emptying (Robinson et al, 1985, 1988a and below), supports a peripheral site of action for the satiety effect of CCK in the neonate. It has been proposed that CCK affects feeding in the monkey by inhibiting gastric emptying thereby facilitating gastric distension (Moran and McHugh, 1982). The receptors demonstrated in the present study could act as a substrate for inhibition of gastric emptying and so mediate suppression of ingestive behaviour in the neonatal rat.
ABSTRACT
In 8 hour-deprived day 1 rats, sulphated cholecystokinin inhibited independent milk ingestion. The threshold effective dose for inhibition of intake rose with age up to day 10. Observed ingestive, but not non-ingestive, behaviour was suppressed by s-CCK. Desulphated CCK had no apparent effect on intake or behaviour. Day 3 rats subjected to hypertonic dehydration drank more milk than non-dehydrated, 2 hour deprived rats, but CCK inhibited milk intake only in the latter. Gastric emptying of normal saline measured in 1 and 3 day rats and corrected for secretions was inhibited by CCK and the threshold effective dose was 0.5 mcg/kg. Inhibition of gastric emptying is suggested as an important contributory mechanism in the satiety effect of CCK in neonatal rats.

Introduction
Inhibition of ingestive behaviour by cholecystokinin (CCK) was first established in the adult rat (Gibbs et al, 1973). A satiety effect of CCK in the developing rat remains, however, controversial. CCK was found, by Blass et al (1979) to inhibit suckling in the rat only after the age of 15 days, suggesting that the neonate is resistant to the satiety action. However, Houpt & Houpt (1979) using a higher dose of CCK, found suckling to be inhibited in rats aged 3 to 7 days. An alternative model of intake in the neonate, was described by Hall and Bryan (1980). They showed that 3 day old rat pups, in a warm incubator on a milk-soaked towel, will ingest milk and that
such independent ingestive behaviour shows some characteristics of adult feeding, including increasing intake with longer periods of deprivation and a decline in ingestive activity over the course of a thirty minute feeding test, suggesting the gradual onset of satiety. Neonatal rats consuming milk under these conditions stop taking the milk at a degree of gastric distension far lower than that at the termination of suckling if milk supply is unlimited (Hall and Rosenblatt, 1977). Independent ingestion might therefore be more readily inhibited by CCK than suckling and was investigated during the present experiments.

The mechanism of CCK satiety is uncertain. It has already been suggested that CCK acts on pyloric receptors to inhibit gastric emptying and, by promoting gastric distension, provoke satiety (Moran and McHugh, 1982, Smith et al, 1984). As documented above, CCK receptors have been described in the neonatal rat stomach (Robinson et al, 1985) but, at the same stage of development, the brain has been reported to bear only low levels of CCK binding (Hays et al, 1981). Hence, although a central action of CCK may contribute to inhibition of feeding in the adult (Della-Fera and Baile, 1979) a satiety effect of CCK in the neonate would be more likely to be mediated at peripheral receptors, perhaps involving inhibition of gastric emptying.

In the present study, the effects of CCK octapeptide on independent ingestion and gastric emptying in the
developing rat were investigated in order to establish whether the receptors that had been identified in the work already described could be linked with physiological and behavioural actions attributable to CCK.

**General methods**

Pregnant Sprague-Dawley white rats obtained from Charles River, were housed individually in hanging cages. Lights in the colony were maintained on a 12:12 hr light-dark cycle. Food and water were available *ad libitum* Rat pups aged 1, 3, 6 and 10 days were removed from the litters for testing. Not more than four pups from the same litter were used for each age and dose of CCK tested and each animal was used only once. During deprivation, pups were placed in a humidified incubator at 37°C.

**Experiment 1: Effect of CCK on independent ingestion in neonatal rat pups**

**Methods**

Ingestive behaviour:

The testing chamber (Hall and Bryan, 1980) was a 20 gallon aquarium with a plastic lid maintained at a constant temperature of 33°C and high humidity. A plastic mouse cage (30 x 19 x 12 cm) was placed in the chamber and lined with three paper towels soaked with full cream cows' milk at 37°C. The amount of was such that the paper towels were
soaked in milk and puddles would form when the towel was pressed by a pup's foot. Unless otherwise specified, animals were maintained in a humidified 37°C incubator for 8 hours (8 hour deprivation) prior to testing.

Animals (n=394) were paired according to age and body weight and numbered for identification. Micturition and defaecation were induced by gentle stroking of the perineum with a tissue. The pups were reweighed, to the nearest 0.01g, injected intraperitoneally with the peptide under test (CCK octapeptide, sulphated or desulphated (Squibb)) or an equal volume of physiological saline vehicle, and a dab of petroleum jelly applied to the injection site to prevent leakage. Of each matched pair, one was given CCK and the other, saline, and experimenters were blind to drug condition. Pups were tested in littermate pairs to control for between-litter differences or differences in intake due to variability in the length of the deprivation period or the amount of milk on the floor of the testing chamber. Five minutes after the injection, the pups (a maximum of three pairs per session) were placed in the testing chamber for thirty minutes. At the end of that time the pups were removed from the chamber, dried carefully with tissue paper and reweighed. The percentage increase in body weight over the testing period was used as a measure of ingestion. In a test of this length, weight gain is an accurate measure of intake, since little weight is lost in faeces or urine as pups need to be anogenitally stimulated for evacuation and insensible
loss is minimal (Hall and Bryan, 1980). For each pair of pups, data were used only if, in the control pup, intake equalled or exceeded 3% of body weight. This procedure is similar to that developed by Hall and Bryan (1980).

CCK administration:
The dosages of CCK (in micrograms/kg) varied according to the age of the pups:

day 1: 0.05, 0.1, 0.5, 1.0 and 8;
day 3: 0.1, 0.5, 8;
day 6: 0.25, 1.0, 8.0;
day 10: 0.5, 1.0, 8.0.

The effect of desulphated CCK (d-CCK) was examined in 1-day old rat pups at doses of 0.5 and 8.0 micrograms/kg.

Behavioural observations:
Every five minutes each animal was observed for one minute and the presence or absence of various behaviours noted. These were a group of behaviours associated with ingestion (mouthing, lick and gape) and a further group of non-ingestive activities (locomotion, roll, stretch, and paw).

They were defined as follows:

*Mouthing;* movements of the mouth sustained for at least 3 seconds.

*Licking;* protrusion of the tongue so that contact was made with the milk.

*Gaping;* extension of the neck and wide opening of the mouth, sustained for at least 3 seconds.

*Locomotion;* movement involving relocation of both front
Roll; the pup rolls over so that one side, the back and the other side touch the floor of the cage.

Stretching; characteristic extension of neck and all four limbs, usually observed during suckling in response to a milk let-down.

Pawing; the pup draws a paw over any part of the head.

Scoring of these behaviours and their association with ingestion has been demonstrated to be reliable (Moran et al, 1983, 1983a). The behavioural observations were performed during about half of the experiments (n=196). Intake data only were obtained from the remaining animals.

Statistics:

The effects of CCK on milk ingestion were assessed by repeated measures analysis of variance (ANOVA), using the littermate control as the repeated factor. For each age, the threshold dosage of CCK for affecting ingestion was determined by planned t comparisons using a pooled error term when overall significance was found on the ANOVA. The effects of CCK on behavioural measures were examined using analyses of variance with repeated measures on two factors (CCK/vehicle and time during the 30 minute ingestion test).

Behaviours were collapsed into two categories: ingestive behaviours (mouthing, licking, gaping and stretching) and non-ingestive behaviours (locomotion, pawing and rolling). The effect of CCK on the incidence of ingestive and
noningestive behaviours was analyzed across ages for three dosages of CCK; subthreshold, threshold and 8μg/kg., which was the maximum dosage tested at each age.

Results: Eight hour deprived rats treated with sulphated CCK-8

Milk intake (Figures 11,12):

Control animals consumed 5.76 ± 0.8% of initial body weight during the thirty minutes of the test. There was no significant effect of age on consumption. However, sulphated CCK-8 had a significant inhibitory effect on milk intake at all ages tested, and the threshold dosage for this inhibition, as determined by planned t comparisons, increased with age. At day 1 (doses 0.05-8 μg/kg), the threshold dose for significant inhibition due to CCK was 0.1 μg/kg (Figure 11). At day 3 (doses 0.1, 0.5, 1 and 8μg/kg), the .01 μg/kg dose was ineffective but 0.5μg/kg produced a significant inhibition of intake (Figure 11). At day 6 (doses 0.1, .05, 1 and 8μg/kg), 0.5 μg/kg was again the threshold dose for significantly inhibiting ingestion (Figure 12), and at day 10 (doses 0.5, 1, 2 and 8μg/kg), the threshold dose for significantly inhibiting intake was 1.0 μg/kg (Figure 12).

There was no significant effect of d-CCK on milk ingestion at either 0.5 or 8 μg/kg in day 1 rat pups.
(F_{1,14}=0.33), p>0.5).

Behavioural changes after CCK administration:

The effect of CCK on ingestive behaviour (Figure 13) and noningestive behaviour (Figure 14) varied depending on the dose tested. Dosages of CCK that were subthreshold for affecting intake did not affect either ingestive or noningestive behaviours. Also, at these dosages, no significant effect of sampling time was found. At the threshold doses, CCK significantly inhibited ingestive behaviours relative to control values (F_{1,26}=16.51, p<0.001) and the effect of CCK varied over time. That is, pups receiving CCK-8 demonstrated a decrease in ingestive behaviour sooner than pups receiving saline. Although there was a decline in ingestive behaviours in control pups in sample 3 (third 10 minutes) relative to samples 1 and 2 (first and second 10 minutes), this decline occurred earlier (in sample 2) in the CCK-treated pups (Figure 13). Threshold dosages of CCK did not affect the frequency of non-ingestive behaviour (Figure 14). A dose of 8 μg/kg of CCK reduced ingestive behaviour relative to control values right from the outset and across the first three sampling intervals (Figure 13) (F_{5,105}=4.01, p<0.002). Again there was no significant effect of CCK on the incidence of noningestive behaviours (Figure 14).

Total ingestive behaviour and mouthing both showed significant positive correlations with intake (Pearson's r: 0.26, 0.30, n=87, p<.01). Locomotor behaviour was
negatively related to intake (r=-.21, p=.02). No other behavioural measure was significantly correlated with intake.

Desulphated CCK had no significant effect on any measure of behaviour.

Conclusions:
Sulphated CCK therefore inhibits milk intake in a test of free ingestion. The effect is dose dependent and the sensitivity of rats to the inhibitory effects of CCK falls with age between 1 and 10 days. At the threshold dose for significant reduction of intake, ingestive, but not non-ingestive behaviours decrease earlier in the test after CCK than after control, indicating that CCK accelerates the onset of satiety in this experimental situation. A high dose of CCK reduced ingestive behaviour throughout the test. Desulphated CCK was ineffective in altering milk intake or observed behaviour.

Experiment 2: Effect of CCK on milk ingestion in dehydrated rat pups.
In order to determine the specificity of the effects of CCK on ingestion the intake of milk was measured in animals for which the stimulus was dehydration rather than food deprivation. Wirth and Epstein (1976) showed that rats do not change their water intake following dehydration induced by injections of hypertonic saline until 2-3 days of age. Bruno et al (1981) have further
demonstrated that pups of this age will avidly consume milk from the surface of a test arena in response to intracellular dehydration. Because a drinking response to a thirst stimulus is not sensitive to CCK in adult animals (Gibbs et al, 1973), this would test whether the ingestive behaviour of pups could be similarly differentiated.

**Methods**

14 three-day rat pups were injected with hypertonic saline (2.5 mosmol/100g sc) two hours prior to testing (dehydrated condition). A control group of 14 three-day rats was injected with physiological saline (non-dehydrated condition). These rats were then tested for the effect of CCK on independent ingestion of milk as in Experiment 1. For analysis, a group of rats consisted of four animals, matched for initial weight. One pair was subjected to dehydration and the other pair received physiological saline. Within each pair, one received CCK, 0.5 micrograms/kg ip., and the other received an injection of saline vehicle. Observers were blind to hydration and drug condition.

**Statistics:** Each group of four matched animals was regarded as a unit, and analyses of variance performed using repeated measures on two factors (CCK/vehicle, and hydration state).

**Results**

**Effect of dehydration on milk ingestion:**

Milk ingestion in dehydrated day 3 rats was significantly greater than in non-dehydrated (two
hour-deprived) animals (Analysis of variance, hydration effect: $F(1,6)=22.8, p<0.005$). (figure 15).

Effect of dehydration on the action of CCK (figure 15):

In the same analysis, a significant effect of drug (CCK/vehicle) was found ($F(1,6)=23.1, p=.003$), but no drug by hydration interaction. Paired t-tests to examine the effect of CCK on intake suggested however, that, while in non-dehydrated animals, CCK inhibited milk intake ($t=3.56, df=5, p<0.02$), no significant suppression of intake occurred in dehydrated animals ($t=1.75, df=5, p>0.1$).

Conclusions:
The results suggest that while CCK inhibits milk intake after deprivation ("hunger") intake is not reduced after dehydration ("thirst").

Experiment 3: Effect of CCK on gastric emptying in rat pups.

Methods:
34 day 1 rat pups and 34 day 3 pups were matched in pairs for age and body weight, fasted for eight hours in a humidified 37°C incubator and orogastric intubation performed using a fine (PE 10) plastic cannula. They were injected intraperitoneally with either sulphated CCK octapeptide or physiological saline vehicle under blind conditions and, 10 minutes later, physiological saline, 25ml/kg labelled with phenol red dye, 93.75 mg/l was infused over one minute into the stomach. After five
minutes the animal was decapitated, the abdomen rapidly dissected and the pylorus tied with silk thread exactly two minutes after death, followed immediately by oesophageal ligature. The stomach was removed, weighed and the contents were removed using a micropipette and saved. The stomach was blotted on filter paper and reweighed. Gastric contents were corrected for secretions by the dye dilution technique (Moran and McHugh, 1982). Gastric emptying was expressed as a percentage of the infused volume emptied over five minutes and the volume of secretions determined by subtracting corrected from total end gastric volume. Day 1 rats were tested using 0.05, 0.1 and 0.5 micrograms/kg and day 3 rats at 0.1, 0.5 and 8 micrograms/kg sulphated CCK-8.

Results
(Figure 16)

After vehicle injections, a mean of 55.18 % +/- 2.86 (se) (n=32) of the original gastric contents had emptied after five minutes. CCK administration resulted in an overall inhibition of gastric emptying at day 1 ($F_{1,13}$=15.8, p<0.001) and at day 3 ($F_{1,13}$=14.1, p<0.001). The threshold dosage for producing this inhibition was different at days 1 and 3. As shown in figure 16, the threshold dose for inhibiting gastric emptying was 0.1 μg/kg in 1-day-old pups, whereas in 3-day pups the threshold was 0.5μg/kg.

Upper gastro-intestinal secretions after vehicle were 34.6±3.3μl. No significant effect of age or of CCK was observed on secretions.
Conclusions: This experiment demonstrates that CCK inhibits gastric emptying in the neonatal rat, and that the thresholds for that inhibition at the two ages tested are identical to the threshold doses for inhibition of milk intake.

Discussion

The present work has demonstrated that sulphated cholecystokinin octapeptide inhibits independent ingestion in neonatal rat pups. The satiety effect of CCK was dose dependent and the minimum effective dose was observed to increase with increasing age. Reference to the present results and those of previous investigators allows comparison of the effects of CCK on ingestion under three different experimental conditions. Independent ingestion at day 3 under control conditions, terminates after ingestion of about 5.7% of body weight. CCK, 0.5 micrograms/kg, significantly inhibits this independent ingestion (this study). Normal suckling (in which milk supply is restricted to the volume provided in milk let-downs by the dam) is inhibited at 3-7 days by 80 U/kg (about 3.2 micrograms/kg) (Houpt and Houpt, 1979). This form of intake can be inhibited readily by intragastric pre-loads (Houpt and Epstein, 1973) and terminates, in 5 day pups, at about 6.4% of body weight (Hall and Rosenblatt, 1977). Suckling behaviour in 5 day rats, in the presence of an unlimited milk supply (Hall and
Rosenblatt, 1977) does not cease until ingestion of over 10% body weight, when gross gastric distension has developed, and under these conditions CCK, 40 U/kg (about 1.6 micrograms/kg) was ineffective (Blass et al, 1979). The three conditions therefore present a series with increasing resistance to inhibition by gastric distension and, in the case of the first two conditions, increasing resistance to inhibition by CCK. This conclusion is in accord with the view that suckling and independent ingestion are regulated by distinct sets of regulatory mechanisms (Hall, 1985, Hall and Bryan, 1980, Hall and Rosenblatt, 1977, Phifer et al, 1986). The only previous study in which the effect of CCK on independent feeding in immature rats was examined was that of Bernstein et al (1976) who found that s-CCK, 40 U/kg (about 1.6 micrograms/kg) inhibited intake of both solid and liquid food in 21 day rats, but found no effect on water intake in water deprived rats.

CCK binding sites in the rat undergo marked developmental changes during the neonatal period. Brain CCK receptor levels increase from very low levels at birth to a peak at around day 12, (Hays et al, 1981). A rise in brain receptors does not, however, provide a ready explanation for an increasing resistance to the satiety effects of CCK. In the gut, CCK receptors, at birth, are observed in gastric mucosa, antral circular muscle and pyloric circular muscle (as described in the previous set of experiments) (Robinson et al, 1985, 1987). However, in
the adult rat, CCK receptors in the upper gastro-intestinal tract are confined to the pyloric sphincter (Smith et al., 1984). Thus, changes in gastric CCK receptor distribution provide a possible basis for the increasing resistance to the satiety effects of CCK seen with age. The CCK binding results indicated, however, that although receptor distribution decreased between birth and day 10, the density of receptors in the antrum increased to a peak between the same ages, and the physiological basis for the increasing resistance to CCK inhibition of ingestion and gastric emptying remains unknown. CCK receptors in two other tissues, the pancreas (Innis and Snyder, 1980) and the vagus nerve (Zarbin et al., 1981) have not been subjected to systematic developmental study, although our results suggest that pancreatic CCK receptors are not present at birth but develop during the first two weeks of life.

Our observations of the behaviour of rat pups demonstrate that CCK inhibits ingestive behaviour but does not affect non-ingestive activity. The time course of behavioural responses during testing was altered by CCK. Animals receiving vehicle showed a fall in ingestive behaviour over the first 20 minutes of the test - a behavioural correlate of satiety. Rats given CCK showed a reduction of ingestive behaviour that was confined to the first third of the test period, suggesting that the effect of CCK on behaviour is confined to the fifteen minutes following i.p. injection. CCK therefore reduced
ingestive behaviour earlier, and at lower levels of milk intake.

Evidence presented here suggests that the inhibition of independent ingestion by CCK is mediated by interaction with physiological mechanisms already in place at birth which may have a role in control of normal feeding. The specificity of the response was established by studying the effect of CCK on ingestion induced by dehydration, the effect of desulphated CCK on ingestion, and the effects of CCK on specific behaviours. As reported by Bruno (1981) it was found that day 3 rats increased independent ingestion of milk after hypertonic dehydration - in contrast to Wirth and Epstein (1976) who found no increase in milk ingestion in dehydrated day 3 rats fed by oral infusions. In dehydrated day 3 rats, CCK did not inhibit milk ingestion, while ingestion was inhibited in matched non-dehydrated controls. Thus, ingestion in response to a deprivation stimulus ("hunger") and intake in response to dehydration ("thirst") can be differentiated by CCK at day 3. This result can be compared with the effect of CCK in adult rats, in which food intake in hungry animals, but not water intake in thirsty animals, was reliably inhibited by the peptide (Gibbs et al, 1973).

Desulphated CCK had no effect on intake or any measure of behaviour in day 1 rats. Gibbs et al (1973a) found that d-CCK failed to inhibit sham feeding in the adult rat, in contrast to s-CCK which showed considerable potency.

Inhibition of milk intake by CCK is therefore produced
only by the sulphated analogue, is accompanied by suppression of ingestive behaviour but not by changes in non-ingestive behaviours, and is observed when ingestion is stimulated by deprivation but not by a thirst stimulus. This evidence indicates that the reduction of milk intake is accompanied by a specific change in behaviour and is not part of a general inhibitory effect of the peptide.

CCK inhibition of feeding in the rhesus monkey may be mediated by inhibition of gastric emptying (Moran and McHugh, 1982). We have shown, in the neonatal rat stomach, an extensive system of CCK receptors which could form part of a similar mechanism for the suppression of independent ingestion in immature rats. In the present study, CCK has been shown to inhibit gastric emptying in the neonatal rat in a dose-dependent fashion. Our results differ from those of Houpt & Houpt (1979) who found no effect of CCK on gastric emptying of saline in rats aged from 3 to 7 days. In their experiments, gastric emptying was measured 50 to 81 minutes after CCK, 80 IDU/kg ip. By 50 minutes, the injected CCK would have undergone enzymatic degradation (Deschodt-Lanckman, 1985) and its effect attenuated. In the present experiments gastric emptying was measured 15 minutes after administration of CCK. As in experiment 1, it was demonstrated that CCK exerts behavioural effects on day 1 and day 3 rats in a test ending thirty five minutes after injection, the time studied for inhibition of gastric emptying was within the known duration of action of CCK. The inhibition of gastric emptying by CCK,
with threshold doses at each age that were identical to thresholds for inhibition of milk intake, suggest that the two phenomena are linked. It has been demonstrated that, for day 6 rats being fed by oral infusions, milk intake is dependent entirely upon the degree of gastric distension (Phifer et al, 1986). CCK inhibits independent ingestion and gastric emptying during the first few days of life when CCK receptors are demonstrable in the neonatal stomach. This evidence points to a substantial role for gastric distension in the inhibition of independent ingestion by CCK in the neonatal rat.
FIGURE 15
**Figure legends**

Figure 11: Volume of milk consumed (expressed as % increase in body weight) in 1- (top) and 3-day-old (bottom) rat pups receiving saline vehicle.
* Significant differences from saline control, p<0.05.

Figure 12: Volume of milk consumed (expressed as % increase in body weight) in 6- (top) and 10-day-old (bottom) rat pups receiving saline vehicle.
* Significant differences from saline control, p<0.05.

Figure 13: Frequency of ingestive behaviours (mouthing, licking, gaping and stretching) in 1 minute sampling intervals every 5 minutes during a 30 minute ingestion test in pups receiving a subthreshold (top), threshold (middle) or 8 µg/kg doses of CCK or littermate controls receiving saline vehicle. Data are collapsed across ages.
* Significant differences from saline control, p<0.05.

Figure 14: Frequency of non-ingestive behaviours (locomotion, pawing and lordosis) in 1 minute sampling intervals every 5 minutes during a 30 minute ingestion test in pups receiving a subthreshold (top), threshold (middle) or 8 µg/kg doses of CCK or littermate controls receiving saline vehicle. Data are collapsed across ages. No significant difference was found.

Figure 15: Effect of CCK on milk intake (mean + se) in day 3 rats pretreated with either hypertonic saline, (dehydrated) or physiological saline (control). Dehydration significantly increased milk intake. CCK
reduced intake in non-dehydrated but not in dehydrated rats. * Significant differences between pups receiving CCK and littermate saline-treated control, p<0.05.

Figure 16: Effect of various dosages of CCK on emptying (expressed as % of initial volume) of a gastric load of saline, 25 ml/kg, given 10 minutes after the CCK and measured after five minutes of emptying in 1- (top) and 2- day-old (bottom) rat pups.

* Significant differences between pups receiving CCK and littermate saline-treated control, p<0.05.
3. GENERAL DISCUSSION

Gastric emptying and the action of cholecystokinin:

The intention of the present series of studies was simply to establish whether CCK receptors were present in the neonatal pylorus, and whether the rat neonate showed ingestive and gastric inhibition following administration of CCK similar to the responses obtained in the adult. Specific CCK receptors were, indeed, demonstrated in the stomach of the neonatal rat, although their extent was much greater than observed in the adult, and while adult receptors were confined to smooth muscle, in the immature animal they were also found in gastric mucosa. The findings that neonatal rats reduce their intake of milk following CCK, that CCK inhibits gastric emptying and that the inhibition of ingestion is specific for intake provoked by a hunger stimulus, are all consistent with the CCK receptors found in the neonatal stomach, and, in view of the paucity of CCK binding in the neonatal rat CNS, suggest that inhibition of ingestion in the neonate is mediated by a gastric mechanism, most likely inhibition of gastric emptying. The increased sensitivity of the neonate to CCK may be related to the more extensive distribution of the receptors which were located, not only in pylorus as in the adult, but in the muscle of the gastric antrum, where they could participate in the inhibition of gastric emptying provoked by CCK. Further studies should be performed to characterize the neonatal receptors and to establish changes in gastric physiology that accompany their
development. The functional significance of the developing receptor system is open to debate. It is unlikely that they are concerned with satiety, as feeding at these early stages is by suckling, and the quantity of milk ingested is, in rats, controlled by the dam, by varying the volume of milk delivered in let-downs. Given unlimited supplies of milk, the neonatal rat will continue to suckle until its stomach is grossly distended. As the present results suggest that in the neonate, CCK acts by inhibiting gastric emptying, it is not surprising, therefore, that the peptide does not inhibit suckling until 15 days of age. CCK has been suggested as a trophic factor in the growth of the pancreas (Logsdon, 1986) and stomach and intestine (Balas et al, 1985) and the receptors identified in the present work might represent the mediating links between peptides and cells in the process of morphogenesis. Lastly, CCK receptors have been identified in human leiomyosarcoma tissue (Miller, 1984). If the development of human CCK receptors in the stomach resembles that of the rat, receptors on malignant gastric muscle cells could mark the re-emergence of immature binding sites following neoplastic change.
CHAPTER 5

GASTRIC EMPTYING IN EATING DISORDERS AND IN RATS ON A RESTRICTED FEEDING REGIMEN.

1. DETERMINANTS OF DELAYED GASTRIC EMPTYING IN ANOREXIA NERVOSA AND BULIMIA NERVOSA

Introduction

Gastric distension is a potent inhibitor of feeding, and the demonstration of delayed gastric emptying in patients with anorexia nervosa (Dubois et al, 1979, Holt et al, 1981, Russell, et al, 1983, McCallum et al, 1985, Stacher et al, 1986) suggests that gastric retention might contribute to the maintenance of undereating in this condition. The mechanisms underlying delayed emptying, and whether it is a primary disorder or secondary to other disturbances are, however, unknown and in these studies, gastric emptying of various meals was investigated in anorexic patients at different stages of nutritional treatment in an attempt to identify the determinants of delayed gastric emptying in anorexia nervosa. In the closely related condition, bulimia nervosa (Russell, 1979), patients succumb to episodes of massive overeating,
Summary

Gastric emptying was measured using a gamma camera in 22 patients with anorexia nervosa, 10 patients with bulimia nervosa without severe weight loss and 10 controls. Patients with anorexia nervosa were tested 1. while underweight and selecting their own diet (10 patients), 2. underweight, but receiving an adequate diet on an inpatient unit (refeeding diet) (12 patients) and 3. under refeeding diet conditions after weight gain (8 patients). Three isotopically labelled meals were used, 1. a mixed solid meal containing labelled poached egg 2. 200 ml of d-glucose solution and 3. 200 ml of physiological saline. Only gastric emptying of the solid meal and glucose solution were significantly delayed. Gastric emptying of saline was normal. The gastric disturbance was confined to patients with anorexia nervosa selecting their own diet. Patients receiving adequate nutrition on the ward had normal gastric emptying and weight gain in this group had no significant effect on emptying. Slow emptying was observed in patients who maintained a low weight solely by food restriction as well as in patients whose anorexia nervosa was complicated by episodes of bulimia. Thus, slow gastric emptying occurred when the quantity of food reaching the duodenum was sufficiently reduced to result in severe weight loss. Moreover, abnormal gastric emptying was seen only after the two meals that contained calories and were hypertonic to plasma, either of which properties could mediate the disturbance. Gastric emptying in bulimia nervosa was normal. Slow gastric emptying could exacerbate undereating in starving patients with anorexia nervosa by enhancing satiety.

suggesting a failure of satiety. Since an acceleration of gastric emptying has been proposed as a factor mediating overeating in morbid obesity (Wright et al, 1983) gastric function in a group of patients with bulimia nervosa was studied in order to elucidate the role of disturbed gastric emptying in the pathogenesis of this condition.
Methods

Subjects

Anorexia nervosa

Twenty two patients (21 female, one male), with anorexia nervosa were studied. They fulfilled the diagnostic criteria of Russell (1983), all having self-imposed severe weight loss, amenorrhoea of at least six months duration and a morbid fear of fatness. Ten patients were investigated while selecting their own diet either as outpatients (seven patients) or as inpatients undergoing observation without refeeding (three patients). Twelve patients with anorexia nervosa were tested between one and four weeks after admission to hospital (mean 14 days +/-5.77 (sd)) while still underweight but receiving a mixed diet of 2500-3000 Kcal per day. Eight of these sixteen patients were retested when they had regained weight on the ward, while still undergoing in-patient refeeding. Testing of patients was therefore performed under three distinct conditions: 1. Underweight, and selecting their own diet. 2. Underweight, but receiving the refeeding diet. 3. Weight restored, and receiving the refeeding diet.

Among the 22 patients with anorexia nervosa, 10 suffered from episodes of bulimia associated with vomiting, abuse of laxatives or prolonged starvation. Of the twelve patients tested while under supervision and receiving the refeeding diet none admitted to bulimic symptoms in the two to three weeks they had been in hospital, although eight patients had experienced persistent bulimia up to
the time of admission. Of the ten patients tested while selecting their own diet, four had episodes of bulimia at least weekly and all four reported at least one episode in the week before testing.

Bulimia nervosa

Ten female outpatients who were not currently underweight and who fulfilled criteria (Russell, 1979) for bulimia nervosa were tested. They all suffered episodes of massive overeating at a frequency of at least one episode per week. Eight of these patients, all within 15% of mean population matched weight (Metropolitan Life, 1983), practised self-induced vomiting after episodes of bulimia, and of these, five regularly took large doses of laxatives. The other two patients, both weighing more than 115% of MPMW, (123% and 170% MPMW) used self-starvation to compensate for calories ingested during bulimic episodes. All these patients showed a morbid fear of fatness similar to that observed in the patients with anorexia nervosa.

Controls

Ten normal control subjects in the same age range as the patients were recruited, 8 female and two male. All were within 15% of mean population matched weight (Metropolitan Life, 1983) and denied symptoms of eating disorder and of other psychiatric illness. Their scores on the Eating Attitudes Test (Garner and Garfinkel, 1979) and on the
Dutch Eating Behaviour Questionnaire (Van Strien et al, 1985) were within reported normal limits.

Clinical and demographic data on patients and controls are given in Table 6.
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<th>Table 6: Demographic and Clinical Data.</th>
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<td>Means with different superscripts are significantly different (p&lt;.05) in an ANOVA including: controls, bulimia nervosa, low weight, inpatients with anorexia nervosa, (AN, INPT, LOW WT) and low weight outpatients with anorexia nervosa (AN, OUTPT, LOW WT)</td>
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Patients and controls were asked to refrain from taking any medication, apart from benzodiazepine hypnotics, for at least one week before testing, and to fast and avoid smoking from midnight on the night before each test. Subjects were also asked to drink a large glass of water before bed on the night before a test. This was found to encourage gastric emptying of solid particles of food which otherwise reduced the quality of results after ingestion of labelled liquid meals because of adsorption of radioactive label onto the solid.

Gastric emptying tests

All tests were performed using an IGE Maxicamera gamma camera and the Adac image analysis system. Caloric and acaloric liquid meals empty differently from each other (Brener et al, 1983), and liquid meals show different emptying patterns compared to solids (Minami and McCallum, 1984). The emptying of each of these three types of meal was therefore examined separately. A mixed solid meal, a glucose solution and a saline solution were used and each series of three studies was completed within a week. Ethical approval, ARSAC (administration of radioactive substances sub-committee) approval and written informed consent were obtained.

Solid meal

60ml of egg white were mixed with 3.7 MBq (100 microcuries) of Technetium 99m-tin colloid and poached
for ten minutes in a domestic poacher. The egg was then sliced in two directions at right angles using an egg slicer, resulting in roughly cuboidal particles of average volume 0.37 ml. This was served hot with 150ml of fresh orange juice and one slice of medium cut white bread and 2.5g butter. The total caloric value of the meal, determined from tables, was 155 Kcal, its volume, after liquidizing in a domestic blender, was 235 ml and the mean caloric concentration was therefore 1.52 Kcal/ml. An aliquot of the liquidized solid meal was centrifuged twice at 5000 rpm for 5 minutes and the supernatant analyzed using the ion selective electrode technique for electrolytes, depression of freezing point for osmolality, and glucose oxidase. This revealed the following constituents: Sodium 68 mmol/l, potassium 42 mmol/l, glucose 97 mmol/l and osmotic pressure 569 mosmol/l (about 2 x plasma tonicity). After in-vitro incubation for two hours in 0.1N hydrochloric acid a sample of labelled egg retained 90.74% of the original radioactivity, allowing for decay, demonstrating that the label would remain bound to the food particles in the acid environment of the stomach.

The meal was served to the subject and the time taken to complete it noted. Imaging began as soon as the meal ended. A 60 second static anterior scan followed immediately by a 60 second posterior scan were recorded every few minutes in the erect posture until two hours after the beginning of the meal. The two views were taken
to compensate for antero-posterior movement of the meal within the stomach (Christian et al, 1980). Each image was displayed on the computer screen and a region of interest drawn electronically around the gastric outline (Figure 17). The geometric means of anterior and posterior gastric counts were corrected for decay and a time-activity curve generated.

Liquid meals

Two liquid meals were used in each study - 200ml D-glucose, 0.5 Kcal/ml (125 g/l) and 200 ml of physiological saline, 9g/l, both at room temperature and both labelled with Tc99m-tin colloid, 3.7 Mbq. After ingestion of the meal imaging was performed using continuous, dynamic, anterior acquisition for 60 minutes with the subject lying on a couch, propped up at 60 degrees to the horizontal. Images were acquired at 15 second intervals and counts within the gastric region of interest for the 240 images were corrected for decay and plotted against time.

Statistics

The four groups of subjects (anorexia nervosa at low weight (self selected diet), anorexia nervosa at low weight (refeeding diet), bulimia nervosa and controls) were compared with each other using one way analysis of variance. No subject appeared more than once in these analyses. Four patients were tested under both in-patient
refeeding and self-selection conditions. Their results were included only in the latter group. For each analysis, the overall F-test result is given, and, if appropriate, post-hoc analysis was performed using Duncan's test. The influence of differences between subjects (e.g. the presence of bulimia, self-induced vomiting or laxative abuse) was determined using multivariate analysis of variance. The effect of weight gain was studied using paired t-tests. In all comparisons differences were taken as significant if \( p \geq 0.05 \). One anorexic patient on the refeeding diet had the solid meal but refused to take the liquid meals.

Results

Subject characteristics (Table 6)

One way analysis of variance revealed no significant difference between the groups in age. Body mass index (in kg/metre\(^2\)) was significantly lower in both groups of patients with anorexia nervosa compared to controls and patients with bulimia nervosa (\( F(3,41)=24.6, p<0.0001 \)). After in-patient treatment, lasting an average of 62 days, anorexic patients gained a mean of 10.33 kg in weight, a significant increase (Paired t-test: \( t=8.28, df=6, p<0.001 \)), although they remained significantly lighter than controls (t-test: \( t=4.25, df=16, p<0.01 \)).

Meal duration (Table 7)

Control subjects completed the solid meal in a mean time

168
of 4.35 minutes. Low weight patients on both the self selection and the refeeding diets took significantly longer to finish the solid meal than controls (One way analysis of variance: F(3,41)=4.68, p=.007). In contrast, there was no significant difference in the time taken to finish the liquid meals, although self-selecting anorexic patients tended to consume the glucose at a slower rate than controls F(3,36)=2.6, p<.07). Comparing anorexic patients before and after weight gain showed no significant change in the time taken to complete any meal (paired t-tests, p> .1). Glucose was consumed faster than saline (Repeated measures ANOVA; within-subject differences in liquid meal duration: F(1,35)=7.65, p=.009).

Gastric emptying (Table 7)

The emptying of all three meals was preceded by a lag phase during which no gastric emptying was seen. The length of this phase was determined by noting the first scan on which activity was observed in the duodenum.

The shape of gastric emptying curves of the three meals varied considerably between subjects. Gastric emptying curves have been described as following various shapes of curve including, commonly, linear and exponential functions (Moore et al, 1981, Brener et al, 1983) and our data were analyzed to establish which of these two
functions best described each curve. Linear regression was performed using untransformed and log-transformed gastric counts. The end of the lag period was used in the regression as the beginning of the curve. Each curve was assigned to either a linear or an exponential function depending on which regression line yielded the best fit (lowest mean squared residuals). For the solid meal, 35.3% emptied linearly and 64.7% according to an exponential function. For the glucose meal, 52.9% emptied linearly, and 47.1% exponentially and for the saline meal, 52.6% emptied linearly, and 47.4% exponentially. This distribution did not vary significantly between groups of subjects. In view of the variation in the shapes of emptying curves, half time calculated from an exponential function or emptying rate in amount emptied per unit time from the slope of a linear function could not be applied consistently. Gastric emptying was therefore calculated in several ways. Emptying of the solid meal was expressed as the percentage that remained 100 minutes after the beginning of the meal, and glucose and saline emptying were expressed as the percentage remaining at 30 min. The percentages remaining were calculated both by interpolation of the best fit curves and by using the decay-corrected counts actually obtained at 30 and 100 minutes. Conclusions were identical using either method and the figures given are those obtained using the latter procedure. In addition, half emptying time was calculated from the curve relating log corrected counts to time, and
the results obtained using this procedure were the same.

Solid meal

During the initial lag phase (mean 22.95 minutes in controls) the solid portion of the meal was distributed from the fundus to occupy the whole stomach. In one control subject, a double isotope scan was performed using Indium 111-DTPA, 3.7 Mbq, to label the liquid phase (orange juice) and Tc99m tin colloid, 3.7 Mbq to label the egg, as in the other studies. This confirmed that the liquid phase occupied the whole of the gastric area immediately after consumption while the egg was initially concentrated in the fundus and was gradually 'fed' towards the antrum. There was no significant difference between groups in the length of the lag phase.

The percentage of the solid meal remaining at 100 minutes was significantly greater in patients with anorexia nervosa who were selecting their own diet compared to low weight patients on the refeeding diet, patients with bulimia nervosa and controls (F(3,41)=4.52, p<.01). There was no significant influence of the duration of the meal on gastric emptying rate, in an analysis of covariance (p>0.88). Time activity curves of control subjects and patients on the self-selected diet are given in Figures 18 and 19, and anterior gastric scans, taken two hours after the beginning of the meal are given in Figure 20. Gastric emptying of the solid meal in patients with anorexia nervosa who were receiving the refeeding diet and normal
or over-weight patients with bulimia nervosa did not differ significantly from controls.

Weight gain among refed anorexic patients had no demonstrable effect on gastric emptying of the solid meal (paired t-test). In no control subject was gastric retention at 100 minutes greater than 50%. If delayed gastric emptying is defined as greater than 50% retention at 100 minutes, it was found in 1 patient with bulimia nervosa (10%), 2 low weight anorexic patients on the refeeding diet (16.67%) and 5 anorexic patients on the self-selected diet (50%) This distribution is significantly different from expected. (Chi-squared=9.14, df=3, p<.03). The delay in gastric emptying among the self selected diet patients was observed both in anorexic patients who suffered bulimic episodes and in patients who did not. Of the five patients in this group whose gastric emptying was delayed, by the above criteria, three suffered from bulimia and two did not. In order to assess the effect of bulimic symptoms on gastric emptying, multivariate analysis of variance was performed. This confirmed the significant difference between diagnostic groups (F(3,46)=4.21, p=.011) but showed no effect of bulimia, vomiting or laxative abuse (p>.4). The proportion of the variance in solid emptying accounted for by diagnostic group in this analysis was 23.85%, while the combined contribution of the other three variables was 2.2%. In four patients, gastric emptying of the solid meal was determined both under refeeding diet and self
select conditions. In the analyses, they are included only in the "self-selected" group. In two of these, delayed gastric emptying, as defined above, was found, with 51.9 and 69.9% remaining at 100 minutes. After approximately two weeks of inpatient refeeding, the gastric abnormality reversed (Table 8). One out of the eight weight restored patients on the refeeding diet also had delayed emptying. In the twelve patients who received inpatient refeeding, there was no significant correlation (Pearson's r) between gastric emptying rate of any meal and duration of refeeding (i.e. interval between onset of refeeding and initial testing).

**Glucose emptying**

The emptying of glucose was preceded by a shorter lag phase than that observed for solids (Repeated measures analysis of variance: Pillais' test, p<.001, for within-subject differences in lag phase). It did not vary between groups. Analysis of variance showed that the percent of the meal remaining at 30 minutes was significantly greater for self-selecting anorexic patients compared to controls, patients receiving the refeeding diet, and patients with bulimia nervosa (F(3,39)=3.20,p=.03). Time activity curves for one control and one patient with anorexia nervosa selecting her own diet are given in Figures 21 and 22. No other diagnostic group had abnormal glucose emptying and there was no correlation between duration of refeeding and glucose emptying among the sixteen refed low weight anorexic
patients. The effect of bulimic symptoms was assessed using multivariate analysis of variance. This confirmed the effect of diagnostic group (F(3,43)=3.6, p=.022), showed that the presence of bulimia or vomiting did not influence gastric emptying rate, but revealed that in patients with bulimia nervosa and self-selected diet patients with anorexia nervosa, laxative abuse was associated with reduced glucose emptying rate (F(1,43)=5.08, p=.03).

Among controls, mean retention of glucose at 30 minutes was 73.88% +/- 11.97(sd). Using the control mean + 1 standard deviation subjects were classified as having "slow" (= or >85% retention at 30 minutes) or "fast" (< or = 85%) glucose emptying. "Slow" emptying was found in 2 controls (20%), 1 patient with bulimia nervosa (10%), 1 low weight anorexic patient on the refeeding diet (8.3%), and 7 self-selecting anorexic patients (70%). (Chi squared=13.46, df=3, p<.004). One weight restored patient on the refeeding diet also showed delayed glucose emptying. Of the four patients tested under both refeeding and self-selected diet conditions, all had delayed gastric emptying of glucose, as defined above, before treatment (Table 8). After two to three weeks of refeeding, three had improved to under 85% 30 minute retention and one had even slower glucose emptying.

Saline emptying

Lag phase and emptying rate showed no significant
variation among groups (p>.5) and were not influenced by the presence of bulimic symptoms. The lag phase for saline was significantly shorter than the solid lag phase (Repeated measures analysis of variance, within subject differences in lag phase, Pillais' test, p<.001) but not significantly different from the lag phase for glucose.

Discussion

The present results confirm that delayed gastric emptying is a feature of anorexia nervosa. This study was designed to identify the determinants of delayed gastric emptying in anorexia nervosa: weight loss, acute starvation, and bulimic symptoms were all considered as possible factors contributing to its pathogenesis. It was also considered that delayed emptying might be a specific physiological anomaly in patients with anorexia nervosa, independent of other factors, and perhaps predisposing susceptible individuals to the condition. Our data show, however, that while patients who were selecting their own diet had significantly delayed gastric emptying, anorexic patients receiving a refeeding diet did not. Weight gain was not found significantly to influence gastric emptying. It seems likely that the delay in gastric emptying observed in the "self-selected" patients was associated with their recent dietary intake. The ten patients in this group reported two patterns of food intake. Six patients
engaged in consistent restriction of food intake while the others alternated periods of starvation with episodes of bulimia followed by self-induced vomiting, laxative abuse or prolonged starvation. Delayed gastric emptying was seen in both of these groups, but not in bulimic patients who were not underweight, indicating that the presence of bulimic symptoms alone does not affect gastric emptying. The results suggest that delayed gastric emptying occurs when there is a reduction of nutrient entering the duodenum sufficient to result in severe weight loss. This reduction may be due simply to inadequate oral intake of food, or alternatively to elimination of food from the stomach by vomiting before it has passed through the pyloric sphincter. It is possible that these changes would be noted in any starving organism, animal or man, and, if this is found to be the case, the regulation of gastric emptying rate would be seen to be determined, in part, by the amount of food consumed. This is often, in practice, a function of food supply, implying that post-prandial satiety might be enhanced under conditions of famine when prolonged intervals between meals are likely to occur.

The delay in gastric emptying was only observed after ingestion of the solid meal and the glucose solution, showing that the abnormality is not restricted to either solid or liquid meals. However, emptying of physiological saline was normal. The solid and glucose meals differ in at least two ways from the saline: they contain caloric
nutrient and are both hypertonic. Both caloric and osmotic stimuli have been found to delay gastric emptying (Hunt, 1956, Hunt and Stubbs, 1975) although the mechanisms by which this inhibition is effected are obscure. McCallum et al (1985) reported that emptying of a hypotonic liquid meal (water) was normal in anorexia nervosa. Since water would stimulate duodenal osmoreceptors, the latter study suggests that duodenal feedback by osmotic stimuli is not disturbed in anorexia nervosa. However, the emptying rates of hypertonic saline and isotonic glucose liquid meals have not been determined, and a disturbance of sensitivity to hypertonic stimuli cannot therefore be excluded.

Inhibition of gastric emptying by nutrients entering the duodenum may involve secretion of gut peptides such as cholecystokinin (Moran and McHugh, 1982) and this mechanism might be disturbed in patients with anorexia nervosa. In favour of a disturbance of duodeno-gastric feedback is our finding, in anorexia nervosa, of a normal lag period for all three types of meal. An abnormal delay in gastric emptying was only observed after some gastric contents had entered the duodenum. On present evidence, a disturbance in duodenal sensitivity to caloric or, perhaps, osmotic, stimuli secondary to severe reduction of food intake seems the most likely mechanism leading to delayed gastric emptying in anorexic patients. The delay in gastric emptying might be mediated by increased pyloric sphincter tone, or decreased gastric contractions. Pyloric activity
has not been studied in anorexia nervosa, and a manometric study of gastric contractions in anorexic patients (Silverstone and Russell, 1967) yielded normal findings. In the latter study, the recent dietary intake of the subjects was not reported and they may not have been tested under the dietary conditions we have found to lead to delayed gastric emptying.

Laxative abuse, among the two groups of patients who were not receiving the inpatient treatment, was found to be associated with significantly slowed emptying of glucose. Purgatives are known to have toxic effects on the autonomic nerves in the intestinal wall (Smith, 1968) and the present results suggest that the stomach may also incur damage as a result of laxative ingestion. Gastric retention in anorexic patients may well exacerbate the characteristic hypophagia, although it did not protect two patients (Patients 1 and 4 in Table 8) from severe, intractable episodes of bulimia. The degree to which undereating is dependent on enhanced satiety due to gastric stasis might be assessed by reversing the abnormality, perhaps pharmacologically, and observing the resulting eating patterns. Although the results suggest that gastric stasis is secondary to undereating, it remains possible that some individuals may develop delayed emptying more readily than others after the onset of dieting, and this sensitivity (perhaps genetically determined) could act as a predisposing factor, allowing common dieting to develop into anorexia nervosa.
A proportion of patients with anorexia nervosa suffer episodes of bulimia followed by self-induced vomiting. The "efficiency" of this process in reducing calorie absorption presumably depends on the duration of the lag period and the rate of gastric emptying, on the interval between ingestion of the food and self-induced vomiting and on the degree to which the stomach can be emptied of food. Slow gastric emptying might, therefore, contribute to the caloric deficit suffered by bulimic anorexic patients, promote further weight loss and lead to the particularly resistant form of illness seen in this group of patients. One subject (patient 1 in Table 8) regularly ate a green apple skin as the first item of her bulimic episode, and was reassured when she saw it return after prolonged vomiting. No evidence of accelerated gastric emptying which might contribute to the pathogenesis of the hyperphagia of bulimia nervosa was found in these studies.

A common symptom of patients with anorexia nervosa is slowness of eating and this was observed during the present study. Whether it is related to fear of weight gain, obsessional symptoms, starvation or oesophageal dysfunction (Stacher et al, 1986) is uncertain, but it did not improve significantly with inpatient treatment, and, surprisingly, it was no less marked among bulimic anorexic patients, when compared with "restrictors".

A rare, but serious, complication of anorexia nervosa, and of malnutrition generally, is acute gastric dilatation (Russell, 1966, Markowski, 1947). The aetiology of this
condition, which has also been described in a patient with bulimia nervosa (Mitchell et al, 1982) is obscure, but it occurs in severely malnourished patients soon after the beginning of in-patient treatment perhaps as a result of rapid refeeding in the presence of delayed gastric emptying. In the present studies delayed emptying was not observed after a mean of two weeks of inpatient refeeding. While the precise time course of the recovery of delayed gastric emptying during refeeding remains to be determined, acute gastric dilatation seems unlikely to occur as a result of refeeding after two weeks of nutritional treatment.
FIGURE 17
FIGURE 18

Graph showing % Gastric counts remaining over time (minutes) for a control subject.
Anorexia nervosa: self selection diet

FIGURE 19
Figure 21

% Gastric Counts Remaining

Control Glucose

Time (Minutes)
ANOREXIA NERVOSA

GLUCOSE

% GASTRIC COUNTS REMAINING

0 30 60

TIME (MINUTES)

FIGURE 22
**Figure legends**

Figure 17: Control subject, after mixed solid meal. Photograph of computer screen showing radioactive counts remaining within the gastric outline. A gastric region of interest has been drawn using a light pen and will be used by the computer to determine the counts remaining within the stomach.

Figure 18: Control subject, after mixed solid meal. Percent of total counts remaining within the stomach have been plotted against time. (Time-activity curve). The initial (lag) phase lasting about 20 minutes is shown, and subsequent emptying approximates to an exponential function.

Figure 19: Time-activity curve for patient with anorexia nervosa selecting her own diet. The lag phase is similar to control, but subsequent emptying is much slower.

Figure 20: Photographs of the computer screen 120 minutes after the beginning of the test in a control subject (left) and one with anorexia nervosa (right) and grossly delayed gastric emptying.

Figure 21: Time activity curve for glucose emptying. Control subject. Rapid linear emptying is demonstrated. Large fluctuations at beginning and end of curve are artefactual, due to movement of subject.
Figure 22: Time activity curve for glucose emptying. Patient with anorexia nervosa, selecting her own diet. Emptying is grossly slowed.
TABLE 8. GASTRIC EMPTYING RESULTS ON FOUR PATIENTS TESTED WHILE ON A SELF SELECTED DIET AND WHILE RECEIVING THE REFEEDING DIET.

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>SOLID MEAL</th>
<th>GLUCOSE MEAL</th>
<th>SALINE MEAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OWN DIET</td>
<td>HOSPITAL DIET</td>
<td>OWN DIET</td>
</tr>
<tr>
<td>1</td>
<td>51.91</td>
<td>17.4</td>
<td>98.47</td>
</tr>
<tr>
<td>2</td>
<td>25.55</td>
<td>29.17</td>
<td>87.64</td>
</tr>
<tr>
<td>3</td>
<td>29.83</td>
<td>18.06</td>
<td>88.51</td>
</tr>
<tr>
<td>4</td>
<td>69.9</td>
<td>23.9</td>
<td>100</td>
</tr>
</tbody>
</table>

S-S D: Self-selected diet. R-F D: Refeeding diet. Gastric emptying expressed as percent of original gastric activity remaining at 100 minutes (solid) or at 30 minutes (liquid meals).
<table>
<thead>
<tr>
<th></th>
<th>CTRLS (10)</th>
<th>BN (10)</th>
<th>AN, INPT LOW WT (12)</th>
<th>AN, INPT NML WT (8)</th>
<th>AN, OUTPT LOW WT (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOLID MEAL MEAL DURATION</td>
<td>4.35±1.36^a</td>
<td>5.16±1.56^a</td>
<td>6.95±2.6^b</td>
<td>5.5±1.31</td>
<td>7.9±3.15^b</td>
</tr>
<tr>
<td>LAG PHASE min</td>
<td>23±10.2</td>
<td>23.8±5.47</td>
<td>19.3±9.5</td>
<td>26.1±5.5</td>
<td>31.3±17.9</td>
</tr>
<tr>
<td>PCREM 100</td>
<td>21.1±14.4^a</td>
<td>22.6±14.5</td>
<td>30±18.1^a</td>
<td>28.4±18.2</td>
<td>45.8±20.4^b</td>
</tr>
<tr>
<td>GLUCOSE MEAL DURATION</td>
<td>0.9±.2</td>
<td>0.96±.47</td>
<td>1.28±.88</td>
<td>0.83±.42</td>
<td>1.85±1.08</td>
</tr>
<tr>
<td>LAG PHASE</td>
<td>2.4±1.9</td>
<td>2.42±1.8</td>
<td>3.81±3.13</td>
<td>3.92±2.8</td>
<td>3±1.88</td>
</tr>
<tr>
<td>PCREM 30</td>
<td>73.88±12^a</td>
<td>72.86±13.4^a</td>
<td>73.7±13.6^a</td>
<td>77.3±6.61</td>
<td>87.2±9.46^b</td>
</tr>
<tr>
<td>SALINE MEAL DURATION</td>
<td>1.05±.5</td>
<td>1.87±1.21</td>
<td>1.91±1.5</td>
<td>1.01±.44</td>
<td>3.35±4.6</td>
</tr>
<tr>
<td>LAG PHASE</td>
<td>2.01±1.55</td>
<td>3.25±2.33</td>
<td>4.6±5.75</td>
<td>4.36±6.1</td>
<td>3.31±1.8</td>
</tr>
<tr>
<td>PCREM 30</td>
<td>37.3±26.2</td>
<td>39.2±24.4</td>
<td>46.6±19.5</td>
<td>57.7±27</td>
<td>48.2±28.3</td>
</tr>
</tbody>
</table>

Means with different superscripts are significantly different in an ANOVA including: controls, bulimia nervosa, anorexia nervosa, (refeeding diet, low weight) and anorexia nervosa (self selected diet).
Summary

In the present experiments, it was found that in rats a feeding schedule designed to restrict body weight to 80% of free-feeding weight markedly slowed gastric emptying of poached egg white, glucose, 0.5 kcal/ml, and physiological saline labelled with 60 Mbq of technetium-99m tin colloid visualized using a gamma camera. In controls, the percentage remaining in the stomach at 120 minutes for each of the three meals was 19.5 (egg), 15.5 (glucose) and 27.1 (saline). After restriction, the corresponding figures were 75.2, 81.5 and 70.3. After three months of free feeding, emptying of the solid and saline meals was not significantly different from normal, but glucose emptying, although more rapid than after restriction, was still significantly slower than in controls. Rats maintained on this diet will be a suitable animal model in which to investigate the pathogenesis of the clinical condition of anorexia nervosa and, furthermore, delayed gastric emptying might be expected to occur in other situations characterised by undereating. Availability of food may be an important determinant of post-prandial satiety because of its effects on gastric emptying and hence on gastric distention.

Introduction

The studies described above show that in anorexia nervosa delayed gastric emptying occurs primarily in patients who are currently starving, the gastric disturbance reversing within about two weeks of intensive refeeding. Moreover, while solid and caloric liquid meals were delayed, emptying of an isotonic, acaloric meal of physiological saline was not affected. A better understanding of the physiological changes underlying the delay in gastric emptying associated with anorexia nervosa could
lead to useful treatments for the disorder. In the present studies, an animal model of delayed emptying was produced in the rat using dietary restriction, the emptying of different types of meal was compared and the effects of refeeding observed.

**Methods**

Gastric emptying was measured in two groups of adult male Wistar rats (9 rats per group, 195-210 gm body weight at start of experiment). One group (control) had free access to food and the other group had access restricted to a 2-hour period each day (09.00 - 11.00 hours) for 16 weeks (water was available at all times). Food used for these studies was a standard rat diet (Rat and Mouse no. 3, STS BP nutrition). Both groups of animals were then allowed free access to food, and gastric emptying measured 12 weeks later. In order to assess the effect of the experimental feeding schedule on body weight another group of nine was deprived in the same way for four months, refeed for three months and weights compared with nine controls given free access to food throughout the experiment.

To measure gastric emptying rats were deprived of food for 8 hours before being given a test meal labelled with technetium\(^{99m}\) tin colloid (60 Mbq). Three types of meal were tested on different days, a solid meal of chopped egg white (mixed with radiolabel before cooking) or, by gavage, D-glucose solution (5 ml of glucose 0.5 kcal/ml) or saline (5 ml of 0.9% NaCl solution). Gastric contents were visualized by holding the
rat in a prone position over a gamma camera (IGE Maxicamera) for a 5 second exposure. This procedure was repeated at intervals over 2 hours and from the gastric image decay-corrected time-activity curves of the stomach contents were generated. In order to determine the influence on gastric emptying of handling the animals, emptying was measured using exposures every 5 to ten minutes and compared with the emptying curve obtained when several exposures were made at each of three times over two hours (Figure 23). The resulting curves were identical and, because the latter method allowed more animals to be tested in a session, it was used for the remaining studies.

For each gastric emptying study, the percent of the original meal remaining in the stomach was plotted against time and a simple regression analysis performed. In these analyses, the zero time point was included, associated with 100% gastric contents. The regression was repeated using the log, and the square root of the gastric data, and the analysis yielding the lowest value for mean squared residuals selected. In this way, the model which accounted for the most of the variance in the particular emptying curve was determined. Regressions were not used if, in the analysis of variance, p>0.05.

Because there was some variation in the most appropriate curve to describe gastric emptying, the slopes of the emptying curves could not be directly compared. Thus, to derive a measure of gastric emptying which used all the data that were collected, the regression which gave the best fit curve was selected and the predicted fraction of the meal remaining at 120 minutes was calculated. In practice this was always very close to the actual
proportion remaining at 120 minutes. In cases in which a
regression line could not be derived, because of a very slow
emptying rate, (see Table 9) the observed fraction of the meal
remaining at 120 minutes was used. Analysis of variance was used
to compare emptying rates derived in this way.

Results

Body weight:

In the two groups of rats in which weight was regularly
monitored, the restriction diet led to a slower rate of growth.
The initial weights of the control and experimental groups were
198.13 ± 3.36g (mean ± se) and 200.0 ± 3.19g respectively (t-
test, df=16, p>.1 compared with 581.0 ± 12.14 for the control
group and 491.1 ± 10.09g for the experimental group after four
months on the restriction diet (t=4.05, p<.001). After three
months of free feeding, weights of the two groups of animals were
594.0 ± 13.6 (controls) and 539.1 ± 10.1 (experimental), (t=2.32,
p<0.05). Thus, the deprivation conditions resulted in a slower
rate of growth in experimental subjects. Free feeding led to an
improvement in weight although the animals were still
significantly lighter than controls after three months.

Gastric emptying curves:

The methodology used resulted in excellent scans with clear
separation of the gastric outline from oesophageal and duodenal
images. Results from the regression analyses are presented in
Table 9. In controls, and in refed rats, 85% of curves were best
analyzed using log-transformed data. However, in rats on

193
restricted diets, the curves were more often non-exponential, and, because of very slow emptying, could, in five cases, not be analyzed, as a non-significant regression curve was obtained.

**Gastric emptying rates.** (Figure 24)

Determination of gastric emptying rate of the control rats (n=9) showed that 2 hours after giving labelled egg-white, glucose or saline, only 19.54± 5.6% (mean ± sem), 15.49 ± 6.21% and 27.1 ± 7.4% respectively of meals remained in the stomach. In the group of rats which had been on the restriction diet for 16 weeks, the amounts remaining in the stomach 2 hours after administering similar meals were increased to 75.2 ± 4.14% (egg-white), 81.5 ± 4.7% (glucose) and 70.3 ± 5.9% (saline). In the same rats, refed for 12 weeks, the amount remaining at 2 hours was 34.9 ± 7.2% (egg), 44.9 ± 5.5% (glucose) and 38.4 ± 7.1% (saline). Sample time activity curves for the three meals under control and restricted feeding conditions are given in Figures 25a-c and gastric scans immediately after the solid meal and at 120 minutes are demonstrated in Figure 26 under the same two feeding conditions.

Analysis of variance including controls, rats on dietary restriction and refed rats, showed a significant effect of diet for all meals (P<0.001). Post hoc analysis using Duncan's test (with alpha set at p= or <0.05) demonstrated that, for all meals, dietary restriction significantly delayed gastric emptying. When

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3These figures represent actual percentages observed. The points in Figure 24 are derived from a regression analysis, and therefore differ slightly.
compared with control, rates of emptying in rats tested after refeeding did not differ significantly for the saline and solid meals although the observed difference between control and refed rats in the rate of glucose emptying (Figure 24) was significant ($t=2.51$, $df=16$, $0.01 < p < 0.05$).

**Discussion**

In these experiments it has been shown that moderate dietary restriction such as is commonly used in rat behavioural studies markedly delays the gastric emptying of solid, glucose and physiological saline meals. Emptying of all meals improved after three months of refeeding. These results are similar but not identical to those obtained from patients with anorexia nervosa in whom saline emptying was found to be normal (Robinson et al, 1988, and above) suggesting that delayed gastric emptying associated with food restriction may be mediated by more than one mechanism.

Delayed gastric emptying associated with dietary restriction may play an important part in the exaggerated satiety observed in anorexia nervosa. The finding that the weights of experimental animals had not fully recovered after three months of free food intake suggests that feeding in these subjects was disturbed and that this disturbance may be associated with the delayed gastric emptying, itself, apparently a result of dietary restriction. As well as pointing to the aetiological mechanisms involved in delayed gastric emptying associated with dietary restriction, study of this animal model may help elucidate the
significance of delayed emptying in modulating feeding patterns under different conditions of food supply.
Figure 23: Gastric emptying in rats
Comparison of intermittent and frequent sampling
Figure 24: Dietary Restriction and Refeeding

Effect on Gastric Emptying in Rats

% Gastric counts at 2 h

EGG WHITE  GLUCOSE  SALINE

CONTROL  RESTRICTED  REFED

Sig diff from control and refed
Figure 25a: Gastric emptying of egg white
Effect of dietary restriction

% gastric contents remaining

Time (minutes)

Control Restricted

0 20 40 60 80 100 120
Figure 25b: Gastric emptying of glucose
Effect of dietary restriction

% gastric contents remaining

Time (Minutes)

Control
Restricted
Figure 25c: Gastric emptying of saline

Effect of dietary restriction

% gastric contents remaining

Time (Minutes)

Control  Restricted
Figure 26

Figure Legends

Figure 26: Time-activity curves of three control rats on two different occasions. First, a curve was generated by scanning at 5-minute intervals for 120 minutes, and the following week, the study was repeated on each rat. After regression analysis, it was shown that the activity was significantly lower in the second week, although the basal rhythm remained the same. Figure 26 shows the gastric region of three control rats (left) and after 4 months of dietary restriction (right), in control subjects (upper) and in rats after 4 months of dietary restriction (lower). Delayed gastric emptying of the meal is observed in the restricted animals.
Figure legends

Figure 23: Time-activity curves of three control rats on two different occasions. First, a curve was generated by scanning at 5 minute intervals for 120 minutes, and the following week, the studies were repeated with 5 closely spaced scans obtained at each of 4 time points: after the meal, and at 30, 60 and 120 minutes. The two methods give almost identical results.

Figure 24: Gastric contents at 120 minutes, derived from regression analyses, expressed as a percentage of the original meal and compared among groups for each of three meals. Gastric emptying is significantly delayed for all three meals. After 3 months of free feeding, emptying of all three meals has recovered although glucose emptying is still significantly slower than normal (0.01<p<.05).

Figure 25a-c: Time activity curves of three control rats and three rats after 4 months of dietary restriction. Marked slowing of gastric emptying of all three meals is observed.

Figure 26: Gastric images depicting counts remaining within the gastric region of interest immediately after the solid meal (left) and after 120 minutes (right), in control subjects (upper) and in rats after 4 months of dietary restriction (lower). Delayed gastric emptying of the meal is observed in the restricted animals.
TABLE 9: For each study, the best fit, of the three models tested, is indicated.

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General Discussion

Delayed gastric emptying in eating disorders: pathogenesis and implications for control of food intake in malnourished individuals

In the present studies, gastric emptying in acutely starving patients with anorexia nervosa was found to be delayed. However, an average of two weeks of intensive refeeding was associated with normal emptying rate suggesting that the disturbance is closely linked to the nutritional status, particularly recent food intake. The experimental studies using rats support the idea that delayed gastric emptying is a phenomenon secondary to starvation, as it was readily demonstrated in animals subjected to a dietary regimen that is commonly employed in experiments in which food is used as a reward. In one respect the rat studies diverged from the clinical work: in anorexic patients egg and glucose emptying was found to be disturbed, but not saline emptying. In contrast, all three meals were affected in the rats. It may be that different mechanisms underlie the delayed gastric emptying observed in rats and patients with anorexia.
nervosa. However, in human subjects the variance observed for saline emptying was much greater than for glucose and egg, reflecting the tighter control mechanisms on the emptying of these latter two meals, and it is possible that delayed emptying of saline would be observed in anorexia nervosa if large enough numbers of patients were tested. The mechanism of delayed emptying in both animals and man requires to be clarified. A number of possible mechanisms exist, including disturbances in gastric muscle anatomy and physiology, serotonin and noradrenergic, and in peptide physiology. In view of the studies reported here and elsewhere, relating cholecystokinin function to control of gastric emptying and food intake, a disturbance in CCK physiology should be considered and investigated. The studies required to investigate this phenomenon would include measurement of upper gastro-intestinal pressures, blood levels of peptide, gastric sensitivity to exogenous agents, both agonists and antagonists of putative factors controlling gastric function and studies of the time course of the development of and recovery from delayed gastric emptying during undernutrition. Data thus far reported is conflicting. Geracioti and colleagues (1989) reported abnormally low levels of CCK in the plasma after a meal in bulimia nervosa, but normal levels in anorexia nervosa (1992). By contrast, Phillipp et al (1991) and Harty et al, (1991) found elevated or delayed CCK secretion in anorexia nervosa, but normal levels in bulimia nervosa.
CHAPTER 6

PERCEPTIVITY AND PARACEPTIVITY DURING MEASUREMENT OF GASTRIC EMPTYING IN ANOREXIA AND BULIMIA NERVOSA

SUMMARY

Gastric emptying of a mixed meal was measured in 22 patients with anorexia nervosa, 10 with bulimia nervosa and 10 controls. Visual analogue scale (VAS) ratings were made during the tests. Patients with anorexia nervosa felt fuller and less hungry than controls, although satiety was not related to gastric emptying rate. Controls showed a correlation of about 0.6 between gastric contents and reported hunger and satiety. Patients with anorexia nervosa had significantly lower correlations between gastric contents and hunger, but normal correlations with fullness. Significant correlations were often observed between gastric contents and symptoms of eating disorder in both anorexic and bulimic groups, but not in controls and this perceptual disturbance may be termed paraceptivity.

Introduction

Anorexia nervosa is a complex disorder in which disturbances are recognized in physiological, cognitive, psychodynamic and family function. In an early attempt to explain some clinical manifestations of the illness, Bruch (1966), suggested that patients were unable to perceive or
interpret somatic and visceral sensory information, and
that this led to abnormalities of body image and of the
states of hunger and satiety that normally contribute to
the control of food intake. The disturbances of body image
perception have been confirmed (Slade, 1985), although not
all studies have demonstrated differences between patients
with eating disorders and normal women. However, studies
of the perception of internal state (interoception) in
patients with anorexia nervosa have been lacking, although
reports of delayed gastric emptying in these patients
suggest that disturbed satiety may play a part in
perpetuating the disorder (Robinson, et al 1988, and
Chapter 4, above). Visceral stimuli of relevance to food
intake are intra-gastric pressure and stomach fullness.
Cannon and Washburn (1912) suggested that perception of
gastric contractions is fairly accurate, although this has
not been confirmed in more extensive studies (Stunkard and
Koch 1964, Whitehead and Drescher, 1980). An attempt to
measure the ability to detect changes in gastric fullness
(Coddington and Bruch, 1970) by introducing different
volumes of nutrient into the stomach via a gastric tube
suggested that some normal individuals can do so with fair
accuracy. Subjects with morbid obesity, as well as two
patients with anorexia nervosa, were less accurate than
normal controls on this measure. Other studies have relied
on questionnaire and analogue scale reports of anorexic
patients without measuring gastric contents, and suggest
disturbances in the reporting of satiety alone (Garfinkel,
or both hunger and satiety (Owen et al, 1985). Studies of responses to eating in patients with bulimia nervosa have demonstrated a tendency to report decreased satiety compared to controls (Robinson et al 1983) and increased hunger and urge to eat post-prandially (Robinson et al 1985a). No study of interoceptive ability has, however, been reported in bulimic subjects. In order to investigate interoception in eating disorders, visual analogue scale ratings were compared, in the present study, with simultaneous measures of gastric contents derived scintigraphically at different times after a meal in patients with anorexia nervosa, bulimia nervosa and controls.

**Methods**

**Subjects**

Twenty two patients who fulfilled accepted criteria for anorexia nervosa (Russell, 1983) were tested. Ten of these patients were selecting their own diet, either as outpatients (7 patients) or as inpatients undergoing observation without active treatment, and twelve were investigated after a mean of two weeks of active refeeding on an inpatient unit, during which they received at least 2500 Kcal/day. These patients form the same group as those described in Chapter 4. They all suffered severe self-imposed weight loss, with secondary amenorrhoea for at least six months, and manifested a morbid fear of fatness. These patients were aged between 18 and 40, one was male, and they suffered a variety of symptoms of anorexia nervosa.
including self-starvation, bulimia and self-induced vomiting (Table 10). Eight patients were re-tested after they had reached a healthy weight.

Ten female patients with bulimia nervosa, according to the criteria of Russell (1979) were tested. Clinical information pertinent to these patients, none of whom was severely underweight, is also summarised in Table 10.

Normal controls were drawn from the same age range as the patients, with a similar sex distribution. Controls scored within the normal range on the Eating Attitudes Test (Garner and Garfinkel, 1979) and the Dutch Eating Behaviour Questionnaire (Van Strien et al, 1985).

**Gastric emptying**

The methods for determining the gastric emptying of a mixed solid meal have already been documented in Chapter 5.

**Rating scales**

Before and 10 minutes after the meal, subjects completed, respectively, the hunger and satiety questionnaires of Garfinkel (1974), modified to apply to meals of fixed size. These checklists include ratings of gastric sensations, overall sensations, mood, urge to eat and preoccupation with food. Patients with anorexia nervosa have been found to differ from controls on the satiety, but not the hunger questionnaire (Garfinkel, 1974). On two occasions before the meal, and whenever an anterior scan was acquired after the meal, 8 visual analogue scale (VAS) ratings were made.
Because of the position of the gamma camera, a traditional pencil and paper VAS was impractical, so an electrical equivalent was devised. Forty light emitting diodes (LEDs) were mounted in a 10cm line labelled at the ends with the words NOT AT ALL or EXTREMELY. Above the line of LEDs a label was displayed.

The labels used were:

- HUNGRY
- SAD
- FULL
- TENSE
- URGE TO EAT
- FAT
- NAUSEA
- URGE TO BE SICK
- DROWSY

Subjects (who were instructed to regard the extremes of the scale as the greatest and least degrees of the feeling they could imagine) adjusted a rheostat until the illuminated LED represented the position of the scale reflected their feelings at that moment. The distance of that light from the NOT AT ALL end of the scale was then recorded by an observer (the author). Except for two (FAT, URGE TO BE SICK) all of these scales are represented in the Garfinkel questionnaires by analogous items.
Statistics

Results of categorical measures were assessed using chi-square tests and visual analogue ratings were compared between groups of subjects with repeated measures analysis of variance, using ratings made at three times: immediately before and after the meal, and at 100 minutes from the beginning of the meal.

Correlations between gastric contents and scale ratings were derived using Spearman's rank test, and values of Spearman's rho within each subject were calculated separately for each scale. Correlations were compared between groups of subjects using one way analysis of variance with post hoc Duncan's tests to indicate significantly different pairs of means.

Linear regression analysis was performed on the same data in an attempt to establish the characteristics of the curves relating gastric contents to each scale for individual subjects. It was found, however, that, in a number of cases, particularly within the group of patients with anorexia nervosa, regression could not be performed, even after log data transformation, and using relatively liberal criteria for entry into the analysis (P=or< 0.2), and simple correlation coefficients were therefore used as a measure of perceptivity in the subjects.

Because results from patients with anorexia nervosa on self selecting and refeeding diets were not significantly different from each other, data from all low-weight patients with anorexia nervosa were pooled and
compared with those from control subjects and patients with bulimia nervosa. Results from subjects tested before and after weight gain were analyzed using paired t-tests.

Results

Hunger and satiety questionnaires

Responses on the hunger questionnaire were not significantly different from controls in either group. On the satiety questionnaire, patients with anorexia nervosa were significantly (p<.05) more likely to report the feelings of having a full stomach, or of being bloated, and less likely to report feeling calm or contented than controls. Compared to control subjects, patients with bulimia nervosa reported more 'bloating' and less 'contentment' post-prandially.

Absolute visual analogue scale ratings

In the repeated measures analyses of variance, significant effects of the meal on hunger, fullness and urge to eat were observed, in the expected direction. That is, subjects felt relatively hungry before eating, the feelings declined after the meal and rose again by 100 minutes (Figure 27a-d). A significant effect of diagnostic group reflected lower scores in anorexic patients on hunger and urge to eat (hunger scales) and higher scores on fullness (satiety). Patients with bulimia nervosa did not show significant deviations from normal on these eating
related scales. However, both anorexia nervosa and bulimia nervosa patients had higher overall scores for nausea, urge to be sick, sadness, fatness, tension and drowsiness.

Group by meal interactions were observed for urge to eat, nausea and urge to be sick. For urge to eat, the interaction reflected poor recovery of the scores with time in patients with anorexia nervosa compared to the other two groups (Figure 27d). Similarly, poor recovery of scores is seen for hunger and fullness, although the effects were not significant. Patients with anorexia nervosa therefore showed prolonged satiety which might be related to slow gastric emptying. However, ratings from patients with fast gastric emptying (less than 50% retained at 100 minutes) were not significantly different from those with slow emptying (50% retention or more at 100 minutes), and analysis of covariance showed no significant effect of gastric emptying rate on hunger and satiety scale scores, or on any other scale. Prolonged satiety therefore occurred in patients with rapid as well as those with slow gastric emptying.

Controls had low scores for nausea with little change over time, patients with anorexia nervosa had higher scores which rose after eating and rose a little further at 100 minutes, while patients with bulimia nervosa had high scores, rising after eating and then falling to normal values by 100 minutes. Ratings of urge to be sick were low throughout in controls. However, patients in both anorexic and bulimic groups showed significant increases after
eating which declined by 100 minutes (Figure 27c). These changes in nausea and urge to be sick were observed both in patients who practised self-induced vomiting and in those who did not, and multivariate analysis of variance revealed no significant effect of bulimia, vomiting or abuse of laxatives on these two scales.

Correlations between gastric contents and VAS ratings (Table 11, Figure 28a-c)

Spearman rank correlations were determined between the percentage of the original counts remaining in the stomach and the score for each scale at every time point available, in order to provide a measure of the relationship between reported experience and actual gastric contents. Correlations between the three normal feeding scales; HUNGRY, FULL and URGE TO EAT were also determined. Analysis of variance was then used to determine whether differences in mean correlations between groups of subjects were significant.

Controls

In control subjects significant correlations were generally only observed between gastric counts and one or more of the three normal feeding scales. The average value of Spearman's rho for these scales was ± 0.60 (with the sign in the expected direction) and, of the ten controls, all had significant (p<0.01) correlations between gastric contents and at least one of these scales. Of the other
scales, a significant correlation was noted between gastric contents and sadness in one subject.

Anorexia nervosa

Patients with anorexia nervosa had significantly lower correlations between gastric contents and urge to eat (p<0.05) while correlations with hunger showed a trend in the same direction (p=0.057) reflected in a significant post hoc difference (Duncan's test) between controls and anorexic patients. Correlations between hunger and urge to eat and between fullness and urge to eat were significantly poorer in patients with anorexia nervosa compared to controls. Two scales (fatness and urge to be sick) showed significantly higher correlations in anorexic patients than in controls. The relationship between gastric contents and the scale FULL was not significantly poorer in anorexia nervosa compared to controls. Thus, while a measure of satiety appeared to be normally correlated with gastric contents, measures of hunger were not, and an abnormal association between symptoms of anorexia nervosa and gastric contents was noted. In eight patients, in whom the studies were repeated after weight gain, (a mean of 10.3 kg over 9 weeks) no significant change was observed in correlations between gastric contents and any visual analogue scale.

Bulimia nervosa

The ten patients with bulimia nervosa differed
significantly from controls on four scales; sadness, fatness, nausea and urge to be sick and, in every case, correlations for patients were higher than for controls. For other scales, there was no significant difference between patients and controls.

Discussion

In this study, disturbances in the experiences of hunger, satiety and in their relationship with gastric function have been demonstrated in anorexia nervosa. Moreover, anomalous links were observed between gastric contents and eating related symptoms in both anorexia nervosa and bulimia nervosa.

Using questionnaires, differences between controls and eating disordered patients were observed for satiety, but not for hunger, results similar to those reported by Garfinkel (1974) who found that patients with anorexia nervosa were more likely to indicate unpleasant gastric sensations after a standard meal than controls. Using visual analogue scales in the present study, patients with anorexia nervosa were found to report feeling less hungry but more full than controls, particularly some time after the end of the meal. However, scores of gastric fullness at 100 minutes in patients found to have delayed gastric emptying (Chapter 5 and Robinson, et al, 1988) were not significantly different from scores of patients with normal
gastric transit. Repeated testing of the same subjects at different gastric emptying rates might show changes in perceived satiety not apparent in the present study, in which a between-subjects design was used. However, the results indicate a cognitive disturbance reflecting the observed delay in gastric emptying but persisting when gastric emptying has returned to normal. Whether this cognitive disturbance antedates the development of anorexia nervosa is unknown.

Patients with anorexia nervosa therefore over-estimate gastric contents in a way apparently analogous to the over-estimation of body image that has been well documented in this disorder (Slade, 1985). Several symptoms were also found to be increased relative to controls in both anorexia nervosa and bulimia nervosa patients, reflecting their general and specific psychopathology (Russell, 1979, Fairburn and Cooper, 1984). Normal subjects showed reasonably good correlations between gastric contents and scales measuring hunger and satiety, but not with other scales. In patients with anorexia nervosa, correlations for satiety were not significantly abnormal, but correlations for urge to eat were, and patients often related gastric contents to symptoms. Patients with bulimia nervosa showed normal correlations for hunger and satiety, but, like the anorexic patients, showed significant correlations between gastric contents and symptoms.

Coddington and Bruch (1970) suggested that some normal
subjects are able to discern with fair accuracy what is placed in the stomach, while Whitehead and Drescher (1980) found that normals were rather poor at detecting gastric contractions. Using a different approach, the present study suggests that normal subjects can often relate changes in gastric contents to feelings of hunger and satiety, and that the average correlation between the psychological and physiological measures is about 0.6. The gastric scan measures only part of the mixed meal, that is, the egg, and may not have reflected accurately its other constituents, nor could it give information on upper gastro-intestinal secretions or intra-gastric pressure. It is, therefore, an imperfect measure of gastric contents, and an incomplete assessment of gastric function, and the results obtained should be interpreted with caution. Moreover, changes observed in subjects' rating of fullness or hunger may have been influenced by the subjects' own expectations and learned responses to eating.

It may, however, be concluded that, in anorexia nervosa, although gastric fullness is rated higher than controls, after a similar meal, it changes in the expected fashion with gastric emptying, that is, by a gradual decline. Analogous changes in the patients did not occur with the scales describing hunger and urge to eat, which often bore no relation at all to gastric contents. One possible interpretation is that while perception of gastric afferent signals is intact, further processing into behavioural strategy (the urge to eat) does not occur normally. This
suggestion, which is consistent with the patient's view of eating as a feared precursor of weight gain, is supported by significantly poorer correlations between fullness and urge to eat in anorexic patients compared to controls. The finding also suggests that, at least in pathological states, hunger and satiety can be dissociated. Gastric sensations also appeared to be related to feeling states some of which are symptoms of anorexia nervosa. A similar disturbance was also observed in patients with bulimia nervosa. Patients with eating disorders often report intense sensitivity to gastric sensations, such that feelings of gastric distension lead to distress, associated with "bloating" or feeling fat. The present results are consistent with these reports and suggest that, in anorexia and bulimia nervosa, gastric sensations are processed in such a way that they modulate unpleasant experiences recognized clinically as symptoms. This apparent "misrouting" of gastric sensations may be termed PARACEPTIVITY, in the sense of distorted perception, and, it is suggested that, in eating disorders, it may be an important way in which physiological changes are translated into psychiatric symptoms. The precise psychological and physiological mechanisms that underlie paraceptive experiences await further study.

Bruch (1966) suggested that patients with anorexia nervosa show disturbances in the perception of sensory information arising in the gastro-intestinal tract, or in its interpretation. The present evidence favours the
second of these alternatives.
Figure 27a. Visual analogue scale ratings
Effect of eating on ratings of FULLNESS

Control Anorexia nervosa Bulimia nervosa

p<.001
Figure 27b. Visual analogue scale ratings
Effect of eating on ratings of HUNGER

- Control
- Anorexia nervosa
- Bulimia nervosa

Rating (mm)

Time (min)

p < .05

p < .001

meal
Figure 27c. Visual analogue scale ratings

Effect of eating on ratings of URGE TO BE SICK

- Meal

p < .05

Control  Anorexia nervosa  Bulimia nervosa

Time (min)
Figure 27d. Visual analogue scale ratings

Effect of eating on ratings of URGE TO EAT

Time (min)

Rating (mm)

p<.05

p<.05

Control  Anorexia nervosa  Bulimia nervosa

meal
Figure 28a. Visual analogue scale ratings

Gastric emptying and VAS ratings

One control subject

Gastric counts remaining %

Correl with gastric counts: +0.90  -0.93  0.0
Figure 28b. Visual analogue scale ratings

Gastric emptying and ratings.

One patient with bulimia nervosa

Gastric counts remaining %

Correl with gastric counts: +0.94  -0.66  +0.94
Figure 28c. Visual analogue scale ratings

Gastric emptying and ratings

One patient with anorexia nervosa

Gastric counts remaining %

Correl with gastric counts: FULL +0.93 URGE TO EAT +0.03 URGE TO BE SICK +0.91
Figure legends

Figure 27a-d: Mean visual analogue ratings in mm on 4 scales by controls (solid lines) and patients with anorexia nervosa (dots) and bulimia nervosa (dashes) 10 minutes before and after and 100 minutes after a small meal. Results of one-way ANOVA at each time are indicated. A significant effect of time was found in the repeated measures ANOVAS for all four scales.

Figure 28: Time activity curves for gastric emptying from 3 subjects with superimposed VAS scores from the same subjects. The control subject's results are given in Figure 28a, the patient with bulimia nervosa in Figure 28b and the patient with anorexia nervosa in Figure 28c. Correlation coefficients (Spearman's rho) between gastric counts and VAS scores are given. Gastric contents are indicated by asterisks. Fullness is accurately perceived by the three subjects. Urge to eat is appropriately rated by control and bulimic subjects and urge to be sick is related to gastric contents for patients but not the control.
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**TABLE 10**: Demographic and clinical information (mean (se) or frequencies) on patients and control subjects. Body mass index; weight/height squared, normal range 19-24 kg/m². Means with different superscripts are significantly different.
CONTROLS | ANOREXIA NERVOSA | BULIMIA NERVOSA
---|---|---
Mean correlations (± SEM) between gastric counts and VAS ratings
HUNGRY | -.59±.11^a | -.24±.09^b | -.43±.11
FULL | .57±.15 | .46±.08 | .62±.09
URGE TO EAT | -.58±.14^a | -.02±.07^b | -.39±.16^a
FAT | -.18±.08^a | .30±.07^b | .38±.12^b
NAUSEA | -.07±.07^a | .23±.08 | .51±.14^b
URGE TO BE SICK | -.08±.09^a | .41±.07^b | .61±.19^b
SAD | -.01±.12^a | .11±.09^a | .42±.11^b
TENSE | .01±.08 | -.04±.09 | .12±.15
DROWSY | .05±.11 | .08±.08 | .13±.16

Mean correlations between different VAS ratings
HUNGRY/FULL | -.55±.15 | -.34±.08 | -.64±.08
HUNGRY/URGE TO EAT | .62±.12^a | .27±.07^b | .71±.08^a

**TABLE 11:** Mean values (se) for within-subject Spearman's rank correlation coefficients between visual analogue scales (VAS) and gastric contents assessed using isotope scanning of a solid mixed labelled meal. Correlations between scales are also given. Means with different superscripts are significantly different (analysis of variance, Duncan's test).
CHAPTER 7
DISCUSSION

DELAYED GASTRIC EMPTYING AND MEDIATION OF REDUCED FOOD INTAKE

In the preceding chapters, an assessment has been made of the contribution of delayed gastric emptying to undereating associated with fenfluramine and cholecystokinin administration, and in anorexia nervosa. Fenfluramine was shown to reduce food intake in bulimia nervosa, and, in the rhesus monkey, was demonstrated to act, in part, by inhibition of gastric emptying. Cholecystokinin binding sites were found in the stomach of the rat in the newborn period, a stage at which such sites are reputedly at low levels in the central nervous system. Moreover, CCK was found to inhibit independent milk intake, oral behaviours and gastric emptying in newborn rats, suggesting a link between CCK-induced inhibition of ingestion and gastric emptying at that early stage of development. Lastly, patients with anorexia nervosa were found to have delayed gastric emptying while they were acutely starving, but not during nutritional rehabilitation, and distorted perceptions of gastric state were observed which appeared analogous to the distortions of body image previously reported in anorexia nervosa. In the present chapter, each
finding will be examined in the context of other research bearing upon it, and, where possible, further investigations suggested.

1. dl-FENFLURAMINE, EATING DISORDERS AND GASTRIC EMPTYING

Bulimia nervosa

Present findings

It was found that intake of food under laboratory conditions in patients with bulimia nervosa was reduced by dl-fenfluramine, 60mg orally, taken two hours before. Patients with bulimia nervosa therefore respond to fenfluramine in a way that has been demonstrated in normal human subjects (Kyriakides and Silverstone, 1979). The time at which eating behaviour was observed (2 hours) was appropriate given the half absorption time of 1 hour for the drug (Pinder et al, 1975) and the duration of side effects noted after the active preparation was close to its known plasma half-life (Campbell, 1971). dl-Fenfluramine was also shown to inhibit bulimic symptoms, including bulimic episodes and self-induced vomiting, without increasing the tendency to restrict carbohydrate that is a feature both of bulimia nervosa (Russell, 1979) and fenfluramine treatment (Wurtman and Wurtman, 1984).

Therapeutic implications

The findings suggests that a more prolonged course of dl-fenfluramine might be of therapeutic benefit in the
treatment of bulimia nervosa. A controlled trial of the dextro isomer, d-fenfluramine (which is more active in the inhibition of feeding (Hirsch et al, 1982) but less likely to result in side effects) has been reported in patients with bulimia nervosa (Russell et al, 1988). The study demonstrated a probable therapeutic effect of d-fenfluramine in bulimia nervosa, although there was a high rate of dropouts in the study and the conclusions are preliminary.

It is possible that further studies will demonstrate that fenfluramine reduces the incidence of bulimic episodes in patients with bulimia nervosa. It is important to consider a number of drawbacks of the drug before contemplating its use in clinical settings. First, treatment with dl-fenfluramine has been associated, in patients with morbid obesity, with several dangers and disadvantages. Although the drug is effective in reducing weight, the benefit is usually lost after medication ceases, and to produce lasting weight reduction, administration of the drug needs to be long term (Douglas et al, 1983). It remains to be shown whether, in bulimia nervosa, any therapeutic benefit can be maintained after drug therapy stops. Several reports have indicated that, in treatment of obesity with fenfluramine, depression can be a complication, either during treatment or after its termination (Garrow et al, 1972). Moreover, in a few cases, pulmonary hypertension has occurred, (Douglas et al 1981). Lastly, in animal studies very large doses of fenfluramine have been
associated with neuropathological changes and apparently irreversible loss of neurones (Dastur et al, 1985). As the majority of studies have been performed using dl-fenfluramine, it remains to be established whether the more recently introduced d-isomer lacks the disadvantages suggested for the racemic mixture.

**Mechanism of action of fenfluramine on feeding**

**Hunger or satiety?**

These two constructs are poorly differentiated. When studied using visual analogue scales, they are inversely correlated (see chapter 6), and they are probably best distinguished behaviourally - the tendency to initiate a bout of ingestion or to work for a food reward, against the tendency to slow and stop an ingestive episode. The latter process, satiety, is associated with a set of behaviours said to be characteristic of the recently fed state, including grooming, exploration and resting (Antin et al, 1975).

**Previous studies**

Several mechanisms of action have been postulated for fenfluramine and the therapeutic effect noted in bulimic patients may depend on one or more of these mechanisms or, indeed, on a quite different mode of action, perhaps specific to bulimia nervosa. The effect of fenfluramine on motivation to initiate eating is demonstrated by its reduction of self ratings of hunger and urge to eat before food is presented (Kyriakides and Silverstone, 1979a) and, in rats, by its reduction of running speed to a food reward.
on a laboratory runway (Thurlby et al, 1985). Moreover, sham feeding, in rats in which ingested food drained from gastric fistulas, is reliably inhibited by fenfluramine (Rowland and Carlton, 1986). The effect of fenfluramine on behaviours linked to feeding can therefore be demonstrated without any gastric distension cues or systemic absorption of nutrient. However, fenfluramine also appears to enhance the satiating effect of ingested food. Hunger ratings are suppressed by fenfluramine, more reliably in relation to the second and subsequent post-drug meals in man (Blundell et al, 1979), fenfluramine satiety is enhanced by gastric preloads in monkeys (Foltin and Schuster, 1983) and norfenfluramine, injected into the paraventricular nucleus of the rat, reduces meal size but not meal frequency (Shor-Posner et al, 1986), again indicating an effect on meal termination rather than initiation.

Findings presented here:
The facilitatory effect of fenfluramine on satiating stimuli could be mediated by the gastric inhibitory effects of the drug, demonstrated in studies described in Chapter 3. Evidence has been provided for a disorder in patients with bulimia nervosa in post prandial ratings of hunger, which did not decline normally. Fenfluramine treatment partially corrected this abnormality so that hunger ratings tended to fall after eating, as occurred in controls. This evidence suggests that, in the patients, the drug interacted with post-ingestive signals - gastric or post-
gastric - to enhance satiety and reduce food intake.

Central or peripheral?

The intention of the second study, in rhesus monkeys, was to investigate a gastric mechanism for the feeding inhibitory effect of dl-fenfluramine, and to estimate the proportion of the total inhibition that could be accounted for by the reduction of gastric emptying rate.

Previous studies

The mechanisms that have been proposed for the feeding inhibitory effect of fenfluramine are a central action mediated by midbrain raphe serotonergic neurones, and an enhancement of satiety by a reduction in gastric emptying rate. The body weight reduction produced by fenfluramine has also been attributed, in part, to an increase in the rate at which fats and carbohydrates are metabolised, and a fall in the efficiency with which fuels are utilized (Bray and Lupien, 1984, Turner, 1979). These latter actions probably have no significant role in short-term reduction of food intake and may well not influence weight loss which has been shown to occur primarily by means of a reduction in caloric intake and not an increase in utilization (Garrow, et al, 1972). The hypoglycaemic effect of fenfluramine (Hamet et al, 1986) would be expected to have the effect of increasing rather than decreasing food intake (Hoebel and Teitelbaum, 1966, Kanarek et al, 1980) and is likely to be irrelevant to the present discussion, although, perhaps, of therapeutic importance in obese diabetic patients.
The central mechanism for fenfluramine inhibition of feeding may be mediated by the serotonergic neurones of the midbrain raphe. Destruction of these neurones by electrolytic lesions is said to reduce or abolish fenfluramine inhibition of feeding (Samanin et al, 1972). The widely accepted conclusion that midbrain raphe serotonergic neurones mediate fenfluramine inhibition of food intake has, however, been challenged (Rowland and Carlton, 1986) because of the lack of corroboration of the results from studies using the more potent and specific serotonin neurone toxin, 5,7 DHT (Carlton and Rowland, 1984). It is therefore not certain that fenfluramine acts by modulation of central serotonin pathways. Pharmacological studies do, however, lend support to the idea that serotonin is involved in the effect of dl-fenfluramine on feeding. The reduction of runway speed by fenfluramine is reversed by metergoline, a serotonin inhibitor that acts both peripherally and centrally (Thurlby et al, 1985). In the same series of experiments, xylamidine, a peripherally active serotonin antagonist, was ineffective in reversing the effect of fenfluramine, indicating that central serotonin neurones are probably involved in this action. Similarly, d-fenfluramine induced reduction of feeding in rats trained to eat for four hours per day was antagonized by metergoline, but not by xylamidine (Carruba et al, 1986). Serotonin (5HT) receptors have been classified into a number of subtypes (Bradley et al, 1986). 5HT1 receptors, mediating among
other actions, smooth muscle contraction (and further subdivided according to central responses into 5HT1a, b and c), 5HT2 mediating, for example, smooth muscle relaxation and 5HT3 which mediate depolarization of peripheral neurones. 5HT1 and 5HT2 responses are antagonized by methysergide, ketanserin and cyproheptadine block 5HT2 receptors and cocaine blocks the 5HT3 receptor. The feeding inhibition of fenfluramine is antagonized by ketanserin, (Hewson et al, 1988) indicating a mechanism involving 5HT2 receptors. dl-Fenfluramine also antagonizes the hyperphagia induced by isapirone, a stimulator of 5HT1a receptors (Wong and Reid, 1987). dl-Fenfluramine causes reduced levels of serotonin in the blood and reduction of the serotonin metabolite 5 hydroxy indole acetic acid (5 HIAA) in the cerebro-spinal fluid in monkeys (Raleigh et al, 1986). However, in the short term, fenfluramine enhances serotonin function, and the evidence is substantial that this action depends, in part on an increased tissue level of serotonin secondary to prevention of neural reuptake (Fuxe et al, 1975).

The site of action of fenfluramine may be in the central nervous system, in the periphery, or both. Small doses of dl-fenfluramine, injected into the paraventricular nuclei or the lateral hypothalamus, reduce feeding (Angel et al, 1987, Blundell and Leshem, 1973), suggesting these sites as central loci for the action of the drug. The attenuation of the feeding inhibitory effect of dl-fenfluramine by electrolytic lesions of the midbrain raphe serotonergic
system suggest a central site of action, although this attenuation is only observed when rats are tested after a lengthy (18hr) fast, when the stomach is empty. If raphe lesioned rats are allowed to eat freely, dl-fenfluramine can be shown to exert an inhibitory effect in a test meal equivalent to the inhibition demonstrated in non-lesioned rats (Davies, et al, 1983). The inhibition of feeding observed under these circumstances may be mediated by the inhibition of gastric emptying demonstrated in the rat (Rowland and Carlton, 1984), hamster (Rowland and Carlton, 1986a), in the experiments reported here, in the rhesus monkey and in man (Horowitz et al, 1985). In the present studies saline and glucose emptying rates were found to be significantly reduced, both after a constant load delivered intragastrically, and during sucrose feeding. Moreover, the inhibition of gastric emptying was found to correlate highly with the suppression of sucrose feeding. The more gastric emptying was inhibited, the more was feeding suppressed. It is suggested that the two effects are intimately linked so that a substantial portion of the suppression of feeding by fenfluramine can be explained in terms of delayed gastric emptying. By measuring the gastric volume at satiety, it was estimated that the animals ceased consumption, after fenfluramine, at a gastric volume that was about 90% of that after vehicle, and that, in consequence, 90% of the feeding inhibition after the drug could be explained by inhibition of gastric emptying. This postulate is, however, open to question.
It is possible that the two effects occur in parallel, perhaps mediated by a common pathway. For example, intracerebro-ventricular fenfluramine inhibits gastric emptying as well as feeding (Rowland and Carlton, 1984) in the rat and the two effects might be quite independent, save for a common central mechanism. The hypothesis is, however, testable, and a number of experimental methods are available which could improve understanding of the link between inhibition of gastric emptying and other mechanisms in fenfluramine induced reduction of feeding. Fenfluramine exists in two forms, dextro and laevo, and most published work to date has been performed using the racemic mixture. d-fenfluramine is much more active in reducing food intake than the l-isomer (Hirsch et al, 1982), and determination of the relative effects of the two forms on gastric emptying could clarify its role in suppression of feeding. If, for example, inhibition of gastric emptying was only found for the l-form, the gastric effects of fenfluramine would be seen to be largely irrelevant to the feeding effects. As the l-form may be an antagonist of dopamine (Bettini et al, 1987), it would be predicted to accelerate gastric emptying, like other dopamine antagonists such as domperidone or metoclopramide (Mimmo et al, 1973, Ricci and McCallum, 1988). Gastric preloads of normal saline would be expected to interact with fenfluramine by enhancing its effect on short term food intake. Normal saline itself does not influence feeding (Moran and McHugh, 1982). However, if combined with fenfluramine, which inhibits
gastric emptying, saline preloads would be expected to shift the dose response curve for the feeding inhibitory effects of fenfluramine to the left. These experiments could be performed by delivering the preloads in animals via an indwelling gastric cannula or, in man, by means of a naso-gastric tube.

**Mechanism of gastric inhibition by fenfluramine:**

**Interaction with neurotransmitter systems**

It might be possible to dissociate the gastric and feeding effects of fenfluramine using specific receptor blocking drugs. The effect of peripherally administered serotonin, for example, is to inhibit gastric emptying and to reduce the size of eating bouts (Fletcher and Burton, 1986). However, it appears that these two actions are separable. The feeding effect can be inhibited by methysergide, a serotonin antagonist, although the gastric inhibitory effect was unaffected by methysergide. This class of experiment would be best designed with a detailed understanding of the pharmacological action of dl-fenfluramine on gastric function. The precise mode of action of dl-fenfluramine in the inhibition of gastric emptying is unknown. The finding that centrally administered dl-fenfluramine inhibits gastric emptying (Rowland and Carlton, 1984) suggests that a central may
contribute to the effect. Although dl-fenfluramine acts on serotonin systems it has not been found to alter levels of serotonin in gastric tissues (Costa et al, 1971). It does affect the motility of the small intestine, however, and this action is antagonized by cyproheptadine, a serotonin (5HT²) antagonist (Mottram and Patel, 1979). Of the other classical neurotransmitters, dl-fenfluramine may have dopamine antagonist actions (Bettini et al, 1987), which would be expected to accelerate gastric emptying (Ricci et al 1988), and weak sympathomimetic (Sipes et al, 1971) and anticholinergic activity (Waton and Kasim, 1975), both of which might contribute to its gastric inhibitory effect. Recently, d-fenfluramine slowing of gastric emptying was found to be reversed by metergoline, a non-specific 5-HT antagonist, but not by more specific blockers of 5-HT2 and 5-HT1C activity (Samanin et al, 1991). This demonstrates that an effect on 5-HT function mediates the effect of d-fenfluramine on gastric emptying, but excludes 5-HT2 and 5-HT1C receptors as the site of action of the drug.

Other possible mechanisms and experiments

Liquid emptying is reportedly less affected by dl-fenfluramine than solid emptying (Rowland and Carlton 1986a) and, as a marked effect on liquid emptying was observed in the experiments reported here, both gastric functions appear to be inhibited by dl-fenfluramine. Liquid emptying is said to be controlled mainly by activity of the proximal stomach, while the distal stomach, namely
antrum and pylorus, are most active in the control of the emptying of solids (Kelly, 1980). An elucidation of the site of action of dl-fenfluramine has, therefore, to account for an effect on two sets of structures in the stomach. dl-Fenfluramine may act centrally to inhibit gastric emptying, or it may interact directly with receptors in the upper gastrointestinal tract. A third possible mode of action is a modulation of other ligands which themselves affect gastric emptying. For example dl-fenfluramine releases endorphins from rat duodenum (Majeed et al, 1987) and as morphine has been shown to have a potent, probably centrally mediated, gastric inhibitory action (Galligan and Burks, 1983) fenfluramine could act by facilitating release of endorphins. Endogenous peptides such as cholecystokinin and secretin also affect gastric emptying (Valenzuela and Defilippi, 1981, Scheufler and Zetler, 1981) and might be important mediating factors in fenfluramine gastric inhibition. The mode of action of dl-fenfluramine on gastric emptying remains to be investigated and it will be necessary to use a number of experimental designs. First, the two isomers of dl-fenfluramine will need to be tested separately, as they have widely differing spectra of action (Garattini et al, 1987). Secondly, it will be necessary to attempt to antagonize the effect of each isomer on gastric emptying using specific drugs blocking the various serotonin receptors, dopaminergic and other monoaminergic and cholinergic receptors, and receptors for endorphins and endogenous peptides. In each
case, consideration will need to be given to whether the drug is acting peripherally or centrally and, if antagonists that do not enter the brain are unavailable, more information will need to be obtained by the use of isolated organ bath preparations.

2. CHOLECYSTOKININ, FEEDING, GASTRIC EMPTYING AND ONTOGENY

In studying the ontogeny of CCK receptors and ingestive behaviour in the rat it was intended to show simply, whether CCK receptors are present in the stomach and to establish the responsiveness of ingestive and gastric function in the neonatal rat to exogenous CCK. In view of the finding that the brain contains few CCK receptors at birth (Hays et al, 1981), it was reasoned that any effect of CCK demonstrated in the neonatal rat could be attributed to peripheral mechanisms. This approach proved fruitful, although the answers it provided were not as simple as the methodology appeared to promise. First, investigation of the upper gastro-intestinal tract revealed widely distributed CCK receptors in the foetal oesophagus and stomach, in contrast to the adult, in which receptors were confined to the pyloric sphincter. Secondly, examination of the effect of CCK on ingestive behaviour in the neonate showed that, at this early age, CCK affects ingestive behaviour at doses that do not influence adult feeding. While tending confirm the original hypothesis that suppression of ingestion in the neonatal rat after CCK is likely to be mediated peripherally, the studies raise a
number of questions that remain to be addressed.

**Ontogeny of CCK systems in the rat**

From the autoradiographic studies it appears that sites on upper gastrointestinal mucosa and smooth muscle bind $^{125}$I-CCK and that the radioligand can be displaced by unlabelled CCK-8. The specificity with which binding occurred was not, however, established. The different classes of CCK receptor currently recognized (A and B, or peripheral and central) have already been described in Chapter 1, and the CCK receptors demonstrated in the upper gastrointestinal tract of the developing rat need to be characterized in terms of the ability of different analogues of CCK to displace radio-labelled CCK from the binding sites in the different tissues in which receptors were identified.

Developmental changes have here been demonstrated in CCK binding in the upper GI tract of the rat. The degree to which this observation can be generalized to other species is, however, unknown while autoradiographic studies of the ontogeny of CCK binding in other peripheral tissues have yet to be performed. Developmental studies of receptor densities in the brain of the rat have shown (Hays et al, 1981) detectable levels at birth around 25% of adult receptor binding. No comparable study in the gut has been reported. However, several authors have investigated the concentration of CCK in the brain and gut of developing rats. In an immunohistochemical study of the rat, Larsson (1977) noted that, in the gut, no cholecystokinin-positive cells were observed in the stomach, that, in the duodenum,
a few were present at birth, and that, these increased in number to a maximum around post natal day 15 after which their numbers declined. Using a bioassay, Brand (1982) found levels of CCK in the neonatal rat gut at 126% of adult levels, while Noyer et al (1980), using radioimmunoassay, reported that neonatal levels of CCK in the rat were 4 times those observed in the adult. The point in development at which maximum levels of CCK have been observed varies between 3 days prenatally (Goldman, et al, 1985) and 4 days postnatally (Noyer et al, 1980). The consensus appears to be that CCK is found in the rat upper small intestine, that neonatal levels are higher than those found in the adult, although the difference between reported neonatal and adult concentrations varies with the methods of extracting and measuring CCK. In the rat brain, by contrast, neonatal concentrations of CCK are much lower than found in the adult. Brand (1982) apparently found very little CCK in the brain at birth. However, Hays et al (1981) found neonatal brain CCK at 9.6% of adult concentrations, Beinfeld et al (1983) found 11% and Goldman et al (1985) reported 2% adult levels in the telencephalon and 5% in the brain stem. Maximum concentrations of CCK have been measured in the brain at 30 days post natally (Noyer, 1980), 35 to 42 days postnatally (Goldman et al, 1985) and 28 days in the forebrain and 41 days in the hindbrain (Beinfeld et al, 1983). CCK develops in the brain much later than that in the gut, and this conclusion, together with the delayed appearance of brain CCK
receptors, and the present findings of early CCK receptor development in the gut, supports a general postulate that gut CCK systems develop early, and undergo maturational changes in the first two weeks of life, while brain CCK systems develop postnatally. In the present experiments CCK binding was not detected in the pancreas until 10 days of age. This observation requires confirmation, but it can be compared with physiological studies demonstrating a lack of responsiveness to CCK in neonatal pancreatic acini in vitro until 12 hours of age; a linear increase of receptors was noted over the next 72 hours followed by a plateau and a further increase to maximum levels which were attained at 21 days post natally (Werlin et al, 1987). The other structure in the rat that has been shown to bear CCK receptors is the vagus nerve (Zarbin et al, 1981). The vagus was not examined in the present experiments, and given the small size of the animals, it would be technically very difficult to study in the newborn rat. Apart from the implications of the early development of upper GI receptor systems for gastric function and feeding, their appearance in the foetus suggests a possible morphogenetic role. Cholecystokinin has been shown, in the adult, to exert trophic effects on pancreas (Logsdon, 1986) and stomach (Balas et al, 1985), and might, at the sites demonstrated, act to influence gastric morphogenesis. This postulate could be studied by injecting CCK and specific CCK antagonists such as L364,718 (now known as MK 329) (Lotti et al, 1987) at different times during
gestation and to observe the effect on upper GI development. A further area of possible relevance is in the development of CCK receptors in gastric neoplasms. The ontogeny of CCK binding in the human stomach has not been studied. However, CCK receptors have been demonstrated on gastric leiomyomata and leiomyosarcomata (Miller, 1984) and their appearance might represent the expression of a receptor previously only present during early development. This makes a number of assumptions that can only be tested by appropriate studies. Human stomachs at different stages of gestation and early development require to be examined for the presence of CCK binding, and the sites of gastric tumours manifesting CCK binding related to the anatomical sites bearing CCK receptors during development.

Ontogeny of ingestive responses to CCK in the rat

The second objective in the experimental work on CCK was to establish whether ingestive behaviour and gastric emptying could be influenced by the peptide. Using the independent ingestion of milk by rat pups in an environment in which they could ingest and exhibit other behaviours while apart from the dam, as pioneered by Hall and Bryan, (1980) it was shown that the pups did consume substantial quantities of milk, and that this consumption could be inhibited by CCK the day after birth, in a dose-dependent manner. The specificity of the response was established in a number of ways. The inhibition of ingestion was accompanied by a specific reduction in observed ingestive responses, but not in non-ingestive behaviours. Secondly, the response was
not observed after large doses of desulphated CCK, indicating that the response was probably mediated by CCK-A receptors. Lastly, no inhibition of milk intake was observed after a hypertonic saline stimulus leading to increased drinking due to dehydration. Thus, when the stimulus to ingestion was one of thirst, no effect of CCK was observed. When the stimulus to ingestion was produced by deprivation, an inhibitory effect of CCK was seen. A decrease in the sensitivity of the pups to CCK was noted from day 1 to day 10, with the relatively low dose of 0.1mcg/kg being effective at the earlier age, and only the highest dose used, 8μg/kg, inhibiting the ingestion of 10 day pups. It is proposed here that this change in sensitivity may be linked to the changes in gastric CCK receptors observed during the autoradiographic study. In support of this suggestion is the finding that CCK inhibits gastric emptying at ages 1 and 3 days. A number of alternative explanations for the results are admissible, and could be tested using suitably designed experiments. The gastric effects of CCK might be unrelated to the effects on ingestive behaviour. A fuller exploration of the inhibition of gastric emptying by CCK at different ages might reveal that the age-related changes in ingestive responses followed a different pattern to changes in gastric inhibition. Gastric distension in the one or three day pup could be prevented by means of a gastric fistula which would allow stomach contents to escape (Phifer et al, 1986). If an inhibitory effect of CCK at the same doses
could be demonstrated in such a preparation, the gastric distension theory for the action of CCK in independent ingestion would be severely challenged. CCK might be acting, in the neonate, at extragastric sites. Brain CCK receptors, although at lower concentrations in the newborn than in adults, are, nevertheless, measurable at around 10% of adult values, and CCK could be exerting an inhibitory effect on feeding by acting at a localised receptor collection, for example in the ventromedial hypothalamus, where, in the adult rat, high concentrations of CCK receptors have been described (Day et al 1986). A detailed autoradiographic study of the neonatal brain is needed, with characterization of receptors as "central" (B) or "peripheral" (A) type (Moran et al, 1986). The present studies, in which desulphated CCK showed no inhibitory activity on ingestion suggests that the response is mediated by CCK-A receptors. The pancreas did not manifest CCK binding until 10 days of age, and would therefore seem to be an unlikely site for the ingestive effects. The vagus nerve also contains CCK receptors in the adult rat (Zarbin, et al, 1981) and they are of the CCK-A type (Moran et al, 1987). No developmental study of vagal CCK receptors in the rat has been performed, and, as already noted, such a study would be technically difficult. The vagus is a possible site of action for CCK satiety (Smith et al, 1981) and CCK inhibition of gastric emptying (Raybould et al 1987) in the adult rat, and a knowledge of the role of the vagus in the reduction of independent
ingestion in the neonate would contribute significantly to understanding of the action of CCK on neonatal independent ingestion.

The neonatal rat was chosen because of its potential as a model of peripherally mediated CCK effects. As we have seen, however, CCK receptors are not confined to the periphery in this animal, and further studies are required to establish the precise locations and roles of other populations of CCK receptors. It is also important to consider how far (if at all) results obtained in this animal model can be extrapolated to the adult rat.

Independent ingestion as a precursor of adult feeding

Three types of feeding behaviour have been extensively studied in the neonatal rat: suckling, ingestion during intra-oral infusions and, as in the present experiments, independent ingestion. It has been argued (Hall, 1985) that suckling has little in common with adult feeding other than the resulting ingestion of nutrient. During suckling, intimate social intercourse takes place between the pup and its mother, milk delivery is intermittent, during "let downs" and this, as well as the pup's response, recognized behaviourally as the "stretch" response, during these brief periods of milk ejection, determine intake. Stretch responses and behavioural activation occur when milk is delivered via a cannula ending inside the back of a rat pup's mouth, and, using this procedure combined with attachment to the nipple, it can be shown that, given unlimited supplies of milk, rat pups 5 or 10 days old will
consume over 10% of body weight, only interrupting consumption when gross gastric distension has occurred and milk has generally refluxed into the nares (Hall and Rosenblatt, 1977). When the milk supply is intermittent, for example during normal suckling, gastric fill does appear to act to inhibit intake (Houpt and Houpt, 1975). Therefore, the special social interactions, the intermittent nature of the delivery of food, the specific behavioural response to suckling and the difficulty inhibiting intake when milk supply is unlimited distinguish suckling behaviour sharply from adult feeding. Independent ingestion occurs when pups are placed in contact with a nutrient in an incubator. Although it presumably occurs seldom if at all during the normal development of a suckling rat, as nutritional needs are met by the dam, independent ingestion has a number of features in common with adult feeding (Hall and Bryan, 1980) 1. The motor responses observed during ingestion (licking, mouthing and lapping) resemble adult oral behaviours during feeding, 2. Intake increases with length of deprivation, in contrast to rats obtaining milk by suckling, in which increasing deprivation does not proportionately increase intake (Houpt and Houpt, 1975) 3. During a test session ingestion terminates with a phase of increasing periods between episodes of oral behaviour, shorter bouts of ingestion and decreased activity. This pattern has been described, in adult rats, as the "behavioural sequence of satiety" (Antin et al, 1975). There is substantial evidence, therefore,
that independent ingestion represents, in the neonatal rat, the premature expression of a complex of behaviours that are normally exhibited only during weaning, at 15 to 20 days of age, and that this form of ingestion has more in common with adult feeding than does suckling behaviour. It has been demonstrated that, in the six day pup fed via a tongue cannula, termination of milk intake is determined entirely by gastric fill (Phifer et al., 1986). Hypoglycaemia does not influence intake until day 20 (Houpt and Epstein, 1973) so that the neonatal rat, ingesting independently, is manifesting a behaviour closely related to adult feeding, but occurring before other influences on feeding, such as that of blood glucose, have developed.

**Mechanism of action of CCK-induced inhibition of ingestion Aversion?**

Cholecystokinin might be reducing intake in neonatal rats by producing an aversive response, a mechanism that has been proposed for the action of CCK in reducing adult feeding (Deutsch, 1983). Evidence presented here demonstrates that the inhibition of feeding by CCK in neonatal rats is accompanied by a specific reduction in oral, but not general motor activity. Secondly, CCK inhibited ingestion which followed a deprivation but not a dehydration stimulus. Neither of these results would be expected if CCK were inhibiting ingestion by inducing an aversive response.

**Inhibition of gastric emptying?**

The response of the neonatal rat to exogenous CCK during
independent ingestion may, therefore, improve understanding of the response to CCK in the adult. Because of its greater simplicity, ingestive behaviour in the neonate provides an opportunity to isolate specific components of the satiety response, perhaps with relevance to adult feeding. The system upon which CCK could act in the adult, that is, the complex of loci which have been shown to bear CCK-A receptors, is extensive, and the hypotheses raised to explain CCK satiety are correspondingly numerous. Pyloric CCK-A receptors (Smith et al., 1984, Moran et al., 1985) could act by mediating the effect of CCK on gastric emptying, thereby facilitating gastric distension and accelerating the onset of satiation during feeding. The evidence suggests that this occurs when CCK is administered to rhesus monkeys during feeding (Moran and McHugh, 1982) and the present developmental work indicates that a similar mechanism could be operating soon after birth. CCK-A receptors have been located on vagal terminals (Zarbin, et al., 1981, Moran et al., 1987) and, as selective afferent gastric vagotomy completely eliminates the inhibitory effect of CCK on feeding (Smith et al., 1981), these receptors might be directly stimulated by circulating CCK to carry a satiety message to the brain. The "gastric emptying" theorists counter that the importance of the vagus is in carrying the sensory gastric distension signal to the brain after it has been provoked by premature gastric distension due to slow gastric emptying.

A serious problem for the gastric hypothesis is the
finding that "sham feeding", during which food is ingested but, in one model, drains from a gastric fistula onto the cage floor, is also inhibited by CCK (Gibbs et al, 1973a). Higher doses of CCK are required to inhibit sham feeding than reduce "normal" feeding, but, as the gastric fistula removes, not only the gastric distension signal, but all the post-absorptive events that could influence feeding, it is predictable that sham feeding is more resistant to inhibition than normal feeding. The possibility is therefore raised that CCK might be acting at more than one site to influence feeding: the pylorus to delay gastric emptying, and the vagus, directly, to explain the sham feeding effects. According to this proposal, the vagus would still be necessary for mediation of the effect of CCK in both types of test. Vagotomy, which, blocks the effect of CCK on normal feeding, was found not to influence the effect of CCK on sham feeding (Kraly, 1984). This observation accords with the theory that CCK acts at multiple sites to reduce food intake, including at least one to inhibit normal feeding and one to reduce sham feeding.

Central action of CCK

CCK receptors have been located in the brain, and good evidence in the sheep (Della Fera and Baile, 1979) and much weaker evidence in the rat (Willis et al 1986) suggests that a central action is an important component of the CCK effect, although peripherally injected CCK, in the sheep,
appears to inhibit feeding by acting outside the CNS (Grovum, 1982). Two problems beset a central theory for the action of CCK administered peripherally (the problem of whether physiologically released CCK could modulate feeding will be addressed presently): CCK would not have access to central sites of action (Passaro et al, 1982) and central CCK receptors have the wrong specificity for the inhibition of feeding. Central (Type B) CCK receptors distinguish rather poorly between sulphated (s-) and desulphated (d-) CCK (Innis and Snyder, 1980), while d-CCK is inactive in feeding tests (Gibbs et al, 1973a, this study). Peripheral CCK receptors, (Type A) in pancreas, pyloric sphincter and vagus, distinguish more sharply between the two analogues of CCK (Innis and Snyder, 1980, Moran et al, 1985, 1987) and are therefore better candidates for the feeding inhibitory action of CCK. However, as already noted, CCK-A receptors do exist at specific loci in the rat brain, (Moran et al, 1986), and one or more of these loci could form part of the mediating system for CCK satiety. The area postrema is of particular interest as it has a poorly developed blood-brain barrier, the receptor sites there could be accessible from the peripheral circulation and it has been suggested (van der Kooy, 1984) that this structure may be important in mediating the effects of CCK on feeding, although contrary evidence has also been obtained (Edwards et al, 1986).

Multiple sites of action

There are, therefore, at least three sites at which CCK
could act on feeding directly from the peripheral circulation: the pylorus, the vagus and the area postrema. It is also possible that the same chemical modulator acts independently at different levels of a unitary feeding control system. Thus, systemically administered CCK may be acting at the peripheral sites mentioned, while centrally injected CCK may be acting at different structures, which have been shown to bear CCK-A receptors, such as the nucleus tractus solitarius. This theory of multiple sites of action both within and outside the nervous system seems to be required by the large amount of experimental evidence indicating the importance of different structures (not exhaustively reviewed here) in the satiety action of CCK.

**Physiological relevance of CCK satiety**

Determining how exogenously administered CCK acts does not provide information on whether its action is relevant physiologically. Blood levels of CCK in man are below the levels required to influence feeding (Brugge and Praissman, 1984), although the gastric inhibitory action of CCK have been regarded as of physiological relevance by some authors (Debas et al, 1975). Because of the complexity of the actions of CCK, measurement of blood levels might not give enough information to establish its physiological role, just as peripheral blood levels of hormones trophic to anterior pituitary cells would not reveal their key modulatory roles. The use of drugs which specifically block the action of CCK in the periphery and in the brain.
have added considerably to the evidence in favour of a physiological role for CCK in feeding. Proglumide, an early CCK antagonist, was found to accelerate gastric emptying (Shillabeer and Davison, 1987) and increase feeding in rats in one (Shillabeer and Davison, 1984) out of two (Schneider et al, 1986) studies. A more specific and potent antagonist, L364,718 (MK 329), was found to increase food intake in the rat (Hewson et al, 1988), and to accelerate gastric emptying in the rat (Dektor, et al, 1988) but not in dogs (Pendleton et al 1987). At present, it is not possible to state that CCK, released from the small intestine, or centrally, has a proven physiological role in the control of food intake. If intestinally released CCK were acting via the general circulation, injection of CCK into the portal venous system would be expected to be a particularly potent intervention in feeding. In fact, hepatic portal injections of CCK do not influence feeding in adult rats (Greenberg et al, 1987). This important finding raises a number of questions. For example, do intraperitoneal injections of CCK act after absorption into the portal circulation (apparently, according to the latter study, not) or locally on vagal or pyloric receptors in the upper abdominal cavity. Where, then, do intravenous injections of CCK act? Lastly, if CCK released after a meal is important physiologically in controlling the size of the meal, how does it get to the relevant receptors? Not, apparently via the portal system. It is conceivable that CCK is transported to pyloric and
vagal receptors by an, as yet unidentified, local transport system, perhaps vascular, by analogy with the hypothalamic-pituitary portal system.

3. GASTRIC EMPTYING AND GASTRIC PERCEPTIVITY IN EATING DISORDERS

The most important determinant of delayed gastric emptying in anorexia nervosa was nutrition. Patients who were underweight as a result of either consuming little or vomiting their meals were likely to have delayed gastric emptying of either the solid meal, the glucose solution or both. This group of patients who are suffering from both long term starvation (low weight) and short term starvation (poor recent intake, or vomiting of most of the recent intake) require further and specific study. The present results need to be replicated using larger groups homogeneous for variables such as bulimia and vomiting. From the present results, it would be predicted that patients with anorexia nervosa who maintained a low weight by pure dietary restriction would show delayed gastric emptying, as would patients who kept their weight low, in spite of episodes of bulimia, by vomiting all or nearly all they had eaten. These patients would show delayed gastric emptying only during periods of food restriction or severe vomiting: when being treated effectively by an expert medical and nursing team, gastric emptying would be normal. Glucose emptying was found to be slower in the patients taking laxatives in an attempt to control weight. These
patients were not identified as a separate group in the study and they come from all patient groups at various weights and stages of treatment. In further studies, homogeneous groups of patients on laxatives for this purpose, particularly those taking large doses, require to be investigated separately, in order to check this finding.

A potentially fatal complication of refeeding in anorexia nervosa is acute gastric dilatation, (Russell, 1966) and it is reasonable to hypothesize that this disorder may develop as a consequence of excessively rapid refeeding in a patient with delayed gastric emptying. If this is the case, then a knowledge of how rapidly delayed emptying recovers during refeeding, and of the dietary programs that are most likely to lead to rapid recovery, would be of great potential benefit to patients. In order to study this, a group of patients with known delayed emptying would be tested at frequent intervals during standard dietary treatment. From the study reported here, it appeared that a period of two weeks was sufficient to produce recovery. However, in this study, the patients on the self-selection diet and those on the inpatient refeeding diet were different groups. Further studies would observe the effect of refeeding on patients initially selecting their own diet, but being retested at intervals. When the duration of the gastric disorder after the beginning of refeeding treatment has been determined, the nutritional components that preferentially improve gastric function could be
isolated by feeding patients for a week with, for example, a liquid diet, and comparing it with a solid diet. In this way, the most therapeutic dietary regime for a patient with delayed gastric emptying due to anorexia nervosa could be determined. It would also be useful to know how quickly delayed emptying reoccurred after discharge, if patients reduced their intake. This could be determined by measuring gastric emptying in patients who have been discharged from the intensive feeding regimen and are found to be reducing their food intake at different times following the withdrawal of active nursing observation.

If delayed gastric emptying in anorexia nervosa results in prolonged post-prandial satiation and an exacerbation of the eating disorder, then a pharmacological approach which accelerated gastric emptying might be of therapeutic benefit. A number of drugs accelerate gastric emptying by different mechanisms, and some have been given to patients with anorexia nervosa, including metoclopramide (McCallum et al, 1985), bethanechol (Dubois et al, 1980), domperidone (Russell et al, 1983) and cisapride (Stacher et al, 1985), sometimes with apparent benefit (Russell et al, 1983). A more logical approach, however, is to determine the pathogenesis of the clinical condition and then treat it appropriately, depending on which physiological system shows the major disturbance.
Investigation of the pathogenesis of delayed gastric emptying in anorexia nervosa

The development of an animal model of delayed gastric emptying of dietary origin is of great importance in the establishment of its pathogenesis. The rat in which access to diet is restricted to 2 hours per day develops delayed gastric emptying and puts on weight more slowly than free-feeding controls. The time course of the development of delayed emptying requires to be established but it appears that this animal reproduces some of the characteristics of the gastric disturbance found in restricting anorexia nervosa. Preliminary investigations should be performed in this animal model, and pharmacological approaches developed. Appropriate medication could then be tested in patients.

The control of gastric emptying is complex, and a large number of experiments will need to be performed. Liquid emptying is reportedly dependent on the proximal stomach for control, while solid emptying requires the activity of the antro-pyloric region (Kelly, 1980). Some modification to this traditional teaching may be necessary, as pyloric activity has been shown to exert important influences on liquid emptying (Schulze-Delrieu, 1985). Since, in both human and animal experiments, gastric emptying rates of both solid and liquid meals were delayed, both functions of the stomach appear to be disturbed... This was suggested in a study of patients with anorexia nervosa (Abell et al, 1987) showing abnormal gastric electrical activity and disturbed antral contractions at the onset of treatment.
The numerous factors that influence gastric emptying and which might be disturbed in anorexia nervosa include autonomic function, both adrenergic and cholinergic, 5 hydroxy tryptamine, histamine, dopamine, peptides and prostaglandins. In the study referred to above, neurotensin and noradrenaline secretion were both found to be abnormally reduced, and autonomic underactivity was noted. However, beta-endorphin, insulin, glucagon, gastric inhibitory polypeptide, gastrin, pancreatic polypeptide and CCK levels were found to be normal. In view of the results obtained in the present studies, the different functions will need to be investigated both during acute food deprivation and during refeeding treatment in patients, and in animals under different dietary conditions. Where possible, specific antagonists should be used to characterize pharmacologically the physiological disturbance, using, in the rat, the influence of agonist and antagonist on gastric function and feeding in vivo and on the activity of muscle strips in vitro.

**Biological significance of delayed gastric emptying induced by dietary restriction**

Although delayed emptying was observed in starving patients with anorexia nervosa, it is not clear whether other malnourished subjects would manifest a similar disturbance of gastric function. The observations in the
rat suggest that delayed gastric emptying is likely to occur as long as the intake of food is sufficiently restricted, and the classic studies by Keys et al (1950) showed that, in otherwise healthy men undergoing semistarvation, a delay in the gastric emptying of a barium meal can be demonstrated. McCallum tested a group of patients with weight loss due to Crohn's disease and found normal gastric function. In this latter study details of dietary intake were not supplied. However, the prediction from the results obtained in the present studies would be that the patients were consuming adequate amounts of food and that their weight loss was related to other aspects of the disease such as malabsorption. A study of patients with physical conditions associated with weight loss, such as thyrotoxicosis, cancer and major abdominal surgery would be of interest for a number of reasons. If patients with very poor oral intake and loss of weight were found to show delayed gastric emptying, then the general application of the present results would be supported. Such a finding would also suggest the possibility that patients with weight loss and delayed gastric emptying might suffer abnormally increased feelings of satiation which could compromise nutritional rehabilitation by reducing meal size and frequency. It would also be contributory to carry out, in normal subjects, a study analogous to that already performed in rats, by measuring gastric emptying after a period during which food intake is restricted to, say, two hours per day.
Aside from the significance of gastric inhibition in the rehabilitation of patients with eating disorders and other wasting conditions, the disturbance may have more general biological implications. It may reasonably be asked what survival value exists in a mechanism which, during periods of starvation, results in increased satiety and, perhaps, a reduced motivation to seek out food. Food supply for the laboratory rat is unlike that of its wild counterparts because, in the cage, food is continually present, while in the wild, long periods may pass during which no food is available to the animal. The very slow gastric emptying observed in the rats fed for 2 hours per day might be, therefore, more typical of gastric function in the wild. It is not known whether slow gastric emptying is associated with an increase in the nutrient extracted from a given meal, although it would not be difficult to test this, using radiolabelled nutrient. A second biological function for the physiological change demonstrated is the enhancement of the role of the stomach as reservoir. Food in the stomach is available to be completely utilized. When absorbed, although fat and carbohydrate can be stored, protein stores are limited, and excess protein is excreted as urea. Food in the stomach is protected against this wasteful process, and slow gastric emptying might thereby serve as an energy conservation mechanism during times of limited food supply - most of the time, in the wild, and in many human societies. Lastly, prolonged satiation would tend to prevent an organism from engaging in food-seeking.
behaviour during times of intermittent supply. Gastric function may therefore be "tuned" to the rate of food supply, and this mechanism would also prevent wastage of energy during fruitless food-seeking behaviour. This postulate could also be tested readily by varying intermeal interval and observing the effect on gastric function. The present theory would predict a mathematical relationship between rate of gastric emptying and rate of food supply. Although simple delay in gastric emptying is the most likely alteration in dietary restriction, it is also possible that confining intake to one part of the day could lead to a slowing of emptying after eating, with an increase in the rate of emptying in the hours before a meal, in other words the development of a circadian pattern of gastric emptying rate. This suggestion could be tested by measuring emptying at different times during the 24 hours.

**Gastric delay and disturbed interoception in eating disorders**

It is clear, from the results presented, that patients with eating disorders report experiences in association with eating that differ markedly from those of normal subjects. The questionnaires used to assess hunger and responses have been used in one previous study (Garfinkel, 1974), and results obtained here are in broad agreement with those of that study. Patients with anorexia nervosa respond no differently from controls when tested before the
meal, but, after eating, report more unpleasant epigastric sensations and more dysphoria than control subjects. Visual analogue scales have been used frequently to assess hunger, satiety and mood states (Stevens, 1966, Silverstone et al, 1978, Hill et al, 1984, Rolls, et al, 1985). In the present studies, elevated scores in patients with anorexia nervosa, on the scales intended to measure satiety (fullness) and psychiatric state (sadness and tension) confirm the results obtained using the satiety questionnaire, in that patients with anorexia nervosa reported more fullness and dysphoria. In addition, lower hunger scores on the VAS, a result which reflected, in some patients, complete denial of hunger or urge to eat (ie, zero scores throughout), extend the range of disturbance. Denial of hunger has been reported in female patients with morbid obesity (Stunkard & Koch 1964), and the present findings indicate a similar disturbance in anorexia nervosa. High and prolonged scores of gastric fullness would be expected if gastric emptying were delayed, as it was in a number of the patients. However, the scores did not vary with gastric emptying rates, so that a high score on the fullness scale was often associated with a small amount of food remaining in the stomach. The finding that patients consistently rated themselves as fuller than controls, whatever the actual gastric contents, suggests a general overestimation of stomach contents in anorexia nervosa, analogous to the over-estimation of body image in this disorder (Slade, 1985).
Ratings of hunger and satiety in patients of normal or high weight with bulimia nervosa did not differ from those of controls. This finding is in contrast to the results obtained during the studies with fenfluramine in which hunger ratings often rose after eating when placebo had been taken, and, on average, did not show the "satiety" response observed in controls. The difference may have been in the presentation of the meal. In the fenfluramine study, the amount of food was in excess and the type was selected to be the type of food that would be most likely eaten during a bulimic episode. The occasion therefore bore similarities to a bulimic episode. In contrast, the meal provided during the gastric emptying studies was low in calories, and therefore relatively "safe" for a patient with bulimia nervosa, and was limited in size to a small breakfast. The latter meal presumably resulted in less anxiety, although the measurements required to test this hypothesis were not made in a comparable way in the two studies.

In an attempt to measure gastric perceptivity, the proportion of the radioactivity remaining in the stomach at each time measured was correlated with the VAS rating at that time. The validity of this measure is open to question and investigation. Reasonably high correlations (about 0.6) were found between gastric counts and normal eating scales in controls. If the subjects were responding to another variable, such as time, or blood glucose, then altering gastric emptying rate with, say, metoclopramide or
secretin would not change an individual's pattern of scores with relation to time, (or blood glucose) although the association with gastric emptying would change. This approach should be used in order to establish whether the VAS ratings of fullness or hunger actually reflect gastric contents or some other variable. Gastric fullness is not the only stimulus that might be sensed as a satiety signal arising in the stomach. Gastric pressure, which could be measured barometrically, may be an important stimulus. A feasible test of this hypothesis would be for control subjects to swallow a gastric balloon, to vary inflation pressure and volume, and to ask subjects to rate their gastric sensations, using VASs. Cannon, in 1912 (Cannon and Washburn, 1912) suggested that gastric hunger contractions were important signals for satiety, and Stunkard and Koch (1964) and Griggs et al (1964) appeared to demonstrate that perception of gastric contractions was significantly poorer in obese subjects compared to controls. This work was, however criticised, (Penick et al, 1967), and the subsequently published view of Stunkard and colleagues (Stunkard and Fox, 1971) was that gastric contractions exert only a weak effect on hunger. In one previous report (Russell et al, 1983) visual analogue scales were used in association with gastric scanning, and it is suggested that ratings of "bloating" were related to delayed gastric emptying in the patient described. No previous study has, however, addressed the problem of gastric perceptivity in eating disorders using gastric
scintigraphy. Two classes of disturbance were found: patients with anorexia nervosa had poor correlations between ratings of hunger or urge to eat and gastric counts, and those with either anorexia nervosa or bulimia nervosa had abnormally high correlations between gastric counts and scales describing symptoms. This phenomenon, here called paraceptivity, in which patients rate a physiological function closely with a symptom, may be an important mechanism by which physiological signals are mislabelled as symptoms. In the management of a patient with an eating disorder, understanding of disturbed gastric emptying and gastric perceptivity could contribute to psychotherapy and monitoring of interoception, perhaps following the commencement of dietary changes intended to correct delayed gastric emptying, might form part of a behavioural approach to anorexia nervosa. Studies repeated within individuals, at different phases of treatment, might provide evidence of improved interoception, which could be associated with better control over food intake. In bulimia nervosa, disturbed interoception was confined to marked "paraceptivity". These patients appeared to be well aware of their stomach contents, to rate them normally in terms of hunger and satiety, but frequently linked stomach contents to symptoms: patients often felt sadder or fatter depending on how much food remained in their stomachs. Bruch (1966) posited, anorexia nervosa, a "disturbance in the accuracy of perception or cognitive interpretation of stimuli arising within the body". These results imply
that, for patients with anorexia nervosa and bulimia nervosa, cognitive interpretation of visceral stimuli is, indeed, grossly disturbed. Of the sequence of physiological events that constitute the supply side of energy homeostasis, gastric distension is a traditional and well studied component. From Cannon's observations (Cannon and Washburn, 1912) stimulating the view that gastric function provided an essential contribution to control of human feeding, through the studies of Richter (1943) demonstrating the ability of an animal to monitor and regulate caloric intake to the experiments of Hunt, McHugh and Moran, showing the regulatory capabilities of the upper gastrointestinal tract, the place of the control of gastric emptying, has been established in the physiology of the regulation of feeding. The studies described here have addressed questions within the realms of pharmacology, developmental biology, pathophysiology and psychopathology from the viewpoint of the stomach, using methods and approaches which spring directly from these and other classic studies.
CHAPTER 8
CONCLUSIONS

In the series of studies described in this thesis, a wide variety of methodologies has been employed, ranging from receptor autoradiography to observations of psychological reactions during meals. The common factor in all these diverse studies has been the hypothesis that slowing of gastric emptying is an important mediating physiological change in the reduction of food intake observed in a variety of circumstances. The different settings in which reduced food intake was observed included both studies in human subjects, (controls and patients with eating disorders) and animal experiments.

dl-fenfluramine

It was found, first, that dl-fenfluramine reduced food intake in patients with bulimia nervosa. It was postulated that this reduction in feeding and bulimic symptoms might be mediated by slowing of gastric emptying by the drug, a phenomenon that had already been reported in normal human controls (Horowitz et al, 1985). In planning the second set of experiments, in the rhesus monkey, it was the intention to study the interaction of the feeding with the gastric inhibitory actions of fenfluramine, in order to test the hypothesis that feeding inhibition was a consequence of gastric inhibition, not merely an additional action of the drug with no significant effect on food
intake. The results in the monkeys showed clearly, first, that glucose and saline emptying were strongly inhibited by fenfluramine, and secondly, that the feeding effects of fenfluramine on sucrose ingestion were closely correlated with the gastric inhibitory effects. This was not conclusive proof of a causal relationship, but was certainly compatible with such an explanation. In terms of the overall hypothesis linking gastric inhibition with feeding inhibition, these findings were in support. In the course of the fenfluramine study in bulimia nervosa, an unexpected observation was a post-ingestive increase in reported hunger in these patients, the first time, as far as this author is aware, that such an abnormality has been described in bulimic patients. This finding was taken up in subsequent studies in which gastric emptying was measured and compared with contemporaneous measures of hunger and satiety.

Gastric emptying in eating disorders
Having explored the relationship between feeding and gastric function after administration of an anorectic agent, it was decided to study patients with eating disorders, a group in which disturbed gastric emptying had already been established (Holt et al 1981, Dubois et al, 1979). This change in physiology could be linked firmly to a prominent symptom observed in patients with anorexia nervosa, namely prolonged feelings of excessive stomach fullness after meals. The pathogenesis of the disturbance
was, however, unknown. It was postulated that, in anorexia nervosa, delayed gastric emptying would be confirmed and that it would be found to vary with another characteristic such as body weight, diet or the presence of bulimic symptoms. The findings and conclusions were clear: gastric emptying was indeed delayed, but only in patients who were acutely starving. Of the other factors only abuse of laxatives, which was associated with delayed gastric emptying of glucose, could be posited as a potential determinant of delayed gastric emptying in anorexia nervosa. The major determinant was, however, current nutritional intake and the importance of this factor accords with observations in volunteers on extremely restrictive diets, made during the second world war, in the classic studies of Keys et al (1950) who noted delayed emptying of a barium meal in these subjects. It was also noteworthy, in the studies described in Chapter 5, that delayed gastric emptying was not confined to patients who lost weight by pure starvation: those who overate but vomited sufficiently to produce significant weight loss, also had delayed emptying. As a result of these findings, it can be stated that delayed gastric emptying occurs in eating disorders in the context of severe starvation, and that intensive refeeding treatment rapidly reverses the disordered gastric function. However, the detailed pathogenesis of the delayed emptying was not determined. In order to study the central and peripheral mechanisms that might be relevant to the disturbance, it was decided
to try and develop a model of delayed gastric emptying in the laboratory rat. This attempt was rapidly successful. Delayed emptying was produced in the rat after a few weeks of a restricted diet. This animal model will prove useful in studying both the determinants and behavioural consequences of delayed gastric emptying. In view of the effect of fenfluramine on gastric emptying observed in Chapter 3, the study of transmitters such as serotonin will be especially relevant. While it was predicted that in anorexia nervosa delayed gastric emptying would be observed, the opposite result was anticipated for bulimia nervosa. The massive overeating of bulimia nervosa can be seen as a failure of normal satiety, and it was reasoned that a marked acceleration of gastric emptying might contribute to this. In the event, gastric emptying was found to be normal in bulimia nervosa, unless weight was very low, indicating concomitant anorexia nervosa, in which case delayed emptying was observed.

**Gastric perceptivity in eating disorders**

The studies on patients with eating disorders are compatible with the hypothesis that, in anorexia nervosa, restriction of food intake is determined, at least in part, by gastric slowing and exaggerated satiety. It was hoped that the results of the psychological measures, the visual analogue scales, would address this hypothesis by providing information on satiety both during starvation, when gastric emptying was slow, and during refeeding, when it was
normal. Support for the hypothesis was not, however, forthcoming because although as would be expected, patients rated themselves as excessively full for a prolonged period after the meal when gastric emptying was slow, they rated themselves as equally full for just as long when gastric emptying was rapid. This is difficult to interpret, but suggests an enduring disorder of satiety, perhaps initiated by delayed gastric emptying, but not remediable by its acceleration during nutritional rehabilitation. The correlations between gastric contents and eating disorder symptoms observed in the course of these studies, which gave rise to a neologism, "paraceptivity", were quite unexpected, but accord with the view that patients with eating disorders have distortions of many aspects of self perception, including body image, internal state and self evaluation.

Cholecystokinin

The studies of gastric perceptivity represented an attempt to provide information on the consequences of delayed gastric emptying in patients with anorexia nervosa. The following studies, on cholecystokinin, were performed to investigate in laboratory animals, the hypothesis that cholecystokinin might be an important mediator in the production of delayed gastric emptying in anorexia nervosa. The animal work can be regarded as preliminary exploration of the physiology of CCK, and, in particular, its possible role in the control of food intake and gastric emptying.
the link between the two and the most important loci of action in producing its effects on these functions. The neonatal rat was chosen because of its lack of CCK receptors in the brain. Their presence was established in the stomach and further studies suggested that CCK effectively suppressed both feeding behaviour and gastric emptying in these animals. These findings are of interest in themselves. However, they provide encouragement to investigate CCK function in anorexia nervosa and in rats on restricted diets, in order to examine the role of CCK in the delayed gastric emptying observed in both patients and diet-restricted rats.

Conclusion
The general approach in this thesis has been to establish a finding in patients, the feeding response to fenfluramine in bulimia nervosa or delayed gastric emptying in anorexia nervosa, then develop an animal model of the finding to elucidate possible mechanisms. The role of delayed gastric emptying in mediating a part of the feeding inhibition associated with fenfluramine administration appears established. CCK function might be disturbed in anorexia nervosa, and studies in the diet-restricted rat will point to physiological processes that should be investigated in order to clarify the disturbances that lead to delayed gastric emptying in these patients. The studies in animals can, of course, only act as pointers to possible mechanisms in man. The physiology of the rhesus monkey is
unique, and the relevant studies require to be performed in human subjects in order to test the hypotheses suggested by the experiments in monkeys. It is even more apparent that investigation of the neonatal rat may tell us little about the physiology of the adult rat, while studies in rats may have little relevance to the situation in normal or clinical human populations. Each finding stands alone as a contribution to the understanding of the physiology of a particular organism. Implications for the functioning of other organisms may only be taken as questions or hypotheses to be addressed by means of specific studies in those populations.
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