ON THE HUMORAL MEDIATION OF THE INTESTINAL PHASE OF GASTRIC SECRETION

Thesis for the Degree

of

Doctor of Medicine

in

The University of Cape Town

by

Ralph Charles Kester

January 1973

To

Ilse

Anthea

Bruce

CONTENTS

"The fact that the peptic glands can be excited at all from the bowel was first observed by myself. In our analysis of the effects of food-stuffs on the stomach it therefore became necessary not only to exclude influences arising in the mouth, but also to study the results independently of events occurring in the intestine."

I.P. PAVLOV (1902)

CHAPTER I

SUMMARY OF THE THESIS

SUMMARY OF THE THESIS

The existence of a stimulatory intestinal phase of gastric acid secretion has been suspected for some time, and recently the importance of this phase has been recognized. The intestinal phase is of particular interest in relation to the profound gastric hypersecretion associated with portacaval anastomosis. The results of many studies in dogs, and recently in man, have demonstrated conclusively that shunt-related gastric hypersecretion is due to unmasking of the intestinal phase by hepatic bypass of a humoral stimulant that is normally inactivated by the liver. Definitive experiments have shown that this humoral agent is a hormone that arises in the jejunum. Elaboration of the hormone is triggered both by the entry of food into the jejunum and by a brief period of jejunal distension with a balloon.

In order to understand the sequence of events in this important stimulatory pathway and to isolate the hormone from portal blood, the time course of hormone release has been determined in shunted dogs. Studies demonstrate that the intestinal phase hormone is released/

released within 30 minutes of the application of the stimulus, and rapidly reaches peak concentration in the portal blood. Intravenous infusion of active portal plasma harvested from a shunted intestinally-fed dog stimulates gastric acid secretion after a delay of approximately one hour, and requires a mean $1\frac{1}{2}$ hours to stimulate peak secretion, which suggests that intermediary steps may be necessary before the hormone can effectively stimulate the parietal cell mass.

Attempts were made to extract the hormone by three simple methods from portal plasma harvested at the optimal time from secreting donor dogs. The acid response of assay dogs with Heidenhain pouches to intravenous infusion of such crude extracts was insignificant and therefore disappointing.

It is revealed for the first time that the pig develops portacaval shunt-related gastric acid hyper-secretion in response to food comparable to that observed in the dog and in man. Porcine jejunal mucosa is thus an appropriate source for isolation of the intestinal phase hormone.

Using/

Using the same principles employed by Gregory and Tracy (1964) for their isolation of gastrin from hog antra, it is demonstrated that pig intestinal mucosa extract contains a heat-stable acidic peptide which is a potent stimulator of gastric acid secretion. Administration of crude intestinal mucosa extract elicits gastric acid secretion after a brief delay, indicating that some intermediate reactions occur before the target organ - the parietal cell mass - is stimulated. It is concluded that the hormone responsible for the intestinal phase of gastric secretion and for portacaval shunt-related gastric hypersecretion is present in pig intestinal mucosa, and can be extracted by a method that selectively isolates heat-stable acidic peptides.

CHAPTER II

INTRODUCTION

INTRODUCTION

Although Pavlov in 1898 was the first to observe stimulation of gastric acid secretion by the intestinal feeding of meat extracts through a duodenal fistula, he failed to mention the intestine in his final statement concerning the factors controlling gastric secretion. In 1925, Ivy, Lim, and McCarthy were the first to demonstrate clearly that a denervated gastric pouch secreted acid when an intestinal meal was given. They made the important proposal that there exists an intestinal phase of gastric secretion. Their proposal was subsequently confirmed by numerous workers (Gregory and Ivy, 1941; Sircus, 1953; Dragstedt, 1959) who obtained indirect evidence to indicate that the intestinal phase is mediated by a humoral factor.

After Lebedinskaja (1933) had observed the occurrence of marked gastric acid hypersecretion following porta-caval anastomosis in normal dogs, the work of many investigators (Dubuque, Mulligan and Neville, 1958; Clarke, Hart and Ozeran, 1958b; Guth, 1962) has served to define and characterize this phenomenon in experimental animals. Furthermore, the gastric hypersecretion seen in shunted animals has been cited as evidence in support of/

of a clinical association between peptic ulcer and portacaval anastomosis in man (Dubuque and others, 1958; Clarke and others, 1958b; Leger, Cachin and Pergola, 1960). Since the acid response is more impressive when the small intestine contains food, the logical suggestion has been made that shunt-associated acid hypersecretion is a manifestation of the unmasking of the intestinal phase of gastric secretion (Brown, Faustina and Orloff, 1967; Orloff, Villar-Valdes, Rosen, Thompson and Chandler, 1969b).

The identity of the intestinal phase stimulus is not fully known. Some have speculated that it may be a gastrin-like material (Ivy and others, 1925; Babkin, 1950), while others have proposed that it is a gastric secretagogue absorbed from food (Gregory and Ivy, 1941; Dragstedt, 1959). Assumptions implicating the role of histamine (Irvine and Code, 1958) have been proved to be unwarranted (Windsor, Thompson and Orloff, 1965; Hubens, 1967; Thompson, 1969a). Studies in which the intestinal mucosa was stimulated by simple balloon distension have shown that the humoral agent is not a secretagogue absorbed from food, but that it is rather a hormone of endogenous origin (Villar-Valdes, Thompson, Rosen,/

Rosen, Chandler and Orloff, 1969; Orloff, Abbott and Rosen, 1970a) that is very likely identical to the agent responsible for the intestinal phase of gastric secretion.

A number of studies have been performed to determine the site of origin of the humoral mediator of the intestinal phase. Experiments involving stimulation of isolated intestinal segments have demonstrated that the hormone arises mainly from the jejunum of both man (Abbott, Rosen and Orloff, 1970) and the dog (Orloff, Villar-Valdes, Abbott, Williams and Rosen, 1970b). Normally, after release from the intestine, the hormone is rapidly degraded by the liver, but when the portal blood bypasses the liver in the presence of a portacaval anastomosis, the hormone is free to stimulate the target organ - the parietal cell mass - to secrete acid significantly.

The present thesis is based on information derived from several concurrent studies concerning the stimulatory intestinal phase hormone. An effort was made to isolate the humoral factor both from the portal blood of the dog and from its site of origin, the intestinal mucosa. The time course of hormone release following application of/

of a stimulus to the jejunum was determined in order to pinpoint the time of maximum hormone concentration in portal blood.

To this end, 58 dogs were subjected to an antrectomy, construction of a Heidenhain pouch, a Roux-en-Y jejuno-cutaneous fistula, a portacaval anastomosis (Eck fistula) and insertion of an indwelling portal vein catheter. After a standard meal had been administered into the jejunal loop, 500 ml. of portal blood was harvested (with isovolaemic replacement) from the donor dogs at various time intervals. The portal blood was centrifuged in the cold, and the plasma was infused over a 15-minute period into an assay dog with a Heidenhain pouch. The 4-hour acid secretory response of the assay dog to the test plasma was compared with its response to control plasma obtained from a fasted normal donor.

The data obtained was crucial to the isolation of a humoral agent from plasma collected at the optimal time from the portal blood of the dog. Such information would also assist in future undertakings to extract the hormone from plasma harvested from the portal vein of cirrhotic patients with portacaval anastomosis.

Preliminary/

Preliminary attempts were now made to extract the hormone in crude form by simple biochemical procedures from portal plasma harvested at the optimal time from secreting donor dogs. Eighteen samples of portal plasma which demonstrated gastric-stimulating activity were treated by three different methods which respectively involved heating, extraction with isopropyl alcohol, and dilution and heating. These crude extracts were tested by intravenous infusion into assay dogs with Heidenhain pouches.

A more promising line of investigation is to isolate the hormone from the intestinal mucosa of the pig, an omnivore already widely used as a source of digestive hormones. Since shunt-related gastric hypersecretion has not been demonstrated previously in the pig, six weanling pigs with Heidenhain pouches, well-conditioned to the laboratory, were given test meals before and after end-to-side portacaval anastomosis. The pouch acid response was measured in thirty-four samples. After the anastomosis, all animals showed a significant increase in the acid secretory response to a meal. It was concluded that the pig, like man, has an intestinal phase of gastric secretion, develops shunt-related acid hypersecretion,/

hypersecretion, and therefore should serve as a suitable species for studies aimed at isolating the hormone responsible for the intestinal phase of gastric acid secretion.

To isolate the hormone from its source, fresh intestinal mucosa was obtained from a local Pacific Coast abbatoir as a slurry squeezed from the entire pig intestine* within several minutes of death. On the reasonable assumption that the hormone is a low-molecular weight peptide, the intestinal mucosa was boiled to precipitate large proteins and peptides, and the supernatant was incubated with diethylaminoethyl (DEAE) cellulose to absorb the active agent. The active material was then removed from the cellulose with sodium hydroxide. Subsequent adjustment of the pH to 4 precipitated a substance which returned to solution at pH 7.4, and was soluble in isopropanol. This material was re-dissolved in saline, sterilized by filtration, and tested by intravenous infusion over 15 minutes into fasting dogs with Heidenhain pouches. The acid secretion of the pouch/

*Because of difficulties with labour protocol, it was impossible to obtain mucosa only from the jejunum.

pouch was determined for a basal hour and for three hours after infusion of the mucosal extracts. Control studies using diluent were done before each test. Twenty-five extracts were tested over a wide dosage range of protein, and a typical dose-response relationship was demonstrated.

The significance of the results of these various studies concerning the humoral mediation of the intestinal phase is discussed, and the role of the intestinal phase hormone in gastric acid secretion is ascertained.

CHAPTER III

HISTORICAL BACKGROUND TO THE INTESTINAL PHASE OF GASTRIC SECRETION

HISTORICAL BACKGROUND TO THE INTESTINAL PHASE OF GASTRIC SECRETION

The Intestinal Stimulus.

The existence of a stimulatory intestinal phase of gastric acid secretion has been recognized for almost a half-century, but, until very recently its role in normal digestion and in gastrointestinal disease has been minimized or ignored. Substantial evidence now confirms the fact that under certain circumstances, the introduction of food into the proximal portion of the small intestine stimulates the parietal cells of the stomach to secrete hydrochloric acid. Although such an observation was first made by Pavlov at the turn of the last century, he paid scant attention to the phenomenon, as did several other early investigators (Chittenden, Mendel and Jackson, 1898; Le Conte, 1900; Sokolov, 1904; Gross, 1906; Lönnquist, 1906).

Preliminary studies by Ivy and McIlvain (1923) demonstrated the excitation of gastric acid secretion by the application of substances (including O.IN hydrochloric acid and 20 per cent ethyl alcohol) to the mucosa of the duodenum and first part of jejunum. It/

It then fell to Ivy and others (1925) to prove beyond doubt the existence of an intestinal phase of gastric secretion. In their classic experiments, dogs with an oesophago-duodenostomy and a denervated total gastric pouch developed profound acid hypersecretion when fed a meat or protein meal. They offered the hypothesis that the gastric response was due to secretagogues absorbed from food into the bloodstream.

Armour and Webster (1932) corroborated the findings of Ivy and his colleagues, while working in Babkin's laboratory. They demonstrated that a variety of agents (e.g. water, meat extracts, products of protein digestion, alcohol, histamine, adrenaline, O.IN hydrochloric acid, magnesium sulphate) when applied to the intestinal mucosa of the dog, induced gastric secretion after a latent period of 1 - 3 hours, whilst application of atropine and fat to the mucosa inhibited the flow of gastric juice. It was also noticed by them, and independently by Holinger, Kelley and Ivy (1932), that dogs whose nutrition were maintained for long periods by pure intestinal feeding, lost all appetite. It was surmised that the gastric secretion observed in their experiments was an expression of a pure intestinal phase.

In/

In 1941, Gregory and Ivy observed that feeding a dog an intestinal meal stimulated acid production from a denervated gastric pouch transplanted to the subcutaneous region. They believed that the intestinal phase was humorally mediated by means of a secretagogue. During the same series of experiments, although they proved that distension of the antrum triggered the release of a hormone (gastrin), no such manouvre was attempted with the small bowel. Babkin, in 1950, although believing that the mechanism whereby the intestine influenced the secretion of gastric juice was through secretagogues, never-the-less kept an open mind, suggesting that "the problem of the occurrence of an 'intestinal gastrin' awaits further investigation."

Previous misconceptions concerning humoral mediation of the intestinal phase by secretagogues were dispelled by Sircus (1953) who stimulated secretion from Heidenhain pouches by the distension of Thiry-Vella small bowel loops. Such gastric acid secretion was abolished if the small bowel mucosa was anaesthetized with procaine prior to distension. He thereby established indirectly that the proximal intestine released a hormone when it was distended. His work was confirmed by others (Nagano, Johnson,/

Johnson, Cobo, Oberhelman and Dragstedt, 1960) who believed that the small bowel stimulatory hormone was controlled by the same factors influencing gastrin secretion.

Augmentation of Intestinal Phase Secretion by Portacaval Shunt.

In the meantime, a series of important discoveries paralleled the above-mentioned events. In 1933, Lebedinskaja, working in Pavlov's laboratory reported that two normal dogs with Eck fistulas (Eck, 1877) developed increased gastric secretion from Pavlov pouches in response to test meals. Since the dogs subsequently developed liver damage, she thought that normal liver function was a prerequisite for normal gastric secretion, and that her findings could explain the increased acid output observed in cirrhotic patients. Her study was confirmed by Gerez and Weiss (1937) who suggested that gastric secretagogues released from the gut were normally partially destroyed by passage through the liver.

Dubuque and others (1958) found that the acid output from canine Heidenhain pouches increased from 21 mEq. per day prior to portacaval shunting, to 63.9 mEq. per day after operation. They noted that portal vein ligation/

ligation caused a similar rise from a control level of 24.9 mEq. daily to 68.3 mEq. daily. Subsequently, numerous investigators have duplicated these observations (Clarke, Ozeran, Hart, Cruze and Crevling, 1958c; Kohatsu, Gwaltney, Nagano and Dragstedt, 1959; Silen and Eiseman, 1959). Thereafter, experiments studying gastric acid hypersecretion following portacaval shunt have been concerned with determining the spectrum of the effect of shunting on gastric secretion, and identifying the agent responsible for hypersecretion.

Although gastric hypersecretion in dogs with a portacaval shunt occurs in the fasting state, it is particularly pronounced following eating (Clarke and others, 1958c; Kohatsu and others, 1959; Silen and Eiseman, 1959; Rex, Code and ReMine, 1964). Hypersecretion occurs despite vagotomy (Kohatsu and others, 1959); antrectomy (Clarke and others, 1958c; Gregory, 1958; Kohatsu and others 1959; Cornish, Silen, Eiseman and Woods, 1960; MacPherson, Miller, Nishikawa, McKissock and Clarke, 1962); total gastrectomy after construction of a Heidenhain pouch (O'Sullivan, Cantlin, Sweeney, Rosteing and Foster, 1960); splenectomy and pancreatectomy (Silen and Eiseman, 1959). Gastric hypersecretion/

hypersecretion is not abolished by jejunectomy or ileectomy (Castaneda, Griffin, Nicoloff, Stone, Leonard and Wangensteen, 1961), colectomy (Silen and Eiseman, 1959) or the administration of oral neomycin (Clarke and others, 1958c).

Studies on the hypersecretion following mesenterico-caval, splenocaval and portacaval shunts have shown acid outputs more than double that of the control values (Hayashi, Rheault, Semb and Nyhus, 1968), but mesenterico-caval anastomoses resulted in the highest, and splenocaval shunts in the lowest degree of hypersecretion. A substance - "pouvoir secrétagogue urinaire (P.S.U.)" - which will stimulate gastric hypersecretion in the rat has been recovered from the urine of patients with portacaval anastomosis only when these are patent (Bonfils, Bader, Leger, Boury, Bernades and Dubrasquet, 1967). This substance is not histamine and it has been shown to have gastrin-like properties.

Substantial indirect evidence has led to the suggestion that the increased acid production associated with portacaval shunting is a manifestation of the unmasking of the intestinal phase of gastric secretion. In support of this proposal are observations that shunting of/

of venous blood from the stomach, proximal duodenum, pancreas and spleen does not produce gastric hypersecretion, while diversion of blood from the jejunum, ileum, or from the colon into the systemic circulation brings about elevated levels of acid output (Clarke, McKissock and Cruze, 1959; Leger and others, 1960; Clarke, Miller and McKissock, 1966). Postshunt gastric hypersecretion can be inhibited by agents which are known to affect the intestinal phase (Cornish and others, 1960), viz. acid perfusion of a duodenal loop; intravenous injection of secretin, phenergan or atropine.

Nature of the Stimulant.

A. The controversy over Histamine.

On the basis of these previous observations, many investigators, commencing with Gerez and Weiss (1937), have postulated that portacaval shunt, by permitting portal blood to bypass the liver, prevents hepatic destruction of a potent secretagogue or hormone absorbed from the intestine (Gregory, 1957; Clarke and others, 1958b; Kohatsu and others, 1959; Silen and Eiseman, 1959). Experimental studies aimed at identifying such a secretagogue have largely focussed on the possibility of the role of histamine. Indirect evidence to support the humoral role/

role of histamine arose in the following manner.

In 1958, Updike, Code and Hallenbeck noticed that the feeding of a meal directly into the jejunum was followed by elevated gastric acid levels, accompanied by an increased output of free urinary histamine. They suggested that histamine liberated into the portal vein may be partially inactivated by the liver. Their suggestion was supported by the observation that the stimulatory effect of histamine on gastric acid secretion was found to decrease greatly when histamine was infused into the portal circulation rather than into the systemic circulation (Irvine, Duthie, Ritchie and Waton, 1959b; Silen and Eiseman, 1959).

Histamine is normally absorbed from the intestine into the portal bloodstream following ingestion of protein (Silen and Eiseman, 1961); the urinary excretion of free histamine after a meat meal is increased in both man (Mitchell and Code, 1954) and the dog (Updike and others, 1958; Irvine and Code, 1958; Irvine, Duthie and Waton, 1959a). Histamine is formed in the gut from L-histidine by bacterial decarboxylation (Ackermann, 1911; Irvine and others, 1959a), and its formation can be reduced by/

by the administration of antibiotics (Wilson, 1954; Irvine and others, 1959a). The liver inactivates histamine by means of histamine methyl transferase, which has been identified in large quantities in the livers of many species (Lindahl, 1960). Both isolated and intact livers of the dog and pig have been observed to remove large histamine loads efficiently (Anrep, Barsoum and Talaat, 1953; Drapanas, Adler, Vang and McMenamy, 1965; Eiseman, Moore and Normell, 1964). Administration of an antihistaminase drug, aminoguanidine, produces a rise in the basal secretion of gastric acid, and potentiates threshold doses of histamine (Irvine and others, 1959b).

Exogenous histamine introduced into the gastrointestinal tract of a laboratory animal and of man does not normally influence gastric secretion but stimulates acid hypersecretion in the presence of a portacaval shunt (Irvine and others, 1959b; Bendett, Fritz and Donaldson, 1963). Silen and Eiseman (1961) observed that the histamine content of postprandial blood taken from the portal vein was greater than in arterial blood. Furthermore they found a progressive increase in peripheral arterial plasma histamine after eating in both intact dogs/

dogs and dogs with portacaval shunts, with greater levels in the latter. However, the differences in histamine elevations after feeding between normal and shunted animals were not statistically significant; in 3 of 8 dogs, there was no change in the histamine response to eating before and after shunt; and there was a poor synchronisation between an increase in the acid secretion from a Heidenhain pouch and the rise in arterial plasma histamine.

Rutherford, Mehlman and Brickman (1966) demonstrated a rise in the postprandial level of arterial plasma histamine after a protein meal in animals with portacaval shunts. In addition, they found that the increased histamine response to eating did not occur in animals with shunting of venous blood from the stomach, duodenum, pancreas and spleen, but did occur when blood from the jejunum and ileum was shunted. Some doubt is cast on the validity of their work, because firstly, gastric secretion was not measured; their results were based on a single sample of arterial blood taken three hours after eating, and they did not record whether the patency of the small and variously constructed shunts had been confirmed.

Despite/

Despite these earlier findings suggesting histamine as the humoral mediator of the intestinal phase of gastric secretion, strong evidence to the contrary began to mount. In the fasting animal, comparison of portal and peripheral blood histamine levels yielded inconsistent results (Anrep and others, 1953; Silen and Eiseman, 1961). Rex and others (1964) fed a meat meal to dogs with and without portacaval transpositions, and then examined the effect on the acid secretion into a Heidenhain pouch and the urinary excretion of free histamine. Although they found a significant increase in acid output during fasting and following eating in dogs with portacaval shunts, no significant difference occurred in histamine excretion in animals studied before and after making the shunt. These authors proposed that an increase in responsiveness of the gastric mucoša rather than increased quantities of circulating histamine was the cause of "shunt-related hypersecretion".

Initial studies in the rat suggested that the effect of portacaval anastomosis was to alter the metabolism of histamine at the site of the end-organ, namely the gastric mucosa. It was shown by Day, Skoryna, Webster and MacLean (1963) and Fischer and Snyder (1965) that the/

the level of histamine was indeed increased in the gastric mucosa of the rat after portacaval anastomosis probably because of an increase in the histamine-synthesizing enzyme, histidine decarboxylase. They were able to suppress the increase in histamine by inhibiting this enzyme with a substance, NSD-1055. The choice of the rat was perhaps unfortunate since this animal has a unique histamine metabolism, and has been described as the "maverick of histamine metabolism" (Code, 1965), so that rat studies on the role of histamine in gastric physiology generally are not applicable to other species, including man.

In sharp contrast to these observations, Newman, Reeder, Davidson, Schneider, Miller and Thompson (1969) found no significant increase in the mucosal histamine concentration in the dog after "shunting". There was no significant difference in the amount of histidine decarboxylase in the gastric mucosa of the dog before or after portacaval transposition, and the administration of NSD-1055 did not influence gastric hypersecretion after portacaval shunt. Using more refined fluorometric techniques for the measurement of histamine, Thompson (1969a) found no difference in the histamine content of portal/

portal and peripheral venous blood after feeding. If histamine were responsible for hypersecretion after "shunting", one would only have expected the effect to be demonstrable after feeding, but in fact the basal rate of acid secretion is also increased (Clarke and others, 1958c; Silen and Eiseman, 1959; Rex and others, 1964; Olbe, 1966; Hubens, 1966 and 1967).

Further evidence against the proposed role of histamine as the intestinal mediator of gastric secretion came from Hubens (1967) who found that the increased gastric secretory response to intestinal stimuli detectable after shunting was blocked by agents that do not influence histamine - stimulated gastric secretion, namely atropine, locally and vagally-applied anaesthetic agents. No increase in blood histamine was detected after a meal in control, cirrhotic and shunted dogs, and there was no correlation between the level of blood histamine and the amount of acid secreted (Windsor and others, 1965).

In addition to the suggestion that histamine may be the intestinal humoral factor, other secretagogues have been proposed, including ammonia, amino acids, proteins or protein derivatives (Clarke and others, 1958c; Silen and Eiseman, 1959; Griffen, Slesh and Mooney, 1969).

B./

B. Hormonal Mediation of the Intestinal Phase.

For the many reasons mentioned above, it is clear that the active agent is not histamine or a secretagogue absorbed from food. It has been proposed that the stimulant is either gastrin or a gastrin-like substance arising from the intestine (Sircus, 1953; Dragstedt, 1957; Castaneda, Griffen, Nicoloff, Leonard and Wangensteen, 1960; Jordan, 1967). There are major arguments against gastrin as the intestinal hormone. Gastrin is almost or completely resistant to inactivation by the liver (Olbe, 1960; Gillespie and Grossman, 1962; Amure and Ginsberg, 1964; Lick, Welsch, Hart, Brückner, Balser and Gürtner, 1967; Thompson, Reeder, Davidson, Charters, Brückner, Lemmi and Miller, 1969). Gastrin is degraded by the kidneys (Maxwell, Moore, Dixon and Stevens, 1971; Hjelmquist, Reeder, Brandt and Thompson, 1972), whereas the portal blood stimulant clearly is very efficiently inactivated by the liver. Moreover, gastrin levels measured by radio-immuno assay after portacaval shunt in dogs have shown a decline rather than a rise (Clendinnen, Reeder, Jackson, Miller and Thompson, 1970).

At this stage it seemed possible that a humoral gastric/

gastric secretagogue is released from the tissues by the intestine into the portal circulation following entrance of food. Brown and others (1967) investigated this possibility by feeding antrectomized dogs with an intestinal meal. The acid secretion from each dog's Heidenhain pouch was measured during isovolaemic anto-transfusion of blood from the portal vein to the thoracic aorta. The combination of an intestinal meal and hepatic bypass of portal blood by portal-systemic autotransufsion produced a significant increase in acid and volume secretion from each denervated gastric pouch.

In a succeeding study, after receiving an intestinal meal, antrectomized donor dogs underwent cross-transfusion of their peripheral venous blood, before and after portacaval anastomosis, into the thoracic aortas of recipient donor dogs (Orloff and others, 1969b). The volume of cross-transfused portal blood was carefully replaced simultaneously with bank blood. Cross-transfusion between a shunted donor given an intestinal meal and an intact recipient resulted in sustained, highly significant increases in gastric output and secretory volume in both donor and recipient animals.

These two studies demonstrated conclusively that a potent humoral agent of intestinal origin mediates shunt-related gastric hypersecretion. To determine the relevance of these experimental findings to man, studies of the gastric acid secretory response to an intestinal meal were conducted in normal humans, patients with alcoholic cirrhosis, and patients with cirrhosis who had undergone portacaval shunts. The entrance of food into the intestine of both normal and cirrhotic subjects produced a small depression of gastric secretion. In contrast, administration of an intestinal meal to cirrhotics with portacaval shunts produced a marked and prolonged outpouring of gastric acid in every patient (Orloff, Chandler, Alderman, Keiter and Rosen, 1969a). Thus it was clear that hepatic bypass of the liver by portal blood unmasks an intestinal phase of gastric secretion in man, just as it does in the dog.

That the humoral agent was not an exogenous secretagogue derived from food, but a hormone of endogenous origin was established by balloon distension experiments in normal subjects and in cirrhotic patients with and without portacaval shunts (Orloff and others, 1970a). Jejunal distension for 20 minutes with a balloon inflated/

inflated to 50 mm. Hg produced a massive and prolonged hypersecretion of gastric acid comparable to that produced by an intestinal meal in the same (or similar) patients.

Site of Origin of the Intestinal Phase Hormone.

Since shunting of portal blood draining the intestine distal to the ligament of Treitz brings about an increase in gastric secretion, several investigators have attempted to pinpoint the site of origin of the humoral agent. Clarke and others (1959) demonstrated that acid secretion from Heidenhain pouches in dogs was increased after superior mesenterico-caval shunting and returned toward normal levels after subsequent portacaval transposition. Systemic shunting of the venous outflow from the stomach, proximal duodenum, pancreas and spleen failed to alter normal acid production. Their findings supporting the view that the mediator of shunt-related gastric hypersecretion arises from the gut distal to the mid part of the duodenum were confirmed by Leger and others (1960).

Silen and Eiseman (1959) found that colectomy did not affect shunt-related gastric hypersecretion in a small series of 5 dogs with portacaval transposition. Castaneda and others (1961) demonstrated that resection of/

of the jejunum or ileum did not eliminate the source of the humoral mediator. However, their findings are open to question since the number of studies were small, their results were not submitted to statistical analysis, and the effect of small bowel resection on gastric secretion (Landor and Baker, 1964; Santillana, Wise, Schuck and Ballinger, 1969) was not recognised at the time of their report.

Clarke and others (1966) observed an increase in the 24-hour acid output both following systemic shunting of blood from the colon and after shunting of blood from the small intestine, and they concluded that the humoral stimulus arose throughout the small and large bowel. However, in 4 of the 6 dogs studied, it appears that the levels of free acid were in fact higher when blood was shunted from the small bowel. In 1968, McPhedran, Bett, Stone and Goldberg reported that aliquots of blood withdrawn from the acutely obstructed portal vein of dogs induced gastric acid secretion when injected into test animals with Heidenhain pouches. In sharp contrast to the findings of previous investigators, only portal blood draining from the stomach and colon had a stimulatory effect, whilst blood from the superior mesenteric vein (draining the small bowel) produced no such response.

In contrast, more recently, Orloff and others (1970b) have examined the gastric secretory responses to the introduction of food into isolated segments of jejunum, ileum and colon, both before and after portacaval shunt in dogs. Portacaval shunt did not greatly enhance the gastric secretory response to the presence of food in the distal half of the small intestine, and food in the proximal three-fourths of the colon failed to produce any response either before or after portacaval shunt. In contrast, in the presence of a portacaval anastomosis, a jejunal meal stimulated massive and highly significant gastric hypersecretion. These results indicated that most, if not all, of the humoral stimulus originates in the jejunum. Comparable experiments were conducted in patients; the gastric secretory responses to the introduction of food into the jejunum and into the ileum were compared in cirrhotic patients with and without portacaval anastomosis (Abbott and others, 1970). As in the dog, it was demonstrated conclusively that the hormone responsible for the intestinal phase of gastric secretion and for portacaval shunt-related acid hypersecretion in humans originates in the jejunum.

Gastric Secretion in Cirrhosis.

The/

The suggestion that there is a causal relationship between gastric and hepatic disease is not a new one (Leva, 1893; Hayem, 1898; Lebedinskaja, 1933). Many studies concerning the influence of cirrhosis on gastric secretion have almost all demonstrated either a depression of acid production, or no change from normal. Although most of these have involved indirect or qualitative determinations of acid secretion, seven quantitative studies have been done in chronic liver disease.

Clarke, Costarella and Ward (1958a) found no significant differences between cirrhotic patients and normal subjects in basal acid secretion, the acid secretory response to a sucrose test meal, and the acid secretory response to an appetizing breakfast. Ostrow, Timmerman and Gray (1960) measured the output of basal acid and basal pepsin, histamine-stimulated acid and pepsin secretion, and the concentration of 24-hour urine pepsinogen and blood pepsinogen in compensated ("healthy") and decompensated ("sick") cirrhotic patients. They reported that all components of the secretion were significantly reduced in both groups, suggesting that chronic illness itself was not the cause of the impaired gastric/

gastric function. Uropepsin and blood pepsinogen values were as low in postnecrotic and biliary cirrhosis as in alcoholic cirrhosis, indicating that alcoholism itself was not responsible for the decreased gastric secretion.

Significant reduction of basal and maximal (post-histamine) acid outputs were recorded in 26 cirrhotic patients (Scobie and Summerskill, 1964). Gastric hypo-secretion was not related to the aetiology or severity of the hepatic disease, nor to the extent of naturally-occurring portal-systemic anastomoses. Tabaqchali and Dawson (1964) demonstrated the normality of gastric secretion in 28 patients with cirrhosis. Schmidt and Martini (1969) reported a reduction in basal acid output in a series of 30 cirrhotic patients who were compared with 13 normal subjects. The mean of the results for histamine-stimulated secretion showed no change from the normal, although the scatter of results was wider.

Baddeley (1968) found no difference in basal secretion between 26 cirrhotics and controls, whereas maximal volume and acid secretion of ascitic cirrhotic patients were significantly impaired when compared with non-ascitic cirrhotics and control patients. A group of investigators from/

from this laboratory (Alderman, Keiter, Chandler, Rosen and Orloff, 1969) revealed that compensated cirrhotic patients have a small but significantly higher basal secretion (mean output of 4.2 m Eq./L) when compared to normal subjects (mean output of 2.5 m Eq./L). The secretory response to histamine was similar in both groups of subjects.

Simulation of Liver Disease in Animal Models.

Because of the clinical association of peptic ulcer with cirrhosis and portacaval anastomosis, experimental models have been designed to reproduce the human situation in animals. Four groups of animal models simulate the human situation:

(1) Production of Liver Damage

(a) Oral Administration of Carbon Tetrachloride. Guth (1962) reported that the increased gastric secretion of dogs rendered cirrhotic with carbon tetrachloride was not affected by subsequent portacaval shunting. Contrary to his findings, Hein, Silen, Skillman and Harper (1963) pointed out that the gastric hypersecretion found in dogs with experimental cirrhosis was augmented up to five-fold after portacaval transposition. In Stelzner's (1965) experiments, liver damage was characterized by hepatic centrilobular necrosis, fibrosis and bile-duct proliferation.

proliferation. There followed such an increased outpouring of gastric acid that all the animals developed anastomotic ulcers within two months.

(b) Hepatic Vein Ligation (Orloff, Baddeley, Nutting, Ross, Halasz and Sloop, 1966) in dogs resulted not only in liver cell necrosis and fibrotic changes, but also in portal hypertension and ascites. In contradistinction to those animals dosed with carbon tetrachloride, these animals developed a diminished output of acid from their Heidenhain pouches.

(c) Combination of Carbon Tetrachloride and Hepatic Venous Ligation (Baddeley and Fejfar, 1968). These authors observed an increased acid output during thecirrhotic phase (with pepsin secretion unchanged), but during the ascitic phase, there was a significant reduction in the production of gastric acid and pepsin, accompanied by changes in the make-up of mucin. They concluded that peptic ulceration in cirrhosis may be due to changes in gastric mucosal resistance rather than acid-pepsin aggression.

(2) Portal Vein Ligation (Dubuque and others, 1968; Gregory, 1958) in dogs with healthy livers soon resulted in the development of portal-systemic shunting. Increased acid secretion was subsequently obtained from a gastric pouch/

pouch, particularly after a test meal. A potent gastric secretagogue was detected in blood taken from the acutely obstructed portal vein, which was neither histamine nor gastrin, and was present in blood from the stomach and colon, but not in blood draining from the small bowel (McPhedran and others, 1968).

(3) Portal Blood Bypassing the Liver:

(a) Eck fistula (Lebedinskaja, 1933; Gerez and Weiss, 1937), or end-to-side configuration.

(b) Portacaval anastomosis, side-to-side (Lee and Fisher, 1961).

(c) Portacaval transposition (Child, Barr, Holswade and Harrison, 1953; Clarke and others, 1958b; Kohatsu and others, 1959). In contrast to dogs with an Eck fistula, portacaval transposition has the advantage of excluding the liver from the portal bloodstream without the production of detectable liver damage. If an arterial blood supply was anastomosed to the hepatic end of the divided portal vein in a shunted dog, thereby improving the oxygenation of the bypassed liver, there appeared a great increase in gastric acid output despite an excellent hepatic blood supply and normal liver function tests (Nicoloff, Doberneck, Leonard, Peter and Wangensteen, 1962). The

latter/

latter investigators concluded that liver dysfunction does not play a role in postshunt hypersecretion.

(4) Biliary Obstruction

Berg and Jobling (1930) and Bollmann and Mann (1932) reported that ligation of the common bile duct in dogs producing complete obstructive jaundice, incurred gastric and duodenal ulcers in most of the animals. Unfortunately, no gastric secretory studies were performed. Silen, Hein, Albo and Harper (1963) noticed that the gastric hypersecretion which developed after ligation of the common bile duct persisted after removal of the obstruction if irreversible liver damage had occurred. Gastric secretion returned to normal levels if hepatic dysfunction was reversible. Such an observation persuaded them to believe that the cirrhotic liver released a secretagogue. Windsor, Fejfar and Woodward (1969) have reported that in patients undergoing massive small bowel resection, there is an association between acute gastric hypersecretion and transient post-operative jaundice in the immediate postoperative period.

Peptic Ulcer Associated with Cirrhosis

The association of peptic ulcer and cirrhosis has been the subject for clinical investigation by many groups of investigators (see Table 1). Clinical radiological and autopsy evidence has been offered to support

an/

TABLE 1: Incidence of Peptic Ulcer in Cirrhotic Patients

First Author		No. of Patients	Peptic Ulcer*			
			No.	Per cent	DU	GU
Schnitker	1934	72	14	19.4	-	-
Ask-Upmark	1939	38	9	24.7	-	-
Ratnoff	1942	386	14	3.6	-	-
Lipp	1952	432	30	6.9	-	-
Dagradi	1955	92	12	13.1	11	1
Swisher	1955	417	58	13.9	51	7
Fainer	1955	94	16	17	-	-
Koide	1958	252	13	5.2	12	1
MacDonald	1958	221	20	9	4	16
Leger	1960	242	25	10.5	16	9
Schriefers	1963	324	15	4.6	-	-
Scobie	1964	26	2	7.7	2	-
Tabaqchali	1964	290	33	11.3	25	8
Schmidt	1969	30	5	16.7	3	2

* DU - Duodenal ulcer

GU - Gastric ulcer

an increased evidence of peptic ulcer in cirrhotic patients. The very diversity of criteria for patient selection and peptic ulcer diagnosis in relation to cirrhosis have so invalidated the arguments presented, that depending upon the references cited, it is possible to conclude that peptic ulcer is either more frequent or less frequent than in a comparable noncirrhotic population. Neither conclusion is valid, and the facts remain to be determined by prospective studies. All the studies have been retrospective in nature, and have suffered from a lack of suitable controls. The reported incidence of peptic ulcer in cirrhotic patients has ranged from 3.6 to 24.7 per cent. It has also been suggested on slender evidence (see Table 2) that there is a greater risk of the development of peptic ulcer in patients with primary biliary cirrhosis than in those with portal cirrhosis (Schmidt and Martini, 1969).

TABLE 2: Incidence of Peptic Ulcer in Primary Biliary Cirrhosis

First Author		No. of Patients	Peptic Ulcer	
			No.	Per cent
Lipp	1952	21	3	14.3
Tabaqchali	1964	42	14	33.3

Gastric Secretion in Portacaval Shunt

Several/

Several investigators have proposed that shunt-related hypersecretion may be due to an increased reactivity of the gastric mucosa rather than to a specific humoral stimulant (Gillespie and Grossman, 1962; Rex and others, 1964; Hubens, 1967). As part of this proposal, it has been suggested that portacaval shunt produces an increase in the parietal cell mass (Landor, Porterfield and Wolf, 1966; Hubens, 1967). Reports of an elevated postshunt response to histamine stimulation (Clarke, Hoffs and El Farra, 1960; Hein and others, 1963; Rex and others, 1964; Orloff and Windsor, 1966; Hubens, 1967), although not consistent, (Guth, 1962; Hayashi and others, 1968; Newman and others, 1969) have been cited as evidence for increased mucosal reactivity. However, the consistent onset of acid hypersecretion within a few days after portacaval shunt, the demonstration of augmented acid production following acute portal-systemic autotransfusion (Brown and others, 1967) and during cross-transfusion of blood from shunted dogs to normal recipients (Orloff and others, 1969b) provide strong evidence against the role of an increased parietal cell mass or mucosal reactivity in postshunt hypersecretion.

Role of Gastrin It is clear from the studies mentioned that/

that if gastrin has any relationship to postshunt hypersecretion, it is a minor role. Antrectomy performed before portacaval anastomosis does not prevent the development of hypersecretion after shunting. Similarly, the hypersecretion after shunting is not abolished by subsequent antrectomy, but it does result in a reduction in gastric secretion by 43 per cent (MacPherson and others, 1962). Increased gastric secretion after portacaval shunt has also been attributed to the improved circulation of gastric mucosa consequent on diminished portal pressure (Ostrow and others, 1960). Although blood flow to the stomach is increased following portacaval anastomosis, particularly to the antrum where the flow is more than doubled (Delaney, Goodale, Cheng and Wangensteen, 1965), serum gastrin levels, both fasting and postprandial, are diminished following portacaval transposition (Clendinnen and others, 1970).

Role of the Vagus Another set of mechanisms have been proposed for the observed action of humoral stimulators of gastric secretion, namely that such substances can sensitize the parietal cells to vagal stimuli. The facility to potentiate direct vagal action on the parietal cells/

cells is a characteristic of gastrin, and is possibly also held by the intestinal stimulatory hormone. Responses to vagal stimuli were reported to be augmented by portal vein occlusion (Gregory, 1958). Using insulin-stimulation in two dogs with Pavlov pouches as a model of vagal stimulation, Olbe (1966) noticed that gastric secretion increased over three-fold after portacaval transposition. In the presence of a mesenterico-caval shunt, sham-feeding of antrectomized dogs produced an increase of 7 - 20 fold in acid output from Pavlov pouches. Despite such evidence supporting a vagal influence of shunt-related secretion, a more direct vagal stimulant, 2 - deoxy - D - glucose, had no effect on Heidenhain pouch acid secretion either before or after portacaval transposition (Newman and others, 1969).

Clinical Studies Until recently, the applicability of many experimental observations regarding shunt-related hypersecretion to humans with portacaval anastomosis has been uncertain. Prior to the investigations by Orloff and others (1969a), all studies of gastric secretion in shunted patients were confined to measurements of basal, and histamine - or caffeine-stimulated acid production, and ignored the intestinal phase of gastric secretion. Clarke/

Clarke and others (1958a) revealed that basal acid output and the secretory response to a sucrose meal were similar in normal controls, cirrhotics and shunted cirrhotics. In contrast, Ostrow and others (1960) reported that the gastric output of pepsin, free and total acid, was significantly higher, both basally and after histamine in 11 shunted cirrhotic patients than in 29 cirrhotics without shunt. However, inconsistent results were obtained in the only two patients studied both before and after shunt.

Bendett and others (1963) found that the basal acid output and histamine-stimulated secretion were not significantly different before or after portacaval shunt in 11 patients. Tabaqchali and Dawson (1964) observed inconsistent changes in basal acid secretion and a reduction in the response to histamine after portacaval shunt in two patients. Schriefers, Schreiber and Esser (1963) studied basal and caffeine-stimulated gastric secretion before and after portacaval shunt in 17 patients and found no significant changes. Furthermore, their studies in 130 cirrhotic patients with and without shunts failed to show significant differences in acid output, although there was a tendency toward hyperacidity in the shunted/

shunted patients. Scobie and Summerskill (1964) reported similar levels of basal and histamine-stimulated acid secretion in control subjects and four patients with portacaval anastomosis.

Wilkinson and Riddell (1965) compared overnight and histamine-stimulated acid secretion before and after portacaval shunt in 17 patients and found no significant differences. Ferrarese and Ronzini (1966) detected no consistent differences in the acid secretory response to histamine before or after portacaval shunt in 10 patients. In contrast to all these previous studies of basal and stimulated gastric acid secretion, Orloff and others (1969a) have demonstrated sustained and highly significant hypersecretion in response to an intestinal meal in all patients with portacaval shunts, but no response in normal subjects and nonshunted cirrhotics. For the first time, shunt-related gastric hypersecretion was demonstrated in man. The gastric hypersecretory response to an intestinal meal in humans with portacaval shunts has been so consistent that the response may be a useful indicator for patency of the anastomosis.

Peptic Ulcer Associated with Portacaval Shunt

Since/

Since the initial report by Dubuque and others (1958), there have been several studies on the subject, all retrospective and without suitable controls. These investigations reflect an incidence of peptic ulcer in shunted patients of approximately 10 per cent, with a range of 1.6 - 38 per cent (Table 3). Five small prospective studies (Table 4) show an average incidence of 12.6 per cent, with a preponderance of gastric ulceration. It should be noted that in the first postoperative month of these seriously ill patients, there was an incidence of 21 per cent of acute stress ulcer (Orloff and others, 1969a). The true relationship of portacaval shunt to peptic ulcer remains to be determined by careful prospective studies with suitable controls.

TABLE 3: Incidence of Peptic Ulcer Following Portacaval Shunt

First Author		No. of Shunt Cases	Peptic Ulcer*			
			No.	%	Du	Gu
Child	1958	100	5	5.0	-	-
Clarke	1958c	62	5	8.0	3	2
Dubuque	1958	60	9	15.0	-	-
Ludington	1958	8	3	38.0	1	2
Linton	1961	137	22	16.0	-	-
McDermott	1961	237	35	15.0	-	-
Wantz	1961	101	9	9.0	6	3
Mikkelsen	1962	230	16	7.0	13	3
Bendett	1963	11	1	9.1	0	1
Hallenbeck	1963	106	6	5.7	-	-
Rousselot	1963	104	6	5.7	-	-
Schriefers	1963	125	2	1.6	-	-
Liebowitz	1964	50	8	16.0	6	2
Tabaqchali	1964	35	5	14.3	4	1
TOTAL		1366	132	9.7		

* Du = Duodenal ulcer

Gu = Gastric ulcer

TABLE 4: Prospective Studies - Peptic Ulcer Associated with Portacaval Shunt

First Author		No. of Shunts	Peptic Ulcer*			Follow-up Time
			No.	Du	Gu	
Clarke	1958c	62	5	3	2	2 years
Ostrow	1960	2	1	-	1	2 years
Bendett	1963	6	1	-	1	6 months
Wilkinson	1965	65	3	1	2	2 years
Orloff	1969a	58	14	4	10	2-6 years
TOTAL		193	24	8	16	

* Du = Duodenal ulcer

Gu = Gastric ulcer

CHAPTER IV

DETERMINATION OF THE TIME COURSE OF RELEASE OF INTESTINAL PHASE HORMONE INTO PORTAL BLOOD

DETERMINATION OF THE TIME COURSE OF RELEASE OF INTESTINAL PHASE HORMONE INTO PORTAL BLOOD.

It has been shown recently by Orloff and his group (1969a, 1969b) that the intestinal phase of gastric secretion is mediated by a potent hormone elaborated mainly in the jejunum. The present studies were planned to determine the kinetics of hormone release following application of the stimulus to the intestine, and to pinpoint the time of maximum hormone concentration in portal blood for subsequent isolation of the hormone. With these ends in view, portal blood was harvested at 15 minute intervals from suitably-prepared donor dogs following administration of an intestinally-fed meal.

The plasma portion of the harvested portal blood was tested for gastric stimulatory activity by intravenous administration to assay dogs with Heidenhain pouches.

MATERIALS AND METHODS

Fifty-eight satisfactory experiments divided into 5 groups were completed at 15, 30, 45, 60 and 90 minutes respectively (Table 5) in order to determine the optimum time for sampling portal blood from the intestinally-fed donor.

TABLE/

TABLE 5:: Number of studies for portal blood harvesting

Harvest Time (mins.)	15	30	45	60	90
No. of Tests	11	17	10	10	10

SURGICAL PREPARATION OF ANIMALS

(1) Donor Dog (Fig. 1)

(a) Establishment of Antrectomy, Heidenhain Pouch and Jejunal Fistula.

Healthy adult mongrel dogs were selected which were conditioned to the laboratory environment and which weighed between 25-30 kg. After 24 hours of fasting, each dog was anaesthetized with 0.5 mg./kg Nembutal (sodium pentobarbitone) given intravenously. Endotracheal intubation was established. The animal was shaved and prepared for laparotomy. A catheter for intravenous infusion was inserted into the left jugular vein through a cut-down. Histamine phosphate 2.75 mg. was injected subcutaneously to allow subsequent mapping out of the antrum.

The abdomen was opened through an upper midline incision. The spleen was mobilized and approximately 10 ml. of 1: 10,000 solution of adrenaline was injected into a tributary of the splenic artery. Haemostasis was secured/

secured by a silk ligature on either side of the needle puncture. When the spleen had contracted, the vessels in the hilum were ligated as close to the organ as possible in order to preserve the vascular connections to the left gastro-epiploic artery. Splenectomy was now performed at this stage, as this procedure would have been necessary during the second stage of the surgical preparation when the portal vein was cannulated through the stump of the splenic vein.

Through a small distal gastrotomy, the extent of the antrum was determined with pH paper (Hydrion AB pH1-11).* The alkaline-secreting antrum is clearly demarcated from the acid-producing body of the stomach by a green to red colour change. Antrectomy was now performed, so that the proximal limit of resection was 2 cm. above the line of pH change. A Heidenhain pouch was constructed with its vascular pedicle arising from the splenic vessels. In order to maintain the animal's nutrition by retaining as large a gastric remnant as possible, the length of the pouch was not more than 8 cm. along the greater curvature.

After the cut edge of the gastric stump adjacent to/

* Micro Essential Lab., Inc., Brooklyn, N.Y. 11210, U.S.A.

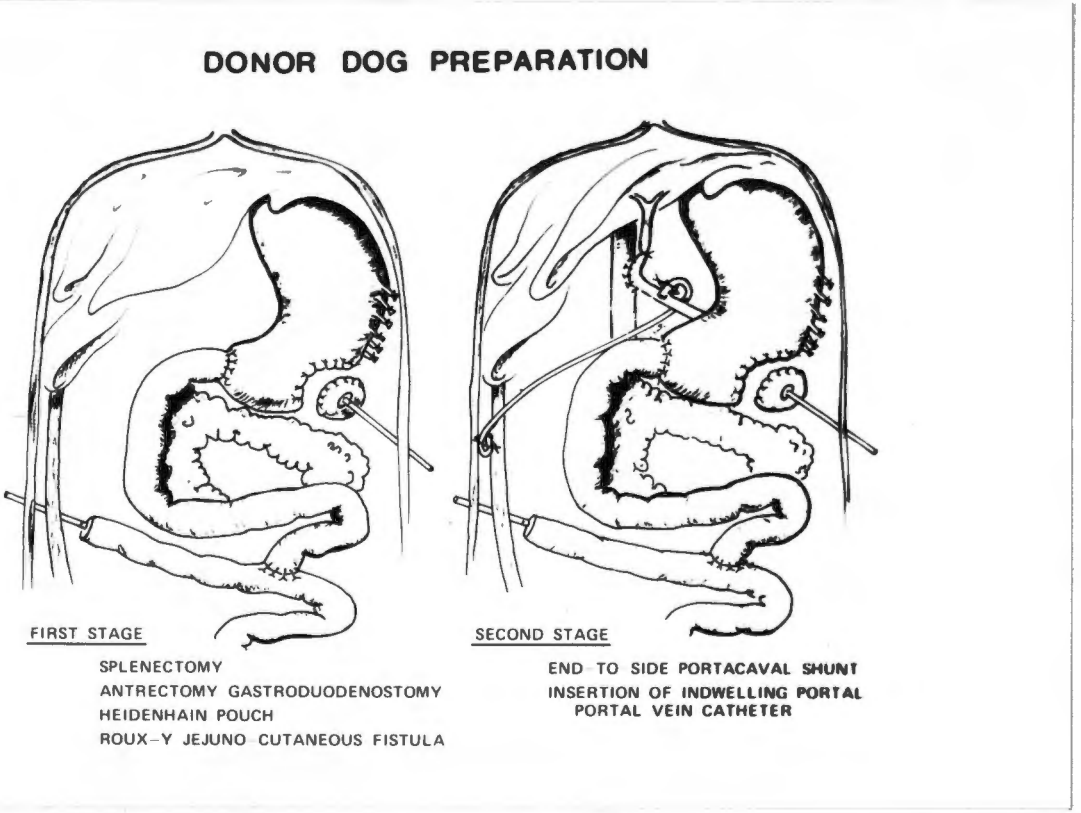


Fig. 1. Surgical preparation of donor dog for harvesting portal blood with intestinal phase hormone.

to the lesser curvature had been sutured, a Billroth I anastomosis was constructed ensuring a large gastro-duodenal junction. Since this operation has been succeeded by a considerable mortality rate and weight loss in the past (Orloff and others, 1969b) because of outlet obstruction to the stomach, a larger gastro-duodenal anastomosis was now accomplished by making a longitudinal incision, 2.5 cm. in length, in the anterior wall of the first part of the duodenum at its midpoint.

Twelve cm. distal to the duodeno-jejunal junction, a Roux-en-Y intestinal anastomosis with an iso-peristaltic loop of 20 cm. in length was constructed. After a stainless steel cannula had been inserted through a stab incision near the proximal end of the Roux-en-Y jejunal loop, the bowel end was closed. The jejunal cannula was brought out through the anterior abdominal wall via a stab incision located 4 cm. lateral to the midline, and 10 cm. caudal to the right costal margin. The cannula was plugged with a metal bolt. A second stainless steel cannula, inserted into the Heidenhain pouch, was led out through a stab wound in the abdominal wall 2 cm. lateral to the midline and 4 cm. caudal to the left costal margin.

On the day of operation, and for 5 days postoperatively, the/

the dog was given Ampicillin 1 G. by intramuscular injection. On each of the first 3 postoperative days, the animal was infused intravenously with 1500 ml. 10 per cent dextrose in 0.45 per cent saline. Water was offered to drink on the third postoperative day, and soft tinned dog food ("Kal Kan M.P.S. Chunk") from the fourth day. The neck catheter was removed on the fourth post-operative day if the soft food had been accepted. On the eighth day, regular hard food (Purina Dog Chow)* was introduced. Commencing on the first postoperative day, the animal was exercised outdoors at least every other day up until the time of the intestinal meal test.

(b) Portacaval Shunt and Insertion of Portal Vein Catheter

About 3 weeks after the first operation, the dog was anaesthetized lightly with Pentothal 5 per cent (0.5 ml./kg.) and its trachea was intubated. A catheter was inserted into the right jugular vein by means of a cut-down. After positioning the animal on its left side, and preparing the skin for laparotomy, a right subcostal incision was made. The upper inferior vena cava was displayed, and the portal vein was mobilized from its trifurcation caudally to the junction of the pancreatico-duodenal/

* Ralston Purina Co., St. Louis, Missouri 63188, U.S.A.

duodenal vein. The termination of the splenic vein was mobilized next.

A side-to-side portacaval anastomosis, 2.5 - 3.0 cm. long was constructed; the hepatic limb of the portal vein between the shunt and the pancreatico-duodenal vein was ligated, converting the shunt to an end-to-side configuration (Eck fistula). The splenic vein was ligated 1.0 cm. away from its junction with the portal vein, and a 13 gauge saline-filled silastic catheter was passed through a small nick in the termination of the splenic vein, so that just the tip of the catheter protruded into the portal vein. The catheter was secured by a second ligature around the splenic vein to secure haemostasis. The first ligature was tied firmly around the catheter, and both catheter and ligature were passed beneath the portal vein so that the free end of the catheter was directed to the right side of the animal's body. The catheter was again secured with the first ligature. Free flow from the catheter was checked for after each manipulation. The catheter was led out below the incision through a stab wound in the abdominal wall into the subcutaneous space. Heparin 0.5 ml. (2500 units) was injected into the catheter, and its/

its end was ligated, and left in the subcutaneous space at the dorsal end of the wound.

During the operation, the animal was transfused with 500 ml. whole blood, and an intramuscular injection of Ampicillin 1 G. was given. The antibiotic was continued for 4 postoperative days. On the first post-operative day, 1500 ml. 10 per cent dextrose in 0.45 per cent saline was infused, and soft food and water was offered. On the third postoperative day, the dog was given a further transfusion of 500 ml. whole blood 24 hours in advance of the test day. The animal was exercised outdoors on the first and third days.

(2) Preparation of Assay Dog.

Small healthy conditioned adult mongrel dogs weighing 10 - 15 kg. were selected. After 24 hours fasting, an upper midline incision was made under intravenous Pentothal anaesthesia, and a vagally-denervated pouch (Heidenhain) was constructed from the body and fundus of the stomach, making the pouch as large as possible. A stainless steel cannula, inserted into the pouch, was brought out through a stab incision in the anterior abdominal wall, as close to the midline and costal margin as possible in order to minimize acid burns.

Postoperation, during the first and second days, the dog was given a liquid meal, and on the third and fourth days, soft food was offered. Thereafter, regular dog food was given. The animal was allowed to recover over three weeks, and then a histamine test was performed to establish the functional status of the pouch. The histamine test consisted of a one hour basal period, followed by subcutaneous injection of 2.75 mg. histamine phosphate. Gastric juice was collected every half hour for two hours. Only those animals whose Heidenhain pouches secreted at least 1.0 mEq. acid in the two hours after the histamine injection were used as assay dogs. Since the assay dogs were used repeatedly over long periods of time, histamine tests were repeated every 4 - 6 weeks (Table 6) to confirm that the gastric mucosa of the pouch remained responsive to humoral stimuli.

Sampling and Assay of Portal Blood (Fig. 2)

Groups of 3 - 6 dogs were used for each experiment. The donor dog was anaesthetized lightly with intravenous Pentothal 5 per cent following a fasting period of 24 hours. The outflow end of the portal vein catheter was mobilized from beneath the skin. Gastric juice

was/

TABLE 6: Results of periodic histamine tests in assay dogs

Assay Dogs	MEq. Acid per Monthly Test											
	1	2	3	4	5	6	7	8	9	10	11	12
1	3.12	4.34	5.06	2.20	2.61	4.57	3.45	3.67	1.67			
2	1.82	1.70	1.13	1.07	1.53	2.29	1.48	1.48	1.44			
3	2.90	1.91	2.73	1.43	2.28	1.14	6.76	2.62	2.46	3.50		
4	4.63	1.46	3.97	3.64	2.31	1.92	1.56	4.43	1.75			
5	1.26	2.03	3.44	3.37	2.46	1.71	2.28	4.88				
6	5.38	1.03	5.04	3.83	1.88	1.72	6.83	2.82	2.22	3.15		
7	1,44	5.17	4.02	5.30	3.09	3.10	3.38	6.08	1.22			
8	2.53	1.20	1.49	1.53	1.51	1.83	2.98	2.99	2.49			
9	2.46	1.08	3.35	1.54	1.08	1.32	1.67	2.41	5.0	5.10	1.90	1.05
10	2.33	2.85	3.23	3.39	2.54	2.22	3.26	2.72	1.52	4.04	1.5	3.9
11	4.09	9.79	1.81	5.98	6.25	2.10	3.70	4.27	2.58	1.55	3.17	
12	4.01	6.63	5.94	1.06	1.05	1.30	4.0	1.55	1.26	1.70	1.32	
13	5.98	1.26	1.70	3.17	2.58	3.97	2.62	2.74	4.69	4.91	2.62	
14	5.94	1.06	3.16	3.40	2.65	3.22	2.15	7.14	4.80	2.86	8.91	
15	1.59	2.17	2.15	2.90	1.75	3.55	2.65	1.42	2.35	4.10		

was collected under basal conditions for one hour from the Heidenhain pouch. Next, the animal was fed through the jejunal fistula, the meal containing 48 G of protein, 19 G of fat and 84 G of carbohydrate, made up with milk to a volume of 500 ml. The meal was instilled over a period of 15 minutes. Gastric juice samples were collected every half-hour after the start of the meal for $5\frac{1}{2}$ hours. The volume of each collection was measured and the acid content was estimated by titration with O.1N sodium hydroxide to pH 4.5 using Topfer's reagent as the indicator. Results were expressed as mEq. per test period.

At predetermined periods after the start of the meal, 500 ml. portal blood was collected by siphonage over a 15-minute period into sterile bottles to which 0.5 ml. (2500 units) heparin had been added. The bottles stood in a mixture of ice and water. Simultaneously, the blood volume was maintained by transfusion of fresh bank blood into a peripheral vein. At the end of the experiment, the dog was killed and the patency of the portacaval shunt and location of the portal vein catheter was determined at autopsy. Only experiments in which the donor dog developed shunt-related hypersecretion, defined as an acid output of 3 mEq. or more during the test, were/

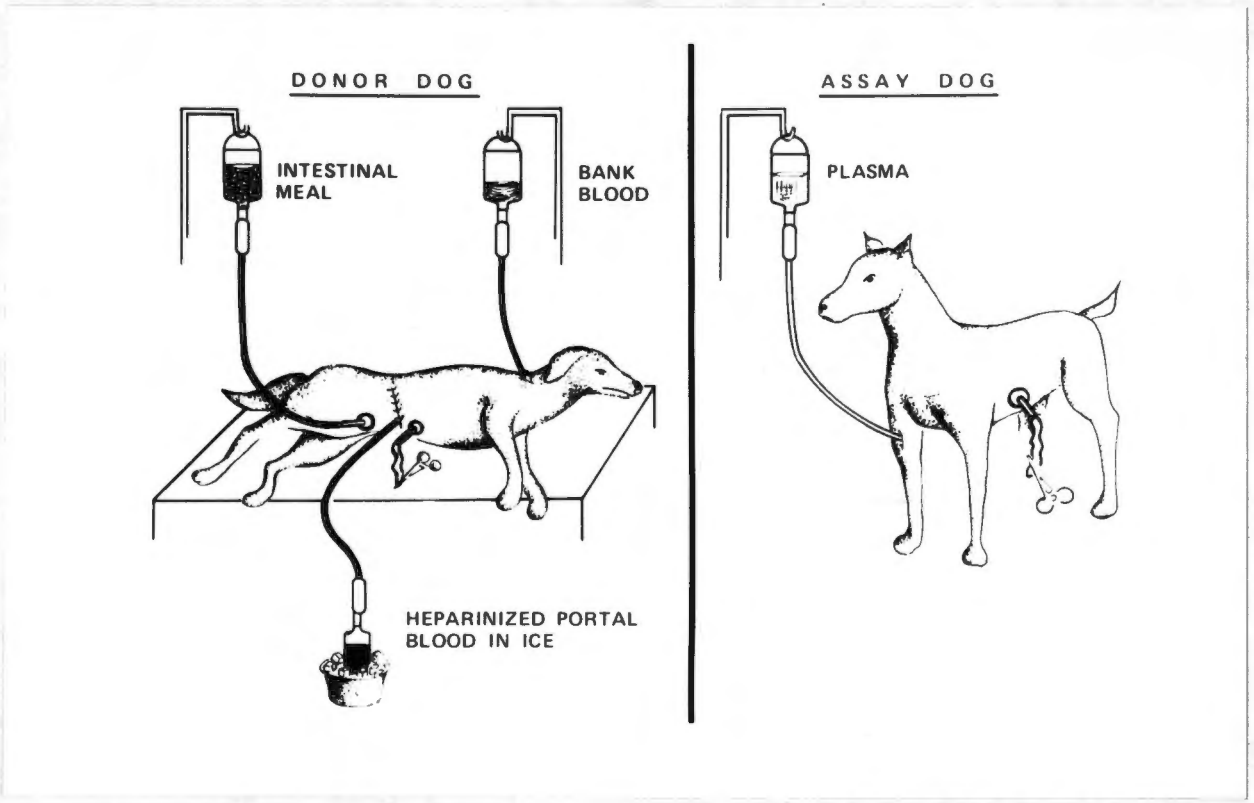


Fig. 2:: Sampling and Assay of Portal Blood Plasma with Intestinal Phase Hormone.

were included in the evaluation. Autopsy confirmed that all the secreting donor dogs had patent porta-caval shunts and that the portal vein catheter was sited satisfactorily. Approximately 10 - 20 per cent of the surgically-prepared dogs failed to show shunt-related hypersecretion and therefore could not be used to evaluate the time course of intestinal hormone release. Such dogs were either cachectic or showed haemorrhagic inflammation of the pouch.

PREPARATION AND TESTING OF PORTAL BLOOD PLASMA

Immediately after collection, the portal blood was centrifuged at 4°C., and the plasma was bio-assayed in a dog which had been fasted over the previous 24 hours.

Bioassay of Portal Blood for Intestinal Phase Hormone

The portal plasma from a dog undergoing intestinal stimulation, approximately 350 ml. in volume, was infused over a 15-minute period into an assay dog held in a Pavlov stand. In order to minimize extraneous stimulation of gastric secretion, the test was conducted in a quiet room, the gastric juice was collected by the same individual and efforts were made to keep the dog as calm as possible. The acid secretion of the Heidenhain pouch was measured for one hour before and half-hourly for/

for four hours after administration of the test plasma. The acid secretory response in mEq. to portal plasma from a stimulated dog was compared with the response of the same assay dog to the same volume of normal bank plasma collected from a fasting dog. The sequence of administration of the control and test plasma was alternated from one dog to the next for each time of sampling. Pouch acid secretion was allowed to return to the basal level between trials of the control and test material.

Since the assay dogs were used repeatedly over several months, it was not surprising that many animals developed hypersensitivity transfusion reactions which were manifested by retching and/or vomiting, restlessness, muzzle swelling, urticaria. The absence or presence of plasma hypersensitivity reactions was noted in the records of each experiment.

In addition to this personally-obtained data, records of previous comparable experiments were studied with the kinetics of hormone release in mind. The material was previously reported by Villar-Valdes and others (1969) and Orloff and others (1970b) in shunted dogs, and by Orloff and others (1969a, 1970a) and Abbott and others (1970)/

(1970) in cirrhotic patients with portacaval shunts.

RESULTS

Following surgical preparation of the donor dogs, approximately one-third of the series had to be excluded from the study because of malnutrition, and/or pneumonia, peritonitis, gastric hyposecretion. If a dog was not tested within one week of end-to-side portacaval anastomosis, then the animal was rejected because with passage of time, it developed progressive liver damage.

The secretory pattern of each donor and assay dog was examined to time the onset of acid response to the stimulus of a meal or infusion of plasma, and to time the hour of peak secretion. The onset of gastric secretion following feeding was arbitrarily defined as the time taken to the collection of the first half hourly sample which contained more than twice the acid content of any of the basal samples. The amount of peak hour secretion was taken as the sum of two consecutive half hour samples which achieved the maximum value in mEq. of acid. The time of the hour of peak secretion was defined as the time taken to reach the completion of the hour of peak secretion.

A./

A. GASTRIC ACID RESPONSE IN THE DONOR DOGS.

(1) Portacaval Shunt-related Gastric Hypersecretion.

The acid secretion from the pouches of the donor dogs, following the stimulus of an intestinally-fed meal, are shown in Tables, 8, 10, 12, 14 and 16 respectively for each time interval and are summarized in Table 7. Fifty-eight shunted donor dogs developed profound gastric hypersecretion, with a mean total gastric acid output of 11.18 mEq. at the completion of the $5\frac{1}{2}$-hour test period, and were therefore suitable for the full experiment. For all of the 58 animals, the mean peak hourly secretion of 3.79 mEq. was highly significant at a p value of less than .001 when compared to the mean basal secretion of 0.16 mEq. (Fig. 3).

When the mean secretory response of the shunted dogs to an intestinal meal was plotted as a graph (Fig. 4), it can be seen that within the first two hours of the test there was a rapid rise in gastric output to reach a maximum rate of secretion, which was succeeded by a persistent outpouring of acid at the rate of approximately 0.9 - 1.2 mEq. per half hour during the test period.

(2) Kinetics of Hormone Release.

The/

The mean time of onset of the meal response; the mean time of the hour of peak secretion; and the mean of the total acid secretion during the peak hour expressed as per cent above mean basal secretion, for each of the five groups of donor dogs, is shown in Table 18. The mean peak hourly secretion, now expressed in an alternative manner, showed an enormous 12 to 57-fold increase above the basal secretory rate, demonstrating the expected gastric hypersecretion in the shunted dogs following administration of an intestinally-fed meal. Gastric secretion began a mean 45 minutes after the start of the meal, with the onset ranging from 30 to 150 minutes. Peak gastric secretion was delayed on the average for 3 hours, with a range of 1 to 5.5 hours.

B. ACID SECRETION IN THE ASSAY DOGS.

The mean acid secretory response of the assay dogs to infusion of portal plasma harvested between 30 and 45 minutes after the start of the meal in the donor dogs, was increased 9-fold over the secretion stimulated by the control plasma (Table 19). The mean total acid secreted was 0.75 mEq. (Table 11), and this value was significant at a p value of less than .02 when compared to the mean control secretion of 0.08 mEq.

When/

When the portal plasma was sampled beginning 15 minutes after the start of the meal (Table 9), there was a 3-fold increase above the control secretion, and the difference was significant with a p value of less than .05. Portal plasma harvested after 45 minutes stimulated even less gastric secretion (Tables 13, 15 and 17). For each of the five groups of assay dogs studied, the mean gastric acid response following infusion of harvested portal plasma is summarized in Table 7.

The data indicate that the level of intestinal phase hormone in the portal blood rises significantly by the end of 30 minutes after the start of an intestinally-fed meal; the active agent reaches maximum concentration between 30 and 45 minutes after the commencement of the stimulus. After 45 minutes, hormone activity in the portal blood decreases markedly.

The secretory response of the assay dog to infusion of harvested portal plasma was delayed for a mean of one hour, with a range of 30 to 180 minutes (Table 20). Peak gastric secretion was attained at an average of $1\frac{1}{2}$ hours after the start of the infusion, ranging from 1 to 3 hours.

The/

The secretory patterns following single balloon distension, multiple balloon distensions and administration of an intestinal meal in 37 shunted dogs with Heidenhain pouches were examined in the data recorded in previous studies conducted in this laboratory (Villar-Valdes and others, 1969; Orloff and others, 1970b). The onset of gastric secretion was delayed for a mean 49 minutes following the start of the stimulus, with a range from 30 to 150 minutes, whereas peak gastric secretion was only achieved at a mean 3.9 hours later, with a range of 1 - 5.5 hours (Table 21).

When the kinetics of hormone release was examined, in a group of shunted cirrhotic patients studied earlier (Orloff and others, 1969a; Abbott and others, 1970; Orloff and others, 1970a), a comparable response to that in the shunted dog was found (Table 22). The acid secretory response occurred at a mean 69 minutes following the start of an intestinal meal or simple balloon distension of the jejunum, with a range of 30 - 180 minutes. Peak gastric secretion was again delayed until a mean 2.6 hours after the start of the meal, with a range from 1 to 4 hours.

Hypersensitivity Reactions to Plasma (Table 23)

In/

In the 58 assay dogs tested, a hypersensitivity reaction to intravenous infusion of control bank plasma was observed in 32, while test portal plasma produced reactions in 24 animals. Although the mean acid secretory response to control plasma was reduced three-fold when a reaction was apparent the acid output after infusion of test portal plasma was doubled in hypersensitive dogs. The only conclusion that can be drawn from these equivocal results is that the presence or absence of a hypersensitivity reaction following infusion of control and test plasma does not influence gastric acid secretion to a significant extent. When a severe reaction did present, it was more frequently associated with hyposecretion.

DISCUSSION

Studies performed in this laboratory over the past six years have demonstrated conclusively that bypassing the portal blood directly into the systemic circulation unmasks a potent humoral agent that stimulates gastric acid secretion. Dogs prepared with Heidenhain pouches were stimulated with an intestinally-fed meal (Orloff and others, 1970b) and simple balloon distension of the intestine (Villar-Valdes and others, 1969) through a Roux-en-Y jejuno-cutaneous fistula. Gastric acid secretion was determined at half-hour intervals for $5\frac{1}{2}$ hours after the stimulus. Three weeks later, the dogs underwent an end-to-side portacaval shunt, and then the tests were repeated.

As expected from previous studies using classic autotransfusion (Brown and others, 1967) and cross-transfusion techniques (Orloff and others, 1969b) the intestinal meal stimulated a marked gastric acid secretory response following portacaval shunt significant at the p of less than .001 level when compared to the acid secretion elicited by the meal before shunt. In the larger group of dogs now studied, the present results emphatically confirmed the previous findings, by again obtaining/

obtaining a highly significant acid secretory response to an intestinal meal in dogs with portal -systemic shunting.

Balloon distension of the jejunum produced a remarkably similar acid secretory response. In the presence of a portacaval anastomosis, simple distension of the jejunum stimulated highly significant gastric acid secretion when compared to the preshunt values.

The introduction of food into isolated, jejunal, ileal and colonic intestinal loops in shunted dogs stimulated highly significant gastric hypersecretion only when the jejunum was the site of the stimulus (Orloff, and others, 1970b). It was concluded that the humoral agent unmasked by portacaval anastomosis was not a secretagogue absorbed from food, but a hormone of endogenous origin elaborated by the intestine in response to either food or simple distension.

Comparable studies were performed in normal humans, compensated cirrhotics, and cirrhotics who had undergone portacaval shunt (Abbott and others, 1970). An intestinal meal or balloon distension was administered using a double-lumen naso-intestinal tube positioned fluoroscopically/

fluoroscopically in the jejunum and ileum. In the absence of a portacaval shunt, the meal produced no discernible acid secretory response. In marked contrast, the shunted subjects had a significant and prolonged stimulation of their gastric acid secretion in response to the meal comparable to that observed in the laboratory dog. Balloon distension in the shunted patients produced a very similar gastric acid secretion to that elicited by an intestinal meal. Thus in man, as well as in the dog, the proximal intestine elaborates a potent hormone capable of stimulating marked gastric acid secretion. Normally, this hormone is rapidly degraded by the liver, but in the presence of a portacaval shunt it bypasses the liver so that its effects on the parietal cell are unmasked.

When the patterns of secretion in the previous investigations in intact shunted dogs (Villar-Valdes and others, 1969; Orloff and others, 1970b), shunted cirrhotic patients (Orloff and others, 1969a; Abbott and others, 1970; Orloff and others, 1970a), and in the present studies of shunted donor dogs, were now analysed, there was revealed a consistent delay of 45 - 90 minutes before gastric acid secretion responded to an intestinal stimulus./

stimulus. Peak hourly gastric secretion was not achieved until 1.4 - 4.4 hours after administration of the stumulus. Although gastric hypersecretion in the assay dogs in response to harvested portal plasma was very significant when the plasma had been collected beginning at 30 minutes after the start of the meal, yet significant results were obtained when portal plasma was sampled as early as between 15 to 30 minutes following introduction of the meal into the jejunum.

These results indicate that the main locus for the delay in shunt-related gastric hypersecretion is not in the elaboration and release of the intestinal phase hormone from the jejunal mucosa, but rather in the effector limb of hormone action, and that intermediary steps may be necessary before the hormone can effectively stimulate the target organ - the parietal cell mass - to secrete acid.

The reason for such a delay is open to speculation. One possibility is that the hormone is released in the form of an inactive precursor or substrate requiring the presence of an "activator" to alter its chemical form and uncover its hormonal function. A further mechanism/

mechanism to be considered is the inactivation offered by inhibiting hormones and reflexes. Because of paucity of knowledge, one can only speculate at this juncture about the interrelationship between the stimulatory intestinal phase hormone and known inhibitors of gastric secretion e.g. secretin, cholecysto-kinin-pancreozymin, and enterogastrone if it truly exists (Lucien, Itoh and Schally, 1970; Johnson and Grossman, 1971). Furthermore, the small intestine itself is believed to release a humoral inhibitor of gastric acid secretion, whose influence is removed by massive small intestine resection (Frederick, Sizer and Osborne, 1965; Santillana and others, 1969; Ruderman and Kamel, 1970). Nothing much is known about the factors controlling the elaboration and release of such a humoral factor from the small bowel, or its full role in gastric physiology.

A further suggestion advanced is that the intestinal phase hormone may activate an intracellular messenger such as adenosine - 3', 5' - monophosphate (cyclic AMP). Since its discovery in 1957 by Rall, Sutherland and Berthet, the demonstration of the wide distribution of cyclic AMP in tissues, and its involvement in the production of the effects of many hormones on their target/

target organs, has led to the concept that hormones act by a two-messenger system (Sutherland, Oye and Butcher, 1965; Butcher, Robinson, Hardman and Sutherland, 1968). The first messenger is the hormone which is released from the cell of origin and travels in the blood stream to the target tissue. At the target tissue the hormone stimulates the formation of a second (intracellular) messenger which triggers a cell-specific sequence of events that leads to the physiological response associated with the hormone. Cyclic AMP has been clearly identified as the only second messenger discovered thus far, and it has already been suggested that cyclic AMP may serve as a mediator for the hormone gastrin at the level of the target cell (Rosen, Chandler, Multer and Orloff, 1971).

The secretory mechanism of the intestinal phase hormone is of a fragile nature since its elaboration can easily be inhibited by general debility in the experimental animal. The construction of a donor dog involves major surgery and its subsequent survival requires careful postoperative care. A donor dog in poor general condition due to pneumonia, localized peritonitis or cachexia, invariably failed to secrete the/

the qualifying minimum of 3 mEq. acid at the end of the 5½-hour test period, requiring it and its recipient assay dog to be withdrawn from the experiment.

During harvesting of portal blood, if the donor dog's blood volume was not restored ml. for ml. with bank blood then it was observed that the target value of 3 mEq. was frequently not achieved, presumably because of hypotension which inhibited hormone formation. Finally, if there was an unavoidable delay of over one week after portacaval anastomosis (Eck fistula) prior to the portal blood harvesting experiment, then the dog's general condition deteriorated because of the progressive liver damage associated with an Eck fistula, and it was wiser not to use such a sick animal.

Two other factors require consideration during evaluation of the meal response in the present investigation. The first is whether removing a sample of hormone-rich blood from the portal blood stream will likely influence the magnitude and secretory pattern of gastric acid secretion in the donor dog. Whether or not such an effect will be significant has not been determined in these studies, but it has been assumed that because of the massive stimulation of gastric secretion/

secretion by an intestinal meal, the removal of a small volume of portal blood over a short period will have a negligible effect on the acid response. The second factor is that heparin is known to inhibit gastric secretion (Thompson, 1966). During cross-transfusion experiments such as the present portal plasma transfer studies, the same amount of heparin and the same regimen for heparinization must be used throughout the investigations.

The intestinal phase hormone which is elaborated by the jejunum in response to food or simple distension produces profound and prolonged gastric hypersecretion for at least 5.5 hours when it escapes hepatic degradation through a portacaval anastomosis. The nature of the food is not important - it is brief distension of the jejunum that triggers off prolonged elaboration and release of the hormone (Orloff and others, 1969b and 1970a). The pressure of a balloon distended for 20 minutes at 50 mm Hg. is comparable to physiological pressures developed in the lumen of the jejunum during normal digestion (Orloff and others, 1970a).

In man and the dog, following ingestion of food, the intact stomach releases small amounts of food into the/

the upper small bowel over a few hours. Such intermittent stimulation leads to prolonged release of intestinal phase hormone over many hours. Presumably, several mechanisms operate to protect the organism against gastric acid hypersecretion. The first mechanism is that the bulk of the hormone in the portal venous blood is rapidly degraded by the liver, and only a small amount escapes hepatic inactivation and becomes free to stimulate the parietal cell mass to secrete approximately 10 per cent of the total gastric acid output in the dog (Dragstedt, Woodward, Storer, Oberhelman and Smith, 1950). A second mechanism has a teleological basis, which is that the stimulatory intestinal hormone is released at a time when the stomach contains food which dilutes and buffers any acid hypersecretion.

With stimulation of gastric acid hypersecretion in the assay dog, acid released from the stomach remnant would reduce the duodenal pH and incur the release of secretin. Despite such inhibition offered to gastric acid secretion, the acid response of the Heidenhain pouch persisted at a remarkable secretory rate. In the studies performed by Kelly, Nyhus and Harkins (1965), the increased acid secretion in response to chyme suggested/

suggested to them that duodenal inhibition of the secretion of gastric acid secondary to a decreased duodenal pH was not of great importance in their laboratory preparation.

Since the gastric acid response of the shunted dog to an intestinal meal (Fig. 4; Tables 18 and 21) shows on the average a peak within the first four hours after feeding, it is likely that such a peak may be attributed to the small amount of hormone which escapes hepatic inactivation during the time of maximal hormone concentration in the portal bloodstream - 30 to 45 minutes after application of the stimulus. The amount of hormone escaping hepatic inactivation at this optimal time very likely triggers off the intestinal phase of gastric secretion.

Figure 3:

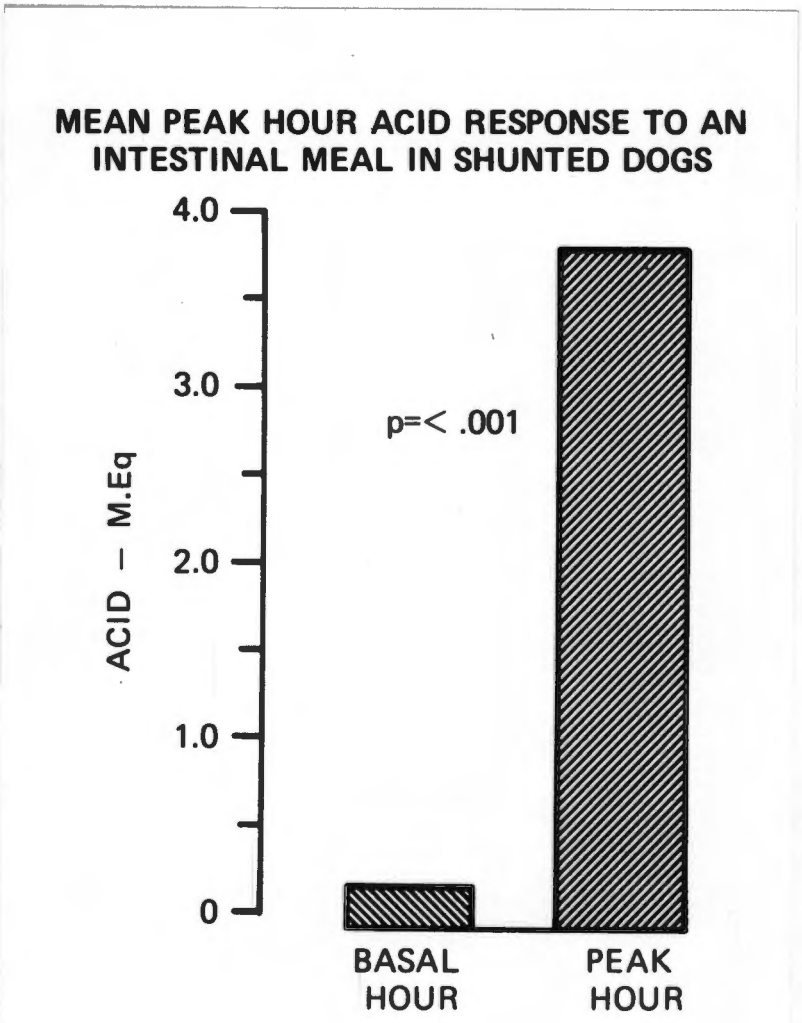
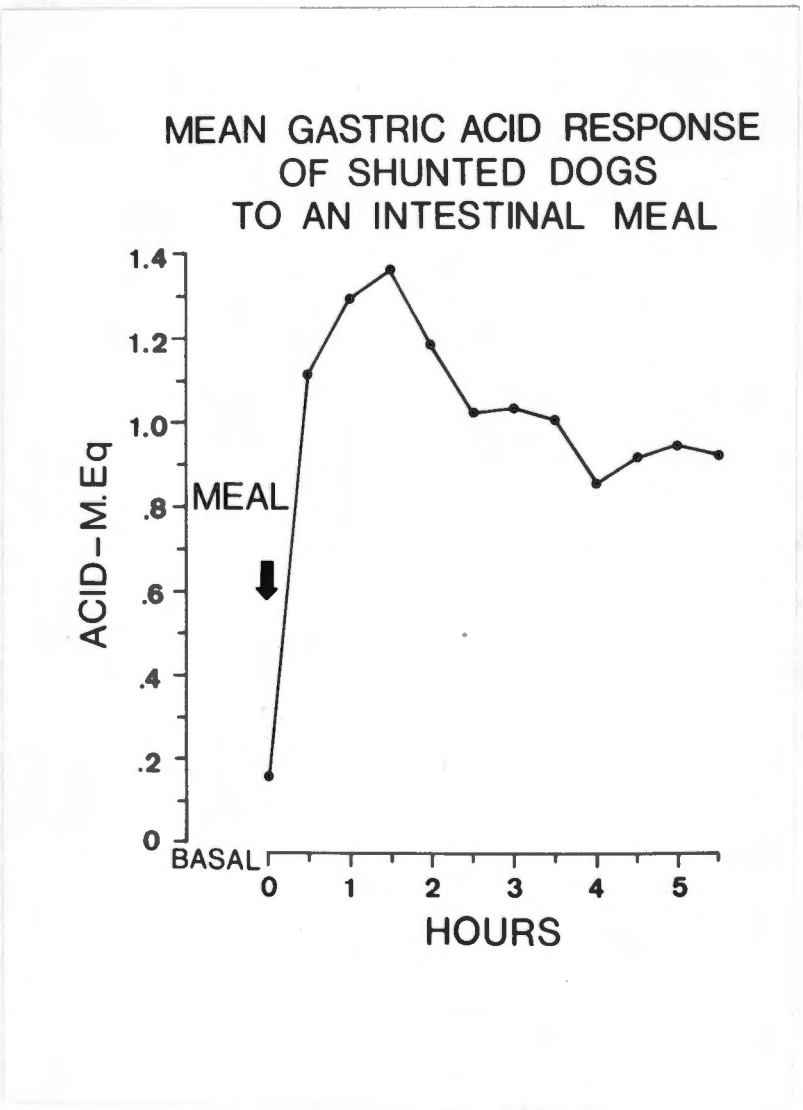

MEAN PEAK HOUR ACID RESPONSE TO AN INTESTINAL MEAL IN SHUNTED DOGS
4.0
3.0
2.0
1.0
0
ACID – M.Eq
p=< .001
BASAL HOUR
PEAK HOUR

Figure 4:


MEAN GASTRIC ACID RESPONSE
OF SHUNTED DOGS
TO AN INTESTINAL MEAL
ACID-M.Eq
1.4
1.2
1.0
.8
.6
.4
.2
0
MEAL
BASAL
0
1
2
3
4
5
HOURS

		Donor Dogs M.Eq Acid				Assay Dogs M.Eq Acid		
Time of Harvest (mins)	No. of Dogs	Basal Hour	Peak Hour	Test $5\frac{1}{2}$ hrs		Control Plasma 4 hrs	Test Plasma 4 hrs	Test Peak Hour
15- 30	11	.1	5.16	16.11		.04	.12	.09
30- 45	17	.15	2.95	8.35		.08	.75	.63
45- 60	10	.13	3.83	11.46		.22	.29	.23
60- 75	10	.20	4.18	12.36		.38	.55	.49
90-105	10	.24	3.0	9.13		.09	.22	.16

TABLE 7: Summary of the series of portal plasma transfer experiments (m.Eq of acid represent mean values).

No.of Dogs	Basal Half Hours		Test - M.Eq Acid Per Half Hour											Total	Peak Hour
			1	2	3	4	5	6	7	8	9	10	11		
1	0	0	.92	4.4	2.2	2.33	1.7	1.5	1.1	1.05	.9	.5	.6	17.2	6.6
2	0	0	.5	1.85	4.8	4.33	2.02	4.8	2.3	2.9	1.2	4.0	4.8	33.5	9.13
3	0	.01	1.65	1.22	1.52	1.28	.6	.9	.56	.36	.35	.28	.18	8.9	2.87
4	0	0	1.2	1.35	2.88	1.53	1.8	3.1	3.91	1.5	1.2	1.08	.9	20.45	7.01
5	0	0	2.2	4.7	4.32	2.88	3.2	3.4	2.89	2.72	2.2	2.0	1.6	32.11	9.02
6	.2	.28	1.5	2.52	1.8	.33	.8	.4	.42	.42	.48	.28	.52	9.47	4.32
7	0	0	0	0	0	.45	.01	.91	.19	.28	1.2	.39	1.35	4.78	1.74
8	0	0	.02	.68	1.82	.45	.7	.6	.15	0	.16	.18	.42	5.18	2.5
9	0	.02	.55	1.68	1.65	1.05	.7	.75	.98	.75	.4	.86	.55	9.94	3.33
10	0	0	.35	2.24	.98	.35	.24	.39	.65	.68	.59	.85	.35	7.67	3.22
11	.2	.4	2.25	4.25	2.72	2.24	4.2	2.55	2.24	2.05	2.25	2.25	1.05	28.05	6.97
MEAN			1.01	2.26	2.24	1.57	1.45	1.75	1.4	1.16	.99	1.15	1.12	16.11	5.16

Basal hour = .1 Peak hour = 5.16 $p = < .001$

TABLE 8: Donor dogs - response of Heidenhain pouch to an intestinal meal in mEq. acid (portal plasma harvested at 15 mins.)

TABLE 9: Acid response of assay dogs after infusion of control plasma and of portal plasma harvested at 15 mins.

No. of Dogs	Control Plasma M.Eq/4 hrs	Test Plasma - M.Eq. Acid per Half Hour								Total M.Eq.
		1	2	3	4	5	6	7	8	
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	.04	.02	0	0	0	0	.06
3	0	.01	0	0	0	0	0	0	0	.01
4	0	.02	0	0	0	0	0	0	0	.02
5	0	.09	.01	0	0	0	0	0	0	.1
6	.17	.01	.02	.08	.15	.04	.02	0	0	.32
7	0	0	0	0	0	0	0	0	0	0
8	.01	0	.17	.21	.03	0	0	0	0	.41
9	.16	.01	.09	.02	0	.04	.02	0	.01	.19
10	.05	.06	.06	.05	.04	.03	.01	0	0	.24
11	0	0	0	0	0	0	0	0	0	0
MEAN	.04	.02	.03	.04	.02	.01	0	0	0	.12

Mean Control Plasma = .04 Mean Test Plasma = .12 $p \leq .05$

No. of Dogs	Basal Half Hours		Test - M.Eq. Acid Per Half Hour											Total	Peak Hour
			1	2	3	4	5	6	7	8	9	10	11		
1	.09	.09	.07	1.82	1.12	1.43	1.52	1.28	1.76	1.5	1.84	1.65	.98	14.97	3.49
2	.03	.02	.01	.35	1.96	.29	.03	.91	.6	.56	.56	.21	.16	5.64	2.31
3	.1	.4	1.05	1.92	1.96	1.58	1.47	1.43	1.54	1.44	2.15	1.89	1.82	18.25	4.04
4	.04	.02	.04	1.5	.66	.46	.45	.22	.42	.27	.5	.96	.84	6.32	2.16
5	.01	.02	0	.02	.28	.09	.23	.38	.42	.77	.68	.44	.75	4.11	1.45
6	0	0	.04	.02	.35	.27	.5	.51	.35	.41	.26	.34	.39	3.44	1.01
7	0	0	.1	1.02	.99	.22	.19	.1	.11	.06	.15	.21	.21	3.36	2.01
8	.22	.41	0	.32	1.85	.23	.13	1.38	.55	.42	1.14	2.1	1.0	9.12	3.24
9	.23	.30	.35	.55	.62	.4	.18	.15	.15	.15	.15	.36	.8	3.86	1.16
10	.02	.05	3.22	1.78	.38	.80	.35	.76	1.65	1.81	.5	1.24	0	12.49	5.0
11	.04	.03	0	2.08	1.28	.98	.8	.26	.35	.78	.14	.09	.05	6.81	3.36
12	.1	.2	1.02	.28	.42	.6	.2	.2	.25	.14	1.2	1.5	.95	6.76	2.7
13	.0	.03	.8	1.1	3.6	.01	1.22	.9	2.6	0	1.5	0	.04	11.73	4.7
14	0	0	.75	1.35	.05	.95	.05	2.3	.9	.31	.6	.18	.26	7.7	3.2
15	.01	.02	.35	.48	.75	1.08	.75	.9	1.44	1.65	2.25	1.95	3.99	15.59	5.94
16	.01	.02	.85	1.62	.3	.72	.6	.85	1.26	.97	.28	0	.44	7.89	2.47
17	0	0	0	0	.45	1.5	0	1.2	.22	.2	.1	.08	.08	3.83	1.95
MEAN			.51	.95	1.0	.68	.51	.81	.86	.67	.82	.78	.75	8.35	2.95

Basal hour = .15 Peak hour = 2.95 $p = < .001$

TABLE 10: Donor dogs - pouch acid response to an intestinal meal in mEq. acid (portal plasma harvested at 30 mins.)

TABLE 11: Acid response of assay dogs after infusion of control plasma, and of portal plasma harvested at 30 mins.

No.of Dogs	Control Plasma M.Eq/4 hrs.	Test Plasma - M.Eq. Per Half Hour								Total M.Eq.
		1	2	3	4	5	6	7	8	
1	.04	0	.1	.07	0	0	0	0	0	.17
2	.11	.02	1.11	.1	.02	0	0	0	0	1.25
3	.01	0	0	0	0	0	0	0	0	0
4	0	.11	.08	.05	0	0	0	0	0	.24
5	.13	.05	.62	.09	0	0	0	0	0	.76
6	.43	0	0	0	0	0	0	0	0	0
7	.02	.25	.65	.51	.01	0	0	0	0	1.42
8	0	0	.56	2.24	1.05	0	0	0	0	3.85
9	.1	0	.04	.16	.1	0	0	0	0	.3
10	0	0	0	.19	.02	.02	0	0	0	.23
11	.22	0	.21	.08	.01	0	0	0	0	.3
12	.2	.01	.21	.98	.6	.03	0	.01	0	1.84
13	0	0	0	0	0	.02	0	0	0	.02
14	0	.01	.2	0	.07	.05	.01	0	0	.34
15	0	.1	.2	.2	.06	.11	0	0	0	.67
16	0	.01	.56	.28	.06	.04	.01	0	0	.96
17	.02	.08	.15	.05	.01	.07	.01	0	0	.37
MEAN	.08	.04	.28	.29	.12	.02	0	0	0	.75

Mean Control Plasma = .08 Mean Test Plasma = .75 $p = < .02$

No.of Dogs	Basal Half Hours		Test - M.Eq. Acid Per Half Hour											Total	Peak Hour
			1	2	3	4	5	6	7	8	9	10	11		
1	0	0	.12	1.2	.9	1.42	.9	1.03	.55	.42	0	.32	.45	7.31	2.32
2	0	0	.25	1.7	1.05	1.48	1.45	1.32	1.4	.9	.9	.75	.9	12.1	2.93
3	.07	.06	1.4	1.05	.6	1.87	1.15	.52	.16	.36	1.92	2.9	2.8	14.73	5.7
4	.1	.13	.3	.56	1.12	1.36	1.25	1.06	1.5	1.44	1.4	1.45	.6	12.04	2.94
5	.07	.1	.8	.52	1.28	.6	.35	.15	.12	.44	1.26	.9	.6	7.02	2.16
6	.06	.06	.06	.01	.1	.05	.22	.28	.6	1.2	.75	.64	.8	4.71	1.95
7	.01	.03	.14	.12	1.6	1.05	.53	.3	.26	.13	.25	.06	.08	4.52	2.65
8	.07	.14	.75	3.3	4.32	5.5	2.24	2.48	3.22	2.4	2.2	1.8	2.45	30.61	9.82
9	.03	.04	.54	1.2	1.12	1.2	2.0	.65	.52	.65	.52	.42	.13	8.95	3.2
10	.1	.15	1.19	1.92	1.36	1.03	2.7	1.95	.46	.28	.44	.72	.6	12.65	4.65
MEAN			.56	1.16	1.35	1.56	1.28	.97	.88	.8	.96	10.0	.94	11.46	3.83

Basal hour = .13 Peak hour = 3.83 $p = < .001$

TABLE 12: Donor dogs - pouch acid response to an intestinal meal in mEq. acid (portal plasma harvested at 45 mins.)

TABLE 13: Acid response of assay dogs after infusion of control plasma and of portal plasma harvested at 45 mins.

No.of Dogs	Control Plasma M.Eq/4 hrs	Test Plasma - M.Eq. Acid Per Half Hour								Total M.Eq.
		1	2	3	4	5	6	7	8	
1	.16	.1	0	.03	0	0	0	0	0	.13
2	.18	.04	0	.02	.03	.07	.07	0	0	.41
3	.35	0	0	.2	0	.02	0	0	0	.22
4	.52	.05	.33	.04	.02	.01	.05	0	0	.5
5	.01	.01	0	.01	0	0	0	0	0	.02
6	.26	0	0	.05	.04	0	0	0	0	.09
7	.32	.2	.6	.04	.01	0	0	0	0	.85
8	.41	.08	.05	.01	0	0	0	0	0	.14
9	0	0	.12	.19	.07	.09	.01	.01	.01	.5
10	.03	.03	0	.01	.01	0	.02	.01	0	.08
MEAN	.22	.05	.11	.07	.02	.02	.01	0	0	.29

Mean Control Plasma = .22 Mean Test Plasma = .29 $p = < .8$

No.of Dogs	Basal Half Hours		Test - M.Eq. Acid Per Half Hour											Total M.Eq.	Peak Hr.
			1	2	3	4	5	6	7	8	9	10	11		
1	.1	.2	.74	2.34	2.75	1.47	1.17	1.66	2.04	3.5	3.52	3.68	2.24	25.11	7.2
2	.1	.2	1.21	.96	1.12	2.4	.6	.6	.07	.01	.3	0	0	7.27	3.52
3	.06	.25	3.36	3.22	2.24	1.2	.4	.26	.44	.33	.6	.93	.76	13.74	6.58
4	0	0	.04	.27	.5	2.7	.6	.23	.4	.49	.57	1.07	1.16	8.03	3.3
5	.02	.1	.7	.46	.94	.9	.7	1.29	2.07	1.42	1.21	1.15	1.5	12.34	3.49
6	.25	.16	.23	.15	,52	.9	.6	.63	.85	1.1	.62	.32	.51	6.84	1.95
7	.1	.11	.37	.14	.05	.36	.37	.54	.88	.87	.25	.55	1.98	6.36	2.53
8	.07	.1	.02	2.05	1.72	1.76	1.76	1.26	1.12	.56	.61	.4	1.07	12.33	3.77
9	.03	.05	.8	2.72	2.55	2.4	2.88	2.1	1.82	1.05	.75	.59	.34	18.0	5.28
10	.04	.05	.44	.65	1.05	1.28	1.95	1.95	2.24	1.5	1.05	.77	.68	13.56	4.19
MEAN			.79	1.3	1.34	1.54	1.1	1.05	1.19	1.08	.95	.95	1.02	12.36	4.18

Basal hour = .2 Peak hour = 4.18 $p = < .001$

TABLE 14: Donor dogs - acid response to an intestinally-fed meal in mEq. acid (portal plasma harvested at 60 mins.)

TABLE 15: Assay dogs - acid response after infusion of control plasma, and of portal plasma harvested at 60 mins.

No.of Dogs	Control Plasma M.Eq/4 hrs	Test Plasma - M.Eq. Acid Per Half Hour								Total M.Eq.
		1	2	3	4	5	6	7	8	
1	0	.08	0	.18	0	0	0	0	0	.25
2	0	.66	1.26	.17	0	0	0	0	0	2.08
3	0	0	0	.2	.13	.01	0	0	0	.34
4	.22	.06	.02	.05	.15	.24	.1	0	0	.62
5	2.57	0	0	0	0	.08	0	0	0	.08
6	.37	.55	1.3	.1	0	0	0	0	0	1.95
7	0	.04	.02	.03	.05	0	0	0	0	.12
8	.07	0	.01	0	0	0	0	0	0	.01
9	.5	0	.02	.01	0	0	0	0	0	.03
10	.02	0	0	0	0	0	0	0	0	0
MEAN	.38	.14	.26	.07	.03	.03	0	0	0	.55

Mean Control Plasma = .38 Mean Test Plasma = .55 $p = < .7$

No. of Dogs	Basal Half Hours		Test - M.Eq. Acid Per Half Hour											Total	Peak Hour
			1	2	3	4	5	6	7	8	9	10	11		
1	.15	.1	.16	.63	.88	.39	.3	.26	.3	.3	.78	.6	.35	4.95	1.51
2	.04	.06	.61	1.19	1.4	1.2	1.6	1.35	.6	.83	.98	.9	1.25	11.91	2.95
3	.22	.33	1.43	2.62	3.52	1.72	3.56	3.96	3.6	3.8	5.12	6.55	5.6	41.48	12.15
4	.05	.1	.09	.3	.48	.6	1.3	.49	.52	.42	.42	.4	.34	5.36	1.9
5	.05	.08	.08	.13	.48	1.05	.73	.32	.2	.18	.42	.31	.19	4.09	1.78
6	.02	.02	.04	.02	.08	.41	.38	.36	.38	.42	.3	.3	.36	3.05	.8
7	0	0	0	.8	.6	.6	.33	.27	.19	.13	.18	.04	.06	3.2	1.4
8	.01	.02	.06	.98	.48	.56	1.21	1.32	1.26	.46	.21	.13	.3	6.97	2.58
9	0	0	.04	1.42	1.25	.8	.11	0	.03	.02	.08	.14	.15	4.04	2.67
10	.6	.54	.62	1.24	1.05	1.17	1.05	0	0	0	0	0	0	6.27	2.29
MEAN			.31	.93	1.02	.85	1.06	.63	.71	.66	.85	.94	.86	9.13	3.0

Basal hour = .24 Peak hour = 3.0 $p \neq < .001$

TABLE 16: Donor dogs - pouch acid response to an intestinal meal in mEq. acid (portal plasma harvested at 90 mins.)

TABLE 17: Assay dogs - pouch acid response after infusion of control plasma, and of portal plasma harvested at 90 mins.

No.of Dogs	Control Plasma M.Eq/4 hrs	Test Plasma - M.Eq. Acid Per half Hour								Total M.Eq.
		1	2	3	4	5	6	7	8	
1	.07	0	0	0	0	0	0	0	0	0
2	.11	0	.32	.02	0	0	0	0	0	.34
3	0	0	0	0	0	0	.02	.01	0	.03
4	.02	0	0	0	.02	0	0	0	0	.02
5	.23	0	0	.32	.04	0	0	0	0	.36
6	.42	0	.11	.11	.06	0	.03	.04	.06	.41
7	0	0	0	0	0	0	0	0	0	0
8	.08	0	.18	.22	0	.15	0	0	.1	.65
9	0	0	0	0	0	0	0	0	.01	.01
10	0	0	.02	.07	.11	.14	0	0	.02	.36
MEAN	.09	0	.06	.07	.02	.03	.01	.01	.02	.22

Mean Control Plasma = .09 Mean Test Plasma = .22 $p = < .1$

Portal Blood Harvest Time-min.	No. of Dogs	Time to onset of Gastric Secretion-min. Mean	Range	Time to peak Gastric Secretion-hrs Mean	Range	Mean peak Hourly Secretion % Above Basal
15	11	39	30-120	2.0	1-5.5	5600
30	17	50	30- 90	3.1	1-5.5	2240
45	10	42	30-150	3.4	2-5.5	2846
60	10	39	30- 90	3.2	1-5.5	1990
90	10	54	30- 90	2.7	1.5-5.5	1150
MEAN	58	45 min.	30-150 min.	2.9 hrs	1-5.5 hrs	2765

TABLE 18: Time Course of Action of Intestinal Phase Hormone in Donor Dogs With Portacaval Shunts.

TABLE 19:

MEAN GASTRIC ACID RESPONSE OF ASSAY DOGS TO INTRAVENOUS INFUSION OF PORTAL BLOOD PLASMA WITH INTESTINAL PHASE HORMONE

Portal Blood Harvest Time (minutes)	No. Dogs	Control Plasma mEq acid/4 hr	Test Plasma mEq acid/4 hr	Increase (%)
15	11	40	120	200
30	17	80	750	838
45	10	220	290	32
60	10	380	550	45
90	10	90	220	144

TABLE 20:

RESPONSE OF ASSAY DOG GASTRIC MUCOSA TO INTRAVENOUS INFUSION OF PORTAL BLOOD PLASMA WITH INTESTINAL PHASE HORMONE

Portal Blood Harvest Time (minutes)	Number of Dogs Secreting >.01 mEq acid	Time of Onset of Gastric Secretion (minutes)		Time of Peak Gastric Secretion (hours)	
		Mean	Range	Mean	Range
15	7	51	30–90	1.4	1–2
30	15	57	30–150	1.4	1–3
45	9	48	30–90	1.4	1–2
60	8	57	30–150	1.9	1–3
90	7	90	60–180	2.0	1–3
MEAN	46	61	30–180	1.6	1–3

Type of Study	No. of Dogs	Time to onset of Gastric Secretion-min. Mean	Range	Time to peak Gastric Secretion-hrs Mean	Range
Single Balloon Distension	11	45	30-150	3.1	1-5.5
Multiple Balloon Distension	18	45	30-120	3.7	1-5
Jejunal Segment Meal	8	57	30-120	4.4	3-5
MEAN	37	49 min.	30-150 min.	3.9 hrs	1-5.5 hrs

TABLE 21: Time Course of Action of Intestinal Phase Hormone in Dogs with Portacaval Shunts.

TABLE 22: Time Course of Action of Intestinal Phase Hormone in Cirrhotic Patients with Portacaval Shunts.

Type of Study	No.	Time of onset of Gastric Secretion-mins. Mean	Range	Time of Peak Gastric Secretion-hrs. Mean	Range
Intestinal Meal	15	75	30-180	2.7	2-4
Jejunal Meal	10	63	30-120	2.4	2-3
Balloon Distension	10	69	30-180	2.9	1-4
MEAN	35	69 min.	30-180 min.	2.6 hrs.	1-4 hrs.

Harvest Time mins.	Nonreactions				Reactions			
	Control		Test		Control		Test	
	No.	mEq.	No.	mEq.	No.	mEq.	No.	mEq.
15	7	.02	11	.12	4	.06	0	0
30	5	.05	6	.29	12	.09	11	.99
45	4	.32	5	.15	6	.16	5	.44
60	4	.77	3	.32	6	.12	7	.65
90	6	.15	9	.20	4	.01	1	.36
MEAN	26	.26	34	.22	32	.09	24	.49

TABLE 23: Mean gastric acid response of hypersensitive and nonsensitive assay dogs following infusion of control and test (portal) plasma.

CHAPTER V

PRELIMINARY EXTRACTION OF THE INTESTINAL PHASE HORMONE FROM THE PORTAL BLOOD OF THE DOG

PRELIMINARY EXTRACTION OF THE INTESTINAL PHASE HORMONE FROM THE PORTAL BLOOD OF THE DOG.

The investigations described in the previous chapter have demonstrated that in the dog the time of maximum concentration of the intestinal phase hormone in the portal blood is between 30 to 45 minutes after the start of an intestinally-fed meal. It seemed logical to attempt extraction of the hormone in crude form by simple biochemical procedures in the first instance from portal plasma harvested at the optimal time from secreting donor dogs. The potency of the crude extracts was bioassayed in dogs with Heidenhain pouches.

MATERIALS AND METHODS

A group of donor dogs (with splenectomy, antrectomy, Heidenhain pouch, Roûx-en-Y jejunal fistula, end-to-side portacaval anastomosis and an indwelling portal vein catheter) was prepared for portal blood harvesting in the usual manner. After one hour of basal gastric secretion, the anaesthetized animal was stimulated with an intestinally-fed meal, containing 48G of protein, 19G of fat and 84G of carbohydrate made up to 500 ml. with milk, given through the jejunal fistula over 15 minutes. The gastric secretion from the Heidenhain pouch was collected every half-hour for

$5\frac{1}{2}$/

5½ hours after the meal, and the acid content of each sample was estimated by titration with 0.1N sodium hydroxide to pH 4.5 using Topfer's reagent as the indicator. Results were expressed as mEq. per test period.

At 30 minutes after the start of the meal, 500 ml. portal blood was collected over 15 minutes by siphonage into sterile bottles, containing 2,500 units (0.5 ml.) of heparin, which were standing in an ice-water mixture. The portal blood was centrifuged at 4°C, obtaining approximately 350 ml. of plasma from each unit of portal blood. Only plasma collected from a secreting donor dog (producing at least 3 mEq. of hydrochloric acid in 5½ hours) was considered for further biochemical processing. Eighteen units of active portal plasma were collected from secreting donor dogs and these were separated into three groups for chemical extraction.

After processing the portal plasma, the extract was administered by intravenous infusion over 15 minutes into a fasted assay dog restrained in a Pavlov stand set in a quiet environment. The acid secretion was measured for one hour before and half-hourly for four hours after the administration of the extract. The results in mEq. of acid were/

were compared in the same dog with those associated with administration of an extract prepared in an identical manner from 350 ml. bank plasma. The bank plasma, which served as a control, was freshly-obtained from the peripheral venous blood of a fasted normal dog. The three extraction procedures applied to the samples of control and test plasma were as follows:

1. HEATING. The control and test portal plasma from five donor dogs were heated in an autoclave set at 270°C. for 20 minutes, and during this period the plasma boiled for at least five minutes. After the plasma had been cooled in the autoclave, it was centrifuged at 4°C. to remove the heat-coagulable precipitate, which was discarded, and the supernatant measuring 150 - 200 ml. was kept for testing.

2. ISOPROPANOL TREATMENT. Eight samples of control and test plasma were treated with isopropyl alcohol, in the ratio of two volumes of plasma to one volume of alcohol. A precipitate formed which was removed by centrifugation. The supernatant was treated with three volumes of ether to remove any residual alcohol. The mixture was allowed to settle into two phases, with a chiefly ether fraction at the top, and a mainly aqueous fraction at the bottom. The/

The top phase was siphoned off and discarded; residual ether in the aqueous phase was blown off with an air-stream, and about 250 - 300 ml. of extract from each sample was retained for testing.

3. HEATED DILUTE PLASMA. In the first procedure which employed heated plasma, a much-reduced volume of fluid was obtained by centrifugation. Because it appeared that the available method of centrifugation at 10,000 r.p.m. was not powerful enough to extract all of the fluid entrapped in the mass of coagulated protein, it was decided to dilute the plasma in an attempt to increase the efficiency of centrifugation. An equal volume of normal saline was now added to each unit of control and test plasma, and the sample was heated in the autoclave and centrifuged as before, obtaining approximately 350 ml. of supernatant for infusion into the assay dog.

RESULTS.

The results obtained during preliminary attempts at extraction of the intestinal phase hormone from the portal plasma of the dog proved to be disappointing, despite the fact that only plasma which demonstrated gastric-stimulating activity was used. Although the mean gastric acid secretion in the assay dogs following intravenous infusion of/

of heated active portal plasma increased three-fold over the control value (Table 24), and the mean acid secretion following infusion of isopropanol-treated portal plasma was doubled (Table 25), the quantities of acid secreted were small and insignificant, and in no way did these compare with the results achieved by administration of untreated fresh portal plasma. An attempt to increase the yield of hormone in the supernatant by diluting the plasma prior to heating and centrifugation also proved to be fruitless (Table 26) as the results were meaningless.

DISCUSSION

In the light of previous unpublished experience with material isolated from hog mucosa in this laboratory, it now appears that the active hormone is probably a low molecular weight acidic protein or peptide, and that it is stable despite considerable heating, which are properties held by the three known digestive tract hormones, viz. gastrin, secretin and cholecystokinin-pancreozymin. Isolation of the hormone from the portal blood of the dog seemed feasible now that the time of hormone release into portal venous blood, and the time of peak hormone concentration had been established.

Proteins/

Proteins can be fractionated according to electrical charge (iso-electric precipitation, electrophoresis, altered celluloses and Rivanol); molecular size (treatment with ammonium sulphate, passage through a Sephadex gel column, and dialysis); solubility (alcohol precipitation); salt interaction ("salting out") and denaturation by heat and other agents. Each method has its own specificities and difficulties. Since handling of large quantities of plasma and speed of fractionation appear to be vital, all methods are not equally applicable. Of these, denaturation of the bulk of plasma proteins by heat appeared to be the logical initial step in the extraction procedure especially since this method was used early in the protocol for the isolation of gastrin (Gregory and Tracy, 1964), secretin and cholecystokinin-pancreozymin (Jorpes, 1968).

When the assay dog is used as an indicator system, alternative methods of extraction incur several hazards. Since alcohol is a stimulant of gastric acid secretion, it had to be ensured that all the residual isopropyl alcohol had been removed from the precipitate by washing with ether. If treatment of the portal plasma with ammonium sulphate is to be employed, then the residual ammonium sulphate must be removed by time-consuming dialysis, before the extract can be administered to the assay dog. Precipitation of/

of a protein fraction with trichloracetic acid was considered, but the difficulty is that any residual acid in the precipitate would be lethal to the dog. Dialysis of the portal plasma was feasible, but again would involve a length of time inconvenient to the laboratory. Instead of utilizing assay dogs, an alternative test system for a gastric acid stimulator would be to measure the potency of the crude extracts with the isolated gastric mucosa of the frog (Harris, Nigon and Alonso, 1969), but the drawbacks are that substances lethal to the dog would probably kill the frog mucosa, and also that the tests can only be done in midsummer, because the frog develops gastric acid hypersecretion during winter hibernation.

Although the results of these preliminary attempts at isolation of the intestinal phase hormone have proved to be disappointing, further attempts at its isolation from plasma will be pursued. Development of a rewarding method of extraction would favour isolation of the hormone responsible for the intestinal phase of gastric secretion from portal blood harvested at the optimal time (after an intestinal meal) from cirrhotic patients with portacaval anastomosis.

TABLE 24: Gastric acid secretion in assay dogs following intravenous infusion of heated control and portal plasma

Test No.	Donor Dogs Basal mEq/Hr.	Donor Dogs Test mEq/5½ Hr.	Assay Dogs Plasma - mEq/4 Hr. Control	Assay Dogs Plasma - mEq/4 Hr. Portal
1	.01	3.36	.03	.15
2	0	3.16	0	0
3	.1	26.15	0	.01
4	.15	12.45	.02	0
5	.01	17.11	0	0
Mean	.05	12.45	.01	.03

TABLE 25: Gastric acid secretion in assay dogs following intravenous infusion of isopropanol-treated control and portal plasma

	Donor Dogs		Assay Dogs	
Test No.	Basal mEq/Hr.	Test mEq/5½ Hr.	Plasma - mEq/4 Hr. Control	Portal
1	.05	23.75	.02	.07
2	.01	14.73	0	.35
3	0	11.36	0	.06
4	.1	11.05	0	.13
5	.05	4.48	.01	.02
6	.12	29.78	0	.01
7	.06	9.63	.29	.06
8	.23	9.40	.04	.02
Mean	.08	14.27	.04	.09

TABLE 26: Gastric acid response in assay dogs following intravenous infusion of heated dilute control and portal plasma

	Donor Dogs		Assay Dogs	
Test No.	Basal mEq/Hr.	Test mEq/5½ Hr.	Plasma - mEq/4 Hr. Control	Portal
1	.12	22.71	.06	0
2	.25	9.30	.08	.08
3	.25	17.73	.12	.01
4	.12	7.01	.08	0
5	.11	6.93	.13	.01
Mean	.17	12.74	.09	.02

CHAPTER VI

PORTACAVAL SHUNT-RELATED GASTRIC HYPERSECRETION IN THE PIG

PORTACAVAL SHUNT-RELATED GASTRIC HYPERSECRETION IN THE PIG

Studies performed in this laboratory over the past six years have demonstrated conclusively that the gastric acid hypersecretion associated with a portacaval shunt in the dog, and in man, is due to unmasking by hepatic bypass of a potent hormone elaborated by the jejunum (Orloff and others, 1969a, 1970a; Villar-Valdes and others, 1969). A dual effort to isolate and identify this hormone is currently in progress. In preliminary experiments the active agent has been recovered in crude form from the portal blood of shunted dogs during intestinal feeding, but the amount available from this source is limited. A potentially more promising line of investigation is to isolate the hormone, or its precursor, from the jejunal mucosa where it originates. Large quantities of hog mucosa are available as an alternative to pursuing these studies in dogs, but shunt-related gastric hypersecretion has not been demonstrated in the pig. The following investigation was designed to determine if the pig develops gastric hypersecretion after a portacaval shunt in response to food comparable to that observed in the dog and in man.

MATERIALS AND METHODS

Six/

Six 6-week old weanling pigs weighing 18 - 20 Kg. were dewormed and then were allowed three weeks to become accustomed to standard Purina pig chow, to be trained to walk on a leash, and to stand quietly in a Pavlov stand. Body weight was recorded twice weekly and at the end of the conditioning period, all the animals were eating well and gaining weight. Each pig was now subjected to the first of two surgical procedures:

(a) Construction of a Heidenhain Pouch

After the pig had been fasted overnight, the animal was premedicated with Atropine 0.5 mg. and *Innovar Vet 4 ml. injected intramuscularly. One hour later, the animal was anaesthetized lightly with 2.0 ml. Pentothal 5 per cent administered through a scalp vein needle inserted into an ear vein. As soon as the pig was induced, intubation of the trachea was accomplished using a long straight-bladed laryngoscope, and respiration was assisted with mechanical ventilation. The anaesthesia was carefully monitored throughout the procedure, since unpredictable respiratory depression is the rule rather than the exception in the pig. A slow intravenous infusion/

* (Each ml. contains Fentanyl 0.4 mg. and Droperidol 20 mg. Prepared by Pitman-Moore, Fort Washington, PA 19034)

infusion of 1000 ml. 10 per cent Dextrose in 0.45 per cent saline was commenced, and supplementary Pentothal 5 per cent was administered as necessary.

The abdomen was opened through an upper midline incision. The stomach was delivered into the wound and a greater curvature gastric pouch was constructed, supplied by a single vascular pedicle arising from the splenic vessels. The Heidenhain pouch was at least 7 cm. long in its greatest dimension. A stainless steel cannula was inserted into the pouch, secured with a silk purse-string suture and brought out through a left upper quadrant stab wound close to the midline, to avoid acid burns.

Food was offered on the first postoperative day, and Ampicillin 1 G was given intramuscularly on the day of operation and for 3 postoperative days. During a recuperative period of three weeks, each pig was placed daily in a Pavlov stand to maintain its conditioning to the test environment. Following the recovery period, the acid secretory response of the pig was determined at one half-hour intervals for a control basal hour and for four hours following a standard oral meal*. Each pig was given three/

* 14 oz. "Kal Kan M.P.S. Chunk" dog food

three test meals in a quiet room with a 48 hour rest period between tests. The volume of each half-hour sample of gastric juice was measured and the acid content was determined by titration with 0.1N sodium hydroxide to pH 4.5 using Topfer's reagent as the indicator. The results were expressed as mEq. of acid per test period.

(b) Portacaval Shunt Operation (Eck fistula)

Upon completion of the three control test studies, the pig underwent a portacaval anastomosis. The animal was anaesthetized as in the first procedure, placed in the left semi-lateral position, and a right subcostal incision was made. The portal vein was mobilized from its trifurcation to the pancreatico-duodenal vein, and the splenic vein was cleared for 1.0 - 2.0 cm. No attempt was made to clean the caudate lobe of the liver from the inferior vena cava. On completion of the shunt, the hepatic limb of the portal vein was ligated between the shunt and the pancreatico-duodenal vein.

Ampicillin 1 G was injected intramuscularly on the operation day, and daily thereafter for three days. Soft food was offered on the first postoperative day. The stand training continued during the recovery week. One week after the shunt, each animal was tested again for its/

its response to a standard oral meal in exactly the same manner as before the shunt.

RESULTS.

The gastric acid responses to oral meals given to the six conditioned pigs before portacaval anastomosis are given in Table 27, and the acid outputs after shunt are shown in Table 28. Using these data, the mean values for the basal, peak hourly, and total acid secretion in response to thirty-four test meals before and after portacaval anastomosis were calculated (Table 29). All of the meals produced a significant increase in pouch acid secretion presumably because of cephalic and direct antral stimulation, but after hepatic bypass of the portal blood, and unmasking of the humoral stimulator from the intestine, the acid secretory response nearly doubled. The total secretory response to the meal before and after portacaval shunt differed significantly with a p of less than 0.02.

Peak hourly secretion after shunt was increased above the preshunt value, differing significantly with a p of less than 0.05. The cumulative acid secretory response to the meals before and after portacaval anastomosis is shown in Fig. 5. The curves are similar in the first hour, but diverge as the effect of the intestinal hormone on the parietal/

parietal cells progressively becomes more dominant.

When the secretory pattern of response to a standard oral meal fed to the shunted pigs was examined (Table 30), it was found that the time of onset of gastric secretion was delayed for a mean 34 minutes, with a range of 30 - 60 minutes. The mean peak hourly secretion occurred at 2.5 hours after the start of the meal, with a range of 1 to 4 hours.

DISCUSSION.

The preparation used in this study did not exclude the effect of antral stimulation, but the output of gastrin should have been similar in response to both the preshunt and the postshunt meals. In fact, there is evidence that bypassing portal blood into the systemic circulation actually decreases the amount of gastrin produced by the antrum in response to an ingested meal (Clendinnen and others, 1970). The influence of gastrin on the parietal cells also should not have been affected by hepatic bypass of the portal blood because gastrin is not sufficiently degraded as it passes through the liver in the portal blood (Thompson and others, 1969). Thus, the nearly two-fold increase in acid secretion observed after portacaval anastomosis cannot be attributed to variations in gastrin production/

production or destruction. Rather, shunting the portal blood directly into the systemic circulation permitted a second potent humoral agent, which normally would have been degraded by prior passage through the liver, to exert its additive effect on the parietal cell test mass.

The influence of this second hormone of intestinal origin became predominant as the meal left the stomach and began to distend the proximal jejunum. The cumulative acid secretion in response to the meals reflected the change in balance of the effects of gastrin and the intestinal humoral agent. Initially the preshunt and postshunt secretory rates were similar under the dominant influence of gastrin, but as more of the meal moved into the intestine and the second humoral agent exerted its effect, the acid secretion of the shunted animals increased at a much greater rate. In the unshunted preparation the same stimulus of food in the intestine was present, and the humoral agent also should have been stimulated. However, without hepatic bypass the intestinal hormone was largely inactivated by passage through the liver before it came in contact with its target organ, the parietal cell mass.

When the patterns of secretion in the pigs after portacaval shunt were examined, it was found that in the relatively/

relatively small number of animals studied, the onset of gastric secretion began at 34 minutes after the meal, occurring a little earlier than in the dog or man, even though the stimulus was food taken by mouth. As before, peak gastric secretion was delayed for 2.5 hours, indicating that some intermediary steps may take place before the parietal cells are effectively stimulated.

It has been demonstrated for the first time in these investigations that the pig develops portacaval shunt-related gastric acid hypersecretion in response to food comparable to that observed in the dog and man. Therefore, porcine jejunal mucosa is an appropriate source for isolation of the intestinal phase hormone and efforts to isolate and characterize this hormone are now currently in progress.

Pre-Shunt		CONTROL-½ hrs		TEST - ½ hours								TOTAL
		1st	2nd	1st	2nd	3rd	4th	5th	6th	7th	8th	
Pig.1	Meal 1	0	0	0	.35	.40	.48	.43	.25	.39	.02	2.32
	2	0	0	.12	.88	.39	.11	0	0	0	.43	1.93
	3	0	0	0	0	.36	.32	2.16	1.3	1.26	.91	6.31
Pig.2	Meal 1	0	0	0	0	0	.15	.06	.41	.21	0	.83
	2	0	0	0	.14	.1	0	.18	.36	.28	.08	1.14
	3	0	0	0	.05	.27	.14	.16	.16	.28	.16	1.22
Pig 3	Meal 1	0	0	.5	.63	.6	.21	.2	.08	.02	.02	2.26
	2	0	0	.15	.2	.1	.1	.04	.05	.21	.24	1.09
	3	0	0	0	0	0	0	0	0	0	0	0
Pig 4	Meal 1	.01	.01	.27	.05	.14	.28	.22	.2	.1	.4	1.66
	2	0	.01	.1	.37	1.16	.35	.23	.27	.2	.18	2.86
	3	0	.01	.05	.25	.07	.16	.25	.4	.63	1.14	2.95
Pig 5	Meal 1	0	0	4.0	3.45	1.82	1.95	3.84	3.6	3.83	3.94	26.43
	2	0	0	.99	1.04	1.65	1.3	1.82	1.8	1.92	2.4	12.92
	3	0	0	.45	.8	.36	.72	1.04	2.16	2.1	1.43	10.06
Pig 6	Meal 1	0	0	.88	.9	1.17	.78	1.44	.83	.14	.55	6.69
	2	.03	.04	1.65	1.48	.96	.84	1.12	.91	1.32	1.08	9.36
	3	.01	.02	.28	.33	.04	.36	.03	.44	.36	.33	2.17

TABLE 27: Gastric acid secretory responses (m.Eq) to meals given to six pigs (pre-shunt).

Posthunt		CONTROL-½ hrs		TEST - ½ hours								TOTAL
		1st	2nd	1st	2nd	3rd	4th	5th	6th	7th	8th	
Pig 1	Meal 1	0	.01	.48	1.1	1.42	1.0	1.45	1.28	.4	.45	7.58
	2	0	0	.25	.24	.5	.55	.41	.32	.5	.43	3.2
Pig 2	Meal 1	0	.01	.28	2.1	.9	1.2	1.9	.37	.46	.57	7.78
	2	0	.01	1.18	1.21	.5	1.5	1.58	1.45	2.2	4.05	13.67
	3	0	0	.25	.42	.56	1.2	1.0	.99	1.4	2.16	7.98
Pig 3	Meal 1	0	0	0	.4	1.2	.6	.75	.85	.53	.3	4.63
	2	0	0	.76	.75	.5	.23	.18	.15	.15	.28	3.0
Pig 4	Meal 1	0	0	0	.65	1.18	1.56	.96	.77	.45	.54	6.11
	2	.02	.04	.63	.99	1.08	1.44	1.43	1.17	1.22	1.08	9.04
	3	0	0	.2	1.11	1.5	.6	1.28	.66	.61	.26	6.22
Pig 5	Meal 1	0	0	.1	1.68	1.69	2.1	2.73	3.15	2.17	3.08	16.7
	2	0	0	.28	1.54	3.3	3.6	4.8	4.95	4.35	3.4	26.22
	3	0	0	.6	.7	1.82	1.35	3.0	2.55	2.25	2.75	15.02
Pig 6	Meal 1	0	.02	.28	.66	.51	.65	.44	.38	.6	.3	3.82
	2	.02	.03	.39	.95	.55	.35	.33	1.54	1.69	1.85	7.65
	3	0	.01	.52	.9	1.04	1.6	1.05	1.0	1.12	1.04	8.27

TABLE 28: Gastric acid secretory responses (m.Eq) to meals given to six pigs (postshunt).

Pig No.	BEFORE PORTACAVALSHUNT			AFTER PORTACAVAL SHUNT		
	Basal Acid mEq/hr	Peak Acid mEq/hr	Total Acid mEq/4 hr	Basal Acid mEq/hr	Peak Acid mEq/hr	Total Acid mEq/4 hr
1	0	1.88	3.52	.01	1.89	5.39
2	0	.57	1.06	.01	4.3	9.81
3	0	.56	1.12	0	1.66	3.82
4	.01	1.27	2.49	.02	2.74	7.12
5	0	5.45	16.14	0	7.06	19.31
6	.03	2.07	6.07	.02	2.45	7.37
Mean	.01	1.97	5.07	.01	3.35	9.33

TABLE 29: Mean values for basal, peak hourly and total acid secretion in response to thirty-four test meals before and after portacaval shunt.

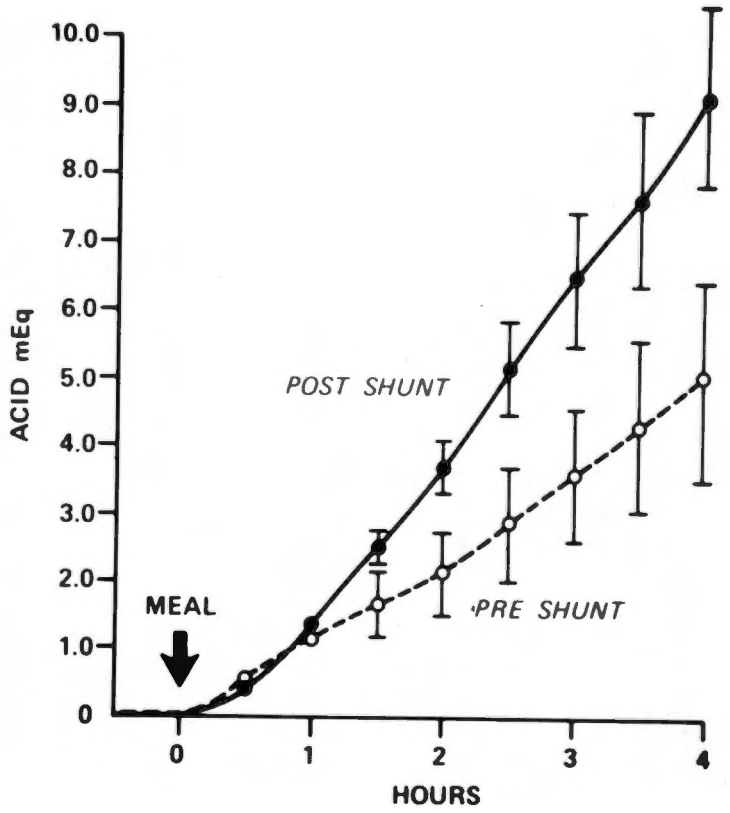


Fig. 5:: Cumulative acid secretion before and after portacaval shunt in pigs.

TABLE 30: Time course of action of intestinal phase hormone in pigs with portacaval shunts.

Pig No.	Time of onset of Gastric Secretion-mins. Mean	Range	Time of peak Gastric Secretion-hrs. Mean	Range
1	30	30	2.5	2-3
2	30	30	3.0	3-4
3	45	30-60	1.5	1-2
4	40	30-60	2.3	2-3
5	30	30	3.0	3
6	30	30	2.7	2-4
Mean	34 min.	30-60 min	2.5 hrs	1-4 hrs

CHAPTER VII

ISOLATION OF A GASTRIC SECRETORY HORMONE FROM PIG INTESTINAL MUCOSA

ISOLATION OF A GASTRIC SECRETORY HORMONE FROM PIG INTESTINAL MUCOSA

A programme for isolation and identification of the intestinal phase hormone logically must involve efforts to extract the agent or its precursor from the mucosa of the small intestine. After preliminary experiments had demonstrated that the pig develops shunt-related gastric hypersecretion similar to that found in the dog and in man, the pig was selected as the source of intestinal mucosa because no other species of intestine is available locally in sufficient quantity. The gastric acid response of assay dogs with Heidenhain pouches to crude extracts isolated from hog intestinal mucosa was now examined.

MATERIALS AND METHODS

In general, hormones isolated from endocrine tissue of mesodermal origin have been found to be steroids, while those obtained from ectodermal or endodermal structures have proved to be amino acid residues. All of the digestive tract hormones that have been isolated in pure form, viz. secretin, gastrin and cholecystokinin-pancreozymin, have been found to be low molecular weight peptides devoid of carbohydrate or other moieties. Therefore/

Therefore, it seemed reasonable to assume that the intestinal phase hormone would prove to be a peptide, and as a first step, to attempt to extract it from its site of origin by a method similar to that used by Gregory and Tracy (1964) for the isolation of gastrin from hog antral mucosa. Their procedure involved boiling of the mucosa to denature the heat-sensitive proteins, absorption on to an anion-exchange cellulose, extraction of the active material with alkali, precipitation with acid, extraction with isopropanol and ether separation on a Sephadex column, and further purification by electrophoresis.

The mucosa from the entire small and large bowel* was purchased on a twice-weekly basis in 500 gal. batches from Clougherty Brothers Packing House (an abbatoir for Farmer John brand pork products) in Los Angeles. Within minutes of the death of the animal, the intestinal mucosa was extruded by machine from the entire length of the jejunum, ileum and colon, and was collected with its wash water as a slurry to which the preservative 0.5 per cent benzyl alcohol was continuously added at the abbatoir to inhibit bacterial growth. A batch of 500 gal. slurry represents a/

* It was not possible to rearrange labour protocol so that only the jejunal mucosa was collected.

a half-day's slaughtering of 2500 - 3000 pigs and yields up to 2 - 3 G of crude extract. The slurry was processed in the laboratory within 12 hours of collection. The steps in the extraction procedure were as follows (Fig. 6):

1. The intestinal mucosa slurry was boiled at 100°C. for one hour. After cooling overnight, the heat-coagulable precipitate was removed by centrifugation and it was discarded.

2. Assuming that the agent was an acidic peptide, the supernatant was treated with large quantities (10 kg.) of diethyl-amino-ethyl (DEAE) cellulose, a basic anion exchange resin which selectively absorbs acidic peptides. After three hours, the cellulose was separated by centrifugation and it was washed repeatedly.

3. The active humoral agent was eluted from the cellulose by treatment with 0.1N sodium hydroxide. The mixture was centrifuged and the pH of the alkaline supernatant was adjusted to 4 in 11N hydrochloric acid. While the mixture was allowed to stand at 4°C. overnight, a voluminous white precipitate formed and settled.

4. The clear supernatant liquid was decanted and discarded. The/

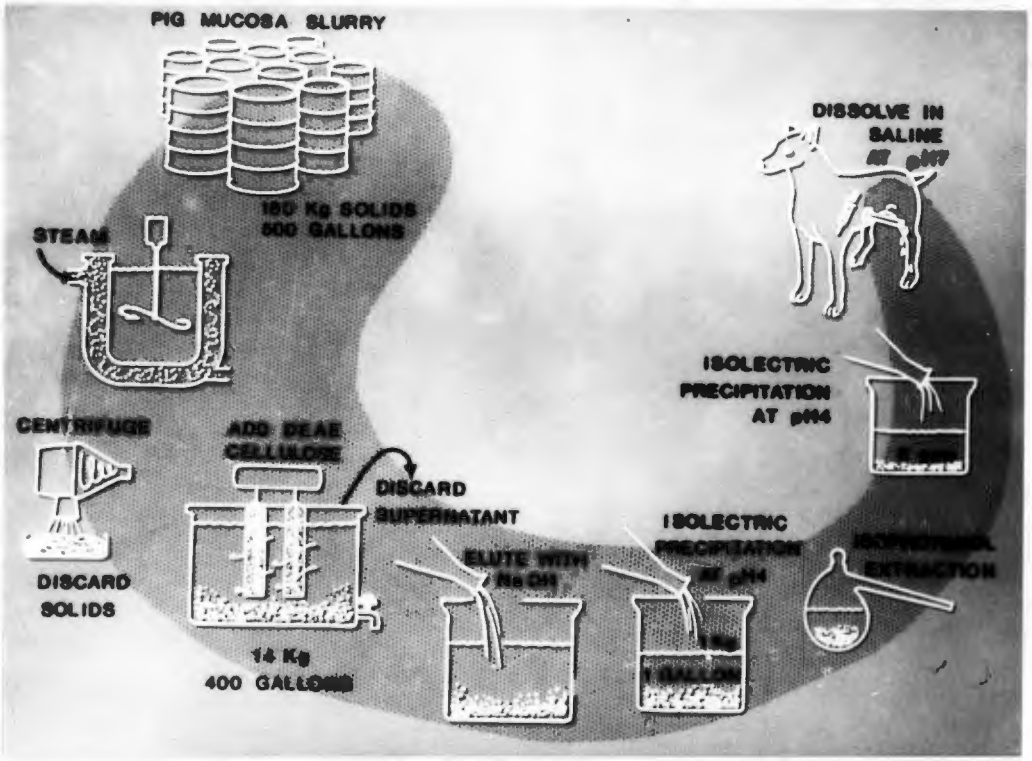


Fig. 6: Isolation of the hormone responsible for the intestinal phase of gastric secretion from pig intestinal mucosa.

The pH of the remaining mixture was adjusted to 7 with 0.1N sodium hydroxide. The insoluble material was removed by centrifugation at 4°C. and was discarded.

5. The supernatant was adjusted to pH 4 with 5N hydrochloric acid, and it was allowed to stand at 4°C. overnight. The supernatant was siphoned off and discarded. The remaining precipitate was resuspended in an equal volume of water, and dibasic potassium phosphate (300 G per 600 ml. precipitate) was added.

6. The solution was treated with isopropyl alcohol (450 ml. per 600 ml. aqueous solution) which extracted the active agent. The mixture was stirred vigorously for 30 minutes and allowed to stand until three layers eventually separated from the emulsion:

(a) top - alcohol-water phase, with alcohol predominating.

(b) a central denatured protein phase

(c) bottom - water-alcohol phase with water predominating.

The top phase was aspirated off and the remaining two phases were discarded.

7. For every litre of the alcohol-water phase, 100 ml. water/

water was added. An equal volume of ether was added to the alcohol-water solution, the mixture was stirred vigorously, and the phases were allowed to separate. The lower aqueous phase was aspirated and the ether was discarded.

8. The pH of the aqueous phase was adjusted to 4 and while it was allowed to stand overnight, a precipitate formed.

9. The supernatant was siphoned off and discarded. The remaining solids were centrifuged and the resultant precipitate was taken up in 0.1N sodium hydroxide. When the precipitate had been completely dissolved, the pH was adjusted to 7.4, and the volume was made up to 250 ml. with sterile 0.9N saline.

10. The solution was sterilized by passage through a millipore filter which had a membrane of 0.22 microns. Aliquots were withdrawn for gastrin assay and total protein determinations.

The crude mucosal extract from each batch of intestinal slurry was administered as an intravenous infusion over 15 minutes to fasting (assay) dogs with Heidenhain pouches. Pouch acid secretion was measured at/

at half-hourly intervals for one hour before, and for three hours after infusion of the mucosal extract. Paired control studies using diluent were run in each test in the same dog. Twenty-five extracts were tested over a wide dosage range of protein.

RESULTS

The secretory response of the Heidenhain pouches to pig intestinal mucosa extracts is displayed in Table 31. For a scale of doses from .003 - 3.0 G., the range of total acid output varied between nil to 2130 µEq. over three hours. The basal secretion and the acid response to the control solution was zero in all of the dogs. The doses were arranged into four groups by weight (Table 32), and as can be seen, there was a typical dose-response relationship. A commensurate rise in gastric acid output was evident as the dose of mucosal extract was increased. A threshold response of 397 µEq. was elicited by a dose of approximately 1 G. Above 2G., there was a highly significant mean total acid secretory response of 1115 µEq.

Examination of the pattern of pouch mucosal response (Table 33) revealed the onset of gastric acid secretion to occur at a mean of 45 minutes after the application of the/

the stimulus, with a range of 30 - 90 minutes. The mean time of peak gastric secretion was 1.25 hours after the commencement of the infusion, with a range of 1 - 3 hours. The mean secretory pattern of the assay dogs in response to six doses of extract ranging between 2 - 3 G. is shown in Fig. 7. Gastric acid secretion reached a peak within the first hour after the start of the infusion, and the rate of secretion dropped to zero at the end of two hours.

Radioimmuno assay of the six largest doses of intestinal mucosa extract for gastrin obtained a mean 0.09 µG per dose (Table 34).

Assay Dog	Dose in G.	Test - mEq. Acid Per Half Hour						Total mEq.
		1	2	3	4	5	6	
1	.003	0	.005	.005	0	0	0	.01
2	.003	0	.02	.01	0	0	0	.03
3	.004	0	0	0	0	0	0	0
4	.006	.01	.03	.04	0	0	0	.08
5	.006	.09	.07	.03	.01	0	0	.2
6	.0075	0	0	0	0	0	0	0
7	.007	0	0	0	0	0	0	0
8	.0075	.03	.03	0	0	0	0	.06
9	.017	0	0	0	0	0	0	0
10	.017	.02	.05	.01	0	0	0	.08
11	.028	.07	.04	0	0	0	0	.11
12	.035	.04	.05	0	0	0	0	.09
13	.04	.05	0	0	0	0	0	.05
14	.04	0	0	0	0	0	0	0
15	.12	.01	.02	0	0	.01	.07	.11
16	.12	0	0	0	0	0	0	0
17	.7	0	.53	.03	.01	0	0	.57
18	1.4	.13	.04	.02	0	0	0	.19
19	1.5	.21	.19	.02	.01	0	0	.43
20	2.0	0	.6	1.5	.03	0	0	2.13
21	2.3	1.0	.07	.01	.02	0	0	1.1
22	2.5	.5	.43	.03	0	0	0	.96
23	2.5	.51	.41	.02	.02	0	0	.96
24	3.0	.42	.03	0	0	0	0	.45
25	3.0	.72	.33	.04	0	0	0	1.09

TABLE 31: Acid secretory response of assay dogs with Heidenhain pouches to intravenous pig intestinal mucosa extract.

TABLE 32:

RESPONSE OF ASSAY DOG GASTRIC MUCOSA TO INTRAVENOUS INFUSION OF EXTRACT OF PIG INTESTINAL MUCOSA

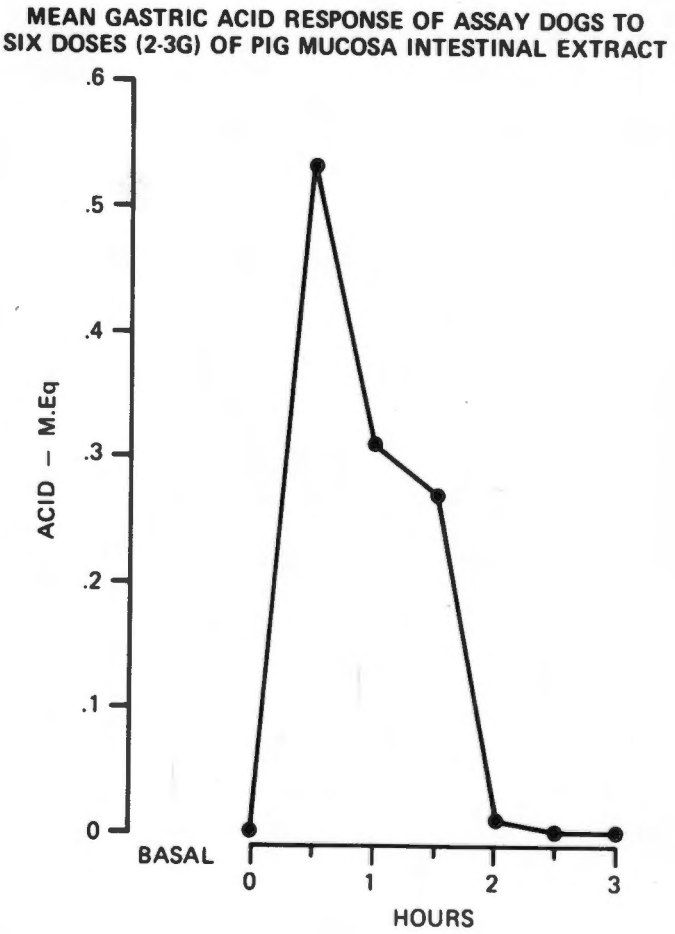
DOSE IN GRAMS	NUMBER OF EXTRACTS	MEAN ACID RESPONSE μEq/3 Hr	
		CONTROL DILUENT	MUCOSA EXTRACT
.003– .008	8	4	49
.017– .120	8	6	55
0.70 –1.50	3	0	397
2.00 –3.00	6	0	1115

TABLE 33:

TIME COURSE OF ACTION OF INTRAVENOUS INTESTINAL MUCOSA EXTRACT IN ASSAY DOGS WITH HEIDENHAIN POUCHES

DOSE IN GRAMS	NUMBER OF EXTRACTS > .01mEq	TIME OF ONSET OF GASTRIC SECRETION-min. MEAN	RANGE	TIME OF PEAK GASTRIC SECRETION-hrs. MEAN	RANGE
.003– .008	4	45	30–60	1.0	1.0
.017– .120	5	60	30–90	1.8	1-3
0.70 –1.50	3	40	30–60	1.0	1.0
2.00 –3.00	6	36	30–60	1.2	1-2
	18	45	30–90	1.25	1-3

Figure 7:


MEAN GASTRIC ACID RESPONSE OF ASSAY DOGS TO
SIX DOSES (2-3G) OF PIG MUCOSA INTESTINAL EXTRACT
ACID – M.Eq
.6
.5
.4
.3
.2
.1
0
BASAL
0
1
2
3
HOURS

	Mucosal Extract G.	Gastrin Assay µG.
	2.0	0,24
	2.3	0.13
	2.5	0.02
	2.5	0.08
	3.0	0.03
	3.0	0.06
MEAN	2.6 G	0.09 µG

TABLE 34:: Content of gastrin assayed in six doses of pig intestinal mucosa extract.

DISCUSSION.

The results in these investigations demonstrate that crude extracts isolated from pig intestinal mucosa contain a heat-stable acidic peptide which is a potent stimulator of gastric acid secretion. Administration of crude intestinal mucosa extract elicits gastric acid secretion after a brief delay, indicating that some intermediate reactions occur before the target organ - the parietal cell mass - is stimulated. The hormone responsible for the intestinal phase of gastric secretion is present in hog intestinal mucosa and can be extracted by a method that selectively isolates heat-stable acidic peptides of low molecular weight.

Although the proximal jejunum contains small amounts of immunoreactive gastrin (Berson and Yalow, 1971), the amount of gastrin assayed in the extracts of pig intestinal mucosa was too small to account for the gastric acid output elicited in the assay dogs since the gastrin content would need to be increased at least ten-fold in order to stimulate minimal rates of acid secretion (McGuigan, Isaza and Landor, 1971).

Isolation and Identification of Gastrointestinal Hormones.

As early as 1946, Harper had shown that an alcoholic extract/

extract of the mucosa of the upper part of the small intestine stimulated acid secretion by the stomach of a cat. Extracts of the lower part of the small bowel were inactive. However, further interest was concentrated on the isolation and purification of gastrin, secretin and cholecystokinin-pancreozymin. The biochemical epoch in the history of gastrointestinal hormones began in 1964 when Gregory and Tracy isolated gastrin. Their method involved boiling of hog antral mucosa to denature heat-sensitive proteins, absorption of heat-stable proteins on to an anion exchange cellulose, extraction of the active material with alkali, precipitation with acid, extraction with isopropanol and ether separation on a Sephadex column, and further purification by electrophoresis. Gregory and Tracy demonstrated that hog gastrin is an acidic peptide made up of 17 linearly-arranged 1 - amino acids, with a molecular weight (MW) of 2114, occurring in two forms, Gastrin 1 and Gastrin 11. Following the discovery of secretin in intestinal mucosa extracts by Bayliss and Starling in 1902, many distinguished scientists failed in their attempts to isolate the hormone over a period of 50 years. Success awaited the development of new methods for the purification of peptides, including ion exchange chromatography./

chromatography. Jorpes and Mutt began work on the isolation of secretin from the upper three feet of hog intestinal mucosa in 1952 (Jorpes and Mutt, 1961), and by 1966, they had succeeded in identifying the full amino acid sequence (Mutt, Jorpes and Magnusson, 1970). The extraction procedure for secretin was similar in principle to that of gastrin, and the hormone was found to be a strongly-basic positively charged polypeptide made up of 27 amino acid residues, and having a MW of approximately 3500.

Isolation and purification of cholecystokinin-pancreozymin (CCK-PZ) was accomplished by Jorpes and Mutt by 1968 (Jorpes, 1968) from the upper small bowel of the pig. The extraction procedure was performed on the methanol-insoluble material that remained after extracting secretin from hog intestinal mucosa, and involved ion exchange absorption, organic solvent extraction, and a series of chromatographic separations. CCK-PZ consists of 33 amino acid residues and has a MW of approximately 4300.

Extraction of Intestinal Phase Hormone.

The isolation of the intestinal factor required a number/

number of critical preliminary decisions:

(a) what animal should serve as a source of starting material,

(b) what procedures of purification would be most likely to yield active fractions, and,

(c) how to set up a plant for preparation of the hormone on a large scale.

Since the pig develops portacaval shunt-related gastric acid hypersecretion in response to food, and has an intestinal phase of gastric secretion comparable to man and the dog, practical considerations of availability made the small intestinal mucosa of this omnivore as the most likely choice of starting material for isolation of the hormone. Starting with the assumption that the hormone was a small heat-stable protein or peptide similar to the other three known digestive tract hormones viz. gastrin, secretin and CCK-PZ, preliminary (and unpublished) procedures were conducted in this laboratory on small batches (50 gals.) of hog mucosa obtained at the slaughter house using similar methods of extraction to that of Gregory and Tracy (1964) for the isolation of gastrin. These pilot ventures later became the basis for/

for the present method of extraction presented in this study.

In these pioneer explorations, the mucosal slurry was kept frozen with dry ice during transportation from the abbatoir to the laboratory. After thawing, the batch was heated to 98°C. for one hour. The precipitated mass of protein was filtered by pressure through twill cloth using a converted wine-press. The filtrate was stirred for one hour with 100 G. DEAE cellulose, and the cellulose was washed twice with distilled water. Absorbed acidic material was eluted with 0.1N sodium hydroxide, and the clear alkaline solution was acidified with hydrochloric acid. From the resultant precipitate, three distinct fractions could be identified:

(1) A diffusely precipitating material between pH 7 - 5.

(2) A fraction with a sharp isoelectric point at pH 3.8 - 4.3.

(3) A large mass of material appearing at pH 2.8.

Further consideration revealed that the first fraction was a consequence of insufficient washing of the cellulose/

cellulose, while the third fraction consisted entirely of nucleic acids and their breakdown products, and heparin-like material. The second fraction thus became the main focus for further attention. This fraction was redissolved at pH 7 and extracted with isopropyl alcohol from a potassium phosphate-saturated solution at pH 9. A great mass of the material became denatured at this point, and was discarded. The isopropanol-soluble fraction was then extracted with ether, and the small water residue was separated and again acidified to pH 4. The precipitate was collected, washed, redissolved at pH 7.4 with buffered saline and sterilized by filtration. Yields by this procedure were of the order of 50 mg. Only minimal secretory responses or none at all were elicited in the assay dog by such small doses, and it became apparent that the programme would have to be conducted on an industrial scale. Accordingly, large boiling and storage tanks, pumps and a continuous centrifuge were purchased and these became the nucleus of the extraction equipment.

The first month that the plant was in function was spent ironing out the many technical problems involved in translating the process from 50 gals. per week to 1000/

1000 gals. per week. Engineering problems were solved of the kind that are not usually met with in the laboratory, ranging from how to get 500 lb. drums off a truck without a liftgate, to the disposal of 500 gals. of obnoxious-smelling liquid. The process is now functioning smoothly, and is yielding approximately 3 G. of hormone per 500 gals. mucosal slurry. With further experience gained, it now appears that repetition of some steps yields significant improvements in purity, and it is possible that a change in their order of performance will increase the yield. Certainly, the yield would be increased by introducing changes in the protocol at several points, such as, for instance, trying to arrange with the abbatoir to have the mucosal slurry boiled immediately after slaughter of the animals, in order to forestall proteolytic destruction. There is a further loss of active factor during centrifugation of hormone-absorbed DEAE cellulose, as the type of industrial centrifuge used was not as efficient as desired.

The fact that prolonged boiling does not appear to destroy activity of the hormone in these preliminary studies, suggests that the active agent is a small molecule with a simple structure, since heat usually denatures complicated molecules/

molecules or those held together by many hydrogen bonds. Heating denatures proteins by uncoiling the molecule, ranging from "fragile uncoiled" to "totally uncoiled", and it is possible that passing the DEAE cellulose-treated material through the second centrifugation may further denature the active factor by rough agitation.

In addition to precipitation of proteins at the isoelectric point of pH 4, gastric-stimulating fractions may precipitate at other isoelectric points such as pH 3, 5 and 6. Although only acidic material has been arbitrarily selected out and subsequently followed up since it afforded an active factor, basic compounds isolated on carboxy-methyl cellulose at an early stage may yield another or other active fractions.

CHAPTER VIII

THE RELATIONSHIP OF THE INTESTINAL PHASE HORMONE TO GASTRIC ACID SECRETION

THE RELATIONSHIP OF THE INTESTINAL PHASE HORMONE TO GASTRIC ACID SECRETION

"The early history of medical milestones includes, in most cases, a latent period between the first observation of a phenomenon and the definitive investigation of it, as well as the medical application of the knowledge thus gained. Usually it has been so that at the time of the original discovery techniques and resources for the further investigation of the problem were not available" (Jorpes, 1968). Since the discovery of the existence of the intestinal phase of gastric secretion, the subsequent elucidation of its physiological characteristics is a striking example of such inertia in development.

The Identity of the Intestinal Hormone.

Gastric acid secretion is stimulated by the sight, smell, or suggestion of food via the vagus nerves, by the presence of food in the stomach, and by food distending the upper portion of the small intestine. The first two stimulatory phases are relatively well understood. In the first or cephalic phase, impulses travelling along the vagus nerves directly affect the acid-secreting parietal cells, and also stimulate the specialized cells in/

in the antral portion of the stomach, to secrete the hormone - gastrin. In the second or gastric phase, the antral mucosa is stimulated to secrete gastrin directly by distension or food within the stomach. In both cases, gastrin is released into the portal venous system, passes through the liver, and then reaches the stomach through the systemic arterial circulation to directly stimulate the parietal cells.

Studies conducted in this laboratory over the past six years have demonstrated conclusively that the third phase, the intestinal phase of gastric secretion, also involves a hormone. This hormone is secreted by the mucosal lining of the jejunum in response to food in the upper intestine, or in response to simple distension. The intestinal phase hormone also is released into the portal blood, but unlike gastrin, it is largely inactivated when it passes through the liver, and therefore exerts only a minor influence on gastric secretion in normal animals and in man. When the liver is bypassed by a portacaval shunt experimentally in the laboratory, or clinically in the treatment of portal hypertension, the intestinal phase hormone escapes destruction by the liver and its profound effect on gastric acid secretion becomes/

becomes unmasked.

The term "hormone" was first introduced by Bayliss and Starling in 1902 to describe an extract of intestinal mucosa, which they named secretin, that stimulated pancreatic secretion. Three years later, Edkins applied this same term to a gastric stimulator substance that he had extracted from the pyloric mucosa. In 1964, this second hormone, gastrin, was isolated in pure form, and identified and synthesized by Gregory and Tracy. Since then, two other gastro-intestinal hormones, secretin and cholecystokinin-pancreozymin, have been isolated, identified and purified. All three of these hormones have proven to be low molecular weight peptides. Over seventy years ago, Pavlov was the first of several investigators to observe the secretion of acid from the intact stomach when food entered the small intestine. Following the classic experiments of Ivy and his colleagues (1925), the existence of a stimulatory intestinal phase of gastric acid secretion has been recognized for almost a half-century. Recent findings in this laboratory have suggested that the hormone responsible for the intestinal phase of gastric secretion and portacaval shunt-related gastric hypersecretion is a small peptide similar to the other/

other digestive hormones that have been isolated thus far.

It has been suggested that this hormone might be "intestinal gastrin" (Ivy and others, 1925; Babkin, 1950; Dragstedt, 1957). If it is a gastrin-like compound, it is distinctly different from the gastrin secreted by the antrum of the stomach, since antral gastrin is not degraded by the liver (Lick and others, 1967; Thompson and others, 1969), whereas the intestinal phase hormone is almost totally inactivated by the liver (Gerez and Weiss, 1937; Dubuque and others, 1958; Orloff and others, 1969b), and very little of it gets into the systemic circulation in intact subjects. It is possible that the intestinal phase agent contains the active C-terminal tetrapeptide moiety of gastrin to which is attached a tag that facilitates efficient processing by the liver (Thompson, 1969b).

The studies described in this thesis have demonstrated that persistent gastric acid hypersecretion proceeds for several hours long after an intestinal meal has been fed, and it is indeed an enigma why such a potent gastric stimulator should be released in great quantities from the intestinal mucosa, only to be almost wholly destroyed by/

by the liver. Gastric hypersecretion occurs in the fasting state in shunted dogs, but it is profoundly increased after eating (Clarke and others, 1958c; Kohatsu and others, 1959; Silen and Eiseman, 1959; Rex and others, 1964). Acid hypersecretion is not abolished by vagotomy (Kohatsu and others, 1959), antrectomy (Clarke and others, 1958c; Gregory, 1958; Kohatsu and others, 1959; Cornish and others, 1960; MacPherson and others, 1962), and splenectomy and pancreatectomy (Silen and Eiseman, 1959), and it occurs in a Heidenhain pouch in the absence of the remainder of the stomach (O'Sullivan and others, 1960).

Colectomy (Silen and Eiseman, 1959) and the administration of oral neomycin (Clarke and others, 1958c) does not prevent the development of the intestinal phase. The acid response is inhibited by known intestinal phase inhibitors such as acid perfusion of the duodenum, and intravenous injection of secretin, phenergan and atropine (Cornish and others, 1960). Although it has been demonstrated by Castaneda and others (1961) that gastric hypersecretion is not abolished by jejunectomy or ileectomy, their evidence was based on the results of studies which were conducted with small numbers/

numbers of animals, and in which the statistical significance of their results had not been determined.

The stimulatory effect of massive small intestinal resection on gastric acid secretion was not recognized by them at that time. Recent investigations in which 75 per cent of the small bowel was excluded during feeding now implicate the remaining small intestine as the source of increased stimulation to gastric acid secretion (Buxton, Wasunna, Saunders and Gillespie, 1972). The ability of small doses of gastrin to inhibit histamine-induced gastric secretion in dogs was greatly exaggerated after portacaval shunt, supporting an idea that hepatic bypass is associated with the reduced activity of an inhibitor normally influencing gastric secretion (Wilken, Hunt, Lowe, Billups and Hardy, 1969).

Portacaval shunt-related gastric hypersecretion is evident as a manifestation of the intestinal phase when venous blood from the jejunum and ileum is shunted into the systemic circulation but does not occur following systemic shunting of blood from the stomach, duodenum, pancreas and spleen (Clarke and others, 1959; Leger and others, 1960; Clarke and others, 1966; Hayashi and others, 1968). Although previous studies have/

have indicated that the colon may release a gastric-stimulating hormone (Clarke and others, 1959 and 1966; Wise, 1969), recent evidence has been presented which opposes such claims (Orloff and others, 1970b).

Nahrwold and Grossman (1967) observed that in dogs there was a decrease in acid secretion from Heidenhain pouches in response to feeding when bile was diverted from the intestine. They suggested that the presence of bile salts in the intestine facilitates the intestinal phase of gastric secretion.

Magnitude of the Intestinal Phase.

Previous studies performed by Dragstedt and his group (Dragstedt and others, 1950) have indicated that in normal dogs the nervous phase accounts for approximately 45 per cent of the gastric juice secreted in a 24-hour period; the gastric phase accounts for about 45 per cent; and the intestinal phase is responsible for 10 per cent or less. Buxton, Wasunna, Bedi and Gillespie (1970) have suggested that synergism exists between the intestinal phase and the other two phases of the meal response, and they concluded that the intestinal component represents between 18 and 38 per cent/

per cent of the total meal response. Since innervated fundic (Pavlov) pouches were used, the influence of extraneous stimuli via the vagi were not excluded by them.

Synergism with other Phases of Gastric Secretion.

Olbe (1960) demonstrated that the secretory response to insulin hypoglycaemia from vagally-innervated gastric pouches increased between 165 and 560 per cent following portacaval transposition. He also found that the secretory response to sham feeding increased 100 per cent following portacaval transposition. However, his conclusions were based on studies performed with very small numbers of dogs, and the statistical significance of the results was not proved. No conclusive evidence has been obtained to prove that vagal stimulation may effect the release of the intestinal humoral factor in an amount sufficient to produce significant gastric acid secretion.

The possibility that gastrin may be involved in the third phase of gastric secretion has drawn the attention of several investigators. Woodward, Lyon Landor and Dragstedt (1954) were unable to inhibit gastric secretion associated with the intestinal phase by/

by acidification of the isolated antrum. This result influenced them to believe that the hormones mediating the gastric and intestinal phases of gastric secretion were not identical. However, Margolus and Harrison (1957) demonstrated profound inhibition of Heidenhain pouch secretion following food when the totally-denervated antral pouch was irrigated with acid.

In 1962, Thompson, Tramontana, Lerner and Stallings demonstrated antral inhibition of the intestinal phase in two preparations. In one study, the response of a Heidenhain pouch to an intestinal meal was suppressed by 80 per cent after acidification of a denervated antral pouch. In the second study, acidification of the isolated antrum resulted in 79 per cent inhibition of gastric secretion stimulated by the distension of an isolated Thiry-Vella fistula with a balloon.

Studies by Jordan and de la Rosa (1964) suggested that gastrin may potentiate the intestinal phase. They reported that the inhibition of intestinal phase secretion found after antrectomy or after infusion of acid or cocaine into the isolated antrum could be abolished by a threshold dose of exogenous gastrin. Jordan (1967) thought that the mechanism responsible for the intestinal/

intestinal phase of gastric secretion was dependent on the antrum and exerted its effect by releasing gastrin from the antrum or potentiating the action of gastrin.

The observations made by Jordan and de la Rosa (1964) were confirmed by Curt, Isaza, Woodward and Dragstedt (1971) who have suggested that the effect of the intestinal hormone on the parietal cell mass is potentiated by the presence of antral gastrin. However, in their studies, the dogs were fed oral meals, so that the influence of duodenal inhibition was not excluded, and since animals without portacaval shunts were tested, the effect of hepatic degradation of the intestinal hormone was not taken into consideration.

The Delay in Action by the Hormone.

The intestinal phase hormone is released when the jejunum is distended by food (Orloff and others, 1969a, 1970a, 1970b) or by simple pressure from an inflated balloon (Villar-Valdes and others, 1969; Orloff and others 1970a). The pressure of a balloon distended for 20 minutes at 50 mm Hg is comparable to physiological pressures developed in the lumen of the jejunum during normal digestion (Orloff and others, 1970a). The gastric secretory/

secretory response to balloon distension of the small bowel is abolished by the prior application of 5 per cent procaine to the intestinal mucosa (Sircus, 1953).

Only two other studies have been performed where the effect of balloon distension on the small intestine was observed. In the studies by Sircus (1953), there was a latent period of 15 to 30 minutes before a secretory response occurred, while Nagano and his colleagues (1960) recorded a delay of 30 to 120 minutes when the duodenum was distended. However, in their investigations, the statistical significance of their results was not determined, and the number of tests performed was not mentioned.

The kinetics of action of the intestinal phase hormone was now examined in the records of previous investigations of humans (Table 22) and dogs (Table 21) in this laboratory, and in dogs (Table 18) and pigs (Table 30) in the present studies. The results are summarized in Table 35. Although the pigs were fed oral meals, intestinal phase secretion commenced soonest in this group, occurring at a mean 34 minutes after the start of the meal. Following the administration of/

of an intestinal meal, the onset of gastric secretion began at a mean 45 minutes in dogs with portacaval anastomosis, and was delayed until 69 minutes after application of the stimulus in shunted cirrhotic patients, with a range varying between 30 and 180 minutes. The time of peak gastric secretion was delayed in all of the groups on the average for three hours, with a range from 1 to 5.5 hours. As early as 1950, Babkin had emphasized that there was a very long latent period (one to three hours) before gastric acid secretion followed the stimulus of an intestinal meal.

There is thus a consistent time lag between the application of the stimulus and the gastric secretory response. This suggests that there are intermediary steps between the application of the stimulus and the ultimate effect on the parietal cell. The present studies have indicated that the site of delay is not during the elaboration and release of the hormone, but rather at the level of the target organ. One can only speculate about the reasons for such a delay, which may be due to activation of a precursor; competition with inhibiting hormones (Lucien and others, 1970; Johnson and Grossman, 1971) of gastric secretion; or an effect on a second messenger/

messenger system through cyclic AMP (Sutherland and others, 1965; Butcher and others, 1968). Such a time lag and the persistence of the response long after the stimulus is withdrawn are characteristics of endocrine action, although the physiological response to many other hormones, such as gastrin, and secretin, is quite prompt.

Exaggerated Intestinal Phase and Peptic Ulcer.

During gastric resection, it was once common practice for some surgeons to establish a gastrojejunostomy 30 cm. or more beyond the suspensory duodenojejunal ligament of Treitz. Such a long afferent duodenojejunal loop has been shown to be an important predisposing factor in the production of stomal ulceration (Merendino, Lannin, Kolouch, Baronofsky, Litow and Wangensteen, 1945). Gastrojejunal ulceration as a consequence of an exaggerated intestinal phase has been attributed to the establishment of such long-loop Billroth II or Finsterer-Devine exclusion types of partial gastrectomy (Dragstedt, 1959).

TABLE 35: Time Course of Action of Intestinal Phase Hormone in Humans, Dogs and Pigs with Portacaval Shunts.

Type of Study	No.	Time of onset of Gastric Secretion Minutes		Time of Peak Gastric Secretion Hours	
		Mean	Range	Mean	Range
Humans	35	69	30-180	2.6	1-4
Dogs	37	49	30-150	3.7	1-5.5
Dogs	58	45	30-150	2.9	1-5.5
Pigs	6	34	30- 60	2.5	1-4

REFERENCES

REFERENCES

Abbott, A.G., Rosen, H. and Orloff, M.J. (1970) — Site of origin of the hormone responsible for gastric hypersecretion in humans with portacaval shunts. Surg. Forum, 21, 340-343.

Ackermann, D. (1911) — Über das B-Alanin als bakterielles Aporrhegma. Z. Biol., 56, 87-90.

Alderman, S.J., Keiter, J.E., Chandler, J.G., Rosen, H. and Orloff, M.J. (1969) — Basal and stimulated gastric secretion in man with cirrhosis with and without portacaval shunt. Surg. Forum, 20, 363-365.

Amure, B.O. and Ginsburg, M. (1964) — Inhibitors of histamine catabolism and the action of gastrin in the rat. Br. J. Pharmacol., 23, 476-485.

Anrep, G.V., Barsoum, G.S., Talaat, M. (1953) — Release of histamine by the liver. J. Physiol. (Lond.), 120, 419-426.

Armour, J. and Webster, D.R. (1932) — The influence of pyloric obstruction on gastric secretion. Canad. Med. Assoc. J., 27, 61.

Ask-Upmark, E. (1939) — Quoted in: The natural history of Laennec's cirrhosis of the liver by Ratnoff, O.D. and Patek, A.J. Medicine, 21, 207-268, 1942.

Babkin/

Babkin, B.P. (1950) Secretory Mechanism of the Digestive Glands, p. 495-503. Hoeber: New York, 2nd Ed.

Baddeley, R.M. (1968) Gastric Acid and Pepsin Secretion in Human Cirrhotic Ascites. Br. J. Surg., 55, 865-866.

Baddeley, R.M. and Fejfar, J. (1968) Gastric Secretory Changes in Experimental Cirrhosis and Ascites. Br. J. Surg., 55, 866.

Bayliss, W.M. and Starling, E.H. (1902) The mechanism of pancreatic secretion. J. Physiol. (Lond.), 28, 325-353.

Bendett, R.J., Fritz, H.L. and Donaldson, R.M. (1963) Gastric acid secretion after parenterally and intragastrically administered histamine in patients with portacaval shunt. New Engl. J. Med., 268, 511-516.

Berg, B.N. and Jobling, J.W. (1930) Biliary and hepatic factors in peptic ulcers. Arch. Surg., 20, 997-1015.

Berson, S.A. and Yalow, R.S. (1971) Nature of immunoreactive gastrin extracted from tissues of gastrointestinal tract. Gastroenterology, 60, 215-222.

Bollman, J.L. and Mann, F.C. (1932) Peptic ulcer in experimental obstructive jaundice. Arch. Surg., 24, 126-135.

Bonfils/

Bonfils, S., Bader, J.-P., Leger, L., Boury, G., Bernades, P. and Dubrasquet, M. (1967)
Étude du pouvoir secrétagogue urinaire (P.S.U.) aprés anastomose porto-cave.
Presse Med., 75, 2395-2396.

Brown, G.E., Faustina, G.E. and Orloff, M.J. (1967)
Humoral mediation of the intestinal phase of gastric secretion.
Surg. Forum, 18, 298-300.

Butcher, R.W., Robinson, G.A., Hardman, J.G. and Sutherland, E.W. (1968)
The role of cyclic AMP in hormone actions.
Adv. Enzyme Regul., 6, 357-389. Ed. by G. Weber. Pergamon Press: London.

Buxton, B.F., Wasunna, A.E.O., Bedi, B.S. and Gillespie, I.E. (1970)
The Magnitude of the Intestinal Phase of Gastric-acid Secretion.
Br. J. Surg., 57, 857-858.

Buxton, B., Wasunna, A.E.O., Saunders, J. and Gillespie, I.E. (1972)
Site of the abnormal stimulus of gastric secretion after small bowel exclusion.
Gut, 13, 539-543.

Castaneda, A., Griffen, W.O., Nicoloff, D., Leonard, A.S. and Wangensteen, O.H. (1960)
Antral hyperfunction following portacaval shunt.
Surg. Forum, 11, 349-351.

Castaneda, A., Griffen, W.O., Nicoloff, D.M., Stone, N.H., Leonard, A.S. and Wangensteen, O.H. (1961)
The Role of the Small Intestine in Gastric Hypersecretion Following Portacaval Shunt.
J. Surg. Res., 1, 221-224.

Child, C.G., Barr, D., Holswade, G.R. and Harrison, C.S. (1953)
Liver regeneration following portacaval transposition in dogs.
Ann. Surg., 138, 600-608.

Child/

Child, C.G. (1958) In Discussion: Peptic Ulcer Following Portacaval Shunt. J.S. Clarke and others. Ann. Surg., 148, 564.

Chittenden, R.H., Mendel, L.B. and Jackson, H.C. (1898) A further study of the influence of alcohol and alcoholic drinks upon digestion, with special reference to secretion. Am. J. Physiol., 1, 164-209.

Clarke, J.S., Costarella, R. and Ward, S. (1958)a Gastric secretion in the fasting state and after antral stimulation in patients with cirrhosis and with portacaval shunts. Surg. Forum, 9, 417-420.

Clarke, J.S., Hart, J.C. and Ozeran, R.S. (1958)b Increase in Heidenhain Pouch Secretion after Portacaval Transposition in the Dog. Proc. Soc. Exp. Biol. Med., 97, 118-121.

Clarke, J.S., Ozeran, R.S., Hart, J.C., Cruze, K. and Crevling, V. (1958)c Peptic Ulcer Following Portacaval Shunt. Ann. Surg., 148, 551-563.

Clarke, J.S., McKissock, P.K. and Cruze, K. (1959) Studies on the site of origin of the agent causing hypersecretion in dogs with portacaval shunt. Surgery, 46, 48-54.

Clarke, J.S., Hoffs, M. and El Farra, S. (1960) The effect of portacaval transposition on Heidenhain pouch secretion in response to various stimuli. Surg. Forum, 10, 134-138.

Clarke/

Clarke, J.S., Miller, I. and McKissock, P.K. (1966)
Increased Acid Secretion From Heidenhain Pouches by Shunting Colonic Venous Blood Around the Liver. Arch. Surg., 92, 653-656.

Clendinnen, B.G., Reeder, D.D., Jackson, B.M., Miller, J.H., Thompson, J.C. (1970)
Effect of portacaval shunting on postprandial serum gastrin levels in dogs. Surg. Forum, 21, 339-340.

Code, C.F. (1965)
Histamine and gastric secretion: a later look, 1955-1965. Fed. Proc., 24, 1311-1321.

Cornish, P.G., Silen, W., Eiseman, B. and Woods, L.P. (1960)
Further studies on the mechanism of gastric hypersecretion after portacaval shunts. Surg. Forum, 11, 351-353.

Curt, J.R.N., Isaza, J., Woodward, E.R. and Dragstedt, L.R. (1971)
Potentiation Between Intestinal and Gastric Phases of Acid Secretion in Heidenhain Pouches. Arch. Surg. 103, 709-712.

Dagradi, A., Sanders, D., and Stempien, S.J. (1955)
The sources of upper gastrointestinal bleeding in liver cirrhosis. Ann. Intern. Med., 42, 852-855.

Day, S.B., Skoryna, S.C., Webster, D.R. and MacLean, L.D. (1963)
Gastric hypersecretion and histamine levels in liver, stomach, duodenum, and blood following portacaval shunt in rats. Surgery, 54, 764-770.

Delaney/

Delaney, J.P., Goodale, R.L., Cheng, J. and Wangensteen, O.H. (1965) — Portacaval Shunting and Upper Gastrointestinal Blood Flow. JAMA, 192, 125-126.

Dragstedt, L.R., Woodward, E.R., Storer, E.H. Oberhelman, H.A. and Smith, C.A. (1950) — Quantitative studies on the mechanism of gastric secretion in health and disease. Ann. Surg., 132, 626-640.

Dragstedt, L.R. (1957) — The Physiology of the Gastric Antrum. Arch. Surg., 75, 552-556.

Dragstedt, L.R. (1959) — The Intestinal Phase of Gastric Secretion. Arch. Surg., 78, 498-499.

Drapanas, T., Adler, W., Vang, J.O. and McMenamy, R.H. (1965) — Primary Regulation of Histamine Metabolism by the Liver. Ann. Surg., 161, 447-455.

Dubuque, T.J., Mulligan, L.V. and Neville, E.C. (1958) — Gastric secretion and peptic ulceration in the dog with portal obstruction and portacaval anastomosis. Surg. Forum, 8, 208-211.

Eck, N.V. (1877) — Concerning Ligation of the Vena Porta. Translated by Child, C.G. Surg. Gynecol. Obstet., 96, 375-376, 1953.

Edkins, J.S. (1905) — On the Chemical Mechanism of Gastric Secretion. Proc. R. Soc. Lond. (Biol.) 76, 376.

Eiseman/

Eiseman, B., Moore, T.C. and Normell, L. (1964) — Histamine metabolism in the isolated perfused pig liver. Surg. Gynecol. Obstet., 118, 69-74.

Fainer, D.C. and Halsted, J.A. (1955) — Sources of upper alimentary tract hemorrhage in cirrhosis of the liver. J.A.M.A., 157, 413-414.

Ferrarese, S. and Ronzini, V. (1966) — La secrezione gastrica nei cirrotici dopo shunts porto-sistemici. Gaz. Int. Med. Chir., 71, 882-889.

Fischer, J.E. and Snyder, S.H. (1965) — Increased gastric synthesis of histamine: a possible mechanism for the gastric acid hypersecretion following porta-caval shunt. Fed. Proc., 24, 1334-1340.

Frederick, P.L., Sizer, J.S., and Osborne, M.P. (1965) — Relation of massive bowel resection to gastric secretion. New Engl. J. Med., 272, 509-514.

Gerez, L. and Weiss, A. (1937) — Über die Magensaftseksetion bei Eckscher Fistel. Z. Gesamte Exp. Med., 100, 281-288.

Gillespie, I.E. and Grossman, M.I. (1962) — Gastric secretion of acid in response to portal and systemic venous injection of gastrin. Gastroenterology, 43, 189-192.

Gregory, R.A. (1957) — The effect of portal venous occlusion on gastric secretion. J. Physiol. (Lond.), 137, 76P-77P.

Gregory/

Gregory, R.A. (1958) Gastric secretory responses after portal venous ligation. J. Physiol. (Lond.), 144, 123-137.

Gregory, R.A. and Ivy, A.C. (1941) The humoral stimulation of gastric secretion. Q.J. Exp. Physiol., 31, 111-128.

Gregory, R.A. and Tracy, H.J. (1964) The constitution and properties of two gastrins extracted from hog antral mucosa. Gut, 5, 103-117.

Griffen, W.O., Slesh, M.Z. and Mooney, C.S. (1969) Gastric secretagogues following portacaval shunting. Surgery, 66, 111-117.

Gross, W. (1906) Beitrag zur Kenntnis der Sekretionsbedingungen des Magens nach Versuchen am Hund. Arch. Verdaukr., 12, 507-516.

Guth, G. (1962) Uropepsinogenausscheidung und pH-Werte des Magensaftes bei experimentell erzeugter leber zirrhose mit nachfolgender portokavaler shuntoperation. Bruns. Beitr. Klin. Chir., 204, 133-143.

Hallenbeck, G.A., Wollaeger, E.E., Adson, M.A. and Gage, R.P. (1963) Results after portal-systemic shunts in 120 patients with cirrhosis of the liver. Surg. Gynecol. Obstet., 116, 435-442.

Harper/

Harper, A.A. (1946) The effect of extracts of gastric and intestinal mucosa on the secretion of HC1 by the cat's stomach. J. Physiol. (Lond.), 105, 31P.

Harris, J.B., Nigon, K. and Alonso, D. (1969) Adenosine - 3', 5' - monophosphate: intracellular mediator for methyl xanthine stimulation of gastric secretion. Gastroenterology, 57, 377-384.

Hayashi, K., Rheault, M.J., Semb, L.S. and Nyhus, L.M. (1968) The effect of splenocaval shunt on gastric secretion and liver function as compared with other shunting procedures in the dog. Surgery, 64, 1084-1091.

Hayem, G. (1898) Sur une variété particulière d'ictére chronique. Ictére infectieux chronique splénomégalique. Presse Med., 6, 121-125.

Hein, M.F., Silen W., Skillman, J.J. and Harper, H.A. (1963) The effect of portacaval shunting on gastric secretion in cirrhotic dogs. Gastroenterology, 44, 637-641.

Hjelmquist, U.B.E., Reeder, D.D., Brandt, E.N. and Thompson, J.C. (1972) Effect of the kidney on endogenous gastrin. Surg. Forum, 23, 318-320.

Holinger, P.H., Kelley, E.H. and Ivy, A.C. (1932) The Vagi and Appetite. Proc. Soc. Exp. Biol. Med., 29, 884-885.

Hubens, A. (1966) Contribution à l'étude de l'hypersécrétion acide de l'estomac après transposition porta-cave, chez le chien. Acta Gastroenterol., Belg., 29, 1009-1037.

Hubens, A. (1967) — Contribution à l'étude de l'hypersécrétion acide de l'estomac après transposition porto-cave, chez le chien. Acta Gastro-enterol., Belg., 30, 7-55.

Irvine, W.T. and Code, C.F. (1958) — Gastric Secretion and Free Histamine in Urine. Am. J. Physiol., 195, 202-208.

Irvine, W.T., Duthie, H.L. and Waton, N.G. (1959a) — Urinary output of free histamine after a meat meal. Lancet, 1, 1061-1064.

Irvine, W.T., Duthie, H.L., Ritchie H.D. and Waton, N.G. (1959b) — The liver's role in histamine absorption from the alimentary tract. Its possible importance in cirrhosis. Lancet, 1, 1064-1069.

Ivy, A.C. and McIlvain, G.B. (1923) — The excitation of gastric secretion by application of substances to the duodenal and jejunal mucosa. Am. J. Physiol.,63, 418-419.

Ivy, A.C., Lim, R.K.S. and McCarthy, J.E. (1925) — Contributions to the physiology of gastric secretion. II The intestinal phase of gastric secretion. Q. J. Exp. Physiol., 15, 55-68.

Johnston, L.R. and Grossman, M.I. (1971) — Intestinal hormones as inhibitors of gastric secretion. Gastroenterology, 60, 120-144.

Jordan, P.J. (1967) — Relationship Between Stimulating Mechanisms of Gastric Secretion in Dogs. J.A.M.A., 199, 399-405.

Jordan/

Jordan, P.H. and De la Rosa, C. (1964) — The regulatory effect of the pyloric gland area of the stomach on the intestinal phase of gastric secretion. Surgery, 56, 121-134.

Jorpes, J.E. (1968) — The isolation and chemistry of secretin and cholecystokinin. Gastroenterology, 55, 157-164.

Jorpes, J.E. and Mutt, V. (1961) — The Gastrointestinal Hormones, Secretin and Cholecystokinin - Pancreozymin. Ann. Intern. Med., 55, 395-405.

Kelly, K.A., Nyhus, L.M. and Harkins, H.N. (1965) — A Reappraisal of the Intestinal Phase of Gastric Secretion. Am. J. Surg., 109, 1-6.

Kohatsu, S., Gwaltney, J.A., Nagano, K. and Dragstedt, L.R. (1959) — Mechanism of gastric hypersecretion following portacaval transposition. Am. J. Physiol., 196, 841-843.

Koide, S.S., Texter, E.C. and Borden, C.W. (1958) — Perforation of Peptic Ulcer Following Paracentesis in Patients with Cirrhosis. Am. J. Dig. Dis., 3, 24-37.

Landor, J.H. and Baker, W.K. (1964) — Gastric Hypersecretion Produced by Massive Small Bowel Resection inDogs. J. Surg. Res., 4, 518-522.

Landor, J.H., Porterfield, J.F. and Wolff, W.S. (1966) — The parietal cell response to chronic gastric secretory stimulation. Surg. Gynecol. Obstet., 122, 61-65.

Lebedinskaja/

Lebedinskaja, S.I. (1933) — Über die Magensekretion bei Eckschen Fistelhunden. Z. Gesamte Exp. Med., 88, 264-270.

Le Conte, P. (1900) — Fonctions gastro-intestinales. Étude physiologique. Cellule, 17, 285-318.

Lee, S.H. and Fisher, B. (1961) — Portacaval shunt in the rat. Surgery, 50, 668-672.

Leger, L., Cachin, M. and Pergola, F. (1960) — Ulcéres gastro-duodénaux aprés anastomose porto-cave. Presse Med., 68, 63-66.

Leva, J. (1893) — Ueber das Verhalten der Magenfunctionen bei verschiedenen Leberkrankheiten. Virchow's Arch. (Pathol. Anat.), 132, 490-501.

Lick, R.F., Welsch, K.H., Hart, W., Brückner, W., Balser, D. and Gürtner, T. (1967) — Zur sekretorischen Function des Magens nach Injektion von Histamin, Gastrin und synthetischem Tetrapeptid in den groBen Kreislauf und in die Pfortader. Z. Gastroenterol., 5, 7-12.

Liebowitz, H R. (1964) — Peptic Ulcer Following Portacaval Shunt. Am. J. Dig. Dis., 9, 605-616.

Lindahl, K.M. (1960) — The Histamine Methylating Enzyme System in Liver. Acta Physiol. Scand., 49, 114-138.

Linton/

Linton, R.R., Ellis, D.S. and Geary, J.E. (1961) Critical Comparative Analysis of Early and Late Results of Splenorenal and Direct Portacaval Shunts Performed in 169 Patients with Portal Cirrhosis. Ann. Surg., 154, 446-457.

Lipp, W.F. and Lipsitz, M.H. (1952) The clinical significance of the co-existence of peptic ulcer and portal cirrhosis, with special reference to the problem of massive hemorrhage. Gastroenterology, 22, 181-189.

Lönnquist, B. (1906) Beiträge zur Kenntniss der Magensaftabsonderung. Skand. Arch. Physiol., 18, 194-257.

Lucien, H.W., Itoh, Zen. and Schally, A.V. (1970) Inhibitory effects of a purified enterogasterone, secretin, and cholecystokinin on histamine- stimulated gastric acid secretion. Gastroenterology, 59, 707-711.

Ludington, L.G. (1958) A study of 158 cases of esophageal varices. Surg. Gynecol. Obstet., 106, 519-526.

McDermott, W.V., Palazzi, H., Nardi, G.L. and Mondet, A. (1961) Elective portal systemic shunt. New Engl. J. Med., 264, 419-427.

MacDonald, R.A. and Mallory, G.K. (1958) The Natural History of Post-necrotic Cirrhosis. A study of 221 Autopsy Cases. Am. J. Med., 24, 334-357.

McGuigan/

McGuigan, J.E., Isaza, J. and Landor, J.H. (1971)
Relationships of gastrin dose, serum gastrin, and acid secretion.
Gastroenterology, 61, 659-666.

McPhedran, N.T., Bett, H.D., Stone, R.M. and Goldberg, M. (1968)
Some observations on the cause of the gastric hypersecretion following portacaval shunt.
Bull. Soc. Int. Chir., 27, 286-290.

MacPherson, W.A., Miller, I., Nishikawa, W.Y., McKissock, P.K. and Clarke, J.S. (1962)
The importance of the antrum in gastric hypersecretion after shunt.
Surg. Forum, 13, 271-273.

Margolus, B.D. and Harrison, R.C. (1957)
Inhibition of the intestinal phase of gastric secretion by antral inhibitor substance.
Surg. Forum, 7, 360-362.

Maxwell, J.G., Moore, J.G., Dixon, J. and Stevens, L.E. (1971)
Gastrin levels in anephric patients.
Surg. Forum, 22, 305-306.

Merendino, K.A., Lannin, B.G., Kolouch, F., Baronofsky, I., Litow, S.S. and Wangensteen, O.H. (1945)
II Length of Afferent Duodeno jejunal loop in Gastric Resection, a Factor in Stomal Ulcer.
Proc. Soc. Exp. Biol. Med., 58, 226-230.

Mikkelsen, W.P., Turrill, F.L. and Pattison, A.C. (1962)
Portacaval Shunt in Cirrhosis of the Liver.
Am. J. Surg., 104, 204-214.

Mitchell, R.G. and Code, C.F. (1954)
Effect of Diet on Urinary Excretion of Histamine.
J. Appl. Physiol., 6, 387-392.

Mutt, V., Jorpes, J.E. and Magnusson, S. (1970)
Structure of Porcine Secretin. The Amino Acid Sequence.
Eur. J. Biochem., 15, 513-519.

Nagano/

Nagano, K., Johnson, A.N., Cobo, A., Oberhelman, H.A. and Dragstedt, L.R. (1960)
The Effect of distension of the duodenum on gastric secretion.
Surg. Forum, 10, 152-155.

Nahrwold, D.L. and Grossman, M.I. (1967)
Effect of exclusion of bile from the intestine on gastric acid secretion.
Gastroenterology, 52, 667-669.

Newman, P.H., Reeder, D.D., Davidson, W.D., Schneider, E., Miller, J.H. and Thompson, J.C. (1969)
Acid Secretion Following Portacaval Shunting.
Arch. Surg., 99, 369-375.

Nicoloff, D.M., Doberneck, R.C., Leonard, A.S., Peter, E.T. and Wangensteen, O.H. (1962)
The Effect of Arterialization of the Liver on Gastric Secretion of Dogs with Portacaval Shunts.
Am. J. Dig. Dis., 7, 1034-1038.

Olbe, L. (1960)
Influence of portacaval transposition on gastric secretion in dogs.
Acta Physiol. Scand., 50, Suppl. 175, 110-111.

Olbe, L. (1966)
Potentiation of Acid Sham Feeding Response in Pavlov Pouch Dogs by Histamine and by Mesenteric-Caval Vein Anastomosis.
Acta Physiol. Scand., 66, 473-480.

Orloff, M.J., Baddeley, R.M., Nutting, R.O., Ross, T.H., Halasz, N.A. and Sloop, R.D. (1966)
The effects of experimental liver disease and portal hypertension on gastric acid secretion.
Surg. Gynecol. Obstet., 122, 19-27.

Orloff/

Orloff, M.J. and Windsor, C.W.O. (1966) Effect of Portacaval Shunt on Gastric Acid Secretion in Dogs with Liver Disease, Portal Hypertension and Massive Ascites. Ann. Surg., 164, 69-80.

Orloff, M.J., Chandler, J.G., Alderman, S.J., Keiter, J.E. and Rosen, H. (1969a) Gastric Secretion and Peptic Ulcer Following Portacaval Shunt in Man. Ann. Surg., 170, 515-524.

Orloff, M.J., Villar-Valdes, H., Rosen, H., Thompson, A.G. and Chandler, J.G. (1969b) Humoral mediation of the intestinal phase of gastric secretion and of acid hypersecretion associated with portacaval shunts. Surgery, 66, 118-130.

Orloff, M.J., Abbott, A.G. and Rosen, H. (1970a) Nature of the humoral agent responsible for portacaval shunt-related gastric hypersecretion in Man. Am. J. Surg., 120, 237-243.

Orloff, M.J., Villar-Valdes, H., Abbott, A.G., Williams, R.J. and Rosen, H. (1970b) Site of origin of the hormone responsible for gastric hypersecretion associated with portacaval shunt. Surgery, 68, 202-208.

O'Sullivan, W.D., Cantlin, M.L., Sweeney, R.D., Rosteing, H.M. and Foster, W.C. (1960) Role of residual stomach in hypersecretion of Heidenhain Pouch after portacaval transposition. Surg. Forum, 11, 347-348.

Ostrow, J.D., Timmerman, R.J. and Gray, S.J. (1960) Gastric secretion in human hepatic cirrhosis. Gastroenterology, 38, 303-313.

Pavlov/

Pavlov, I.P. (1898) — Die Arbeit der Verdauungsdrüsen". Bergmann: Wiesbaden. Quoted by W. Gross, Arch. Verdaukr., 12, 507-516, 1906.

Pavlov, I.P. (1902) — In: The Work of the Digestive Glands. Translated by W.H. Thompson. 1st Ed. Griffin: London p114.

Rall, T.W., Sutherland, E.W. and Berthet, J. (1957) — The relationship of epinephrine and glucagon to liver phosphorylase. IV. Effect of epinephrine and glucagon on the reactivation of phosphorylase in liver homogenates. J. Biol. Chem., 224, 463-475.

Ratnoff, O.D. and Patek, A.J. (1942) — The natural history of Laennec's cirrhosis of the liver. Medicine, 21, 207-268.

Rex, J.C., Code, C.F. and ReMine, W.H. (1964) — Gastric Secretion of Acid and Urinary Excretion of Histamine in Dogs with Portacaval Transposition. Ann. Surg., 160, 193-201.

Rosen, H., Chandler, J.G., Multer, F. and Orloff, M.J. (1971) — The role of cyclic adenosine 3' 5' monophosphate in gastric secretion. Surg. Forum, 22, 291-293.

Rousselot, L.M., Panke, W.F., Bono, R.F. and Moreno, A.H. (1963) — Experiences with Portacaval Anastomosis. Am. J. Med., 34, 297-307.

Ruderman/

Ruderman, R.L. and Kamel, I. (1970) — The effect of intestinal resection on gastric acidity in the rat. Surg. Forum, 21, 312-313.

Rutherford, R.B., Mehlman, B., Brickman, R.D. (1966) — Regional contributions to portal blood histamine in the dog. Surgery, 60, 159-168.

Santillana, M., Wise, L., Schuck, M. and Ballinger, W.F. (1969) — Changes in gastric acid secretion following resection or exclusion of different segments of the small intestine. Surgery, 65, 777-782.

Schmidt, H.A. and Martini, G.A. (1969) — Gastric Secretion in Chronic Liver Disease. Ger. Med. Mon., 14, 246-254.

Schnitker, M.A. and Hass, G.M. (1934) — A histologic study of the liver in patients affected with peptic ulcer. Am. J. Dig. Dis., 1, 537-543.

Schriefers, K.H., Schreiber, H.W. and Esser, G. (1963) — Zur Frage der Magensaftsekretion und des Magen-Duodenalulcus beim Pfortaderhochdruck der Lebercirrhose und nach porto-cavalen Shunt-Operationen. Langenbecks Arch. Chir., 302, 702-715.

Scobie, B.A. and Summerskill, W.H.J. (1964) — Reduced gastric acid output in cirrhosis: Quantitation and relationships. Gut, 5, 422-428.

Silen, W. and Eiseman, B. (1959) — The nature and cause of gastric hypersecretion following porta-caval shunts. Surgery, 46, 38-47.

Silen/

Silen, W. and Eiseman, B. (1961) — Evidence for histamine as the agent responsible for gastric hypersecretion after portacaval shunt. Surgery, 50, 213-219.

Silen, W., Hein, M.F., Albo, R.J. and Harper, H.A. (1963) — Influence of liver upon canine gastric secretion. Surgery, 54, 29-35.

Sircus, W. (1953) — The intestinal phase of gastric secretion. Q. J. Exp. Physiol., 38, 91-100.

Sokolov, A.P. (1904) — Analysis of the secretory work of the stomach in the Dog. Thesis, St. Petersburg. Quoted in: Babkin, B.P., Secretory Mechanisms of the Digestive Glands. 2nd Ed. 1950. Paul Hoeber: New York.

Stelzner, F. (1965) — Die Frage des hepatogenen Ulkus. Munch. Med. Wochenschr., 107, 773-780.

Sutherland, E.W., Oye, I. and Butcher, R.W. (1965) — The Action of Epinephrine and the Role of the Adenyl Cyclase System in Hormone Action. In: Recent Progr. in Horm. Research, ed. G. Pincus, 21, 623-642. Academic Press, New York.

Swisher, W.P., Baker, L.A. and Bennett, H.D. (1955) — Peptic ulcer in Laennec's cirrhosis. Am. J. Dig. Dis., 22, 291-294.

Tabaqchali/

Tabaqchali, S. and Dawson, A.M. (1964) — Peptic ulcer and gastric secretion in patients with liver disease. Gut, 5, 417-421.

Thompson, J.C., Tramontana, J.A., Lerner, H.J. and Stallings, J.O. (1962) — Physiologic Scope of the Antral Inhibitory Hormone. Ann. Surg., 156, 550-569.

Thompson, J.C. (1966) — In Discussion: The question of an antral chalone, p.193-228. Gastrin, Proceedings of a Conference, Sept. 1964, UCLA, ed. M.I. Grossman, Butterworths: London.

Thompson, J.C. (1969a) — Alterations in Gastric Secretion after Portacaval Shunting. Am. J. Surg., 117, 854-866.

Thompson, J.C. (1969b) — In Discussion: Humoral mediation of the intestinal phase of gastric secretion and of acid hypersecretion associated with portacaval shunts, by Orloff, M.J., Villar-Valdes, H., Rosen, H., Thompson, A.G. and Chandler, J.G. Surgery, 66, 118-130.

Thompson, J.C., Reeder, D.D., Davidson, W.D., Charters, A.C., Bruckner, W.L., Lemmi, C.A.E., Miller, J.H. (1969) — Effect of Hepatic Transit of Gastrin, Pentagastrin and Histamine Measured by Gastric Secretion and by Assay of Hepatic Vein Blood. Ann. Surg., 170, 493-503.

Updike/

Updike, E.H., Code, C.F. and Hallenbeck, G.A. (1958)
Excretion of Free Histamine in Urine During Gastric Secretion Stimulated by Humoral Mechanisms. Am. J. Physiol., 195, 197-201.

Villar-Valdes, H., Thompson, A.G., Rosen, H., Chandler, J.G. and Orloff, M.J. (1969)
Endogenous origin of the humoral agent responsible for gastric hypersecretion associated with portacaval shunt. Surg. Forum, 20, 360-362.

Wantz., G.E. and Payne, M.A. (1961)
Experience with portacaval shunt for portal hypertension. New Engl. J. Med., 265, 721-728.

Wilken, B.J., Hunt, D., Lowe, C.F., Billups, W.A., and Hardy, J.D. (1969)
Gastrin inhibition of gastric secretion before and after portacaval shunt. Surg. Gynecol. Obstet., 129, 22-26.

Wilkinson, F.O.W. and Riddell, A.G. (1965)
Studies on gastric secretion before and after portacaval anastomosis. Br. J. Surg., 52, 530-535.

Wilson, C.W.M. (1954)
The matabolism of histamine as reflected by changes in its urinary excretion in the rat. J. Physiol. (Lond.), 125, 534-545.

Windsor, C.W.O., Thompson, J.C. and Orloff, M.J.O. (1965)
Effects of Experimental liver disease and portacaval shunts in gastric acid secretion and blood histamine. Surg. Forum, 26, 322-324.

Windsor/

Windsor, C.W.O., Fejfar, J. and Woodward, D.A.K. (1969) Gastric secretion after massive small bowel resection. Gut, 10, 779-786.

Wise, L. (1969) In Discussion: Humoral mediation of the intestinal phase of gastric secretion and of acid hypersecretion associated with portacaval shunts by Orloff, M.J., Villar-Valdes, H., Rosen, H., Thompson, A.G. and Chandler, J.G. Surgery, 66, 118-130.

Woodward, E.R., Lyon, E.S., Landor, J. and Dragstedt, L.R. (1954) The physiology of the gastric antrum. Gastroenterology, 27, 766-785.

ACKNOWLEDGEMENTS

ACKNOWLEDGEMENTS

The investigations which form the basis of this thesis would not have been possible but for the encouragement that I received from many people. I am particularly thankful for the opportunity of spending one year from July 1971 in the surgical laboratory of Dr. Marshall J. Orloff, Professor and Chairman, Department of Surgery, University of California, San Diego, and I am deeply indebted to him for permitting me to be solely responsible for the conduct and data-recording of these studies.

I wish to thank Drs. James Chandler and Hymen Rosen for their interest and support, and my fellow surgical residents, Drs. Masumi Nozawa, Tom Modafferi and Stuart Levine for their valuable assistance. I am sincerely grateful to Mrs. Ruth Valleau, medical artist; Mr. Tom King, photographer; and Mrs. Jean King for typing the manuscript.

During my tenure in San Diego, I was supported financially by the Eastern Regional Hospital Board of Scotland; The Board of Management, Dundee General Teaching Hospitals; a Fulbright Travel Scholarship; and/

and the J.W. Duncan Baxter Scholarship (University of Cape Town); for all of these, I am especially thankful.

QUESTIONS POSED BY EXAMINER 1 on the Thesis entitled "On the Humoral Mediation of the Intestinal Phase of Gastric Secretion" submitted by Dr R.C.Kester for the degree of Doctor of Medicine.

1. Although it seems very reasonable to look for a direct correlation between the maximal concentration of circulating stimulant hormone arising from the small intestine, and the peak acid response in time, need these two factors of necessity correlate? It might be, for example, that variations in synergy or potentiation from other stimuli, or the local cholinergic background in the fundic gland area might be more crucial to the timing of the peak response, than the concentration per se of the humoral agent. This might particularly apply to these experiments which are conducted under general anaesthesia, during which a reduction in cholinergic activity would likely be present.

2. In the experiments using plasma derived from portal vein blood were steps taken to remove or discount histamine and other secretagogues? This would seem important before concluding that any stimulation of secretion from the administration of this plasma was due to an additional material.

3. While it is admitted that the demonstration of acid hypersecretion in pigs following portacaval shunt is a welcome addition to the background knowledge to the present work, particularly when extracts of mucosa have been made from this species, can we be absolutely convinced that the hypersecretion is really arising from a source in the small intestine? After all, these animals, unlike the dogs, still retained their antra, and the meals were administered orally, rather than by tube directly into the small intestine. It is admitted that it is very likely the mechanism of this acid hypersecretion is strictly comparable to that in the dog experiments, but it seems that absolute proof of this fundamental conclusion is lacking. Would it not have been better more closely to mimic the experimental situation of the dogs when doing the experiments in the pigs?

4. Again when it comes to the preparation of extracts of the mucosa of the intestine, it would clearly have been preferable to use as starting material for these extracts just the mucosa from the small intestine without the added colon. Although the excuse is presented that it was not possible to alter the technique used in the abattoir, surely it is possible by direct personal attendance, for the medical investigators and technicians to secure the mucosa of the appropriate segment for themselves by simply separating the mucosa of the two organs at the time of collection. Even if a small sample of one individual batch were obtained in this way this might have given further strength to the argument that the peptide isolated in such a manner was relevant. An attempt at drawing up a profile or distribution of the peptide in different segments of the small intestine would also, of course, be of interest.

5. There is a considerable amount of repetition in the introduction and the historical background chapters, and this comment applies to several parts of the text.

6. How does /...

6. How does one reconcile the finding that the acid hypersecretion induced by portacaval shunting in animals was not abolished by resection of either the jejunum or ileum? It doesn't seem altogether a complete answer simply to say (page 31) that the experimental evidence was on a small number of observations, or that the effects of small bowel resection introducing a certain degree of hypersecretion may have confused the issue. It is presumed that the latter comment suggests that acid hypersecretion resulting from the partial resection of small bowel may have more than compensated for the removal of a certain amount of source of intestinal humoral stimulant.

7. One slight cause for concern, to which mention has already been made, is that many of the current observations were made under general anaesthesia. It is generally known that most forms of general anaesthetic will suppress gastric secretion both under basal conditions and in response to a variety of stimuli, and the pattern of response may be somewhat different from that obtained in the fully conscious state. The general anaesthesia alone may be responsible for both induced and delayed responses, and may be an important factor in the long latent period which is apparent in many of the present series of experiments.

8. From the results in Table 6 it appears that there is quite a considerable degree of variation in the Heidenhain pouch responses with the passage of time. For example, Dog 1 shows a range from 1.67 to 5.06 mEq, Dog 7 shows a range from 1.22 to 6.08 mEq, and these two extremes are within one month of each other. These considerable variations are perhaps worthy of comment.

9. Why was titration carried out to a pH of 4.5 rather than the neutral point of 7.0?

10. Turning next to page 62, can we be sbsolutely certain that <u>all</u> dogs which did not show acid hypersecretion following the portacaval shunt, solely had some other specific or non-specific explanation for this failure? Why did these dogs show cachexia or haemorrhagic inflammation of the pouch, or some other complication, and was there any other possible explanation for the failure of a proportion to show the customary hypersecretion? What about the autopsy findings in these dogs who did not hypersecrete - did these have blocked shunts?

11. With regard to the animals which showed "Hypersensitivity" types of reaction following repeated administration of portal vein plasma, were the secretory responses in these animals used or discarded? There always must be a little uncertainty about the interpretation of secretory responses in the presence of such reactions. First a possibly significant release of histamine or other secretory stimulants might artifically increase responses, and on the other side, it is well-known that nausea, pyrexia, and other non-specific indicators of general systemic upset may quite distinctly suppress secretory responses. From the data presented in Table 23 it appears that the hypersensitive dogs had lower basal secretion, but higher test responses to portal plasma. How, therefore, can it be confidently concluded, as stated on page 69, that the presence or absence of a reaction had no effect, when test responses admittedly doubled, i.e. increased by 100 per cent!

12. On page 64/...

12. On page 64 it is stated that tests had to be done within a week of the portacaval anastomosis, since with the passage of further time liver damage developed. Does this not mean that all the animals were already sick on account of this damage, and presumably the rate of the damage developing varied from one animal to another. Therefore a considerable contribution to the secretory changes observed could well be due to this liver upset rather than primarily to the alteration in blood flow diverted past a supposedly normal liver.

13. It is not absolutely clear what was the source of the bank blood used in the control experiments, and it would seem important particularly to know if the blood was withdrawn in dogs which had been fasted for a sufficient length of time to avoid the inclusion of any small intestine stimulatory hormone following feeding.

14. Can we be absolutely sure that the nature of the food introduced into the jejunum is not important in eliciting this small intestinal phase of secretion? It sounds just a little unlikely that the only mechanism of triggering off the release of this hormone is mechanical distension, and I wonder if sufficiently thorough analyses of the different diet components irrigated through segments of the small intestine with the avoidance of significant distension, have been made.

15. In Table 9, although the mean control plasma level is less than that of the harvested portal vein blood, two dogs gave large acid responses to the control plasma, and indeed the outputs to the control were grossly in excess of anything achieved with the test plasma. Indeed these responses to the control plasma are so large as to invite comment and possible explanation.

16. If we compare the results in the assay dogs presented in Tables 11, 13, 15, 17 and 19, we see that there is a marked difference in the secretory outputs during the control plasma tests. In the controls for the tests using blood harvested at 45 and 60 minutes particularly the controls were very high indeed, 0.22 and 0.38 mEq as compared to 0.04 and 0.08 in the two series with blood collected at an earlier stage. It seems entirely possible that these unusually high control values may have defeated any statistical significance which might be associated with the secretory responses to the test portal vein plasma.

17. It is also interesting in this series of experiments with portal blood harvested at different times after the meal, that when the control values were high there is almost an impression of "inhibition" following the portal blood infusion. Is it a possibility that this portal vein blood might contain in addition to the accepted humoral stimulant, an additional inhibitor agent?

18. In Tables 27 and 28 there is quite a large variation in the meal responses in repeat tests in the same animals. This is particularly marked in the first animal in each table.

19. I wonder /...

19. I wonder why the effects of the pig extracts were not tested in the same species, i.e. pigs, particularly when this group of investigators clearly had developed expertise with these animals. This would have immediately got over any major argument of possible species difference.

20. Under number 10 on page 130 it is stated that aliquots of the extract were withdrawn for gastrin assay. I wonder if assays of other possible stimulants, e.g. histamine, were also performed?

21. In the summary of mechanisms of stimulation of gastric secretion on page 148 I think it would have been appropriate also to mention the possibly very important contribution of direct distension of the fundic gland area.

22. As is commented, the earliest responses were to meals which were given orally, and does this not add considerable support to the notion that the small intestine stimulating hormone of gastric acid secretion depends for its full and prompt response on co-operation with other phases of stimulation of the secretion?

23. Finally, I think that perhaps one of the greatest questions with which the reader is left is what evidence is there that the factor released in the dog experiments is necessarily in any way related to the material which has been extracted in the pig mucosa experiments? It may be possible to extract a very large range of peptides with considerable power to stimulate gastric acid secretion, by a variety of extraction processes, but this is a very long way indeed from proving that such factors have any physiological role, and particularly that they play a part in the mediation of the stimulation arising from the small intestine. It is well known that many powerful biological effects can be manifest by extracts prepared from a variety of techniques, and they may or may not have any physiological significance. I do not feel that any evidence has been presented either for or against this particular material extracted in this series of experiments being a normal physiological mediator of the small intestinal phase of gastric acid secretion. After all, this material was collected primarily from fasting pigs, and in the fasting situation as illustrated bythe earlier work in this thesis, it has not been possible to demonstrate effective quantities of the stimulating agent. Against this line of argument, however, it could be stated that the material is accumulating in the cells of origin in the mucosa of the small intestine, but I just believe we have no good evidence one way or the other. It certainly would be interesting to extend this work in an attempt to build up a more complete picture of the nature, source, distribution, and mechanisms for release enactivation etc. of this material, and it is possible that this is under way. Meantime, from the evidence presented in this thesis, we are still clearly a long way from knowing whether this substance is of importance or not.

QUESTIONS POSED BY EXAMINER 2 on the Thesis entitled "On the Humoral Mediation of the Intestinal Phase of Gastric Secretion" submitted by Dr R.C.Kester for the degree of Doctor of Medicine.

1. In showing the gastric hypersecretion in a denervated pouch after portacaval shunt and instillation of food into the jejunum he draws heavily on previous work done by the department and does

(a) not present any control dogs without portacaval shunting
(b) has failed to establish whether the pouches are completely denervated by insulin testing and
(c) glosses over the possibility that the product may be histamine by reference to previous authors
(d) does not adequately explain why 10-20% of dogs showed no such secretion - a factor which might have changed the statistics quite drastically
(e) does not mention the Cape Town work showing that bile duct ligation or liver damage alone will cause gastric hypersecretion, and as his dogs were investigated in the first week after operation, whether this might not be a factor.

2. He rather abruptly dismisses the previous work showing that total small bowel resection and even colectomy still causes acid hyper-secretion and that various manoevres on the liver and bile ducts will cause hypersecretion. He also fails to mention the possible or probable release of cholecystokinin which is a known stimulant of gastric acid secretion.

3. Consequently, in the absence of being able to extract an hormone, the evidence rests very heavily on a group of experiments which show that portal vein plasma causes hypersecretion at 30 and 45 minutes after infusion to an assay dog preparation. Although significant at the 1 in 50 and 1 in 20 level these differences are rather slight and had there been a minor variation in 1 or 2 results at each of these interval times, the results might well have been inconclusive.

4. Finally, Dr Kester has not described his statistical methods thus greatly loading the reviewer with an added burden of checking some of the statistics by a number of methods.

QUESTIONS POSED BY EXAMINER 3 on the Thesis entitled "On the Humoral Mediation of the Intestinal Phase of Gastric Secretion" submitted by Dr R.C. Kester for the degree of Doctor of Medicine.

1. The gastric acid secretion was measured from denervated (Heidenhain) pouches. No mention was made whether the pouches' response to insulin was ever tested to confirm they were truly denervated. Neurogenic factors thus cannot be entirely excluded. Secondly, the pouches' response to a known stimulator of gastric acid secretion (e.g. histamine or pentagastrin) would have given much better perspective as to how significant any intestinal phase of gastric acid secretion might be. The intestinal phase is described as "profound", but how does this compare with the actual and theoretic maximal acid response?

2. The dogs used in the first part of the study were said to develop progressive liver damage after a period of one week following portacaval shunting. It would be important to know the state of their livers when the study was done at four days. A liver biopsy could have been done as the dogs were under light anaesthesia, and in fact it seems as if they were sacrificed after the study. If this were so, renal histology would have been useful as gastrin is largely excreted by the kidneys.

3. The results of the studies are defined in tables. A clearer picture might emerge by plotting graphs of control and test data. In chapter 4 the total acid output of the 4 hours of control infusions was given as a single figure; it would be useful to know how the pattern of secretion during the control period differed from that during the test infusion.

4. No mention is made of the statistical methods used. Most of the data are skewed and seem more fitted to non-parametric than parametric statistical analysis. In fact using a Mann-Whitney two-tailed U-test, the data in table 9 becomes not significant (P 0.10), but, using the same method, data in table 11 remains highly significant.

5. It is difficult to understand the reason for the time delay between stimulation and effect. In donor dogs the mean time of onset of gastric acid secretion was 45 minutes after the stimulus, with peak stimulation at 2.9 hours. In assay dogs, the mean time of onset of gastric secretion was 61 minutes with a peak at 1.6 hours. If the onset of secretion is delayed in the assay dogs, it is difficult to fathom why the peak of secretion should occur earlier than in the donor dogs. Furthermore, the assay dogs had a normal circulation and, presumably normal livers. It has been claimed that the intestinal phase hormone (IPH) is rapidly inactivated by the liver. If this is in fact so, why was it not inactivated during the inordinately long time it took to act in the assay dog. It was suggested that the delay in action took place at the effector site (the parietal cell); this may be so, but to postulate this might be due to time required for activation of adenyl cyclase and the cyclic AMP system is unrealistic as this is extremely rapid, the effect usually being noticeable in minutes. It is noted that the infusion of crude gut extract into pigs resulted in a far more rapid effect - onset usually is in the first half hour period. This is far more in keeping with a true hormonal effect.

6. Transfusion /...

6. Transfusion reactions were understandably experienced with repeated infusions of plasma. When reactions were severe, hyposecretion was more common than hypersecretion. Thirty-two control and twenty-four test infusions were associated with reactions. Assuming an equal proportion of reactions to be severe in each group, this would bias the figures against the control infusions.

7. It is not permissable to assume that delayed onset of secretion in an orally fed pig results from gut stimulation. A pig's stomach is notorious for its slow emptying with constant bile reflux into the gastric antrum. No attempt was made to ensure when and to what extent the stomach had actually discharged its contents into the duodenum.

The next weighty evidence that there might be such a substance as IPH is provided in chapter 7 where a dose-related response is achieved with intravenous administration of crude extract of pig intestinal mucosa. This was indeed a major undertaking. It would have been interesting to see whether known extracts of gut mucosa (e.g. cholecystokinin) would have produced a similar response, and perhaps what the extract would do to secretion stimulated by a background submaximal dose of gastrin. This would have enabled a clearer definition of some of its properties.

ANSWERS TO QUESTIONS POSED BY EXAMINERS on the Thesis entitled "On the Humoral Mediation of the Intestinal Phase of Gastric Secretion" submitted by Dr R.C. Kester for the degree of Doctor of Medicine.

Examiner 1, Questions 1 and 7

I believe that any influence of anaesthesia was eliminated by the use of controls that were also anaesthetized. In the previous experiments on cross circulation that were done under anaesthesia (Orloff, Villar-Valdes, Rosen, Thompson and Chandler, 1969), the secretory pattern was similar to that found in subsequent experiments. Moreover, studies have been performed in both humans (Orloff, Abbott and Rosen, 1970) and dogs (Villar-Valdes, Thompson, Rosen, Chandler, and Orloff, 1969) without anaesthesia, and the pattern of response has been identical.

Examiner 1, Question 2.
Examiner 2, Question 1c.

The question was put whether histamine could be responsible for the stimulation, and was it looked for. Since the portal blood is being shunted into the systemic circulation, it should make no difference whether one looks for histamine in the portal blood or in systemic blood. In previous studies on histamine, blood was sampled from a catheter in the inferior vena cava just opposite the portacaval shunt, and no differences in the levels were found when compared to systemic arterial blood (Windsor, Thompson and Orloff, 1965). Since it had been shown conclusively that histamine is not the humoral agent responsible for the gastric hypersecretion which accompanies portacaval shunt, it did not seem warrantable to evaluate its significance in the present investigations.

Examiner 1, Question 3.

The studies of shunt-related gastric hypersecretion in the pig have been conducted along three lines. The first plan was to determine if the pig develops portacaval shunt-related gastric hypersecretion in response to food comparable to that observed in the dog and man. The results presented in this thesis indicate that such gastric hypersecretion after hepatic bypass does occur.

The remaining 2 studies in pigs are still in progress. They are examining:

(a) The gastric secretory response to intestinal distension by an indwelling jejunal balloon catheter before and after end-to-side portacaval anastomosis. Each pig undergoes intestinal meal and balloon inflation tests.

(b) Gastric secretory response to food instilled into isolated segments of the intestine before and after portacaval shunt. The segments of bowel isolated in the form of Thiry-Vella fistulas are the duodenum, jejunum ileum, and proximal three-fourths of the colon.

Examiner 1, Question 4.

In an attempt to improve the extraction procedure of the active agent from the gut mucosa of the pig, extracts are now made solely from the small bowel. This change in procedure was made following the first group of 25 extracts, and I am not in a position to give first-hand details of the results.

Examiner 1 /...

Examiner 1, Question 8.

Although the assay dogs were well-conditioned to laboratory conditions, variation in the gastric acid response to successive monthly histamine tests was apparent. The dogs were starved for 24 hours prior to the histamine test, and were tested in groups of 3 - 4 in a quiet room. There may have been a more uniform response if each dog was tested in isolation. Dogs 1 and 7, and indeed Dog 9, were withdrawn from the batch when it was obvious that their gastric pouches were not responding satisfactorily to histamine. In the definitive experiment, each dog acted as his own control.

Examiner 1, Question 9.

Most indicators used for titration of acid change at a pH of 3.5 to 4.5, rather than pH 7.0. The choice of indicator is arbitrary, but any "acid" that would be picked up between pH 4.5 and 7.0 would be insignificant.

Examiner 1, Question 10.

Autopsy of the donor dogs who failed to show gastric hypersecretion after portacaval anastomosis demonstrated that their shunts were still patent. These dogs were noted to be in poor condition due to cachexia, in marked contrast to the secreting dogs, because following antrectomy and construction of a Heidenhain pouch, the remaining gastric stump was insufficient to maintain adequate nutrition. If the Heidenhain pouch was not fashioned with careful attention to the preservation of the blood supply, then mucosal bleeding at the time of the test rendered the gastric sample unsuitable for colorimetric titration.

Examiner 1, Question 11.
Examiner 3, Question 6.

The effect of hypersensitivity reactions on the acid responses to control and portal plasma was looked at with interest. No definite secretory pattern could be established, as reactions and nonreactions were randomly scattered throughout the low and high acid responses, and there was also no correlation between the degree of reaction and the level of acid secretion.

Examiner 1, Question 12.
Examiner 2, Question 1e.
Examiner 3, Question 2.

Perhaps I made too fine a point about emphasizing carrying out the intestinal meal tests soon after establishment of a portacaval shunt, i.e. portacaval shunt made on a Monday, and the intestinal meal test and portal transfer study carried out on the succeeding Friday. Although liver biopsies were not done to assess possible liver damage, the livers of the sick animals at autopsy, and the healthy shunted animals (inspected during retrieval of the cannulae) were always macroscopically normal. With this short interval after shunting, we believed that liver damage was insignificant.

Examiner 1, Question 13.

For the source of bank plasma for the control experiments, mongrel dogs weighing between 15-25 kg were used. They were starved for at least 24 hours prior to collection of their blood, which was by exsanguination of the lightly-anaesthetized animal on the afternoon before the test day. The blood was centrifuged, and the plasma was stored at 4°C.

Question 14 /...

Question 14.

Regarding the nature of the substances introduced into the jejunum, a number of investigators have studied the effect of protein and non-protein foodstuffs, organic and inorganic chemical agents on the intestinal phase. No studies that I am aware of have specifically avoided distension during the introduction of the substances, or have compared the secretory responses by statistical analysis.

Questions 15 - 16.

I am not able to give a reasonable explanation why the control values for the 15, 45 and 60 minute (plasma transfer) tests were higher than usually obtained. Each dog served as his own control, and the differences in acid response were analysed.

Question 17.

Since circulation of the intestinal humoral factor would stimulate the gastric remnant (apart from the pouch), acidification of the duodenum would release secretin, an inhibitor of gastric secretion, and such an inhibitor would be collected in the harvested portal blood, together with other possible unidentified humoral intestinal inhibitors.

Question 18.

It is admitted that the individual responses for each pig varied with successive tests, despite patient conditioning of the animal to the laboratory environment, and seclusion of the animal in a quiet room with the same investigator on each occasion. The pig in a Pavlov stand was much more restless than the docile assay dogs, and this may account toward the variability of the results. (See also answer to Question 3).

Question 19.

The question of testing pig intestinal mucosal extracts in pigs is important, bearing in mind species specificity: some of the problems preventing such testing were mainly economical and logistic in keeping a set of pigs with gastric pouches at hand for a prolonged period.

Question 20.

During extraction of the active peptide from the pig mucosa, histamine was left attached to the DEAC resin during elution of the peptide with sodium hydroxide, and was removed by centrifugation. A routine check of the final product confirmed the absence of histamine.

Question 22.

It is highly likely that the intestinal phase bears a synergistic relationship to the other phases of gastric secretion, and this possibility has drawn the attention of several investigators. A study would need to be made to compare the acid secretory response to oral and intestinal feeding in order to answer one aspect of the question asked. The answer may come when the results appear from current investigations of shunted pigs with Thiry-Vella fistulas.

Question 23/...

Question 23.

One cannot be certain that the factor released in the dog is related to the material extracted from the pig mucosa. In the experiments conducted, the pig and the human respond to an intestinal meal in a manner identical to that observed in the dog. From a biological standpoint, it would be highly unusual for the pig and the dog to be different.

Examiner 2, Question 1a.

It was not deemed necessary to study control dogs without portacaval shunting, since this would have repeated previous studies in this laboratory employing such controls (Orloff and others, 1969; Orloff, Villar-Valdes, Abbott, Williams and Rosen, 1970).

Examiner 2, Question 1b.
Examiner 3, Question 1.

Most workers in the field of gastric physiology do not feel that it is necessary to test so standard a preparation as the Heidenhain pouch for completeness of denervation. Regardless, the key to all studies is the inclusion of parallel controls that do not show the response.

Examiner 2, Question 2.
Examiner 3, Question 7.

It is possible that cholecystokinin is released during intestinal feeding. However, cholecystokinin is only a mild stimulant of gastric secretion, and in a relative sense, is not nearly as potent as the intestinal phase hormone. Moreover, the main source of cholecyctokinin is the duodenum, while the main source of the intestinal phase hormone is the jejunum.

Examiner 2, Question 4.
Examiner 3, Question 4.

With regard to the statistical method used, in the absence of enough data to decide whether the results were skewed or not, the statistical method employed was the paired test. With reference to Table 9, a non-parametric test (Sign test)* shows the probability to be .008, so that this confirms the significance of the results. Furthermore, the Wilcoxon Matched-Pairs-Signed-Ranks Test* shows the data to be significant at the level of .01.

Examiner 3, Question 3.

A set of graphs are enclosed which plot the control and test data to show the patterns of secretion. Although the pattern of secretion in response to control plasma somewhat resembles that following infusion of the test plasma in the 45, 60 and 90 minute tests, the pattern seen after infusion of portal plasma collected between 30 and 45 minutes shows the most striking difference.

* Nonparametric Statistics for the Behavioural Sciences by Sidney Siegel, p.68 and 75. McGraw Hill Book Co., London, 1956.

REFERENCES

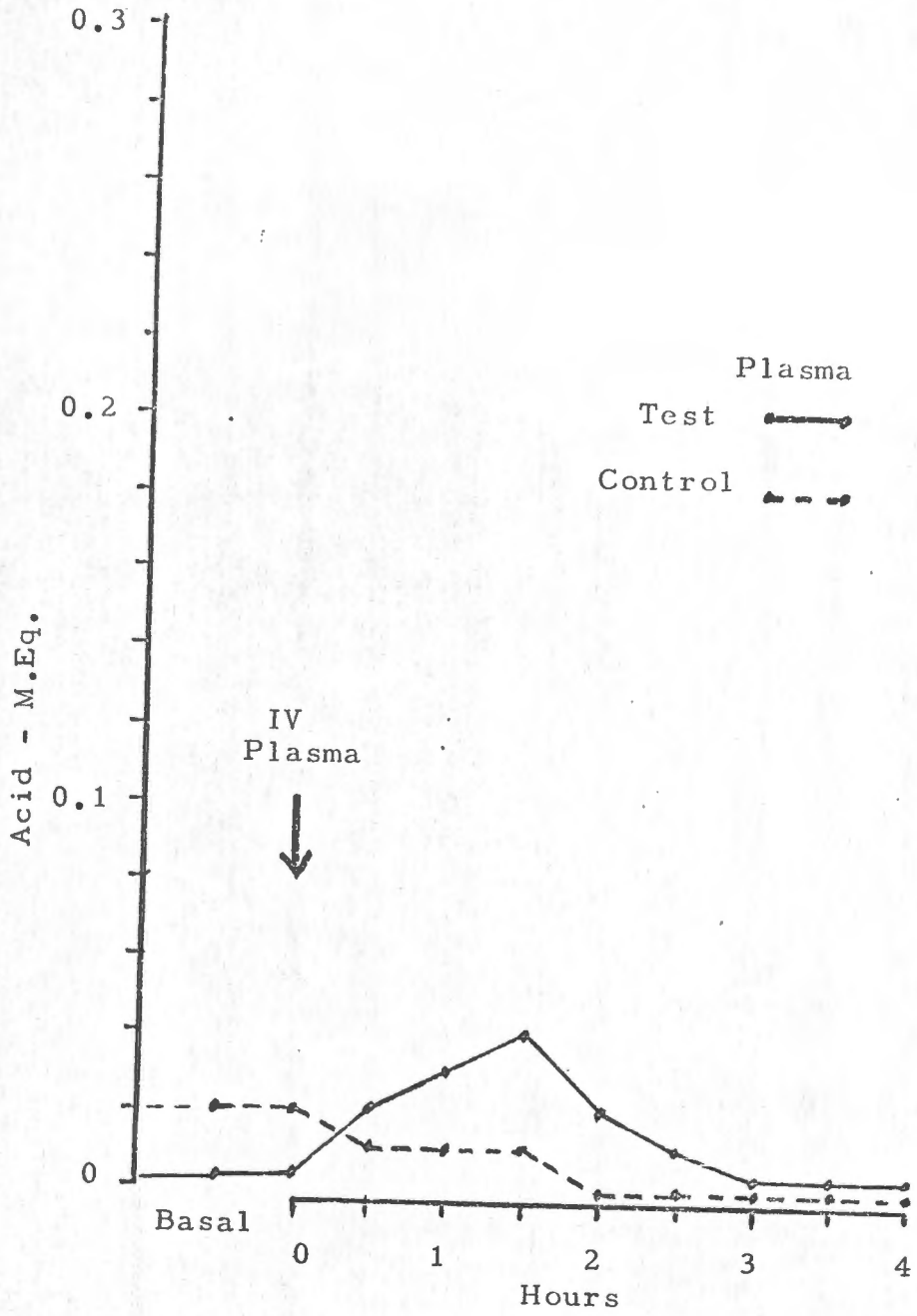
Orloff, M.J., Villar-Valdes, H., Rosen, H., Thompson, A.G. and Chandler, J.G. (1969). Surgery, 66, 118.

Orloff, M.J., Abbott, A.G. and Rosen, H. (1970). Am. J. Surg., 120, 237.

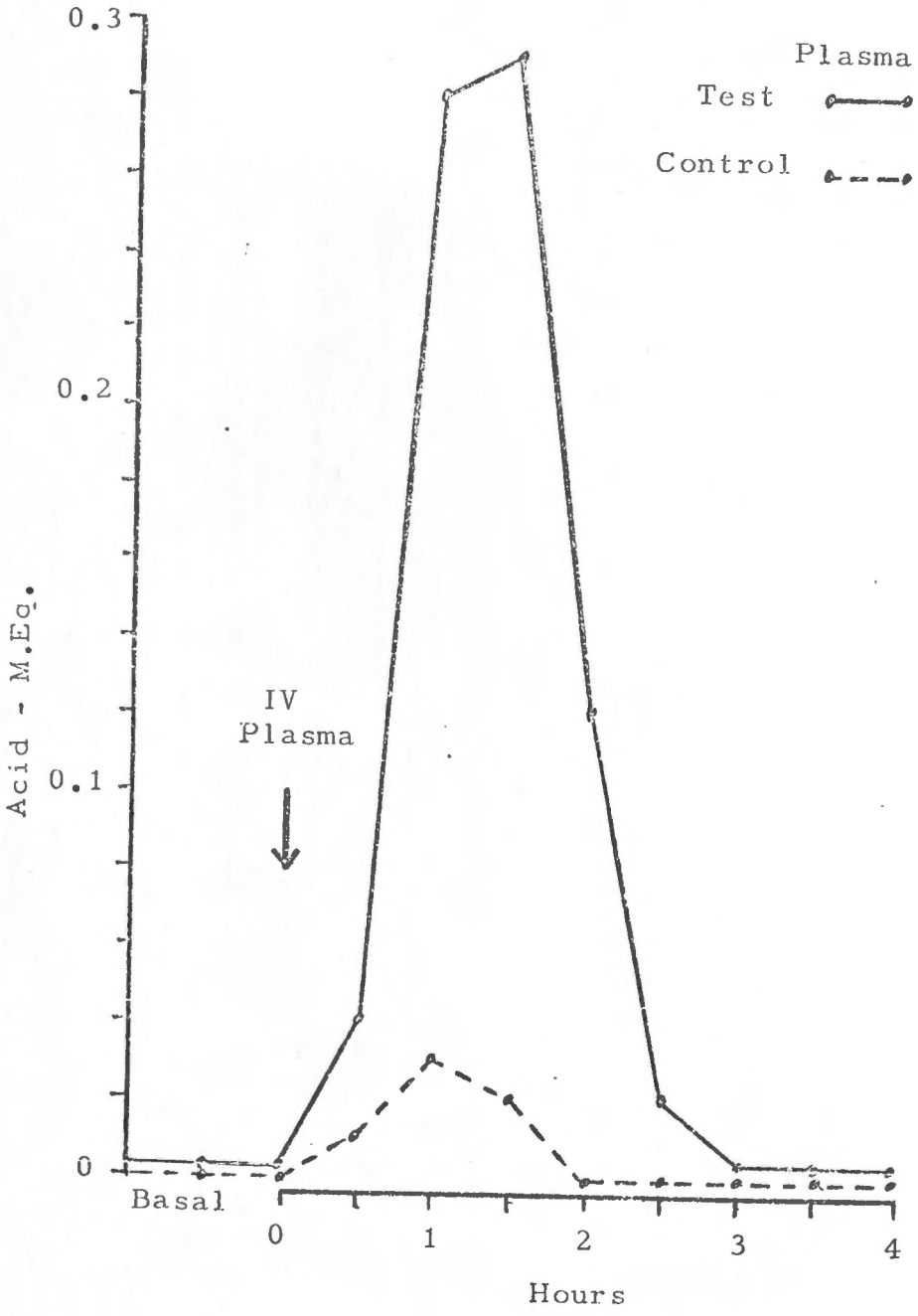
Orloff, M.J., Villar-Valdes, H., Abbott, A.G., Williams, R.J. and Rosen, H. (1970). Surgery, 68, 202.

Villar-Valdes, H., Thompson, A.G., Rosen, H., Chandler, J.G. and Orloff, M.J. (1969). Surg. Forum, 20, 360.

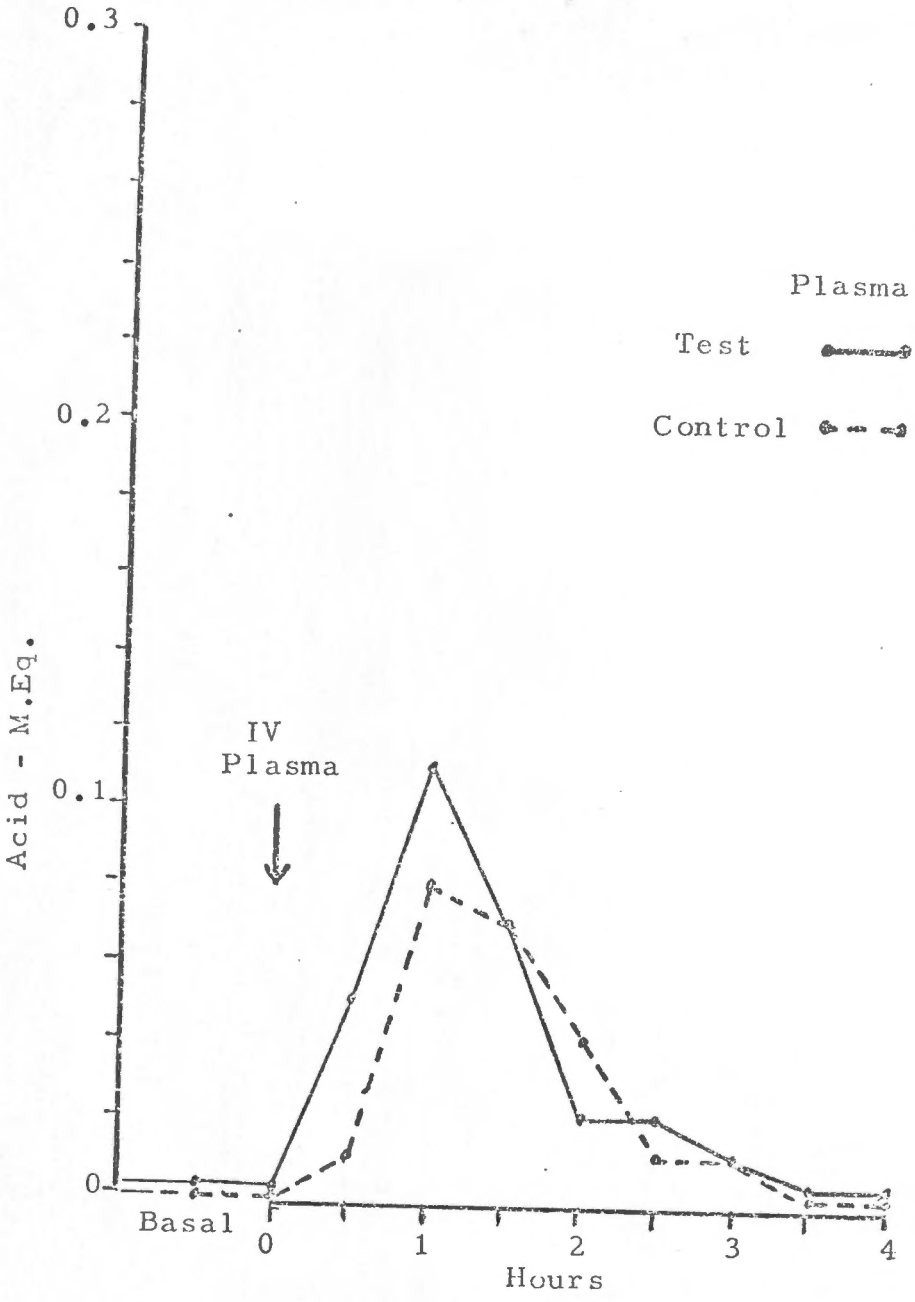
Windsor, C.W.O., Thompson, J.C. and Orloff, M.J.O. (1965). Surg. Forum, 26, 322.



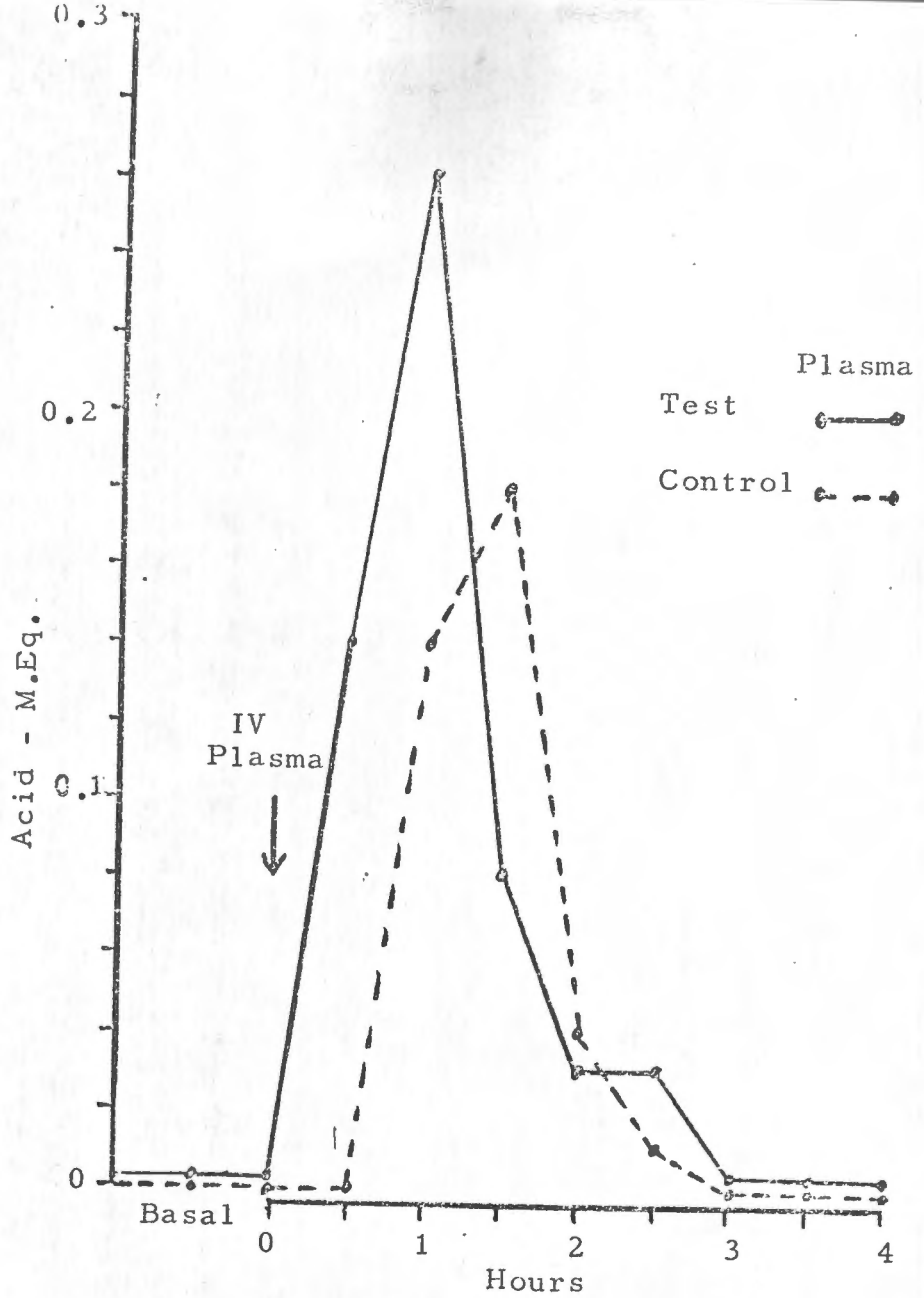
Secretory pattern to infusion of control and portal plasma (harvested at 15 minutes).



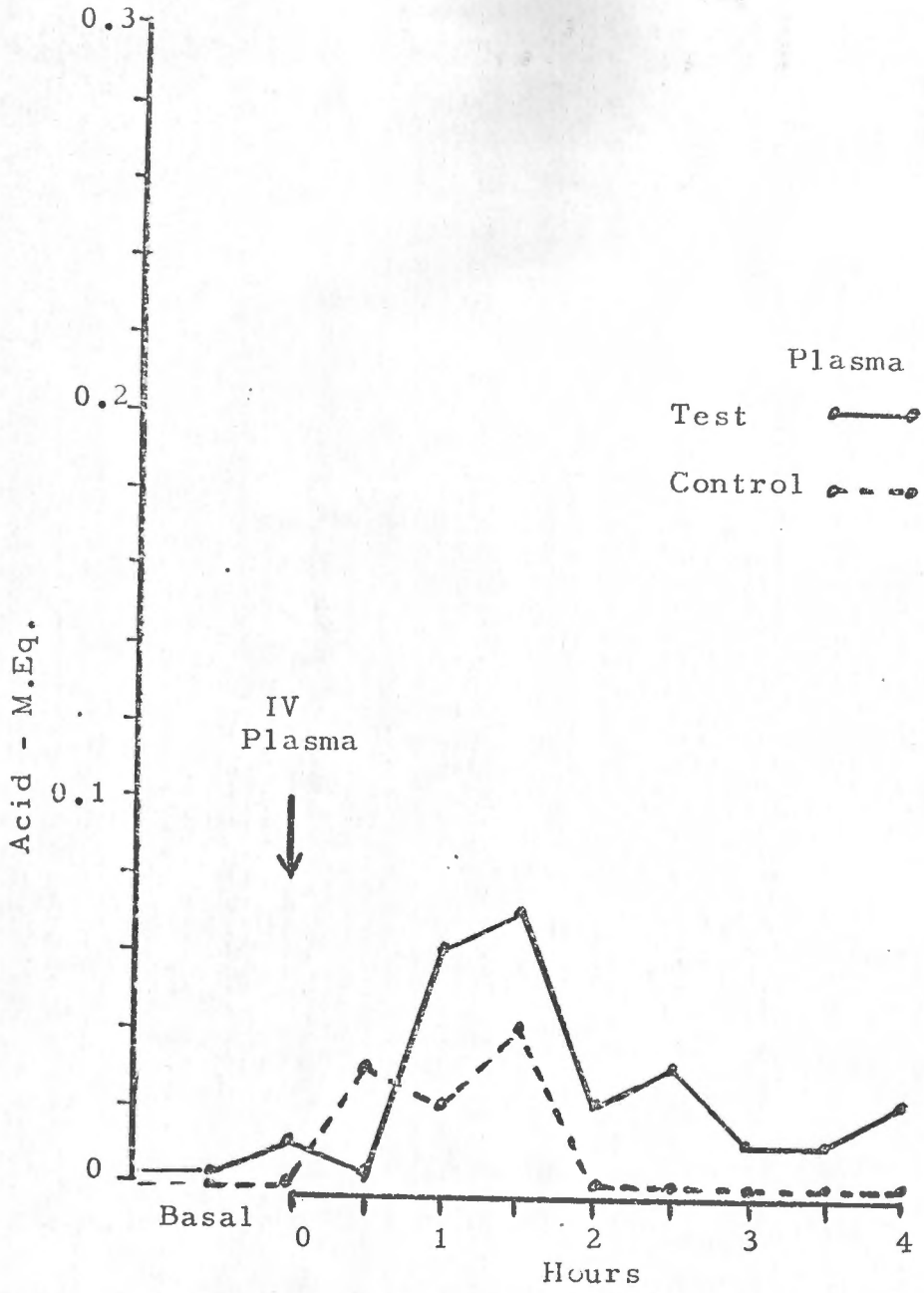
Secretory pattern to infusion of control and portal plasma (harvested at 30 mins.).



Secretory pattern to infusion of control and portal plasma (harvested at 45 mins).



Secretory pattern to infusion of control and portal plasma (harvested at 60 mins).



Secretory pattern to infusion of control and portal plasma (harvested at 90 mins).