CONTROL OF ABOMASAL SECRETION IN THE SHEEP

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T O M Y P A R E N T S

A C K N O W L E D G E M E N T S

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PREFACE

This thesis is concerned with an experimental investigation of abomasal secretion in sheep.

The abomasum is the most caudal region of the ruminant stomach and is the only truly secretory part of it.

The experimental work considered includes the study of hydrochloric acid secretion, the electrolyte composition and the peptic activity of abomasal secretion.

Simultaneous observations have been made of abomasal acid secretion and motility of the reticulum and rumen recorded from partial exteriorisations of these structures. These observations have been made under a variety of conditions including periods when animals were feeding and fasted, and during rumination.

The effect of intraduodenal infusions of fatty acids and oil on abomasal acid secretion and reticulum and rumen motility and of intravenous infusions of the same fatty acids has been studied.

Observations have also been made of the intravenous infusion of various substances reported to influence gastric secretion. Extracts exhibiting 'enterogastrone' activity have been tested; the effects of insulin studied; and the action of a synthetic gastrin (ICI 50, 123) which exhibits gastrin-like properties has been investigated.

These observations were undertaken in the hope that it would be possible to elucidate further, factors which control or affect abomasal acid secretion. An attempt has been made to determine whether any clear cut relation could be established between abomasal secretory activity and reticulo-rumenal motility.

The observations presented below have been discussed in relation to the literature.

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CHAPTER I

INTRODUCTION

The ruminant stomach consists of four discrete compartments: the reticulum, rumen, omasum and abomasum.

Ruminants display two types of gastric digestion of ingested food material; one of these is a fermentative digestion due to the activity of the symbiotic micro-organisms established and maintained in the reticulum and rumen. The other is a hydrolytic digestion due to the hydrochloric acid and pepsin secreted in the abomasum.

In this thesis the gastric secretory activity of the adult ruminant is the prime consideration, that is the secretion which contributes to the hydrolytic digestion.

The Reticulum and Rumen

The reticulum and rumen communicate through a large opening, the reticulo-rumenal orifice, and may be regarded effectively as potentially a single fermentation reservoir. It is in this reservoir that the exploitation of structural carbohydrates takes place. The bacteria and protozoa present in the reticulum and rumen contribute in this respect; the bacteria are largely responsible for the breakdown of cellulose and starch while the protozoa are mainly starch consumers. (Annison and Lewis, 1959).

The activities of the micro-organisms include the following:

1. The production of short chain volatile fatty acids from carbohydrates. These products are absorbed to a large extent from the rumen.
2. The conversion of non-protein nitrogen into amino acids; carbohydrates serve as an energy source in this process.
3. The hydrolysis of triglycerides and hydrogenation of unsaturated fatty acids. Only small amounts of triglyceride and unsaturated

fatty acids enter the duodenum (Garton, 1965): the proportion probably increases with high fat diets (Hill, 1965).

4. Increasing the biological value of ingested protein: this is brought about by processes of degradation and resynthesis.

5. The synthesis of vitamins, which are essential for the well being of the host, but whose production depends on the activity of the symbiotic micro-organisms.

The development from an exclusively hydrolytic gastric digestion in the young suckling animal to a combination of both fermentative and hydrolytic digestions in the adult may be rapid. This transition is stimulated to a considerable extent by the ingestion of plant material (Warner and Flatt, 1965).

In the suckling or milk fed young animal, the ingested food material passes directly to the abomasum by way of the oesophageal groove. The mechanisms controlling this structure have been discussed by Watson (1944) and Comline and Titchen (1951, 1961).

In the young animal the reticulum and rumen are relatively poorly developed (Tamate, 1957) and their motility is characteristically irregular (Benzie and Phillipson, 1957). Dzuik and Sellers (1955) provided evidence that suggested the essential basic neurological mechanisms were present but the occurrence of normal patterns of motility in terms of force and frequency of contractions, depended on muscular development and the presence of solid feed in the reticulum and rumen. However, the irregular motility in milk fed calves compared with that of calves on solid feed may have been due to a deficiency of sensory stimulation, sensory receptors and efferent innervation. The effects should not, without additional evidence, be attributed solely to an under developed musculature.

In the course of the transition of the reticulum and rumen towards their mature form their capacity increases, musculature and epithelium develop and both their circulation and metabolic activity increase (McGilliard, Jacobson and Sutton, 1965). These factors appear to be closely correlated and are markedly altered by the continued ingestion of solid feed.

In young ruminants feeding on milk, the ingested material flows directly to the abomasum. Presumably with progressive maturation of an animal (in an endocrinological and neurological sense) conditions prevail which make it more probable that the maximum response to the stimulus accorded by ingesta will occur. With the regular ingestion of plant material most of which passes to the reticulum and rumen, a potent stimulus to the development of these structures is delivered: the fibrous nature of plant material and the VFA produced from this material are believed to be of particular importance (McGilliard et al, 1965, Warner and Flatt, 1965).

The appropriate neurological development may be governed by local environmental conditions, including the composition of the blood: this overtly changes mainly in respect to blood sugar (a relative hyper to hypoglycaemia) and an increased steam volatile fatty acid (VFA) concentration.

A feature in the development of the reticulum and rumen, is that the symbiotic micro-organisms become essential for digestion to occur. The animal itself aids with its copious secretion of saliva with considerable buffering power and high electrolyte (particularly sodium) content which contributes to the maintenance of a favourable medium for fermentation. The regular muscular contractions enable the reticulum and rumen to act as a continuous fermentation system. These contractions contribute by:

1. Mixing the digesta and so distributing the micro-organisms throughout the 'system'.

2. Enabling rumination to occur whereby material is further broken down.

3. Assisting removal of gaseous by-products of the fermentation.

4. Their role in promoting the passage of ingesta out of the reticulum and rumen for further digestion and absorption in caudal parts of the alimentary tract.

The co-ordinated cyclic activity of the reticulum and rumen depends on their extrinsic innervation via the vagus nerves. In the absence of this innervation and in the absence of such cyclic motility, death follows; as it does after bilateral vagotomy (Duncan, 1953).

The Omasum

The functions of the omasum are not well understood. It possesses a particularly high internal surface area to volume ratio and has attributed to it as one of its functions an important role in absorption (Oyaert and Bouckaert, 1961). It is also thought to contribute to the passage of digesta into the abomasum by pumping or aspiration of the more fluid digesta from the reticulum and rumen (Stevens, Sellers and Spurrell, 1960). Particle size and their density appear to determine to some extent the quantity and nature of the material which passes into the omasum from the reticulum and rumen.

Ekman and Sperber (1952) have remarked on the diminution of bicarbonate which occurs during the passage of digesta through the omasum. This is thought to decrease the neutralising power of digesta flowing into the abomasum. Barcroft, McAnally and Phillipson (1944) have reported that absorption of VFA occurs in the omasum.

The Abomasum

In the abomasum the digesta is subjected to the hydrolytic phase of digestion. The exocrine secretion of the abomasum contains the following components:

1. The proteolytic enzymes pepsin and rennin, the latter's clotting power is of greater importance than its proteolytic activity. Berridge, Davis, Kon, Kon, and Spratling (1943) found that the young milk fed calf secretes only rennin, the secretion of pepsin first became pronounced with the ingestion of solid feed. Henschel, Hill and Porter (1961) could not substantiate these findings. Their results indicated pepsin could be secreted in calves as early as the seventh day. As the ruminant matures rennin secretion diminishes while that of pepsin increases until it is virtually the sole proteolytic enzyme.(Hill, 1956).

   Passive immunity is conferred on the newly born animal by the antibodies attached to the globulin proteins in colostrum. Peptic hydrolysis of these globulins would be detrimental to the animal's health. It would appear from the findings of Hill (1956) that there is little abomasal secretion in the newly born animal. This would increase the opportunity the globulins may have to pass into the small intestine where they are absorbed unchanged.

2. Hydrochloric acid both provides a suitable medium for peptic digestion (optimum pH 2.1) and contributes to digestion by acid hydrolysis.

3. Mucus which acts as a buffer in preventing the destruction of the mucosa by acid, and also as a lubricant in protecting the mucosa from harmful objects.

The abomasum of the adult ruminant possesses the characteristic feature of continuous secretory activity. Due to this the contents of the

abomasum are always of high acidity and remain so even with the frequent passage into the abomasum of near neutral reticulo-rumen contents (the omasum reduces the neutralising power to some extent because of its capacity in reabsorbing CO_2 or HCO_3^-).

In comparison to the situation in animals with a simpler stomach, gastric proteolysis may occur relatively quickly because of the pH conditions and the nature of the material which enters the abomasum. The main feature is the finely divided physical form of the material enabling the rapid penetration of the secretions.

In the sheep it has been estimated the abomasum secretes about 5 - 6 litres and that of the cow or ox 30 - 35 litres of gastric juice/24 hours (Hill, 1965). The volume of saliva secreted/24 hours for a 25 kg sheep is about 6 litres. It is interesting to compare these volumes of acid and saliva with the water content of blood. For a 25 kg sheep this would be in the vicinity of 1.5 litres.

The large volume of acid secretion is produced by an extensive area of gastric mucosa: the surface area of the mucosa is increased by the presence of numerous large folds of it (Hill, 1965). The secretory activity of the cells maintains a continuous flow of juice. Both resynthesis and depletion of 'zymogen' granules has been detected histologically in different cells in small areas of the abomasal mucosa. It is not known whether both processes - resynthesis and secretion - occur at the one and same time in the same cell (Hill, 1965). Presumably they may.

It can be argued that the most important contribution abomasal digestion makes in the ruminant is by its proteolytic activity. This would not occur with any degree of efficiency in the absence of HCl secretion. A consideration of enzyme-substrate reactions makes it appear probable that

it is a more controlled and graded reaction than that which would be accorded simply by acid hydrolysis. Some workers hold the view that peptic digestion in the abomasum is of little significance (Annison and Lewis, 1959) because of the rapid passage of digesta through this region. It should be realised, however, that there is a rapid acidification of digesta entering the abomasum, that pepsin is autocatalytic below pH5, and that consequently the maximum level of peptic activity is likely to be attained within a short time of the entry of digesta into the abomasum. Even though the digesta may not remain long in the abomasum, these considerations suggest that considerable proteolysis may occur in it.

The abomasum could have an absorptive function. This has been postulated in the case of VFA (Phillipson and Ash, 1965) but has not been demonstrated for VFA and such substances. The relative amounts of lipids and their fatty acid compositions do not change markedly in the digesta passing through the omasum and abomasum (Garton, 1965). In the abomasum most of the micro-organisms disintegrate (Smiles and Dobson, 1956), facilitating subsequent digestion of their structural lipids in the small intestine. This disintegration is probably brought about by the unfavourable medium resulting in death of the micro-organisms which are subsequently destroyed by the combined acid and peptic activity.

The presence of a lipase in gastric juice obtained from animals with a simple stomach was suspected as early as 1880 (Cash cited by Luciani, 1913), but this was generally attributed to contamination. Laqueur (1904 cited by Luciani, 1913) using Pavlov pouches provided evidence of a "lipolytic ferment in the gastric mucosa". This enzymic activity was not aided by bile in contrast to pancreatic lipase. Recent evidence of a gastric lipase present in the secretions of simpler stomachs has been reported by Bank, Krut, Marks, Bronte-Stewart and Uys (1964). Argument

continues however, about the existence of a true gastric lipase.

The Gastric Mucosa

Individual constituents of the secretion of the abomasum are produced by individual cells, brief descriptions of which follow.

Parietal Cells: These are large spheroidal cells with a round nucleus (Zimmerman, 1898, cited by Babkin, 1950). The cells contain intra-cellular capillaries or canaliculi. They stain readily with acid aniline dyes.

Parietal cells are regarded as the site of hydrochloric acid formation; evidence for this conclusion has been provided from the work of Linderstrøm-Lang (1934, 1935) Glick (1934, 1935) (cited by Conway 1953). Linderstrøm-Lang investigated the micro-chemical distribution of hydrochloric acid and enzymes in the mucous membrane of pig stomachs. A correlation was revealed between acid secretion and parietal cell counts and also between pepsin content and numbers of chief cells. Supporting evidence for the assignation to parietal cells of acid secretion was obtained from pigments and histological examination. This evidence first appeared from the use of the Prussian blue reaction. FitzGerald (1910) has reviewed the literature on this reaction in this connection.

More specialised investigations have indicated the wall of the parietal cell canaliculi is the site of acid formation. (Hollander 1943, Golgi 1893, Langendorff and Laserstein 1894, Hoerr and Bensley 1936, all cited by Conway 1953). A more detailed discussion of the relation between the parietal cell and acid secretion has been undertaken by Conway (1953).

Peptic Cells: These cells are smaller than the parietal cells and possess a spherical nucleus; they are situated close to the lumen of the gland and lack secretory capillaries. According to Babkin (1950) the parietal cells in the body of the gland are separated from the lumen by peptic cells.

The parietal cells are connected to the lumen by intercellular capillaries. Due to the disposition of the parietal cells, it appears that the peptic cell secretion is perhaps washed out in, or by, the acid secretion.

The peptic cells have the characteristics of gland cells producing an organic secretion. They possess granules which are larger than those of parietal cells and have a higher refractive index. The smaller granules of parietal cells are thought to be mitochondria, while those of the peptic cells have been related to zymogen granules.

Peptic cells are the only cells of the gastric glands which do not divide by mitosis, but are formed from mucous cells in the neck (Babkin, 1950). This process has been supposed to be reversible in that in certain pathological conditions a reduction of peptic cells is observed, and the cell numbers are made up by an increase of mucous cells. Whether this occurs as an actual transformation of the highly specialised peptic cells, or just the cessation of manufacture of these cells in favour of mucous cells is not known.

Peptic cells are responsible for the formation and liberation of pepsinogen, the precursor of pepsin. When the secretion contains a high enzyme content there is a concomitant diminution of the granules in the peptic cells. (Bowie and Vineberg 1935, Bowie 1936, cited by Babkin 1950). This is not well seen in ruminants because of the continuous nature of the gastric secretion.

Mucous Neck Cells: These cells characteristically contain flat nuclei occupying the base of the cell. The cytoplasm is granular and possesses characteristic staining properties and a higher mucin content than the surface epithelium cells (Bloom and Fawcett, 1962).

Surface Epithelium Cells: These are tall regular columnar cells which

contain granules believed to be mucigen which has specific staining properties (Bloom and Fawcett, 1962). Upon their release, these granules yield an alkaline mucus which protects the surface of the mucosa.

Other cells found in the gastric mucosa include the Argentaffine cells, thought to be concerned with the production of intrinsic factor. These cells are more numerous in the intestine, particularly the duodenum.

The composition of the gastric secretion from different regions of the stomach varies according to the types of cells present in the secretory tubules. In the cardiac region of the stomach the secretory cells are mainly of a mucous nature, with only a small number of peptic and parietal cells. Although the abomasal secretory glands of the ruminant are similar to the secretory glands in the simpler stomachs of some other animals (Hill, 1951, Sommerville, 1956) the existence of a cardiac zone of the abomasum was first proposed on the basis of evidence provided by Bensley (1902) and later confirmed by Hill (1951). The omaso-abomasal junction was suggested as a region which is analagous to the cardiac zone in the simpler stomach of some animals. The fundic region of the stomach contains the largest number of parietal and peptic cells. The secretion from this area is normally highly acid and rich in enzyme.

In contrast in the pyloric region parietal cells are sparse or absent. The secretion from this area of the abomasum is largely of a mucous nature. (Sewell 1878, Pauli 1884, both cited by Hill 1961). There is in the pyloric region a small number of peptic cells. The most significant function of the pyloric mucosa is its probable role in providing an excitatory stimulus to the parietal cells of the fundus. This is achieved through the liberation of the hormone gastrin. The mucous nature of the secretion may contribute towards the neutralisation of material entering the duodenum and serve to prevent to some degree, the acidification of the pyloric mucosa

which has been shown to inhibit gastrin release in dogs (Longhi, Greenlee, Bravo, Guerrero and Dragstedt, 1957).

The Study of Gastric Acid Secretion

In the study of gastric acid secretion a variety of techniques have been used in an endeavour to elucidate the mechanisms involved in this unique phenomenon of the body.

(i) The Test Meal (James 1957):

Cannon (1898) was one of the first investigators to use the Test Meal. Cannon fed alkaline bread, aspirating the stomach contents from different regions so as to obtain an indication of the degree of acidification. He also fed cats bread and bismuth and followed the passage through the alimentary tract radiologically. Gianturco (1934) used a similar approach by feeding cats meat coated with barium sulphate.

The Fractional Test meal was used as the principal method of obtaining a measure of gastric emptying time until Hunt and Spurell (cited by James, 1957) developed the Serial Test meal, whereby the complete stomach contents were aspirated after a certain time interval, on different days. The technique is most applicable to fully co-operative subjects i.e. to man.

(ii) Fistulae:

A further aid in the investigation of digestion has been the fistulated stomach. By this means samples of stomach contents may be obtained at any stage of digestion, allowing subsequent analyses to be carried out. Beaumont, Carlson, and Wolf and Wolff contributed a great deal to an understanding of human gastric function from their studies of subjects with fistulae (for a review see Wolf and Wolff, 1943).

Unfortunately the fistula is of limited value in the study of

stomach secretions as severe contamination occurs by the ingested food material, saliva, and reflux from the duodenum. This disadvantage may be overcome in acute experiments to some extent by emptying the stomach and isolating it by ligation of the oesophagus and duodenum - these techniques are not applicable where the subject must recover.

(iii) Pouches:

The preparation of certain regions of the stomach into isolated pouches has greatly facilitated the study of gastric secretion. Hollander (1951) has reviewed the history of several varieties of gastric pouch. The following provides a brief survey of these developments:

(1) Heidenhain Pouches: Heidenhain (1879) devised the original fundic pouch which is completely separated from the rest of the stomach. This pouch is regarded as possessing a blood supply only, but many Heidenhain pouches have some degree of vagal innervation.

(2) Pavlov Pouches: Pavlov modified the Heidenhain pouch in a manner which contributed to the preservation of the vagal nerve supply to the gastric glands. He constructed a mucosal septum between the pouch and main stomach in such a way that the pouch largely retained its vagal and sympathetic nerve supplies.

(3) Denervated and Auto-transplanted Gastric Pouches: Ivy and Farrell (1925) introduced the auto-transplanted pouch. A Heidenhain fundic pouch was transplanted to the mammary region where it eventually obtained a new blood supply from the very vascular mammary gland. When this occurred the original blood supply was removed by ligation and section of the original pedicle containing arteries, veins and nerves. The use of this pouch has been of some moment in providing definitive evidence of a hormonal role in gastric secretion. Other forms of this type of pouch have been prepared with the muscle layer

of the pouch removed (Klein and Arnheim 1932, Gregory and Ivy 1941).

(4) A Pouch of the Entire Stomach: This type of pouch with an oesophogeal-duodenal anastomosis was first used by Ivy, Lim and McCarthy (1925). This pouch is of limited value: its main use has been in the definition of the intestinal phase of gastric secretion.

(5) Innervated Antral Pouches: These were probably first used by Zeliony and Savich (1911). In these a mucosal septum is constructed between the antral region and the rest of the stomach. Continuity of the alimentary tract may be re-established by gastroduodenostomy of gastrojejunostomy.

(6) Isolated Antral Pouches: Are those which are completely divided from the rest of the stomach; the extent of their vagal innervation is in many cases uncertain (Wohlrabe and Kelly, 1959). Difficulty has been experienced in constructing an antral pouch so as to contain only antral mucosa on one side and fundic mucosa on the other.

A wide variety of pouches are used in the study of gastric secretion. These have been unfortunately, in some respects, of variable construction and composition. This no doubt, is responsible for a major part of the conflicting data present in the literature. Despite this criticism it must be admitted that many clues on the control of gastric secretion have been provided by different workers using different preparations and their seeking to resolve difficulties arising from conflicting results. This has served as a stimulus to further work in that attempts have been made to resolve apparently conflicting results obtained with the different techniques, and provide a "complete" understanding of the control of gastric secretion.

A common test for the innervation of a pouch was its response to an insulin induced hypoglycaemia. Secretion produced in response to insulin administration was supposed to occur by vagal stimulation of the acid and pepsin secreting cells. Recent evidence (Uvnas, Emas, Fyro and Sjodin, 1966) has shown that gastrin may be released by insulin hypoglycaemia, and gastrin may stimulate pepsin secretion (Gregory and Tracy, 1964). It is possible therefore, that secretion may occur from a denervated pouch in response to an insulin induced hypoglycaemia. Insulin has also been shown to inhibit gastric secretion (Hirschowitz, 1966a) (this aspect will be discussed in detail later).

Inter-relationships between Abomasal Secretory Activity and Reticulo-rumenal Motility

Inter-relationships between abomasal secretory activity and reticulo-rumenal motility have been demonstrated by Ash and Hill, who have emphasized the importance of the passage of digesta to the abomasum (Hill 1955, 1960, Ash 1961a). Ash (1961b) also provided evidence of the stimulation of HCl secretion by steam volatile fatty acids in the fluid digesta entering the abomasum.

However, it has been recognised that conditions in the abomasum per se influence the activity of the reticulum and rumen. Phillipson (1939) demonstrated that abomasal distension caused an inhibition of reticulum and associated rumen movements. This finding was confirmed by Dussardier (1955) and by Titchen (1954, 1958). In decerebrate preparations reflexly stimulated reticulum contractions were prone to inhibition by distension of the abomasum if the splanchnic nerves were intact. Inhibitory afferents have also been demonstrated in the vagus nerves; this observation has only been made in decerebrate preparations (Titchen, 1958).

Stimulation of reticulum and rumen motility by a reduction in abomasal pH to 1.0 has been cited by Titchen as a physiological stimulus. He suggested that abomasal acid secretion could give increased reticulum contractions. This has not been established since it has been shown only in decerebrate preparations in which the slowly declining secretion of HCl after decerebration was accompanied by a decline in the force and frequency of reticulum contractions. Iggo (1957) in studies of single afferent fibres of the vagus nerve has demonstrated the presence of acid receptors in the cat's stomach.

Ash (1961a) has suggested that the inflow of digesta into the abomasum, the secretion of acid, and the flow of material from the abomasum are integrated, with the abomasum controlling these functions. However, as yet no clear cut relationship can be defined between reticulo-rumen motility and abomasal acid secretion.

Apart from the preparation of, and study of the effects of gastrin extracts from sheep and cattle abomasal mucosa, (Anderson, Fletcher, McAlexander, Cohen, Pitts and Harkins 1961, Anderson, Fletcher, Pitts and Harkins 1962) the hormonal control of acid secretion in ruminants has not been systematically investigated.

CHAPTER II

NORMAL SECRETION IN THE FED-FASTED STATE

Introduction:

Many physiologists have endeavoured to separate the roles played by nervous and humoral mechanisms in the control of digestive secretions. Earlier workers concerned with gastric secretion explained their discoveries solely in terms of reflex responses. Foster (1889) realised that intact vagi were not essential for gastric secretion. He stated that secretion was probably due to a local mechanism. Heidenhain (cited by Foster) in studies of a fundic pouch obtained evidence of a secretatogogic effect of ingesta on acid secretion.

In contrast secretion of acid in response to the direct stimulus of food was attributed to a local nervous mechanism induced by chemical stimulation of peripheral nerve endings (Khizhin 1895, cited by Luciani, 1913 p 111). Von Mering (1899, cited by Luciani) found that atropine and pilocarpine had similar effects to those demonstrated on salivary secretion. Pavlov in sham feeding experiments demonstrated a reflex secretion of gastric juice: upon section of the vagus nerves this reflex secretion ceased.

It was not until after Bayliss and Starling (1902) had discovered secretin that Edkins (1905) formulated the gastrin theory. Edkins showed that extracts of the pyloric mucosa stimulated gastric secretion. He attributed this activity of such extracts to a hormone which he named gastrin. Since that time considerable attention has been paid to the possibility of hormonal mechanisms of stimulation of gastric secretion.

Controversy as to the existence and nature of an excitatory antral hormone persisted for many years. Ivy (1930) discussed the possibility

that histamine was the gastric hormone. He concluded, "In the light of our present knowledge, we are warranted in stating that histamine is closely related to, but not identical with the active principle of pyloric mucosa extracts."

Almost 60 years after Edkins' first preparation of a "gastrin" extract, Gregory and Tracy (1964) described the preparation and properties of "pure gastrin".

Recent work has been particularly concerned with the combined actions of neural and humoral mechanisms. It has emphasized the importance of permissive, additive and synergistic relationships between these two forms of stimulation.

Digestive glands situated in the most cranial regions of the alimentary tract, for example the salivary glands, are mainly or even wholly under neural control. In some lower regions humoral control is predominant. Considerable integration of the two occurs however, and in many cases they are interdependent. Evidence that vagal activity might influence the response of the digestive glands to hormones was provided by Uvnäs (1942). Experiments performed in anaesthetised cats indicated that:

1. vagal excitation could cause a release of gastrin.
2. concurrent vagal excitation was an important factor in determining the response of the fundic glands to gastrin. This observation has been supported by Andersson and Olbe (1964).

Evidence on the participation of the vagus in the release of gastrin has been reviewed by Gregory (1962), who also advanced strong support for the view that cholinergic excitation sensitises the oxyntic cells to gastrin.

The synergistic relationship between vagal activity and gastrin has been further elucidated by the work of Olbe (1964, 1964a). Using dogs with vagally innervated fundic pouches, Olbe found the response to sham

feeding ceased upon excision of gastrin producing regions. Intravenous infusions of a gastrin extract, at subthreshold doses, restored the sham feeding response in "antrectomised" dogs. This potentiation of the direct vagal action on gastric acid glands by gastrin is shared with other secretatogogues. A similar response has been obtained with the infusion of subthreshold amounts of histamine or after mesenteric-caval venous anastomosis, a procedure which may result in a polypeptide, histamine or other such agents released from the small intestine escaping hepatic breakdown (Olbe, 1966).

In the ruminant, Krinitsin (1935, cited by Hill 1961) in studies on the secretory response of an innervated and subsequently denervated abomasal pouch, considered the vagus nerves fulfilled an adaptive and trophic function in intensifying the metabolic processes of the secretory cells thus rendering them more responsive to excitatory stimuli.

The strongest contender for the final common mediator of gastric acid secretion is at the present time considered to be histamine. Evidence for this view has been obtained from studies of: the histamine forming capacity (histidine decarboxylase content) of the rat gastric mucosa (Kahlson, Rosengren, Swahn, and Thunberg, 1964), the inhibition of histamine formation by specific inhibitory agents (Levine 1965), histamine destruction (Code 1965), the relationship between histamine and vago-mimetic drugs (Shore 1965) and of the relationship between histamine, gastrin and other secretatogogues (Haverback, Stubrin and Dyce 1965).

This view is not held by all. Clark, Curnow, Murray, Stephans and Wyllie (1964) found in man that hexamethonium and atropine inhibited a histamine induced gastric secretion. They concluded that histamine acted through nerves which in turn produced the local chemostimulator, acetylcholine.

Supporting evidence in monkeys has been provided by Rosato, Smith, Gelfand and Brocks (1966) and Rosato, Smith and Brooks (1966) where a histamine stimulated acid secretion was inhibited by atropine. However, atropine is in some respects no more specific than any other pharmacological agent used to blook responses. Mellanby and Pratt have drawn attention to the side effects of atropine which limit its usefulness as an analytical agent (Mellanby and Pratt, 1939).

The classical concept of gastric stimulatory mechanisms has been regarded as consisting of three well defined stages: cephalic, gastric and intestinal. A brief review of acid secretion from the abomasum in terms of these phases is presented below.

1. Cephalic phase of abomasal acid secretion:

It has been stated that in the adult ruminant the cephalic phase of vagally stimulated gastric acid secretion is absent (Hill, 1965). This conclusion has been largely reached through the observations of Hill (1960). In sham fed sheep with oesophageal fistulae, no secretory response was obtained. However, some reservations were held as to the condition of the animals used. There was also in these animals no response in acid secretion on feeding with the reticulo-rumen empty. Vagal stimulation of the stomach caused by an insulin induced hypoglycaemia produced an increase in acid and pepsin output in the secretion of the abomasum. On emptying the reticulo-rumen prior to insulin administration, the hypoglycaemia produced an increase in pepsin output only. Espe and Cannon (1937) obtained results from milk fed calves with Pavlov pouches that indicated the cephalic phase of gastric secretion was absent or of very little significance.

That a conditioned hypermotility of the reticulum and rumen may occur in response to feeding or in anticipation of feeding in cattle and sheep has been shown by Quin and Van der Wath (1938), Dedashev (1959) and Reid (1962).

Bogdanova (1950, cited by Hill 1961) noticed diurnal changes in abomasal secretion in calves, and an increase in secretion associated with the time of feeding. Hill (1961) interpreted the secretory response as arising from the increased passage of digesta to the abomasum caused by the hypermotility of the reticulum and rumen.

2. Gastric phase of abomasal acid secretion:

In the ruminant this has been held by Hill (1961a) to be the most important phase; the main controlling factor is believed to be the passage of digesta from the reticulo-rumen and omasum to the abomasum.

Savich and Tichomirov (1911), Popov (1932) Belgowski (1912) (all cited by Hill, 1961) showed in sheep and cattle, that abomasal acid secretion is continuous. Hill (1955) undertook an experimental investigation of the relative contributions of humoral and nervous stimulation in the maintenance of this continuous "spontaneous" acid secretion by the abomasum. When digesta was prevented from entering the abomasum, gastric acid secretion ceased. From this he concluded that the continuous nature of abomasal secretion was due to specific stimuli - and that these arose from the entry of digesta into the abomasum.

Ash (1961a) measured acid secretion from an abomasal pouch and flow of digesta from the abomasum simultaneously. He observed a close correlation between acid secretion and flow of digesta. Hill (1960) supports this evidence with studies of insulin induced hypoglycaemia in sheep. No acid secretory response was obtained when the reticulo-rumen had been emptied prior to the insulin administration. Ash (1961b) discussed distension effects of the abomasum in the production of acid secretion. He found an increased secretion occurred upon distension of the abomasum.

A copious secretion of acid from the oxyntic cells is produced by the hormone gastrin. In the monogastric animal the stimuli to gastrin

release from the pyloric or antral region of the stomach are:

1. vagal excitation of the antrum.
2. local stimulation of the antrum by swallowed food. The form of stimulus afforded by the food may be one of distension, of a tactile nature or of chemical origin.

That the vagus nerves participate in gastrin release has been realised for some time, but the importance of this in the ruminant is not known. Tactile stimulation and distension of the abomasum by digesta is probably a potent form of stimulus to gastrin release. Hill (1955, 1960, 1961a, 1965) emphasizes the importance of the flow of digesta to the abomasum in its secretion of acid.

In the exploitation of structural carbohydrates, the ruminant produces an abundant supply of volatile fatty acids. These are absorbed through the reticulo-rumen and omasal epithelia (Barcroft, McAnally and Phillipson 1944). However, some VFA are still contained in the digesta entering the abomasum (Ash 1961a). Popov (1932, cited by Hill 1965) found that the addition of acetic acid to the abomasum stimulated gastric secretion. Ash (1961a, 1961b) carried out a series of experiments on the introduction into the abomasum of buffered solutions of various proportions of acetic, propionic and butyric acids. These substances markedly stimulated the secretion of acid from the abomasum. The excitatory mechanism is not clear: even when a small volume of acetate was added to an empty rumen (where there was little chance of overflow into the abomasum) acid secretion was stimulated. An acid secretory response was also obtained upon intravenous administration of sodium acetate (Hill 1965 p224).

3. Intestinal phase of abomasal acid secretion:

There has been little study on the intestinal phase of abomasal acid secretion. The main emphasis has been on the inhibitory effects of

conditions in the duodenum on abomasal acid secretion.

Inhibition of gastrin release from the antral regions of the simple stomach when the gastric contents are more acid than pH 2.0 - 2.5 has been shown by Longhi, Greenlee, Brave, Guerrero and Dragstedt (1957). In the abomasum a similar situation appears to operate. Ash (1961b) used distension of the abomasum with a saline filled balloon as a stimulus to acid secretion from an innervated pouch. As the acidity of the abomasal fluid increased, the secretion of acid by the pouch began to decline. The pH of the digesta which leaves the abomasum remains within a fairly narrow range. Ash (1961b) compared this situation with the fluctuating acidity of the pouch secretion. He interpreted these two findings as reflecting a relationship between gastrin stimulation and inhibition.

The duodenum actively participates in the control of abomasal acid secretion. The presence of fat or its digestion products and other food products in the duodenum causes an inhibition of abomasal acid secretion and motility, with a concomitant inhibition of reticulum and rumen motility. These effects have been attributed to the release of the hormone enterogastrone. Acidification of the duodenal mucosa is effective in inhibiting abomasal acid secretion (Ash 1961b). It is thought this effect is mediated by the vagus.

In the following experimental study the possibility of there being a relationship between reticulo-rumenal motility and abomasal secretion has been investigated. It was thought that the existence of such a relationship might be demonstrated by simultaneously recording movements of the reticulum and rumen from partial exteriorisations and collecting secretion from an abomasal pouch.

Methods:

A. Preparation of Animals:

Young Romney ewes or their crosses (about 25 kg) were used. These sheep were kept in an animal room and fed red clover chaff and water ad lib. After some months this diet was supplemented by a fixed amount of a protein rich (min. 20%) concentrate ('Primrose' vitalized sheep nuts, Manawatu Mills N.Z.).

Gastric secretion was obtained from an isolated pouch of the abomasum.

Pre-operative preparation of the animals included habituation for as long as possible before the operation. The animals were fasted 24 - 48 hours but were allowed access to water throughout the fasting period. The abdomen and neck were clipped closely a few hours before the operation.

Premedication in the form of a morphine substitute, pethidine HCl ('Pethilorfan', Roche) was given intra-muscularly in earlier operations. This practice was discontinued in an endeavour to facilitate rapid recovery from the anaesthetic. Benzyl-Penicillin ('Crystapen', B.P. Glaxo) 500,000 units and atropine sulphate (BDH) 5 mg were injected intravenously prior to anaesthesia which was induced with thiopentone sodium ('Intraval', May and Baker). Anaesthesia was maintained with ether administered from a Boyle's vaporizer via a cuffed endotracheal tube. The depth of anaesthesia was judged chiefly by the degree of muscular relaxation, the nature of respiration, the strength of the heart beat and assessed blood pressure. In some operations changes in heart rate were recorded using a cuff and an electrical transducer on the foreleg.

Surgical Procedures:

Aseptic precautions were adopted in all operations. The skin was sterilized with 70% ethyl alcohol and tincture of iodine, and draped with

sterile linen.

The type of pouch prepared may be described as a modification of that illustrated by Hill and Gregory (1951) as their Heidenhain pouch type C. The pouch remains attached to both mesenteries, receiving a blood supply from vessels on the greater and lesser curvatures of the abomasum.

The approach used was either through a lateral laparotomy wound about 3 - 5 cm caudal to and parallel with the costal arch or through a midline laparotomy incision. After the abomasum had been located a region of the fundus was selected in which to prepare a pouch about 12 - 15 cm cranial to the pylorus. The area to be contained in the pouch was carefully chosen so that it possessed a large arterial blood vessel. It was hoped nerves might course in the close vicinity of the blood vessels.

The abomasum was transected completely but the two mesenteries with their blood and nerve supply were kept intact. The first line of sutures in the pouch stitched mucosa to mucosa. A second line of inverting sutures was applied over the first, suturing serosa to serosa. This formed a water tight seal and prevented any protrusion of the acid secreting mucosa. The other side of the pouch was treated similarly. In producing continuity of the abomasum by rejoining the caudal and cranial regions, the technique of suturing mucosa to mucosa followed by serosa to serosa was again applied.

A stainless steel cannula (Fig. 1) was inserted into the pouch either along the line of sutures or in a region with no sutures present; this position depended on the position of the pouch and the likely ease of its delivery through a stab wound in the abdomen. The cannula was covered with omentum and brought out through a stab wound or the abdominal midline wound. The abdominal wound was closed by suturing muscle and peritoneum to muscle and peritoneum. The skin was joined with a line of Michel clips. The cannula was held in place with the aid of an elastrator ring

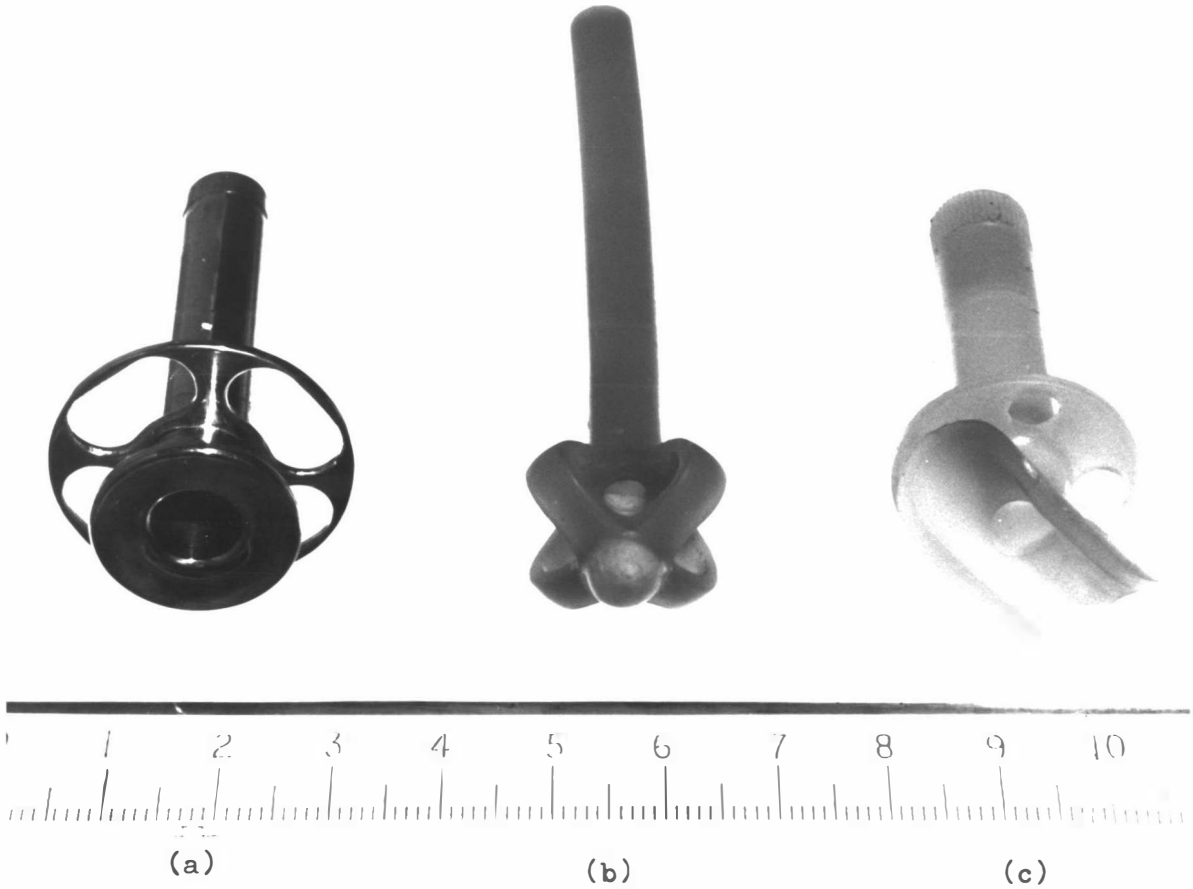


Fig. 1: Types of cannulae used in the preparations. From left to right: (a) stainless steel cannula inserted into the abomasal pouch, (b) latex mushroom de Pezzer catheter used to facilitate free drainage of the pouch; the stem was cut to project about 2 cm out of the stem of (a), (c) poly-propylene duodenal cannula, centimetre scale.

(Elastrator N.Z.). Free drainage of the pouch was facilitated by insertion into the cannula of a latex mushroom or urethral de Pezzer catheter (Bardex 640, F.G. 22) (Fig. 1).

The wounds were covered with "tulle gras" (a vaseline based ointment) and the animal given an intramuscular injection of long acting penicillin ('Prolopen', Glaxo 850,000 units). The Michel clips were removed 7 - 8 days later.

A feature of the operative procedure was the large amount of saline used to wash away digesta contaminating viscera and to keep the structures warm and moist. The liberal use of saline was also thought to aid in haemostasis.

Post-operative care:

The animals were suspended in canvas slings of a corset type design, whereby the sling closely fitted the body, but allowed generous movement of the animal within the crate. Food and water were offered as soon as the animal had stood, could swallow and exhibited co-ordinated movements of its head and neck. Eating usually followed within 4 hours of the operation. This initial feeding phase was transient and loss of appetite, and perhaps complete cessation of feeding for up to 6 days frequently occurred.

2 - 4 weeks after the pouch operation, according to the condition of the animal, partial exteriorisations of the reticulum and rumen, and the insertion of a duodenal cannula were undertaken. For this purpose the preparation and anaesthesia was similar to that explained as before. The technique of the partial exteriorization of the reticulum was that of Titchen (1958a). The partial exteriorizations of the rumen were carried out by the technique as described by Reid (1962).

The duodenal cannula was inserted at a point about 10 cm from the

pylorus. These cannulae were originally of perspex but this type was later discarded in preference to poly-propylene cannulae (Fig. 1), thought to be of a less irritant nature. The duodenal cannula was brought out through a stab wound in the abdominal wall on the right side, 2 cm caudal to the costal arch and about 8 - 10 cm from the mid-line. Recovery from these operations was considerably faster than from that of preparation of a pouch.

Records:

Abomasal pouch secretion was recorded at 15 min intervals throughout the collection periods. A latex finger cot was tied over the stainless steel cannula and projected freely into a plastic funnel suspended under the animal. A polythene tube passed from the plastic funnel to a glass filter funnel from which a tube led into one of a series of test-tubes contained in a fraction collector. The fraction collector was placed beneath the crate in an elevated position, so as to reduce the dead space of tubing from the pouch cannula to the test-tubes. The fraction collector was modified to operate from a time clock so that 15 min samples could be collected rather than fixed volumes. The changing of the tubes was signalled on the kymograph by the introduction of a switch into a signal circuit; the switch was activated each time an empty tube was advanced after a 15 min period of collection in another tube. When experiments were not in progress the secretion was diverted to a large collecting flask.

Motility of the reticulum and rumen was recorded as the contractions of partial exteriorizations of the:

(i) reticulum

(ii) cranial dorsal rumen

(iii) main ventral rumen

Records of these contractions were obtained by the mechanical system of recording briefly reported by Reid and Titchen (1959) and later discussed

and described by Reid (1962). The strings operated frontal writing levers on a smoked 10" kymograph paper. Thus the kymograph (Palmer, London) was used to record the following:

(1) time (usually in 60 sec)

(2) change of the tubes in which secretion was collected (every 15 min)

(3) activity of the three exteriorizations

(4) jaw movements

Jaw movements were recorded by a closed air-tambour system. This method distinguished between eating, rumination and inactivity.

Analyses of Gastric Secretion:

(i) Volume: Volumes of 15 min samples were measured in a measuring cylinder and read to the nearest 0.5 ml graduation. Volume was always measured on 15 min samples although subsequent analyses were frequently performed on pooled 30 min samples.

(ii) Acidity: Free and total acidity estimates were obtained by titrating with 0.025 N sodium hydroxide using Topfers Reagent for an indicator of free acidity and phenolphthalein for total acidity (James 1957 p 31). 1.0 ml or 0.5 ml of gastric juice was the usual quantity titrated.

(iii) pH: The pH of the abomasal secretion was determined electrometrically with a Beckman pH meter. The volume of 15 min collection periods did not in all cases, permit the estimation of pH.

(iv) Sodium and Potassium: 1 ml of gastric juice was diluted to 1:100 by distilled water; the resulting concentration of sodium and potassium was determined by a Flame Photometer (Evans Electro-selenium Ltd). In cases of very low acid secretion, higher dilutions of Na were required.

(v) Enzyme activity, pepsin: Pepsin activity of the secretion was determined

by a modification of Hunt's method (1948) in which:

1. 1.0 ml of gastric juice was allowed to react for 10 min with an acidified (pH 2.1) 2% solution of haemoglobin at 37°C. The haemoglobin used was 'Bacto-Haemoglobin', Difco.

2. The reaction was stopped after 10 min by the addition of 10 ml of 5% trichloroacetic acid (TCA).

3. The resulting mixture was centrifuged; the supernatant contained digestion products of the haemoglobin. Tyrosine and phenylalanine containing peptides were estimated colorimetrically, the amount of these substances present was regarded as an index of peptic activity in the gastric juice.

4. 0.5 ml of the supernatant was reacted with 1.0 ml of Folin-Ciocalteau reagent and 4 ml of 0.5 N NaOH. The intensity of the resulting blue colouration was measured with the aid of a spectrophotometer. The values obtained were compared with a set of tyrosine standards.

A blank was obtained by the addition of 10 ml TCA to the gastric juice before the haemoglobin. A duplicate for each sample was determined. The estimates obtained by this method are relative only. The pepsin potency gradually diminished even though the juice was frozen stored, so that a valid comparison of peptic activity can only be made within the one experiment. An exception to this is where analyses were carried out directly after collection - this proved impractical in experiments which involved collection of samples over prolonged periods.

Results:

Secretion from isolated abomasal pouches and the motility of exteriorizations of the reticulum and rumen were studied in fed and fasted animals. Observations made involved studies on the motility and secretory activity when animals, first ate after a fast; when they ate food which was continuously available; when both fed and fasted animals were ruminating; and at times when both the fed and fasted animal had no characteristic pattern of feeding activity. For convenience of description the latter state may be referred to as the "inactive" condition.

In all five animals in which the secretory activity of isolated pouches of the abomasum was studied, there was a continuous secretion; the volume, acidity and pepsin content of which varied according to the condition which maintained at the time. Relationships were established between the volume and composition of secretion to the fed, feeding and fasted condition of the animals. No such clear relationship was evident in the case of rumination.

A detailed consideration of these relationships is given below. Much of this detailed information was obtained from prolonged observation in two animals in which continuous periods of collection of secretion and of recording of the activity from exteriorizations were continued for up to 100 hours at a time. In general these observations have been confirmed in all the animals. The results obtained are discussed under the headings of 'feeding', 'rumination', 'inactivity' and 'fasting'.

1. Feeding

Continuous secretion from the abomasum was observed in all five sheep in which abomasal pouches had been prepared. Although continuous in nature, the pouch secretion varied greatly in its volume and acid content. Volumes of secretion ranged between 1 ml to 20 ml/15 min and acid concentration

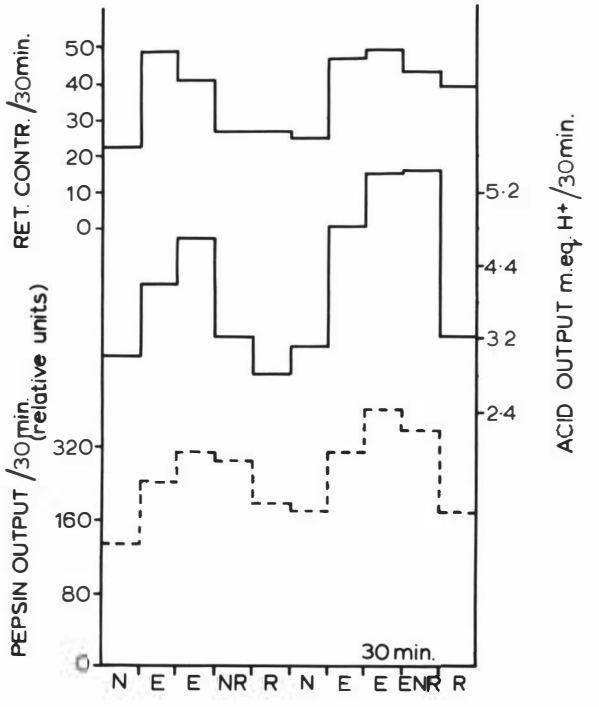


Fig. 2: Relationships between an animal's feeding activity, abomasal pouch secretion and reticulum movements in a sheep fed ad lib. Graph from above downwards: reticulum contractions /30 min, acid output from the pouch in m-equiv H^+/30 min, pepsin output from the pouch in relative units/30 min, 30 min time intervals, activity of animal (N = nothing, i.e. neither feeding nor ruminating; E = eating; R = ruminating). A clear increase in the frequency of reticulum contractions, acid and pepsin output followed feeding. This was so in a number of experiments in different animals.

varied from 60 - 160 m.equiv H^+/litre. The lowest values for the volume and acid content were obtained in sheep subjected to prolonged fasting.

In two of the animals detailed and prolonged observations were made of the way in which secretion varied, both in volume and composition; according to whether they were feeding or fasted and under both conditions (i.e. fed or fasted) when they were ruminating. The use of a jaw recorder enabled the determination of the animals' activity during the 15 min collection periods of pouch secretion. Similarly the records of motility from the partial exteriorizations enabled the correlation of reticulum and rumen movements with abomasal pouch secretion. (See Tables 1 and 2).

Eating was consistently followed by an increase in volume, acidity and pepsin output from the pouches. This response was evident in animals whenever they fed; it was observed when food which was continually available was eaten (fresh supplies were made available at 9.00 a.m. and 4.30 p.m. daily), and when food was offered to end a period of fasting.

In every case a clear cut relationship between eating and pouch secretion (in terms of acid and pepsin outputs and volume of secretion) was established (Fig. 2). These responses were obtained in animals both when they were fed red clover chaff and concentrate nuts. Acid output from the pouch usually reached a peak within 60 min of first eating (except after a fast); the pepsin concentration of the secretion usually reached a peak within 15 min. The increase in pepsin output was not simply due to a maintenance of pepsin concentration in an increased volume of secretion. There was both an increase in the volume of secretion and the pepsin concentration. This contrasted with the situation of times other than feeding when if there was an increase in the volume of secretion the concentration of pepsin fell.

As well as the definite abomasal secretory responses, an increased

frequency of contractions of the reticulum and rumen was observed when the animals ate. While feeding on the red clover chaff, reticulum and associated rumen movements ('A' sequences, as termed by Reid 1962) occurred at a frequency of 22 - 26 contractions/15 min; in contrast the frequency of 'A' sequences when eating the concentrate nuts increased even more greatly - to or in excess of 44 contractions/15 min. Rumen movements alone ('B' sequences, as termed by Reid 1962) were not measured during feeding because the records of contractions of the exteriorizations of the dorsal and ventral rumen often could not be distinguished from movements of the animal.

Rumination:

In contrast to the responses to feeding no consistent relationship could be established between the state of rumination and secretion from abomasal pouches. On occasions a definite relation appeared to exist between secretion and rumination. With long periods of rumination (60 - 90 min) there was, during the first 30 min, a gradual decrease in secretion volume and acidity and thereafter an increase in both these parameters. No such relation was detected during the shorter periods of rumination. There were increases and decreases in the volume of pouch secretion and in its acid concentration with rumination in both animals. In some instances the commencement of rumination was accompanied by an increase in pepsin output and concentration (despite an increased volume of secretion).

Inactivity: (this term has been used as a convenient expression to refer to the absence of either eating or ruminating.)

Under conditions of ad lib feeding, when the animals were eating, reticulum and rumen contractions were frequent. Upon the cessation of eating, a period of inactivity ensued in which reticulum and associated

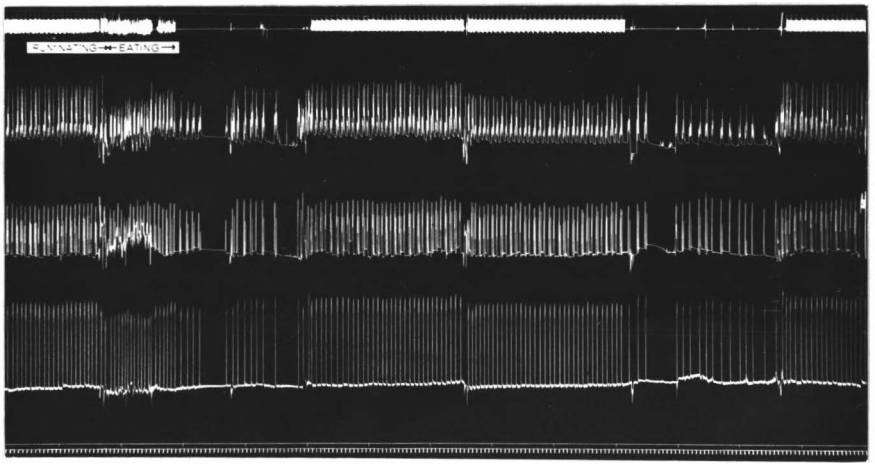


Fig. 3: Record of the activity of an animal and movements of the reticulum and rumen in a sheep fed ad lib. Records from above downwards: jaw movements, contractions of the main ventral rumen, contractions of the cranial dorsal rumen, contractions of the reticulum, signal for the change in collection of samples of abomasal pouch secretion at 15 min intervals, 60 sec time-marker.

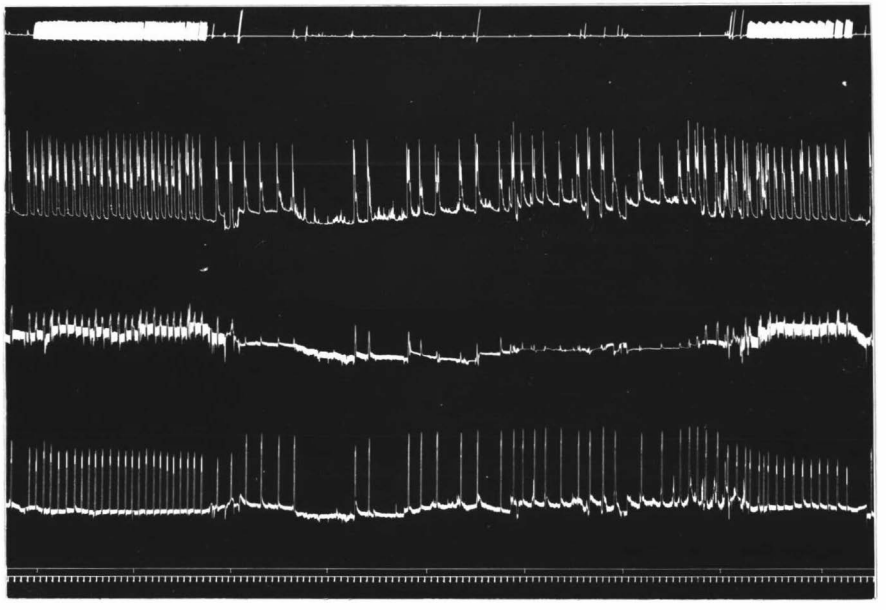


Fig. 4: The effects of fasting on movements of the reticulum and rumen. Records from above downwards: jaw movements, contractions of the main ventral rumen, contractions of the cranial dorsal rumen, contractions of the reticulum, signal for the change in collection of samples of abomasal pouch secretion at 15 min intervals, 60 sec time-marker. There was a reduced frequency of 'B' sequences of contraction of the rumen and weak contractions of the cranial dorsal rumen in the 'A' sequences. Food was withdrawn 7 hr previous to this record being obtained. Same animal used as in Fig. 3.

rumen movements were reduced in frequency (to about 8 - 12 contractions/ 15 min). The secretion from pouches during this period varied considerably. In one animal the secretory response of the abomasal pouch followed a similar trend to that of reticulum and rumen motility, in that in this animal acid output decreased during the inactive period. In contrast in another animal, abomasal pouch secretion was maintained or even increased upon the cessation of eating. Stomach movements were not recorded from this animal, but subsequent observations when recordings of motility were undertaken showed a decrease in reticulum and rumen motility as reported in the previous preparation. Periods of inactivity which occurred after rumination were characterised by an increase in acid secretion from the pouch but reticulum and rumen contractions were slowed in frequency. In general periods of inactivity were associated with a decreased frequency of stomach movements (Fig. 3).

In the fasted state, in all animals studied inactive periods which occurred after rumination were frequently characterised by an almost complete cessation of reticulum and rumen movements and a very low volume of pouch secretion - this low level of activity continued for periods of 10 - 15 min.

Fasting:

In the preparations studied it was found that abomasal pouch secretion and reticulo-rumenal motility waned in an apparently related fashion as a fast (11 - 12 hr) progressed. In the earlier stages of fasting long periods of rumination occurred: these were replaced by shorter periods of rumination (15 - 30 min) and extensive periods of inactivity (60 - 75 min). As the fast continued, the frequency of stomach movements decreased. This was particularly true of the 'B' sequences in which contractions of the dorsal rumen became very weak (Fig. 4).

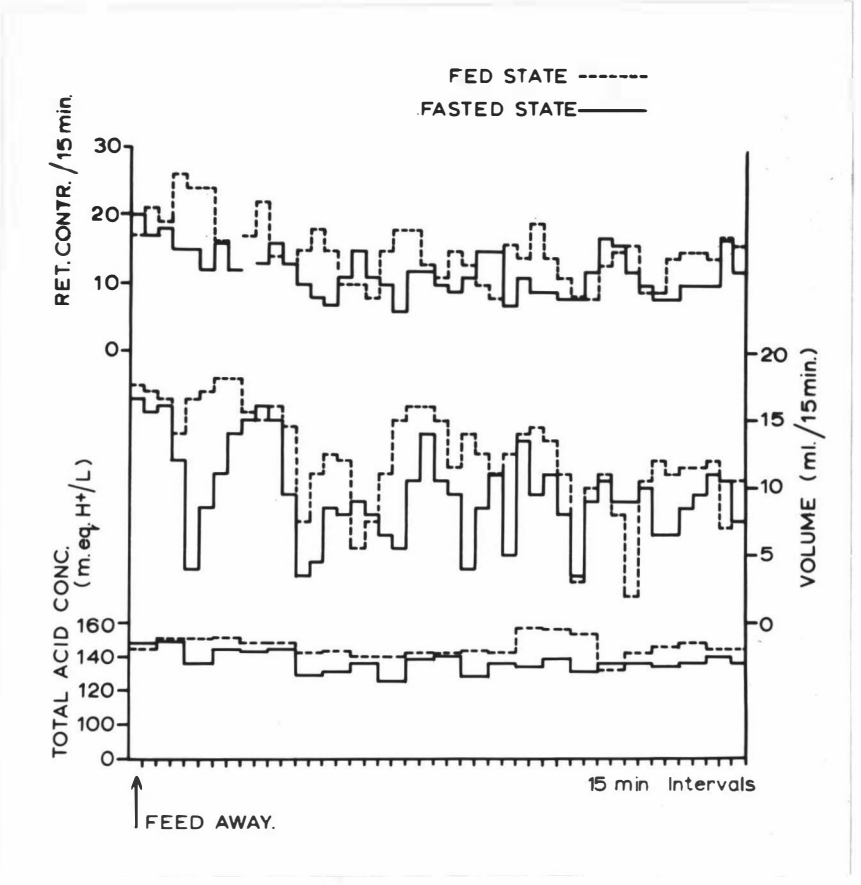


Fig. 5: A comparison in a fed and fasted state in the same sheep of reticulum activity and abomasal pouch secretion. The observations recorded here were made in both instances at the same time of day (i.e. 10 p.m. to 9.30 a.m.). Graph from above downwards: reticulum contractions/ 15 min, volume of acid secretion in ml/15 min, total acid concentration in the secretion in m-equiv H^+/litre, 15 min time intervals. Note the lower levels of reticulum contractions and pouch secretion in the fasted condition.

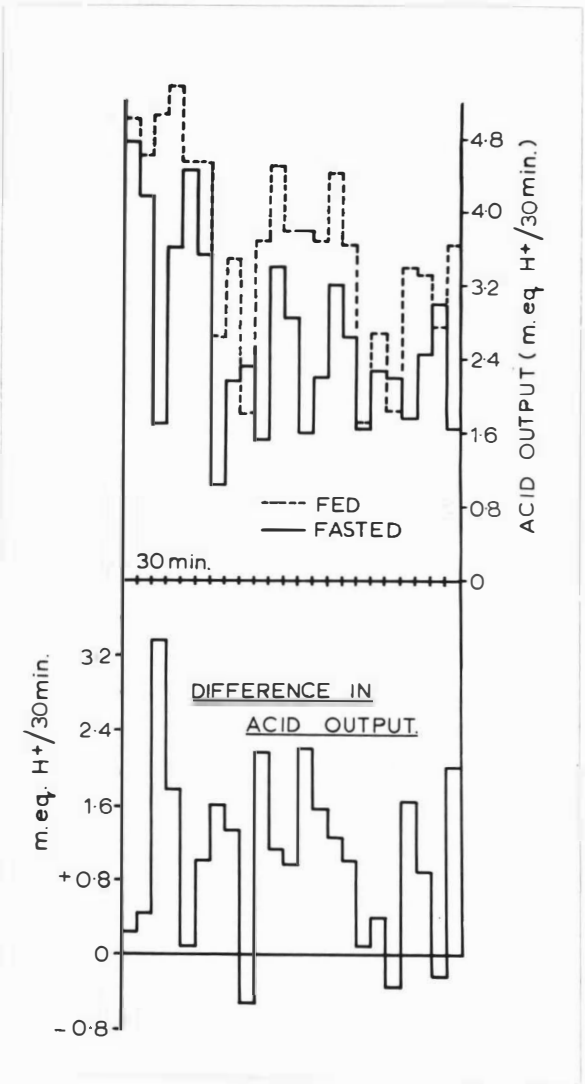


Fig. 6: A comparison between the acid output from an abomasal pouch in the same sheep fed and fasted over the same time period on different days. The lower graph shows the difference in acid output under the two conditions when the fasting level of pouch secretion for each 30 min period has been subtracted from the output recorded during a corresponding period in the animal in the fed state. Ordinate is acid output expressed as m-equiv H^+/30 min, abscissa time in 30 min intervals. Same Experiment as Fig. 5.

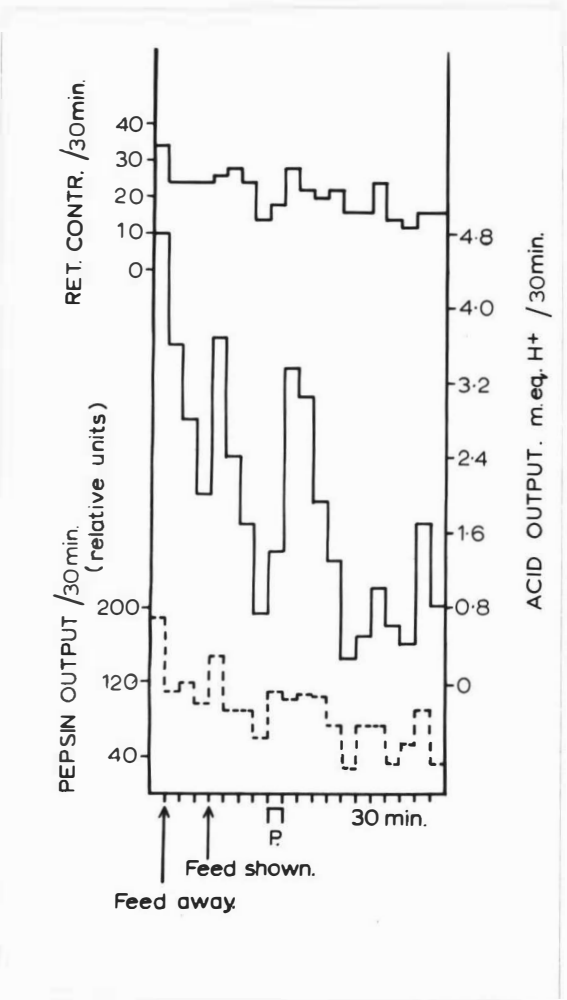


Fig. 7: Effects of fasting recorded in a sheep, showing the progressive development of a considerable reduction in the frequency of contractions of the reticulum and secretion from an abomasal pouch. Graph from above downwards: contractions of the reticulum/30 min, acid output from the pouch in m-equiv H^+/30 min, pepsin output from the pouch in relative units/30 min, 30 min time-intervals. Note the increase in secretion on teasing the animal with food and with the presence of a person (P, known to the animal) in the same room.

The volume of acid secretion from the abomasal pouch gradually lessened as the fasting progressed, but fluctuated considerably during its decline (values ranged from 1 - 14 ml/15 min): in some cases there appeared to be a direct relationship between the frequency of stomach movements and the volume of secretion (Fig. 5). The acid concentration of the secretion did not alter markedly: it lay between 125 - 148 m-equiv. H^+/litre. A comparison in the animals when fasted and fed over the same time period indicated that fasting resulted in lower amounts of abomasal secretion and a decreased frequency of reticulum and associated rumen movements (Fig. 5). Both reticulo-rumenal motility and pouch secretion varied widely at these lower levels of activity. The difference in acid output of a pouch in the same sheep fasted and fed over the same time period is shown in Fig. 6.

In the animals to which the above description refers it was found occasionally that the response to fasting differed: in some cases within a few hours of the fast, the acid secretion became meagre in volume (falling from 12 - 14 ml/15 min to 1 - 7 ml/15 min) and reduced in acidity (60 - 120 m-equiv H^+/litre); this resulted in a rapid decline in acid output from the pouch (Fig. 7). At these times of reduced gastric secretion there were less frequent and less strong contractions of the reticulum and rumen.

In the present study less systematic observations on the effect of fasting and pepsin secretion from abomasal pouches were made. In general there was a decline in pepsin output which was largely related to the decline in volume of secretion.

Responses to feeding after fasting:

Different forms in the response to feeding after a 12 hour fast were

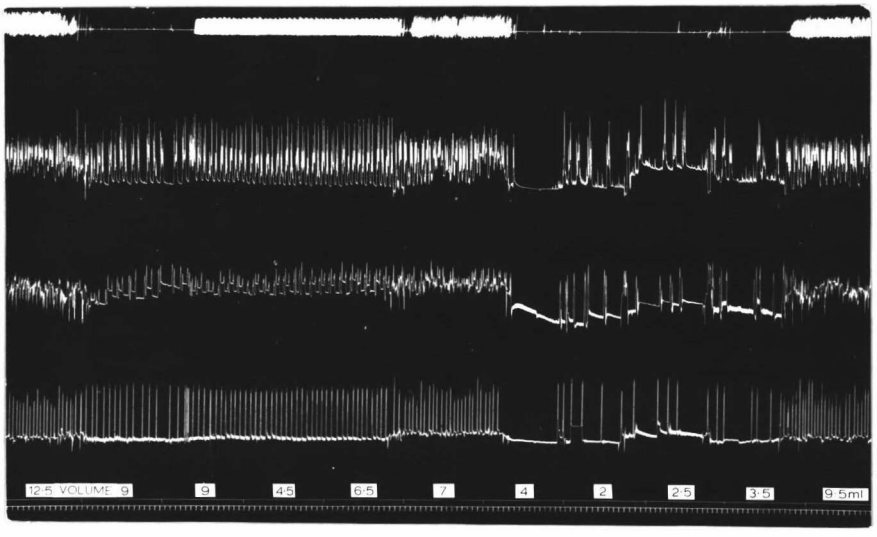


Fig. 8: The responses recorded 2 hr after a sheep first fed following a 12 hr fast. From above downwards: jaw movements, contractions of the main ventral rumen, contractions of the cranial dorsal rumen, contractions of the reticulum, Volume in ml of 15 min samples of abomasal pouch secretion, signal for the change in collection of samples of abomasal pouch secretion at 15 min intervals, 60 sec time-marker. In this animal at this time there were larger volumes of abomasal pouch secretion and more frequent reticulum contractions whilst the animal was actually eating. Lower volumes of secretion were associated with reduced frequencies of reticulum and rumen contractions.

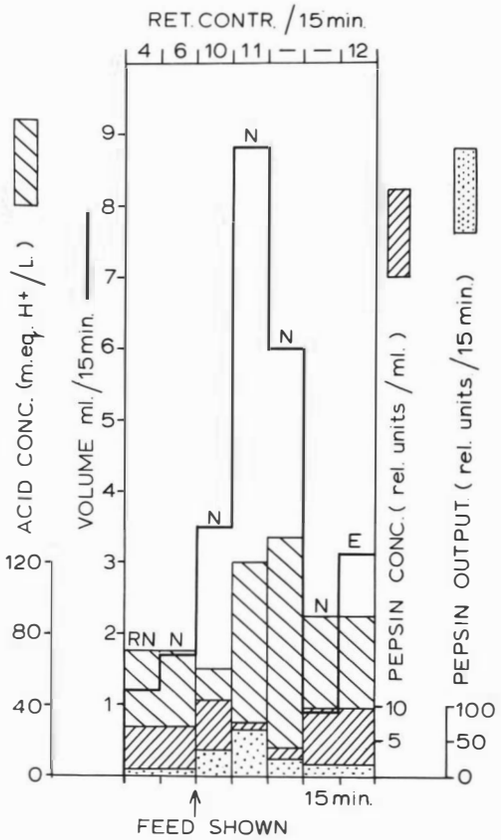


Fig. 10: The stimulation of abomasal pouch secretion accorded by teasing a fasted animal with food. Graph from above downwards: reticulum contractions/15 min, activity of the animal (R = ruminating; E = eating; N = nothing), thick black line = volume of secretion from the pouch in ml/15 min, wide shading = total acid concentration in the secretion in m-equiv H^+/litre, narrow shading = pepsin concentration of the secretion in relative units/ml, dotted area = pepsin output from the pouch in relative units/15 min, 15 min time intervals.

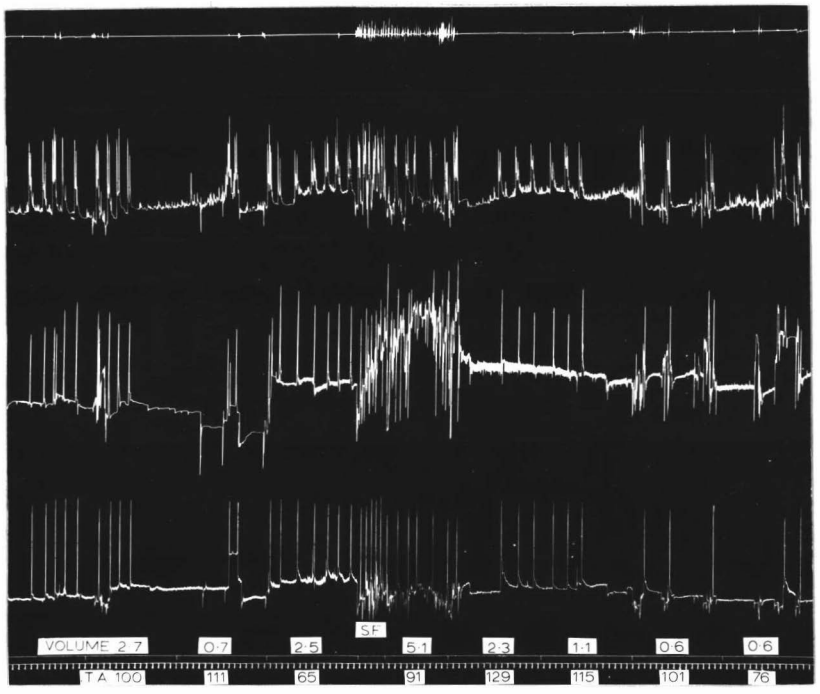


Fig. 11: The effects on reticulo-rumenal motility and abomasal secretion of teasing a fasted sheep with food. Records from above downwards: jaw movements, contractions of the main ventral rumen, contractions of the cranial dorsal rumen, contractions of the reticulum, S.F. = teasing with food, Volume in ml of 15 min samples of abomasal pouch secretion, signal for the change in collection of samples of abomasal pouch secretion at 15 min intervals. 60 sec time-marker, T.A. = total acid concentration in m-equiv H^+/litre of the 15 min samples of abomasal pouch secretion. There was a marked increase in pepsin concentration and output after teasing with food.

observed. The amount of feed consumed, the frequency of contractions of the reticulum and rumen and the level of secretion maintained by the abomasal pouches varied in the different animals studied. A high level of secretion in one animal was associated with continual activity in the form of eating or rumination. In another animal, lower levels of secretion were associated with a smaller food intake, and long periods of inactivity (after feeding) in wh reticulum and rumen contractions occurred at a low frequency (Fig. 8). Levels of abomasal pouch secretion and reticulo-rumenal motility approaching those normal for the fed animal were sometimes not attained until 10 - 12 hours after feeding commenced (Fig. 9).

Cephalic phase of abomasal acid secretion:

The simultaneous recording of abomasal pouch secretion and contractions of the reticulum and rumen from the partial exteriorizations enabled the clear demonstration of a cephalic phase of abomasal acid secretion. Preparations to feed spread over 5 - 10 min without actually feeding fasted animals (teasing with food), resulted in an increased output of acid and pepsin in the secretion from the abomasal pouches (Fig. 10). During the period of teasing an increased frequency of reticulum and associated rumen contractions was evident (Fig. 11). Following this period of stimulation, secretion and motility (unless rumination occurred) decreased. Although the volume increased to a maximum 15 - 30 min after the start of teasing with food, the acid concentration was at its greatest 30 - 45 min from the initiation of teasing, that is at a time when the volume of secretion had begun to decline (Fig. 10). Pepsin concentration increased within the first 15 min but was not maintained subsequently. The maximum pepsin output occurred with the maximum volume of secretion. The maximum pepsin concentration thus occurred before the maximum output was attained (Fig. 10).

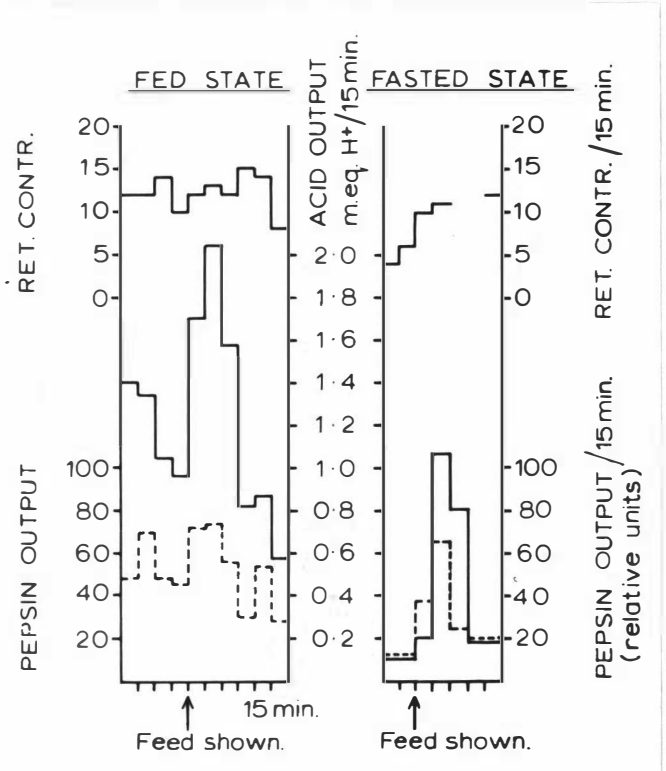


Fig. 12: The response to teasing with food of the same sheep when fed (a) and fasted (b). Graph from above downwards: reticulum contractions/15 min, acid output from the pouch in m-equiv H^+/15 min, pepsin output from the pouch in relative units/15 min, 15 min time intervals.

Similar responses in pouch secretion and reticulo-rumenal motility to teasing with food were also obtained in fed animals (Fig. 12).

In one experiment during complete inhibition of abomasal acid secretion and reticulum and rumen motility produced by the intraduodenal infusion of oleic acid, a teasing response was obtained in the form of an increased acid output from the pouch. It is noteworthy that at this time there was a complete absence of contractions of the reticulum and rumen. Unfortunately the volume of juice was insufficient to permit pepsin estimations to be undertaken.

An inhibition of a "psychic" nature was also detected on some occasions. In one instance the high level of pouch secretion in response to feeding was abruptly halved in volume on the appearance of strangers in the animal room; when they had left the room, the volume of secretion returned to normal.

Electrolyte concentration of abomasal acid secretion:

In the pouch secretion investigated a definite relationship was found to exist between the electrolytes, sodium and potassium, and the acidity of the secretion.

Sodium ion concentration varied inversely with the H^+ concentration of the secretion. Under ad lib conditions of feeding Na^+ concentration ranged from 1 - 25 m-equiv/litre. Under fasted conditions Na^+ concentration varied from 3 - 75 m-equiv/litre. The lower value of Na^+ was associated with the teasing response.

In contrast to Na^+ concentration, potassium ion concentration showed a direct relationship with acid concentration. Under ad lib feeding K^+ concentration ranged from 4 - 15 m-equiv/litre, while under fasting conditions K^+ concentration values were of the order 2 - 11 m-equiv/litre. As with the lower value of Na^+, the higher value for K^+ (11 m-equiv/litre)

obtained under the fasted condition was associated with the "sham feeding" response. At times a low acidity did not have an associated rise in Na^+ concentration. An example of this was an acidity of 111 m-equiv H^+/litre (pH 1.11) when the Na^+ concentration was 2 m-equiv/litre (see Table 2 #31, 32).

Discussion:

The continuous secretion of gastric acid is a characteristic feature in ruminants. Hill (1955) provided evidence that the continuous secretion of abomasal acid was not spontaneous in the sense of an inherent property of the secretory cells (c.f. parotid salivary gland secretory activity of ruminants, Babkin 1950) but was due to specific stimuli occasioned by the entry of digesta into the abomasum.

In the abomasal pouches of sheep studied in the present work, continuous secretion was evident even during long periods of fasting. The secretion from the pouches (which were estimated to be of about 80 ml capacity), was voluminous and the acid in a concentration which approached the maximum which has been calculated (Babkin 1950, Conway 1953) and demonstrated in other species (James, 1957). The acidity of the secretion of the pouches studied here was as high as that reported by Masson and Phillipson (1952), Ash (1961a - b) and Hill (1960, 1965).

A marked secretory response to feeding was revealed. This was particularly so when new feed was offered. Secretory responses to feeding were also demonstrated on other occasions. Ash (1961a) collected abomasal pouch secretion at 60 min intervals from ad lib fed sheep. He found variation from hour to hour in acid secretion, but no consistent effect of feeding. However, when these animals were subjected to twice daily feeding, definite secretory responses were revealed. In experiments in which pouch secretion was collected for successive periods of 15 min, Ash reported a definite increase in acid secretion when fasted animals fed; he found little if any enhanced secretion in feeding animals which had continuous access to food. In other experiments Ash followed both abomasal outflow and the secretion from an abomasal pouch. An increase in daily food intake was associated with higher levels of secretion of, and outflow from, the

abomasum: both decreased when the food intake was reduced. Phillipson and Ash (1965) have reported that outflow of digesta from the omasum in relation to feeding is variable, but on restricted diets an increased outflow may be observed within 30 min of first feeding; this outflow reached a peak 1 - 3 hours after feeding. Phillipson and Ash (1965) have also investigated the effects of feeding on the flow of digesta from the abomasum. Flow from the abomasum was high during feeding, but subsided when feeding had finished. They argued that if a depression in the outflow of digesta from the abomasum corresponded to a period of high outflow from the omasum, the abomasum would become distended. The abomasal distension could eventually cause a reduction in the frequency of reticulum and associated rumen contractions and this in turn could lead to a reduced flow of digesta into the abomasum. The inhibitory effects of abomasal distension on reticulo-rumen motility have been recognised (Phillipson 1939, Titchen 1958). An inhibition of outflow from the abomasum was presumed to have been a possible consequence of distension of the duodenum by the initial large flow of digesta into this region. Ash (cited by Phillipson and Ash 1965) has shown that distension of the duodenum with an inflated condom reduced the force and frequency of contractions of the reticulum. Running abomasal contents through an isolated duodenal loop had virtually no effect on the reticulum. Ash interpreted these findings as suggesting that the inhibitory action on the reticulum arose from the physical stimulus of a distension, rather than anything of a chemical nature.

Ash (1959) showed acid secretion was stimulated when rumen fluid or buffered solutions were introduced into the abomasum of sheep in which the rumen had been emptied. The responses were potentiated when the VFA concentration of the solutions was increased. The distension resulting from the rapid flow of digesta into the abomasum, together with the chemical stimulation accorded by the digesta (e.g. VFA's) would be expected to

provide a potent stimulus to acid secretion. Either a physical or chemical stimulus alone it can be argued, is potentially capable of causing an increase in the abomasal secretory activity. It appears reasonable to suggest that the secretory responses obtained on feeding are due in large part to the increased passage of digesta into the abomasum, thereby promoting secretion by distension, chemical and possibly tactile stimuli. In the fasted animal decreases in acid output which occurred during periods of inactivity following rumination, could possibly be explained in terms of motility of the reticulum and rumen. In a fasted animal small amounts of digesta may pass into the abomasum during rumination, thus producing a stimulus for acid secretion. In the following inactive periods when motility dropped to almost nil, there would be little passage of digesta and consequently little acid secretion.

Hill (1965) has discussed the increased concentration and output of pepsin from abomasal pouches during the secretory response to feeding. In some animals, Hill failed to detect an increased secretory response. There are at least two explanations of this. One of these is a lack of vagal innervation of the abomasal pouch. Although a basal secretion of pepsin from abomasal pouches may result from intrinsic cholinergic mechanisms, Hill has suggested that reflex vagal excitation of the pepsin secreting cells must occur intermittently if not continuously. However, another explanation might be provided from Schofield's experiments whereby inhibitory mechanisms arising from the small intestine and antrum have been postulated as causing a reduction in pouch motility and pepsin output following feeding. In studies of Heidenhain and transplanted pouches in dogs, Schofield (1959) demonstrated a decrease in pouch motility and a depression of pepsin output following feeding. It was suggested from studies made of the effects of atropine and hexamethonium, that both motility and pepsin secretion were dependent on the same activity of the intramural nerve plexuses. As the

inhibitory response was obtained in extrinsically denervated pouches a humoral mechanism appears to have produced the effect. In the present experiments in fasted animals, on feeding a slight delay in the secretory response in terms of the acid and the volume of secretion occurred, but the response gradually increased to a peak and was maintained or fluctuated about a higher level. Both motility and secretion varied together, but it was not possible to separate cause and effect. The definite secretory response to feeding after a fast confirms previous work (Ash 1961a, Hill 1960).

In the light of the present experiments it is not possible to agree with Hill (1961a, 1965) that there is no cephalic phase or a cephalic phase of minor importance in the ruminant. In the present experiments hypermotility of the reticulum and rumen and acid secretion from the abomasal pouch occurred on teasing the sheep with food. The greatest acid and pepsin output occurred 15 - 30 min after the start of the teasing period. The pepsin concentration of the secretion was greatest within the first 15 min. Motility usually decreased markedly a few minutes after the stimulation period ended.

Preshaw and Webster (1967) in a comparison of sham feeding and teasing as stimuli for gastric acid secretion in dogs with gastric fistulae demonstrated that the duration of stimulation was an important factor in determining the volume and acidity of the secretory response. Teasing with food produced a much smaller response than that of sham feeding, whose magnitude was comparable to the maximum secretory response of gastrin or histamine. The differential effect between teasing and sham feeding was attributed to a lack of oral stimulation. The teasing may be regarded as a true psychic component of the cephalic phase of gastric acid secretion.

Whether this cephalic phase of abomasal acid secretion observed was

the result of a direct vagal stimulation of the abomasal secretory cells, or it occurred indirectly in some other way has not been determined. The responses in the pepsin concentration of the secretion and the pepsin output from the pouch suggest a direct vagal stimulation. However, an explanation of the cephalic phase of abomasal acid secretion in the ruminant could be a vagal stimulation of pepsin secretion and stimulation of acid secretion by gastrin liberated by the increased passage of digesta into the abomasum from the resultant hypermotility of the forestomachs. The longer latency of the oxyntic cells to vagal stimulation, as compared with the peptic cells is well known. Linde (1954) produced a constant level of acid secretion in anaesthetised cats by a series of subcutaneous injections of histamine. On the additional stimulation of the vagus, pepsin output increased immediately but acid and water output did not increase for 10 - 15 min. In view of these results it would be unwise to conclude that on the basis of the latency of the oxyntic cells' response that the increased secretion from the abomasal pouch was due solely to the passage of digesta from the reticulum and rumen.

A particularly interesting observation was that an increased acid output occurred despite the absence of motility of the reticulum and rumen in response to teasing during the inhibition produced by the intraduodenal infusion of oleic acid. In this case, movement of digesta from the reticulum and rumen appears unlikely to have occurred, so the secretory response was probably due to a vagal release of gastrin, or a direct vagal stimulation of the abomasal secretory cells.

Recent findings in monogastric animals (Andersson and Olbe 1964) indicate that tonic vagal activity of the oxyntic cell is of great importance in potentiating its response to gastrin. In animals with a simpler form of stomach, the cephalic phase of gastric acid secretion is presumed

to facilitate the initial gastric digestion required by the sudden entry of large amounts of food into the stomach (Gregory 1962). Hill (1960) in experiments with sheep failed to demonstrate a cephalic phase of abomasal acid secretion by sham feeding in animals with oesophagostomies, or by feeding animals with an empty reticulo-rumen. Hill suggested the apparent absence of a cephalic phase was probably due to the continuous digestion of ruminants; the continual passage of digesta into the abomasum eliminated the requirement of an auxillary acid secretion in the form of a cephalic component. Although a continual passage of digesta from the reticulum and rumen and omasum into the abomasum occurs, the volume of material passed varies in proportion according to the animal's activity. Phillipson and Ash (1965) have reported that the flow of digesta into the abomasum increases on feeding.

In the present experiments, in contrast to previous workers' results, secretory responses from the abomasal pouches were obtained with feeding and also in response to teasing the animals with food. There would appear to be some justification in assuming that just as feeding a simple stomached animal results in an increased gastric secretion of which the cephalic phase contributes greatly, feeding a ruminant may also result in an increased abomasal acid secretion by a similar mechanism. This cephalic phase of abomasal acid secretion would occur simultaneously with the increased passage of digesta into the abomasum and thus contribute in no small way to the acid secretion required at this time. The cephalic phase of vagal stimulation could facilitate the abomasal secretory response by sensitising the oxyntic cells to gastrin, increasing the pepsin secretion of the juice, and potentiating the liberation of gastrin.

Hill (1965) has suggested the existence of reflex control, from the forestomachs of pepsin secretion. In non-ruminant animals reflex pathways

originating in mechanoreceptors and chemoreceptors in the stomach and duodenum have been shown to exert effects on the stomach through vagal efferent fibres (Harper, Kidd and Scratcherd 1959). Proof of the reflex control of pepsin secretion by the forestomachs has not been demonstrated, although Hill offers as evidence an experiment where an increase in pepsin concentration and output occurred during rumination. He thought it unlikely that stimulation of pepsin secretion arose via an orogastric reflex as eating a meal with the reticulo-rumen empty was without effect. It should be realised, however, that emptying the reticulo-rumen is a severe procedure and may consequently interfere with responses, particularly reflex responses in a non-specific manner.

The results presented here support previous work from which it was concluded that an increased pepsin concentration and output were encountered on feeding. No consistent relationship could be established between rumination and pepsin secretion. The increased pepsin output on feeding might be taken as indirect evidence of a vagal innervation of the abomasal pouch.

The control of stomach motility and secretion in the ruminant is a complex one. Emphasis in this chapter has been placed on the nature of the secretory mechanisms of the abomasum, and in particular the possible causes in the fluctuation in the volume and acidity of the pouch secretion. Responses in abomasal acid secretion to feeding have been demonstrated at all times.

A cephalic phase of abomasal acid secretion has been established by teasing the animals with food. This form of stimulus has been shown in simple stomached animals to be of a much smaller magnitude than the acid secretion produced in response to sham feeding. It remains to be seen

whether sham feeding (carried out under suitable conditions) may result in an even greater secretory response from the abomasum.

CHAPTER III

INTRADUODENAL AND INTRAVENOUS INFUSIONS OF FAT, AND INTRAVENOUS INFUSIONS OF ENTEROGASTRONE

Introduction:

In 1886 Ewald and Boas (cited by Gregory 1962) discovered that the addition of olive oil to a meal inhibited gastric secretion and slowed gastric emptying. These actions of fat were studied intensively by Pavlov and co-workers. They held the view (cited by Code 1951) that the inhibition occurred as a reflex and that it was probably a vagal reflex between the stomach (effector) and duodenum (source of afferent stimulation).

Lim, Ivy and McCarthy (1925) showed that the vagus was not essential for this inhibition to occur. They found that the secretion, induced mechanically in vagotomised stomachs (separated from the oesophagus and intestines) was inhibited by the presence of fat in the duodenum. Farrell and Ivy (1926) found that feeding inhibited the motility of a completely transplanted fundic pouch of a dog. This inhibition was most marked if the meal contained fat. Feng, Hou and Lim (1929) obtained evidence that the presence of fat in a meal also inhibited gastric secretion. In dogs provided with denervated or transplanted fundic pouches, 50 ml of olive oil added to a meat meal led to a failure to obtain the secretory response normal with such a meal.

Babkin (1950) stated that there are two phases of the action of fat on the gastric glands; one inhibitory, the other excitatory. The volume of secretion, the acidity and peptic activity are all lowered in the inhibitory phase. The excitatory phase is usually manifest as a prolonged excitatory period following the inhibition and may even, with larger quantities of fat digested, result in a hypersecretion by the gastric glands. This excitatory phase is characterised by an increase in the volume and

acidity of the juice, but a low pepsin content is still maintained. Babkin (1950) also stated that fat inhibits chiefly the cephalic phase of gastric secretion and there is little inhibition of gastric secretion when fat is present in the stomach and is prevented (by experimental means) from passing to the duodenum.

The inhibitory effect of fat on gastric secretion stimulated by histamine appears to be determined by the ratio of histamine to fat, and their respective times of administration (Code 1951). An illustration of the dose response relationship was provided by Lim et al (1925). On the introduction of 100 mg histamine into a stomach pouch, the gastric glands were stimulated to secrete despite the previous profound inhibition due to fat: in contrast, in similar circumstances the secretory response to 50 mg histamine was diminished by the fat induced inhibition.

Alley and Babkin (1939, cited by Babkin 1950) investigated the effects of histamine and pilocarpine on gastric secretion inhibited by fat in dogs provided with Pavlov or Armour pouches. Both drugs were given subcutaneously. Histamine phosphate elicited a copious secretion of gastric juice despite the previous ingestion of fat. Histamine stimulated gastric secretion even though there was partial paralysis of the vagal innervation of the gastric glands. Pilocarpine caused a gastric secretion whose volume was similar to the control (pilocarpine without fat) but whose pepsin content was on average one third of that of the secretion of the control animals. This was taken to indicate that pilocarpine stimulated the oxyntic cells directly, whereas for the peptic cells to respond to pilocarpine, the participation of the parasympathetic nervous system appeared necessary. Babkin concluded:

> "The facts that fat inhibits only to a slight extent the gastric secretion evoked by histamine and diminishes the ability of

pilocarpine to stimulate the output of pepsin without interfering with the production of the acid and liquid parts of the juice support the theory that the digestive glands are compound structures, formed of various sets of secretory epithelia, the work of each set being regulated independently by different nerves, hormones, absorbed chemical substances, or drugs."

The part played by the nervous system in the inhibition of gastric secretion and motility by the presence of fat in the duodenum remains obscure. Quigley and Meschan (1938) investigated the effects of fats and fatty acids on the motility of the pyloric antrum and pyloric sphincter of conscious dogs. In chronic double vagotomised animals, the depressant action of the fats was greatly diminished. A vagal reflex was not believed to be exclusively involved, but instead the idea of an increased sympathetic discharge, or the discharge unopposed by the vagus resulting in an interference with the production of enterogastrone, or leading to a decrease in the sensitivity of the pyloric region to enterogastrone was favoured. Recent studies by Halvorson, Middleton, Bibler, Harkins and Nyhus (1966) have shown that vagotomy has no effect on the inhibitory action on gastric secretion of fat in the duodenum. Their results indicated that the release of an inhibiting humoral substance by fat in the duodenum was not related to the vagal innervation of the duodenum. These workers also interpreted their results as suggesting that the inhibitory action of fat in the duodenum acted directly on the parietal cell as subcutaneous injections of histamine failed to stimulate acid secretion.

Grechishkina and Skliarov (1959) found that in dogs with Pavlov pouches the inhibitory effect of fat on gastric secretion was preserved after destruction of the parasympathetic nerve supply to the pouch. The inhibition of gastric secretion by fat became less marked when the coeliac

plexus was removed, that is after the destruction of the sympathetic nerve supply. These results are apparently a contradiction of the theory advanced by Quigley and Meschan (1938), provided that pyloric motility and gastric secretion are inhibited by the same mechanism. Fields and Duthie (1965) studied the effect of vagotomy on the intraluminal digestion of fat in man. They concluded that the diminished amounts of bile acids and lipase found in the intestinal lumen after vagotomy was due to the withdrawal of a parasympathetic stimulus in the case of bile delivery. This could result in a reduction in the formation of the micellar phase of fat digestion and absorption. There is little support in the literature for the idea that the vagus is in any way concerned in the control of the secretion of bile. There is little other evidence on the possible role of the nervous system in the mediation of the fat inhibition.

Many workers have endeavoured to elucidate the conditions in the duodenum which govern the inhibition of gastric secretion and motility brought about by fat. Sircus (1958) carried out experiments on dogs with duodenal cannulation and innervated or denervated pouches. Infusions of fat emulsions into the duodenum inhibited gastric secretion in response to both a meal and histamine. No difference was observed between the effects in denervated and innervated pouches. When similar fat emulsions were perfused through an isolated duodenal loop, inhibition of the secretory response of the innervated pouch failed. On incubating olive oil with pancreatic secretion, inhibition of the gastric secretory response to meals was obtained. Perfusion of glycerol, oleic acid and linoleic acid through a duodenal loop deprived of pancreatic secretion resulted in a slight augmentation of gastric secretion from the innervated pouch.

Menguy (1959) found that fat introduced into the small intestine of the intact rat inhibited gastric secretion. This inhibitory activity was

decreased when bile was previously diverted from the small intestine. When bile salts were added, the same degree of inhibition as in the intact rat occurred. In a later study Menguy (1960) demonstrated in the rat, that the inhibitory effect of fat in the duodenum on gastric motility was dependent upon the presence of both bile and lipase. Long and Brooks (1965) compared the inhibitory properties of oleic acid with its parent triglyceride, triolein. In dogs with innervated pouches the duration of inhibition was longer and the absorption slower with oleic acid than with triolein.

The inhibition of gastric secretion and motility by the presence of fat in the duodenum has been attributed to the liberation of a duodenal hormone, enterogastrone. The existence of this hormone has been supported by the extraction of an inhibitory principle from the duodenal mucosa. Conjecture still exists however, as to the nature and physiological properties of enterogastrone, and the possibility remains that gastric secretion and motility are inhibited by two separate entities.

It is appropriate at this stage to review the events leading up to the postulation of an inhibitory duodenal hormone, and the subsequent failure to isolate and characterise such an entity. Feng, Hou and Lim (1929) demonstrated in dogs, that fat inhibited gastric secretion as well as gastric motility. Feeding 50 cc of olive oil half an hour before a 150 gm meat meal inhibited secretion from Bickel gastric pouches (pouches with both their parasympathetic and sympathetic nerve supplies removed) for a period of about 2 hours. 50 cc of olive oil given with 200 gm meat inhibited secretion from autotransplanted pouches, although the inhibition was not always marked, and at times did not extend beyond the first hour. Paraffin oil had no inhibitory effect on gastric secretion. The intravenous administration of thoracic duct lymph from animals previously fed fat, and

the intravenous administration of an ethereal extract of thoracic duct lymph failed to inhibit the gastric secretory responses of the pouches. Diversion of the thoracic duct lymph from the circulation via a cannula failed to alter the inhibitory response to orally administered olive oil.. These results suggested a humoral agent was responsible for the inhibition and that it was not an absorbed digestion product of fat, or at least it was not one which was transported by the lymphatic system. It was concluded that fat excites the production, from the intestine, of a chalone (for a definition of this term see Babkin, 1950 p 560) for gastric secretion. At that time Feng et al (1929) favoured bile as the inhibitory agent. Kosaka and Lim (1930) studied the role of bile and cholecystokinin in the inhibition of gastric secretion by fat. The intravenous injection of dog's gall bladder bile (0.3 cc/kg body wt.) did not cause inhibition of gastric secretion. The administration of 30 cc of bile by stomach tube immediately before a meal did not influence acid secretion. Following cholecystectomy fat still caused an inhibition of gastric secretion and in some cases this inhibition appeared to be more intense and prolonged. In the cholecystectomised animal, the ingestion of bile with the oil definitely reduced the degree and duration of the inhibition on gastric secretion accorded by the oil.

The intravenous injection of 2.5 mg/kg/body weight of cholecystokinin (prepared by Ivy) produced no effect on gastric secretion. 5 mg/kg of cholecystokinin inhibited secretion in response to a meal. The inhibitory action of cholecystokinin persisted after removal of the gall bladder, indicating that the cholecystokinin effect did not depend upon its gall bladder action. Kosaka and Lim concluded that cholecystokinin may include or may actually be the chalone for gastric secretion.

Kosaka and Lim (1930a) obtained saline extracts of intestinal mucosa

which inhibited the meal response in Heidenhain pouch dogs. The active principle was named enterogastrone (entero = intestine; gastr = stomach; one = chalone). The significance of the intestinal extracts was questioned as colonic extracts also exhibited considerable inhibitory activity. Kosaka and Lim in 1933 (cited by Babkin 1950, p 636) used a picric acid method of extraction to obtain active preparations of enterogastrone from the blood of an animal that had been fed with olive oil. Gray, Bradley and Ivy (1937) prepared and biologically assayed enterogastrone. The preparation of the hormone involved the extraction of hormones from the duodenal mucosa with dilute hydrochloric acid and their subsequent precipitation with sodium chloride. Enterogastrone was further concentrated from this first or "A precipitate" as it was termed by Gray et al (1937), by precipitation with tannic acid.

An arbitrarily designated gastric secretory unit of enterogastrone was defined by Gray et al (1937) as follows:

> "A unit of enterogastrone is that quantity, which when injected intravenously in a 12 - 14 kg dog with a pouch of the entire stomach receiving sufficient histamine subcutaneously at 10 minute intervals to maintain a uniform flow of 1 cc of gastric juice (5 mg HCl) per minute, causes a 50 per cent reduction in the secretion of hydrochloric acid during two hours following its injection."

One unit of enterogastrone was contained in about 50 mg of tannic acid preparation. 100 mg of the preparation produced a fall in blood pressure of about 10 mm Hg. Although the extract had inhibitory effects on both motor and secretory activities of the stomach, small doses completely inhibited motility for a period proportional to the dose, but such doses had no effect on gastric secretion in total pouch dogs during the continuous

administration of histamine. A characteristic of small doses of the enterogastrone preparation was that it lowered the secretory rate to a constant reduced level, which persisted for several hours. The secretory response to a meal of meat was more readily inhibited than the secretory response to a single dose of histamine: Gray et al attributed this to a quantitative difference in the character of the two stimuli. The inhibitory effect of the extract on a gastric secretory response initiated and maintained by continuous administration of histamine increased with the size of the dose of enterogastrone and varied inversely to the strength of the secretory stimulus. When extracts were administered subcutaneously, four times the dose of enterogastrone had to be given to obtain an effect equal to that obtained with a dose administered by the intravenous route.

Greengard, Atkinson, Grossman and Ivy (1946) presented a communication summarizing a series of investigations on enterogastrone which culminated in a method of preparation of the chalone which was suitable for injection in man. The method of extraction was similar to that of Gray et al (1937), but contained a further purification step in the form of a picric acid precipitation. The product was colourless, easily soluble in water and judged by its effects on a histamine induced gastric secretion in the total pouch dog was of a potency of 1 unit/25 mg. It possessed little if any inhibitory action on gastric motility in contrast to the cruder product prior to the picric acid treatment. Visscher (1948) subjected preparations of enterogastrone to electrophoretic separation at pH 7.5 and tested the fractions obtained in anaesthetised rats in which the pylorus was ligated. The slowest moving fraction showed the greatest inhibition. In dialysis experiments it was found that activity was recovered from the external solution and this anti-secretory activity was equal to that of the starting material. The soluble fraction of the non-dialysable material was nearly

inactive.

Öbrink (1947) studied the electrophoretic properties of a purified enterogastrone preparation. He found the secretory depressing factor had no or at least a very slight electrophoretic mobility between pH 2.06 and 7.42, and the components showing mobility appeared to be ineffective in inhibiting a histamine induced gastric secretion. Winberg (1947) reported on the use of dialysis for the preparation of enterogastrone. Winberg improved the procedure involved in the extraction of enterogastrone by the method of Greengard et al (1946). Dialysis through a cellophane membrane was an essential step in the new method. Several inactive preparations were "activated" and purified by dialysis although only small amounts of active material were obtained. Dialysis was suggested as a method for separating the stimulatory substances of high molecular weight from the inhibitory substances of low molecular weight.

Winberg and Öbrink (1948) investigated the possibility that enterogastrone might be a protein by studying the effect of proteolysis on their preparations. The experiments performed showed that the inhibiting power of an enterogastrone extract on a histamine induced gastric acid secretion was unaffected by incubation with either pepsin or trypsin. Öbrink and Winberg (1950) exposed two different enterogastrone preparations to heat. The preparation with a lower content of organic material was the more thermosensitive one and did not withstand temperatures above 40°C. Although each preparation had equal biological activity it was thought that organic impurities had a protective effect on the thermolabile enterogastrone molecule. Öbrink and Winberg (1950a) reported that an enterogastrone preparation showed a characteristic absorption curve in the ultraviolet region. This curve had a maximum at 2600Å and a minimum at 2350Å. Evidence was obtained that this absorption curve was due to the active enterogastrone molecule;

a decrease in biological activity was accompanied by a diminution of the absorption curve at 2600Å. Öbrink and Winberg concluded from their investigations that the chemical and physical properties of the enterogastrone molecule indicated that it was unlikely that enterogastrone was a protein but could be related to a nucleic acid. No further advances have been made in the elucidation of the structure of enterogastrone. Emphasis has recently become focussed on the discrepancies between the manner in which enterogastrone extracts inhibit gastric secretion and the inhibition accorded by the presence of fat in the intestine.

Linde, Öbrink and Ulfendahl (1952) have studied the mode of enterogastrone-action on HCl secretion. They concluded that enterogastrone inhibits the volume output as well as the primary and secondary acidity in Heidenhain pouch dogs, but that it has no specific action on the acid concentration per se. The changes in acidity after enterogastrone administration have been regarded as secondary to alterations in the secretory rates.

In 1932, Kosaka, Lim, Ling and Liu (cited by Gregory 1962) offered the first comparison of the inhibitory effects on a histamine secretory response in a Heidenhain pouch dog of (a) an enterogastrone preparation, and (b) 50 ml of olive oil given by mouth before injection of the histamine. The extract produced a considerable inhibition of the histamine response, but the olive oil was almost ineffective. Gray et al (1937) used dogs with denervated pouches of the entire stomach to compare the inhibition of secretion caused by a unit of enterogastrone with the inhibition caused by 50 ml olive oil administered orally. The extract was about twice as effective as the oil in bringing about an inhibition of hydrochloric acid production.

Grossman, Greengard, Woolley and Ivy (1944) studied the action of an enterogastrone preparation on pepsin secretion in dogs. They found pepsin

output was moderately depressed by enterogastrone in the absence of vagal influences, but no definite inhibition of pepsin concentration was detected. A depression of pepsin concentration after fat administration occurred only in those pouches which were vagally innervated. The experiments indicated that vagal nervous influences are extensively concerned in the inhibition of pepsin secretion by fat, and that enterogastrone preparations have no direct inhibitory action on pepsin secretion.

Harris, Grossman and Ivy (1947) investigated the role of the vagus nerves in the inhibition of gastric motility by fat, and by intestinal (enterogastrone) and urinary (urogastrone) extracts. Their results showed that fat in the intestine inhibited gastric motility induced by distension in both the vagally innervated and vagally denervated stomach. Intravenously administered urogastrone and enterogastrone inhibited the distension induced motility of the vagally innervated stomach, but neither affected motility in the vagally denervated stomach. Sircus (1958) reported that denervation of a fundic pouch abolished the inhibitory effect of fat in the duodenum on acid secretion stimulated by histamine: he had found that fat in the duodenum could inhibit the acid secretion of an innervated fundic pouch in response to histamine.

Uvnäs (1948) discovered that enterogastrone prepared according to the method described by Greengard et al (1946) did not inhibit in the anaesthetised cat gastric secretion stimulated by the intravenous infusion of histamine. As enterogastrone preparations had been reported to inhibit secretion in dogs Uvnäs suggested that the discrepancy may have been due to fundamental differences in the gastric secretory mechanism in the dog and cat. He apparently overlooked the absence of reports on enterogastrone in anaesthetised dogs, i.e. of reports in the two species under comparable experimental conditions. Howat and Schofield (1954) obtained inhibitory responses with

enterogastrone preparations on gastric secretion stimulated by histamine in anaesthetised cats. Pancreatic secretion stimulated by secretin was also inhibited by the preparation of enterogastrone. Their active preparations were provided by Ivy, as they had failed to prepare potent extracts in their own laboratory. These inactive preparations of enterogastrone stimulated gastric and pancreatic secretion, and as such exhibit the same properties of the extracts prepared by Uvnäs (1948). It is probable that Uvnäs's results were due to inactive preparations.

The relationship between the effects of fat administered intraduodenally and of the effects of enterogastrone extracts has been further complicated by the finding of Grossman, Woolley, Dutton and Ivy (1945) that nausea produced by mechanical stimulation of the oesophagus or duodenum inhibited a histamine response in vagotomised pouches of the entire stomach and in transplanted fundic pouches of dogs. Gregory (1956) undertook a careful study to determine whether fat inhibited the gastric secretory response to histamine. In dogs, transplanted fundic pouches were stimulated to secrete at a constant and slow rate by the continuous administration of histamine. Fat introduced into the duodenum in such a way as to minimise nausea caused slight secretory inhibition on the low rates of secretion and no inhibition on higher rates of secretion. Enterogastrone was presumed to have been liberated as in similar experiments in which motility was recorded, a profound inhibition of tone and motility of the pouch occurred. The view that enterogastrone has a physiological action in inhibiting the "antral phase" of the gastric secretory response to a meal, was strongly supported by the finding of Gregory and Tracy (1959) that fat in the duodenum inhibited the secretion of a denervated fundic pouch when this pouch was stimulated by gastrin liberated from a pyloric pouch irrigated with acetylcholine. The subsequent discovery that the introduction of fat into

the duodenum did not inhibit the response of denervated fundic pouches to injections of gastrin was taken to indicate that enterogastrone inhibited the release of gastrin from the antrum. Recent work (Bibler, Harkins and Nyhus 1966) has indicated that enterogastrone may act at the oxyntic cell level. A discussion of this work and the contrary conclusion drawn from it will follow later in the chapter.

No enterogastrone extracts so far produced have had precisely the same effects as fat infused into the duodenum, but a secretin preparation of Greenlee, Longhi, Guerrero, Nelsen, El-Bedri and Dragstedt (1957) has been reported to possess properties characteristic of an endogenous enterogastrone release. Gregory (1962, p 131) has cited results which indicate that the effects observed were not due to secretin, but may have been due to the presence in the extract of the hormone enterogastrone.

As well as inhibiting the secretory and motor responses of the stomach, enterogastrone has also been implicated in the control of food intake. Maclagen (1937) reduced the feed intake of rabbits by the subcutaneous and intravenous administration of an enterogastrone preparation prepared by Lim. This may have been a non-specific effect as the preparation was relatively crude and the effects were transient. Janowitz and Grossman (1951) in an investigation of the regulation of food intake in dogs, found prefeeding small doses of carbohydrate, fat or protein hydrolysate was without significant effect on subsequent food intake. Larger quantities inhibited food intake. As the smaller prefeeding doses were assumed to cause the release of endogenous enterogastrone the experiment was presumed to provide physiological evidence that enterogastrone per se does not contribute directly to satiety. It should be realised, however, that the small prefeeding doses may not have been of sufficient quantity to cause the release or a significant increase in the liberation of enterogastrone and that enterogastrone may still be

involved in the regulation of food intake.

Although the supposed liberation of enterogastrone is best demonstrated by the presence of fat or its digestion products in the duodenum, other products of digestion have also been implicated in the release of this chalone. Sircus (1958) showed carbohydrate, protein digestion products and hyper and hypotonic solutions inhibited pouch secretion of dogs. He discussed the possibility that an osmoreceptor mechanism was involved.

In the ruminant few studies have been undertaken to elucidate the influence of duodenal digestion products or duodenal extracts in the control of forestomach motility and abomasal secretion and motility.

Singleton (1951) inhibited abomasal motility by infusing 5 ml of a 50% emulsion of olive oil into the duodenum. The inhibition was characterised by "a short latent period" and periods of inhibition alternated with bursts of activity.

Reid (1957) administered mineral oils in the form of light and heavy paraffins into the rumen or omasum of cows. A depression of food intake was obtained only with light paraffins. When these light paraffins were emulsified or administered into the omasum, the latency of the effect was decreased and the depression of food intake was intensified and prolonged.

Hill (1965, p 225) cited unpublished results from experiments in which 5% palm oil added to the diet had no significant effect on the acidity of secretion or flow from the abomasum. 10% palm oil caused a reduction in the flow of digesta from the abomasum. A differential effect of triglycerides in the feed was obtained: 5% tallow produced an increase in the flow of digesta from the abomasum.

Phillips (1965) cited unpublished results from his laboratory on the infusion of oleic and linoleic acids into the duodenum. It was found that amounts greater than 1.5 - 2 ml/hour of these acids caused severe diarrhoea

and loss of appetite.

Titchen, Reid and Vlieg (1966) carried out studies on the effects of intraduodenal infusions of fat on the food intake of sheep. 100-150 ml of peanut oil infused into the duodenum over 17 hr (0.18 - 0.49 ml/kg/hr) depressed food intake, caused a reduction in stomach movements and led to diarrhoea. 50 ml of peanut oil administered over the same time did not have any of these effects. 11.5 to 23 ml infusions over 30 min (0.80 - 1.84 ml/kg/hr) of peanut oil, olive oil, soya bean oil, a hydrolysate of peanut oil and of oleic acid caused a reduction in the movements of the reticulum and rumen. 'B' sequences were less affected than 'A' sequences. The hydrolysate of peanut oil and the oleic acid had more profound inhibitory effects than did peanut or olive oil. Slow infusions of heavy liquid paraffin failed to produce the effects obtained with peanut oil. The faster infusion of heavy paraffin if anything, it was suggested, appeared to increase the frequency of reticulum and rumen movements.

Following on from the work of Titchen et al (1966), a detailed investigation has been undertaken of the effects of the introduction of fat into the duodenum on abomasal secretion and forestomach motility. As enterogastrone has been implicated in mediating these inhibitory effects, a comparison was made between the inhibitory responses on abomasal secretion and reticulo-rumenal motility of the intraduodenal administration of fat and of the intravenous administration of enterogastrone extracts.

Methods:

A constant speed infusion apparatus (Sage Instruments Ltd. N.Y.) was used for the intravenous and intraduodenal administration of oleic acid (May & Baker), the intraduodenal administration of olive oil (Gilseal, pharmaceutical quality) and for the intravenous administration of enterogastrone extract. A gas-liquid chromatographic analysis of the oleic acid (kindly undertaken by the Biochemistry Dept.) showed the sample of oleic acid to contain 68% oleic acid, 8.6% palmitoleic acid, 5.3% myristic acid, 4.7% palmitic acid, 4.0% linolenic acid, 2.4% linoleic, 3.1% lauric acid, 3.1% myristoleic and a small quantity of other acids. Behr, Fuson and Snyder (1959) have offered the following as the composition of olive oil; oleic acid 83%, linoleic acid 7%, palmitic acid 6% and stearic acid 4%.

The methods of collection, estimation and recording applied to abomasal secretion, and motility of the exteriorizations were the same as those described previously. At times of severe inhibition of abomasal secretion the low volumes collected made it necessary to pool samples so that the 15 min period of sampling was extended effectively in some cases to as long as 90 min.

The method used in the preparation of an enterogastrone concentrate was that Greengard, Atkinson, Grossman and Ivy described in Gastroenterology 7, 1946. Freshly slaughtered pigs' intestines were obtained from the abattoir of a local bacon factory and taken to the Biochemistry and Physiology laboratories at the University where the complete extraction process was carried out. 6 kg of intestine yielded approximately 700 mg of extract. The final extract took the form of a white powder which retained biological activity over several months when stored at $4^{o}C$.

The extract was dissolved in 0.9% saline at a concentration of 5 mg/ml a few hours before its administration to the animal. In an endeavour to

reduce bacterial contamination the solution was passed through a sterile millipore filter (Millipore, USA grade GSWP 025,00; GS 0.22 μ). Prior to its intravenous infusion the enterogastrone solution was warmed to 37°C in a water bath.

All intravenous infusions were introduced into the jugular vein via a polythene cannula. The jugular vein was cannulated 24 hours before the experiment. Heparinized saline was used as a means of preventing clotting in the polythene cannula. Control infusions of saline were made before and in some cases after the administration of the extract.

An attempt was made to assay the level of depressor substances present in the extract. The intravenous injection of 25 mg of material into a spinal preparation of a cat had no significant effect upon blood pressure. 50 mg of the extract produced a decrease in blood pressure 15 - 20 sec after its administration. 0.05 mg of histamine intravenously produced an entirely different response in that an initial small drop in blood pressure was followed by a marked increase. It was assumed that the depressor activity of the extract was not due to histamine.

Results:

The intraduodenal infusion of 0.016 ml/kg/min of oleic acid resulted in a reduction in the volume, acidity and pepsin content of abomasal pouch secretion. Olive oil did not cause an inhibition of the pepsin content of the juice. Reticulum and rumen motility was inhibited to a variable degree depending upon the substance infused and the rate of infusion.

Infusions of 10 ml oleic acid into the duodenum over 30 min (0.016 ml/kg/min) caused, within 15 min of the start of the infusion, a reduction of 'A' sequences of the reticulum and rumen contractions, and a diminution to 50% of the resting level of the volume of acid secretion. The inhibition of motility of the reticulum and rumen, and secretion from the abomasal pouch persisted for up to 6 - 7 hr.

2 ml of oleic acid infused into the duodenum over 20 min (0.005 ml/kg/min) led to a marked reduction in the volume of secretion within 30 min of the start of the infusion. An 80% inhibition of acid secretion persisted for about 90 min. The frequency of reticulum and rumen contractions was slowed slightly but did not exhibit any marked inhibition. Olive oil at the same dose rate, after a longer latency, caused an inhibition of secretion of shorter duration than did oleic acid, and was without significant effect upon reticulum and rumen motility.

The intravenous administration of oleic acid in the form of the free fatty acid resulted in a sudden (apparent within 8 min and 0.1 ml) and complete inhibition of reticulum and associated rumen movements. The acid concentration of the secretion was not inhibited with equal rapidity despite the rapid reduction in the volume of secretion.

Infusions of 10 ml of oleic acid into the duodenum: (See Table 3)

In fed and fasted animals, infusions of 10 ml oleic acid over 30 min (0.016 ml/kg/min) into the duodenum reduced the volume of secretion from the

abomasal pouch to 50% of its pre-infusion level within 15 - 20 min. The acid concentration was maintained at pre-infusion levels for the first 15 min of the infusion. Thereafter, the volume of secretion progressively declined as did the acid concentration of the secretion and the frequency of reticulum and rumen movements. An effect on food intake was evident at this stage. This was because the infusions were usually undertaken at the time when fresh feed was routinely offered, and at this time a prolonged period of continuous feeding of 90 min or more usually took place. However on those occasions when an infusion of oleic acid was undertaken, the usual period of feeding was reduced to 30 - 50 min. When feeding occurred reticulum and rumen movements increased to a frequency in the vicinity of that normal for the feeding animal on clover chaff (about 26 contractions/ 15 min). The frequency of these movements gradually slowed as eating became less avid. In contrast, neither the volume of secretion, nor its acidity were changed appreciably by feeding. When feeding ceased, a complete inhibition of the 'A' sequences was apparent, but 'B' sequences continued. Acid output from the abomasal pouch remained inhibited, and an inhibition of pepsin output was detected.

The animals showed a marked change of demeanour within 60 min of the infusion. Most of the sheep subjected to these intraduodenal infusions of oleic acid spent some time (4 - 5 hr) slumped in their slings; on standing 'B' sequences commonly occurred. Short periods of eating were followed by reticulum and associated rumen contractions but there was no concomitant rise in the volume and acidity of secretion.

The effect of offering feed 90 min after the infusion commenced to an animal fasted for 12 hr was examined. In this animal there was a complete inhibition of reticulum and associated rumen movements with the oleic acid infusion. It ate for 50 min. 'A' sequences were initially, when the

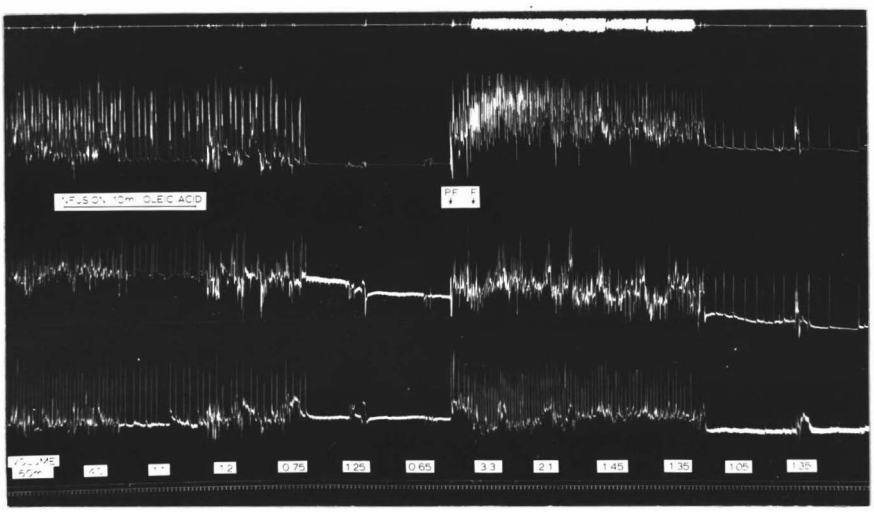
animal ate, of a high frequency (26 contractions/15 min), but gradually dwindled (Fig. 13). The volume of secretion increased slightly, but there was no increase in acidity. In contrast pepsin secretion was elevated markedly on feeding.

With 10 ml infusions of oleic acid intraduodenally, motility and secretion remained inhibited for 7 - 9 hr; the volume of secretion was reduced for about 5 - 6 hr to the extent of 90% (0.2 - 3 ml) and acidity for 2 - 3 hr to the extent of 95 - 98% (2 - 4 m-equiv H^+/litre) of the resting levels - see Fig. 14.

Motility on its return from the inhibition accorded by the intraduodenal infusions of oleic acid exhibited after-effects of the inhibition in that:

1. contractions of the ventral rumen were absent or much reduced in force in the 'A' sequences for up to 30 min after reticulum contractions had returned.
2. contractions of the reticulum of a diphasic and monophasic nature occurred between the triphasic contractions associated with the regurgitation of rumination (Fig. 15).
3. contractions of the reticulum frequently occurred on top of a prevailing level of sustained reticulum tonus during the first period of rumination observed after the infusion.
4. contractions of the reticulum showed an exaggerated relaxation between the 2nd and 3rd phases of the triphasic contraction.
5. contractions of the reticulum associated with regurgitation were increased in number as shown by up to 29 "triphasic" contractions of the reticulum per 15 min in comparison with the usual 14 - 18 contractions per 15 min during periods of rumination (Fig. 15).

An increase in the volume and acidity of the abomasal secretion preceded the resumption of feeding or rumination and anything approaching



Volume as above 6.0, 4.0, 1.1, 1.2, 0.75, 1.25, 0.65, 3.3, 2.1, 1.45, 1.35, 1.05, 1.35

Fig. 13: The effects of an intraduodenal infusion of 10 ml oleic acid over 30 min on reticulo-rumenal motility and abomasal pouch secretion. Records from above downwards: jaw movements, contractions of the main ventral rumen, "infusion of oleic acid", contractions of the cranial dorsal rumen, contractions of the reticulum, Volume in ml of 15 min samples of abomasal pouch secretion, signal for the change in collection of samples of abomasal pouch secretion at 15 min intervals, 60 sec time-marker. Note the marked inhibition on motility and volume of secretion which was reversed by preparation (P.F.) to and actual feeding (F.). Feeding did not increase the acid concentration of the secretion (see Table 3 exp 27/6, #15).

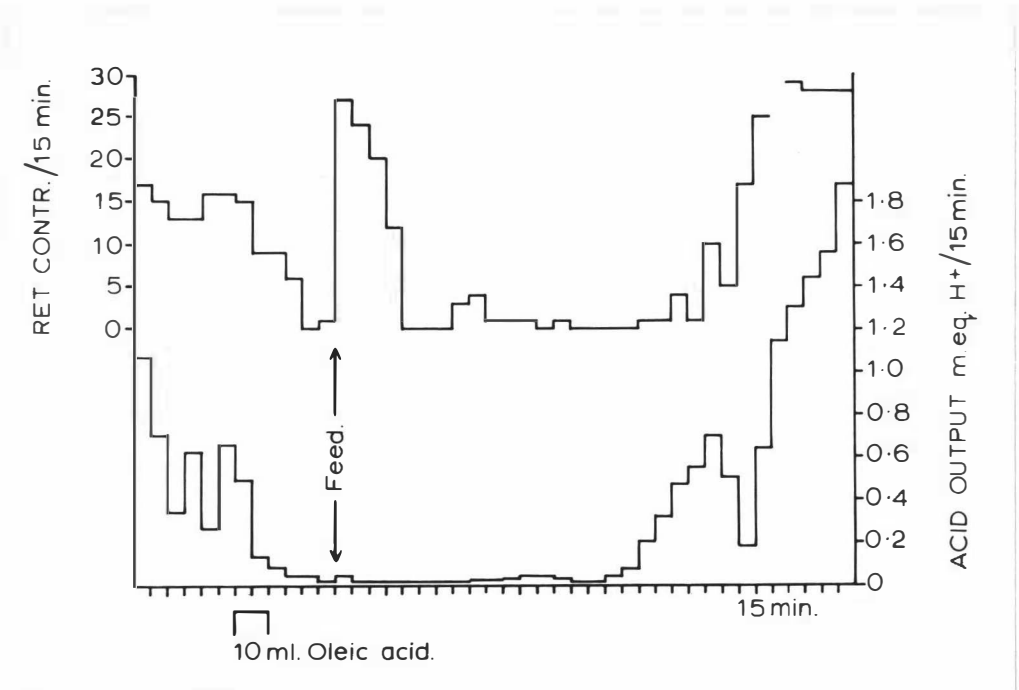


Fig. 14: The effects of 10 ml oleic acid infused over 30 min into the duodenum of a sheep which had been fasted for 12 hr. The sheep was fed 90 min after the infusion commenced; it ate for about 50 min. The oleic acid infusion inhibited reticulum contractions and acid output. On feeding there was a temporary reversal of the effect on reticulum contractions but no such effect on acid output. Graph from above downwards: reticulum contractions/15 min, acid output from the pouch in m-equiv H^+/15 min, 15 min time intervals.

normal motility of the reticulum and rumen. As movements of the reticulum and rumen approached normal frequencies, the secretion from the pouches quickly rose even higher in volume and acidity and thus to pre-infusion levels. During the maximum period of inhibition of secretion the sodium concentration increased to around 140 m-equiv/litre, while that of potassium fell to 1 m-equiv/litre. The return of acidity was characterised by an unusually high K^+ concentration (e.g. 16 m-equiv/litre).

In one experiment undertaken in an animal from which food had been removed and in which motility of the reticulum and rumen and secretion from the abomasal pouch were completely inhibited by the intraduodenal infusion of 10 ml oleic acid, the animal was teased with food. The sheep appeared to show no interest in the feed, and no change of reticulo-rumenal motility was detected, but the volume of secretion doubled and the acid output in successive 15 min periods increased over six-fold from 0.012 to 0.078 m-equiv H^+/15 min. The period of teasing appeared to stimulate recovery from the oleic acid induced inhibition, as after teasing with food the volume and acidity of the pouch secretion steadily increased until normal levels were eventually attained.

In a similar experiment, various substances were used in an endeavour to overcome the inhibition of secretion and motility. Infusions into the abomasal pouch of 20 ml of saline at 37°C and 20 ml of 0.15N HCl at 37°C were without effect on motility. Infusions into the pouch of 0.5 mg methacholine chloride (Amechol, S.A. Moore Ltd., London) in 20 ml saline at 37°C, 1.0 mg Amechol in 5 ml of saline and 0.1 mg histamine acid phosphate in 10 ml of saline had no demonstrable effect on motility or secretion. All of these doses may be considered as being too small (compared with those found efficacious in other species by other workers). Distension of the pouch with 130 ml of acid secretion failed to stimulate reticulum contractions.

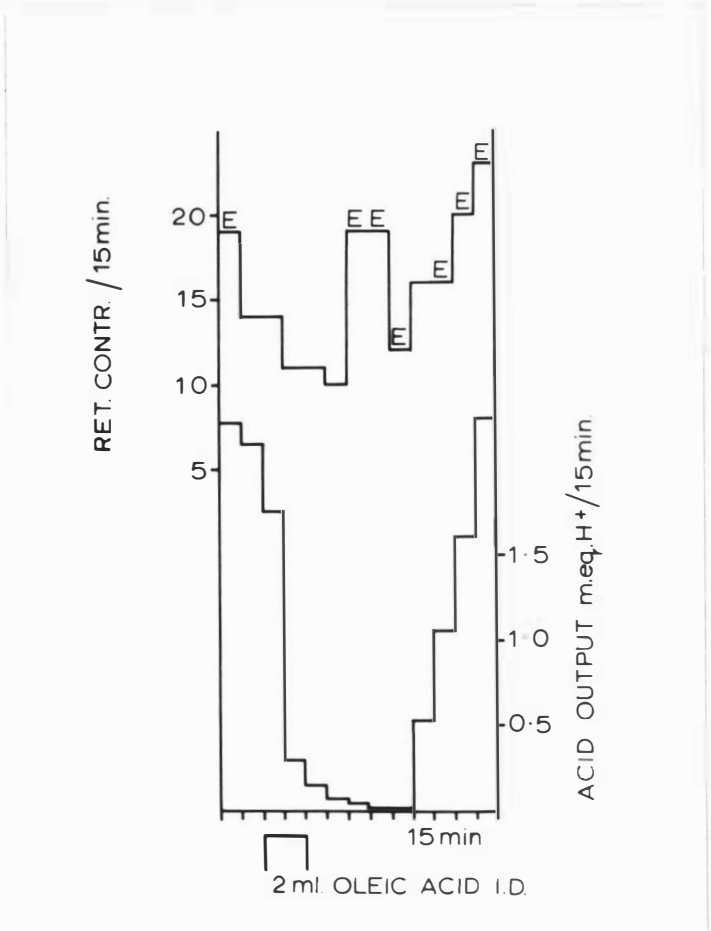


Fig. 16: The effects of an intraduodenal infusion of 2 ml oleic acid over 30 min in a sheep fed ad lib. An inhibition of pouch secretion was evident within 30 min. Graph from above downwards: periods in which the animal was eating (E), reticulum contractions/15 min, acid output from the pouch in m-equiv H^+/15 min, 15 min time intervals, time of oleic acid infusion. Note the increase in reticulum contractions when the animal first ate after the infusion had been completed, and the absence of a similarly immediate secretory response on eating.

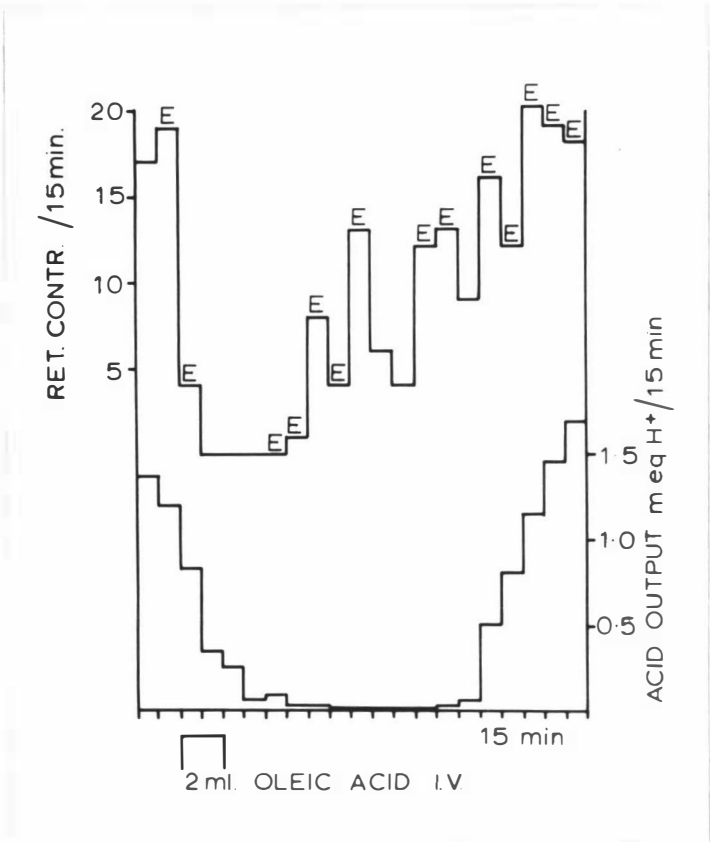


Fig. 17: The effects of an intravenous infusion over 30 min of 2 ml oleic acid in a sheep fed ad lib. Graph from above downwards: periods during which the animal ate (E), reticulum contractions/15 min, acid output from the pouch in m-equiv H^+/15 min, 15 min time intervals. Note the rapidity with which the maximum inhibitory effects became apparent in the case of the reticulum contractions compared with the effects on gastric secretion. The oleic acid infusion was commenced during the most vigorous period of eating of the day: the cessation of eating for 45 min (15 min during and 30 min after the infusion) is an effect which can be ascribed to the infusion.

0.25 mg histamine acid phosphate given subcutaneously produced no change in secretion or motility. In contrast 0.5 mg histamine administered intravenously over 30 min (0.8 µg/kg/min) resulted in a burst of secretion 15 min after the infusion commenced. The acid concentration of this secretion increased markedly from 2 - 88 m-equiv H^+/litre. If further increased to 100 m-equiv H^+/litre, although the volume of secretion had dropped to the pre-histamine levels. The increase in the volume and acidity of the pouch secretion was transient; the volume increase lasted for only 15 min and the acid concentration for 30 min. There were no effects on reticulum and rumen motility comparable to those on acid secretion. The acid concentration of the secretion was subsequently maintained at a level greater than that which had been present prior to the administration of histamine.

A comparison of the intraduodenal and intravenous administration of oleic acid:

An infusion of 2 ml oleic acid into the duodenum over 30 min (0.003 ml/kg/min) resulted in a marked drop in the volume of and concentration of acid in pouch secretion within 30 min of the start of the infusion. An 80% inhibition of acid secretion persisted for about 90 min (Fig. 16). In contrast to infusions of larger doses of oleic acid the pepsin concentration in the secretion from the abomasal pouches was not greatly affected. Reticulum and rumen motility slowed slightly despite continued feeding, and declined markedly if there was a cessation of eating. In other experiments where feeding did not occur during and after the infusion, little effect on motility was evident, (see Table 4).

An infusion of 2 ml oleic acid into the jugular vein over 30 min (0.003 ml/kg/min) led to a reduction in the volume of pouch secretion within 15 min of first entering the blood stream (Fig. 17). An 80% inhibition of the acid secretion lasted for 3 hr. The acid concentration of the secretion

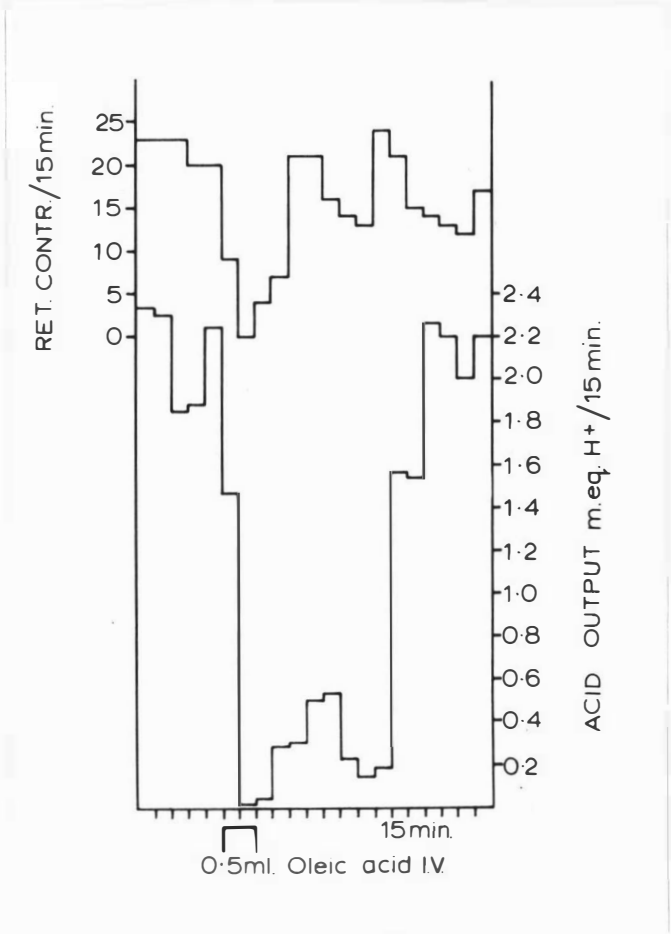


Fig. 18: The effects of the intravenous infusion of 0.5 ml oleic acid over 30 min. The infusion was made into the jugular vein of a sheep fed on an ad lib basis. Graph from above downwards: reticulum contractions/15 min, acid output from the pouch in m-equiv H^+/15 min, 15 min time intervals. Note the marked inhibition of reticulum contractions (culminating in their disappearance for a period of 15 min) and the inhibitions of acid output from the pouch.

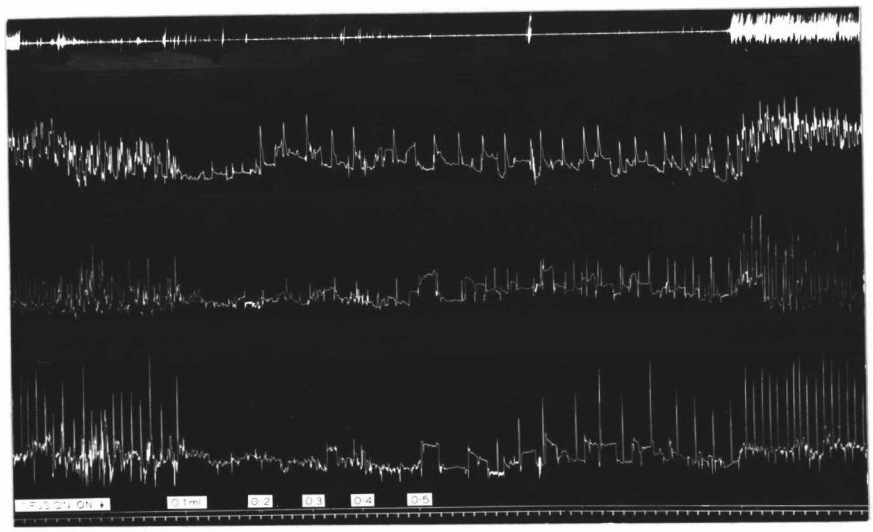


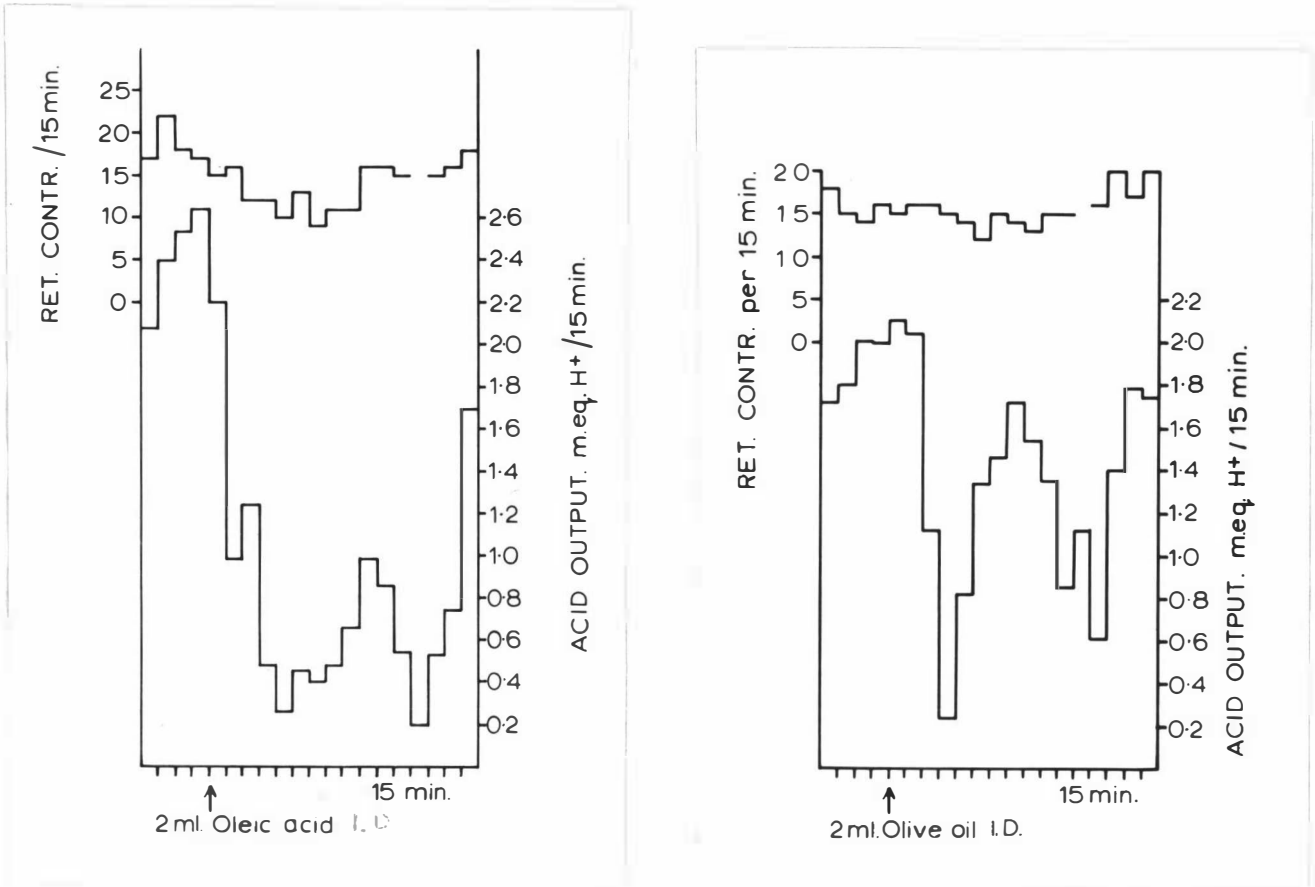
Fig. 19: The inhibitory effects of an intravenous infusion of 0.5 ml oleic acid over 30 min in a sheep fed ad lib. Records from above downwards: jaw movements, contractions of the main ventral rumen, contractions of the cranial dorsal rumen, contractions of the reticulum, quantities of oleic acid infused, signal for the change in collection of samples of abomasal pouch secretion at 15 min intervals, 60 sec time-marker. Note the persistence of 'B' sequences of contraction of the rumen during the inhibition.

decreased slowly to a minimum of 2 m-equiv H^+/litre (from the original 130 m-equiv H^+/litre) 2 hours after the infusion started. This level was maintained for 75 min; thereafter the acid concentration slowly increased as the volume of secretion increased. There was a return to the pre-infusion levels about 4 hours later (Fig. 17). Pepsin output from the pouches was significantly depressed, but the pepsin concentration was not reduced, in contrast to reductions observed after the intraduodenal infusions at higher dose rates (0.016 ml/kg/min).

In the experiment shown as Fig. 17, reticulum and rumen contractions were totally absent for a period of 60 min. The onset of this inhibition occurred within 4 min and when 0.3 ml of the oleic acid had been infused. At one stage despite short periods of eating, no reticulum contractions occurred. After 2 - 3 hours groups of contractions of the reticulum and rumen followed each period of eating. Normal motility did not return until 6 hr after the infusion (see Table 7).

The intravenous infusion of 0.5 ml oleic acid over 30 min (0.0008 ml/kg/min) caused after 15 min an almost complete inhibition of secretion from the pouch. After a further 15 min of this profound inhibition the volume of secretion slowly rose over 2 hr to normal levels. 45 min after the start of the infusion a drop in the acid concentration of the secretion from the pouch was evident: this drop in acid concentration became more marked in subsequent samples (see table 7, exp. 4/10). The acid output of the pouch reached pre-infusion levels $2\frac{1}{2}$ hr after the infusion ended (Fig. 18). No definite effects on the pepsin concentration of the secretion were evident but pepsin output was significantly depressed for about 2 hr.

Within 8 min of the start of the infusion (when 0.1 ml of oleic acid had been infused) a complete inhibition of reticulum movements was observed, this continued for 30 min (Fig. 19). A few rumen contractions ('B' sequences)



(a) (b)

Fig. 20: A comparison of the effects of the intraduodenal infusion over 20 min of 2 ml oleic acid (a) and olive oil (b) in the same sheep fed ad lib. Graph from above downwards in each figure: reticulum contractions/15 min, acid output from the pouch in m-equiv H^+/15 min, 15 min time intervals, substance infused into the duodenum. Note the longer latency, lesser intensity and shorter duration of the inhibition produced by olive oil.

persisted. Reticulum and rumen motility returned and the frequencies of these contractions were normal within 75 min of the start of the infusion (Fig. 19).

Intraduodenal infusion of olive oil:

2 ml of olive oil was infused into the duodenum over 20 min (0.005 ml/kg/min) at a time when the animal was secreting at a high level in response to the daily feed. The acid output from the abomasal pouch was not inhibited within 30 min of the start of the infusion: after this time there was a reduction in acid output from the pouch which lasted for 75 - 90 min. No inhibitory effects on pepsin secretion from the abomasal pouch, or on movements of the reticulum and rumen were detected.

An increase in acid output, subsequently followed by another depression in acid output occurred in some cases during the "inhibitory" period. This form of response was observed with infusions of both olive oil and oleic acid (See Fig. 20, and Tables 5 and 6).

A comparison between the effects in the one animal of 2 ml infusions of oleic acid and olive oil into the duodenum over 20 min has been made (Fig.20) and the results are summarised in the following tabular form:

	Latency of Response	Duration of inhibition of acid output	Depth of inhibition	Motility of reticulum and rumen
Olive oil	30 min or more	75 min	Maximum inhibition of acid output > 90%	No definite effect
Oleic acid	15 - 30 min or less	225 min	Maximum inhibition of acid output > 90%	Slowing in frequency of 'A' sequences of reticulum and rumen contractions

Intraduodenal infusions of oleic acid, linoleic acid and linolenic acid:

A comparison was made of the effects produced by 2 ml infusions into

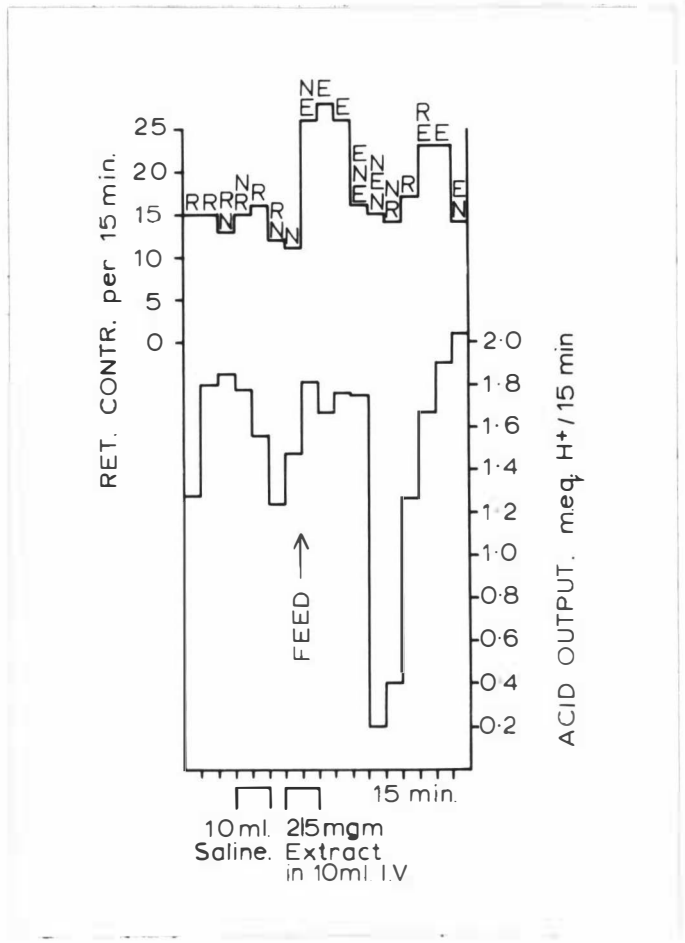


Fig. 21: The intravenous infusion of an enterogastrone extract in a fed sheep. Feed had been removed just before the experiment commenced; new feed was offered 15 min after the infusion commenced. A control infusion of saline had been given. Graph from above downwards: feeding activity of the animal (N = nothing or no feeding or ruminating activity; R = rumination; E = eating), reticulum contractions/15 min, acid output from the pouch in m-equiv H^+/15 min time intervals, time and dose of substances infused intravenously. Note the delayed effect of the extract on acid output from the pouch.

the duodenum over 20 min (0.005 ml/kg/min) of oleic, linoleic and linolenic acids. Although no differential effect could be distinguished in their actions on abomasal acid secretion, a definite relationship between the degree of unsaturation of the fatty acid and the inhibition of reticulum and associated rumen motility was evident. The higher the unsaturation of the fatty acid, the greater appeared to be the inhibition of motility. Further experiments are required to allow a more precise statement to be made.

The intravenous infusion of enterogastrone extracts:

The intravenous infusion of 50 mg of enterogastrone extract over 30 min (0.083 mg/kg/min) in sheep, caused an inhibition of abomasal pouch secretion and a reduction in the frequency of reticulum and rumen movements. No consistent effects were observed as the degree and duration of the inhibition of both pouch secretion and reticulo-rumenal motility varied with different enterogastrone preparations, some of which failed altogether to exhibit inhibitory properties.

In two experimental investigations undertaken before the morning change in feed 50 mg and 215 mg of enterogastrone extract in 10 ml of saline was infused intravenously over 30 min (0.083 mg/kg/min and 0.358 mg/kg/min respectively). With the larger dose after 15 min of the infusion (110 mg), the frequency of reticulum and associated rumen movements was slightly reduced, and secretion from the abomasal pouch was slightly increased. New food offered midway through the infusion was taken and an increase in reticulum and rumen motility and abomasal pouch secretion followed.

The cessation of feeding (130 g feed consumed) after 60 min was accompanied by a transient period of irregular motility of the reticulum and rumen, and a sudden decrease in both the volume and acidity of secretion (Fig. 21). The return to normal motility was almost immediate but

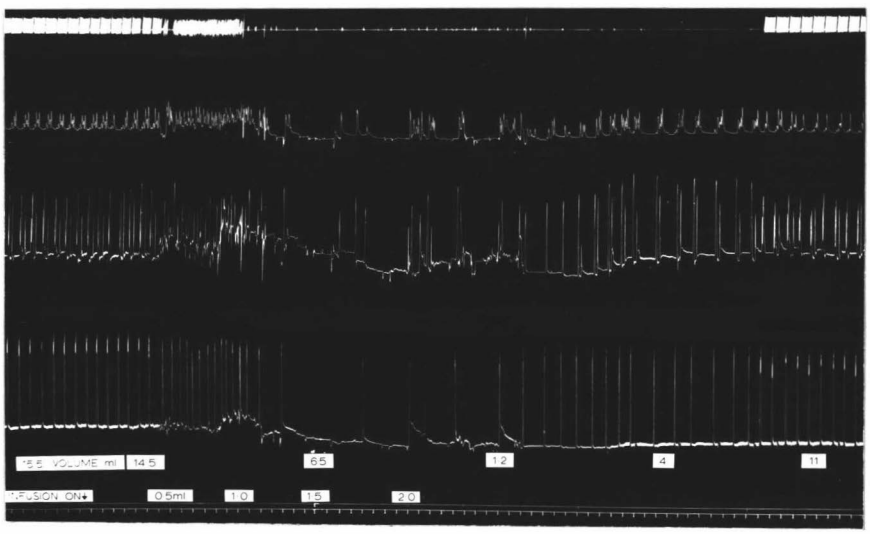
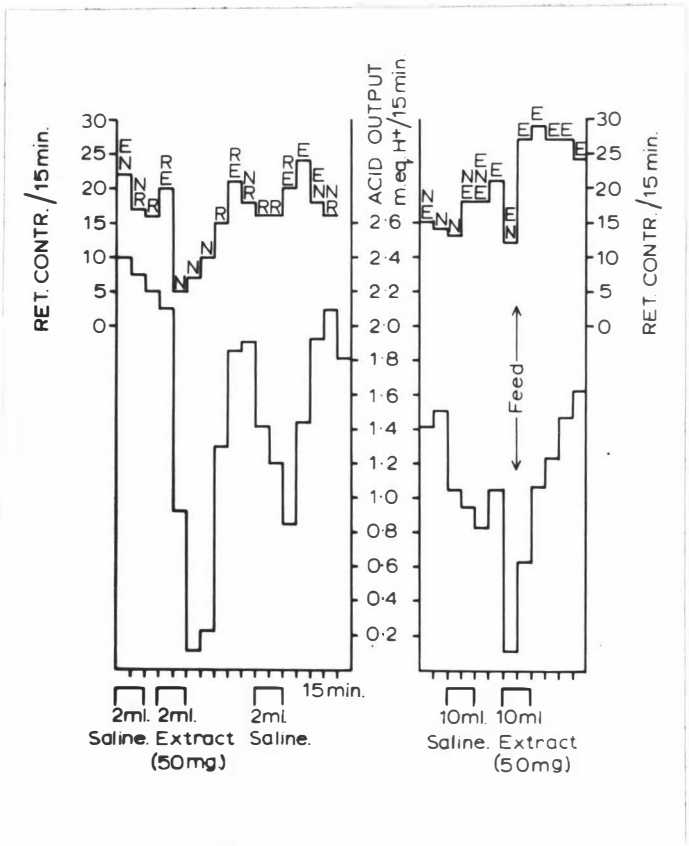


Fig. 22: The inhibitory effects on reticulum and rumen motility and abomasal pouch secretion of the intravenous infusion over 30 min of 50 mg of an enterogastrone extract in 2 ml saline. Records from above downwards: jaw movements, contractions of the main ventral rumen, contractions of the cranial dorsal rumen, contractions of the reticulum, Volume in ml of 15 min samples of abomasal pouch secretion, volumes of the infusion of the enterogastrone solution (0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml), signal for the change in collection of samples of abomasal pouch secretion at 15 min intervals, 60 sec time-marker. Note the inhibition of food intake, reticulo-rumenal motility and abomasal pouch secretion within 20 min of the start of the infusion.



(a) (b)

Fig. 23: Effects on the frequency of contractions of the reticulum and acid output from a pouch of intravenous infusions of extracts and enterogastrone. The sequence of administration (by intravenous infusion in a jugular vein) of control volumes of saline and of the two extracts tested is shown at the bottom of the graphs. Graphs from above downwards: feeding activity (N = no activity; R = rumination; E = eating), reticulum contractions/15 min, acid output from the pouch in m-equiv H^+/15 min, 15 min time intervals.

secretion remained inhibited for 30 min, see Table 8.

In the second study, a different batch of enterogastrone was used. Within 15 min of the start of the infusion of 25 mg of the extract eating was inhibited, the frequency of reticulum and associated rumen contractions slowed, and a marked inhibition of the output of secretion from the pouch occurred (Fig. 23b). Acid concentration was somewhat slower in being reduced by the extract. Consumption of the new feed offered midway through the infusion provided a stimulus of sufficient strength to overcome the inhibition of both secretion and motility (Fig. 23b). Feeding ceased when the animal had ingested 200 g of the red clover chaff.

In another study with a third batch of an enterogastrone preparation, the infusion was given in the early afternoon 4 - 5 hr after the animal's feed had been changed. 50 mg of enterogastrone extract in 2 ml of saline was administered over 30 min (0.083 mg/kg/min). At the commencement of the infusion the animal was ruminating and subsequently began to feed. Feeding ceased after the introduction of 25 mg of the preparation. Reticulum and associated rumen movements ('A' sequences) were markedly inhibited for 25 min (Fig. 22). Movements of the rumen alone ('B' sequences) were affected to a lesser degree. In those 'A' sequences which did occur there were characteristically weak contractions of the main ventral rumen. A partial marked inhibition of the frequency of reticulum and rumen contractions persisted for a further 20 min until rumination occurred (Fig. 22).

After the administration of 50 mg of extract over 30 min the volume of the pouch secretion dropped to about 50% of its original level, but no change in acid concentration was detected. 15 min later the volume of secretion fell to 10% of the resting level, while acid concentration decreased to 40% of its resting level. Acid output from the abomasal pouch was reduced for about 50 min (Fig. 23a).

In the experiments reported pepsin output from the pouch decreased during and following the infusion of enterogastrone, the fall in output was related to the decrease in volume of secretion, not to a decrease in pepsin concentration. In one experiment the intravenous infusion of enterogastrone extract was followed by a definite lowering of feed intake. Normally when feed was changed at 9.30 a.m. the animal consumed 250 - 300 g of the chaff. After 215 mg of extract in one experiment it was reduced to 130 g and in another after 50 mg of extract 220 g of feed was eaten in a comparable period during and after the infusion.

Extracts of enterogastrone prepared by the method of Greengard et al (1946) were on occasions obtained in the form of a brown gum, in contrast to the usual white powder. Upon intravenous administration this amorphous material exhibited no inhibitory effects upon secretion and motility.

Discussion:

There have been no reports of studies recording simultaneously the effects of intraduodenal infusions of fat on both reticulum and rumen motility and abomasal acid secretion. Other effects of the intraduodenal administration of fat and other substances have been studied and reported. Hill (1965) described changes in acidity of abomasal secretion and the flow of digesta from the abomasum resulting from the addition of triglyceride to the diet. Singleton (1951) infused carbohydrate, protein and fat into the duodenum and reported effects on abomasal motility. Phillips (1965) reported experiments in which the infusion of C18 unsaturated fatty acids into the duodenum caused diarrhoea and inappetence. Titchen, Reid and Vlieg (1966) investigated the effects of intraduodenal infusions of fat on the food intake of sheep. Movements of the reticulum and rumen were recorded from partial exteriorizations of these regions.

In the experiments discussed here, the intraduodenal infusions of oleic acid in the higher dose range led to a definite inhibition of abomasal secretion, reticulum and rumen motility, and food intake. Abomasal pouch secretion was usually inhibited before reticulo-rumenal motility, but in some cases a decrease in the frequency of reticulum and rumen contractions preceded a lowering in acid output from the pouch. The short latency before secretion fell, however, made it unlikely the effect was a direct result of the decreased frequency of stomach movements. The inhibitory effect on abomasal pouch secretion exhibited a short latency even with high levels of secretion and was manifest as a reduction of volume, acid and pepsin output from the pouch. Presumably higher levels of secretion are associated with stronger stimuli to secretion. Abomasal acid secretion was more susceptible to the inhibition in the early stages of feeding or of the infusion than was reticulo-rumenal motility. Feeding soon after the

infusion provided a stimulus of sufficient strength to overcome the inhibitory influence on movements of the reticulum and rumen but not the inhibition of pouch secretion. As the inhibitory effect waned an increase in acid secretion from the pouch preceded the return of normal movements of the reticulum and rumen. Smaller doses of oleic acid produced marked reductions in acid secretion from the pouch but had little detectable effect on motility of the reticulum and rumen. This may be taken to suggest that it is unlikely that the inhibition of abomasal acid secretion and reticulum and rumen motility are necessarily brought about by the same mechanism.

The magnitude of the response to the intravenous infusion of oleic acid in the form of the free fatty acid was most marked. Movements of the reticulum and rumen were completely inhibited within 4 min (0.3 ml) of the start of the infusion. An immediate and complete reduction in the delivery of the abomasal secretion, but a maintained acidity which occurred in one experiment suggested an inhibition of pouch motility may have preceded that of its secretion. A visible side effect of the intravenous fatty acid infusions was transient "snuffing" by the animals. This may have been a reaction to the formation of pulmonary fat emboli; if it was, it was short lived (20 min). This type of experiment must be regarded as of a preliminary nature - the intravenous administration of fatty acids or of oils in an appropriate form which does not cause pulmonary or other side effects is a necessary adjunct to the present experiments. In man emulsions of fat have been formulated which possess minimum side effects and allow their introduction intravenously to provide a high caloric intake in a minimum amount of fluid.

In contrast to the situation in the simpler stomached animal, in the

ruminant at least a very high proportion of the dietary fat is modified before reaching the duodenum. In the rumen, ingested triglycerides are hydrolysed and the resultant unsaturated fatty acids hydrogenated (hydrogenation may occur before hydrolysis). The principal fatty acid entering the duodenum of the ruminant is the saturated C18 acid (stearic acid), although a small quantity of C18 unsaturated fatty acids also occurs (Ward, Scott and Dawson, 1963). The effects resulting from the introduction of different fatty acids in the ruminant should not be presumed to be the same as in simple stomached animals.

Two major theories have been proposed as to the absorption of fat from the intestine. Verzár (1936) in his Lipolytic Hypothesis modified an even older theory of Pflüger and suggested that fat was split in the lumen of the intestine by lipase to liberate glycerol and the constituent free fatty acids. These fatty acids were "solubilised" in an aqueous phase by bile and absorption took place from this phase. Frazer (1946) contested this hypothesis and proposed that triglyceride or neutral fat was not completely hydrolysed to glycerol and fatty acids. His theory was derived from the observation that if triglyceride was emulsified to a particle size of less than 0.5 μ, absorption of the triglyceride was possible without prior hydrolysis. Frazer suggested neutral triglyceride was hydrolysed to a mixture of mono, di and triglycerides and free fatty acids. The glycerides were absorbed into the lacteals and eventually travelled into the thoracic duct lymph. The free fatty acids passed directly into the portal blood stream and were taken up by the liver.

Subsequent investigations have supported both theories in part. Hoffman (1966) has discussed a physico-chemical approach to the intraluminal

phase of fat absorption. He stressed the solubility of the hydrolytic products in the bile salt micelle and indicated much more lipid should be absorbed in the form of monoglyceride and fatty acid than as diglyceride and triglyceride. Electron microscopic studies of triglyceride absorption by pinocytosis have been provided by Palay and Karlin (1959). Rubin (1966) however, advanced the view that there is little basis for most of the mechanisms proposed for the process of triglyceride absorption.

The route of fat absorption at any time has been shown to depend on conditions existing in the small intestine. Wiseman (1964) has reviewed the process of absorption of fat from the intestine. Evidence has accumulated that the absorption of long chain free fatty accids or their triglycerides occurs almost entirely via the lymph (Wiseman p 134). Short chain fatty acids are absorbed mainly via the portal blood and exist in the free form. Fatty acids of 10 carbon atoms or less do not appear to travel via the lymph in any great amount, whereas those of longer chain length do. This partitioning between portal and lymphatic routes of absorption can be attributed to the greater permeability of lymphatic capillaries. Borgström (1953) noted that in the absence of bile from the intestine only one quarter of the absorbed palmitic acid was transported in the lymphatics. This led him to suggest the existence of an alternative pathway of fat absorption. This suggestion was supported by Saunders and Dawson (1963) who fed C^{14} oleic acid to rats with biliary fistulae and thoracic duct fistulae. Only 7% of the oleic acid absorbed was recovered in the lymph and 28% of the lymph lipid radioactivity was free fatty acid. Attempts to correct the defects of absorption in rats deprived of bile by giving the oleic acid emulsified in taurocholate, in polyoxy-ethylene sorbitan monolaurate (Tween 20, an emulsifying agent) or as sonicated emulsions (emulsified in an ultrasonic disintegrator without the aid of chemical emulsifying agents)

were made. All three treatments led to the appearance of an increased proportion of the fatty acid in the lymph. Tween 20 and the sonicated emulsions did not correct the deficiency in esterification as shown by the high percentage of oleic acid in the free fatty acid form in the lymph. Taurocholate in a concentration similar to that found in the intestinal lumen partially corrected the deficiency in the esterification of oleic acid.

The importance of the particle size of the digestion products in the lumen of the intestine in determining the ultimate fate of these substances has been indicated. Rubin (1966) has reviewed electron microscopic studies of triglyceride absorption in man. He has discussed the confusion which has arisen over the term pinocytosis and has suggested this process is best expressed by the recognition of three classes of pinocytosis as proposed by Fawcett (1965). In this concept macropinocytosis has been defined as a process that is sufficiently gross to be resolved by light microscopy. Smaller invaginations which may only be seen by electron microscopy have been designated as occurring by micropinocytosis. This latter process has been further divided into two types; one involving smooth surfaced thin membranes, the other involving heavier membranes with specialized coatings. Rubin has reported that accumulating evidence suggests the heavier "fuzzy" membranes have properties for selective binding of particular classes of ions or molecules which can then be absorbed by micro-pinocytosis. Absorbed digestion products have been shown to enter the intestinal columnar cell in different ways. Rubin (1966) has provided evidence for the micropinocytotic entry of protein into the intestinal cells of new born suckling puppies. In puppies fed whole protein (colostrum) and corn oil simultaneously, the pinocytotic entry of whole protein was reported to be demonstrated, but no clear morphological evidence of pino-

cytosis of fat was evident. The membrane of the invagination thought to be associated with the entrance of the protein was thicker than the thin membranes surrounding fat particles. Shiner (1966) has submitted evidence to show that fat droplets may enter the intestinal cell through the membranes of the microvilli without the requirement of a pinocytotic mechanism. Droplets of 250Å diameter were observed close to the inner of the double membranes surrounding the microvilli. In contrast droplets absorbed by pinocytosis between the microvilli were of about 500Å diameter. In a comparison between the intra-microvillous transport and the pinocytotic entry of fat, Shiner suggested that the process of intramicrovillous transport, by the great area available for absorption was compatible with the observed speed with which fat enters the epithelial cell.

Saunders and Dawson (1963) have discussed the effects on the metabolism of fatty acids entering the intestinal cell by pinocytosis. Fat globules entering the cell in this way were surrounded by a membrane of the endoplasmic reticulum which contains many of the enzymes necessary for triglyceride synthesis. It was thought that fatty acids entering the cell by pinocytosis may be converted to triglycerides while those entering by other pathways may remain unesterified.

It would appear from the results cited that fat and other digestion products may be absorbed into the intestinal cell in a variety of ways; the mode of absorption probably determines to some extent the immediate environment into which the absorbed substances come into contact and this in turn could affect the subsequent metabolism of an absorbed digestion product in the intestinal cell. The route taken by the material absorbed from the lumen of the intestine probably reflects the form and particle size (molecular or otherwise) in which it is discharged by the cell. In an animal subjected to abnormal amounts of fat in the intestine, pathways of fat

absorption not usually of any consequence in the transport of digestion products from the lumen of the intestine may become of moment. For example it seems probable, although no definite evidence has been provided, that in the normal animal a small amount of long chain fatty acids pass into the portal vein and hence into the general circulation (unless they are completely metabolised by the liver). This pathway could conceivable become of greater importance when large amounts of fat were present in the intestine.

The relationship between fat absorption and the inhibitory effect of fat on gastric secretion and motility is emphasized when one considers the profound influence that conditions in the duodenum exert on this inhibitory action; of particular importance are the pancreatic lipolytic enzyme lipase, and bile. Heath and Morris (1962, 1963) have shown that the absorption of fat, and the role of bile and pancreatic juice in the absorption of fat in sheep and lambs is essentially the same as that which occurs in simple stomached animals. Menguy (1959) analysed some of the inhibitory effects on gastric secretion in the intact rat by the introduction of fat into the small intestine. This inhibitory activity was decreased when bile was diverted from the small intestine. When bile salts were administered, the same degree of inhibition as in the intact rat occurred. In previous experiments, (Menguy and Koger, 1959) it had been found that the parenteral administration of bile salts inhibited gastric secretion in rats; this same dose intraduodenally failed to alter gastric secretion. Unpublished results quoted, showed that oleic acid intraduodenally was not as effective as olive oil in inhibiting acid secretion in the rat. Menguy (1960) in further studies in rats found that the introduction of olive oil into the duodenum suppressed gastric motility. In rats in which the bile was kept out of the small intestine by "shunting" it to the rectum, the same amount of oil consistently failed to alter gastric motility. When the oil was

mixed with bile salts, complete and immediate suppression of gastric motility occurred. In rats with diversion of both pancreatic and biliary secretions, gastric motility was unaltered by either oil alone or oil mixed with bile salts. Oleic acid failed to alter gastric motility in "bile shunted" rats. In a study of fat absorption in rats deprived of their exocrine pancreatic secretions, Masarei and Simmonds (1966) observed that virtually no triglyceride was absorbed in the absence of both bile and pancreatic juice. The triglyceride had been administered as a fine emulsion with most of the particles less than 0.3 μ in diameter, and this was taken to suggest that the experiments provided strong evidence against the absorption of triglyceride by pinocytosis. In contrast the absorption of finely emulsified fatty acid was not significantly decreased in pancreatic fistula animals (lipase and bile absent). These workers concluded that bile or pancreatic juices are essential for the absorption of triglyceride from the small intestine, but not for the absorption of fatty acid.

Kern and Borgström (1965) have discussed the effect of a conjugated bile salt on oleic acid absorption in the rat. They state that conjugated bile salts participate in the intestinal absorption of fatty acid and suggest they do by the following mechanisms:

1. the "solubilisation" of fatty acids and monoglycerides within the lumen of the intestine by the formation of micelles; it is in this form that the lipids are thought to be absorbed normally.
2. within the intestinal epithelial cell they facilitate the synthesis of triglyceride and stimulate the incorporation of glucose and glycerol into lipid.
3. they exert a poorly understood and controversial effect upon gastrointestinal motility.

Kern and Borgström (1965) showed that oleic acid is very slowly

absorbed by rats with biliary fistulae; when a pure conjugated bile salt was fed simultaneously with oleic acid the rate of absorption of the oleic acid was increased. They suggested that their data indicated bile salts increased the rate of gastric emptying, but no conclusive evidence of this was obtained. The addition of bile salts to the diet increased the percentage of the labelled oleic acid (used to detect the fate of oleic acid) in the triglyceride fraction extracted from the small intestinal mucosa of biliary fistula rats. This effect of bile was not present in control operated rats. The rate of absorption of oleic acid seemed to be dependent upon the amount of bile salt present. Bile salt in excess of the amount usually present did not increase the percentage of labelled oleic acid in triglyceride in the mucosa. It was suggested that excess bile salt increased absorption by an intraluminal effect, perhaps by leading to an increased micellar solubilisation.

The presence of bile and lipase in the intestine is probably of importance in determining the osmolality of the intestinal contents. The possibility of the existence of a mechanism sensitive to the osmolality of the intestinal contents which initiates an inhibition of gastric secretion and motility has been proposed (Hunt 1956, Sircus 1958). Sircus (1958) showed in dogs that hypertonic saline, glucose, fructose, polysaccharide complexes and peptone inhibited gastric secretion when introduced into the duodenum in concentrations so as to raise the osmolality beyond certain levels. There was a notable similarity of inhibition produced by these different substances in different pouch and duodenal preparations in respect of latent period, duration of action and degree of effect. This suggested the possibility that a single common osmoreceptor mechanism operates in the small intestine. The osmoreceptor was postulated to act by the release of an inhibitory agent or perhaps by interfering with the vasculature of the

gastric mucosa. Support for the latter view has been provided by Menguy. He cited unpublished results (Menguy 1960) in which it was found that an inhibition of the gastric secretory activity produced by fat in the intestine was accompanied by a decrease in blood flow to the fundus of the stomach.

It seems probable that both the osmotic and pH sensitivity (Hunt 1956, Sircus 1958) and the capacity to respond to these forms of stimulation are present in the duodenum. Fat, however, is effective in causing an inhibition even when instilled into the jejunum (Sircus 1958).

Although the absorption of fat from the intestine has been definitely implicated in the response of gastric secretion and motility, the exact mechanism of the inhibition has not been established. Since both secretion and motility have been inhibited in transplanted pouches, a humoral agent has been generally accepted to be responsible for the inhibitory actions. Feng, Hou and Lim (1929) concluded that fat per se was not the inhibitory agent responsible for the reduction in secretion from autotransplanted pouches. Intravenously administered thoracic duct lymph collected before and after a fat meal was without effect on secretion. Defatted lymph (ether extract) appeared to cause a slight stimulation of secretion. The experimental details provided are meagre and it is difficult to regard their experiments as providing a final answer on the problem. Quigley, Zettleman and Ivy (1934) analysed the factors involved in gastric motor inhibition by fats. Their results showed that no gastric motor inhibition followed the intravenous injection of emulsified fat (egg yolk) or the products of fat digestion (soap, glycerine and intestinal lymph). However, after the introduction of egg yolk the dogs still exhibited a voracious appetite despite the existing lipaemia. This suggests the fat was not in a suitable physiological form to produce motor inhibition, as satiation would be expected to have occurred. 100 mg sodium oleate in 10 cc of saline administered intravenously failed

to inhibit motility. An olive oil emulsion was tried but proved too toxic for intravenous administration. It appears unlikely from these studies that thoracic duct lymph contains the inhibitory principle. Clear experimental evidence is lacking that an absorbed digestion product of fat serves as an inhibitory factor. However, in the present undertaking, the inhibition of reticulum and rumen motility resulting from the intravenous infusions of oleic acid, suggests that the entrance of long chain free fatty acids (abbreviated below to LCFA) into the general circulation (either via the lymphatic or portal route) could be the inhibitory mechanism exerted by the presence of fat in the duodenum. The decrease in the esterification of LCFA by the intestinal mucosa in the absence of bile results in a higher level of blood LCFA in the free form (Saunders and Dawson 1963). In the normal animal sudden loading of the intestine could conceivably cause (due to a temporary deficiency of bile) an elevated blood FFA level both from lymphatic and portal vein transport. The long duration of an inhibition induced with the larger doses of oleic acid (10 ml) used in the present experiments was probably due to stasis of the gut contents. During such an inhibition, reticulum and associated rumen movements ('A' sequences) were affected to a greater degree than rumen movements alone ('B' sequences). A number of 'B' sequences (which are usually associated with eructation), frequently occurred in groups when the animals stood. This event could have been the result of reflex stimulation of rumen contractions by the exposure of receptors to gas. Reid (1962) has reported that the insufflation of gas into the reticulum and rumen resulted in a stimulation of 'B' sequence contractions.

In studies on the parental administration of fat emulsions in the rat, Baume, Meng and Law (1966) found these infusions had dose related depressant effects on the volume and acidity of gastric juice. No acute

microscopic mucosal lesions were located, although neutral fat accumulation occurred in the gastric mucosa and submucosal arteries. One explanation offered of the effect was the intestinal or biliary excretion of intravenously administered fat with the subsequent release of enterogastrone. Burr, McPherson and Tidwell (1960) administered fat emulsions intravenously to rats and followed their appearance in the contents of the intestine. Fat was administered either as labelled fat emulsions, labelled chyle and as labelled abumin fatty acid complex. It was calculated 11% of the intestinal lipid originated from blood lipid secretion. This work supported the hypothesis that blood lipid re-enters the intestinal tract. Baume et al (1966) also offered as possible mechanisms for the diminution in gastric secretion, either a reduction in gastric blood flow or a direct action of the intravenous fat on the oxyntic cells. However, the results obtained may have been due in part to central effects. Cirpili and Ridley (1966) have obtained evidence in rats of an association between the ventromedial nuclei of the hypothalamus and the acid and pepsin secreting cells of the stomach. Changes in the blood composition following intraduodenal infusions of oils and fatty acids may influence higher centres in the brain. Mayer (1955) and Kennedy (1953) have advanced, respectively, long term and short term lipostatic theories on the control of food intake. Circulating levels of non-esterified fatty acids have been considered on low carbohydrate diets to give a better indication of hunger-satiety relationships than the change in arterio-venous glucose (see Anand 1961).

The differential effect obtained with the intraduodenal infusion of oleic, linoleic and linolenic acids on motility indicates that a mechanism may exist whereby unsaturated fatty acids entering the blood stream may inhibit movements of the reticulum and rumen. If one were to argue teleologically this would be held to enable further hydrogenation of unsaturated

fatty acids in the cranial compartments of the ruminant stomach. The effects observed could be a reflection of the rate of absorption of the fatty acids (see discussion below of Roberts' (1931) work). The present experiments provide an indication of the importance of undertaking an extensive comparison of the effects of intravenous and intraduodenal infusions of these fatty acids. Roberts (1931) studied the effects of oils on gastric secretion and motility in man. From an analysis of the actions of the oral administration of different oils and fatty acids, Roberts concluded that possibly a relationship existed between the iodine value of the oils and their inhibitory potency. The greater the degree of unsaturation of the constituent fatty acids of the oils, the greater the inhibition. He suggested the effects might reflect the different rates of absorption of the fats. Quigley and Meschan (1941) showed that oleic or linoleic acids inhibited the frequency and magnitude of contractions of the pyloric sphincter more effectively than cream, palmitic, ricinoleic and myristic acids. The sodium salts of the fatty acids were more effective than the triglycerides. Card (1941) compared the inhibitory action of different fats and fatty acids introduced into the duodenum on gastric contractions. No difference was detected in the inhibition resulting from tributyrin, tricaproin, tripalmitin and triolein when emulsified with bile salts. Linoleic acid was twice as potent in reducing gastric contractions as butyric, caproic, palmitic or oleic acids.

A comparison between the effects of the intraduodenal infusion of oleic acid and olive oil in the sheep were studied in the experiments described earlier. The latency of the inhibition produced by the introduction of 2 ml of oleic acid into the duodenum was shorter than that with 2 ml of olive oil administered in a comparable manner. The duration and intensity of the inhibitory effects on acid secretion were greater with oleic acid.

These results support the findings of Long and Brooks (1965) who found that in dogs with vagally innervated gastric pouches the duration of the gastric inhibition was longer and the absorption more prolonged with oleic acid than with triolein. The longer latency in response to the olive oil is presumably due to the time required for hydrolysis of the triglyceride. In the sheep only traces of monoglyceride are present in the small intestine (Lough, Lennox and Garton, unpublished results, cited by Garton 1965, p 397): the importance of monoglyceride in micelle formation has been mentioned previously by Hoffman (1966). It appears, therefore, the quantitative release of an inhibitory principle from the intestine of sheep might be of moment in relation to an increased duration of fat absorption in these animals.

The graded dose effect of oleic acid on motility of the reticulum and rumen and secretion from the abomasal pouch can be taken to suggest that the two inhibitory effects are not brought about by the same mechanism. In the sheep a 2 ml dose of oleic acid introduced into the duodenum caused a definite decrease in the pouch secretion but had no significant effect on movements of the reticulum and rumen. The 2 ml dose may have been of insufficient amount to raise the blood free fatty acid levels above a postulated critical level; in contrast the absorption of this dose could have been such to cause the production of an inhibitory agent (perhaps enterogastrone) which may affect gastric secretion only and have little or no effect on reticulum and rumen motility. An indirect effect of the inhibitory agent in this case may result from a decrease in abomasal acid secretion with a consequent withdrawal of an afferent stimulus to reticulum and associated rumen movements. Additional evidence to support the view that in sheep, motility of the forestomachs and secretion of the abomasum are differentially affected by the presence of fat in the duodenum is provided by the observation that feeding an inhibited animal soon after the intro-

duction of fat, markedly stimulated movements of the reticulum and rumen, but had no stimulatory effects on abomasal acid secretion.

As a duodenal hormone, namely enterogastrone, is presumed by many to be released from the intestine by the presence of fat, an investigation of the effects of enterogastrone extracts in sheep was initiated. The experiments described above appear to provide the first reports of the effects in ruminants of the administration of enterogastrone on reticulum and rumen motility and abomasal pouch secretion.

In sheep the effects of the extracts were shorter in duration as compared with the responses reported in the cat and dog. The reasons which may be offered to explain the different time course of the response include the following:

1. The extracts prepared could have been less active than those used by Greengard et al (1946). It is possible that in the present case the situation was intermediate between that of Greengard et al (1946) and of Howat and Schofield (1954). Howat and Schofield (1954) failed to prepare active extracts in their laboratory and depended on the provision of potent preparations from Ivy's laboratory.
2. Enterogastrone per se, prepared from pig intestine may exhibit a lower activity in sheep than in other animals. Investigations of the same hormone in different species, have in some cases demonstrated the existence of small differences in the amino acid content and sequence occurring in the active centres of the molecule (Gorbman and Bern, 1962). This point is of importance in relation to the activity of gastrin discussed later.
3. Compared with animals possessing a simpler form of stomach, in sheep a stronger stimulation to acid secretion may exist. This could result from a greater sensitivity of the acid secreting cells to chemical

and nervous stimulation, and might be accompanied by an increase in sensitivity to inhibitory agents.

The abomasal secretory mucosa in its provision of a continuous and at times intensive secretion of acid and pepsin, has perhaps evolved a mechanism such that during these periods, exhaustion of the cells involved does not occur. Anderson, Fletcher, McAlexander, Pitts, Cohen and Harkins (1961) have isolated gastrin extracts from the fundic as well as the pyloric region of the abomasum of cattle. It is noteworthy that these extracts were much longer acting when tested in dogs than those obtained from non-ruminants.

4. The mode of administration of the extracts differed in that in the experiments undertaken above slow intravenous infusions over 30 min were given. Earlier workers gave single injections of material.

Some preparations of enterogastrone had more profound effects on the motility of the reticulum and rumen than did others; this could be ascribed in part, to the activity of the animal at the time of infusion. On the other hand, the degree of purification of the extract may have contributed to the apparent differential actions. A preparation of enterogastrone made by Gray and his colleagues inhibited both pouch secretion and motility (Gray et al, 1937). Another preparation was reported to inhibit pouch secretion but to possess little if any inhibitory effect on pouch motility (Greengard et al, 1946). The recovery of an extract with motor depressant properties from the by-products of purification has not been reported to the date of writing.

One question which arises is whether reticulum and rumen motility are more susceptible to inhibition by enterogastrone extracts than is abomasal motility. In ruminants it has been established that movements of the more cranial regions of the stomach are under the control of the central nervous

system. In animals with a simpler form of stomach the intrinsic innervation or mechanisms are perhaps more dominant in the control of the basic motor activity of the stomach: a fine regulator of this activity is provided by the extrinsic nerves. The dependence of the orderly motility of the reticulum and rumen on the control exerted from the medulla oblongata may mean that a greater susceptibility to inhibitory effects is due to a central effect of enterogastrone rather than solely a peripheral mechanism.

Harris, Grossman and Ivy (1947) investigated the role of the vagus nerves in the inhibition of gastric motility by fat and intestinal extracts. They demonstrated that intestinal extracts failed to inhibit distension-induced motility in the vagally denervated stomach: this was taken to have indicated that the motor inhibitory factor in the extract, which is active in the vagally innervated stomach, is not related to the motor inhibitory chalone released by the presence of fat in the intestine. Harris et al (1947) suggested the inhibitory agent in the extracts may be non-specific in character and point out that the specific chalone for motility released by the presence of fat in the duodenum (capable of inhibiting distension-induced motility in the extrinsically denervated stomach, as well as the innervated stomach) has never been isolated as a separate entity from the intestinal mucosa.

In sheep some similarity in the inhibitory effects on reticulum and rumen movements resulting from intraduodenal infusions of fat, and the inhibition accorded by exogenous enterogastrone was observed. In both cases the frequency of 'B' sequences of contraction was less affected than that of 'A' sequences, and in both cases ventral rumen contractions of the 'A' sequence were greatly reduced in force. This effect however, could be general for a number of inhibitory mechanisms and in the cases above not due to the same active principle. Irregularities in contractions of the

reticulum on rumination such as were observed in the recovery from a long term oleic acid induced inhibition were not observed during or after the administration of enterogastrone extracts.

Uvnäs (1948) prepared an enterogastrone extract according to the method of Greengard et al (1946) for use in the study of gastric secretion of cats. His preparations contained a vasodepressor material which apparently was not histamine - the extract failed to cause contraction of guinea pig ileum. This is akin to the situation found in the present study and commented on in "methods" in this chapter. Uvnäs reported the content of secretin was high in his enterogastrone preparation as the pancreatic secretory response to the extract was 1/10th of that elicited by a commercial secretin material. Uvnäs' preparation failed to inhibit histamine stimulated gastric secretion and in some cases produced a distinct increase. Uvnäs suggested the lack of inhibitory potency and the small potentiating effect of the extract was the result of contamination with gastrin. The possible significance of this in the inhibition of reticulum and rumen movements will be taken up in a later discussion dealing with gastrin. It is possible that in the case of those experiments described above in which inhibitory effects on motility of the reticulum and rumen were not detected, the inhibition was masked by the over-riding effect occasioned by the effect of feeding.

The influence of enterogastrone on abomasal pouch secretion was manifest mainly as an inhibition of the volume of secretion. Acid output was lowered but the acid concentration of the secretion was not greatly inhibited initially; this is in keeping with the view of Linde, Öbrink and Ulfendahl (1952) who contend that the inhibition of acidity appears to be a consequence of the reduction in volume of gastric secretion.

Feeding during a fat-induced inhibition of secretion and motility

resulting from the intraduodenal infusion of 10 ml oleic acid failed to increase the acid output from abomasal pouches, but stimulated reticulum and associated rumen contractions ('A' sequences). Feeding after an intravenous infusion of enterogastrone extract resulted in an increased frequency of reticulum and rumen movements, and an increase in the acid output from the pouch. These observations may be simply due to a quantitative difference in the circulating levels of the inhibitory agent. On the other hand, the infusions of 2 ml oleic acid into the duodenum, where feeding failed to stimulate acid secretion from the pouch, implies that either the content of active enterogastrone in the extract used was low in comparison to the amount liberated from the intestine in response to fat, or the inhibition of secretion produced by the extract occurs by some mechanism other than that accorded by fat.

In one experiment, no response to the intravenous infusion of 215 mg extract was detected until feeding ceased, 45 min after the infusion ended. Movements of the reticulum and rumen became irregular for 7 - 8 min and the volume of pouch secretion dropped from 12 ml/15 min to 1.5 ml/15 min. The decrease in pouch secretion could not be attributed to the irregular motility, but was more likely due to some agent which affected both of these. Whether the inhibitory agent was present in an active state throughout the period of feeding and once the desire for food was partially satisfied the inhibition broke through, or whether the extract was metabolised in some way to liberate active products is not known. With the largest dose used (215 mg) perhaps there were sufficient stimulatory substances present to mask inhibition. No inhibitory effects were detected on the pepsin concentration of the pouch secretion, but pepsin output was lowered considerably. Grossman, Greengard, Woolley and Ivy (1944) in a study of pepsin secretion and enterogastrone found that no inhibition of pepsin concentration occurred in denervated or

innervated pouches in response to the extracts, but a depression of pepsin concentration after fat administration occurred in the vagally innervated pouch.

A non-specific inhibition of pouch secretion and reticulo-rumenal motility must be regarded as a distinct possibility when the actual crudity of the extract is envisaged. Pyrogens are a common contaminant of biological preparations. Temperature recordings were not taken after the entero-gastrone administration as it was thought this further interference might influence secretion anyway. Necheles (1942, 1945, cited by Howat and Schofield, 1954) claimed an inhibition of gastric secretion may be produced by doses of pyrogens too small to affect body temperature. It was hoped that the millipore filtration would eliminate bacterial contamination. It is however, difficult to dismiss the results obtained as being of no significance and due to contaminants.

An indication of the possible importance of contaminants in the preparations was provided by the observation that the infusion of the gummy and presumably less purified material was without adverse effect upon secretion and motility. It would appear from this result that the inhibitory principle in these preparations was either combined with other material in such a way so as to be unable to exert its true effect or else it was entirely absent.

One of the experimental difficulties in intravenous infused experiments undertaken on gastric secretion is the action of heparin on the responses. Thompson, Lerner, Tramontana and Miller (1966) investigated the range of action of heparin in suppressing canine gastric acid secretion. 10,000 U.S.P. units of heparin given intravenously at the time of the secretory stimulus significantly inhibited the responses in gastric secretion to a meal, gastrin, histamine, insulin and acetylcholine in dogs of 20 - 25 kg.

An experiment in the present study was undertaken to test the action of heparin on pouch secretion in sheep. 1000 units given intravenously was without effect on reticulum and rumen motility and the volume of abomasal pouch secretion. A possible inhibitory effect on the acidity of secretion was noticed after one injection, but this was not confirmed by further studies. A detailed investigation as to the potency of heparin in inhibiting acid secretion in the sheep is warranted.

The failure to purify and characterise enterogastrone as a separate entity, and the failure of enterogastrone extracts to exhibit the properties of endogenous enterogastrone has led many workers to regard the existence of the hormone with suspicion. Accumulating evidence over recent years has suggested enterogastrone could be one of the accepted duodenal hormones passing either unrecognised or in another form. Greenlee et al (1957) described a secretin preparation which when tested against pouch secretion in the dog, was found to possess all the physiological properties of enterogastrone liberated by the presence of fat in the duodenum. Gillespie and Grossman (1964) studied the inhibitory effect of secretin and cholecystokinin on gastric secretion in dogs in response to gastrin and histamine. Rapid intravenous injections of secretin and cholecystokinin inhibited the acid response of Heidenhain pouches to continuous gastrin extract. Cholecystokinin caused a greater inhibition than secretin. Responses to small doses of histamine were inhibited by cholecystokinin, but not by secretin. Gregory and Tracy (cited by Gregory 1962 p 129) infused fat into the duodenum (liberating enterogastrone) and found this procedure did not cause an inhibition of pouch secretion stimulated by injections of gastrin. It was concluded enterogastrone interfered with the release of gastrin from the antrum.

Jordan and De la Rosa (1964) investigated the inhibition of gastric

secretion by duodenal mucosal extracts. "Pure" preparations of secretin and cholecystokinin provided by Jorpes were parenterally administered to dogs with denervated fundic pouches. Secretin did not significantly inhibit the stimulation of gastric secretion following feeding unless a dose in the toxic range was used. Secretin did, however, inhibit secretion following a second meal. The data was interpreted to suggest that secretin exerted a greater effect on the intestinal phase of gastric secretion. This view was supported when secretin failed to inhibit gastric secretion (gastric phase) stimulated by perfusion of the antrum with liver (a means of achieving gastrin release). Cholecystokinin preparations inhibited gastric secretion which occurred with both the first and second meals, as well as that stimulated by perfusion of the antrum with liver solution. Diversion of bile to the outside did not diminish the inhibitory effect of cholecystokinin. Johnson and Magee (1965) reported gastric motor inhibition by cholecystokinin-pancreozymin extracts. Secretin extracts and serotonin failed to inhibit gastric motility. Two cholecystokinin-pancreozymin preparations consistently inhibited the motility of denervated pouches and innervated stomachs. Intraduodenal instillation of small volumes of olive oil and peptone inhibited pouch motility in a similar manner to the cholecystokinin-pancreozymin extracts. After boiling in water for 60 min the preparations remained potent in inhibiting motility. Incubation with activated pig pancreatic juice at 37°C for 30 min destroyed the gastric motor inhibitory and cholecystokinetic properties of the extract. In a further study Johnson, Brown and Magee (1966) reported that cholecystokinin-pancreozymin extracts produced an inhibition of gastric motility in man. The extracts were not tested against acid secretion at this stage.

Jorpes and Mutt (1966) took crude extracts of cholecystokinin-pancreozymin and passed them over an acidic ion exchanger, a basic ion exchanger,

a sephadex column and another acidic ion exchanger. This procedure resulted in a 10,000 fold increase in hormonal activity without any observed change in the ratio of cholecystokinin-pancreozymin. It was assumed that both activities were exerted by the same substance. Evidence to support this contention was obtained when both activities were lost by oxidation with H_2O_2 and restored with cysteine.

The idea that enterogastrone, pancreozymin and cholecystokinin are one and the same hormone is an attractive proposal. Pancreozymin stimulates the enzyme output from the pancreas. Cholecystokinin causes contractions of the gall bladder with the subsequent delivery of bile into the duodenum. Enterogastrone inhibits gastric secretion which would have increased the duodenal acidity. Lipase, bile and a less acid pH provide optimum conditions for fat absorption. One simple observation that makes this idea difficult to accept is that peptone which is a particularly effective stimulant to pancreozymin release from the duodenum (Wang and Grossman 1951), has not as great an effect as fat on gastric secretion.

The existence of three separate entities, pancreozymin, cholecystokinin and enterogastrone has not been disproved. As the hormones arise from the same region, the duodenum, and produce their effects on digestive organs, a similarity in their molecular configuration is conceivable. A common property pertaining to hormones with similar sequences of amino acids is an overlapping of their activities; this has been described for example, with adrenocorticotropic hormone and melanocyte-stimulating hormone from the hypophysis (Li, 1963). Hormones possess in their structures certain sequences of amino acids which are essential to the activity of the molecule. These areas are known as the active centre of the hormone. In purification processes part of the molecule which determines the specificity of a hormone for a definite tissue may have become damaged or lost. If the active

centre has remained intact, the resulting molecule may now be active in other tissues and exhibit properties of hormones with a similar active centre.

Enterogastrone has been implicated as the parent molecule of the gastric secretory depressant factor urogastrone, found in urine (Gray, 1941). Kaulbersz, Patterson and Sandweiss (1962) found that urogastrone extracts prepared from the urine of hypophysectomised dogs did not possess the usual secretory depressant characteristics of urogastrone preparations. Enterogastrone extracts prepared from the intestine of hypophysectomised dogs were no different from those of normal dogs. It was suggested this was evidence that urogastrone is unlikely to be excreted enterogastrone. The conclusion may be questioned as hypophysectomy could conceivably have affected some tissue responsible for the degradation of enterogastrone subsequent to its release from the intestine. The writer is particularly sceptical of the physiological significance that is attributed to, and the proposed origins of, biologically active factors isolated from urine.

Although the liberation of enterogastrone appears to be related to the absorption of fat by the intestinal mucosal cell (Menguy 1959, 1960, Sircus 1958) a more general mechanism concerned with enterogastrone release must be envisaged to encompass the inhibitory actions not only of fat but also carbohydrate and protein (Schofield 1959, Sircus 1958, Johnson and Magee 1965). Schofield (1959) has provided evidence which strongly suggests enterogastrone plays a regulatory role in the process of gastric emptying even in the absence of fat. In a dog fed lean meat, the tone and motility of a completely denervated or transplanted fundic pouch was inhibited 15 min after consumption of the meal. The manner of the inhibition was similar to that obtained upon the introduction of fat into the duodenum. It appears that one could regard enterogastrone as being one part of a

mechanism which operates to provide relatively stable conditions in the small intestine - stable conditions say in terms of osmolality, pH and concentrations of products of the hydrolytic digestion of fat, carbohydrate and protein.

The results presented earlier can be taken to suggest that motility of the reticulum and rumen and abomasal acid secretion are not necessarily inhibited by the same mechanism. It is suggested that motility of the forestomachs may be markedly influenced by the presence of long chain fatty acids in the blood stream. In the absence of evidence of a greater sensitivity of the gastric secretory mechanism to circulating fatty acid and in the light of the unsatisfactory role of the effects of vagotomy, it appears that the most likely explanation for the inhibitory effects in the case of secretory responses is that which supposes the liberation of a chalone or inhibitory hormone, from the small intestine. Conclusive evidence to support these suggestions will not be provided until a purified extract of enterogastrone has been obtained; until it has been demonstrated to mimic the effects of the introduction of fat into the duodenum; demonstrated to possess inhibitory effects on gastric secretion, but no direct action on motility of the reticulum and rumen; and its release has been identified by a specific bioassay or better still, chemical assay procedure.

CHAPTER IV

GASTRIN

Introduction:

Although Pavlov stressed the importance of the cephalic phase of gastric secretion, he indicated that this could not be taken to account for the entire secretory response of the stomach to a meal (Pavlov 1910 cited by Gregory 1962). Experiments performed by Pavlov and his pupils showed gastric secretion could be excited by the presence of food in the stomach, and that the stimulus originated in the pyloric not the fundic region: the mechanism was assumed to be a nervous reflex in keeping with the concepts which Pavlov had developed.

In 1905 Edkins proposed the "Gastrin Theory" of gastric acid secretion. Edkins suggested the secretory cells of the stomach were stimulated by a mechanism analagous to that proposed by Bayliss and Starling (1902) for secretin in the secretion of pancreatic juice. This view was based on experimental observations in anaesthetised cats in which extracts of the pyloric mucous membrane on intravenous administration, caused a marked secretion of gastric juice. Fundic extracts did not cause secretion. The active extracts retained their stimulatory properties after boiling. Cardiac extracts from the pig stomach, in general had the same efficiency in promoting secretion as did pyloric extracts. Attempts to demonstrate both biochemically and physiologically, the existence of an antral hormone were unsuccessful. This was in part due to the fact that extracts made from almost any tissue contained a powerful stimulant of gastric secretion - the ubiquitous substance in all tissue extracts is histamine. In 1919 Popielski (cited by Gregory 1962) discovered that histamine was a powerful stimulant of gastric secretion. This observation led to the realisation that in all the extracts histamine was probably a potent stimulant of gastric secretion. Efforts to prove the existence of a stimulatory hormone on

gastric secretion by physiological experiments involved procedures whereby the fundic region was vagally denervated or completely separated from other regions of the stomach. Transplanted fundic pouches provided, potentially, the best vehicle to test a possible humoral stimulus to gastric acid secretion.

Stimulation of the antrum with a variety of agents, failed in most cases to produce a secretory response in denervated and transplanted fundic pouches. It was later realised that these initial failures were due to the related decreased sensitivity of the acid secreting cells and reduction in gastrin release from the antrum, which result from removal of the vagal innervation. A further point which must be considered is that in many cases irrigation of the antrum with acid was used as a means of providing a "stimulus" to gastric acid secretion. In fact a low antral pH is now known to be inhibitory to gastrin release.

Ivy (1930) in reviewing the information gathered, concluded that histamine was closely related to, but was not the active principle of, pyloric mucosal extracts. Komarov (1938, cited by Gregory 1962) showed that the trichloroacetic acid precipitate of an acid extract of pig, cat or dog antral mucosa was a protein fraction free of histamine. This extract stimulated the secretion of acid upon its intravenous administration to anaesthetised cats and conscious dogs. Pepsin secretion was not stimulated and the extracts were not active when injected subcutaneously. In anaesthetised cats atropine failed to suppress the acid secretion maintained by repeated intravenous injections of this extract.

Gregory and Tracy (1959, 1960, 1961) described a method for preparing gastrin which provided a potent and highly purified product. The active part of this extract was free of histamine, dialysable through cellophane and effective in stimulating secretion when given intravenously, intramuscularly and subcutaneously. Pepsin secretion did not appear to be stimulated by it.

In man, responses to the extract were strongly reduced by atropine. The constitution and properties of two gastrins extracted from pig antral mucosa were described by Gregory and Tracy (1964). A new preparation method was evolved that worked easily and cheaply on a large scale. The final product of this method comprised two almost identical peptides, designated as Gastrin I (GI) and Gastrin II (GII).

The amino acid sequence of GII was elucidated as Pyro-glutamyl-Glycyl-Prolyl-Tryptophyl-Methionyl-(Glutamyl)$_5$-Alanyl-Tyrosyl(SO_3H)-Glycyl-tryptophyl-Methionyl-Aspartyl-Phenylalanine amide

It was subsequently found that two gastrin peptides differed only in that in GII the tyrosyl residue was sulphated (Gregory and Tracy 1966 p 98). The N and C terminal groups in both gastrins have been established as pyro-glutamyl and phenylalanine amide respectively.

Gregory and Tracy (1964) have reviewed the physiological properties of antral extracts when these were injected intravenously into anaesthetised animals. The extracts exhibited some or all of the following actions:

1. stimulation of gastric acid secretion
2. stimulation of pepsin secretion
3. slight stimulation of volume or flow of pancreatic secretion
4. stimulation of the addition of enzyme to pancreatic secretion
5. stimulation of gastric and small intestinal tone and motility
6. little or no stimulation of gall bladder tone or of hepatic biliary flow
7. inhibition of gastric acid secretion stimulated by some other means

Gregory and Tracy then presented a preliminary survey of actions on the gastrointestinal tract of GI and GII, a resume of which follows:-

1. Acid and pepsin responses to subcutaneous injections:

In conscious dogs single subcutaneous injections in the range 0.25 - 2.5 µg/kg body weight of GI or GII stimulated acid and volume flow of gastric juice from denervated fundic pouches. Large single subcutaneous injections (4 - 5 µg/kg) of GI or GII gave a small response in acid and volume of secretion, but produced a large amount of pepsin in the juice.

2. Inhibition of oxyntic cells and stimulation of peptic cells by intravenous injections:

In dogs with denervated fundic pouches, repeated small subcutaneous injections of GI or GII established a steady secretory response proportional to the dose. During such a response 10 - 50 µg of GI or GII injected intravenously caused an inhibition of acid and volume output from the pouches. When the flow of juice returned a large and sustained output of pepsin occurred - the output of pepsin was too great to represent accumulation. A similar response in terms of pepsin output was obtained if the background secretion was maintained by histamine. It was further demonstrated that both the inhibition of the oxyntic cells and stimulation of the peptic cells persisted after atropine.

3. Gastro-intestinal motility:

Motor activity of a denervated pouch was stimulated by intravenously administered GI or GII. These induced contractions persisted after the administration of atropine. A Thiry-type jejunal loop was first stimulated to contract and then there was a prolonged period of decreased tone on the intravenous administration of GI or GII. In contrast to the situation with gastric motility, atropine inhibited this response.

4. Stimulation of pancreatic secretion:

The intravenous injection of 50 µg of GI or GII caused a prompt but short lived (15 min) increase in the volume of pancreatic flow from

anaesthetised cats or dogs and conscious dogs.

In anaesthetised animals when a steady flow of enzyme poor juice was produced by continuous administration of secretin, 50 µg GI or GII intravenously, caused an increase in volume flow and a considerable increase in enzyme content of the secretion. These hydrelatic and ecbolic effects of the gastrins persisted after atropine.

5. Biliary tract:

50 µg GI or GII intravenously caused a slight increase in gall bladder tone and flow of hepatic bile. This effect persisted after atropine.

6. Effects on blood pressure:

Rapid intravenous injections of GI or GII in dogs under chloralose anaesthesia caused a prompt fall in blood pressure. 100 µg of gastrin produced a similar response to that of 1 µg histamine base. Repeated injections (after the first) caused smaller responses or had no effect at all. The response to 10 - 20 µg GI or GII was negligible.

Continuing with their previous work, Tracy and Gregory (1964) described the physiological properties of a series of synthetic peptides related to Gastrin I. Studies were made of the effects of the peptides on secretion of the stomach and pancreas and the motility of gastric pouches and the intestine. It was found that the C terminal tetra-peptide sequence

tryptophyl-methionyl-aspartyl-phenylalanine amide

was required for the entire range of physiological activities. Morley, Tracy and Gregory (1965) have reported on the structure-function relationships in the active C terminal tetra-peptide sequence of gastrin.

Konturek and Grossman (1966) undertook a quantitative comparison of histamine, natural porcine gastrin (GII of Gregory), the C terminal tetra-peptide amide and two related peptides, as stimulants of gastric secretion in dogs with vagally innervated and vagally denervated gastric preparations.

The maximum response of the Heidenhain pouch to gastrin and the peptides was lower than to histamine. The maximal response of the fistulated stomach (vagally innervated) was about 20% higher with gastrin than with histamine. The peptides were more potent than histamine on a weight basis but only β alanyl penta-peptide gave a maximal secretory response equal to that of gastrin. Wormsley, Mahoney and Ng (1966) investigated the effects of a gastrin-like pentapeptide (ICI 50,123) on the stomach and pancreas of man. This peptide t-butyloxycarbonyl β alanyl-tryptophyl-methionyl-aspartyl-phenyl-alanine amide exhibited similar physiological properties to gastrin. Constant intravenous infusions in the dose range from 0.01 - 0.1 μg/kg/min produced maximum acid outputs. Greater dose rates were necessary to stimulate bicarbonate and enzyme output of the pancreas. The stimulation of pancreatic activity was only apparent on a continuous background of secretin administration.

The inhibitory action of gastrin extracts on a steady acid secretory response was first reported by Uvnäs in 1943 (cited by Gregory and Tracy 1964). In 1963 Gillespie and Grossman found in dogs with Heidenhain pouches secreting in response to continuous injection of gastrin extract or of histamine, that the rapid intravenous injection of gastrin extract caused a marked inhibition of acid secretion (Gillespie and Grossman 1963). Evidence of a specificity of the effects of antral extracts was obtained in experiments where extracts from the fundic gland area, duodenum, ileum, colon and pancreas were found to produce little or no inhibition of acid secretion.

The observation Gregory and Tracy (1964) made that large doses of their pure gastrin inhibited acid secretion confirmed the suggestion of Gillespie and Grossman (1963) that gastrin may inhibit, as well as stimulate, acid secretion. Gregory and Tracy (1964) however, retained reservations whether the gastrin they used may not be identical to the physiological form found in the animal. Quintana, De la Rosa and Dragstedt (1965) investigated

whether endogenously liberated gastrin would inhibit continuous acid secretion. Irrigation of an isolated antral pouch with a suspension of liver failed to inhibit a plateau of acid secretion from Heidenhain pouches in dogs established by the continuous intravenous injection of histamine. Rather, the two stimuli appeared to summate. Inhibitory effects of exogenous gastrin previously obtained by other workers were attributed by Quintana et al to the effect of an excessive dose or an excessively rapid release of the hormone into the blood stream.

Recently, the possible role of gastrin in the stimulation of pancreatic secretion has been demonstrated (Preshaw, Cooke and Grossman 1965). In dogs with a transplanted pancreas, stimulation of an antral pouch with acetylcholine caused a gastric secretory response and an increase in the protein content of pancreatic secretion. In other dogs with pancreatic fistulae and a transplanted antral pouch a gastric and pancreatic response was obtained on perfusion of the antral pouch with acetylcholine. The pancreatic response was not due to passage of gastric juice into the intestine. Both gastric and pancreatic responses were abolished by acidification of the antral pouch. It was suggested the pancreatic secretory response was mediated by gastrin. Supporting evidence has been provided by Blair, Brown, Harper and Scratcherd (1966) who found that in the cat chemical and mechanical stimulation of the antrum produced a hormonally mediated increase in pancreatic enzyme secretion. The block of these effects by atropine and cocaine indicated that release of the antral stimulant was dependant on a local cholinergic reflex.

Preshaw, Cooke and Grossman (1966) in dogs with pancreatic and gastric fistulae, showed sham feeding caused a marked increase in pancreatic protein output. This pancreatic response to sham feeding was inhibited upon acidification of an innervated pouch of the pyloric gland area. It

was suggested that part at least of the pancreatic response to sham feeding was mediated by the vagal release of gastrin from the pylorus.

In a study of the effect of gastrin and histamine on the secretion of bile, Zaterka and Grossman (1966) showed that both pure gastrin II and histamine increased the rate of flow of bile in dogs subjected to total gastrectomy with oesophago-jejunostomy, cholecystectomy and ligation of the minor pancreatic duct. The choleresis caused by gastrin was similar to that produced by secretin; it was characterised by an increase in the concentration and output of bicarbonate. Histamine produced a decrease in the bicarbonate concentration and an increase in the chloride concentration. Neither gastrin nor histamine produced flow rates equal to those attained by secretin. From such studies, it appears that a rather wider spectrum of actions of gastrin that Edkins (1905) originally postulated has been demonstrated.

In ruminants there have been few reports of the effects of gastrin on abomasal acid secretion. Of moment has been the report that gastrin extracted from the sheep and cow possesses a greater potency than gastrin extracted from animals with a simpler stomach. In the following experiments a preliminary investigation of the effects of gastrin on abomasal acid secretion and reticulo-rumenal motility has been carried out.

Methods:

An analogue of gastrin, t-butyloxycarbonyl β alanyl-tryptophyl-methionyl-aspartyl-phenylalanine amide (ICI 50,123; kindly supplied by Dr. Fitzgerald, Imperial Chemical Industries Ltd, England), was used in the experiments undertaken. The pentapeptide, a white powder, was dissolved in ammonium hydroxide solution (100 mg powder in 0.26 ml N NH_4OH, diluted to 100 ml with distilled water). The required dose was made up and administered in 0.9% saline. The peptide prepared in this way was confirmed by experiment to be potent in stimulating gastric secretion from the fistulated stomach in anaesthetised dogs (dose of gastrin administration = 3 μg/kg body weight intravenously).

Intravenously administered "gastrin" (ICI 50,123) was given into the jugular vein of sheep which had been cannulated with a polythene tube at least 16 hr previously. Infusions of "gastrin" were given by a Syringe Pump (Sage Instruments Ltd, N.Y.). Subcutaneous injections were made in the suprascapular region.

Jaw movements, motility of the reticulum and rumen and abomasal pouch secretion were recorded as described previously.

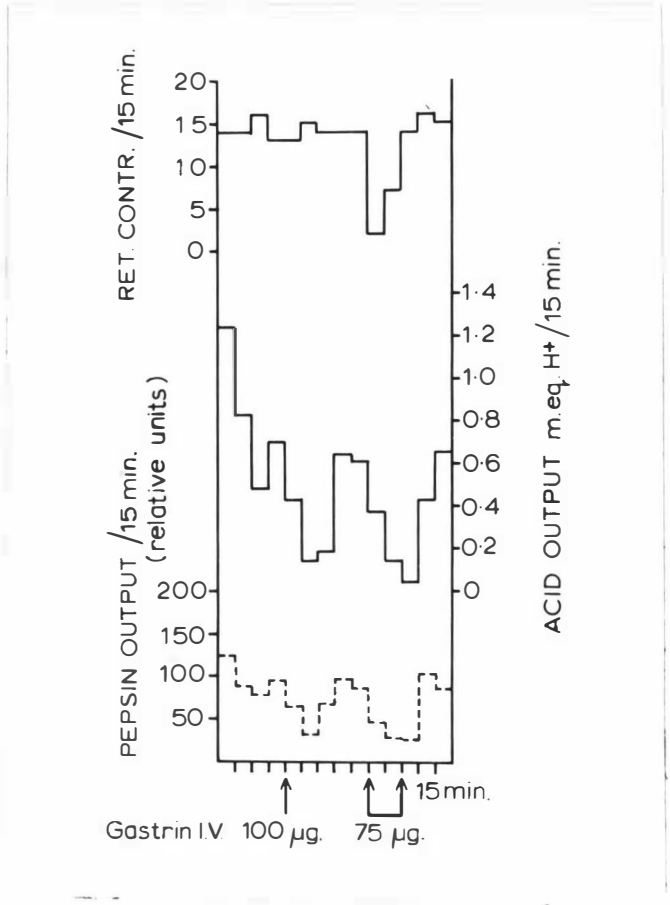


Fig. 24: A comparison between the effects of a single rapid intravenous injection of 100 ug and the slow intravenous infusion over 23 min of 75 µg of ICI 50,123 in a sheep fasted for 16 hr. Graph from above downwards: reticulum contractions/15 min, acid output from the pouch in m-equiv H^+/15 min, pepsin output from the pouch in relative units/15 min, mode of administration of ICI 50,123. Note the injection of the peptide had a negligible effect on reticulum contractions and a small effect upon pepsin output from the pouch. A marked inhibition of motility and secretion occurred with the intravenous infusion of the peptide: the inhibition of secretion was more prolonged with the infusion than with the single injection.

Results:

"Gastrin" (ICI 50,123) administered either intravenously or subcutaneously, failed in sheep in every case to cause a significant stimulation of abomasal pouch secretion. The peptide was administered intravenously in doses ranging from 1 - 100 µg (0.04 - 4 µg/kg body weight), while subcutaneous doses varied between 0.5 and 20 µg (0.02 - 0.8 µg/kg body weight). The higher doses of "gastrin" (ICI 50,123) resulted in an inhibition of acid output from the abomasal pouch and a reduction in the frequency of reticulum and rumen movements. The decrease in acid output was manifest as an inhibition of both volume and acid concentration of the pouch secretion. The intravenous infusion of "gastrin" (ICI 50,123) at high dose rates, caused a sudden and complete inhibition of reticulum and rumen motility. The duration of this inhibition lasted only for the period of infusion and normal motility returned rapidly once the infusion ended.

Intravenous administration of ICI 50,123 ("gastrin"): (See Table 9)

In an animal fasted for 16 hr, a single injection of 100 µg of "gastrin" (ICI 50,123) caused an immediate reduction in the volume of secretion from the abomasal pouch. 15 min after the injection the acid concentration of the juice decreased and fell over the next 30 min from 108 m-equiv H^+/litre to 47 m-equiv H^+/litre. The acid output from the pouch was inhibited for 45 min (Fig. 24). The pepsin output decreased during the period of the volume inhibition, but the pepsin concentration of the secretion was not materially affected. On the injection of "gastrin" (ICI 50,123) reticulum and rumen movements were completely inhibited for about 4 min. This inhibition of the reticulum and rumen was followed by a 4 min period of rumination in which the reticulum exhibited a high degree of tonus. Thereafter no further irregularities in movements of the reticulum and rumen were observed.

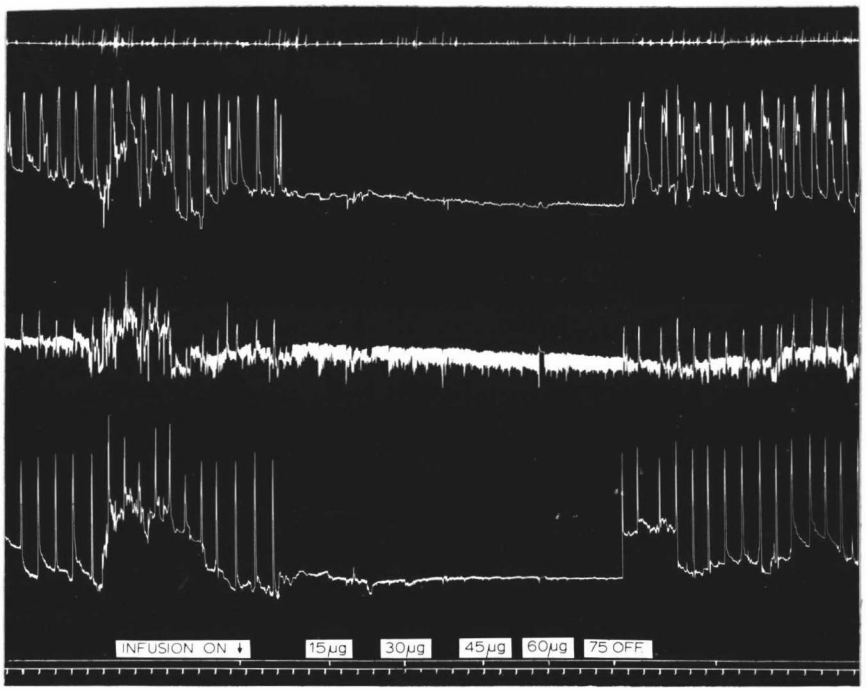


Fig. 25: The inhibitory effects on movements of the reticulum and rumen of an intravenous infusion of 75 µg ICI 50,123 over 23 min in a fasted sheep. Records from above downwards: jaw movements, contractions of the main ventral rumen, contractions of the cranial dorsal rumen, contractions of the reticulum, signal for the infusion of ICI 50,123, 60 sec time-marker. Note the rapid return of normal motility following the inhibition.

In the same animal, 75 µg of "gastrin" (ICI 50,123) was infused over 23 min (0.13 µg/kg/min). A decrease in the volume of the pouch secretion occurred within 15 min, and persisted a further 30 min. The acid concentration of the secretion was reduced 15 min after the commencement of the infusion, and fell from 106 m-equiv H^+/litre to 18 m-equiv H^+/litre within 30 min. The latency of the response in terms of both the volume and acid concentration of the juice was similar to that obtained with the injection of 100 µg "gastrin" (ICI 50,123). The pepsin output from the pouch fell during the inhibitory period. In contrast to the effects of a single injection the pepsin concentration of the secretion was markedly reduced for a period of 45 min. This inhibition of the pepsin content of the juice became evident in the first 15 min of the infusion and was seen as a decrease in the unit concentration of the enzyme in addition to the reduction in the volume of secretion. For a comparison between the rapid injection and the infusion of ICI 50,123 see Fig. 24. Reticulum and rumen movements recorded from the partial exteriorizations exhibited a sudden and complete inhibition from the time the infusion commenced to when it had finished. Normal motility of the reticulum and rumen in terms of the force and the frequency of contractions was recorded before and after the profound inhibition (Fig. 25).

In a similar experiment, but in a fed animal, 75 µg "gastrin" (ICI 50,123) infused over 23 min (0.13 µg/kg/min) caused a slightly different form of response in abomasal secretion and reticulo-rumenal motility. Both the volume and acid concentration of the gastric juice dropped within 15 min of the start of the infusion. Acid output from the abomasal pouch was inhibited for 75 min and pepsin output for 60 min. The acid concentration of the secretion fell over a period of 60 min from 135 m-equiv H^+/litre to 16 m-equiv H^+/litre. The pepsin concentration in the abomasal secretion was definitely inhibited during the infusion period and may have been

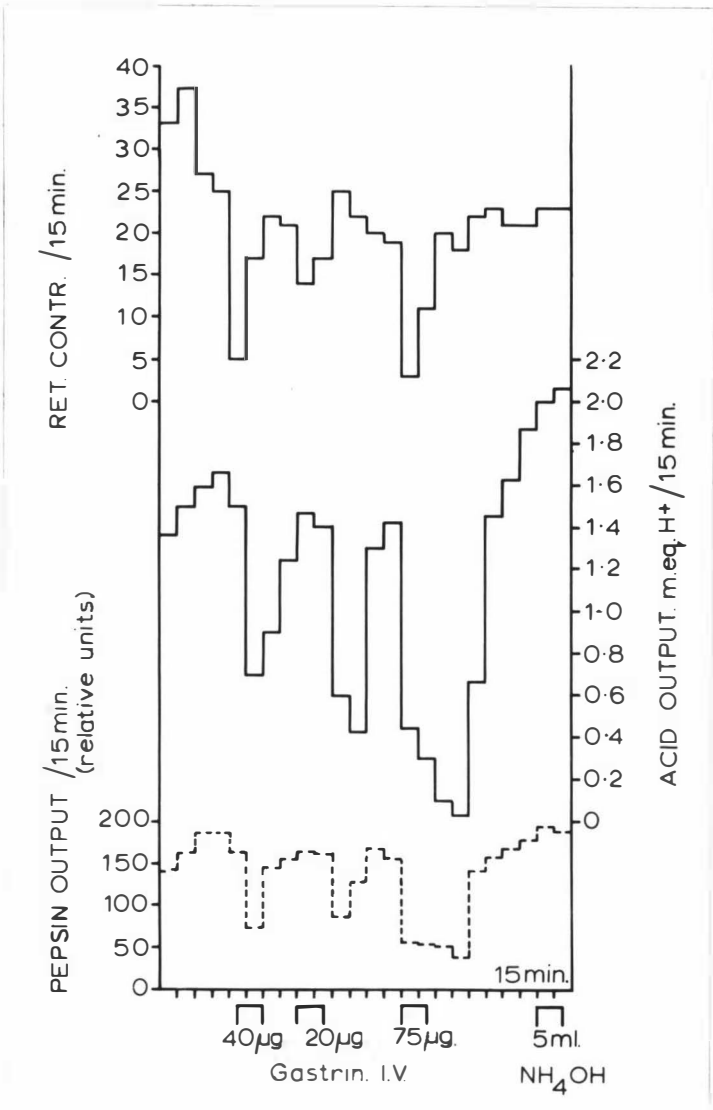


Fig. 26: The intravenous infusion of ICI 50,123 in a sheep fed ad lib. Graph from above downwards: reticulum contractions/15 min, acid output from the pouch in m-equiv H^+/15 min, pepsin output from the pouch in relative units/15 min, 15 min time intervals, substances and the doses and periods of their intravenous infusion. Note the inhibition of reticulum movements, and acid and pepsin output from the abomasal pouch on the infusion of all doses of the peptide. A control infusion of 5 ml of the vehicle was without effect.

reduced for a further 30 min, although this reduction was not as marked as that which occurred during the actual infusion. As in the fasted state, reticulum and associated rumen movements ('A' sequences) showed a complete inhibition over the period of infusion. However, the rumen movements of 'B' sequences were not as grossly affected, although in these, contractions of the main ventral rumen were weak. The frequency of 'B' sequence contractions increased when the animal stood. Near the end of the infusion in this fed state the animal ate a little and this was followed by three reticulum and associated rumen contractions: at this time the reticulum exhibited a marked degree of tonus. Normal motility of the reticulum and rumen returned upon the completion of the infusion.

40 µg "gastrin" (ICI 50,123) infused intravenously over 23 min (0.08 µg/kg/min) in a fed sheep caused a reduction in the volume of the abomasal pouch secretion for 15 - 30 min and a fall in the acid concentration which lasted for 45 min. The decrease in acid concentration was small (136 - 101 m-equiv H^+/litre): in contrast the acid output from the pouch was reduced to 50% of the resting level for 30 min (Fig. 26). The pepsin concentration was reduced for only 15 min. Prior to the infusion of gastrin the animal had been feeding and the reticulum and rumen exhibited a high frequency of contractions. Feeding continued throughout the infusion but the force and frequency of reticulum and associated rumen contractions were reduced, but not completely inhibited. Normal motility of the reticulum and rumen resumed within 120 sec of the cessation of the infusion. A control infusion of saline and NH_4OH solution (the vehicle for ICI 50,123) had no effect on either pouch secretion or motility of the reticulum and rumen (see Fig. 26).

In other experiments 1 µg and 10 µg of "gastrin" (ICI 50,123) infused intravenously over 60 min (0.008 µg/kg/min and 0.0008 µg/kg/min respectively)

had no effect on abomasal pouch secretion or reticulum and rumen motility.

Subcutaneous administration of "gastrin" (ICI 50,123): (See Table 10).

In one animal fasted for 24 hr when 5 μg (0.2 μg/kg) of "gastrin" (ICI 50,123) was given subcutaneously there was no effect on the secretion of the abomasal pouch or movements of the reticulum and rumen.

In contrast a subcutaneous injection of 10 μg "gastrin" (ICI 50,123) led to a rapid fall in the volume and acidity of the pouch secretion (Fig. 27). The acid concentration of the juice decreased from 59 to 28 m-equiv H^+/litre: a weak inhibition of acid output persisted for 20 min (Fig. 27). The pepsin output from the pouch was reduced for a period corresponding to the inhibition of volume (Fig. 27). The pepsin concentration in the abomasal secretion was not affected. A slight slowing in the frequency of reticulum and associated rumen contractions was evident 15 min after the injection.

Repeated 1 μg "gastrin" (ICI 50,123) injections at 15 min intervals failed to produce any significant response in terms of abomasal acid secretion and reticulum and rumen movements (Fig. 27). A single subcutaneous injection of 0.5 μg "gastrin" (ICI 50,123) also failed to produce an effect (Fig. 27); however, there may have been an excitatory action as evidenced by stimulation of triphasic contractions of the reticulum which were not associated with regurgitation. In the fasted animal soon after the administration of "gastrin" (ICI 50,123) triphasic contractions of the reticulum (both associated with and without regurgitation) were occasionally observed. These were never observed at any other time - the control periods occupied more time than did the periods of observation during and after gastrin administration. That rumination which occurred with this event was characterised by frequent small remasticatory periods.

In experiments where abomasal acid secretion and reticulum and rumen movements had been inhibited by an intraduodenal infusion of oleic acid,

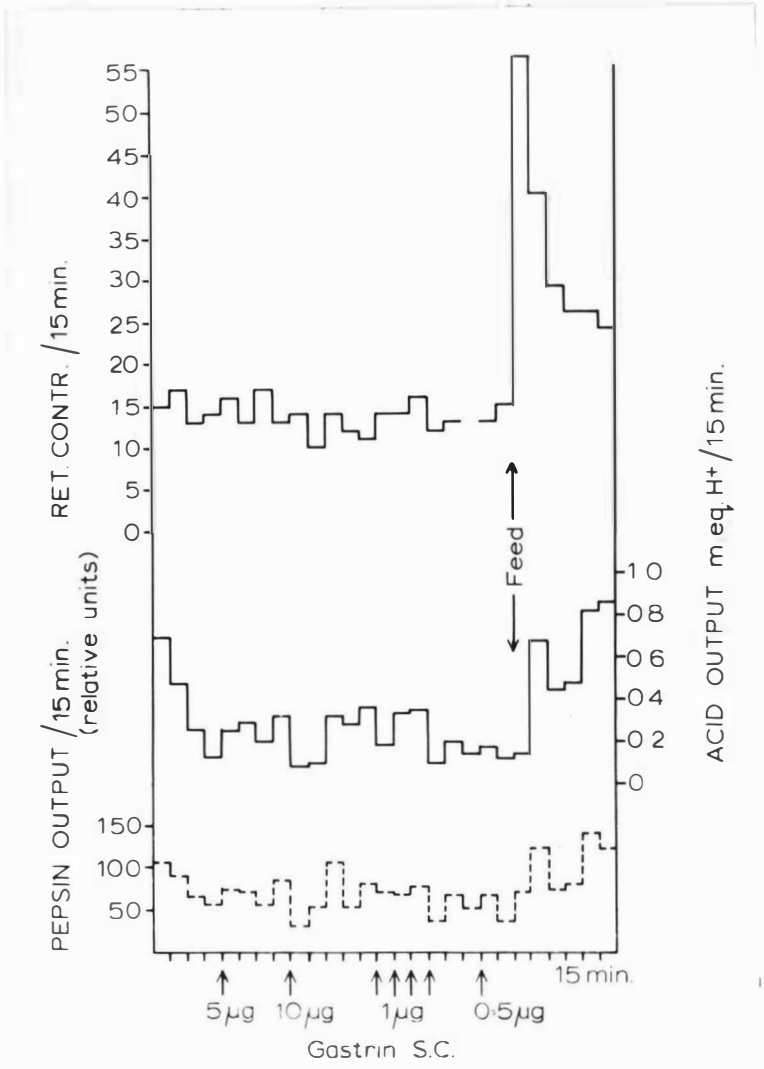


Fig. 27: Effects of the subcutaneous injection of ICI 50,123 in a sheep fasted for 24 hr. A distinct inhibition of acid and pepsin output occurred with the 10 ug dose. Graph from above downwards: reticulum contractions/15 min, acid output from the pouch in m-equiv H^+/15 min, pepsin output from the pouch in relative units, 15 min time intervals, time of each dose of subcutaneously administered ICI 50,123 indicated by arrows. Note the effect on reticulum contractions of feeding (nuts eaten first, followed by chaff).

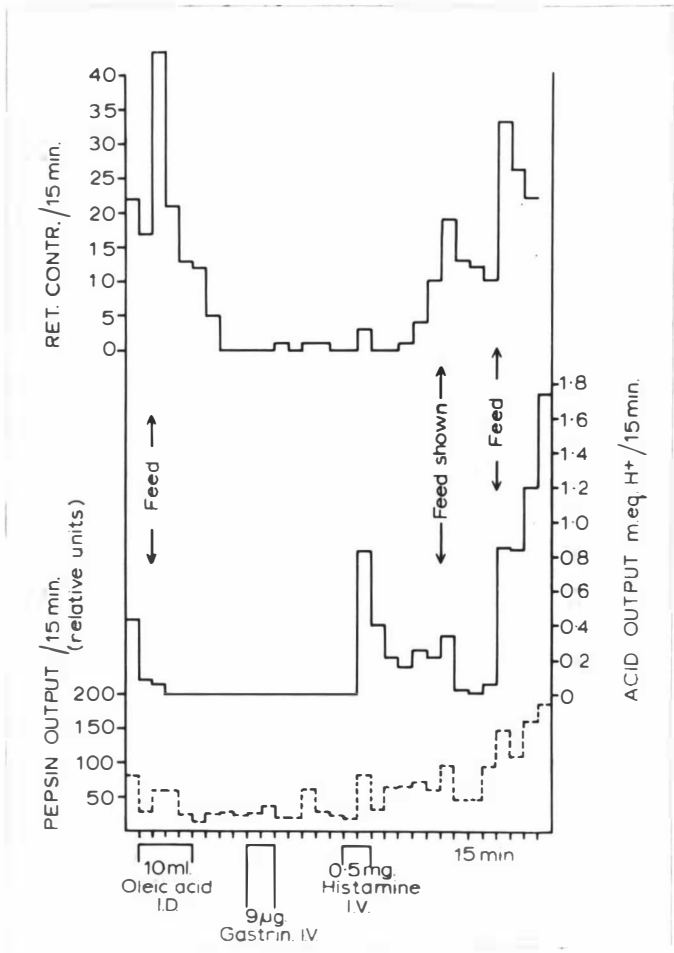


Fig. 28: The inhibitory effects of an infusion into the duodenum of 10 ml oleic acid over 60 min in a sheep fed ad lib. ICI 50,123 failed to stimulate secretion. In contrast histamine had a marked stimulatory effect on secretion. Graph from above downwards: reticulum contraction/15 min, acid output from the pouch in m-equiv H^+/15 min, pepsin output from the pouch in relative units/15 min, 15 min time intervals, substances infused. Note how feeding during the oleic acid infusion stimulated reticulum movements, but had no excitatory effect on acid output from the abomasal pouch.

the intravenous administration of "gastrin" (ICI 50,123: 0.016 µg/kg/min) failed to stimulate either acid secretion from the abomasal pouch or contractions of the reticulum and rumen (Fig. 28). 0.5 µg histamine given intravenously over 20 min (1 µg/kg/min) stimulated abomasal pouch secretion (Fig. 28). The experiment is illustrated in Table 11.

An interesting feature of the secretory response of the abomasal pouch to administered gastrin, was the high content of mucus in the samples. Estimates of the mucus content were not made, but the magnitude of the increase was clearly visible. Another point of interest was the frequent occurrence of diarrhoea a few hours after the introduction of gastrin. This diarrhoea was only transient and appeared only when a number of doses of the pentapeptide had been given over a short period.

Discussion:

The failure of "gastrin" (ICI 50,123) to stimulate abomasal pouch secretion over a wide range of doses was unexpected. Stimulation of acid secretion using the same preparation has been reported in man (Wormsley et al 1966) and an identical pentapeptide has been shown to stimulate acid secretion from Heidenhain pouches and gastric fistulas of dogs (Konturek and Grossman 1965). Similarly Tracy and Gregory (1964) and Morley, Tracy and Gregory (1965) in their studies on a series of synthetic peptides found the pentapeptide alanyl-tryptophyl-methionyl-aspartyl-phenylalanine amide highly active in stimulating acid secretion in dogs. The possibility of an inactive preparation would appear to be ruled out by the secretory response obtained from the fistulated stomach of an anaesthetised dog. Intravenous injections of "gastrin" (ICI 50,123) were made, and an increase in the gastric secretion, volume and acidity was evident within 3 - 5 min of the injection. This response to a single small dose of "gastrin" lasted only for about 15 min.

Since the preparation was active in the anaesthetised dog, but failed to produce secretory responses in anaesthetised and conscious sheep, it is possible that sheep gastrin differs from the synthetic peptide ICI 50,123. Anderson et al (1961) isolated and characterised gastrin from cattle; this gastrin was found to be more potent than pig gastrin in stimulating acid secretion in dogs, and also the secretory response to the extract exhibited a latency of 60 - 90 min in contrast to not greater than 30 min with pig gastrin. Anderson et al (1962) subsequently isolated and assayed sheep gastrin. These extracts exhibited a weaker activity in the dog than either pig or cattle gastrin and in contrast to cattle gastrin no delayed secretory response after injection was observed. Although both fundic and antral preparations were active, the latter were more

potent than the former.

Gregory and Tracy (1966) have reported that attempts have been made in their laboratory to isolate and characterise the peptides present in sheep antral mucosa. The predominant peptide was isolated and its amino acid sequence determined; it was found to differ from pig and human gastrins. Two other similar peptides were also present in small amounts, but these, at the time, were not satisfactorily separated. Apparently no attempts were made to isolate and characterise peptides extracted from regions of the abomasum other than the pylorus.

The observation of Anderson et al (1961) that cattle gastrin was more potent than pig in stimulating acid secretion from the dog, together with the report of Gregory and Tracy (1966) that sheep gastrin differs from pig, supports the view that pig gastrin or its analogs may exhibit a low potency in sheep. The definite inhibitory effect of the pentapeptide suggests that a competitive inhibition of the physiological gastrin may have resulted from the introduction of a peptide with a similar active centre. An alternative explanation for the lack of a secretory response could be offered in that sheep possess a very sensitive secretory mechanism and limited range within which gastrin will stimulate secretion. Gastrin in a dose above this range could cause an inhibition of abomasal acid secretion.

The inhibitory effects of large doses of gastrin in animals with a simple stomach have been documented (Gillespie and Grossman 1963, Gregory and Tracy 1964). Gillespie and Grossman caused a reduction in acid secretion in response to gastrin and histamine, upon the rapid intravenous injection of large doses of gastrin. Gregory and Tracy (1964) found that an inhibition of acid secretion occurred with high doses of pure gastrin.

Quintana et al (1965) failed to inhibit an acid secretory response

to histamine, by the stimulation of the production of endogenous gastrin. They attributed the inhibitory effects of gastrin obtained by other workers to an excessive dose or to a rapid release of the hormone into the blood stream.

It is possible that in the ruminant there is a very critical blood level of gastrin or the abomasal mucosa is sensitive in some other way to gastrin. The observation that the acid inhibition caused by ICI 50,123 in the fed animal was greater than that for the fasted animal could be taken to suggest an interplay between endogenous and exogenous gastrin. Morley, Tracy and Gregory (1965) reported that a very small (1 µg) intravenous injection of Gastrin I or II augmented a background histamine response of acid secretion in dogs. A larger dose (5 µg or greater) led to an inhibition which was usually produced with larger doses of gastrin. This finding is of particular interest with respect to the ruminant in which a continuous acid secretion has been concluded to be occasioned by the constant and specific stimuli of digesta entering the abomasum. The functional state of the abomasal secretory processes may be regarded as analagous to that of a background secretion produced by histamine or gastrin in the simple stomached animal. The introduction of exogenous gastrin in the ruminant could conceivably cause an inhibition in a similar manner as that reported by Morley et al(1965). However, in an oleic acid induced inhibition of abomasal pouch secretion, the slow intravenous infusion of "gastrin" (ICI 50,123), did not cause any stimulatory effects. It would be expected from the results of Gregory and Tracy (1959) that after the liberation of enterogastrone (produced by the presence of oleic acid in the intestine), the circulating levels of gastrin would be reduced so an excessive dose effect of gastrin should not have arisen. However, the recent results of

Bibler, Harkins and Nyhus (1966) in which the introduction of fat into the duodenum resulted in an inhibition of exogenous gastrin stimulated gastric secretion, indicate that the action of the peptide on the oxyntic cell may have been inhibited. The acid secreting cells were still functional as an intravenous infusion of histamine caused a definite acid secretory response from the abomasal pouch.

The transient effects on secretion and virtual absence of effects on reticulo-rumenal motility of a single intravenous injection of 100 µg in comparison to the intravenous infusion of 75 µg of "gastrin" (ICI 50,123) suggests that the pentapeptide was rapidly inactivated. This was particularly the case with the inhibition of reticulum and rumen movements, where, with the injected material the effect was slight. The continuous introduction of gastrin into the blood stream by infusion was sufficient to maintain an inhibition. The method of gastrin inactivation remains obscure. Gillespie and Grossman (1962) demonstrated that the ability of a 'histamine-free' gastrin extract to stimulate acid secretion on intravenous infusion, was undiminished by passage through the liver. It would appear that some other mechanism must be concerned in the inactivation of gastrin. Gregory and Tracy (1966) have proposed that gastrin may be inactivated by removal of the masking amide group or from the oxidation of the S atom in the methionine residue of the C-terminal tetrapeptide. It is quite possible that endogenous gastrin is not inactivated as rapidly as was the pentapeptide, used in these experiments.

The marked effects of the infusions of ICI 50,123 on movements of the reticulum and rumen and to a lesser degree pepsin secretion of the abomasal pouch indicate an interference with vagal mechanisms. Gregory and Tracy (1964) reported pure gastrin stimulated motility of the gastro-intestinal tract of dogs. Logan and Connell (1966) studied the effect of ICI 50,123

on intestinal motility in man. Their results indicated that the activity of the peptide may be selective. ICI 50,123 in doses which provoked a maximal gastric secretion, had a constant motor effect on the rectum and usually a motor but occasionally an inhibitory effect on the sigmoid colon. These workers cited as a possible physiological role for the motor function of gastrin, the initiation of or participation at least in the gastrocolic response. The gastrocolic response has been defined as an augmentation of the segmental activity of the colon and small intestine observed after eating or after distension of the stomach.

Tracy and Gregory (1964) indicated that some of the peptides they studied, on intravenous injection, possessed an inhibitory action on gastric tone and motility which followed their stimulatory effects. This type of action was not consistently observed with natural gastrin. A recent report on the effects of gastrin and its analogues on isolated smooth muscles of different species has been made by Mikos and Vane (1967). These workers studied the actions of synthetic human Gastrin I, natural pig Gastrin II and ICI 50,123 on rat and hamster smooth muscles. In the rat, the descending colon only, showed a high sensitivity to gastrin. GI, GII and ICI 50,123 were equiactive in stimulating contractions of the rat descending colon. These actions of the peptides were substantially decreased or abolished by hexamethonium, hyoscine and morphine. This indicated the actions of the peptides on rat colon were indirect, presumably through stimulation of preganglionic cholinergic nerves.

In contrast hamster fundal strips were contracted by GI, GII and ICI 50,123; ICI 50,123 was twice as potent as either GI or GII. The contractions of the fundal strips were unaffected by hyoscine, mepyramine, hexamethonium, nicotine and morphine in concentrations higher than that required to block the actions of acetylcholine, histamine and ganglionic

transmission. Thus the experiments showed the action of gastrin and related peptides on hamster fundal strips was a direct one (c.f. that on rat colon). The authors postulated that the receptors for gastrin in smooth muscle, nervous tissue and those involved in gastric secretion have similar configurations.

These results support the view of a differential effect of gastrin within and between species. In the sheep both abomasal acid and pepsin secretion and movements of the reticulum and rumen were affected in a similar manner (i.e. inhibited). There may have been a colonic stimulation judging from the diarrhoea produced soon after the administration of ICI 50,123.

In the ruminant orderly movements of the more cranial regions of the stomach depend on the influence of the CNS. Parasympathetic post-ganglionic cholinergic efferents innervate the musculature of the reticulum and rumen. Similarly these same type of efferents are supposed to be concerned in the control of pepsin secretion. The observation that pepsin secretion was inhibited particularly during the infusion indicates a common mechanism of inhibition. Gregory and Tracy (1964) found a large output of pepsin in the secretion from the Heidenhain pouches in dogs following a "gastrin-induced" inhibition. Schoenfield, Siplet and Komarov (1966) studied the effect of a gastrin preparation on gastric secretion in chronic fistula rats. In high doses the gastrin extract (a high potency extract supplied by Gregory) caused an inhibition of total acid output and volume of gastric secretion. These workers regarded the gastrin extract as also responsible for a true inhibition of basal pepsin secretion.

In the phenomenon of gastric secretion, a close association between the vagus and gastrin has been indicated. Uvnäs, Emås, Fyrö and Sjodin (1966) have studied the interaction between vagal impulses and gastrin in the control of gastric secretion: the removal of vagal impulses and its

subsequent importance in the decrease in activity of gastrin has been discussed. The workers concluded, with reference to vagal impulses and gastrin, "Each of the two stimuli may under experimental conditions become intensive enough to break through the stimulatory threshold of the HCl secreting glands. It is doubtful, however, whether they ever do this under physiological conditions."

It is difficult to offer a single explanation for the inhibition of gastric secretion accorded by "gastrin" (ICI 50,123). Since acetylcholine is the chemical transmitter of postganglionic cholinergic efferents, and as the nature of the inhibition due to "gastrin" (ICI 50,123) appears similar to the nicotinic effects of acetylcholine (these effects are described by Goodman and Gilman, 1955) one such explanation could be that gastrin directly or indirectly liberates acetylcholine with the resultant large dose producing nicotinic effects. The observations of Gregory and Tracy (1964) are not in opposition to this view and also provide an indication of the diversity of action of gastrin. These workers reported that gastric motility stimulated by gastrin, peptic activity stimulated by gastrin and oxyntic cell activity inhibited by gastrin were all resistant to atropine (atropine does not antagonise the nicotinic effects of acetylcholine); in contrast the response of intestinal motility to gastrin was inhibited by atropine.

From the results presented and the limited observations reported, it would appear that the inhibition of abomasal acid secretion most likely results from a disturbance of the circulating levels of gastrin. The severe inhibitory effects of the pentapeptide on movements of the reticulum and rumen and the diminution of pepsin secretion which corresponded to the infusion indicate that block of a vagal effector mechanism may account for the inhibition. No definite evidence has been provided to justify the acceptance of any one theory.

An observation of interest was that in the fed animal upon the intravenous infusion of the peptide, 'B' sequences of contractions of the rumen were less affected than 'A' sequences of contraction of the reticulum and rumen. Is this a characteristic of an inhibition of movements of the reticulum and rumen in that 'B' sequences are more resistant than 'A' sequences, or does it indicate that the gastrin and oleic acid induced inhibitions operate through a similar mechanism? If so, is this mechanism central, peripheral or perhaps both? These and many other questions remain to be resolved - perhaps one of the most interesting is whether the actions of the synthetic gastrin preparations provide a clue on the relations between the function and structure of gastrin preparations in different species.

CHAPTER V

GENERAL DISCUSSION

Those animals with a simple form of stomach do not exhibit continuous gastric secretion - the secretion from the stomach occurs in association with the ingestion and/or gastric and intestinal digestion of food. At other times than those of eating or digesting food - the "interdigestive" period - secretion is at a low level or absent.

An indication of the part played by the central nervous system in the regulation of gastric secretion and particularly in relation to blood sugar levels has been obtained from a series of experimental observations made on gastric secretion in rats made hyperphagic by bilateral hypothalamic lesions placed ventromedially (Cirpili and Ridley 1966). When such animals were maintained on a restricted food intake they showed significantly higher levels of gastric acid and pepsin secretion than did normal animals on the same or comparable food intakes.

The administration of 2 deoxy-D-glucose by the intravenous route led to an increased secretion in both normal control and sham fed animals: 2 deoxy-D-glucose did not produce an increased secretion of gastric juice in animals made hyperphagic with ventro-medial hypothalamic lesions, (2 deoxy-D-glucose is not metabolised yet presumably occupies "receptor sites" in metabolic cycles or pathways concerned in glucose transport - it thus prevents glucose becoming available and being utilised and produces a functional cellular deficiency of glucose).

These reactions to 2 deoxy-D-glucose and the earlier finding of a suppression of secretion in hyperphagic rats during insulin hypoglycaemia indicate an association between the ventromedial nuclei and the acid and pepsin secretory responses which occur during hypoglycaemia or interference

with glucose metabolism.

Further evidence of an association between the hypothalamus and gastric secretion has been provided by Mischer and Brooks (1966). Bipolar electrodes were implanted unilaterally into the ventromedial or lateral hypothalamus of rats fitted with chronic gastric cannulae and were used to stimulate discrete regions of the hypothalamus. Electrical stimulation of the hypothalamus through electrodes which were placed in the ventromedial nucleus ("satiety centre") resulted in a significant decrease in volume and output of both acid and pepsin of the gastric contents. No significant change in the acid and pepsin concentration was detected. Animals in which the electrode tips were in the lateral hypothalamus ("feeding centre") exhibited a significantly increased volume of secretion and acid output during periods of electrical stimulation. During the gastric secretory response to an insulin induced hypoglycaemia, electrical stimulation of the ventromedial nucleus caused a reduction in the volume and acid and pepsin output of the secretion. In contrast, electrical stimulation of the lateral hypothalamus had no effect upon the insulin induced response. It was subsequently shown that bilateral vagotomy undertaken in two rats, abolished the increased acid secretion previously obtained in response to electrical stimulation of the lateral hypothalamus. These studies on the effects of ablation and stimulation of areas in the hypothalamus on gastric secretion are consistent with the existence of a localised system within the hypothalamus controlling gastric secretion in a manner similar to the hypothalamic control of food intake.

It is interesting to predict that such a control also exists in the ruminant. This is of moment in the light that definitive evidence has been provided of the control of the CNS over movements of the more cranial regions of the ruminant stomach. Although this control is basically

medullary, the medullary centres are prone to the effect of influence from higher parts of the CNS.

The changes in frequency of movements of the reticulum and rumen observed during the normal periods of feeding, rumination and inactivity deserve special mention. Reid (1963) has discussed the effects of dietary factors on the motility of the reticulum and rumen. The higher frequency of reticulum and associated rumen contractions ('A' sequences) observed with a concentrated protein feed (in the form of nuts) is probably a reflection of the degree of reflex oral, pharyngeal, oesophageal and gastric stimulation. Borgatti (1948) has drawn attention to the greater buccal stimulation accorded when ingested food was chewed for the first time, than that occasioned during the mastication of material regurgitated from the stomach. He suggested this contributed to the different frequencies of reticulum and rumen contractions observed during feeding and rumination. Evidence has been provided in ruminants of a reflex oesophageal stimulation of stomach movements. Clark and Weiss (1952) demonstrated that tactile stimulation of the caudal part of the thoracic oesophagus provokes or increases reticulum contractions and salivary secretion. Distension of the oesophagus in decerebrate preparations and anaesthetised animals has been shown to stimulate reticulum and rumen contractions (Kay and Phillipson 1959, Sellers and Titchen 1959). Reid (1962) has reported that tactile stimulation of the reticulum produced an increased frequency of reticulum and associated rumen contractions. Stretch of the reticulum has also been shown to stimulate reflexly, contractions of itself (Titchen 1958, Reid 1962). In addition, both tactile and stretch stimulation of the reticulo-rumenal fold modify reticulum and rumen movements (Titchen 1960, Reid 1962).

From these reported observations it can be inferred that the sudden drop in frequency of reticulum and associated rumen contractions upon the cessation of feeding is probably due to a combination of factors. These

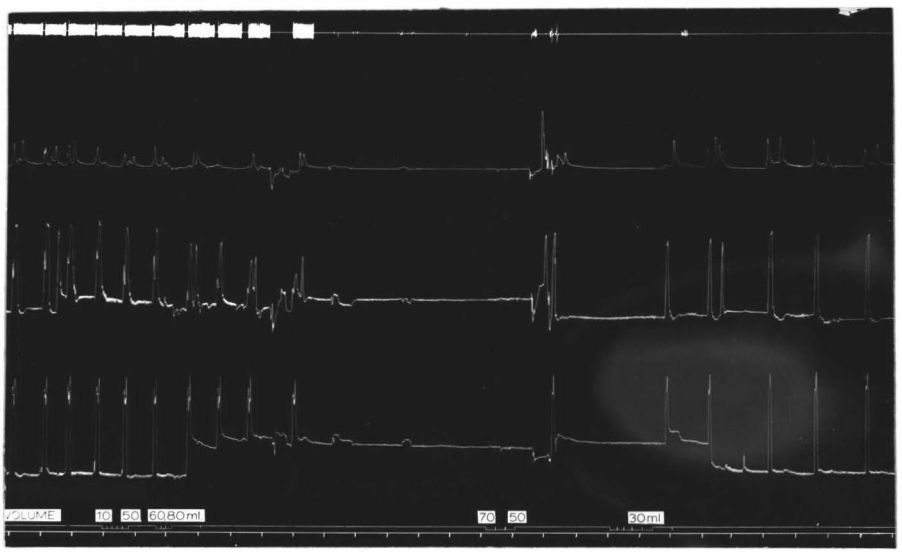


Fig. 29: The inhibitory effects of distension of an abomasal pouch with saline, on movements of the reticulum and rumen. Records from above downwards: jaw movements, contractions of the main ventral rumen, contractions of the cranial dorsal rumen, contractions of the reticulum, signal for the volume of saline in the pouch, 60 sec time-marker. When the pouch was distended with 80 ml saline, a pressure of 45 mm Hg was developed in the pouch. Note the high tonus of the reticulum first seen before its complete inhibition and continuing through the period of inhibition.

could include: a decrease in the afferent impulse input to reflex centres, particularly those impulses arising from oral, pharyngeal and oesophageal sites; the reflex inhibition of reticulum and rumen movements produced by distension of the abomasum (Phillipson 1939, Titchen 1958) and possibly the duodenum (Phillipson and Ash 1965); satiety could also contribute to a decreased motility through a diminution of excitatory efferent impulses arising in the CNS. This latter factor may be reflected in the gradual slowing in frequency of reticulum and associated rumen movements ('A' sequences) as feeding progresses.

A relationship between the central nervous system and gastric secretion and motility in the ruminant has been demonstrated in the experiments presented here by the occurrence of a cephalic phase of abomasal secretion. In the response to teasing a sheep with food, both an increase in the frequency of reticulum and associated rumen movements ('A' sequences) and an increase in abomasal acid secretion (thought to be unrelated, in part at least, to an increased passage of digesta into the abomasum) was observed. Attention should be drawn to the fact that the technique of partial exteriorizations employed in these studies allows the recording of movements of the reticulum and rumen wall only, and therefore an increased frequency of contractions of the reticulum and rumen does not necessarily mean a concomitant increase in the passage of digesta from these regions has occurred.

In an attempt to determine whether an abomasal pouch was innervated, the pouch was distended with 80 ml of isotonic saline at 37°C. This volume of saline produced a pressure within the pouch of 45 mm Hg (measured by an electromanometer) and the distension accorded, after a latency of 30 sec resulted in a tonus increase of the reticulum for 4 min, followed by an inhibition of reticulum and associated rumen movements (Fig. 29). 30 ml of isotonic saline when withdrawn from the pouch was followed by a return of

reticulum movements but tonus of the reticulum was evident during these contractions. Normal motility resumed soon after the withdrawal of the full 80 ml of saline. 80 ml of abomasal acid secretion (140 m-equiv H^+/litre) introduced into the pouch resulted in a lesser degree of inhibition than a similar volume of saline. It was thought this may have been due to the presence of acid receptors in the abomasal mucosa.

Although this experiment supported the conclusion that the abomasal pouch possessed an innervation, the nature of this innervation could not be determined: the inhibition of reticulum and associated rumen movements, may have arisen from inhibitory fibres taking either a course in vagal or sympathetic nerve trunks (Titchen 1958).

In another attempt to determine the innervation of the abomasal pouch 0.5 u/kg of "glucagon free" insulin (Eli Lilley U.S.A.) was administered intravenously to an animal fasted for 24 hr. The insulin caused within 15 min an immediate inhibition of reticulum and associated rumen movements and of abomasal pouch secretion. Apart from a slight stimulatory effect on motility and secretion 45 min after insulin's administration, an almost complete inhibition of reticulum and associated rumen movements lasted for 6 hr until the animal was fed. Rumination did not occur at any stage. Acid secretion from the pouch was inhibited for 9 hr and did not return to normal despite continual eating, until 3 hr after feeding had commenced.

This unexpected profound inhibition of forestomach motility and abomasal secretion demonstrated the danger of using insulin as a determinant of a vagal innervation. Previous reports of an inhibition of pouch secretion have been provided in a review of the action of insulin hypoglycaemia on motor and secretory functions of the digestive tract (Bachrach 1953). Recently Hirschowitz (1966a, 1966b) and Hirschowitz and Robbins (1966) have obtained evidence that the inhibition of gastric secretion by intravenously administered insulin is due to a direct effect of insulin on the

gastric mucosa and is dependant on the dose of insulin given. In a further study, Hirschowitz and Sachs (1966) reported that the inhibitory action of insulin on gastric secretion could be rapidly reversed or largely prevented by the intravenous administration of K^+.

As well as this direct inhibitory action of insulin on the acid secreting cells, Olbe (1964) has produced evidence which has indicated that the response of the oxyntic cell to vagal excitation may be entirely dependant upon the presence of circulating gastrin. In dogs prepared with oesophageal fistulae, sham feeding produced a gastric secretory response. Upon excision of the gastrin liberating regions the response to sham feeding virtually disappeared. Intravenously infused gastrin extract at sub-threshold doses restored the sham feeding response.

Thus the direct inhibitory action of insulin and its probable dependence on gastrin, together with its property of exciting the release of gastrin, make it unsuitable as an agent for determining a vagal innervation. It would appear that 2 deoxy-D-glucose may be a more appropriate vagal stimulator (Feinblatt, Gelfand and Smith, 1966).

In the elucidation of factors concerned in the duodenal control of abomasal secretion and reticulum and rumen motility, the nature of the inhibitory action of fat and its digestion products remains obscure. The experimental studies undertaken have indicated that more than one mechanism exists for the inhibition of abomasal secretion and forestomach motility. It has been suggested from the results obtained that movements of the reticulum and rumen are profoundly affected by the level of long chain free fatty acids in the circulation. Likewise, abomasal acid secretion is also affected by this condition, but there appears to be another mechanism operating at the level of gastric secretion although having little if any effect in low concentrations on motility of the reticulum and rumen. This additional mechanism could be the elusive inhibitory hormone, entero-

gastrone. However, the enterogastrone extracts prepared and administered to the sheep had inhibitory actions on both abomasal secretion and fore-stomach motility. As enterogastrone extracts with properties simulating those of endogenously released enterogastrone have yet to be prepared, it seems as though the true inhibitory agent (termed enterogastrone) is still to be isolated. Recent developments in the field of duodenal hormones has resulted in reports that pancreozymin-cholecystokinin preparations inhibit gastric secretion and motility in a way similar to that of endogenous enterogastrone (Johnson and Magee 1965, Johnson, Brown and Magee 1966, Gillespie and Grossman 1964). Brown and Magee (1967) have shown that cholecystokinin (a commercial preparation, Vitrum) produced a profound inhibition of gastric secretion in Heidenhain pouch dogs secreting in response to endogenous gastrin released by perfusing the antrum with acetylcholine or peptone solutions. Thus, these recent results suggest that cholecystokinin is an inhibitory hormone acting on gastric secretion and motility.

Following up this work on duodenal hormone preparations, Brown and his colleagues studied the effect of duodenal alkalinisation on gastric motility (Brown, Johnson and Magee, 1966). In dogs alkaline buffer solutions and pancreatic juice when introduced into the duodenum were found to stimulate motility of denervated fundic pouches. Pancreatic juice did not increase the pH sufficiently in the duodenum to stimulate motility in transplanted fundic pouches. It was suggested that the solutions either prevented the release of an inhibitory humoral agent, or they released a stimulatory humoral agent. A subsequent study (Brown, Johnson and Magee 1967) showed that the response of transplanted fundic pouches to alkalinisation of the duodenum could be abolished by the intravenous administration of small doses of cholecystokinin-pancreozymin extract. Olive oil, peptone

and HCl when introduced into the duodenum also inhibited the motility response.

Of interest with reference to the above studies is the experiment performed by Chey and Lorber (1966) in which dogs with Heidenhain pouches underwent "total" pancreatectomy or ligation of the pancreatic ducts. After either operation, the daily acid output significantly increased in response to a meal. The instillation into the duodenum of fresh canine pancreatic juice caused an immediate and striking decrease in the acid hypersecretion. This inhibitory effect was abolished by boiling the juice prior to its administration. The inhibitory effect was not due to the alkalinity of the juice as the intraduodenal instillation of sodium bicarbonate in a concentration comparable to that of fresh canine pancreatic juice did not influence the daily acid secretion. It was suggested the acid hypersecretion might have resulted from the impairment or removal of a duodenal inhibitory mechanism.

The evidence presented above is consistent with the view of a tonic release from the duodenum of an inhibitory agent acting on gastric secretion and motility. Under certain circumstances changes (chemical, osmolality, or pH) in the duodenal content (and under many experimental conditions these changes are non-physiological) may result in an increased liberation of the inhibitory agent or agents. How these postulated agents are released remains obscure. It appears unlikely from the different routes of administration used, and the number of substances effecting an inhibition that a single mechanism operates in mediating the effects on gastric secretion and motility: rather a number of mechanisms acting separately, additively or synergistically should be envisaged whether they be blood levels of fatty acids, glucose or amino acids, inhibitory hormones or an inhibition of stimulatory hormones, peripherally or centrally sited

osmoreceptors or even neural reflexes.

It can be seen that the control of stomach motility and secretion in the ruminant is a complex one. Emphasis in this thesis has been placed on the nature of the secretory mechanisms of the abomasum, and the influence of abomasal exocrine secretions on other regions of the stomach. Experimental observations have strongly suggested the occurrence of a cephalic phase (in the sense of a direct vagal stimulation of the secretory cells) of abomasal secretion, and the importance of acid secretion in the control of reticulum and rumen motility in the conscious animal must remain a real possibility.

Attempts to define the relationship between abomasal secretion and reticulo-ruminel motility have not been successful. Certain workers argue to the view that the passage of digesta into the abomasum is the main stimulus to abomasal acid secretion (Hill 1955, 1960, Ash 1961a). Titchen (1958) has cited the secretion of acid by the oxyntic cell as a physiological stimulus to the motility of the reticulum and rumen. Bost and Vérine (1966) in experiments on conscious sheep demonstrated that reticular activity was modified reflexly by distension of the omasum and abomasum: slight distension intensified contractions, while severe distension inhibited contractions. These workers also found that the reticulo-omasal sphincter controlled the passage of ingesta; this passage of ingesta was irregular and was not strictly related to "cycles of reticular activity". Ash (1961a) has suggested that the inflow of digesta into the abomasum, the secretion of acid and the flow of material from the abomasum are integrated, with the abomasum controlling these functions. While no conclusive evidence has been obtained, preliminary experiments indicate that an analogue of gastrin, ICI 50,123 has a differential effect on motility of the alimentary tract of sheep. It is possible that gastrin

per se possesses this effect and contributes to an abomasal control of reticulum and rumen motility.

The experimental studies undertaken have served to illustrate the importance of the duodenal control of abomasal acid secretion and of reticulo-rumenal motility. As well as these more basic control systems, however, higher centres in the brain have been shown to influence motility and secretion; for example the inhibition of abomasal secretion observed with the appearance of strangers.

It is suggested that many control mechanisms concerned with abomasal secretion and forestomach motility, operate as part of a complex co-ordinated series of direct hormonal and reflex responses summing to ensure the continuation of effective fermentative and hydrolytic phases of digestion that are exhibited by ruminants.

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TABLE 1

Experiment: 29/5

72 hour run on No. 2

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
1	N-R	15	11	13)	149	3.28	8.2	180	8	8	1.05
2	R-E	19	-	9)							
3	E	25	-	12.5)	164	4.02	10.2	250	8	10	1.05
4	E-N	17	7	12)							
5	N	8	9	5)	139.5	1.74	9.9	124	17	5	1.10
6	N	9	10	7.5)							
7	N-R	14	9	12.5)	148	3.78	8.3	212	8	11	1.05
8	R	16	8	13)							
9	N-R	12	7	12)	148.5	3.42	9.0	207	9	10	1.00
10	R	13	-	11)							
11	N	10	-	7.5)	152	3.12	10.2	209	12	10	1.05
12	E	22	-	13)							
13	E	23	-	15.5)	176.5	5.30	7.7	231	7	15	1.05
14	E	22	-	14.5)							
15	E-N	17	-	12.5)	148.5	3.79	8.5	217	8	11	1.05
16	N	11	8	13)							
17	R	17	8	10)	151	2.72	8.3	149	9	7	1.00
18	R	15	8	8)							
19	R	16	8	12)	153.5	3.68	8.5	204	8	10	1.05
20.	R-N	11	6	12)							
21	N	12	10	12.5)	146	3.65	9.3	232	7	10	1.05
22	R	16	8	12.5)							
23	R	15	7	10.5)	147.5	2.95	8.2	164	8	8	1.05
24	N	10	5	9.5)							
25	E	19	-	12.5)	152.5	3.74	8.8	216	7	11	1.03
26	E	-	-	12)							
27	N	-	-	11)	147	3.16	9.0	193	7	8	1.05
28	R	-	-	10.5)							
29	R	-	-	3.5)	133.5	1.40	9.0	94	17	4	1.20
30	N	-	-	7)							
31.	R	-	-	11.5)	146	3.50	9.4	226	8	12	1.10
32	R	-	-	12.5)							
33	R	-	-	13.5)	146	3.94	9.9	267	8	10	1.05
34	N-R	-	-	13.5)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15min where meas.	Volume ml/15 min	Total conc. m-equiv H^+/l	Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
35	R	-	-	-)	142.5	-	9.4	-	9	10	1.10
36	R	13	-	13)							
37	N	15	-	10)	146	3.28	12.5	281	8	10	1.05
38	E	25	-	12.5)							
39	E	-	-	15.5)	150.5	4.29	10.6	302	6	13	1.08
40	E	25	-	13)							
41	E	22	-	15)	149	4.32	9.0	261	5	14	1.05
42	E	22	-	14)							
43	N	14	11	12)	149	4.32	8.2	238	6	12	1.10
44	R-E	18	-	17)							
45	E	26	-	14.5)	145	3.91	9.3	251	7	10	1.05
46	E-R	21	-	12.5)							
47	R	19	12	15)	147.5	4.35	9.3	274	6	11	1.10
48	R	19	10	14.5)							
49	R	19	12	15)	148	4.59	9.4	291	5	12	1.08
50	R-E	20	-	16)							
51	E	25	-	16)	146.5	2.78	9.0	171	5	14	1.05
52	E	-	-	3)							
53	--R	-	-	14.5)	153	4.36	7.7	219	5	12	1.10
54	R	>16	>10	14)							
55	E	22	-	13)	148.5	4.01	8.8	238	5	10	1.10
56	E-N	14	>8	14)							
57	N	10	8	13)	149	4.10	8.2	225	6	10	1.05
58	N-R	15	10	14.5)							
59	R	18	-	16.5)	147.5	4.79	8.6	279	5	13	1.10
60	R-E	17	-	16)							
61	R	17	9	15)	148.5	4.01	9.8	265	6	10	1.10
62	R-N	11	9	12)							
63	N	10	5	9.5)	142	2.98	10.9	229	11	7	1.15
64	N-R	15	9	11.5)							
65	R	15	9	13.5)	149	3.87	12.3	320	10	10	1.05
66	R	16	8	12.5)							
*PF 67	N	18	-	13.5)	149	4.17	13.0	364	10	11	1.08
*F 68	E	26	-	14.5)							
69	E	27	-	17.5)	147	5.00	11.4	388	7	13	1.08
70	E	25	-	16.5)							
71	E	21	-	12)	148.5	4.08	10.2	280	7	12.5	1.05
72	E	23	-	15.5)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/30 min	Pepsin conc/ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
73	E-N	20	-	19)	148.5	5.12	10.6	366	7	10.5	1.10
74	N	13	10	15.5)							
75	R	18	10	9)	145.5	1.53	8.6	90	10	6.5	1.10
76	R	19	11	1.5)							
77	R	18	12	8.5)	137	3.08	10.7	240	17	6	1.15
78	R	18	10	14)							
79	R-E	22	-	16)	145.5	4.66	11.7	374	7.5	11.5	0.98
80	E	23	-	16)							
81	E-N	15	-	16)	146.5	4.68	11.2	358	6.5	12.5	1.10
82	N-R	13	9	16)							
83	R-N	16	-	16)	150	4.72	10.7	337	6	11.5	1.05
84	E	22	-	15.5)							
85	E-N	20	-	16.5)	148	4.74	9.8	314	8.5	12	1.10
86	N-R	17	-	15.5)							
87	R	18	11	16)	148.5	4.16	9.6	269	7	11	1.08
88	N-R	15	10	12)							
89	R-N	15	8	4)	136	1.70	9.8	122	18.5	6	1.15
90	N	12	9	8.5)							
91	R	16	8	11)	145	3.62	9.0	225	10	8	1.10
92	N	12	9	14)							
93	-	-	-	15)	143	4.43	11.4	353	8.5	12	1.10
94	N	>13	-	16)							
95	N-R	16	-	15)	144	3.53	10.7	262	8.5	12	1.10
96	R	13	7	9.5)							
97	N	10	6	3.5)	129	1.03	10.2	82	23	2.5	1.20
98	N	8	3	4.5)							
99	N	7	5	8.5)	131	2.16	9.4	155	18	5.5	1.10
100	N	11	6	8.0)							
101	R	15	6	9.0)	136	2.31	8.6	146	14.5	7	1.05
102	R-N	11	4	8.0)							
103	N	10	4	6.5)	126	1.51	9.4	113	21	4.5	1.20
104	N	6	2	5.5)							
105	N-R	12	-	10.5)	139	3.40	9.4	230	11.5	12	1.05
106	R	12	7	14)							
107	N	10	6	10.5)	141.5	2.83	11.2	224	10.5	7.5	1.08
108	N	9	1	9.5)							
109	N	11	5	4)	128.5	1.61	14.1	176	19.5	5.5	1.15
110	N-R	15	7	8.5)							
111	R	15	6	11)	136.5	2.18	10.7	171	12.5	8.5	1.10
112	R-N	7	1	5)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
113	N-R	11	4	13.5)	135	3.10	9.6	221	15.5	7.5	1.10
114	N	9	2	9.5)							
115	N	9	4	11)	139	2.64	9.6	182	10.5	8.5	1.10
116	N	8	1	8)							
117	N	8	2	3.5)	131	1.64	11.8	147	17.5	6.5	1.15
118	Nib.	12	-	9)							
119	R	17	7	10.5)	137.5	2.72	10.2	199	11.5	8.5	1.10
120	R	16	6	9)							
121	R-N	12	-	9)	137	2.60	10.7	203	11	8	1.10
122	N	10	2	10)							
123	N	8	1	6.5)	135	1.75	9.4	122	13	6.5	1.10
124	N	8	2	6.5)							
125	N	10	-	8.5)	136	2.45	8.5	153	12.5	7.5	1.08
126	N	10	-	9.5)							
127	N-R	>10	-	11)	139.5	3.0	10.2	219	10.5	8	1.10
128	R	17	5	10.5)							
129	R-N	12	2	7.5)	136	1.63	10.7	128	12.5	7	1.20
130	R	12	3	4.5)							
*SF 131	N	15	-	4.5))	129.5	1.16	10.2	92	17	8.5	1.20
*SF 132	N	15	-	4.5)							
133	N	13	-	12.5)	142.5	3.14	7.8	172	7	10.5	1.10
134	N	9	1	9.5)							
135	R	15	4	4)	119	0.83	9.4	66	24	3	1.30
136	R-N	11	1	3)							
*F 137	E	30	-	7.5)	130.5	2.81	10.6	228	12.5	11.5	1.10
138	E	29	-	14)							
139	E	27	-	14)	140	4.13	10.7	316	6	13.5	1.00
140	E	27	-	15.5)							
141	E	25	-	16)	143.5	4.52	11.2	353	5	13.5	1.08
142	E	25	-	15.5)							
143	E	21	-	15)	146.5	4.69	9.4	301	4	13	1.10
144	E	22	-	17)							
145	E	23	-	15.5)	147.5	4.57	7.5	232	4.5	12.5	1.10
146	E	23	-	15.5)							
147	E-R	22	-	15.5)	143.5	4.16	8.6	249	4.5	12	1.08
148	R	23	12	13.5)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total conce. m-equiv H^+/l	Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
149	R-E	22	-	16)	147.5	4.72	9.1	291	5	12.5	1.10
150	E	20	-	16)							
151	N-R	22	-	16)	145.5	4.58	10.1	318	4.5	12.5	1.10
152	R	19	9	15.5)							
153	R	17	10	14)	142.5	4.13	11.2	325	5	10	1.10
154	R-N	18	-	15)							
155	E	21	-	17)	145.5	4.80	9.8	323	4.5	12.5	1.08
156	E-N	18	-	16)							
157	R	16	11	15.5)	147	4.12	8.5	238	4	11.5	1.08
158	N	11	7	12.5)							
159	N	11	10	12)	147.5	3.61	7.8	191	7.5	8	1.08
160	R	18	10	12.5)							
161	R-N	12	7	12)	148	3.77	10.2	260	7	8.5	1.10
162	R-E	23	-	13.5)							
163	E	-	-	15)	148.5	4.75	10.1	323	5	12	1.10
164	R	>9	-	17)							
165	R-E	22	-	15.5)	145.5	4.73	11.4	370	6	13.5	
166	E	20	-	17)							
167	E-N	18	-	17)	147	3.90	9.0	238	6	11.5	
168	N	10	6	9.5)							
169	N-R	12	5	12)	149	3.72	8.5	212	7.5	9	
170	R	18	11	13)							
*SF 171 F	N	18	-	15)	147.5	4.87	10.7	353	7	12	
172	E	31	-	18)							
173	E	30	-	18)	153	5.05	10.1	333	6	13.5	
174	E	28	-	15)							
175	E	26	-	16.5)	154.5	4.33	6.9	193	6	10.5	
176	E	25	-	11.5)							
177	E	26	-	13)	151.5	4.40	6.4	186	7.5	11	
178	E	25	-	16)							
179	N	17	-	17.5)	145.5	5.02	7.4	255	6.5	12.5	
180	R	21	12	17)							
181	R	19	14	16.5)	150.5	4.59	8.3	253	6	11.5	
182	E	26	-	14)							
183	E	24	-	16.5)	151	5.06	7.2	241	6.5	12.5	
184	E	24	-	17)							
185	N-E	16	-	18)	151	5.36	8.2	291	6.5	13	
186	E	-	-	17.5)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
187	E-N	17	-	15.5)	148	4.51	7.4	226	7.5	10.5	
188	E	22	-	15)							
189	N	14	-	16)	148	4.51	7.0	213	6.5	8.5	
190	N	13	8	14.5)							
191	N	15	11	7.5)	142.5	2.64	13.4	248	10	8	
192	R	18	10	11)							
193	R	15	12	12.5)	143	3.50	9.8	240	8	8.5	
194	N	10	8	12)							
195	N	10	7	5.5)	140.5	1.83	13.3	173	13	6	
196	N	8	8	7.5)							
197	E-R	15	11	11)	142.5	3.70	9.4	244	9	10.5	
198	R	18	9	15)							
199	R	18	9	16)	141	4.51	10.2	326	7.5	12	
200	N-R	13	9	16)							
201	R-N	11	7	15)	143	3.79	12.3	326	7.5	10	
202	N-R	15	7	11.5)							
203	R-N	13	9	14)	143	3.79	8.3	220	7.5	9.5	
204	N-E	10	8	12.5)							
205	N	8	6	11)	157.5	3.70	7.8	183	8	8	
206	R	16	9	12.5)							
207	R-N	14	9	14)	156.5	4.46	8.5	242	7.5	10.5	
208	E	19	-	14.5)							
209	N-R	14	12	13.5)	154	3.62	8.5	200	8	9.5	
210	R-N	11	8	10)							
211	N	8	6	3)	132.5	1.72	8.2	106	17.5	7	
212	N	8	5	10)							
213	N-R	13	8	11)	142.5	2.71	8.2	156	9	9.5	
214	R	15	8	8)							
215	R	16	8	2)	145.5	1.82	8.0	100	10	10	
216	N	9	7	10.5)							
217	N	9	5	12)	148	3.40	8.5	195	9.5	10	
218	R	14	>4	11)							
219	R	15	8	11.5)	144.5	3.32	8.0	184	9.5	10	
220	N	15	-	11.5)							
221	N-R	14	-	12)	144.5	2.74	7.5	142	8.5	11	
222	R	17	9	7)							
223	R	16	8	10.5)	145.5	3.64	9.0	225	9.5	9.5	
224	E	>12	-	14.5)							
225	E	29	-	15.5)	147.5	4.65	9.0	283	7.5	13	
226	E	28	-	16)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total acid conc. m-equiv H^+/l	Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
227	E	23	-	16.5)	140	4.83	9.0	310	6.5	13	
228	E	23	-	18)							
229	E	24	-	17.5)	149	5.07	6.6	224	7	14	
230	N-R	18	>10	16.5)							
231	R	18	12	17)	146	4.89	7.4	248	6.5	13.5	
232	R	18	11	16.5)							
233	R-E	22	-	17.5)	147	5.29	8.0	288	6.5	14	
234	E	22	-	18.5)							
235	N-E	-	-	18)	147.5	5.31	7.2	259	6.5	12.5	
236	E-R	19	-	18)							
237	R	18	12	12)	146	3.65	5.9	147	8.5	8.5	
238	R-N	11	9	13)							
239	R	17	12	15)	146.5	4.47	8.2	250	-	-	
240	R-N	15	-	15.5)							
241	R	17	11	16)	147	4.41	8.0	240	7.5	10.5	
242	R	17	12	14)							
243	R-N	13	9	12.5)	147.5	3.54	10.7	257	9	8	
244	N	11	5	11.5)							
245	N-E	21	-	14)	145.5	4.22	9.8	284	8	10	
246	E	20	-	15)							
247	E	20	-	18.5)	145	4.86	10.7	358	7.5	11.5	
248	N-R	16	11	15)							
249	R-N	11	8	13.5)	144.5	3.18	9.8	216	8	8	
250	N	10	7	8.5)							
251	N	9	7	10)	140	3.22	16.7	384	11.5	7	
252	R	16	8	13)							
253	R	18	12	12.5)	142	3.62	9.1	232	9	9.5	
254	R	17	10	13)							
255	N	12	6	13)	141	3.45	11.4	279	9	9.5	
256	N	11	3	11.5)							
257	E	-	-	10.5)	142.5	3.49	11.2	274	10	9.5	
258	E	22	-	14)							
259	E-R	18	-	14.5)	145	4.06	8.0	224	7.5	10	
260	R	17	9	13.5)							
261	R	15	9	12.5)	145	3.91	9.8	265	8	8.5	
262	R-N	12	8	14.5)							
263	N	8	6	6.5	144.5	0.94/ 15 MIN	9.0	58/ 15 MIN	9	8	

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total conc. m-equiv H^+/l	Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
*SF											
264	N	15	-	12.5)	139.5	3.82	8.5	234	9	8.5	
265	N-R	15	-	15)							
266	R	18	7	12.5	145.5	1.82/ 15 MIN	9.8	122/ 15 MIN	6.5	10	
F 267	E	26	-	16.5)	131	4.52	8.6	297	7	11	
268	E	29	-	18)							
269	E	27	-	18.5)	146.5	5.13	7.4	259	6	13	
270	E	26	-	16.5)							
271	E	24	-	15.5)	147.5	3.83	7.5	195	7.5	10.5	
272	E	23	-	10.5)							
273	E	24	-	15)	149	4.69	5.9	186	7.5	12	
274	E	24	-	16.5)							
275	E	22	-	16.5)	150	5.10	7.4	252	6.5	13.5	
276	E	23	-	17.5)							
277	N-E	20	-	17.5)	153	5.35	6.7	234	6.5	14.5	
278	E-N	20	-	17.5)							
279	N	13	9	17)	148.5	4.31	9.1	264	6.5	11.5	
280	N	12	7	12)							
281	R	19	11	12.5)	149	3.87	7.8	203	8	10	
282	N-E	11	10	13.5)							
283	E	23	-	16.5)	144	4.90	8.2	279	6.5	13	
284	E	24	-	17.5)							

TABLE 2

Experiment: 4/6

100 hour run on No. 2

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total conc. m-equiv H^+/l	Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
1	E	-	-	13)	142.5	3.99	6.9	193	5	9	1.09
2	E	24	-	15)							
3	E	22	-	17)	142.5	4.84	7.0	238	4	12	1.07
4	E	20	-	17)							
5	N	12	7	16)	140	3.92	5.9	165	4	6.5	1.06
6	N	8	5	12)							
7	R	15	7	12)	141.5	2.97	5.9	124	6.5	5	1.06
8	R	16	9	9)							
9	R-N-E	14	8	4)	131	2.10	7.8	125	9.5	6.5	1.02
10	E	22	-	12)							
11	E-N	19	-	13.5)	141.5	3.82	6.4	173	5	9	1.00
12	N	12	8	13.5)							
13	R	17	10	14.5)	141.5	3.89	6.7	184	5.5	8.5	1.01
14	R	16	8	13)							
15	N-E	16	-	10)	140	3.50	7.8	195	6	8.5	1.01
16	E	23	-	15)							
17	E	21	-	15.5)	127.5	3.70	6.2	180	4.5	8.5	1.08
18	E-N	13	10	13.5)							
19	N	10	5	11.5)	130.5	2.94	5.3	119	5.5	5.5	1.06
20	N	10	3	11)							
21	N-E	13	4	7.5)	137	2.47	10.7	193	9.5	5	1.00
22	E-N	>19	-	10.5)							
23	R	-	-	8)	136.5	2.46	9.8	176	10.5	5	1.01
24	R	>17	-	10)							
25	R-N-R	15	9	10)	134.5	2.02	8.2	123	11	6.5	1.02
26	R-N	12	6	5)							
27	N	7	5	6.5)	124.5	1.93	8.5	132	20	5.5	1.08
28	N-R	>9	>5	9)							
29	R	>6	-	12)	141.5	3.75	7.5	199	6	9	1.00
30	E	24	-	14.5)							
31	E-N	16	-	16)	111	3.50	5.0	157	2	7.5	1.11
32	N	10	-	15.5)							
33	N	8	-	11.5)	139	3.00	5.9	127	5.5	5.5	1.00
34	N-R	12	-	10)							
35	R	14	-	6)	128	1.47	6.9	79	16	5	1.09
36	R	14	-	5.5)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total conc. m-equiv H^+/l	Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
						15 MIN		15 MIN			
37	R-N	11	-	9	131	1.18	7.0	63	-	-	-
38	N	8	-	11.5	136	1.56	5.9	68	7	7.5	1.10
39	N-R	14	-	12	136.5	1.64	6.4	77	5	8	1.05
40	R-E	16	-	12.5	136.5	1.71	5.6	70	5	7	1.08
41	E	21	-	15	139	2.08	6.2	93	4.5	9	-
42	E-N	20	-	15	139.5	2.09	5.8	87	4	10	1.05
43	N	11	7	14.5	140.5	2.04	5.6	81	3	8	1.03
44	N	14	-	14.5	139.5	2.02	6.7	97	4	8	1.01
45	N-R	15	-	14	142	1.99	5.1	71	4	8.5	1.02
46	E	26	-	15	140.5	2.11	6.4	96	4.5	7.5	1.02
47	E	27	-	15.5	143	2.22	7.0	110	3	8.5	1.00
48	E	25	-	13.5	141	1.90	7.4	100	4.5	7.5	1.00
49	E	24	-	13	141.5	1.84	5.8	75	5	7	1.02
50	E	22	-	7.5	135	1.01	5.8	43	9	5	-
51	E-N	16	-	11.5	134.5	1.55	6.4	74	11	6	1.09
52	N	12	-	13.5	139	1.88	5.1	69	5	8.5	1.02
53	N-R	13	7	15	139	2.08	5.8	87	5	9	1.05
54	R-E	18	-	15	141.5	2.12	6.4	96	4.5	8	1.05
55	E	22	-	15.5	144	2.23	8.0	124	5	9.5	1.01
56	E	-	-	15.5	143.5	2.22	6.7	104	4.5	11	1.00
57	N	>10	>5	15	142.5	2.14	6.2	93	4.5	10	1.01
58	N	9	6	13	143.5	1.86	5.6	73	4.5	8	1.00
59	N-E	13	-	10.5	139	1.46	5.6	59	7.5	6	1.03
60	E	23	-	13	141	1.83	6.7	87	7.5	7.5	1.03
61	E	24	-	16.5	144	2.38	6.7	110	4.5	11	1.00
62	E	23	-	17.5	143	2.50	7.0	124	4.5	12	1.02
63	E-N	16	-	15.5	144.5	2.24	6.9	107	4.5	11	1.06
64	N	12	7	14.5	127	1.84	6.9	100	4.5	6.5	1.20
65	R	15	10	13	141.5	1.84	9.6	125	6	8	1.10
66	R	15	7	14	142.5	1.99	8.5	119	5	8	1.02
67	R-N	12	8	8.5	138	1.17	6.9	59	10.5	7	1.02
68	N	9	8	11.0	135.5	1.49	6.9	76	11.5	5.5	1.09
69	N-R-E	16	-	14.5	138.5	2.01	6.4	93	6.5	9.5	1.08

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
						30 MIN		30 MIN			
70	E	20	-	16)	143	4.86	7.0	238	4.5	12	1.00
71	E	23	-	18)							
72	E	23	-	19)	144	5.40	7.4	277	3.5	13.5	0.98
73	E	22	-	18.5)							
74	E-N-R	16	10	18)	154	5.39	7.4	259	3.5	11.5	1.00
75	R	15	8	17)							
76	R	15	9	12)	140.5	3.65	6.4	166	6	8	1.01
77	R-N-R	13	9	14)							
78	R	15	8	16)	139.5	4.60	6.9	228	4.5	10.5	1.03
79	E	23	-	17)							
80	E-N	18	-	17)	142	3.90	5.9	162	5	10	1.01
81	N	8	7	10.5)							
82	N-R	12	8	13)	140.5	3.86	6.4	176	7	7	1.01
83	R	15	8	14.5)							
84	R	16	8	13)	140.5	3.79	7.5	202	7.5	8	1.03
85	R-N-E	14	-	14)							
86	E	22	-	15)	141.5	4.39	7.4	229	5	10.5	1.04
87	E	21	-	16)							
88	N	10	7	10)	138.5	3.25	6.4	150	10.5	6.5	1.03
89	N-R	>7	>5	13.5)							
90	R	14	10	15)	140	4.13	6.9	203	6.5	8.5	1.06
91	R	15	8	14.5)							
92	R	14	8	14)	140.5	3.31	7.4	185	7.5	8	1.00
93	R	14	9	11)							
*PF											
94	N	12	-	11.5)	137	3.56	7.8	203	10.5	8.5	1.03
95	N	12	-	14.5)							
96	R	15	-	13.5)	141	3.45	6.9	169	8	7.5	1.01
97	E	27	-	11)							
98	E	26	-	13)	137.5	3.51	7.4	189	9.5	7.5	1.10
99	E	22	-	12.5)							
100	E	22	-	15.5)	139.5	4.53	5.6	182	5	11	1.05
101	E	23	-	17)							
102	E	22	-	17.5)	143.5	4.81	5.9	198	6	11	1.06
103	N	14	11	16)							
104	N-R	12	10	14)	141.5	4.24	7.4	222	7	9	1.04
105	E	25	-	16)							
106	E	25	-	16.5)	142	4.69	6.7	221	5	11.5	1.03
107	E	25	-	16.5)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
108	E	22	-	18)	142.5	5.06	7.0	248	5	12.5	1.08
109	N-R	16	12	17.5)							
110	R	15	13	15)	144.5	4.48	6.7	208	6	9.5	1.02
111	R-N-R	16	9	16)							
112	R	16	11	18)	140	5.04	6.9	248	5	11.5	1.07
113	R	16	11	18)							
114	E	25	-	18.5)	141.5	5.38	6.7	255	5	14	1.05
115	E	23	-	19.5)							
116(a)	E	25	-	20)	139.5	5.44	6.4	250	4.5	13	1.10
116(b)	E	21	-	19)							
117	N-R	16	14	18)			4.3	144	4.5	10.5	1.00
118	R	16	12	15.5)							
119	R-N	14	10	14.5)			4.8	148	6.5	7.5	1.00
120	N-R	15	10	16.5)							
121	R	15	10	16.5)			5.1	142	7	8.5	1.00
122	N-R	12	9	11.5)							
123	R	-	-	12.5)			5.8	148	8.5	7	1.00
124	E	24	-	13)							
125	E	22	-	15.5)			5.1	174	5	10	1.00
126	E-N	13	11	18.5)							
127	N-R	12	8	17.5)			4.3	132	5	9.5	1.00
128	R	14	7	13)							
129	N	8	4	10.5)			7.4	184	7.5	9	1.00
130	R	15	12	14.5)							
131	R	14	10	15)			6.1	180	9.5	9.5	1.00
132	R-E	18	-	14.5)							
133	E-N	17	-	15)			5.1	132	10	5	1.00
134	N	9	4	11)							
135	N-R	12	9	10.5)			4.5	86	24.5	4	1.00
136	R	14	10	8.5)							
137	R	14	8	4)			6.7	80	11	8.5	1.09
138	R	14	6	8)							
139	R-N-E	13	-	10)			5.8	144	6.5	9.5	1.01
140	E	21	-	15)							
141	N	10	4	15.5)			6.7	160	19	4.5	1.00
142	R	13	10	8.5)							
143	R	15	6	7.5)			7.0	122	11.5	6.5	1.02
144	R-E	17	>4	10)							
145	E	19	-	12.5)			5.6	100	9.5	9	1.00
146	N-R	8	5	5.5)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
147	R	14	7	11)			4.8	92	15	4.5	1.01
148	R	13	6	8)							
149	R-N	9	6	4.5)			5.3	68	25	5	1.07
150	N	11	3	8.5)							
151	N	14	-	14.5)			4.0	120	6.5	10.5	0.99
152	N	13	-	15.5)							
153	E	>17	-	18.5)	148.5	5.12	4.8	166	4.5	12	0.98
154	E	26	-	16)							
155	E	26	-	17.5)	145	5.29	4.6	168	4	13.5	0.98
156	E	24	-	19)							
157	E	20	-	17)	149.5	4.86	3.8	124	4	13.5	0.99
158	E	23	-	15.5)							
159	E	23	-	15)	146.5	4.17	3.8	108	5	9	0.98
160	N	12	9	13.5)							
161	N-R	12	10	14.5)	152	4.64	5.6	170	5	11	0.98
162	R	17	11	16)							
163	R	17	12	13)	159	4.29	6.6	178	7	10	0.99
164	R	15	11	14)							
165	E	25	-	16)	153.5	5.22	4.8	164	5	13.5	0.99
166	E	22	-	18)							
167	N	14	10	17)	146.5	4.69	4.8	154	4.5	11.5	0.98
168	R	15	11	15)							
169	R	14	8	10.5)	145.5	3.71	4.0	102	6.5	8.5	0.99
170	R-N	9	8	15)							
171	N-R	14	8	13)	141.5	4.24	5.1	152	5	11.5	1.00
172	R	13	8	17)							
173	N-E	16	-	15.5)	142	4.19	5.6	168	5	10.5	1.00
174	E-N-R	19	-	14)							
175	R	14	9	9.5)	141	2.82	4.8	96	9	6.5	1.00
176	R-N	10	7	10.5)							
177	N-R	11	8	10)	140.5	2.67	5.1	96	11	6	1.00
178	R-N	11	6	9)							
179	N-R	11	>6	13.5)	140	4.20	5.1	152	8	11	1.00
180	R	14	8	16.5)							
181	E	20	-	17)	143	4.79	5.8	194	5	12.5	1.00
182	R	14	10	16.5)							
183	R	14	11	12)	158	3.40	6.1	131	8.5	9.5	1.00
184	R	14	8	9.5)							
185	R-N	12	10	10)	136	2.99	7.4	162	11	7	1.01
186	E	24	-	12)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
187	E-N	20	-	16.5)	143	4.79	5.6	188	4.5	13	1.00
188	N-R	15	10	17)							
						15 MIN		15 MIN			
189	R	>8	>6	12.5	147	1.84	4.3	54	4.5	7	0.99
190	R	14	7	11.5	150	1.72	4.6	53	8	5	1.00
191	R-N	12	7	9.5	145	1.38	5.1	48	10	5	1.00
192	N-R	12	7	10.5	130	1.36	6.7	70	17	6.5	1.02
193	R	14	8	7.5	139	1.04	6.6	49	8.5	5.5	1.00
194	R-N	10	7	7.5	129	0.97	6.1	46	19.5	4	1.07
F.S. 195	N	12	-	12.5	136	1.70	5.8	72	11.5	9	1.01
196	N	13	-	15.5	132	2.05	4.8	74	5	11	1.00
197	N	12	6	11.0	143	1.57	5.1	56	4	6	1.00
198(a)	R	15	7	6.0	135	0.81	5.1	31	8.5	3.5	-
198(b)	R-N-R	14	9	7.0	124	0.87	7.8	55	19	3.5	1.08
199	R-N	8	4	4.5	130	0.58	6.2	28	20.5	4	-
200	N	9	5	2.5	94	0.23	7.8	19	46	2.5	-
201	N	6	1	5.0	100	0.50	8	40	44	2.5	-
202	N	9	5	8.0	110	0.88	6.7	54	25	6	1.09
						30 MIN		30 MIN			
203	N	13	-	13)	134.5	3.36	4.0	100	9.5	8.5	1.06
204	R	14	6	12)							
205	R-N	12	-	12)	135.5	3.05	4.6	104	10	8	1.01
206	N	9	1	10.5)							
207	N	10	-	6.5)	127.5	1.99	6.6	102	19.5	5.5	1.03
208	N	9	2	9.0)							
209	N-R	9	-	7.5)	126	1.26	7.5	74	17.5	5	1.07
210	R-N-R	12	2	2.5)							
211	R-N	9	2	2.0)	82.5	0.25	8.3	25	52.5	2.5	-
212	N	7	0	1.0)							
213	N	>6	>2	3)	71.5	0.54	9.6	72	66	3.5	-
214	N	9	-	4.5)							
215	N-R	9	2	6)	118.5	1.42	6.1	74	23.5	5.5	1.09
216	R	14	1	6)							
217	R-N	9	1	4.5)	117.5	0.70	5.1	30	26.5	5	-
218	N	4	0	1.5)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total conc. m-equiv H^+/l	Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
219	N	4	2	2.5)	63.5	0.38	8.3	54	69	4	-
220	N	7	2	4.0)							
221	N	6	0	7.5)	119.5	1.70	6.1	88	22	7	1.08
222	N	10	1	7)							
223	R	13	3	5)	124	0.74	5.8	34	20	5.5	-
224	N	2	0	1.0)							
						15 MIN		15 MIN			
*F.S.											
226	N	10	-	3.5	-	-	7.2	25	71	3.5	
227	N	11	-	7.5	-	-	5.8	43	27.5	7	
228	N	8	0	4.5	-	-	3.8	17	16.5	5	
229	N	14	0	5.5	-	-	5.2	27	22.5	5	
230	N-R	9	2	3.4	120	0.41	5.8	20	20	4	-
231	R-N	4	0	1.2)	70	0.20/	7.0	21/	62	2	-
232	N	6	0	1.7)		30 MIN		30 MIN			
						15 MIN		15 MIN			
*SF											
233	N	10	-	3.5	59	0.21	10.6	37	74	2.5	-
234	N	11	-	8.8	120	1.06	7.5	66	16	7.5	1.06
235	N	-	-	6.0	134	0.80	4.0	24	7.5	6	1.02
						30 MIN		30 MIN			
236	N	-	-	0.9)	89	0.36	9.6	38	13.5	4.5	-
237	E	>12	-	3.1)							
238	E	25	-	7.8)	133.5	2.44	5.6	104	12.5	8.5	1.01
239	E	28	-	10.5)							
240	E	25	-	12)	142	3.41	4.5	108	4.5	9.5	1.00
241	E	>13	-	12)							
242	E	24	-	13)	147.5	3.91	4.0	106	3	11	0.98
243	E	25	-	13.5)							
244	E	22	-	12.5)	146.5	3.15	4.5	96	4.5	8	0.98
245	N	13	>5	9)							
246	N-R	14	9	9)	140.5	1.90	4.5	60	6.5	6.5	0.99
247	R	16	8	45)							
248	R	15	-	6.5)	134	1.81	6.1	82	13.5	5.5	1.02
249	E	20	-	7)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
250	E-N	6	-	4)	126	0.76	6.6	38	20.5	4.5	-
251	N	6	4	2)							
252	N	5	5	2.5)	82	0.49	9.3	58	52.5	2.5	-
253	N-E	10	-	3.5)							
254	E	23	-	9.5)	135	2.90	6.6	142	10.5	9.5	1.01
255	E-N	16	-	12)							
256	N	8	6	10)	144	2.52	4.8	84	4.5	6.5	1.00
257	N-R	14	9	7.5)							
258	R	17	9	8.5)	143	1.86	6.2	80	7	5	1.00
259	R-N-E	15	8	4.5)							
260	E	20	-	6.5)	135.5	2.10	6.9	106	13	6	1.02
261	N	9	5	9)							
262	E	22	-	13)	144.5	3.83	5.1	134	4	11	1.00
263	E	22	-	13.5)							
264	E	21	-	12.5)	144.5	2.60	4.3	76	3.5	8.5	0.98
265	N	8	6	5.5)							
266	N	9	5	7.5)	139.5	2.23	5.8	92	8.5	6.5	1.00
267	R	13	9	8.5)							
268	R-N-E	10	4	4)	132	1.98	6.1	92	15.5	5	1.02
269	N	7	3	11)							
270	R	13	7	11)	144.5	3.03	4.6	97	4.5	9	1.00
271	R	16	6	10)							
272	R-E	18	-	11.5)	143.5	3.52	5.8	142	4	10	1.00
273	E	25	-	13)							
274	E	22	-	14.5)	133.5	4.00	5.6	168	1	12.5	1.01
275	E-N	18	-	15.5)							
276	N	9	8	12)	146.5	3.59	3.8	93	2	9	0.99
277	N-R	10	4	12.5)							
278	R	15	9	12)	146	2.34	5.3	84	4	7.5	1.00
279	R-N	14	7	4)							
280	R	>12	>5	9.5)	145	2.90	6.7	134	5	7.5	1.00
281	R-N-E	22	-	10.5)							
282	E	29	-	15.5)	139	4.30	7.0	216	3	13.5	1.01
283	E	27	-	15.5)							
284	E	23	-	16)	142	4.69	4.8	158	4	11.5	1.01
285	E	22	-	17)							
286	E-N	16	-	13)	142	3.83	3.8	103	4.5	13	0.98
287	N	10	8	14)							
288	N-R	11	7	13.5)	141.5	2.62	5.1	54	8	8	1.00
289	R	16	10	5)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
290	R	18	9	11.5)	135	3.04	6.1	138	8.5	7	1.00
291	R	18	10	11)							
292	R-E	23	-	14)	141	4.09	6.1	176	5.5	12.5	1.00
293	E	26	-	15)							
294	E	26	-	15.5)	141	4.02	5.8	166	6	12	1.00
295	N	13	>7	13)							
296	N-R	15	8	13)	141.5	3.11	4.6	102	6.5	8.5	0.99
297	R-N	11	5	9)							
298	E	23	-	12)	154	4.31	6.6	184	8	14	1.02
299	E	28	-	16)							
300	E	25	-	16)	145.5	4.58	4.6	144	5.5	14	1.00
301	E	21	-	15.5)							
302	N	13	11	16)	141	4.44	4.0	126	4.5	11.5	1.00
303	N-R	12	9	15.5)							
304	R	17	8	14.5)	141	3.60	4.6	118	5.5	9	1.00
305	R	17	12	11)							
306	E	>14	-	13)	138.5	4.15	4.8	144	6.5	10.5	1.00
307	E	27	-	17)							
308	E	25	-	17.5)	143.5	4.95	4.5	156	4.5	13.5	0.99
309	E	25	-	17)							
310	N	15	>8	15.5)	144.5	4.05	3.5	98	5.5	10	0.99
311	N	10	6	12.5)							
312	N-R	14	11	12)	143.5	3.87	4.0	108	6.5	9	1.00
313	R	16	10	15)							
314	R-N	12	9	10)	142.5	3.21	4.5	96	8	7.5	1.00
315	R	14	10	12.5)							
316	R	16	8	14)	139.5	3.91	5.3	148	8	10	1.00
317	R-E	20	-	14)							
318	E	21	-	16)	139.5	4.60	5.1	168	6.5	13.5	1.00
319	N	10	7	17)							
320	N-R	13	10	15)	141	4.02	4.6	132	6.5	11.5	0.99
321	R	15	6	13.5)							
322	R-N-R	13	8	15)	142	4.19	4.3	126	6.5	9	0.99
323	R	16	11	14.5)							
324	R	15	10	13)	141	2.82	4.6	92	7	8	1.00
325	N-R	15	8	7)							
326	R	16	11	9)	141	2.54	5.8	104	8.5	5	1.00
327	R-N-E	14	8	9)							
328	E	26	-	16)	140.5	4.71	6.1	204	4.5	9.5	1.01
329	N-E	23	-	17.5)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
330	E	27	-	12)	140	4.27	4.5	138	5	13	1.01
331	E	26	-	18.5)							
332	E	24	-	14.5)	142	4.61	3.8	124	4.5	12.5	1.00
333	E	25	-	18)							
334	E	22	-	16)	142	4.83	3.2	108	5	11.5	0.99
335	N	15	-	18)							
336	N	12	9	21.5)	144	5.40	3.5	132	5.5	8.5	0.99
337	R	>9	>8	16)							
338	R	>16	>9	18)	141	4.80	5.1	174	4.5	10.5	1.00
339	R	19	12	16)							
340	R	17	11	14.5)	142.5	4.27	4.8	144	5.5	8.5	0.98
341	R-N-E	19	-	15.5							
342	E	27	-	18)	139.5	4.95	3.8	134	4	11.5	1.00
343	N-R	18	-	17.5)							
344	R	17	12	15)	142.5	4.28	4.5	134	4.5	8.5	0.99
345	R-N	14	8	15)							
346	N-E	21	-	15)	141	4.51	4.6	148	5	10	0.99
347	E	25	-	17)							
348	E	23	-	18.5)	142	5.25	3.8	140	5	12.5	0.99
349	E-N	17	>8	18.5)							
350	N	12	9	17)	144.5	4.55	3.8	120	4.5	9.5	0.98
351	R	17	11	14.5)							
352	R-N-R	12	9	14)	140.5	4.57	5.1	166	6	9.5	0.99
353	R	18	>10	18.5)							
354	R	17	10	17.5)	140.5	4.64	5.1	198	5	10.5	0.99
355	R-N	15	9	15.5)							
356	N-R	11	8	15)	138.5	4.36	5.1	160	6.5	9.5	0.97
357	R	14	9	16.5)							
358	R-E	22	-	19)	141	5.28	4.8	180	5.5	12.5	0.99
359	N	15	>7	18.5)							
360	N	8	6	14.5)	141.5	3.40	4.5	108	7	8	0.99
361	R	15	12	9.5)							
362	R	15	9	5.5)	127	1.71	5.8	78	18	3.5	1.02
363	R-N-E	12	>6	8)							
364	E	25	-	13.5)	132	3.43	6.1	158	9.5	9	1.00
365	N	15	>6	12.5)							
366	N	10	4	10)	138	2.55	4.5	84	12	5.5	1.00
367	R	12	10	8.5)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
368	R-N	13	>9	11)	142	3.27	6.2	142	3.5	12	0.99
369	R	18	>10	12)							
370	R	16	12	13.5)	138	3.86	4.5	126	9.5	9.5	1.00
371	N	13	>8	14.5)							
372	N-E	15	-	16)	137	4.52	4.5	148	8	12	1.00
373	E	>15	-	17)							
374	E	27	-	16.5)	137	4.66	4.3	146	7.5	11.5	0.98
375	E	>22	-	17.5)							
376	E	24	-	18)	142	5.25	3.8	140	5.5	12.5	0.99
377	E	24	-	19)							
378	E	27	-	18.5)	138	5.17	3.8	142	5.5	13	1.00
279	E	24	-	19)							
380	E-N	20	-	18.5)	142	4.97	3.5	122	4.5	12	0.99
381	N-R	17	13	16.5)							
382	R	18	13	18.5)	137	5.07	4.0	148	6	12	1.00
383	N-R	-	-	18.5)							
384	R	-	-	19.5)	140	5.18	4.5	166	5	12.5	0.99
385	R-E	-	-	17.5)							
386	E-N	21	-	17.5)	139	4.86	4.6	160	5.5	10.5	1.00
387	N-R	14	9	17.5)							
388	R	17	>11	16.5	137	2.26/ 15 MIN	4.6	76/ 15 MIN	5.5	11	0.99

* P.F. = prepare feed

F.S. = feed shown

S.F. = shown feed

TABLE 3

Experiment: 22/6

The intraduodenal infusion of 10 ml oleic acid over 30 min. The animal had access to feed before, during and after the infusion, new feed was changed 15 min after the infusion commenced (N.F.).

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/15 min	Pepsin conc/ ml rel.	Pepsin output/ 15 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
5	R-N	13	-	9.5	145	1.38	6.2	59	14	9	
6	N	14	-	9.0	143.5	1.29	6.4	58	15	8.5	
7	N	11	-	4.3	141	0.61	7.5	32	17	5.5	
10 ml oleic acid	new feed										
8	E	27	-	3.2	117	0.37	8.0	26	35	5	
9	E	13	-	1.6	125	0.20					
10	E-N	4	-	0.95	93	0.09					
11	N	0	5	0.25	73	0.02					
12	N	1	4	0.40	43	0.02	9.3	59/ 120 MIN	88.5	3.5	
13	N	0	8	0.70	-	-					
14	N	1	4	1.05	35	0.04					
15	N	0	1	0.70	8	<0.01					
16	N	0	1	0.65	6	"					
17	N	0	2	0.45	2	"					
18	N	0	5	0.30	2	"					
19	N	0	1	0.30	2	"					
20	N	0	1	0.20	2	"	8.0	26/ 105 MIN	140	1	
21	N	0	1	0.20	2	"					
22	N-E	5	-	0.95	2	"					
23	N	0	1	0.85	2	"					
24	N	0	0	0	-	-	-	-	-	-	
25	N	0	0	0	-	-	-	-	-	-	
26	N-E	10	-	6	2	0.01	7.7	46	138	1	
27	E-N	2	-	0.95	4	0.01)	7.7	15/ 30 MIN	142	1	
28	N	2	0	1.05	10	0.01)					
29	N-E	2	-	2.5	30	0.07)	9.9	54/ 30 MIN	106.5	2.5	
30	E-N	8	-	3.0	62.5	0.19)					

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total conc. m-equiv H^+/l	Acid output m-equiv H^+/15 min	Pepsin conc./ ml	Pepsin output/ 15 min rel.units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l
31	N	3	1	5.0	108	0.54	7.2	36	48	5.5
32	N	3	3	5.8	124	0.72	6.1	35	29	7
33	N-E	7	3	5.5	124.5	0.68	5.9	33	29	7.5
34	R	23	-	1.7	106.5	0.18				
35	R	20	5	1.05	77	0.09	12.8	110/ 45 MIN	47	7.5
36	R	18	6	5.8	102.5	0.59				
37	R-E	22	-	9.5	142	1.35)	11.0	225/ 30 MIN	11.5	14
38	E	25	-	11.0	147	1.62)				
39	E	24	-	12.0	150	1.80)	6.7	162/ 30 MIN	7	16
40	E	27	-	12.0	153	1.84)				
41	E-N	14	-	8.5	149.5	1.27)	5.9	125/ 30 MIN	8	14.5
42	N-E	16	-	12.0	153.5	1.84)				

Experiment: 27/6 - The intraduodenal infusion of 10 ml oleic acid over 30 min. The animal was fasted for 12 hr and fed 90 min after the infusion commenced (F).

3	R-N	16	5	8.0	133.5	1.07)	10.2	138/ 30 MIN	16	8
4	R-N	15	6	5.5	126.5	0.69)				
5	N	13	3	3.0	125	0.37	11.7	35		
6	N-R	13	6	5.5	112.5	0.62	11.2	62	25	4
7	R-N	16	4	2.5	115.5	0.29	11.2	28		
8	N	16	-	6.0	107.5	0.64	13.0	78	28	4.5
→ 10 ml oleic acid 9	N	15	-	4.0	118.5	0.47	11.7	47		
10	N	9	3	1.1	113.5	0.12	-	-	-	-
→ 11	N	9	-	1.2	76	0.09	-	-	-	-
12	N	6	-	0.75	52	0.04				
13	N	0	0	1.25	33	0.04	10.9	29/ 45 MIN		
14	N	1	0	0.65	17	0.01				
*FEED 15	N-E	27	-	3.3	12	0.04	13.9	46		
16	E	24	-	2.1	12	0.02				
17	E	20	-	1.45	12	0.02	14.6	73/ 45 MIN		
18	E-N	12	-	1.35	16	0.02				

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/15 min	Pepsin conc./ ml rel.	Pepsin output/ 15 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l
19	N	0	5	1.05	10	0.01				
20	N	0	5	1.35	6	< 0.01				
21	N	0	2	1.25	4	"	13.0	64/ 60 MIN		
22	N	3	1	1.35	3	"				
23	N	4	5	2.3	2	"				
24	N	1	3	1.7	4	"	12.5	75/ 45 MIN	90	1
25	N	1	2	2.1	8	0.02				
26	N	1	1	1.9	14	0.03				
27	N	0	0	1.5	16	0.02				
28	N	1	3	2.5	12	0.03	9.4	90/ 75 MIN	72	1
29	N	0	0	1.4	12	0.02				
30	N	0	1	2.3	12	0.03				
31	N	0	0	2.5	14	0.03	11.4	62/ 30 MIN	80	1
32	N	0	0	2.9	31	0.09				
33	N	1	1	3.5	58	0.20	9.8	34		
34	N-E	1	3	4.1	79	0.32	10.6	43		
35	E-N	4	-	4.9	92	0.45	8.5	42	44	3
36	N	1	0	5.3	108	0.57	8.0	42	31	4.5
37	N-E	10	-	6.1	114	0.69	6.9	42	25	5
38	E-N	5	-	4.3	116	0.50	6.7	29	23	4
39	N-E	17	-	1.9	100	0.19	12.5	110/ 30 MIN	38	4.5
40	E-N	25	-	6.9	92	0.63				
41	N-R-E	-	-	9.0	128	0.11	10.7	198/ 30 MIN	14	9
42	E	29	-	9.5	137	1.30				
43	N-E-N	28	-	10.5	137	1.44	10.1	106	10	9.5
44	E	28	-	11.0	143	1.57	10.2	260/ 30 MIN	8	11.5
45	E	28	-	12.5	141	1.76				

Experiment: 24/8 - The intraduodenal infusion of 10 ml oleic acid over 30 min. Feed was removed 15 min after the infusion began.

						30 MIN		30 MIN		
1	E	25	-	14.5)	147.5	2.94	10.1	202	10	8.5
2	E	22	-	5.5)						
3	E-N	14	11	14.5)	145	4.28	10.7	216	14.5	8.5
4	N-R	13	9	15)						

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total acid conc. m-equiv H^+/l	Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l
5	R	16	10	15.5)	146	4.68	10.1	324	7.5	12
6	R-E	22	-	16.5)						
7	E	22	-	16.5)	146.5	4.84	9.4	310	11	13
8	E	22	-	16.5)						
9	N	12	11	10.5)	141	3.60	8.2	208	6	8
10	N-R	12	9	15)						
11	R	15	9	16)	143.5	4.52	7.4	232	6.5	12
12	R	14	12	15.5)						
13	R-E	18	-	15.5)	144	4.68	10.2	332	7.5	11
14	E	23	-	17)						
15	E	23	-	17)	146.5	5.06	9.6	332	5	14
16	E-N-E	18	-	17.5)						
17	N	11	9	15	147.5	2.21/ 15 MIN	8.5	127/ 15 MIN	5	13
18	N	12	7	7.5)	136	1.90	13.9	194	21	4
19	N-R	12	9	6.5)						
20	R	13	9	12)	137	3.56	11.8	306	16.5	8.5
21	R	15	8	14)						
22	R	15	11	13)	143.5	4.02	12.8	358	10.5	10.5
23	R	14	9	15)						
24	N	11	8	15.5)	145.5	4.22	12.0	348	7.5	12
25	R	13	12	13.5)						
26	R	13	8	16)	147	4.34	12.6	372	7.5	11
27	R	14	11	13.5)						
28	R	14	10	13.5)	144	3.96	9.8	270	10.5	9.5
29	R	14	9	14)						
30	R	14	10	10.5)	138	2.90	13.4	282	13	7.5
31	R	14	10	10.5)						
32	R-N	14	-	15)	145.5	4.22	10.1	292	9.5	13
33	N-R	>10	>8	14)						
						15 MIN		15 MIN		
34	R-N	12	10	14	153	2.14	-	-		
35	N-R-N	14	11	14	151	2.11	10.2	143		
36	N	13	>8	12.5	150	1.87	9.1	114		
10 ml oleic acid 37	R	15	11	6.5	149	0.97	11.7	76		
38	N	8	8	0.7	122	0.08				
39	N	4	4	0.9	90	0.08	12.0	120/ 150 MIN		
40	N	0	1	0.9	56	0.05				

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/15 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l
41	N	0	1	0.7	35	0.02				
42	N	0	2	0.9	26	0.02				
43	N	0	0	0.8	24	0.02				
44	N	0	1	1.4	13	0.02	12.0	120/ 150 MIN		
45	N	1	3	2.2	4	0.01				
46	N	0	3	1.6	2	<0.01				
47	N	0	0	0.6	2	"				
48	N	0	1	2.5	2	"				
49	N	0	2	1.6	2	"				
50	N	0	1	1.0	2	"	11.2	80/ 75 MIN		
51	N	0	1	1.2	2	"				
52	N	0	2	0.9	2	"				
53	N	0	1							
54	N	0	0	POUCH INFUSIONS OF SALINE,						
55	N	0	0	ACID and						
56	N	0	0	ACID SECRETION						
57	N	0	1							
58	N	0	2							
59	N	0	0							
60	N	0	0	1.0	8	0.01				
61	N	0	0	1.5	6	"				
62	N	0	0	1.5	4	<0.01	8.2	481/ 60 MIN		
63	N	0	0	1.7	6	0.01				
64 FEED SHOWN	N	0	0	1.5	8	"	11.2	50/ 30 MIN		
65	N	0	0	3.0	26	0.08				
66	N	0	1	2.0	26	0.05	12.3	64/ 30 MIN		
67	N	0	0	3.3	32	0.11				
68	N	0	0	2.1	36	0.07	11.8	48/ 30 MIN		
69	N	0	0	2.0	41	0.08				
70	N	0	1	5.7	43	0.24	11.0	63/ 15 MIN		
71	N	0	0	3.3	49	0.16	11.0	82/ 30 MIN		
72	N	0	0	4.1	57	0.23				

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/15 min	Pepsin conc./ ml rel.	Pepsin output/ 15 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l
73	N	0	0	3.9	66	0.26	9.6	37		
74	N	0	0	4.5	67	0.30	9.0	40		
75	N	0	1	4.0	63	0.25	9.1	36		
76	N	0	0	4.9	73	0.36	9.0	44		
77	N	1	0	4.3	82	0.35	9.0	37		
78	N	3	0	3.0	64	0.19	11.2	58/ 30 MIN		
79	R	20	1	2.3	45	0.10				
80	R	29	5	3.7	58	0.21	11.7	43		
FEED 81	N-E	26	-	5.5	87	0.48	13.3	73		
82	E	-	-	10	125	1.25	13.4	134		
83	-	-	-	12	142	1.70	12.0	144		
84	-	-	-	12	147	1.76	13.3	160		

TABLE 4

Experiment: 14/9

The intraduodenal infusion of 2 ml oleic acid over 30 min.
The animal had access to feed before, during and after the infusion.

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/ min	Pepsin conc./ ml rel.	Pepsin output/ min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
						30 MIN		30 MIN			
1	-	-	-	14.5)	149	4.46	11.7	344	17.5	9.5	1.01
2	N	-	-	15.5)							
3	E	-	-	12.5)	139	3.12	14.4	346	22	7	1.05
4	E	35	-	11.5)							
5	E	27	-	6.5)	114	1.76	15.5	240	42	5	1.15
6	E	24	-	9)							
7	N	15	-	16)	140	4.68	13.0	436	16.5	12	1.00
8	N	14	12	17.5)							
9	E	20	-	16.5)	144.5	4.34	13.0	390	14	9.5	1.02
10	N	16	15	13.5)							
11	N	13	10	16.5)	144.5	4.12	13.0	370	16.5	7.5	1.03
12	N-E	14	>7	12)							
13	E	20	-	10)	129	2.96	14.1	352	26.5	5	1.10
14	E-N	15	-	13)							
15	N-R	13	-	15)	140	4.06	13.0	376	17.5	8	1.07
16	R	18	-	14)							
17	N-E-N	15	-	10)	133.5	3.26	13.0	318	23	7	1.08
18	N	>9	-	14.5)							
19	R	20	14	18)	142	5.04	12.3	436	12.5	11.5	1.00
20	R	17	-	17.5)							
						15 MIN		15 MIN			
21	N-E	16	-	18)	143.5	2.58	12.5	225	14	10	1.03
22	N-E	18	-	20	143	2.86	12.3	246	12	12.5	1.04
2 ml oleic acid 23	N	15	-	16.5	142	2.34	11.2	185	13	10	1.01
24	E-N	11	-	3.5	134	0.47	9.6	34	21	6.5	-
25	N	12	-	1.4	78	0.11	-	-	64.5	3.5	-
26	N	9	>7	1.4	47	0.07	-	-	-	-	-
27	N-E	15	-	2.0	25	0.05	12.5	25	-	-	-
28	N-E-N	14	-	3.8	14	0.05	15.2	58	126	1	-
29	N	11	9	6	56.5	0.34	12.0	72	84	3	-

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Acid output m-equiv H^+/ min	Pepsin conc./ ml rel.	Pepsin output/ min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
						15 MIN		15 MIN			
30	N	12	8	10	105.5	1.05	13.0	130	38	6.5	1.18
31	N	14	-	14.5	126.5	1.82	12.0	174	22.5	10	1.10
32	E-N-E	19	-	15.5	134.5	2.08	12.3	191	17.5	11	1.11
33	E-N-E	21	-	14	134	1.88	12.5	175	17.5	10	1.11

Experiment: 21/9 - The intraduodenal infusion of 2 ml oleic acid over 30 min. The animal had access to feed before, during and after the infusion.

						30 MIN		30 MIN			
5	E-N	18	-	18)	134	5.0	13.6	504	16	9	
6	E-N	16	14	19)							
7	N-E-N	15	>10	17.5)	136	4.82	13.6	482	15	8.5	
8	E-N	17	-	18)							
9	N	14	12	27	130	3.51/ 15 MIN	13.6	367/ 15 MIN	24	6	
10	N	14	8	18.5)	136	5.96	13.3	504	19	8	
11	N	14	8	18)							
12	N-R	-	-	20)	131	4.84	13.6	544	22	8	
13	R-N-R	>14	-	17)							
14	R-N	14	8	9.5)	110	1.60	14.6	212	34	4	
15	N	>9	>6	5)							
16	N	12	7	13.5)	118	3.60	13.6	414	31.5	5.5	
17	N	13	9	17)							
						15 MIN		15 MIN			
18	E	20	11	17	130	2.21	11.8	201	20	7	
19	E-N	19	14	17.5	130	2.27	12.0	210	19	7	
20	N	14	11	16.5	130	2.14	11.4	188	19	7	
2 ml oleic acid 21	N-R-N	14	11	13.5	130	1.75	11.4	154	21	7	
2 ml oleic acid 22	N	11	11	3	99	0.30	11.4	34	56	4.5	
23	N	11	11	2.8	59	0.16	12.8	36	81	3.5	
24	N	10	9	2.8	28	0.08	13.6	38	112.5	1.5	
25	E	19	-	3.4	17	0.06	13.4	46	114	1.5	

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/ min	Pepsin conc./ ml rel.	Pepsin output/ min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
						15 MIN		15 MIN			
26	E	19	-	3.0	4	0.01	13.3	40	126	1	
27	E-N	12	11	4.2	4	0.02	13.4	56	120	1	
28	N-R	16	11	8.5	62	0.53	13.4	114	75	4	
29	R-N-E	16	10	10	106	1.06	13.0	130	39	5	
30	E-N	20	-	14	115	1.61	12.0	168	29.5	7	
31	E	23	-	17	135	2.29	11.4	194	14.5	10	
32	E	-	-	18.5	142	2.63	12.0	222	14	10.5	

Experiment: 30/8 - The intraduodenal infusion of 2 ml oleic acid over 30 min. The animal had access to feed before, during and after the infusion.

						30 MIN		30 MIN			
1	R-E	23	-	8)	135	2.36	6.2	108	14	9.5	1.01
2	E	25	-	9.5)							
3	E	25	-	BLOCK 29)	142.5	6.20	4.3	190	8	11	1.00
4	E	25	-	14.5)							
5	E	22	-	13)	140	3.28	4.2	98	9.5	10	1.00
6	E	22	-	10.5)							
7	E	21	-	9.5)	138.5	3.72	4.2	100	12.5	10.5	1.00
8	E	19	-	14.5)							
9	N	15	12	13)	141	3.74	3.7	98	7.5	11	1.00
10	N	12	8	13.5)							
11	N-R	15	13	12.5)	140	3.50	6.4	166	8	10	1.00
12	R	13	12	13.5)							
13	R	14	11	11.5)	98.5	2.16	3.8	88	7	6	1.10
14	R	14	11	11.5)							
15	R	13	12	13.5)	87.5	2.58	3.2	94	5	7.5	1.20
16	N-R	14	12	16)							
17	R	12	12	14.5)	143	3.86	4.6	124	7	11.5	1.00
18	R	13	10	12.5)							
19	R	11	7	11)	142.5	3.20	4.6	104	9.5	7.5	1.00
20	N-R	12	7	11.5)							
21	R	12	9	12.5)	144.5	3.68	4.2	106	8	10.5	1.00
22	R	12	10	13)							
23	R	14	11	14)	58	1.60	2.7	74	4	6	1.37
24	R	14	8	13.5)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
25	R	13	13	13.5)	141	3.74	5.1	134	8	11	1.00
26	R	13	9	13)							
27	R	10	8	6)	130.5	1.24	5.4	52	18	7	1.04
28	N	8	7	3.5)							
29	N-R	13	11	8.5)	137	2.54	5.1	94	17.5	9.5	1.02
30	R	14	9	10)							
31	R	14	10	10)	140.5	2.94	4.6	96	11.5	8	1.00
32	R	14	9	11)							
33	R-N-R	12	8	11.5)	140	3.56	5.1	130	10	10.5	1.00
34	R	13	12	14)							
35	N-R-N	11	-	13)	146	3.64	6.7	168	11	12	1.00
36	R - N	>10	-	12)							
						15 MIN					
37	N-E	21	-	12.5	150	1.87)	4.6	118	8	9	0.95
38	E-N-E	25	-	13	149	1.94)					
2 ml oleic acid 39	E	24	-	12	150	1.80	7.4	89/ 15 MIN	10	11.5	0.92
40	E	23	-	2.3	133	0.31					
41	E	20	-	1.3	88	0.11	9.8	72/ 60 MIN	54	4	-
42	E	16	-	1.3	57	0.07					
43	E	17	-	2.1	52	0.11					
								15 MIN			
44	E-N	15	-	4.3	68	0.29	13.0	56	66	4.5	-
45	N	8	9	6.3	120	0.76	9.9	62	40	5.5	-
46	N	8	7	7	129	0.90	8.2	57	28	5.5	-
47	N-E	11	8	7	132	1.06	7.4	52	23	6	-
48	E	25	-	7.5	123	0.92	13.3	100	29.5	5	1.10
49	E-R	18	-	8	143	1.14	8.5	68	21.5	6	1.10
50	R	16	13	8	134	1.07	9.4	75	21.5	6	1.05
51	R	>11	-	5	135	0.67	9.8	49	25	5	-
52	E	25	-	7	124	0.87	12.3	86	31.5	6.5	1.15
53	E	20	-	11.5	139	1.60	8.2	94	17	10.5	1.02
						30 MIN		30 MIN			
54	R	15	12	13)	142	3.12	7.5	164	12.5	12	1.00
55	R	15	10	9)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
56	R	15	9	9)	136	2.72	9.0	180	14		
57	R-N-E	23	-	11)							
58	E-N	30	-	14)	140	4.06	7.4	214	8	15	1.00
59	N-E	21	-	15)							
60	E-N	21	-	16)	141	4.52	6.9	220	6	16	0.99
61	R	17	15	16)							
2 ml oleic acid 62	E-N	15	-	14)	153	4.16	5.9	160	6.5	14.5	0.98
63	N	14	13	13)							
						15 MIN		15 MIN			
64	N	11	10	5	150	0.75	5.6	28	11.5	7	-
65	N	11	10	5	122	0.61	8.6	43	27	4	-
66	N	12	>10	7	127	0.89	8.8	62	27	5	1.10
67	N	> 9	>8	8.5	137	1.16	7.7	65	18.5	6.5	1.02
								30 MIN			
68	N-R	>13	>11	7	139	0.97)	8.6	124	16.5	5.5	1.00
69	R-N-E	18	-	7.5	139	1.04)					
						30 MIN					
70	E	24	-	12)	139	3.40	7.7	188	12.5	9.5	1.01
71	E	26	-	12.5)							
72	E	23	-	11.5)	139	3.34	8.2	196	11.5	9.5	1.00
73	E	22	-	12.5)							
74	E	22	-	14)	141	3.94	6.2	174	8	11	0.95
75	E	19	-	14)							
76	E-N-E	23	-	14)	141	3.52	6.7	168	8	10	0.95
77	E-N-E	19	-	11)							

TABLE 5

Experiment: 1/3

The intraduodenal infusion of 2 ml oleic acid over 20 min in sheep S.D.M. The animal had access to feed before, during and after the infusion.

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/ min	Pepsin conc./ ml rel.	Pepsin output/ 60 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l
						30 MIN				
117	E	18		11.5)	136	3.2				
118	N-E-R	17		12)			11.5	484	19.5	8.5
119	R-N	15		8)	142	2.70				
120	R-N	11		11)						
FEED										
121	E	35		9.5)	171	3.4				
122	E	26		10.5)			12.3	624	17.5	8.5
123	E	22		14.5)	139	4.30				
124	E-N	21		16.5)						
						15 MIN				
125	N-E-N	21		9	123	1.1				
126	E-N	18		15.5	126	1.95				
2 ml oleic acid 127	N-E	13		15.5	137	2.12	13.6	708	15	9.5
128	N	13		14.5	135	1.96				
129	N	13		16.5)	135	2.23				
130	N-E	13		9.5	128	1.22	13.3	560	18	5.5
131	N	13		7.5	118	0.88				
132	N	> 8		8	115	0.92				
133	N	14		6.5	103	0.70				
134	N-E	14		11.5	124	1.36	14.7	352	26	5
135	E	19		5.5	122	0.67				
136	E	20		2	92	0.18				
137	N-E	17		3	40	0.12				
138	E-N	15		9.5	102	0.97	15.0	568	24	8.5
139	N	14		14.5	150	2.17				
140	N	16		13.5	140	1.89				

Experiment: 3/3 - Oleic acid infusion into the duodenum of sheep S.D.M. - 2 ml/20 min. Fed state.

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total acid conc. m-equiv H^+/l	Acid output m-equiv H^+/15 min	Pepsin conc./ml rel.	Pepsin output/60 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l
FEED										
168	E	>17	>9	14.5	143	2.07				
169	E-N	22	15	16	150	2.4	12.0	768	6.5	12
170	N	18	10	17	150	2.55				
171	N	17	10	17	156	2.65				
2 ml oleic acid										
172	N	15	10	14	157	2.20				
173	N-E	16	13	7.5	131	0.98	11.7	280	15	7.5
174	N-E	12	6	9.5	131	1.24				
175	N	12	6	4	123	0.49				
176	N	11	5	2.5	103	0.26				
177	N	13	9	5.5	84	0.46	15.4	260	48.5	4
178	N	10	6	4	101	0.40				
179	N	12	4	5	93	0.46				
180	N-E-N	12	7	6	111	0.67				
181	N	16	8	8.5	117	0.99	13.6	328	22	5
182	N-E-N	16	9	7	122	0.85				
183	N	15	8	3.5	114	0.40				
184	N-R	>8	>5	2.5	82	0.20				
185	R-E-N	15	8	5.5	99	0.54	15.5	464	38	6.5
186	E	16	8	7.5	100	0.75				
187	E-N	18	12	14.5	117	1.7				

TABLE 6

Experiment: 2/3

The intraduodenal infusion of 2 ml olive oil over 20 min in sheep S.D.M.
The animal had access to feed before, during and after the infusion

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 60 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l
144	R	16	8	12	142	2.54	10.2	572	12.5	10
145	R-N	15	6	13						
FEED										
146	E	29	18	15	131	4.06				
147	E-N	23	14	16						
148	E	24	17	17	141	4.80	11.0	704	8.5	11.5
149	E-N	20	16	17						
						15 MIN				
150	E-N	20	13	15	139	2.08				
151	N-E	19	11	15	138	2.07				
2 ml olive oil 152	E-N	17	11	16	137	2.19	11.0	660	7	8.5
153	E	19	12	15	138	2.07				
154	E-N	14	9	17.5	137	2.40				
155	N	16	8	11	138	1.52				
156	N-E-N	17	-	2	117	0.23	13.6	328	40	5
157	N	14	8	2	65	0.13				
158	Nib	14	8	7	73	0.51				
159	N	12	9	10.5	121	1.27				
160	N	>11	>5	13	131	1.70	10.6	552	10	9
161	N-E	14	11	16	134	2.14				
162	N-E-N	16	9	15	137	2.05				
163	N-E	16	12	9.5	133	1.26				

Experiment: 4/3 - Olive oil infusion into the duodenum of sheep S.D.M. - 2 ml/20 min Fed state.

FEED						
1	E	18	10	13.5	128	1.73
2	E-N	15	8	14	131	1.83
3	N	14	8	15	137	2.05
4	N	16	10	14.5	137	1.99

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/15 min	Pepsin conc./ ml rel.	Pepsin output/ 60 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l
2 ml olive oil 5	N	15	10	15.5	136	2.11				
6	Nib	16	9	15	139	2.08				
7	N	16	8	8.5	136	1.16				
8	N	15	9	2	118	0.24				
9	R-N	14	6	8	103	0.82				
10	N	12	7	10.5	130	1.36				
11	N-E-N	15	9	11	134	1.47				
12	N	14	7	12.5	138	1.71				
13	N	13	8	11.5	137	1.57				
14	N-E-N	15	7	10	134	1.34				
15	N	15	8	6.5	130	0.84				
16	N	>13	>6	9	126	1.13				
17	N-E	16	9	5	118	0.59				
18	E-N-E	20	12	11	127	1.40				
19	E	18	-	13	135	1.75				
20	E	20	15	12.5	137	1.71				

TABLE 7

Experiment: 6/9

The intravenous infusion of 2 ml oleic acid over 30 min.
The animal had access to feed before, during and after the infusion.

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total conc. m-equiv H^+/l	Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
1	N-R	18	14	17)	128	3.40	6.2	166	14.5	12	1.07
2	R	15	11	9.5)							
3	R	15	10	11.5)	124.5	3.12	6.4	160	19.5	6	1.07
4	R	15	10	13.5)							
5	N	13	10	14.5)	134	4.22	6.1	192	13	12	1.02
6	R	14	10	17)							
7	R	14	9	17)	136.5	4.58	6.9	232	8.5	12	1.01
8	R-E	18	-	16.5)							
9	E	24	-	17.5)	132	4.76	6.9	248	7	12.5	1.02
10	E	20	-	18.5)							
11	N	12	11	16)	139.5	1.85	5.6	150	10	10	1.00
12	N	9	7	10.5)							
13	R	12	10	7.5)	124	3.30	6.9	128	22.5	4.5	1.01
14	R	15	9	11)							
15	R	14	10	13)	123.5	3.20	5.9	154	17	7.5	1.04
16	R	14	9	13)							
17	R-N	11	8	15)	120	3.60	5.9	176	14	9	1.04
18	N	9	5	15)							
19	N	11	7	15.5)	126	3.90	5.1	158	12.5	10.5	1.02
20	N-R	11	7	15.5)							
21	R	14	7	6.5)	112	1.12	5.4	54	27	6	1.09
22	R-N	12	8	3.5)							
23	N-R	12	6	12.5)	107	2.88	5.9	160	23	8	1.10
24	R	13	7	14.5)							
25	R	13	6	13.5)	134	3.88	5.1	148	15	10.5	1.01
26	R	14	9	15.5)							
27	R-N	13	7	14)	114	3.20	5.0	140	11	9	1.05
28	R-N	13	6	14)							
29	N-R	12	7	14)	124	3.54	6.4	182	12.5	9	1.03
30	R	14	7	14.5)							
31	R-N-R	12	6	13.5)	127	3.36	6.1	164	13	9.5	1.01
32	R-N	13	7	13)							
33	N	8	3	6)	113.5	1.70	7.2	108	25	5.5	1.05
34	R	14	7	9)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
35	R	14	7	9.5)	120	2.34	6.4	126	18.5	5.5	1.02
36	R-N-R	14	6	10)							
37	N	14	-	15.5)	127	4.06	7.5	240	17	11	1.00
38	N	16	-	16.5)							
39	N	>7	-	13)	133	3.40	8.2	208	18	11.5	1.00
40	N	16	-	12.5)							
						15 MIN		15 MIN			
41	R	17	-	10.5	132	1.39	8.2	87	21	6.5	1.05
42	R-E	19	-	9.5	127	1.21	9.0	85	24	5.5	1.08
2 ml oleic acid IV	43 E-N	4	-	6.5	128	0.83	7.7	50	22	9	1.05
	44 N	0	-	3.1	114	0.35	14.1	96/ 30 MIN	54	5	1.18
	45 N	0	-	3.7	68	0.25					
	46 N	0	-	1.2	55	0.07					
	47 N-E	0	-	2.3	31	0.07					
	48 E-N	1	-	1.5	10	0.015	15.4	140/ 75 MIN	114	2	-
	49 E-N	8	-	2.1	10	0.02					
	50 N	4	-	1.8	2	0.01					
	51 N-E	13	-	2.5	2	0.01	10.4	62/ 30 MIN	132	1.5	-
	52 N	>6	-	3.5	2	0.01					
	53 N	4	1	1.9	2	0.01					
	54 N-E	12	-	1.3	2	0.01	10.9	66/ 45 MIN	126	2	-
	55 N-E-N	13	-	2.7	10	0.03					
								15 MIN			
	56 N	9	5	4.5	12	0.06	11.7	53	120	2.5	-
	57 E-N	16	-	6.5	77	0.50	8.8	56	58	5	-
	58 N-E	12	4	7.5	109	0.82	7.5	57	39	6	-
	59 E	20	-	10	115	1.15	7.5	75	30.5	6.5	1.10
								30 MIN			
	60 E	19	-	11	132	1.45)	7.2	168	18	7.5	1.08
	61 E	18		12	140	1.68)					
						30 MIN					
	62 E-N	14	8	11.5)	122.5	2.52	6.1	126	15.5	6	1.08
	63 N	11	6	9)							

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/30 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
64	N-E	15	9	8.5)	114.5	2.24	6.2	120	20.5	5.5	1.10
65	E	18	-	11)							
66	N	12	4	11.5)	121	2.66	5.4	118	16.5	6.5	1.05
67	N	11	3	10.5)							
68	N	11	5	12)	123.5	2.84	6.2	142	14.5	7	1.07
69	E	13	-	11)							

Experiment: 4/10 - The intravenous infusion of 0.5 ml oleic acid over 30 min. The animal had access to feed before, during and after the infusion.

						15 MIN		15 MIN			
1	E	23	-	14.5	148	2.15	14.1	204	4.5	10.5	0.99
2	E	23	-	14.5	146	2.12	14.9	216	6.5	8.5	0.99
3	N-E	23	-	13	142	1.85	16.0	208	11.5	6	1.00
4	E	20	-	13	144	1.87	15.0	195	8.5	9	0.99
5	N-E-N	20	-	15.5	145	2.25	14.2	220	5	11	0.99
0.5 ml oleic acid IV 6	N	8	-	10	147	1.47	13.8	138	3	12	0.98
7	N	0	-	0.20	150	0.03	-	-	-	-	-
8	N	4	-	0.25	148	0.04	-	-	-	-	-
9	N	7	-	2.7	107	0.29	15.2	41	-	-	-
10	E	21	-	3.5	86	0.30	17.5	61	52.5	6	-
11	E-N-E	21	-	7	74	0.52	20.0	140	84	4.5	1.30
12	N	16	16	7	81	0.57	19.2	134	62	3.5	1.29
13	N	14	12	3.5	63	0.22	19.2	67	82	1.5	-
14	N	13	9	4	39	0.16	18.4	74	96	1	-
15	N-E	24	-	5.5	35	0.19	18.7	103	112	1	-
16	N-E	21	-	14.5	108	1.57	18.4	267	50	7.5	1.12
17	N	>15	-	11	140	1.54	16.3	179	15.5	9	1.00
18	N	14	9	15.5	146	2.26	15.7	243	7	7.5	0.98
19	N	13	-	15	147	2.20	15.0	225	5	8	0.97
20	N	12	8	13.5	149	2.01	14.9	201	4	9.5	0.97
21	N-E	17	-	15	147	2.20	14.4	216	4	10	0.97

TABLE 8

Experiment: 15/7

The intravenous infusion of 50 mg of enterogastrone extract over 30 min. The animal had access to feed before, during and after the infusion.

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/15 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
1	-	-	-	9.5	142	1.35)	6.9	124	14	10.5	1.07
2	R-N	>7	>2	8.5	144	1.22)					
3	N-E	15	-	10	141	1.41)	6.9	142	12	10	1.10
4	N	14	-	10.5	144	1.51)					
10 ml saline 5	N	13	-	8	142	1.14)	7.2	108	13	8	1.02
6	N-E	18	-	7	136	0.95)					
7	E-N-E	18	-	6	136	0.82)	9.8	132	18	8.5	1.02
8	E	21	-	7.5	140	1.05)					
10 ml extract (50 mg) 9	E-N	12	-	0.70	142	0.1)	9.8	58	36	7.5	1.08
10	E	27	-	5.5	115	0.63)					
11	E	29	-	8.5	138	1.17)	6.4	104	16	9.5	1.01
12	E	27	-	8.5	144	1.22)					
13	E	27	-	10	147	1.47)	5.4	114	11.5	11	1.01
14	E	24	-	11	147	1.62)					

Experiment: 20/7 - The intravenous infusion of 215 mg of enterogastrone extract over 30 min. The animal was offered new feed 15 min after the infusion commenced.

1	R	>3	>2	9	142	1.28)	5.6	120	16.5	12.5	1.00
2	R	15	11	12.5	144	1.8)					
3	R-N	13	10	12.5	148	1.85))	5.1	124	9.5	11.5	1.00
4	N-R	15	9	12	148	1.78)					
Saline 5	R	16	10	10.5	150	1.57))	5.6	106	11.5	9.5	1.00
6	R-N	12	7	8	145	1.23)					
Extr. 7	N	11	8	10.5	142	1.49))	6.2	130	11.5	11	1.01
FEED 215 mg 8	N-E	26	-	12.5	145	1.81)					
9	E	28	-	11.5	145	1.67)	6.2	148	12.5	11	1.00
10	E	26	-	12.5	142	1.77)					

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/15 min	Pepsin conc./ ml rel.	Pepsin output/ 30 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
11	E-N-E	16	18	12	147	1.76)	5.0	68	9	12.5	1.00
12	N-E-N	15	13	1.5	139	0.21)					
13	N-R	14	10	4	100	0.40	10.2	41/ 15 MIN	48.5	4.5	-
14	R	17	11	9.5	134	1.27)	6.9	144	16.5	10	1.02
15	R-E	23	-	11.5	145	1.67)					
16	E	>23	-	13	146	1.90)	5.9	156	9.5	13	1.00
17	E-N	>14	-	13.5	151	2.04)					

Experiment: 1/9 - The intravenous infusion of 50 mg of enterogastrone extract over 30 min. The animal had access to feed before, during and after the infusion.

						30 MIN					
1	N-R	14	-	12.5)	130	2.8	8.5	182	21	10	1.08
2	N-E	25	-	9)							
3	E	27	-	12)	136.5	3.54	8.3	216	19.5	12.5	1.08
4	E	26	-	14)							
5	E	26	-	15.5)	142.5	4.42	8.3	258	10.5	13	1.02
6	E	>18	-	15.5)							
						15 MIN		15 MIN			
2 ml saline	7 E-N	22	-	17	139.5	2.37	9.9	168	10.5	14.5	1.11
	8 N-R	17	>14	16.5	141	2.33	10.1	167	9.5	16	1.02
	9 R	16	12	15.5	141	2.18	9.9	153	9.5	14.5	1.01
2 ml Extr. (50 mg)	10 R-E	20	>12	14.5	142	2.06	9.3	135	10.5	13	1.05
	11 N	5	>2	6.5	142	0.92	10.9	71	15.5	10.5	-
	12 N	7	6	1.2	96	0.11)	15.0	78/ 30 MIN	82.5	4.5	-
	13 N	10	10	4	55	0.22)					
	14 R	15	9	11	117	1.29	13.8	151	28.5	12	1.10
	15 R-E	21	-	13	143	1.86	11.7	152	16.5	14.5	1.08
	16 N-R	18	>11	13	147	1.91	10.6	138	13.5	13.5	1.09
2 ml Saline	17 R	16	9	10.5	134	1.41	10.6	111	17.5	9	1.08
	18 R	16	9	9.5	127	1.21	11.8	112	22	6.5	1.10
	19 R-E	20	-	7	119	0.83	13.3	93	30	6	-

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/15 min	Pepsin conc./ ml rel.	Pepsin output/ 15 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l	pH
20	E	24	-	11.5	125.5	1.44	13.9	160	24.5	9	1.10
21	E-N	18	-	14	137.5	1.92	11.8	165	14	14	1.09
22	N-R	16	12	15	139.5	2.09	11.5	172	10.5	14.5	1.07
23	R	>7	>6	13	139	1.81	11.2	146	11.5	12.5	1.05

TABLE 9

Experiment: 21/10

The intravenous injection and infusion of ICI 50,123
Animal fasted 16 hour

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min where meas.	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/15 min	Pepsin conc./ ml rel.	Pepsin output/ 15 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l
1	N	14	-	9	138	1.24	13.6	122	21.5	9.5
2	N	14	-	6.5	128	0.83	13.3	86	22	7.5
3	N	16	-	5	97	0.48	15.5	77	51	5
4	N	13	1	6.5	108	0.70	14.2	92	36	5.5
100 µg "Gastrin" IV (4 µg/kg)										
5	N-R-N	13	-	4	108	0.43	16.0	64	41	5
6	N	15	-	2	73	0.15	16.3	33	-	-
7	N	14	-	4	47	0.19	17.0	68	111	3.5
8	N	14	-	6.5	98	0.64	14.7	95	80	5
9	N	14	-	6	101	0.61	13.8	83	46	4.5
75 µg "Gastrin" IV (0.13 µg/kg/min) 10	N	2	0	3.5	106	0.37	12.2	43	41	6
11	N	7	0	2.5	60	0.15	11.7	29	52	4.5
12	N	14	-	2	18	0.04	13.4	27	122	1.5
13	N	16	-	6.5	64	0.42	15.4	100	90	4.5
14	N	15	-	6	109	0.65	14.1	85	40	6
Experiment: 24/10 - The intravenous infusion of ICI 50,123. Animal fed.										
1	N-E	33	-	10.5	130	1.36	13.3	140	18.5	10
2	E	37	-	11.5	130	1.49	13.8	159	17	10
3	E	27	-	12.5	127	1.59	14.7	184	19	10.5
4	E	25	-	12.5	133	1.66	14.7	184	13.5	12
40 µg "Gastrin" IV (0.08 µg/kg/min) 5	E	5	-	11	136	1.50	14.6	161	12.5	11.5
6	E	17	-	5.5	127	0.70	13.4	74	29	5.5
7	E	22	-	9	101	0.91	16.0	144	47	4
8	E	21	-	10	124	1.24	15.5	155	24	7

Sample (15 min)		Activity	Reticulum Contr. (Aseq) /15 min where meas.	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total conc. m-equiv H^+/l	Acid output m-equiv H^+/15 min	Pepsin conc./ ml rel.	Pepsin output/ 15 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l
20 µg "Gastrin" IV 0.04 µg/kg/min	9	E-N	14	-	11	134	1.47	14.7	162	16.5	7.5
	10	N-E	17	-	10.5	133	1.40	15.2	160	19	7.5
	11	E	25	-	5	118	0.59	17.0	85	33.5	4
	12	N-E	22	-	6	72	0.43	18.0	128	84	3
	13	E-N	20	-	10.5	124	1.3	15.5	163	27	7
	14	N	19	9	10.5	135	1.42	14.7	154	16.5	7.5
75 µg "Gastrin" IV (0.13 µg/kg/min)	15	N	3	5	3.7	120	0.44	15.5	57	28.5	4.5
	16	N	11	11	3.5	87	0.30	15.8	55	60	3
	17	N	20	6	3.0	29	0.09	17.3	52	117	1
	18	N-R-E	>17	>6	2.2	16	0.03	17.6	39	138	1
	19	E	22	-	8.5	79	0.67	16.5	140	78	4.5
	20	E	23	-	11	133	1.46	14.2	156	17	9
	21	E	21	-	12	136	1.63	13.8	166	14	10.5
5 ml NH_4^+ Soln. IV	22	E	21	-	13.5	138	1.86	13.3	179	12	12
	23	E	23	-	14.5	137	1.99	13.3	193	12.5	12.5
	24	E	23	-	15	138	2.07	12.5	187	12.5	13.5
	25	E	>11	-	12.5	137	1.71	12.3	154	14.5	14
Experiment:		28/10 - Animal fasted 16 hours. Intravenous infusions of ICI 50,123 and histamine.									
	4	N-R	19	7	12	121	1.45	13.0	156	26.5	10.5
	5	R-N	16	5	9	113	1.02	13.4	121	35.5	6.5
	6	N	14	4	5	93	0.46	13.3	66	62	5
	7	N	16	-	7	80	0.56	14.6	102	72	4.5
1.2 µg "Gastrin" IV (0.0008 µg/kg/min)	8	N	16	-	8	99	0.79	12.8	102	56	7
	9	N	15	4	8	111	0.89	11.7	94	41	8
	10	N	11	1	4.5	89	0.40	11.7	53	64	4.5
	11	N	15	4	4	47	0.19	12.8	51	100	2.5
	12	N	13	3	4.5	71	0.32	12.8	58	86	5
	13	N	13	4	4	60	0.12	12.2	49	90	3.5

Sample (15 min)		Activity	Reticulum Contr. (Aseq) /15 min where meas.	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total conc. m-equiv H^+/l	Acid output m-equiv H^+/15 min	Pepsin conc./ ml rel.	Pepsin output/ 15 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l
10 µg "Gastrin" IV (0.008 µg/kg/min)	14	N	15	2	7	63	0.44	12.2	85	92	4.5
	15	N	12	1	5.5	77	0.42	11.7	64	68	7
	16	N	11	3	3.5	61	0.21	12.2	43	84	5
	17	N	12	4	4.5	38	0.17	12.2	55	118	3
	18	N	12	3	6.5	66	0.43	12.2	79	88	5
	19	N	>7	-	4.5	68	0.31	10.4	47	80	4.5
	20	N	14	-	4	46	0.18	11.0	44	106	3.5
	21	N	14	-	7.5	78	0.58	11.7	88	76	6
	22	N	15	-	7.5	111	0.83	9.9	74	41	9
0.5 mg Histamine IV (0.5 µg/kg/min)	23	N	21	-	9.5	111	1.05	8.8	84	35	10.5
	24	N	13	-	10.5	124	1.30	5.6	59	20	14.5
	25	N	16	-	7.5	129	0.97	4.5	34	15	13
	26	N	15	-	2.5	81	0.20	12.0	30	64	6.5
FEED											
	27	E	51	-	8	85	0.68	14.2	114	64	9.5
	28	E	31	-	11.5	117	1.34	13.3	153	28	11.5

TABLE 10

Experiment: 9/11

Subcutaneous injections of ICI 50,123
Animal fasted 24 hour

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min where meas.	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total conc. m-equiv H^+/l	Acid output m-equiv H^+/15 min	Pepsin conc./ ml rel.	Pepsin output/ 15 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l
2	N	15	-	7	102	0.71	15.5	108	44	9
3	N	17	-	5.5	87	0.48	16.3	90	64	6
4	N	13	3	4	65	0.26	16.3	65	78	5
5	N	14	4	3.5	37	0.13	16.6	58	104	3.5
5 µg "Gastrin" SC (0.2 µg/kg)										
6	N	16	0	4.5	55	0.25	16.5	74	96	4.5
7	N	13	1	4.5	66	0.30	16.0	72	78	5
8	N-R-N	17	2	3.5	57	0.20	15.8	55	92	4.5
9	R-N	13	-	5.5	59	0.32	15.5	85	88	5
10 µg "Gastrin" SC (0.4 µg/kg)										
10	N	14	2	2	38	0.08	15.2	30	-	-
11	N	10	0	3.5	28	0.09	15.4	54	112	3
12	N	14	2	6.5	49	0.32	16.0	104	96	4
13	N	12	0	3.5	80	0.28	15.4	54	68	7
14	N	11	0	5.5	68	0.37	15.0	82	76	5
1 µg "Gastrin" SC (0.04 µg/kg)										
15	N	14	1	4.5	43	0.19	15.5	70	104	5.5
" → 16	N	14	1	4.5	76	0.34	15.4	69	70	8
" → 17	N-R	16	-	5	70	0.35	15.4	77	74	5.5
" → 18	N	12	-	2.5	42	0.10	15.0	37	94	3.5
19	N	13	-	4.5	45	0.20	15.2	68	96	5
20	N	>9	-	3.5	44	0.15	14.7	51	98	4
0.5 µg "Gastrin" SC (0.08 µg/kg)										
21	N	13	-	4.5	40	0.18	15.4	69	106	3.5
22	'R'	15	-	2.5	51	0.13	14.7	37	96	4.5
FEED 23	E	56	-	4.5	33	0.15	16.0	72	112	5

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min where meas.	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total conc. m-equiv H^+/l	Acid output m-equiv H^+/15 min	Pepsin conc./ ml rel.	Pepsin output/ 15 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l
24	E	40	-	8	85	0.68	15.5	124	64	8
25	E	29	-	5	89	0.44	15.4	77	52	8
26	E	26	-	5.5	85	0.47	14.6	80	62	7.5
27	E	26	-	8.5	97	0.82	13.4	139	50	11
28	E	24	-	8.5	101	0.86	14.2	121	38	13
29	E	26	-	7.5	102	0.76	14.2	106	38	13
30	E	24	-	7	98	0.69	13.4	94	44	10
31	E	20	-	6	86	0.52	14.6	88	66	7.5
32	E	25	-	6.5	90	0.58	14.2	92	54	8.5

TABLE 11

Experiment: 31/10

The intraduodenal infusion of 10 ml oleic acid over 60 min and the subsequent intravenous infusion of ICI 50,123 and histamine in an animal fed ad lib.

Sample (15 min)		Activity	Reticulum Contr. (Aseq) /15 min where meas.	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/15 min	Pepsin conc./ ml rel.	Pepsin output/ 15 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l
1		-	-	-	11	109	1.2	12.5	137	46	10.5
2		N	-	-	8.5	86	0.73	12.8	109	66	5
3		N	22	-	6	72	0.43	13.3	80	82	4.5
10 ml oleic acid ID (0.067 ml/kg/min)	4	N	17	-	2.5	37	0.09	12.5	31	106	4
	5	E	44	-	4.5	14	0.06	13.3	61	138	2.5
	6	E	21	-	7	2	0.01	8.5	59	156	2
	7	E	13	-	3.5	2	0.01	7.4	26	150	2
8		N-E	12	-	3	2	"	6.1	18	156	2
9		E-N	5	>10	4	2	"	7.2	29	156	2
10		N	0	12	4	2	"	7.4	30	156	2
11		N	0	7	3.5	2	"	7.2	25	156	2
9 µg "Gastrin" IV (0.02 µg/kg /min)	12	N	0	5	3.5	2	"	7.4	26	156	2
	13	N	0	5	4	2	"	9.4	38	156	2
14		N	1	3	2.5	2	"	8.6	21	154	2
15		N	0	6	2.5	2	"	8.2	20	-	-
16		N	1	5	6.5	2	0.01	9.4	61	154	2
17		N	1	3	3.5	2	0.01	8.5	30	154	2
18		N	0	4	3.5	2	"	6.9	24	154	2
0.5 mg Histamine IV 1 µg/kg/min	19	N	0	5	3.5	2	"	6.4	22	148	2.5
	20	N	3	-	10.5	79	0.83	8.0	84	72	7
21		N	0	2	4	100	0.40	8.5	34	48	8.5
22		N	0	-	5	44	0.22	12.2	61	88	4.5
23		N	1	-	5	35	0.17	13.0	65	100	3
24		N	4	-	6	43	0.26	12.3	74	96	3
25		N	10	3	5	46	0.23	12.2	61	96	3.5

Sample (15 min)	Activity	Reticulum Contr. (Aseq) /15 min where meas.	Rumen Contr. (Bseq) /15 min where meas.	Volume ml/15 min	Total Acid conc. m-equiv H^+/l	Total Acid output m-equiv H^+/15 min	Pepsin conc./ ml rel.	Pepsin output/ 15 min units	Na^+ conc. m-equiv /l	K^+ conc. m-equiv /l
FEED SHOWN										
26	N	>19	-	8	43	0.34	12.3	98	98	3
27	N	13	1	3	8	0.02	15.2	46	128	2
28	N	12	4	3	2	0.01	15.2	46	132	1.5
29	N	10	1	6	12	0.07	16.0	96	128	2
FEED										
30	E	33	-	10.5	81	0.85	14.1	148	62	7.5
31	E	26	-	8	105	0.84	13.6	109	40	7
32	E	22	-	12	99	1.20	13.4	161	44	7
33	E	>5	-	14.5	121	1.75	12.8	186	30	11.5